ISHAM 2009 Tokyo

ABSTRACTS

PR-01

Congratulatory Remarks on the Opening of the Congress and JSMM Award Ceremony

Hideoki Ogawa
ISHAM2009 in Tokyo Congress President / CEO & Professor Emeritus, Juntendo University, Tokyo, Japan

Representatives, Ladies and Gentlemen, Our dear friends,

On behalf of the Organizing Committee of the 17th Congress of ISHAM 2009 in Tokyo and as the Congress President of the 53rd Annual Meeting of the Japanese Society for Medical Mycology (JSMM), I would like to say many thanks to all who came from numerous parts of the world to join this meeting here in Tokyo. We are very happy and honored to organize such a big medical mycology congress entitled "Medical Mycology in the 21st Century" on this special occasion. As you know, our JSMM has more than 1,000 members mainly inside Japan, but including some from Korea, China etc. In this year, our JSMM has only the General Assembly. We will not hold an independent academic meeting besides the ISHAM meeting. It means our JSMM meeting is included in the ISHAM Congress in 2009. On this special occasion, as the Congress President of JSMM and ISHAM 2009 in Tokyo, I am very happy to show sincere appreciation to the respected scientists who lead the development of medical mycology in the world. In this meaning, we set up a short and smart ceremony to give certificates of appreciation to the past ISHAM Presidents and past Congress Presidents of ISHAM. We are very happy to invite Prof. Urabe Harukuni, the President of ISHAM 1985-1987, Prof. Frank C. Odds, the President of ISHAM 1991-1993, Prof. David H. Ellis, the 12th Congress President of ISHAM 1994, Prof. Johannes Müller, the President of ISHAM 1994-1996, Prof. Luciano Polonelli, the 13th Congress President of ISHAM 1997, Prof. Ricardo Negroni, the 14th Congress President of ISHAM 2000, Prof. Michael G. Rinaldi, the 15th Congress President of ISHAM 2003, Prof. David W. Warnock, the President of ISHAM 2004-2006, and Prof. Bertrand F. Dupont, the 16th Congress President of ISHAM 2006 to receive this certificate. I am honored to first present the certificate of appreciation to Prof. H. Urabe, Prof. F. C. Odds, Prof. D. H. Ellis, Prof. J. Müller and Prof. L. Polonelli. Then after that, Prof. WATANABE Shinichi, President of JSMM will give the certificate of appreciation to Prof. R. Negroni, Prof. M. G. Rinaldi, Prof. D. W. Warnock and Prof. B. F. Dupont. I believe that participation by these distinguished colleagues in ISHAM 2009 will be a significant contribution to the success of the Congress in Tokyo.

PROFILE

Juntendo University (M.D. 1966)/Professor & Chairman, Dept. of Dermatol. (1981-2004)/Dean (1996-2000)/President (2000-2008)/CEO (2004-)
Vice President of ISHAM (1994-1997)/Vice President of ISD-Int. Society of Dermatol. (1994-1999)/President of APSMM-Asia Pacific Society for Medical Mycology (1997-)/President of ISHAM 2009 in Tokyo
Guest Professor/ Duke University , Beijing Medical University etc.
Honorary Member/ADA-American Dermat. Asso./SID/Bri. Asso. Dermat., German Soc. Dermat., Korean Dermat. Asso., Chinese Med. Asso.
Award/ Commander Knight, Thailand (1992)/Honorary Citizenship, Italy (2001)
Editor/ Founding Editor in Chief, J. Dermatol. Sci. (Amsterdam) etc.
Chief Organizer of Int. Dermat. Training Course (for 30 Asian countries)
Publications in English/ 595 (Total Impact Factor: 1,072)

PR-02

Dangerous black fungi are all around us: How come we are still alive?

Sybren de Hoog
ISHAM President, Centraalbureau voor Schimmelcultures Fungal Biodiversity Centre, The Netherlands

Melanized fungi are well-known for causing brain infection, and often take a fatal course. Contrary to expectations, some of these species, although generally taken to be opportunists, are recurrently found in patients without detectable immune disorder or underlying disease. In the phylogeny of the fungal Kingdom these fungi belong to a limited number of groups: frequently the order of black yeasts, the Chaetothyriales is concerned, with Cladophialophora bantiana and Exophiala dermatitidis as main agents. The predilection of these species is different. While C. bantiana nearly always has a cerebral location, E. dermatitidis mostly disseminates with severe mutilation and pronounced neurotropism. Another order, with a second disease category, is found in the Microascales: fungal brain infection resulting from aspiration of polluted water and temporary coma following a near-drowning event. The respective patient category frequently develops a delayed cerebral abscess by Scedosporium species.

In clinical practice, fungal brain infection is difficult to diagnose. The fact that a very small number of species is concerned, which also has a more or less pronounced anamnesis and clinical course, enhances early suspicion of a fungal infection, and enables empirical antifungal therapy. Novel tools for in situ detection are being developed. The sources and routes of transmission of melanized fungi is still poorly understood. Species of black yeasts and allies differ considerably in predilection and virulence. Environmental isolation of some of the species thus far has been successful only by using an animal bait; strains isolated from the environment by direct planting are mostly different from the ones causing infection in the human host. These data suggest that the order Chaetothyriales is in an early phase of pathogenic adaptation to the vertebrate host.

PROFILE

Sybren de Hoog (1948) is senior researcher in at the Centraalbureau voor Schimmelcultures in Utrecht and professor at the Institute of Biodiversity and Ecosystem Dynamics of the University of Amsterdam, The Netherlands and the Research Center for Medical Mycology, Peking University, Beijing, China. He has written over 360 refereed papers, in addition to the (co-)editing of symposia on yeasts, yeast-like and medical fungi in Amersfoort (1987), Atlanta (1992), Adelaide (1993), Vancouver (1994), Veldhoven (1996), Utrecht (2006) and Angers (2007). He was program chairman of the TIFI/ECMM congress in Amsterdam (2003). He has prepared an Atlas of Clinical Fungi (with J. Guarro, Reus, Spain), for which a third edition on CD-ROM appears at this congress, and many chapters on filamentous yeasts for the book "The Yeasts" (eds C.P. Kurtzman et al.). He worked several months as a guest professor at the Research Center for Pathogenic Fungi in Chiba, Japan. Currently he is President of the International Society for Human and Animal Mycology (ISHAM). His teaching activities comprise the international CBS Course Medical Mycology for hospital personnel, and several courses for medical biology students.

OK-01

Intrinsic heteroresistance of *Cryptococcus neoformans* to azoles: A stress survival mechanism of the fungus

Kyung J. Kwon-Chung
Molecular Microbiology Section, Laboratory of Clinical Infectious Diseases, NIAID, NIH, Bethesda, MD, USA

In 1999, Mondon et al described heteroresistance to fluconazole in two strains of *C. neoformans* isolated in 1998. Heteroresistant strains produced cultures with heterogeneous compositions in which most of the cells were susceptible but some were highly resistant to fluconazole (MICs, $\geq$ 64 ug/ml) and were recovered at variable frequencies. Highly resistant homogeneous populations can be obtained by subculturing resistant clones on media with the drug while homogeneous populations of sensitive cultures could never be obtained. Furthermore, the highly resistant cultures derived from a resistant subpopulation are lost during repeated transfer on drug free media without losing the basal subpopulation of resistant cells. Whether this type of unusual resistance to azoles is intrinsic or acquired through exposure to azole drugs has not been conclusively determined. In order to determine whether heteroresistance of *C. neoformans* to azoles is intrinsic, we screened 102 strains of serotype A, 28 strains of serotype D, 21 strains of serotype B and 10 strains of serotype C from patients that were isolated at least 10 to 20 years prior to the birth of azole drugs or from the environment. Surprisingly, all strains tested manifested heteroresistance to fluconazole which indicated heteroresistance to be intrinsic in *C. neoformans*.
We have identified different categories of heteroresistance based on the duration of serial passages required in the drug free media to achieve loss of resistance in the heteroresistant strains. We also observed that the ability of resistant subpopulations to grow on high concentrations of fluconazole is directly associated with chromosomal duplication events. Heteroresistance in clones capable of growing in the presence of fluconazole evolve gradually with aneuploidy in multiple chromosomes. These aneuploids become haploid upon release from the drug stress. These findings suggest that heteroresistance is a mechanism by which *C. neoformans* overcomes the stress exerted by azoles.

PROFILE

Education

1952-1958 Ewha Womans University, Seoul, Korea. Bachelor and Master of Science (Biology)
1961-1965 University of Wisconsin, Madison, Wisconsin. MS and Ph.D. received August 1965

Appointment

1973-1995 Senior investigator, Laboratory of Clinical Investigation, NIAID/NIH
1995-present Head, Molecular Microbiology Section, Laboratory of Clinical Infectious Diseases, NIAID/NIH

Honors and Awards

1977 NIH Director's Award
1978-1979 Chair, Medical Mycology Division, American Society for Microbiology
1982 International Society for Human and Animal Mycology (ISHAM) Award
1982 Lucille George's Award, International Society for Human and Animal Mycology
1982-1985 Vise president, International Society for Human and Animal Mycology
1996 Rhoda Benham Award, Medical Mycological Society of Americas
1995-2001 Burroughs Wellcome Fund, Molecular Pathogenic Mycology Advisory committee

Bibliography

243 peer-reviewed publications; 50 invited publications; 2 Medical Mycology books

OK-02

Dancing with fungus

David Ellis
Mycology Unit, SA Pathology at the Women's and Children's Hospital, Adelaide, Australia

Over the past 20 years medical mycology has undergone rapid changes on a global basis, largely as a result of the dramatic increase of opportunistic fungal infections associated with AIDS and other immunosuppressed patients. The "Great Toe Nail Wars" between terbinafine and itraconazole came to a climax in the 1999 with the publication of the LION study with Glyn Evans as the lead author. However controversy still continues with the taxonomy of the dermatophytes. These fungi have been studied intensely for over 100 years and many species and varieties have been described based on numerous phenotypic characters, many of which may overlap. On the basis of recent molecular studies, I suspect that we may have over classified many dermatophytes, in fact, there may only be a handful of species? The "Living Petri Dish" a term coined by Mike Rinaldi aptly described AIDS patients and the prevalence and diversity of fungal infections associated with them; in particular the yeasts *Candida* and *Cryptococcus*. Fortunately, for many patients, this coincided with the release of fluconazole in the early 1990's, which drove the rapid expansion of mycology into the clinical arena. In this decade, we have witnessed the full impact of the haematology and transplant patient, the 'White Cell Wipe Out' group, with the concomitant emergence of non-albicans yeasts and the dominance of the moulds, like *Aspergillus*, *Scedosporium* and *Fusarium*. We have seen the introduction of new antifungals, like voriconazole, posaconazole and the echinocandins, of new rapid non-invasive diagnostic tests, like glactomannan and PCR, of new imaging technology, like HRCT, MRI and PET scanners, and the publication of new consensus treatment guidelines and protocols. In the laboratory, we are currently undergoing a transition between morphological to molecular identification, with an ever-changing fungal taxonomy and greater demand for antifungal susceptibility testing. This conference will highlight the many challenges facing us in the diagnosis and management of fungal infections. We live in an exciting time for medical mycology.

PROFILE

Associate Professor David Ellis is Head of the Mycology Unit at SA Pathology, Women's and Children's Hospital, Adelaide and an Associate Professor in the School of Molecular and Biomedical Science at the University of Adelaide. He graduated from La Trobe University Botany Department with BSc Hons, MSc and PhD in mycology and has been working in Medical Mycology for the past 30 years. David is an Honorary Fellow of the Royal College of Pathologists of Australasia, and a Fellow and past President of the Australian Society for Microbiology. He is the current President-elect of ISHAM. His current research interests include the epidemiology and ecology of medically important fungi, especially *Cryptococcus*, fungal taxonomy and antifungal susceptibility testing.

KL-01

The *Candida* cell wall: Biosynthesis, immune recognition and adaptation to stress

Neil A.R. Gow
School of Medical Sciences, Institute of Medical Sciences, University of Aberdeen, UK

The fungal cell wall is a dynamic organelle that plays major roles in defining their shapes, and through interactions with other cells, their ecologies and life styles - including pathogenesis. The core components of the cell wall are shared by most fungal species and consequently the immune system has evolved to enable detection of these molecules to induce protective responses. In *Candida albicans* the cell wall and septal cross-walls are composed of a robust chitin-glucan inner skeleton to which an outer shield of highly glycosylated mannoproteins at attached. The robustness of the skeleton is explained in part by the fact that it can be rebuilt and strengthened under conditions of cell wall stress. For example echinocandins induce a protective response that results in a compensatory increase in the cell wall chitin content. We have shown that this increase in chitin can offset the efficacy of echinocandins in a variety of *Candida* species. Under stress conditions the activation of cell wall salvage signalling pathways can also lead to the assembly multiple forms of septa that will enable cell division to continue. The regulation of this process may involve direct phosphorylation of chitin synthase. Cells with high amounts of chitin in their wall are not only physically protected from damage, they seem also to become less recognisable by the surveillance activities of cells of the innate immune system. This complements emerging evidence that has shown that all the major components of the *C. albicans* the cell wall may be recognised, both singly and in combination, by the immune system. However, while some components stimulate immune recognition others attenuate or block it. Therefore the dynamic nature of the cell wall makes it a moving target for the immune system and for clinical intervention.

PROFILE

Professor Gow is a current Vice-President of ISHAM former British Mycological Society President and editor in Chief of the journal Fungal Genetics and Biology. He graduated with a B.Sc. from Edinburgh University and a Ph.D. from Aberdeen Univerwsity. He was a research fellow in Denver, before returning to Aberdeen as a faculty member in 1984. He is a founding member of the Aberdeen Fungal Group that constitutes one of the largest academic centres for medical mycology in the world and is a fellow of Institute of Biology, the Royal Society of Edinburgh and the American Association of Microbiologists. His research is focussed on: (i) the molecular genetics of cell wall biosynthesis in pathogenic fungi - in particular the genetics of glycosylation and the fungus-host interaction in relation to innate immune recognition, (ii) chitin synthesis and the response to antifungal agents; (iii) directional growth responses of fungal cells; (iv) the virulence properties of medically important fungal species; (v) the evolution, genome biology and genotyping of *Candida* species. He has published over 200 research papers and reviews in these areas.

KL-02

The roles of C-type lectins in the host defense against fungal infection

Yoichiro Iwakura
Center for Experimental Medicine, Institute of Medical Science, University of Tokyo, Japan

The C-type lectins form a group of proteins with a lectin-like carbohydrate-recognition domain (CRD) in their extracellular carboxyterminal domains. Some C-type lectin family members recognize the carbohydrate structures of microbes as pathogen-associated molecular patterns in a calcium-dependent way. Dectin-1 was first reported as a dendritic cell (DC)-specific C-type lectin receptor, and is also highly expressed on macrophages and neutrophils. Dectin-1 has a CRD in its extracellular carboxyl terminus and an immunoreceptor tyrosine-based activation motif (ITAM) in its intracellular amino terminus, and is known as the receptor for β-1, 3-linked and/or β-1, 6-linked glucans (β-glucans) found in the cell walls of fungi. Thus, we have generated dectin-1-deficient mice to determine the importance of this molecule in the defense against pathogenic fungi. *In vitro*, β-glucan-induced cytokine production from wild-type dendritic cells and macrophages was abolished in cells homozygous for dectin-1 deficiency ('dectin-1-knockout' cells). *In vitro*, dectin-1-knockout mice were more susceptible than wild-type mice to pneumocystis infection, even though their cytokine production was normal. However, pneumocystis-infected dectin-1-knockout macrophages did show defective production of reactive oxygen species. In contrast to those results, wild-type and dectin-1-knockout mice were equally susceptible to *Candida* infection. Thus, dectin-1 is required for immune responses to some fungal infections, as protective immunity to pneumocystis, but not to *Candida*, required dectin-1 for the production of antifungal reactive oxygen species. I will discuss the roles of C-type lectins in the host defense against fungal infection and the homeostasis of the immune system.

PROFILE

Graduated from the Graduate School of Science, Kyoto University, Kyoto, in 1974, and became a research associate of the Institute of Virus Research, Kyoto University. Associate researcher at Sloan-Kettering Institute for Cancer Research, NY, from 1978 to 1980. Moved to Institute of Medical Science, University of Tokyo, in 1985, and became a professor in 1992. Working on the generation and analysis of animal disease models, especially of rheumatoid arthritis and AIDS, using gene targeting and embryo manipulation techniques.

KL-03

Immunologic risks for fungal infections: Translating knowledge into targeted prevention strategies

Kieren A. Marr
Johns Hopkins University School of Medicine, Baltimore, MD, USA

For several decades, focus has been directed on developing strategies to prevent invasive fungal infections (IFI) in immunosuppressed and hospitalized patients, by providing antifungal drugs as prophylaxis (to everyone at risk), empirically (for treatment of fever), and pre-emptively (directed by results of laboratory testing). Success has been seen in preventing Candida infections, and more recently, Aspergillus infection, especially with use of reasonably-well tolerated azole antifungals. However, there remain limitations to each strategy, with cumulative toxicities and drug interactions ultimately leading to complicated, and sometimes, poor outcomes. One of the problems with developing these strategies is that we are using drugs to prevent complications that have reasonably low prevalence; this effectively 'caps' the positive predictive value of any testing strategy or predictive formulas. Thus, one way to optimize prevention strategies is to better target the population with the highest risks. In the last years, results of several studies have started unraveling the mysteries of why some people develop IFI, outlining pathogen-specific immune reconstitution and inherited factors that dictate innate immunity. This talk with summarize recent developments, with a focus on how this knowledge may be harnessed in development of future strategies to prevent IFI in immunosuppressed and hospitalized people.

PROFILE

Kieren A. Marr received medical training at Hahnemann University, Pennsylvania, Duke University, and the University of Washington. She was faculty at the Fred Hutchinson Cancer Research Center and Oregon Health and Science University before relocating to Johns Hopkins University in 2008, where she currently directs the Transplant and Oncology Infectious Diseases Program. Dr. Marr holds membership in the Infectious Disease Society of America, International Society for Human and Animal Mycology, American Society of Transplantation, and several other professional societies. She serves on the editorial boards for several journals is a faculty contributor for Faculty of 1000 Medicine and an editor for Up to Date in Infectious Diseases. Dr. Marr is well published, with articles in journals that include the *New England Journal of Medicine, Blood,* and *J Immunology*. She has written chapters and edited several books. Active in research and research training, Dr. Marr has served as the principal investigator for several federally and commercially supported studies. She has won numerous awards, most recently, membership to the American Society for Clinical Investigation.

SL-01

A gift from nature: The birth of statins

Akira Endo
Biopharm Research Laboratories. Inc., Tokyo, Japan

Coronary heart disease (CHD) is the major cause of death in modern, developed countries and an increasing cause in developing countries. Elevated blood cholesterol levels are one of the major risk factors for CHD. In the mid-1970s, after testing 6,000 types of fungi by hand, we discovered ML-236B (now known as compactin) from the fungus *Penicillium citrinum* as a potent inhibitor of cholesterol synthesis. Compactin proved to be a selective, competitive inhibitor of 3-hydroxy-3-methylglutaryl (HMG) CoA reductase, the rate-limiting enzyme in cholesterol synthesis. Subsequently, after animal studies with rats, laying hens, dogs and monkeys, we showed that compactin was extremely effective in lowering plasma cholesterol levels in patients with severe hypercholesterolemia with no serious side effects. These results paved the way for the worldwide development of compactin's analogs (collectively called "statin"), and by year 2000, seven statins have been approved in many countries. Fourteen large-scale clinical trials involving 90,000 middle-aged adults for 5 years showed that statins lowered LDL cholesterol levels by 25-35% and reduced the incidence of heart attack by one-third. No major harmful effects of lowering cholesterol were observed in any of these studies. It is estimated that millions of people have extended their lives through statin therapy. Today, an estimated 30 million people worldwide are taking statins. Statins are the largest selling class of drugs currently taken by patients throughout the world. Sales for this one class in 2007 were $34 billion.

PROFILE

Akira Endo, born in 1933, obtained a BA at Tohoku University (Faculty of Agriculture) in Sendai in 1957 and a PhD in biochemistry at the same university in 1966. From 1957 to 1978 he worked as a biochemist at Sankyo Co. He spent two years (1966-1968) at the Albert Einstein College of Medicine in New York as a research associate. From 1979 to 1997 he worked as an associate professor (1979-1986) and later a full professor (1986-1997) at the Tokyo University of Agriculture and Technology (TUAT), and after official retirement, besides becoming the director of Biopharm Research Laboratories Inc., he serves as a Professor on Special Mission at Tohoku University and Waseda University and a Visiting Professor at Kanazawa University. Prizes and honors received include the 1987 Heinrich Wieland Prize (Germany), the 2000 Warren Alpert Foundation Prize (U.S.A.), the 2006 Japan Prize, the 2006 Massry Prize (U.S.A.), the 2008 Albert Lasker~DeBakey Clinical Medical Research Award (U.S.A.), Distinguished Professor Emeritus at TUAT (2008) and Honorary Citizen of Akita Prefecture, Japan (2008).

AL-01

Functional hyper-expression of fungal drug efflux pumps in *Saccharomyces cerevisiae*

Masakazu Niimi
Former Chief, Mycology Laboratory, Department of Bioactive Molecules, National Institute of Infectious Diseases Tokyo, Japan

Fungal azole drug resistance can be a problem in some patient groups. High-level azole resistance in clinical *Candida* isolates is most frequently caused by the over-expression of energy-dependent drug efflux pumps. These pumps usually belong to either the ATP-binding cassette (ABC) family or major facilitator superfamily (MFS) class of membrane transporter. Little is known about how these pumps work and there is an urgent need to develop pump antagonists that circumvent resistance. We have developed a protein hyper-expression system utilizing a *Saccharomyces cerevisiae* host strain deleted in seven major ABC transporters, which thus has a reduced background of endogenous efflux activity. Plasmid pABC3 was engineered to allow functional hyper-expression of foreign proteins in this host. The main advantages of this system are its cloning efficiency and the use of homologous recombination to stably integrate single copy constructs into the host genome under the control of a highly active transcriptional regulator. The expression system not only facilitates the functional analysis of heterologous proteins, including fungal multidrug efflux pumps responsible for azole resistance, but also provides the tools needed to screen for pump inhibitors. Suppressing the activity of these fungal ABC transporters with small molecule multi-drug efflux pump inhibitors could reduce the drug resistance of these pathogenic fungi and therefore help increase the efficacy of antifungal chemotherapy with triazoles. Compounds including FK506, cyclosporine A, enniatin, milbemycins, synthetic D-octapeptides and unnarmicins have been identified as potent inhibitors of fungal ABC transporters. The protein hyper-expression system in *S. cerevisiae* is thus a powerful technological pratform for the discovery and development of new modulators for fungal chemotherapy as well as for studying the molecular mechanisms of ABC transporter activities.

PROFILE

Dr. Masakazu Niimi received a D.D.S. from Kyshu Dental College in 1974 and a Ph.D. in Microbiology from Kyushu University in 1984. He held a research assistant position at Kyushu Dental College and at Kagoshima University, Japan. This was followed by a postdoctoral fellowship at the University of Otago, Dunedin, New Zealand for 9 years. He was the Chief of the Mycology Laboratory, Department of Bioactive Molecules, at the National Institute of Infectious Diseases in Tokyo for the last 9 years. His is currently Honorary Fellow of the Molecular Microbiology Laboratory, Department of Oral Sciences, at the University of Otago since April 2009. He has retained an interest in *C. albicans* since his Ph.D. studies of glucose effect of this pathogen. His current research is in mode of action of antifungal agents and the efflux pump-mediated drug resistance of pathogenic fungi. This reflects an interest in dissecting fungal ABC membrane protein functions and developing new classes of antifungals.

SS-01-1 Current status of echinocandin for invasive fungal infections

Clinical implication of PK-PD (pharmacokinetics-pharmacodynamics) on antifungal agents

Hiroshige Mikamo
Department of Infection Control and Prevention, Aichi Medical University, Japan

Three pharmacokinetics-pharmacodynamics (PK-PD) parameters have been shown to describe the association between antimicrobial dosing and treatment effect. These parameters include the percentage of time that the levels of drug in serum exceed the MIC (T > MIC), the Cmax/MIC, and the area under the serum concentration-time curve (AUC) in relation to the MIC (AUC/MIC). Numerous studies with antifungal drugs have demonstrated that the specific parameter predictive of activity varies for different drug classes and also have each target value. Drugs with static killing activity and long post antibiotic effect (PAE), such as flucytosine, depend on T>MIC. Drugs with cidal killing activity and long PAE, such as polyene and echinocandin, depend on Cmax/MIC. Drugs with static killing activity and long PAE, such as triazole, depend on AUC/MIC. Although researches on PK-PD of antifungal agents have been rather clarified for *Candida* species, there have been not necessarily much data for *Aspergillus* species.

The class of echinocandins represents a milestone in antifungal drug research that has further expanded our therapeutic options. On the other hand, the favorable pharmacokinetic profile of the echinocandins has been elucidated in animal and human studies. Since we also have investigated the association between PK-PD profile on micafungin and clinical outcome, I would shown our data in this seminar.

PROFILE

Educational History

Graduate Nagoya University, The Faculty of Literature at 1978
Graduate Gifu University School of Medicne at 1984
Graduate Gifu Graduate School of Medicine at 1989

Occupational History

1989-1992	Research Associates, Medical doctor of Gifu University Hospital
1992.9-2004.3	Assistant Professor, Gifu University Hospital
2004.4-2007.7	Associate Professor, Gifu University Hospital
	Associate Professor, Division of Anaerobe Research, Life Science Research Center, Gifu University

SS-01-2 Current status of echinocandin for invasive fungal infections

Current status of echinocandin susceptibility and resistance

David S. Perlin
Director and Professor, Public Health Research Institute/UMDNJ, USA

The echinocandins drugs are the first class of antifungal agents to target the fungal cell wall by inhibiting glucan synthase, which catalyzes the biosynthesis of the critical cell wall polymer β1,3-D-glucan. The three principal drugs, caspofungin, micafungin and anidulafungin are highly efficacious, although they have a limited spectrum. They are highly serum protein bound, display favourable PK/PD properties, and have an excellent safety profile. Antifungal resistance to echinocandin drugs remains relatively low. Yet, it can be an important factor in patient management. Global surveillance studies confirm that >99% of clinical isolates are inhibited by all three echinocandin drugs at ≤2 μg/ml, which along with PK/PD data from model systems, formed the basis of the CLSI susceptibility breakpoint. Elevated MIC values up to the breakpoint are more common, but an uncertain correlation exists between MIC and clinical outcome. The development of drug resistance resulting in clinical failure is associated with amino acid substitutions in Fks subunits of glucan synthase. These mutations decrease the sensitivity of glucan synthase to drug by several log orders. The biochemical inhibition properties provide a strong measure of drug response and potential clinical outcome, and can be used to distinguish among drugs. An evaluation of more than 100 clinical isolates of *C. albicans* and *C. glabrata* obtained from patients failing therapy suggests that for strains with fks mutations, the CLSI breakpoint of 2 μg/ml for caspofungin is appropriate. However, a lower breakpoint of ≥ 0.25 μg/ml is more suitable for anidulafungin and micafungin against *C. albicans*, while breakpoints for *C. glabrata* ought to be ≥ 1.0 μmg/ml and ≥ 0.25 μmg/ml for anidulafungin and micafungin, respectively. The Fks mediated resistance mechanism is conserved in *Candida* spp., and *Aspergillus*, and it helps accounts for intrinsic reduced susceptibility observed among the *C. parapsilosis* family.

PROFILE

Education

1976 Brandeis University, BA
1980 Cornell University, PhD
1983 Yale University School of Medicine, Postdoc
1985 University of Rochester School of Medicine and Dentistry, Postdoc

Positions

1985 Assistant Member, Public Health Research Institute, New York, NY
1990 Associate Member, Public Health Research Institute, New York, NY
1992 Scientific Director, Public Health Research Institute, New York, NY
2003 Prof., Microbiology Molecular Genetics, New Jersey Medical School -UMDNJ
2006 Director, PHRI Center, New Jersey Medical School-UMDNJ, Newark, N.J.
2008 Director, UMDNJ Regional Biocontainment Laboratory, Newark, N.J.

Recent Honors

2005 Fellow, New York Academy of Sciences, New York, N.Y.
2009 Visiting Professor, University of Manchester, UK

SS-02-1 Recent advances in diagnosis and treatment of tinea pedis and tinea unguium

Recent strategy of control and management of tinea pedis in Japan

Shinichi Watanabe
Chairman of Dermatology, Teikyo University School of Medicine; Professor of Teikyo University Institute of Medical Mycology, Japan

Due to the launch of many excellent antifungal agents for tinea pedis, the number of patients with the condition was expected to be reduced in Japan. The number, however, has not yet decreased. Although the reason for this is not clear, it may be caused by recurrence and/or reinfections of tinea pedis. The recurrence may be related to incomplete or irregular treatment. First, most patients discontinue the treatment immediately after clinical symptoms disappear even if the dermatopytes have not been completely eradicated. Second, patients tend to apply the topical agents to only symptomatic lesions. However, causative organisms are usually present at not only symptomatic, but also asymptomatic lesions. Third, patients frequently fail to keep up regular application. Therefore, it is important to continue regular application to both soles and all interdigital areas at least one month. It is a Japanese custom to take off the shoes in the home. Because there are a lot of asymptomatic patients with tinea pedis who are unaware that they are suffering from tinea pedis, these patients scatter their foot scales containing dermatophytes on the bathmat, floor and/or straw matting in their houses. In fact, it has been demonstrated that dermatophytes are almost 100% cultured from the bathmat in public bath houses or in the house where patients with fungal infections of the foot live. Therefore, the source of reinfections may be these foot scales. Therefore, it is important to treat not only the patients, but also their family members. In addition, cleaning the floor and/or straw matting and washing bathmat are necessary for the removal of dermatophytes and prevention of infections. If it is difficult to remove all of the scales containing dermatophyes in the house, then I recommend a prophylactic topical application even after tinea pedis cures.

PROFILE

1978	M.D., Faculty of Medicine, University of Tokyo, Tokyo, Japan
1985	Ph.D., Faculty of Medicine, University of Tokyo, Tokyo, Japan
1998-Pres	Chairman of Dermatology, Teikyo University School of Medicine Professor of Teikyo University Institute of Medical Mycology
1994-1998	Professor of Dermatology, Teikyo University School of Medicine
1988-1994	Associate Professor of Dermatology, Teikyo University School of Medicine
1985-1988	Research Fellow, Department of Dermatology, Massachusetts General Hospital, Harvard Medical School, Boston
1984-1985	Chief of Dermatology, Sanraku Hospital
1979-1984	Assistant Professor of Dermatology, Faculty of Medicine, University of Tokyo
1978-1979	Resident in Department of Dermatology, Faculty of Medicine, University of Tokyo

SS-02-2 Recent advances in diagnosis and treatment of tinea pedis and tinea unguium

Fungal identification in onychomycosis

Michel Monod
Olympia Bontems, Philippe Hauser. Centre Hospitalier Universitaire Vaudois (CHUV), Switzerland

Onychomycosis is the most frequent nail disease, and affects all ages and populations. Direct microscopic examination of infected nail material should confirm the clinical diagnosis of fungal infection whenever possible before oral treatment is commenced. Subsequently, fungal culture from nail sampling permits the species involved in infection to be determined. Although dermatophytes are the main cause of onychomycosis, various non-dermatophyte filamentous fungi (NDF) are often isolated from abnormal nails. Whether a NDF is really the infectious agent of onychomycosis, or whether it has to be considered as a casual and transient contaminant, often remains an open question. Direct identification of the infecting agent in nail was shown to be feasible using Polymerase Chain Reaction (PCR) and sequencing or Restriction Fragment Length Polymorphism (RFLP).
Identification of fungi in nails using PCR/sequencing/RFLP provides significant improvement in comparison to results obtained by cultures: (i) NDF can be identified with certainty as the infectious agents of onychomycosis, and discriminated from dermatophytes as well as from transient contaminants. (ii) It is possible to identify the infectious agent when direct nail mycological examination showed fungal elements, but negative results were obtained from fungal culture. This represents a substantial improvement of the sensibility of identification of fungi in nails since the frequency of culture negative results when direct nail mycological examination showed fungal elements is in the range of 40%. (iii) Identification of the infectious agent can be obtained in 24 h with PCR/sequencing/RFLP, whereas results from fungal culture can take as long as 1-3 weeks. The simplicity and the reliability of the assays, and high NDF detection frequency support that PCR/sequencing/RFLP can be routinely and advantageously used to identify infectious fungi in nails, provided that enough nail material is collected by the clinician.

PROFILE

2004-2009	Centre Hospitalier Universitaire Vaudois (CHUV), Dermatology Department, and University of Lausanne (UNIL), Switzerland	Associate Professor
1988-2009	CHUV, Dermatology Department	Group leader of the Mycology Laboratory
1986-1988	Ciba-Geigy (Basel, Switzerland) Biotechnology Department	Post doctoral fellow Yeast Genetics
1984 1986	Public Health Research Institute (New-York, US)	Post doctoral fellow *Bacillus subtilis* genetics
1983-1984	University of Arizona Cell biology Department	Post doctoral fellow *Bacillus subtilis* genetics, Mycology in phytopathology
1981-1983	University of Lausanne Institut de Génétique et Biologie Microbiennes	Post doctoral fellow *Bacillus subtilis* genetics
1975-1981	University of Lausanne Institut de Botanique Systématique et de Géobotanique	Mycology PhD student

Michel Monod studied biology in Lausanne (biochemistry and botany), and obtained a Ph.D degree in mycology. He was postdoctoral fellow at the University of Arizona and at the Public Health Research Institute in New-York where he worked in genetics of gram-positive bacteria and antibiotic resistance. Back in Switzerland, he worked in the biotechnology department of Novartis (Basel), then moved in Lausanne in the department of dermatology of the University Hospital. His scientific research mainly focus on fungal secreted proteases, dermatophytes and onychomycosis.

SS-03-1 Advances in molecular biological diagnosis of mycoses

Application of molecular diagnosis of cutaneous fungal infections

Takashi Mochizuki
Department of Dermatology, Kanazawa Medical University, Uchinada, Ishikawa, Japan

A majority of fungal pathogens isolated from human skin lesions are successfully identified by their characteristic morphology. In some cases, however, identification by culture based methods such as micromorphology, physiological tests, and biological tests is difficult. To identify these isolates without characteristic morphological features, and to confirm the identification based on culture based methods, several molecular methods have been introduced over the last few years. Genetic analysis targeting on mitochondrial DNA, random amplification of polymorphic DNA, and certain genes such as ribosomal(r) RNA genes, chitin synthase I, and topoisomerase II have been successfully used for species level identification of dermatophytes and some other fungal species. Among them, DNA sequence analysis and restriction fragment length polymorphism (RFLP) analysis of internal transcribed spacer regions (ITS) of rRNA genes have been widely used for species level identification of many fungal species including dermatophytes, *Sporothrix schenckii* and some dematiaceous fungi. The methods have been applied to identify causative fungi directly from clinical samples such as infected nails. Recently, highly sensitive methods have been introduced for typing at the subspecies level. Successful subtyping of clinical isolates of *Trichophyton (T.) rubrum*, *T. mentagrophytes*, and *T. tonsurans*, using non-transcribed spacer regions (NTS) of rRNA genes has been reported. Using RFLP analysis of NTS, we have found 8 different molecular types among *T. tonsurans* strains isolated in Japan, but only three types among strains isolated from tinea gladiatorum. The advantages, indications and limitation of molecular methods will be summarized in the presentation.

PROFILE

Education

1975-1981	Hirosaki University, Aomori, Japan, M.D.
1982-1986	Graduate School, Shiga University of Medical Science, Shiga, Japan Ph.D.

Professional experience

2005-Present	Professor, Department of Dermatology, Kanazawa Medical University, Ishikawa, Japan
2005-Present	Professor, Division of Dermatomycology (Novartis Pharma), Research Institute of Medical Science, Kanazawa Medical University, Ishikawa, Japan
1997-2005	Associate Professor, Department of Dermatology, Kanazawa Medical University, Ishikawa, Japan
1986-1997	Instructor, Department of Dermatology, Shiga University of Medical Science, Shiga, Japan
1992-1993	Visiting Professor (Associate), Department of Plant Pathology, University of California at Riverside, California, USA
1991-1992	Visiting Researcher, Department of Botany, University of Texas at Austin, Texas, USA

SS-03-2 Advances in molecular biological diagnosis of mycoses

Advances in molecular biological diagnosis of *Candida* infection

Ruoyu Li
Department of Dermatology, Peking University First Hospital, Research Center for Medical Mycology, Peking University, China

As the number of immunocompromised hosts growing, the fungal opportunistic infection is increasing significantly. *Candida* infection is the most common one that leads to high mortality, especially Candidemia. Other non- *albicans* yeasts infections are emerging and some of them are azole-resisting. More sensitive and accurate methods are in demand to make the early diagnosis. The identification of the pathogenic yeast to species level could help the selection of sensitive drug. The strain typing is helpful to clarify the source of infection. The molecular biological methods mainly include PCR-related methods, DNA sequencing analysis, DNA hybridization and Gene chip. The ribosomal repeat is the gene that is used most frequently. This review presentation will firstly introduce the application of PCR in diagnosis that includes Multiplex PCR and Real-time PCR. Then the gene typing methods that include PFGE, RAPD, RFLP, SSCP as well as MLST will be discussed. The newly developed technology such as DNA microarray-based system will also be addressed.

KEYWORDS: molecular biology; *Candida*; PCR; diagnosis; strain typing.

PROFILE

2005.1-pres.	Chairman, Dept. Dermatology, Peking University First Hospital
1998.6-pres.	Professor, Dept. of Dermatology, Peking University First Hospital
1992.12-1998.6	Associate Prof., Dept. of Dermatology, Peking University First Hospital
1997.3-1997.5	Visiting scholar, Dept. of Pathology, UTHSC at San Antonio, U.S.A.
1991.4-1992.7	Foreign researcher, Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Japan
1987.8-1992.12	Attending physician, Dept. of Dermatology, Peking University First Hospital
1983.1-1986.6	Beijing Medical University, M.S. Medicine
1978.3-1982.11	Beijing Medical College, M.D.
2006-pres.	President, Society of Mycology, Chinese Society for Microbiology
2008-pres.	President, Medical Mycology Society, Mycological Society of China
2008-pres.	Vice president of Asia Pacific Society of Medical Mycology

SS-04-1 Diagnosis, epidemiology and treatment of systemic fungal infections

The epidemiology of invasive fungal infections in transplant recipients: Overview of TRANSNET and OTIP

Tom M. Chiller
Mycotic Diseases Branch, CDC, USA

Invasive fungal infections (IFI) are a major cause of morbidity and mortality among Hematopoietic Stem Cell Transplant (HSCTs) and Solid Organ Transplant Recipients (SOTs). A better understanding of the epidemiology of these infections could significantly improve the prevention and treatment of this important post-transplant complication. Incidence and epidemiology of IFIs in this population has been derived mainly from single-institution studies. Multicenter prospective surveillance for these infections is generally lacking.

Two systems in the US have been developed to perform surveillance for IFIs at multiple centers, TRANSNET, which has completed and OTIP, which is ongoing. TRANSNET consists of a consortium of 23 US transplant centers, where all IFIs from 2001 through 2006 were prospectively recorded. Detailed denominator data were collected at each site, as well as clinical, diagnostic, and outcome information for each IFI case. A total of 983 cases of IFI from16,200 HSCTs and 730 IFIs from 16,808 SOTs were collected. Invasive aspergillosis (IA) was the most common IFI among HCSTs and invasive candidiasis the most common among SOTs. TRANSNET is the most detailed prospective assessment of IFI's following organ transplantation. Insights from these data will be valuable in understanding the incidence for IFI in populations at greatest risk, and designing comprehensive preventive and other interventional strategies to improve outcomes among these pts.

OTIP was designed as a risk factor cohort surveillance study that involves 6 transplant centers following HCSTs and lung transplant recipients for 2.5 years post transplantation. Currently, there are more than 600 patients enrolled and being followed. Data are being collected on infections, drug use, outcomes, and environmental exposures. A specimen bank is also being collected in order that pathogen discovery and diagnostic samples are available for future use.

Surveillance for IFIs in transplant recipients has revealed high morbidity and mortality. Infections are occurring early and also late in the course of transplantation. These data will be of value towards a better understanding of prevention and treatment strategies for IFIs and the design of future studies.

PROFILE

Dr. Chiller received his bachelor's degree from Dartmouth College and his medical and public health degrees from Tulane University. He completed a residency in internal medicine at University of Texas, Southwestern and then worked for a year at UT Southwestern as an attending physician in HIV medicine. He completed a fellowship in infectious diseases and mycotics at Stanford University and then traveled to the Centers for Disease Control and Prevention (CDC) to train in infectious disease epidemiology as an Epidemic Intelligence Service (EIS) officer. He has been at CDC for 7 years and is currently deputy chief of the Mycotic Diseases Branch in the Division of Foodborne, Bacterial, and Mycotic Diseases.

Dr. Chiller is board certified in internal medicine and infectious diseases. He is an adjunct assistant professor in the division of infectious diseases at Emory School of Medicine and is also an adjunct faculty member at the Emory and Tulane Schools of Public Health. He has authored numerous articles and book chapters.

SS-04-2 Diagnosis, epidemiology and treatment of systemic fungal infections

Presentation of the resomyc registry for prospective data collection and analysis of the epidemiology, therapy, and outcomes of invasive fungal infections (IFIs)

Françoise Dromer[1)], Sébastien Becquerel[2)], Karine Sitbon[1)], Etienne Sévin[2)], Olivier Lortholary[1)]

Institut Pasteur Molecular Mycology Unit, Ntl Reference Center for Mycoses and Antifungals, Paris, France[1], EpiConcept, Paris, France[2]

Knowledge on the epidemiology of IFIs is mostly based on population-based surveillance programs that difficult to implement on a long term basis. They often concern specific groups of patients. Likewise, therapy and outcome are usually assessed through randomized therapeutic trials, mostly in adults, and likely to omit the mild or most severe cases because of exclusion criteria. The real life is different. Assessment of diagnostic procedures, populations at risk, therapeutic management and outcomes without the filter of geographical limitations or exclusion criteria can provide valuable data for the medical and scientific community. The French National Reference Center for Mycoses and Antifungals has a broad mission of surveillance. A network of mycologists and clinicians has been implemented over time. They voluntarily notify their IFIs to the NRCMA. While none of the IFIs is a reportable disease, the performances of the cryptococcosis survey were evaluated by the capture-recapture method to be as good as that of some reportable diseases. To improve data collection and feedback to our collaborators, we developed a software called VOOZANOO (free software under GNU GPL) to generate online questionnaires, thus providing real time data update and statistical analysis.

The RESOMYC registry includes 5 questionnaires corresponding to nationwide surveys (cryptococcosis, imported endemic mycoses and rare IFIs due to Fusarium spp, Zygomycetes, *Scedosporium* and other uncommon moulds or yeasts), as well as regional surveys (yeast fungemia and invasive aspergillosis). Proven or probable IFI are notified through the secured website. As of March 2009, the databases contained 6745 cases of IFIs recorded since 2002 (1985 for cryptococcosis) including yeast fungemia (n=2407), invasive aspergillosis (n=824), cryptococcosis (n=2723), rare IFIs (n=533) and endemic mycoses (n=238). The registry represents a gold mine for the analysis of epidemiological trends, therapeutic management and outcome of IFIs occurring in Europe.

PROFILE

Positions

2001-present	Head, Molecular Mycology Unit, Institut Pasteur
2002-present	Director, French National Reference Center for Mycoses & Antifungals, Institut Pasteur
2005-present	Co-director, Medical Mycology course, Institut Pasteur
1993-2001	Deputy Director, French National Reference Center for Mycoses & Antifungals, Institut Pasteur.
1992-present	Assistant Professor then Associate Professor, Institut Pasteur
1989-1991	Assistant Professor ("assistant hospitalo-universitaire", Hôpital Bichat).

Medical Training

1980-1987	Resident in Internal Medicine and Infectious Diseases (Hôpitaux de Paris).
1974-1980	Medical degree (Necker-Enfants Malades Medical School).

Scientific Education

1988	post-doctoral position, Clinical Mycology Section (head Dr. J.E. Bennett), Laboratory of Clinical Investigation, NIAID, NIH, Bethesda, MD.
1984-1987	PhD in Immunology, University Paris 6
1982-1983	Master's degree in cellular and molecular pharmacology, University Paris 6

Honors

2006	Elected member, American Academy of Microbiology

SS-05-1 Recent advances in aspergillosis

Animal models in preclinical trials of aspergillosis

Karl V. Clemons
California Institute for Medical Research, and Department of Medicine, Division of Infectious Diseases, Santa Clara Valley Medical Center, San Jose, CA; Department of Medicine, Division of Infectious Diseases and Geographic Medicine, Stanford University, Stanford, CA, USA

Invasive aspergillosis is a highly lethal disease. In spite of recent advances in therapeutic options with echinocandins and newer azoles, improved therapy remains a goal. Animal models of aspergillosis, particularly murine models, are integral to this search as they enable investigation of novel indications, or combination therapies prior to a clinical trial or when a clinical trial is impractical. This is particularly important now as few new antifungals are in product development. For instance, individual studies of three lipid-formulations of amphotericin B had shown them to be effective against aspergillosis. We addressed the question of comparative activities in a murine model of systemic aspergillosis and found each effective at higher dosages. Although liposomal amphotericin B was nearly equivalent to conventional amphotericin B, no formulation was superior to another. Central nervous system aspergillosis is the most frequent site of disseminated disease and has a high mortality rate. We developed a murine model of CNS aspergillosis in neutropenic mice for the purpose of examining potential therapies. In different studies we found that caspofungin and micafungin were both effective, as well as itraconazole, posaconazole and voriconazole were efficacious, but none resulted in cure. Similarly, liposomal and lipid-complexed amphotericin B were effective at higher doses, but doses of liposomal amphotericin B above 15 mg/kg were less effective. Various combinations of azole or echinocandin with a lipid-formulated amphotericin B were effective, but only voriconazole combinations showed increased reduction of fungal burden in the brain. Different models can show different results, as we have found with micafungin being efficacious models of CNS or systemic aspergillosis, but less effective in a pulmonary model in steroid-suppressed mice. Preclinical trail of other compounds include isavuconazole, partricins, and arylamidine, as well as the potential of collectins for therapy. These examples provide a basis for clinical trial or therapy.

PROFILE

1974	B.S. The Ohio State University
1979	M. S. Miami University
1983	Ph.D. Arizona State University
1984-1987	Post-doctoral Scholar, Stanford University, Santa Clara Valley Medical Center, and Institute for Medical Research
1987-1992	Lecturer in Medicine, Stanford University, Research Associate, Santa Clara Valley Medical Center, and California Institute for Medical Research, San Jose, CA
1992-present	Senior Lecturer in Medicine, Stanford University, Senior Research Associate Santa Clara Valley Medical Center and California Institute for Medical Research
1992-present	Director of Animal Facility and Biosafety Officer - California Institute for Medical Research
2001-2004	Chair-elect, Chair, and Division Advisor, Division F, American Society for Microbiology
2002-present	Editorial Board of Antimicrobial Agents and Chemotherapy
2008-present	Associate Editor of Medical Mycology

SS-05-2 Recent advances in aspergillosis

Filamentous fungal infections and the role of amphotericin B

David W. Denning
Medicine and Medical Mycology, University of Manchester, Manchester, UK

In the neutropenic setting, filamentous fungal infections are usually caused by Aspergillus species, *A. fumigatus* being most common. Less common infections include caused by several Zygomycetes species, *Fusarium* spp, *Scedosporium* spp. and even rarer moulds. Voriconazole is the treatment of choice for invasive aspergillosis and *S. apiospermum* infection, and may be effective for *Fusarium* spp., but is ineffective for any infections caused by the Zygomycetes. Micafungin and other echinocandins are only effective for *Candida* and *Aspergillus* infections, and less effective for invasive aspergillosis during profound neutropenia, than in non-neutropenic patients. Unfortunately azole resistance in *A. fumigatus* is increasing (in Europe), particularly itraconazole resistance, but also cross resistance to voriconazole and posaconazole. *A. terreus* and *A. nidulans* are resistant to amphotericin B.

The role of amphotericin B (including the less nephrotoxic AmBisome (liposomal amphotericin B)) is:

- Zygomycosis
- Azole resistant aspergillosis
- Azole breakthrough infections, including prophylaxis failure, if adequate bioavailaity has been demonstrated with itraconazole
- Major drug interactions, such as concurrent administration of rifampicin, carbamezepine, phenobarbitone and other CYP 51A inducing agents
- Renal failure (if IV voriconazole is required)
- Invasive aspergillosis failing therapy with voriconazole, posaconazole or echinocandin therapy
- Some very rare fungal infections, unresponsive to other therapy

Amphotericin B still has an important role to play in the treatment of invasive fungal infecton, even if diminished compared with 10 years ago.

PROFILE

David Denning is Professor of Medicine and Medical Mycology at the University of Manchester and Director of the National Aspergillosis Centre, Manchester at Wythenshawe Hospital in Manchester, England. An active researcher, Dr Denning' s research interests include the diagnosis and treatment of invasive aspergillosis other life-threatening fungal infections, respiratory fungal allergy, antifungal resistance, the assessment of new antifungal agents and the genomics of the aspergilli. He has authored or coauthored more than 350 peer-reviewed journal articles and book chapters. Dr Denning is a Founder of the antifungal discovery and development company F2G Ltd and the molecular diagnostic company Myconostica Ltd. He consults widely with the pharmaceutical industry. He manages the Aspergillus website at www.aspergillus.org.uk. With Drs Stevens and Steinbach, he co-chairs the Advances Against Aspergillosis meetings (www.AAA2010.org).

SS-06-1 *Malassezia* yeasts and related dermatoses

Luliconazole, a new imidazole, and its effect on *Malassezia*

Ryoji Tsuboi
Department of Dermatology, Tokyo Medical University, Tokyo, Japan

Luliconazole (LLCZ) is a new imidazole antifungal agent developed by Nihon Nohyaku Co., Ltd. The 1% cream and solution have been available in Japan since 2005 for the clinical treatment of superficial mycoses. This substance exerts a wide spectrum of antifungal activity against pathogenic fungi, including dermatophytes, *Candida* spp. and *Malassezia* spp. *In vitro* MICs of luliconazole against these various fungi proved that its antifungal activity surpassed that of the other clinically available topical agents.

The MIC of luliconazole against *M. restricta* was very low (0.004-0.016 μg/ ml) and comparable to that of ketoconazole. We developed a guinea pig seborrheic dermatitis (SD) model with *M. restricta*. *M. restricta* was inoculated to the clipped dorsal skin of male guinea pig once daily for seven consecutive days without occlusion, so that cutaneous lesions with erythema and scaling mimicking SD developed. Topical application of 1%LLCZ cream once daily for three consecutive days macroscopically improved the cutaneous lesions and significantly reduced the number of organisms as measured by PCR. These results point to the clinical utility of luliconazole for the treatment of SD as well as superficial dermatomycoses.

1. Munechika Y, et al: Antifungal activity of luliconazole in a guinea pig seborrheic dermatitis model with *Malassezia restricta*. ISHAM 2009 Poster.
2. Koga H, et al: The durable effect of luliconazole in a guinea pig tinea pedis model. ISHAM 2009 Poster.
3. Koga H, et al: *In vitro* antifungal activities of luliconazole, a new topical imidazole. Med Mycol 2009 in press.
4. Watanabe S, et al: A comparative clinical study between 2 weeks of luliconazole 1% cream treatment and 4 weeks of bifonazole 1% cream treatment for tinea pedis. Mycoses 49:236-41, 2006.

PROFILE

2002-present	Professor and Chairman, Department of Dermatology, Tokyo Medical University
1990-2002	Assistant & Associate Professor, Department of Dermatology, Juntendo University School of Medicine
1987-1989	New York University Medical Center, Postdoctoral Fellow (Cell Biology)
1987	Ph.D., Juntendo University School of Medicine (Dermatology)
1980	M.D., Japan National Defense Medical College

SS-06-2 *Malassezia* yeasts and related dermatoses

Dermatosis and *Malassezia*

Jan N. Faergemann
Department of Dermatology, Sahlgrenska University Hospital, Gothenburg, Sweden

The *Malassezia* yeasts are members of the normal human cutaneous flora in adults but also associated with several diseases. In pityriasis versicolor, under the influence of predisposing factors, *Malassezia* changes from the round blastospore form to the mycelial form. A great problem in pityriasis versicolor is the high rate of recurrence and to avoid this prophylactic treatment is mandatory. *Malassezia* folliculitis is a chronic disease characterised by pruritic follicular papules and pustules located primarily on the upper trunk, neck and upper arms. In direct microscopy clusters of round budding yeast cells are found. The disease responds rapidly to antimycotic therapy. There are now many studies indicating that the *Malassezia* yeasts play an important role in seborrhoeic dermatitis. Many of these are treatment studies showing a good effect of antimycotics paralleled by a reduction in number of organisms. Severe seborrhoeic dermatitis often difficult to treat is associated with AIDS. In skin biopsies from patients with seborrhoeic dermatitis we have found an increase in NK1+ and CD16+ cells in combination with complement activation indicating that an irritant non-immunogenic stimulation of the immune system is important. However, we also found an increase in the production of inflammatory interleukins as well as regulatory interleukins for both T_H1 and T_H2 cells indicating a complex activation of the immune system in the skin. The majority of adult patients with atopic dermatitis localised to the head, neck and scalp react with a positive reaction in skin prick test to a *Malassezia* extract as well as to recombinant *Malassezia* allergens. The majority also have specific IgE antibodies and react with a positive reaction in atopic patch test with a *Malassezia* extract. There are also treatment studies indicating that antifungal treatment may be beneficial in these patients.

PROFILE

2002-Pres. Professor of Dermatology
1979-2002 Associate Professor of Dermatology

SS-07-1 Tinea capitis: Recent advances in diagnosis and treatment

Treatment of tinea capitis in 2009

Boni E. Elewski
Department of Dermatology, University of Alabama School of Medicine at Birmingham, Alabama, USA

Griseofulvin has traditionally been considered the primary antifungal agent for tinea capitis. However, higher dosages and longer durations of therapy have become necessary to achieve clinical success. The current recommended guidelines suggest a dose of 20-25 mg/kg/day for at least 6 to 8 weeks. Longer durations would be required for ectothrix infections with *Microsporum canis*. Not all patients tolerate this dose and compliance is reduced because of the long duration of therapy, and requirements to take with food. Some patients are intolerant of, or allergic to, griseofulvin. Newer antifungal drugs including terfinafine, itraconazole and fluconazole have been studied in tinea capitis. The ideal regimen would be short and simple- once daily with or without food. Desired pharmacokinetics would be persistence after discontinuation. Both terbinafine and itraconazole persist; no detectable levels of griseofulvin are noted within 4 days after discontinuation.

Terbinafine has recently been approved for children with tinea capitis 4 years and older. A multicenter study (n=1286) showed complete and mycological cure rates significantly higher than griseofulvin (45.1% vs.39.2% and 61.5% vs 55.5%, respectively; P< .05). Terbinafine was significantly better than griseofulvin for mycologic, clinical and complete cure rates with *Trichophyton tonsurans*, but not *Microsporum canis* infections.
Dose according to body weight: <25 kg 125 mg once daily, >35 kg 187.5 mg daily, 25 - 35 kg 187.5 mg daily using laminated packets containing 125 mg or 187.5 mg. There were no new safety issues, and no significant effects on liver transaminases.

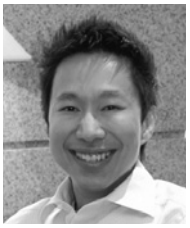
Fluconazole and itraconazole may be efficacious in tinea capitis and have efficacy against *Microsporum* and *Trichophyton* spp. A multicenter study of fluconazole showed a dosage of 6mg/kg/day for 3 or 6 weeks to be effective in some patients.

Topical antifungals are often used as adjunct therapy but are not sufficient to treat the primary infection.

PROFILE

2009-present	Chair of Faculty Counsel, University of Alabama at Birmingham
2005-present	Vice Chair of Clinical Affairs, Department of Dermatology, University of Alabama at Birmingham
2004-2005	Past President, American Academy of Dermatology
2002-present	American Academy of Dermatology Bioterrorism Task Force (Chair, 2001-2003)
2001-2002	Vice President, American Academy of Dermatology
1999-2000	President, Women's Dermatological Society
1999-present	Director of Clinical Research, Department of Dermatology, University of Alabama at Birmingham
1999-present	Professor of Dermatology, University of Alabama at Birmingham, Birmingham, AL
1995-1996	Laboratory Director, Center for Medical Mycology, University Hospitals of Cleveland, Case Western Reserve University
1993-2000	Editorial Board, Journal of the American Academy of Dermatology
1992-1993	Member, Institutional Review Board, Case Western University
1989-1999	Assistant, Associate and Full Professor of Dermatology, Case Western Reserve University, Cleveland, OH
1982-1989	Chief of Dermatology Department, The Akron Clinic Foundation, Inc. Akron, OH

SS-07-2 Tinea capitis: Recent advances in diagnosis and treatment



Tinea capitis: Thailand experience

Rataporn Ungpakorn
Institute of Dermatology, Bangkok, Tahiland

Tinea capitis is common dermatophytic infection mostly seen in children. Among over 130,000 cases of dermatology patients who visited the Institute of Dermatology annually, approximately 100 new cases were identified comprising 0.5% of dermatophytosis patients with a predominant male to female ratio of 2:1. Only 5% of cases were over 14 years of age.

While *Trichophyton tonsurans* has been reported as the major causative organism worldwide, *Microsporum canis* is still the leading pathogen among outpatient cases in Thailand. The incidence of *Trichophyton tonsurans* and *Trichophyton mentagrophytes* are 4.2% and 4.12%, respectively.

Among anthropophilic species, *Microsporum ferrrugineum* is the most common cause of epidemic tinea capitis in Thailand. The main cause of spread was sharing beddings and combs amongst children living in charity homes. Localised grey patchy alopecia was the most common presentation with inflammatory lesions seen in less than 5% of patients. Carriers were not common, but if found were mostly in female staff.

With all available oral antifungals, griseofulvin at 20-25 mg/kg/d is still the drug of choice in most of the cases with no exception to *Microsporum* spp. infections. Minor disadvantage is long-term continuous daily requirement which contributes to the problem of compliance. Present data showed no superiority among newer systemic antifungals in terms of efficacy except for availability of liquid formulation such as oral solution or granules which enhances convenience in pediatric patients.

Inflammatory tinea capitis mandates immediate treatment to prevent permanent scars. The most important prognostic factor is clinical severity at the time of diagnosis. Variability of clinical presentation that may mimic other noninfectious scalp conditions and inadequate laboratory examination are main reasons for delayed diagnosis resulting in inappropriate treatment leading to unwanted complication. Whether corticosteroid is required depends on clinical judgment based on individual cases.

PROFILE

Present	Consultant, Laser and Skin Clinic, Bumrungrad International Hospital
	Dermatology and Mycology Consultant, Institute of Dermatology and Chulalongkorn Medical University, Bangkok, Thailand
	Board Member, Institute of Dermatology Alumni Association
	Board Member, Dermatological Society of Thailand
	Council Member, Asian Dermatological Association (ADA)
	Board Member, Asia-Pacific Society of Medical Mycology (APSMM)
1997	Certified Thai Board of Dermatology
1996, 1998	Research Fellow, Guy's, King's and St. Thomas's Hospitals, London, United Kingdom
1994	Fellow in Dermatology, King Mongkutklao Medical College Hospital, Bangkok, Thailand
1987	Doctor of Medicine University of the East, Ramon Magsaysay Memorial Medical Center, Manila, Philippines
1983	Bachelor's of Science (2nd Honours, Pure chemistry), Chulalongkorn University, Bangkok, Thailand

SS-08-1 Recent advances in yeast research

Molecular epidemiology and pathogenesis of *Candida* infections

Frank C. Odds
Medical Mycology at the University of Aberdeen, Scotland, UK

The epidemiology of infections caused by *Candida* spp. has benefitted from steady advances in molecular strain typing technology. Approaches such as microsatellite typing and multi-locus sequence typing provide highly discriminatory and important tools for the investigation of sources, transmission and phenotypic properties of strain types. Studies of the pathogenesis of *Candida albicans* infections have moved from definition of genes associated virulence to in-depth studies of the fungus-host crosstalk *in vivo* and *ex vivo.* These advances and their implications for future research will be discussed.

PROFILE

Frank C. Odds is Professor of Medical Mycology at the University of Aberdeen, Scotland. He has worked for more than 40 years on aspects of the epidemiology, pathogenesis and treatment of fungal infections, in academia and in industry.
He is currently co-Chairman of the Wellcome Trust Immunology and Infectious Diseases grants Committee. His honours include Fellowship of the Royal Society of Edinburgh, Fellowship of the American Academy of Microbiology, and Honorary Membership of the British and International Medical Mycology societies. He is listed in the ISI "Most Highly Cited Scientists in Microbiology" database.

SS-08-2 Recent advances in yeast research

Candida albicans genomes and genomics

Judith Berman
Depts of Genetics, Cell Biology and Development, University of Minnesota; Dept. of Microbiology, University of Minnesota, USA

The *Candida albicans* genome sequence has been publicly available for almost 10 years with the full diploid assembly first published 5 years ago. This talk will highlight the types of studies that have exploited the genome sequence information to understand the organism, its growth and development as well as the diversity and responsiveness of its genome.

PROFILE

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General Research area: Genome structure, dynamics and function in *C. albicans* and *S. cerevisiae*;
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ABSTRACTS

Symposia

CB-01-1

An atypical isolate of *Paracoccidioides brasiliensis* found in our culture collection

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An atypical type of *Paracoccidioides brasiliensis* isolate IFM54648 (LDR2) was accidentally found in our culture collection (Medical Mycology Research Center, Chiba University, Chiba, Japan). Based on a species-specific PCR testing, the isolate was negative for the major antigen 43-kDa glycoprotein coding gene (*gp43*). Furthermore, an atypical band pattern in loop-mediated isothermal amplification (LAMP) for detection of *gp43* was demonstrated. A partial sequence of *gp43* showed less than 89% in identities to the other *P. brasiliensis* genospecies. Isolate IFM54648 was initially isolated from a mass in the lower jaw of a patient, a 64-year-old Brazilian male who has chronic paracoccidioidomycosis (PCM). Five samples of sera collected from the patient over a period of 7 years did not react with an immunodiffusion test for PCM. In addition, the patient lived in São Paulo, but now lives in Parana, Brazil. Both are endemic areas of PCM. The mycological characteristics of the isolate IFM54648 were similar to that of typical *P. brasiliensis*. A total of 8 genes were sequenced from IFM54648, and the sequences were compared with the new isolate and other reference isolates and database sequences. We analyzed fragments of the gene sequences that code for *gp43*, the internal transcribed spacer regions of ribosomal RNA, the D1/D2 domains of the large subunit ribosomal RNA, glucan synthase, chitin synthase, glyoxalase I mRNA, 70-kDa heat-shock protein mRNA and urease. The gene sequences were 98.9% to 100% identical between IFM54648 and Pb01. When IFM54648 compared to the other typical isolates, the identities were generally lower than 98%. A phylogenetic tree constructed using *gp43* sequences revealed that IFM54648 clustered with Pb01 at a considerable distance from other isolates. Therefore, this isolate is probably related to Pb01, which has recently been shown to be genetically distinct from other isolates of this species.

CB-01-2

The paracoccidioidomycotic granuloma

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We studied the granulomatous lesions characteristic of paracoccidioidomycosis evaluating the effect of drugs that interfere with fibrosis, employing a murine model. We analysed the local expression of collagens, of relevant cytokines (IFN-gamma, TGF-beta and TNF-alfa) and of matrix metalloproteases (MMP) and compared with the pattern of lesions developed. We also quantified fungal dissemination to various organs, NO and MMP concentration and their gelatinolytic activity.

IFN-gamma, found mainly in lymphocytes of resistant mice and TGF-beta, expressed on macrophages and multinucleated giant cells preferentially in susceptible mice may promote, respectively, macrophage activation or deactivation, enhancement or inhibition of *P. brasiliensis* killing and consequently fungal control or dissemination. TNF-alfa expression, detected in ECM, macrophages and giant cells increased during the infection, eventually inducing protective immunity. The expression of active MMP9 decreased throughout the infection, more strikingly in resistant than in susceptible mice, suggesting a tendency from degradation to matrix synthesis.

Susceptible mice were infected with Pb, and treated with IFN-gamma (antifibrotic activity), Tetracycline (extracellular matrix-ECM synthesis inhibition) or Lumiracoxib (ECM components inhibition).

Our results suggest that at an early period of infection, the treatment with IFN-gamma restrained *P. brasiliensis* through segregation within compact granulomas by ECM fibers, causing a decrease of fungal load eventually by an increase of IL-12 and NO production. On the contrary, treatment with anti-inflammatory drugs caused disorganization of the granuloma architecture, facilitating fungal dissemination, with decreased production of NO and GM-CSF. Tetracycline caused marked inhibition of fungal growth, as well as of granuloma formation, in parallel with increased IL-12, GM-CSF and NO production. Collagen was found as organized but not continuous fibers in infected mice, and as continuous fibers in IFN-gamma treated mice. Anti-inflammatory treatment elicited extensive deposition of disorganized fibers, in contrast with the absence of collagen deposit observed in Tetracycline-treated animals.

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CB-01-3

Cell wall α-1,3-glucan synthesis and regulation in *Paracoccidioides brasiliensis*

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In accordance with the exclusive presence of α-1,3-glucan in the yeast-like phase of the fungus, northern analyses show that the *P. brasiliensis AGS1* gene (*PbrAGS1*) is only expressed in the yeast-like phase, and after 48 h into the mycelial (M) to yeast (Y) transition, when most of the culture was in the yeast phase. A faint signal could be detected in the M form of the fungus, whose cell wall is virtually devoid of α-1,3-glucan. Supplementing the growing culture medium with serum has been documented as a way to increase both the amount of cell wall α-1,3-glucan in the Y form of *P. brasiliensis*, and its virulence. Growth of Y phase cultures in YPG supplemented with 5% horse serum (HS) lead to an increase in the α-1,3-glucan content of the cell wall, accompanied by a slight increased in the level of expression of *AGS1* but higher increase in the expression of *RHO2*. This suggests a post-transcriptional regulation of *PbrAGS1*, as in *S. pombe*, where *AGS1* is post-transcriptionally regulated by Pck2 through the product of *RHO2*. Comparison of the levels of expression of *PbrAGS1* and *PbrRHO2* in the M and Y stages of the fungus shows a direct correlation, suggesting a similar role of Rho2 in *P. brasiliensis*. A homology search in the recently released *P. brasiliensis* genome database (Broad Institute, MIT and Harvard, Cambridge, MA), shows that *AGS1* is the only gene in the genome of *P. brasiliensis* related to the synthesis of α-1,3-glucan, which agrees with a single type of cell wall α-1,3-glucan in this fungi. In *S. pombe* and *Aspergillus niger*, multiple *AGS* genes have been reported, and at least two different forms of cell wall α-1,3-glucan have been identified in each fungus.

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CB-01-4

Speciation, recombination and molecular evidence of sex in the *Paracoccidioides* genus

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The species of the *Paracoccidioides* genus are a thermodimorphic fungus that cause paracoccidiodomycosis (PCM), a systemic disease affecting mainly rural workers that are in close contact with the soil, and is endemic to Latin America. This genus was considered to be comprised of a single, clonal species, *P. brasiliensis*, but studies on different isolates revealed a great degree of genetic variability. Pb01 isolate, one of the best studied, is particularly different (Carrero et al., 2008) of the three previously described phylogenetic species S1/PS2 /PS3 (Matute et al., 2006). Using the genealogic concordance method of phylogenetic species recognition (GCPSR) via maximum parsimony and Bayesian analysis, 17 genotypically similar isolates, including Pb01, were found to cluster in a distant clade from the one containing S1/PS2/P3. Consistent with GCPSR, the Pb01-like group can be considered a new species distinct from S1/PS2/PS3 group, since it is strongly supported by all independent and concatenated genealogies (posterior probability of 1.0 and bootstrap of 100%). We have proposed the formal description of the Pb01-like cluster as the new species *Paracoccidioides* lutzii. These two genetically distinct fungal populations present exclusive phenotypic characteristics such as virulence levels, resistance to fungicides and proliferation rates, as previously reported for representatives of both clusters of the species complex until now known as *P. brasiliensis*. In addition, recombination analysis revealed independent events inside both main groups, which is suggestive of reproductive isolation. The identification of complementary mating-type loci (MAT 1-1 and MAT 1-2), the mating machinery genes found in the three Paracacoccidioides sequenced genomes (isolates Pb01, Pb18 and Pb3) and their expression led us to conclude that these pathogens can have a sexual stage in its life cycle.

CB-02-1

The *Candida albicans* Chk1p histidine kinase and Cek1 MAPK are required for mannan biosynthesis

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CHK1 encodes a hybrid histidine kinase of *Candida albicans*. It is required for disseminated infection, which correlates with in vitro defects of chk1 mutants in adherence to mammalian cells and enhanced susceptibility to neutrophil killing. The chk1 mutant also exhibits defects in cell wall mannans associated with a highly flocculant phenotype. A signal pathway to which Chk1p might associate is unknown. We therefore compared mutants in chk1 with 4 MAPK mutants phenotypically, including, cek1, cek2, hog1, and mkc1. Of the in vitro assays we used, the chk1 and cek1 as well as the sho1 mutants resembled each other in regard to Congo red and calcofluor white sensitivities as well as flocculation in M199 medium. In regard to these phenotypes, we determined that Chk1p is part of a parallel and independent signal pathway from that of Sho1-Cek1 MAPK. However, all strains were also examined for mannan profiles. Bulk mannan was prepared from each strain and subjected to GPC-MALLS analysis. The profiles of Wt and the Sho1 mutant were similar, with three Mw types of mannan, designated as high, intermediate and low. In comparison, the chk1 and cek1 profiles of mannan were identical, consisting exclusively of low Mw mannan. Our data thus indicate that Cek1p and Chk1p share functional activity in regard to mannan biosynthesis that is most likely independent of the Sho1p cognate sensor of the Cek1 MAPK pathway.

CB-02-2

Biogenesis and expression of *Candida albicans* beta mannose adhesins

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Since their identification in *C. albicans* phosphopeptidomannan (PPM) by S. Suzuki *et al*. and extensive structural work from the same group, β-1,2 mannosides have generated much interest from the scientific and medical communities. The reasons for that are an anomer type of linkage rare in the living world, conferring a unique spatial configuration, their role as *C. albicans* adhesins through interaction with Galectin-3, whereas their association with a cell wall surface glycolipid named Phospholipomannan -PLM-triggers innate immunity pathways through TLR2; finally, strong cumulative evidence has shown that mounting adaptive humoral response against the same structures confers immunity to rodents against *C. albicans* infections. By contrast, at the *C. albicans* level very little is still known about the mechanisms of β-Man generation and expression. We have identified 9 genes encoding for beta-mannosyltransferases (Bmts) and defined the functions of 6 of them involved in PPM and PLM mannosylation whereas compensatory mechanisms resulting from individual gene deletions seems to act on cell wall mannoproteins (CWMPs) as carrier molecules. Extensive immunochemical analysis suggested that each of CWMPs family i.e. matricial, linked to β-1,3 or β-1,6 glucans could be β-mannosylated according to processes differing from those of PPM. To explore this new model of β-Man distribution, investigations on genes transcription were undertaken. They surprisingly revealed no or little differences between strains of both serotypes grown in conditions inducing dramatic differences in β-Man cell wall surface expression. Therefore β-Man biogenesis and expression appears as model of unforeseen complexity needing to be stepwise deciphered since any gap in our knowledge about this important *C. albicans* specific trait could impair or render elusive any therapeutic approach based on these targets.

CB-02-3

Calcineurin regulation of the *Aspergillus fumigatus* cell wall and hyphal growth

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The calcineurin pathway has been shown to be important for both hyphal growth as well as pathogenicity in *Aspergillus fumigatus*. This pathway is now being dissected to determine the specific molecular mechanisms controlling hyphal growth, in an effort to be able to identify the specific genes of protein domains for optimal antifungal targeting. Here we will review the calcineurin pathway and its putative controls over both cell wall homeostasis and composition as well as hyphal growth and virulence.

CB-02-4

Biochemical and genetic probing of glucan synthase

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Glucan synthase catalyzes the biosynthesis of the major fungal cell wall polymer β1,3-D-glucan. The enzyme complex is comprised of catalytic Fks subunits and the ubiquitous regulatory component Rho1; although, the organization of this complex is not well understood. In recent years, a great deal has been learned about the biochemistry of glucan synthase and genetic regulation of FKS genes by studying the properties of mutant strains of *Candida* and Aspergillus resistant to echinocandin class inhibitors. These potent glucan synthase inhibitors represent the newest class of antifungal agents. Mutations in FKS subunits conferring resistance can increase the drug inhibition constant by 3 log orders, and they can decrease the catalytic capacity of the enzyme 4-fold without affecting cell growth. In such cells, the expression ratios of FKS1 and FKS2 may be altered to maintain a constant level of synthase activity. Echinocandin drugs modified with fluorescent tags can be used as probes, which provide valuable information about drug binding and the distribution of glucan synthase in the cell.

CB-02-5

Beta 1,6 glucan synthesis

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CB-03-1

Mechanisms involved in the resistance of *Candida albicans* biofilms to antifungals

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Candida albicans is capable of forming biofilms that exhibit elevated intrinsic resistance to various antifungal agents, in particular azoles and polyenes. The molecular mechanisms that are involved in the antifungal resistance of biofilms remain poorly understood. We have used transcript profiling to explore the early transcriptional responses of mature *C. albicans* biofilms exposed to various antifungal agents. Mature *C. albicans* biofilms grown under continuous flow were exposed to concentrations of Fluconazole (FLU), Amphotericin B (AMB) and caspofungin (CAS) that, while lethal for planktonic cells, were not lethal for biofilms. Interestingly, FLU exposed biofilms did not show any significant change in gene expression over the 2h course of the experiment. In AMB-exposed biofilms, 11 genes showed a more than 2-fold increase in expression, of which only 3 have been shown to be up-regulated in planktonic cells exposed to AMB. In contrast, CAS-exposed biofilms elicited a rapid and large change in gene expression. There was little overlap between AMB- or CAS-responsive genes in biofilms and those that have been identified as AMB-, FLU- or CAS-responsive in *C. albicans* planktonic cultures. These results suggested that the resistance of *C. albicans* biofilms to azoles or polyenes is not due to the activation of specific mechanisms in response to exposure to these antifungals but rather to the intrinsic properties of the mature biofilms. In this regard, our study lead us to observe that AMB physically binds to *C. albicans* biofilms and to beta-glucans which have been proposed to be major constituents of the biofilm extracellular matrix and to prevent azoles to reach biofilm cells. Thus, enhanced extracellular matrix or beta-glucan synthesis during biofilm growth appear to prevent antifungals such as azoles and polyenes to reach biofilm cells, limiting their toxicity to these cells and the associated transcriptional responses.

CB-03-2

Switching, mating, biofilm formation and pathogenesis in *Candida albicans*

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The majority of *Candida albicans* strains are **a**/α in nature. These cells can not undergo white-opaque switching due to repression by the a1-α2 repressor. When they undergo *MTL*-homozygous to **a**/**a** or α/α, they are relieved of repression and can switch from the dominant, mating-incompetent white phenotype to the mating-competent opaque phenotype. This scenario, when it was discovered, was perplexing, since it was not clear why *C. albicans* had inserted into its mating process so complex a process as white-opaque switching. A hypothesis was developed to explain the role of white-opaque switching in the mating process. It was proposed that the process evolved to facilitate mating in the host through a signaling system that generated a white cell environment that protected chemotropism between minority opaque **a**/**a** and opaque α/α cells. It was proposed that the protective white cell environment was a biofilm. The components of this hypothesis have been demonstrated *in vitro*. The formation of biofilms by *MTL*-homozygous cells, including the synthesis of the extracellular matrix, and the dependency of the process on the pheromone signal transduction pathway in white cells, is described. The relationship of biofilm formation by *MTL*-homozygous cells, the minority genotype in nature, and by pheromone-induced white, *MTL*-heterozygous cells, the majority genotype in nature, is addressed.

CB-03-3

The target of regulation of morphogenesis in *Candida albicans* by farnesol

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Hyphal formation through germination of yeast cells in the pathogenic fungus *Candida albicans* is an important virulence factor. In this regard, the cAMP-PKA signal pathway is known to be one of the key regulatory mechanisms responsible for germination. We have utilized both a GlcNAc medium and a glucose medium to study germination (1992 Cho *et al.*) but find that the cellular cAMP content during germination in these two media fluctuates differently. Further, we have not resolved which signaling pathway is utilized during germination in each medium.

While farnesol, a quorum sensing molecule of *C. albicans*, blocks the induction of germ tubes, it was reported that it also inhibits the cAMP-PKA pathway (2008 Davis-Hannna *et al.*).

To address issues of germination in regard to signaling, we studied *MSI3*, a gene encoding a novel HSP70 protein which is related to the cAMP-PKA pathway (2003 Cho *et al.*). In this session, we discuss our research on the morphological regulation by farnesol using a conditionally regulated *MSI3* mutant grown in the two media mentioned above.

CB-03-4

Assessing *Candida* biofilm formation in a new in vivo non vascular model

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More than half of all human microbial infections can be associated to biofilms, highlighting the impact of biofilm on public health. *Candida albicans* can form biofilms on a wide variety of medical devices such as urinary and vascular catheters, but also dental, joint and voice prostheses, pacemakers, ocular lenses. We have optimized an in vivo biofilm model for *Candida* based on a subcutaneous rat model previously described by Van Wijngaerden et al. (1999). Pieces of three-lumen catheters treated with serum are implanted under the skin of rat after an adhesion phase with *Candida* cells. Biofilms, assessed by colony forming units and scanning electron microscopy, reach an optimal size after two days, but they can remain in the host for up to 9 days. Immunosuppression treatment of the animal host increases the reproducibility of the biofilm generated inside the lumens. A critical step in biofilm production is the adhesion phase: incubating cells in RPMI medium instead of Spider or YNB glucose resulted in an increased biofilm formation, not only in wild type but also in strains defectuous in biofilm development in other models, such as als3. Both wild type and als3 can form biofilm in vitro on 96-well plate polystyrene substrate and on polyurethane disk when grown in RPMI, but the mutant does not when grown in Spider. We also observed biofilm formation in vivo with als3, this mutant being able to adhere and grow as hyphae in RPMI. In contrast, cells of the cph1 efg1 strain are not able to form biofilm in in vitro assays in RPMI, and also do not generate biofilm in vivo. Even in absence of constant flow, adherence is crucial to the in vivo subcutaneous biofilm model.

Van Wijngaerden, E., et al. (1999). J Antimicrob Chemoth 44: 669-674.

CB-03-5

Role of *Candida albicans* surface antigen in adherence in *in vitro* biofilm model

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Candida albicans expresses many immunodominat antigens participating in adherence. The CR3-RP (*C. albicans* CR3-related protein) is related to human adhesion glycoprotein, also known as Mac-l, the iC3b receptor. This research studied a contribution of this protein to adherence using *in vitro* biofilm model. For experiments, standard strain *C. albicans* CCY 29-3-162 and some *C. albicans* clinical catheter isolates were selected. Biofilm was formed in polystyrene 96-well plates according to Li *et al.*, 2003. The expression of the CR3 RP was determined after 1.5-h (period of adhesion) or after 48-h (mature) biofilm and compared with that estimated in planktonic yeast cells employing two polyclonal antibodies (AB): anti CR3-RP Ab or anti CR3-RP-M Ab (immunization of rabbits with synthetically prepared peptide DINGGATLPQ and peptide conjugated with *Candida* mannan). The effective peptide and peptide-mannan conjugate vaccination was demonstrated via spectrum of specific Ig-isotype antibody response. The expression of the CR3-RP was very week in planktonic cells using both antibodies. After period of adhesion, yeasts have started to express this protein detected exclusively after interaction with anti CR3-PR Ab and significant increasing of the CR3-RP expression was observed in 48-h biofilm. It is of interest, that CR3-RP-M antibody did not react with CR3-RP expressed by biofilm. We suppose some inhibitory effect of mannan on CR-RP peptide in conjugate. The CR3-RP Ab was also tested for ability to reduce 48-h biofilm development due to binding to CR3-RP receptor. Experiment was conducted using pre-incubation of the *C. albicans* with anti CR3-PR Ab. In standard strain as well as in selected *C. albicans* catheter isolate, pre-incubation with anti-CR3-RP Ab significantly decreased biofilm. Similar results were observed employing CLSM. In summary, biofilm proved to be a suitable model for adhesion study. Moreover, *C. albicans* CR3-RP seems to be an antigen of interest in design of novel *Candida* vaccine.

CB-04-1

Genetic studies on sterol and mannoprotein biosynthesis in *Candida glabrata*

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Candida glabrata is considered to be a closely related species to *Saccharomyces cerevisiae*. Many *C. glabrata* genes have orthologues of *S. cerevisiae* genes such as those involved in the biosynthesis of sterol and mannoproteins. We have investigated the effects of gene knockout and gene knockdown of putative *C. glabrata* genes involved in the above biosynthesis pathways on cell viability under various culture conditions. Overall phenotypic analyses of the *C. glabrata* mutants revealed mixed results: 1) orthologues of some genes required for *C. glabrata* viability were not essential in *S. cerevisiae*; 2) for other *C. glabrata* genes a requirement for viability was dependent on culture conditions as shown for orthologues in *S. cerevisiae*. Most of the genes encoding mannoprotein synthesis were classified as the former, presumably due to differing redundancy between the *C. glabrata* and *S. cerevisiae* genes. Genes involved in ergosterol biosynthesis were categorized as the latter, because sterol uptake, which does not occur in *S. cerevisiae* due to "aerobic sterol exclusion", would rescue the low level of ergosterol biosynthesis in host tissues. Therefore, genes for ergosterol biosynthesis were not required for growth in host tissues or serum containing medium. Aus1p, whose orthologue in *S. cerevisiae* is thought to be an anaerobic sterol transporter, was responsible for aerobic sterol uptake in *C. glabrata*. On the basis of these results, it was anticipated that other orthologues of genes responsible for sterol uptake in *S. cerevisiae* were differently regulated during aerobic sterol uptake in *C. glabrata*. Thus, genetic studies in *C. glabrata* may provide an alternative view of gene function predicted from studies in the model yeast *S. cerevisiae*, and may contribute to understanding host-fungal interactions including pathogenicity.

CB-04-2

Development of genetic manipulation systems in dermatophytes

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Molecular genetic studies of fungal pathogens have made significant contributions to our understanding of the diseases that they cause. Gene transfer by transformation (genetic transformation) is an indispensable technique for such studies, which has provided powerful tools for evaluating gene functions, such as targeted gene disruption and overproduction of heterologous proteins. Since the first reports of successful production of genetic transformants in model filamentous fungi such as Aspergillus nidulans, polyethylene glycol (PEG)-mediated transformation of protoplasts has been the most common approach for the transfer of exogenous genes to filamentous fungi.

Dermatophytes are human and animal pathogenic fungi that commonly gain access to the host via cornified tissues such as the hair, skin or nails, and cause superficial mycosis. Genetic transformation of these fungi by random integration of exogenous genes has also been carried out by the protoplast/PEG-mediated method, all of which have low transformation frequencies. The difficulty of genetic manipulation due to low transformation frequencies may be reflected by the small number of reports regarding genetic manipulation of these fungi. Therefore, very little is currently known about the details of the mechanism of their host invasion. To overcome the problems and improve the transformation frequency, we developed an Agrobacterium tumefaciens-mediated genetic transformation (ATMT) system for the clinically important dermatophyte, Trichophyton mentagrophytes. Targeted gene disruption was also achieved by ATMT, but the frequency of homologous recombination via ATMT was still lower. It has recently been reported in several filamentous fungi that deletion of Ku70 and/or Ku80, the key molecules of the non-homologous end joining (NHEJ) pathway involved in DNA double-strand break repair, increases the targeted gene disruption frequency. To improve the homologous recombination frequency, we produced Ku80 deletion mutants of T. mentagrophytes. Ku80- mutant strains may facilitate genetic manipulation of dermatophytes.

CB-04-3

Stress-signalling in *Candida albicans*

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Candida albicans encounters a variety of environmental stresses during disease establishment and progression within the host. Consequently, stress responses are important for the virulence of this major fungal pathogen. Several studies have highlighted the importance of the Hog1 Stress Activated Protein Kinase (SAPK) in *C. albicans* stress responses and virulence. For example Hog1 plays an important role in protection against diverse stress conditions including osmotic, heavy metal and oxidative stress, and *hog1* mutants display attenuated virulence in a mouse model of systemic candidiasis, and are more prone to killing by phagocytes. However, despite the importance of Hog1 in *C. albicans* stress responses, little in known regarding the relay of signals to the SAPK module, or the downstream effectors of the Hog1 mediated stress response.

In an attempt to identify upstream regulators and cellular targets of Hog1 we employed tandem affinity purification (TAP) and peptide mass fingerprinting to identify proteins that physically associate with Hog1 in *C. albicans*. *Ten proteins were identified as potential Hog1 interacting partners. One of these was the serine/threonine protein kinase Rck2, homologues of which are well characterized substrates for the Saccharomyces cerevisiae* Hog1 and the *Schizosaccharomyces pombe* Sty1 SAPKs. In contrast, the remaining identified proteins are potentially novel SAPK interacting partners. One of these, the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (Gap1) was chosen for further study, as our parallel proteomic experiments had implicated Hog1 in Gap1 modification and glycolysis. Data will be presented illustrating the role of Hog1 in regulating Gap1 in *C. albicans*. This work, which directly links a key stress signalling pathway to a central metabolic pathway, is significant due to the growing evidence that modulation of metabolism is a key aspect of stress responses.

CB-04-4

Novel functions of the fungal biosurfactant protein in degradation of biopolymers: *Aspergillus oryzae* hydrophobin RolA laterally moves on hydrophobic surfaces and recruits polyesterases

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When fungi grow on plant or insect surfaces coated with wax polyesters that protect against pathogens, the fungi generally form aerial hyphae to contact the surfaces. Aerial structures such as hyphae and conidiophores are coated with hydrophobins, which are surface-active proteins involved in adhesion to hydrophobic surfaces. Hydrophobins are small proteins containing eight conserved cysteine residues, which are ubiquitous among filamentous fungi. When the industrial fungus *Aspergillus oryzae* was cultivated in a liquid medium containing the biodegradable polyester polybutylene succinate-coadipate (PBSA), the rolA gene encoding hydrophobin RolA was highly transcribed. High levels of RolA and its localization on the cell surface in the presence of PBSA were confirmed by immunostaining. Under these conditions, *A. oryzae* simultaneously produced the cutinase CutL1, which hydrolyzes PBSA. Cutinases, which are well known to be produced by pathogenic fungi, hydrolyze cutin, a heterologous complex of wax esters found on the surfaces of plants. Preincubation of PBSA with RolA stimulated PBSA degradation by CutL1, suggesting that RolA bound to the PBSA surface was required for the stimulation. Immunostaining revealed that PBSA films coated with RolA specifically adsorbed CutL1. Quartz crystal microbalance analyses further demonstrated that RolA attached to a hydrophobic sensor chip specifically recruited CutL1. These results suggest that RolA adsorbed to the hydrophobic surface of PBSA recruits CutL1, resulting in condensation of CutL1 on the PBSA surface and consequent stimulation of PBSA hydrolysis. A fluorescence recovery after photobleaching experiment on PBSA films coated with FITC-labeled RolA suggested that RolA moves laterally on the film. We discuss the implications of both novel properties of hydrophobin RolA in making contact to plants, insects, or animals prior to fungal infection, because the PBSA degradation process catalyzed by *A. oryzae* CutL1 in combination with RolA likely mimics the early stage of fungal infection.

CB-04-5

Transcriptional control of carbon metabolism in *Candida albicans*

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We and others have shown that *Candida albicans* cells phagocytosed by macrophages activate an extensive program of transcriptional changes, including a metabolic transition from glycolysis to gluconeogenesis. Some of these pathways, such as beta-oxidation, the glyoxylate cycle, and gluconeogenesis, are required for full virulence in mouse models, indicating that some niches in the mammalian host are carbon-poor. Mutations in these highly conserved pathways, such as icl1 and fox2; have unexpected pleiotropic phenotypes in *C. albicans*; notably, the fox2 mutant (beta-oxidation) fails to utilize ethanol as a carbon source. The Distel group has shown that this is at least partly due to defects in peroxisome function. We identified the transcriptional regulators of alternative carbon metabolism pathways based on S. cerevisiae and Aspergillus nidulans. *C. albicans* contains homologs of the S.cerevisiae Cat8p and Adr1p (FacB and AmdX in A. nidulans) transcription factors that control expression of glyoxylate and gluconeogenic genes. Null mutants in *C. albicans* confer no apparent phenotype in vitro, in contrast to the corresponding S. cerevisiae mutants (but similar to A. nidulans). *C. albicans* contains a homolog (CTF1) of the A. nidulans fatty acid catabolism transcription factors FarA and FarB, but does not have Oaf1 or Pip2, regulators of peroxisome biogenesis genes in yeast. CTF1 is required for growth on oleic acids (as are FarA/FarB) and FOX2 expression is dependent on CTF1. In vivo, the ctf1 mutant showed a mild attenuation in virulence, similar to the fox2 mutant. ctf1 mutant strains did not, however, confer pleiotropic phenotypes. Thus, both phenotypic and genotypic observations suggest that the regulatory network for alternative carbon metabolism in *C. albicans* is more similar to filamentous fungi than budding yeast. We are identifying targets of these regulators to understand more completely the observed pleiotropic phenotypes and the roles of these pathways in vivo.

CB-05-1

Comparison between the closely related species *Candida albicans* and *Candida dubliniensis*

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Candida dubliniensis is the closest relative of *Candida albicans*, the most pathogenic *Candida species*. However, despite this, epidemiological and infection model data show that *C. dubliniensis* is significantly less pathogenic than *C. albicans*. In addition, multilocus sequence typing shows that the population structure of *C. dubliniensis* is significantly less divergent than that of *C. albicans*. The reasons for the lower virulence of *C. dubliniensis* are not obvious as both species share many characteristics that are usually associated with pathogenicity, including the ability to produce true hyphae. The *C. dubliniensis* genome sequence has recently been completed by the Wellcome Trust Sanger Institute (http://www.sanger.ac.uk/sequencing/*Candida*/dubliniensis/). As expected, comparison of the *C. albicans* and *C. dubliniensis* genomes revealed that they are highly similar and that synteny is largely conserved. However, significant differences in the composition of a number of gene families are evident, some of which have been previously associated with virulence (e.g. *SAP* and *ALS* families), while others have no known function (e.g. the telomere-associated *TLO* family and the *IFA* family encoding putative transmembrane proteins). *C. dubliniensis* also possesses a larger number of pseudogenes than *C. albicans*, including several filamentous growth regulator (*FGR*) genes that may play a role in morphogenesis. These data suggest that *C. albicans* has undergone expansions of specific gene families while *C. dubliniensis* may have undergone reductive evolution of redundant loci following restriction to specialized, and as yet unidentified, ecological niche(s). Comparative transcriptomic analysis also suggests that differential expression of specific genes very likely contributes to phenotypic differences between the two species.

CB-05-2

The role of genetic code ambiguity in *Candida albicans* and its impact on proteome diversity

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Alterations to the standard genetic code have been found in both prokaryotes and eukaryotes, demolishing the dogma of an immutable and universal genetic code. Recent studies suggest that evolution of such alterations require structural change of the translation machinery and are driven through mechanisms that require codon decoding ambiguity. In *C.albicans*, a structural change in a novel ser-tRNACAG allows for its recognition by both the LeuRS and SerRS, providing such a device. In order to determine whether this tRNA charging ambiguity results in ambiguous CUG decoding, we have quantified the serine and leucine incorporation at the CUG codon by Mass-Spectrometry. The data showed that 3.0% of leucine and 97.0% of serine are incorporated at CUG codon in vivo under standard growth conditions. Since there are 13,000 CUG codons in *C.albicans* such ambiguity expands the proteome exponentially thus creating a statistical proteome, by synthesis of an array of proteins from an mRNA containing CUG codons. Moreover, this ambiguity increases under stress, indicating that leucine incorporation at the CUG is sensitive to environmental change and manipulated during mRNA translation, raising the hypothesis that leucine incorporation may be higher than that measured. In order to determine its highest levels, we have created highly ambiguous *C.albicans* cells through tRNA engineering. These cells tolerate up to 28% leucine incorporation at CUG, which represents an increase of 28,000 fold in decoding error rate, without significant decrease in growth rate. Such ambiguity resulted in morphological variation, though exposing a hidden phenotypic diversity. Whole genome analysis of CUG distribution unveiled an impressive flexibility of the *C.albicans* proteome since 66% of its genes have CUG codons, which have the potential to generate 283 billion different proteins. This study highlights novel features of *C.albicans* biology and unanticipated roles for codon ambiguity in the evolution of the genetic code.

CB-05-3

Aspergillus fumigatus gene expression in experimental murine lung infections

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Aspergillus fumigatus is the most frequent cause of invasive aspergillosis in immune suppressed human patients. We have developed a murine model for analyzing the early stages of *A. fumigatus* colonization and progression to invasive disease. The model incorporates instillation of conidia into mouse lungs and subsequent harvesting of bronchoalveolar lavage fluid (BALF) samples for analysis. Validated mRNA amplification and analysis protocols have allowed transcriptome analysis of the fungal mRNAs present in the BALFs.

Expression profiling of *A. fumigatus* germlings at 12-14 hours after instillation into neutropenic mouse lungs reveals dramatically altered gene expression relative to growth in laboratory culture. Up-regulated genes are often found in secondary metabolism and other accessory gene clusters such gliotoxin, pseurotin, and siderophore biosynthesis clusters. We found also significant concordance between the observed host-adapted changes in the transcriptome and those resulting from in vitro iron limitation, nitrogen starvation, and loss of the LaeA methyltransferase.

To further elucidate the role of LaeA in *A. fumigatus* virulence, we analyzed temporal gene expression profiles of a wild type and an isogenic laeA-deleted strain, which misregulates gene expression at secondary metabolite gene clusters and is avirulent in a murine model. Growth and differentiation during initiating phases of murine infection were compared between parental and mutated isolates at 4, 8, and 14 hours post-infection in neutropenic mice. Transcriptome analysis of the laeA mutant revealed a major in vivo regulatory deficit of a few secondary metabolite biosynthetic gene clusters and more than thirty accessory gene clusters.

In our continuing studies employing this murine early infection model, we will undertake analysis of hypervirulent A. fumigatus mutants, *A. fumigatus* proteome analysis, and the murine host response to the fungal pathogen.

CB-06-1

Understanding cell cycle control in the pathogenic yeast *Cryptococcus neoformans*

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Cryptococcus neoformans is a pathogenic yeast causing opportunistic infections to immunodeficient patients. Our group has been studying cell regulation processes in this yeast and reported its unique cell cycle behavior which is different from that of the model budding yeast *Saccharomyces cerevisiae*. In contrast to *S. cerevisiae*, very little is known about the molecular regulation of the *C. neoformans* cell cycle. To clarify cell regulation at the molecular level, homologues of cell cycle control genes in *C. neoformans* were cloned and analyzed for their role and function in the cell cycle. We have previously reported the cloning of the CDC28/Cdc2 homologue, *CnCDK1* and three Cdk1 cyclin homologues: two B-type cyclins and a single G1 cyclin gene. In addition we have also cloned the Cdc25 and Wee1 counterpart Candidate genes. We successfully created deletion mutants for *CnCLN1*, *CnCLB2*, and *CnWEE1* but not *CnCLB1* and *CnCDC25*. Complementation tests showed that CnCdk1 and CnCln1 can function in *S. cerevisiae* and CnCdc25 in *Schizosaccharomyces pombe*. Sequence analysis revealed that we have cloned the likely cell cycle gene homologues in *C. neoformans*. For CnCdk1 however, we found a difference in an amino acid residue in the well conserved PSTAIRE motif known to be involved in cyclin binding. In addition, for CnCln1, there was no other sequence that was found to have G1 cyclin similarities despite having multiple G1 cyclins in other budding yeasts such as *S. cerevisiae*. Two upstream ORFs, which are known to affect translational efficiency of many eukaryotic genes, were also found in the 5' leader of *CnCLN1*. *CnCLN1* deletion resulted to a wide range of morphogenetic defects that suggest that it may act as an upstream activator of the complex cell cycle transcription process activated at Start, affecting a variety of downstream reactions that occur during the G1 to S transition.

CB-06-2

The regulation of white-opaque switching and its role in the mating process

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To mate, natural *Candida albicans* a/α strains must undergo homozygosis to a/a or α/α, then switch from white to opaque. This is a more complex scenario than the mating process of S. cerevisiae, and involves a unique signaling process, in which mating-competent opaque cells, through release of mating pheromones, induce mating-incompetent white cells to become adhesive and cohesive, and to form a biofilm that facilitates mating. Induction of white cells involves the unique induction of white genes by pheromone mediated by a cis-acting sequence, WRE, common to the promoters of white-specific, pheromone-induced genes. The configuration of the pheromone responses of white and opaque cells includes both common elements, the unique participation of the first intracellular loop of the common α-pheromone receptor in the white, but not opaque, response, and distinct downstream transcription activators. The evolutionary implications of this complex set of interactions, and the relationship to pathogenesis will be briefly discussed.

CB-06-3

Biogenesis and germination of *Cryptococcus neoformans* spores

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Cryptococcus neoformans is a human pathogen that can cause meningoencephalitis, primarily in immunocompromised individuals. Although a respiratory route of infection by C. neoformans is generally accepted, the identities of infectious particles in nature are unknown. It has been proposed that infections can be mediated by spores, the products of sexual development. Using gradient centrifugation, we have succeeded in isolating pure spore populations in numbers sufficient for biochemical and molecular analyses. We found that spores differ from yeast cells in several respects: spores are ovoid in shape with a crenulated surface, spores are covered by a thick coat that is compositionally distinct from the yeast cell surface, and spores are resistant to numerous environmental stresses. To understand spore biogenesis and germination, we carried out gene expression microarrays of two phases of development: sexual development and spore germination. Sexual development between yeast cells ultimately results in the production of spores, and spore germination results in the production of yeast. We found that many genes expressed at the end of sexual development (when spore chains are formed) start at high levels in spores and decrease over the course germination into yeast. Microscopic visualization of spore germination using fluorescence microscopy, TEM, and SEM has allowed us to correlate changes in gene expression with morphological states during germination. We found that degradation of the spore coat and subsequent emergence of a yeast cell from the spore body coincide with the expression of hydrolytic enzymes, some of which have been shown previously to play no apparent role in yeast growth. We are currently analyzing several of these enzymes for potential spore-specific roles in germination using genomic, genetic, and biochemical approaches. Our goal is to determine the composition of the spore surface and to uncover how the various components are assembled during sporulation and degraded during germination.

CB-06-4

Cyclin/CDKs and hyphal morphogenesis in *Candida albicans*

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The cyclin-dependent kinase (CDK) Cdc28 is known to play key roles in regulating cell shape in both Saccharomyces cerevisiae and *Candida albicans*. Our lab discovered that C. albcians uses a hypha-specific cyclin/ Cdk, Hgc1/Cdc28, for hyphal development.

To identify Hgc1/Cdc28 substrates, we designed a strategy to find proteins undergoing Hgc1-dependent gel mobility shift. This led to the discovery of several key components of the cell polarity machinery.

First, we found that Rga2, a GAP of Cdc42, undergoes Hgc1-dependent hyperphosphorylation. Using an analog-sensitive Cdc28 mutant, we confirmed that Cdc28 can phosphorylate Rga2 in vitro. We established that Rga2 represses hyphal growth, which is relieved by Hgc1/Cdc28 phosphorylation upon hyphal induction. We obtained evidence to propose a model that Rga2 phosphorylation by Hgc1/Cdc28 prevents it from localizing to hyphal tips, thereby leading to localized Cdc42 activation at the growth site. Rga2 also undergoes transient Cdk-dependent hyperphosphorylation at bud emergence, suggesting that similar mechanisms may regulate bud growth as well.

Second, we found that *C. albicans* uses two G1-cyclin/Cdk kinases, Ccn1/Cdc28 and Hgc1/Cdc28, in a temporally controlled manner to rapidly establish and persistently maintain phosphorylation of the septin Cdc11 during hyphal development. We established that upon hyphal induction, Ccn1/Cdc28 binds to septin complexes and phosphorylates Cdc11 on Ser394. And this phosphorylation requires prior phosphorylation on Ser395 by the septin-associated kinase Gin4. Mutating Ser394 or Ser395 blocked Cdc11 phosphorylation and impaired hyphal morphogenesis. We conducted reconstitution experiments using purified Ccn1/Cdk, Gin4, and septins and were able to reproduce phosphorylation on the same residues. Our results have thus uncovered a direct link between the cell cycle engine and the septin cytoskeleton, and provide new insight into mechanisms for polarized growth that appear to be conserved in fungi.

CB-07-1

Candida albicans Ssa1 mediates host cell invasion

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Background. The *C. albicans* HSP70 proteins, Ssa1 and Ssa2, are located on the cell surface where they are targets of antimicrobial peptides. we investigated the roles of these proteins in *C. albicans* host cell invasion and virulence.

Methods. The virulence of *ssa1δ/δ* and *ssa2δ/δ* mutants was measured in mouse models of oropharyngeal candidiasis (OPC) and hematogenously disseminated candidiasis (HDC). Their susceptibility to environmental stressors and killing by the HL-60 neutrophil-like cell line was measured. The capacity of these mutants to bind to N-cadherin and E-cadherin, and to invade and damage endothelial cells and the FaDu oral epithelial cell line in vitro was also determined. Finally, the endothelial and epithelial cell interactions of latex beads coated with BSA, or recombinant Ssa1 were analyzed.

Results. All mice infected intravenously with the *ssa1δ/δ* mutant survived after 21 days compared to a median survival of 7-8 days for mice infected with the wild-type (WT), *ssa2δ/δ*, and *ssa1δ/δ::SSA1* complemented strains. Mice infected with *ssa1δ/δ* mutant also had significantly reduced kidney, liver, and brain fungal burden. Moreover, the *ssa1δ/δ* mutant had markedly impaired virulence in the mouse model of OPC. Mice infected orally with this strain had ~100-fold lower oral fungal burden after 5 days of infection compared to mice infected with the control strains. The *ssa1δ/δ* mutant had WT susceptibility to environmental stressors and killing by HL-60 cells. However, it had significantly reduced capacity to bind to cadherins, and to invade and damage endothelial and epithelial cells. Furthermore, significantly more latex beads coated with recombinant Ssa1 were internalized by these cells compare with beads coated with BSA.

Conclusions. *Ssa1* is essential for normal *C. albicans* virulence during HDC and OPC. Ssa1 functions as an invasin by binding to host cell cadherins and mediating invasion of these cells.

CB-07-2

Iron aquisition of *Candida albicans* during oral infections

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Iron sequestration by host iron-binding proteins is an important mechanism of resistance to microbial infections. Inside oral epithelial cells, iron is stored within ferritin, and is therefore not usually accessible to pathogenic microbes. We observed that the ferritin concentration within oral epithelial cells was directly related to their susceptibility to damage by the human pathogenic fungus, *Candida albicans*. Thus, we hypothesized that host ferritin is used as an iron source by this organism. We found that *C. albicans* was able to grow on agar at physiological pH with ferritin as the sole source of iron, while the baker's yeast Saccharomyces cerevisiae could not. A screen of *C. albicans* mutants lacking components of each of the three known iron acquisition systems revealed that only the reductive pathway is involved in iron utilization from ferritin by this fungus. Additionally, *C. albicans* hyphae, but not yeast cells bound ferritin, and this binding was crucial for iron acquisition from ferritin. Transcriptional profiling of wild-type and hyphal-defective *C. albicans* strains suggested that the *C. albicans* invasin-like protein Als3 is required for ferritin binding. Hyphae of an Dals3 null mutant had a strongly reduced ability to bind ferritin and these mutant cells grew poorly on agar plates with ferritin as the sole source of iron. Heterologous expression of Als3, but not Als1 or Als5, two closely related members of the Als protein family, allowed S. cerevisiae to bind ferritin. Immunocytochemical localization of ferritin in epithelial cells infected with *C. albicans* showed ferritin surrounding invading hyphae of the wild-type, but not the Dals3 mutant strain. This mutant was also unable to damage epithelial cells in vitro. Therefore, *C. albicans* can exploit iron from ferritin via morphology-dependent binding through Als3, suggesting that this single protein has multiple virulence attributes.

CB-07-3

Molecular genetics studies of dermatophytes: Investigation of secreted proteases and other possible virulence-related factors

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Dermatophytes are human and animal pathogenic fungi that commonly gain access to the host via keratinized structures, such as the hair, skin, or nails, and cause superficial mycosis. To penetrate through these solid barriers and maintain growth on host tissues, dermatophytes appear to produce various secreted hydrolytic enzymes both constitutively and inductively. As possible virulence-related factors, a great deal of attention has been focused on the secreted proteases that digest keratins, the major constituents of the cornified tissues. There have been many reports of biochemical and molecular biological characterization of secreted proteases from different dermatophytes. Although little is known about the regulation of protease gene expression in dermatophytes, extensive studies of protease production in other filamentous fungi, Aspergillus nidulans and Neurospora crassa, suggested that their expression may be related to catabolic repression.

High-throughput gene analysis methodologies, such as differential cDNA screening and cDNA-based microarray analysis, have recently begun to be applied for molecular genetics studies of dermatophytes, leading to accumulation of their gene expression profiles under different growth conditions.

On the other hand, due to the difficulty of genetic manipulation in dermatophytes caused by the low transformation frequencies, there have been only a few reports regarding successful production of null mutants by targeted gene disruption via homologous recombination. However, an efficient genetic transformation system for the clinically important dermatophyte, Trichophyton mentagrophytes, via Agrobacterium tumefaciens (ATMT) was recently developed. Inactivation of Ku80, one of the key molecules of the non-homologous end joining (NHEJ) pathway involved in DNA double-strand break repair, also facilitated the production of null mutants by targeted gene disruption in this fungus. The development of such genetic manipulation systems will promote large-scale molecular genetics studies of dermatophytes, leading to a better understanding of their mechanisms of host invasion.

CB-07-4

Extracellular delivery of potential virulence factors in *Paracoccidioides brasiliensis*

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Exosomes have been recognized as important structures related with virulence of microorganisms and modulation of the host's immunity. Exosome-like vesicles have recently been characterized in fungal pathogens such as Cryptococcus neoformans and Histoplasma capsulatum. We describe the isolation and partial characterization of membranous extracellular vesicles in *Paracoccidioides brasiliensis*, a dimorphic fungus that causes human paracoccidioidomycosis (PCM). We compared two *P. brasiliensis* isolates: Pb3, which represents phylogenetic group PS2, and Pb18, from the main species S1 and widely used in experimental PCM. We have previously shown that the progression of disease and pattern of immune responses in B10.A mice differ when comparing infection with S1 (more virulent) to PS2 isolates. Cell-free supernatant fluids from *P. brasiliensis* yeast cells cultivated at 36oC in F-12 defined medium (Gibco)/glucose were concentrated in Amicon and ultracentrifuged (100,000g). Pellets analyzed by electron microscopy showed the presence of 2-layered membranous vesicles sizing 20 to over 200 nm. Intense immunogold labeling with MOA lectin was observed on the surface and inside the vesicles. Sterols have been detected in 100,000g extracellular pellets derived from live, but not dead cells, suggesting that the membranous structures do not result from cell debris. Specific *P. brasiliensis* antigens were present in 100,000g extracellular preparations, as revealed in immunoblots with sera from PCM patients, but not from healthy individuals. Similar reactivity patterns between Pb3 and Pb18 were observed. Enzymatic activities of laccase, phosphatases, and urease were detected in dose-response experiments with extracellular pellets from both Pb18 and Pb3, however the levels of laccase and phosphatase activities were comparatively higher in Pb18. Preliminary data using 100,000g pellets further fractionated in sucrose gradient suggested the induction of at least nitric oxide by J774 cultured macrophages. Proteomic and lipomic analyses of the vesicle fractions are being performed.

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CB-07-5

Putative virulence factors of *Aspergillus fumigatus*

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The number of patients with invasive fungal infection (IFI) has dramatically increased in last 3 decades. *Aspergillus fumigatus*, the most common species recovered from aspergillosis, is one of the most important pathogen of IFI. Although our antifungal armamentarium has been expanded recently, mortality from aspergillosis is still high. Early initiation of therapy seems to improve the survival rate. Study of virulence factors of the fungus may lead to the development of novel diagnostic tools or advancements in therapy.

A. fumigatus is known to produce various immunomodulatory substances. Although many Candidates of the fungal virulence factors have been studied, non of these substances has been confirmed as being tied to the pathogenesis of the fungus. We previously examined the influence of fungal secondary metabolites such as gliotoxin and other low molecular substances on the virulence. Gliotoxin possesses potent immunosuppressive activity on various mammalian cells, but was known to be produced very slowly in a conventional environment. Recently it was learned that, in well-aerated condition, gliotoxin is produced much faster than previously believed. The primary target of infection by *A. fumigatus* is lung, which is well-aerated organ with high oxygen content, and this fact is believed to contribute to gliotoxin production in vivo.

Recently, gliotoxin was found to be detectable in the sera of aspergillosis mice and of aspergillosis patients. Moreover, the δGliP mutant strains (GliP: the gene encodes a nonribosomal peptide synthase that catalyzes the first step in gliotoxin biosynthesis) showed partially attenuated virulence in immunosuppressive mice. These findings indicate that gliotoxin is, at least partly, showed to contribute the pathogenesis of aspergillosis.

Some other virulence factors are expected to play a synergistic role with gliotoxin, and we showed that *A. fumigatus* produces potent cytotoxic substances other than gliotoxin. Studies are in progress to clarify the significance of the unknown substances.

CB-08-1

Molecular characterisation of a second CO2 sensing pathway in the fungal pathogen *Candida albicans*

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Carbonic anhydrases (CA) catalyse the hydration of CO2. CA expression is critical for the yeast human pathogen *Candida albicans* which faces several environmental changes, such as CO2 availability, depending on its infection location. Indeed, inactivation of the CA gene (NCE103) compromises fungal growth in air (0.033% CO2). However, exposure of the mutant to an atmosphere enriched with CO2 (5.5%, physiological conditions), restores growth. NCE103 transcript levels in air are two-fold higher than in 5.5% CO2 demonstrating a clear transcriptional regulation of this gene. Nce103p protein levels show an even more pronounced regulation, suggesting the presence of additional post-translational regulation. In mammals, CO2 is sensed by adenylate cyclase (AC), leading to the activation of the cAMP-PKA pathway. This sensing pathway is conserved in yeast, but NCE103 is found to be regulated by CO2 in an AC- independent manner. These finding imply the presence of a second CO2 sensing pathway. Mammalian carbonic anhydrase IX has been described to be regulated by the transcription regulator HIF-1p, but no ortholog has been identified in yeast. However, we have established that in the model yeast Saccharomyces cerevisiae, the CA (ScNCE103) is regulated by the helix-loop-helix transcription factor Cbf1p. This protein acts as a repressor on ScNCE103 expression in high concentrations of CO2. However, the ortholog of Cbf1p in *C. albicans* is not involved in the regulation of NCE103. We found that the 175 bp in front of the translation start site of *C. albicans* NCE103 are enough to partially complement a mutant strain, but that 284 bp are required to produce a wild-type level and regulation of Nce103p. We have also determined that the N-terminal tail of Nce103p is essential for the function as well as the formation of a complex involving Nce103p.

CB-08-2

Cellular adaptation to host-specific stresses in *Cryptococcus neoformans*

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Cryptococcus neoformans is an important opportunistic human fungal pathogen. The ability to grow at high temperature is an important requirement of *C. neoformans* to establish disease. Previously we found that the *C. neoformans* Ras1 protein is required to maintain cell polarity in response to mild stress, including growth at high temperature, and for sexual differentiation. We also identified downstream components of Ras signaling that mediate this response. Current models suggest that membrane localization is required for Ras protein function and dictates its signaling specificity. To determine the importance of *C. neoformans* Ras1 membrane localization on protein function, we generated *ras1* alleles with mutations altering specific post-translational modifications. Protein farnesylation is required for all Ras1 functions. In contrast, Ras1 palmitoylation is dispensible for mating. However, protein palmitoylation mediates localization of Ras1 to the plasma membrane and is required for normal cell polarity in response to high temperature stress. In addition, likely as a result of its effect on thermotolerance, Ras1 palmitoylation is also required for pathogenesis of *C. neoformans*.

CB-08-3

Profile of microbial volatile organic compounds (MVOCs) in *Aspergillus fumigatus*

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A. fumigatus is a saprophytic filamentous fungus that is responsible for invasive aspergillosis, a life-threatening disease that usually only occurs in immunocompromised patients. *A. fumigatus* is widespread in the environment through asexual spores called conidia. In operating rooms or medical wards, the occurrence of the fungus is visually detectable after the formation of colonies, which makes it too difficult to suppress spreading of the fungal growth. An earlier detection of fungal emergence allows for reducing risks of the fungal infection. Emission of MVOCs, arising either from their metabolism or from degradation of the materials, seems to be an effective Candidate for an indicator of the occurrence of fungal growth in the environment. Some MVOCs are known to be generally synthesized in most of fungi and the others as strain- or species-specific. Examinations of profiles of MVOCs may be applicable to notification of fungal occurrence in the medical environment.

In this paper, we detected MVOCs emitted from two *A. fumigatus* strains, Kuboyama strain (IFM40822) and soil-borne strain (KT0176), during the course of fungal development. The detection was performed by the SPME (solid phase microextraction)/ GC-MS (gas chromatography-mass spectrometry) method. We confirmed the species of MVOCs: some were detected in the early stage of development, others in the later ones.

CB-08-4

Molecular modelling of *A. Fumigatus* signal reception in response to environmental shift

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Appropriate responses to environmental pH govern virulence of numerous fungal pathogens and emerging experimental evidence reveals a complex interplay of transcription factor function during alkaline adaptation. The proteolytically activated *A. nidulans* transcription factor, PacC, is essential for growth at alkaline pH *in vitro*, a phenotype which extrapolates to severe attenuation of virulence in neutropenic mice[1]. Other transcription factors which become important at high pH in *A. nidulans* include the recently characterized SltA and calcium-responsive CrzA proteins, both of which mediate cation tolerance, with differing ion-specificities, CrzA acts downstream of the protein phosphatase, calcineurin, to regulate calcium tolerance in both *A. nidulans* and *A. fumigatus*. An emerging picture of functions under control of these proteins offers insight on normal responses to alkalinisation and ion stress, in particular, the molecular events occurring downstream of transcription factor function.

In order to assess the physiological response of *A. fumigatus* to alkaline stress, and the role of calcium signalling in such environmental adaptation, we have measured temporal gene expression profiles following *in vitro* transfer from acidic to alkaline medium, and in response to calcium exposure. Our analyses identify adaptation mechanisms of vastly different magnitude and longevity. The datasets were analyzed independently and comparatively in order to identify stress-specific responses and examine correlation between the two mechanisms, respectively.

Searching for molecular components upstream of transcription factors, we have scoured the datasets for membrane components of alkaline adaptation. To identify molecular interactions required for initiation of *A. fumigatus* alkaline adaptation we have also used the integral pH-sensing plasma membrane protein PalH to isolate novel protein interactors, from a full length *A. fumigatus* cDNA library, using the yeast membrane two hybrid (split-ubiquitin) system.

1. Bignell, E., et. al. (2005) Mol Microbiol. 55:1072-1084.

CB-08-5

Transcriptome analysis of the *Aspergillus fumigatus* calcineurin

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Calcineurin plays an important role in the control of cell morphology and virulence in fungi. Calcineurin is a serine/threonine-specific protein phosphatase heterodimer consisting of a catalytic subunit A and a Ca2+/Calmodulin binding unit. We have previously shown that calcineurin is not an essential gene in *A. fumigatus*, and presented the roles of calcineurin in regulating differentiation and fitness in this opportunistic pathogen. A mutant of *A. fumigatus* lacking the calcineurin A (calA) catalytic subunit exhibited defective hyphal morphology related to apical extension and branching growth, which resulted in drastically decreased filamentation. Recently, we characterized an *A. fumigatus* CRZ1 homologue, CrzA, and demonstrate its mediation of cellular tolerance to increased concentrations of calcium and manganese. In addition to acute sensitivitiy to these ions, the crzA null mutant suffers altered expression of calcium transporter mRNAs under high concentrations of calcium, and loss of virulence. Here, we extended these studies by investigating which pathways are influenced by *A. fumigatus* calcineurin during proliferation by comparatively determining the transcriptional profile of *A. fumigatus* wild type and delta calA mutant strains. Our results show an important involvement of mitochondrial functions in the delta calA mutant phenotype. Furthermore, we identified several genes that encode transcription factors that have increased mRNA expression in the delta calA mutant and that could be involved in the Cal-CrzA pathway. Finally, we overexpressed the A. nidulans cnaA and cnaB genes and evaluated their influence of mRNA accumulation of selected Candidate genes through real-time RT-PCR experiments. Increased expression of the CnaA induced an augmentation of the germination and proliferation rate. However, this effect is more pronounced on the hyphae extension since none of the mutant strains displayed alterations in the pattern of nuclear kinetics and septa formation.

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CB-09-1

Proteomic approaches to study the many facets of *Candida albicans* biology and pathogenicity

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Candida albicans is an opportunistic pathogenic fungus capable of causing infections in an expanding population of immunosuppressed patients. The implementation of proteomics in the post-genomic era of this organism can provide vital information about its biological complexity and pathogenetic traits. Proteomics is the term generally used to encapsulate all of the technology currently available to analyze global patterns of protein expression and involves the combined application of advanced techniques to resolve, identify, quantify and characterize proteins, as well as bioinformatics tools to store, communicate and interlink protein and DNA sequence and mapping information from the genome project. *C. albicans* proteomic analyses in the laboratory have focused on several processes associated with the host/fungus interactions and pathogenesis, including filamentation, signal transduction, cell wall/adhesion, biofilm formation, secretion, antibody responses and vaccine development. Results have provided important insights into the many different aspects of *C. albicans* biology and pathogenicity. Thus, proteomics is rapidly becoming an indispensable tool in *C. albicans* research, particularly to address problems that cannot be solved by genomic studies. Furthermore, in the near future it is expected that results from proteomic experiments will lead to much needed novel techniques for the management of candidiasis.

CB-09-2

Genome-wide analysis of *Candida albicans* cell wall remodelling

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Fungal cell walls are dynamic organelles that can alter their structures in response to environmental conditions and cell wall perturbing agents. We were interested in identifying the proteins that are important for cell wall remodelling in response to cell wall defects. A non-gel proteomics approach was used to analyse the proteins that are localised to the *C. albicans* cell wall. Comparisons were made between untreated wild type cells and cells treated with agents that interfere with cell wall integrity including Calcofluor White, Congo Red, SDS and the echinocandin class of antifungal drugs. In addition, the cell wall proteome of signalling pathway mutants and mutants with defective cell walls was analysed. The predicted GPI-anchored proteins Phr1, Pga31 and Sap9 were notable in appearing under cell wall stress conditions but were not detected in untreated wild type cells. Phr1 is a member of the Gas family of transglycosidases that modify cell wall beta-(1,3)-glucan. Pga31 is a novel protein that may play a role in chitin assembly as a *pga31* mutant has significantly reduced chitin levels. Sap9 is a member of the Sap family of secreted aspartyl proteinases but is predicted to be GPI-anchored, analogous to the *Saccharomyces cerevisiae* yapsins, which act as sheddases. Transcript profiling by DNA microarray and Northern analysis confirmed that the expression of *PHR1, SAP9* and *PGA31* as well as genes encoding other predicted GPI-proteins was increased in cell wall stress conditions. One cell wall stress-activated, novel, predicted GPI-protein Pga54 was selected for further analysis by generating null mutant and reintegrant strains and by expression in *S. cerevisiae*.

CB-09-3

Transcriptomics and proteomics as a tool for the study of azole antifungal resistance in *Candida albicans*

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Overexpression of *MDR1*, which encodes a multidrug efflux pump of the major facilitator superfamily, and *ERG11*, which encodes the azole target lanosterol demethylase, are both frequent causes of fluconazole resistance in *Candida albicans*. We have used combined gene expression and proteomic profiling to identify the mechanisms by which these genes are constitutively up-regulated and to identify genes and proteins whose expression are under their influence. Using genome-wide gene expression profiling, we identified a zinc cluster transcription factor, designated as *MRR1*, that was coordinately upregulated with *MDR1* in drug-resistant, clinical *C. albicans* isolates (*PLoS Pathog*. 2007;3:e164). Likewise we found *UPC2* and other genes involved in ergosterol biosynthesis to be coordinately upregulated with *ERG11* in a fluconazole-resistant clinical isolate (*Eukaryot Cell*. 2008;7:1180-90). Inactivation of *MRR1* in drug-resistant isolates abolished both *MDR1* expression and multidrug resistance. Sequence analysis of the *MRR1* alleles of matched drug-sensitive and drug-resistant isolate pairs overexpressing *MDR1* showed that resistant isolates had become homozygous for *MRR1* alleles that contained single nucleotide substitutions. Introduction of mutated alleles into a drug-susceptible *C. albicans* strain resulted in constitutive *MDR1* overexpression and multidrug resistance. Likewise, sequence analysis of the *UPC2* alleles of the matched drug-sensitive and drug-resistant isolate pairs overexpressing *ERG11* revealed that the resistant isolate contained a single-nucleotide substitution in one *UPC2* allele. Introduction of the mutated allele into a drug-susceptible strain resulted in constitutive upregulation of *ERG11* and increased resistance to fluconazole. By comparing the transcriptional and proteomic profiles of drug-resistant isolates and *mrr1&* mutants derived from them and of strains carrying wild-type and mutated *MRR1* alleles, we have defined the target genes and proteins that are controlled by Mrr1p. By comparing the gene expression profiles of the fluconazole-resistant isolate and of strains carrying wild-type and mutated *UPC2* alleles, we have identified target genes and proteins that are controlled by Upc2p.

CB-09-4

Transcription activator, AtrR, regulates gene expression of ABC transporters and contributes to azole drug resistance in Aspergilli

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Infections by human pathogenic fungi such as /*Aspergillus fumigatus*/ are commonly treated with the azole fungicides that inhibit the ergosterol biosynthesis in the fungal cell membrane. During the long-term usage of the drug, sometimes emerge azole resistant isolates of /*A. fumigatus*/. A major mechanism of azole resistance in /*A. fumigatus*/ is the upregulation of genes encoding drug efflux pumps, mainly belonging to ABC transporters.

We have already isolated a spontaneous resistant mutant of /Aspergillus oryzae/ for azole drugs and found that at least three ABC transporters were upregulated in the mutant compared to the wild type. Since the expression of several ABC transporter genes was upregulated simultaneously in the mutant, we assumed that azole resistance is caused by mutation of a common transcription factor that controls these gene expressions. Overexpression analyses of transcription factor genes found in /A. oryzae/ genome revealed that upregulation of a zinc cluster gene, designated /atrR/, resulted in increased drug resistance and also induced the gene expression of ABC transporters in /A. oryzae/. Deletion of the /atrR/ reduced the expression level of the three ABC transporter genes and consequently resulted in significant increase in azole drug susceptibility, especially the /atrR/ mutant was also susceptible to fluconazole. Orthologous genes of the /A. oryzae atrR/ have been found widely in genomes of filamentous fungi, including /Aspergillus nidulans, *A. fumigatus*/, and a plant pathogen /Magnaporthe grisea/. Both strains with deletion of these orthologs in /A. nidulans/ and /*A. fumigatus*/ were also hypersensitive to azole drugs. These results indicate that the novel transcription factor, AtrR, regulates gene expression of ABC transporters that would function as drug efflux pumps and contributes to the azole resistance in /Aspergillus /fungi. In addition, transcriptomic study of /A. oryzae atrR/ deletion mutant will be reported.

CB-09-5

Both transcriptomic and proteomic analysis of the *Cryptococcus neoformans* phospholipase C1 mutant indicates a pleiotropic role for PI-PLC

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Background and objectives: Cryptococcal phospholipase C1 (*PLC1*), encoding a phosphatidylinositol-specific phospholipase C (PI-PLC), is essential for growth at 37°C, cell wall integrity, melanin production (via laccase transcription), secretion of the invasin, phospholipase B1 (Plb1) and virulence. *PLC1* regulates at least some of these phenotypes via activation of the PKC/MAPK signalling pathway. The objective of this study is to further investigate the molecular mechanism of *PLC1* in cryptococcal pathogenesis using comparative transcriptomics (microarray) and proteomics.

Methods: Spotted long oligonucleotide microarray and 2D differential in-gel electrophoresis (DIGE) were performed to compare gene and protein expression profiles of the *PLC1* knockout mutant (*d-plc1*) and wild type (WT) *C. neoformans* strain H99. Overrepresented gene ontologies (GO) were used as a basis for determining correlative phenotypic analysis.

Results: In *d-plc1*, 61% of the 491 differentially expressed (DE) proteins identified showed increased expression, compared to WT (301 up-regulated and 190 down-regulated). Similarly, 68% of the 219 DE genes identified in *d-plc1* were up-regulated (149 up-regulated and 70 down-regulated), indicative of a strong correlation between transcription and translation. Many of the DE genes had roles in secretory processes, cell wall homeostasis and nutrient uptake, supportive of previously published phenotypes including reduced secretion of Plb1, a cell wall integrity defect and compromised growth in *d-plc1*, respectively. Other DE genes have roles in fatty acid biosynthesis, transcription regulation, protease and β-glucanase enzyme activities and α-pheromone production, supportive of *d-plc1* phenotypes now presented.

Conclusion:*PLC1,* encoding a PI-PLC, exerts a pleiotropic effect in *C. neoformans* and its molecular mechanisms are complex. Molecular and phenotypic analyses confirm that *PLC1* has an essential role in secretion which is required for host invasion, cell wall homeostasis and integrity, and nutrient uptake and energy utilization. Further epigenetic study in this secretory pathway is warranted.

CB-10-1

Comaparative genomic analysis of mating and virulence in *Candida* species

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The *Candida* clade (defined as species that translate CTG as serine rather than leucine), contains a number of major human pathogens, as well as non-pathogenic species. As part of a international consortium, we contributed to the sequencing and analysis of a second isolate of *Candida albcans* (the most common cause of candidiasis), the major pathogens *Candida* parapsilosis and *Candida* tropicalis, the minor pathogens *Candida* lusitaniae and *Candida* guilliermondii, and Lodderomyces elongisporus, which is rarely isolated from clinical samples. The genomes were compared to the related species Debaryomyces hansenii, a marine yeast, and Pichia stipitis, which is associated with insects and digests woody xylose. The CTG clade species vary significantly in virulence, ploidy and mating ability. We analysed the evolution of the Mating Type-like Locus (MTL) and of genes associated with mating and meiosis in the *Candida* clade. Our analysis shows that the haploid, sexual species form a sub-clade, from which the alpha2 homeodomain protein is missing. The MTL is intact in the diploid species *C. albicans* and C. tropicalis. Surprisingly however, L. elongisporus appears to completely lack an MTL locus, and is also missing other genes involved in the mating signaling pathway. C. parapsilosis, a major cause of infection in neonates, is missing the a1 homeodomain protein, and is also missing an MTLalpha mating partner. In addition, the level of single nucleotide polymorphism is 70-fold lower in C. parapsilosis than in L. elongisporus. Our analysis of the MTL in the closest known relatives of C. parapsilosis (C. orthopsilosis and C. metapsilosis) suggests that C. parapsilosis has undergone species-specific gene loss.

CB-10-2

High Throughput Genetic Approaches For Understanding *Candida albicans* Virulence

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Candida albicans typically resides in the gastrointestinal tracts of humans and other warm-blooded animals. It is also the most common human fungal pathogen, causing a variety of skin and soft tissue infections in healthy people and more virulent invasive and disseminated disease in patients with compromised immune systems.

C. albicans is an obligate diploid with a cumbersome parasexual cycle; both properties have effectively blocked large-scale genetic approaches to understanding its virulence; even targeted mutagenesis is difficult because recessive mutations must be introduced twice to produce an observable phenotype.

Until recently, genetics in this organism was limited to studies of small sets of deletion mutants generally involving genes already suspected to affect the process of interest. In the past several years, however, technological innovations have permitted construction of large numbers of different kinds of mutants for use in forward, non-"candidate-based" genetic screens.

Using a methodology developed in the laboratory, we have constructed a set of approximately 1000 isogenic *C. albicans* deletion strains. Each strain has been extensively characterized, including effects of antifungal drugs and measurements of fitness in the mouse, using the tail-vein injection model. The overall results of these screens will be discussed, as well as specific examples of genes involved in drug resistance and virulence.

References:

Davis, D.A., Bruno, V.M., Loza, L., Filler, S.G., Mitchell, A.P. (2002) *Candida albicans* Mds3p, a conserved regulator of pH responses and virulence identified through insertional mutagenesis. Genetics 162:1573-81.

Fonzi W.A., Irwin M.Y. (1993) Isogenic strain construction and gene mapping in *Candida albicans*. Genetics 134:717-28

Morschhauser J., Michel S., Staib P. (1999) Sequential gene disruption in *Candida albicans* by FLP-mediated site-specific recombination. Molecular Microbiology 32:547-56.

Noble, S., Johnson, A.D. (2005) Strains and strategies for large-scale gene deletion studies of the diploid human fungal pathogen *Candida albicans*. Eukaryotic Cell 4:298-309.

Noble, S., Johnson, A.D. (2007) Genetics of *Candida albicans*, a Diploid Human Fungal Pathogen. Annual Reviews 41:193-211.

CB-10-3

The occurrence of ploidy-shift may be due to aberration of chromosome 5 in *Candida albicans*

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The human pathogenic fungus *Candida albicans* has traditionally been classified as a diploid, asexual organism. However, a set of *C. albicans MTL* genes has been identified that corresponds to the master sexual cycle regulators of the *Saccharomyces cerevisiae* mating-type (*MATa/MATalpha*) locus and mating-competent forms of the organism were described that produced tetraploid mating products (1, 2). A phenomenon of ploidy shift was described by the author in this organism (3). A clinical isolate NUM51 contained both diploid and tetraploid cells and electron microscopy of the culture showed that some diploid cells seemed to be undergoing endoduplication, while some tetraploids showed endomitosis with nuclear structures similar to those of meiosis II in *S. cerevisiae*. Our electrokaryotypic analysis of this isolate showed a monosomic chromosome 5 with normal length (1.23 Mb) carrying an *MTLalpha*, and a shorter homeolog containing *MTLa* as an extra chromosome (0.55 Mb). Neither a hemizygous (*-/MTLalpha*) derivative, which was easily constructed by the loss of the homeolog from NUM51, nor an *MTLalpha*-disrupted one (*MTLa/MTLalpha::* MPA_r) gave the occurrence of ploidy shift. These findings suggest the occurrence of ploidy shift of this strain may be due to both the loss of a part of chromosme 5 and the heterozygosity for the *MTL* locus.

(1) Hull CM & Johnson AD (1999). Science 285(5431):1271-1275.
(2) Magee BB & Magee PT (2000). Science 289(5477):310-313.
(3) Suzuki T, Kanbe T, Kuroiwa T & Tanaka K (1986). J Gen Microbiol. 132:443-453.

CB-10-4

Investigating the relationship between sexual development and pathogenesis of *Cryptococcus neoformans*

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Cryptococcus neoformans is a human pathogen that causes fungal meningitis, primarily in immunocompromised individuals. Although the infectious particles in human disease are not known, one hypothesis is that infections can be mediated by spores, the products of sexual development of *C. neoformans*. To understand how spores are formed, we carried out an analysis of gene expression over time during development using microarrays. We found that gene expression changes occur in temporal cascades corresponding to the different morphological stages of development. We are using these data to identify genes involved in the late stages of development, presumably controlling spore formation. To study the basic properties of spores, we developed a purification strategy that results in large numbers of pure spores. Using a variety of methods, we have determined that spores are covered by a thick coat that is both morphologically and compositionally distinct from yeast cells that appears to provide spore resistance to environmental stress. We are continuing to explore the physical and biochemical properties of spores with particular attention to the composition of the spore surface. Finally, to understand how spores interact with the host immune response, we carried out phagocytosis assays with alveolar macrophages and virulence assays in mice. We have discovered that macrophages in culture phagocytose spores (but not yeast), spore survival is dependent on macrophage activation state, and spores cause fatal disease in a mouse model of cryptococcosis. We are continuing to investigate the specific interactions between spores and macrophages that mediate phagocytosis. Our data represent the first glimpses into the process of spore biogenesis, the basic properties spores, and the host response to these novel particles. This work lays a foundation for future studies of fungal spores and their roles in disease and informs the study of the mammalian innate immune response to pathogenic fungi.

CB-10-5

A method for mating clinical *Candida albicans* isolates

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Candida albicans can be induced to mate in the laboratory. This raises the question of why mating has never been observed in human patients. It also allows, in principle, strains to be crossed for genetic analysis, although the absence of meiosis complicates the interpretation of these crosses. Both the study of *in vivo* mating and the genetic analysis of strains would benefit from efficient methods for mating clinical isolates. Existing mating techniques involve selection by the use of auxotrophic markers, requiring time-consuming sequential disruption of two copies of biosynthetic genes if wild-type isolates are to be crossed. Furthermore, auxotrophy reduces fitness in animal models, and could potentially interfere with assessing the fitness of recombinants in such models. We have developed a method for mating clinical isolates marked with two drug resistance markers, the mycophenolic acid (MPA) resistance-conferring allele of *IMH3* and the nourseothricin (NAT) resistance gene *CaNAT1*, allowing the selection of recombinants on the basis of resistance to both agents. We could obtain, from 6 pairwise combinations of 7 clinical isolates, recombinants, as verified by PCR amplification of mating type loci and drug resistance cassettes and by FACS analysis of DNA content.

IM-01-1

Recognition of fungal DNA by TLR9

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Innate immune cells can sense pathogen-associated molecular patterns (PAMPs) via pattern recognition receptors (PRRs). The sugar components of the *Candida albicans* cell wall have been reported to be important PAMPs that are recognized by the host innate immune system. Furthermore, several PRRs are involved in the detection of these PAMPs, such as toll-like receptor (TLR)2, TLR4, and dectin-1. In contrast, few studies have reported that fungal nucleic acids stimulate host immune cells. Recently, we reported that DNA from *Cryptococcus neoformans* activates bone marrow dendritic cells (BM-DCs) by triggering the TLR9/MyD88 pathway. We also found that DNA extracted from *C. albicans* induced IL-12p40 production and CD40 expression in BM-DCs by activating a TLR9-mediated signaling pathway. Under physiological conditions, TLR9 can recognize *C. albicans* DNA, which may be released from dying fungal cells that are located outside host immune cells or from live fungal cells that are phagocytosed by immune cells. After the phagocytosis of *C. albicans*, DNA may be released from these fungal cells, resulting in the stimulation of the TLR9-mediated signaling pathway. Thus, this pathway may be involved in the host defense response against *C. albicans*. However, the fungal organ burden in TLR9-deficient mice after systemic infection with *C. albicans* was similar to that in control mice. With the recent progress in understanding the mechanism of *C. albicans* recognition, it is suggested that the interactions between multiple PAMPs and PRRs, which enable the recognition of different components of fungal cells, are integrated, making it possible for the immune system to respond to this pathogen in more sensitive and specific manner. Moreover, signals from different PRRs function synergistically or antagonistically under physiological conditions. Understanding the *C. albicans* DNA-TLR9 interaction and downstream signaling pathway could provide insights into the overall mechanism of *C. albicans* recognition by PRRs and the subsequent immune response.

IM-01-2

Cross-talk between PARs and TLRs in fungal infections

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The inflammatory response is characteristic of the innate immune defense against fungi and is controlled by Toll-like receptors (TLR) that, by affecting the balance between oxidative and nonoxidative fungicidal mechanisms and pro- and anti-inflammatory cytokine production, ultimately impact on the quality of microbicidal activity and inflammatory pathology. Although individual TLR activates specific antifungal programmes on phagocytes, cooperation between TLR and other innate immune receptors is key to regulating and shaping innate antifungal immunity. Extracellular proteases can specifically cleave and trigger protease-activated receptors (PARs), a family of four G-protein-coupled receptors that posses specific cleavage sites for serine proteases within their extracellular N-terminal domains. Activated PARs couple to signaling cascades that affect, among others, secretion and inflammatory responses. The involvement of PARs in the host response to fungi and/or fungal proteases has long been suspected but never proved. We have determined that activation of TLRs by fungi unmasked an essential and divergent role for PAR1 and PAR2 in downstream signaling and inflammation. TLRs activated PARs and triggered distinct signal transduction pathways involved in inflammation and immunity to *Candida albicans* and *Aspergillus fumigatus*. Conceptually, that fungal recognition by TLRs implicates the participation of PARs sensing tissue injury and conditioning the activity of TLRs recognizing microbial motifs, defines a binary signalling pathway in mammalian response to fungi.

IM-01-3

Characterization of PMN chemotactic factors involved in susceptibility to vaginal candidiasis

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Background: Vulvovaginal candidiasis (VVC) caused by *Candida* species is a common mucosal infection affecting significant numbers of women during their reproductive years. While adaptive immunity and innate resistance by polymorphonuclear neutrophils (PMNs) have no protective role against VVC, an aggressive PMN migration into the vagina occurs in susceptible women resulting in an aberrant inflammatory reaction associated with symptomatic infection. The migration of PMNs is strongly correlated to the vaginal presence of calcium-binding proteins, S100A8 and S100A9, during symptomatic vaginal infection. The purpose of this study was to characterize the role of the calcium-binding proteins in the immunopathogenesis of VVC using the established experimental mouse model. **Methods:** Supernatants from coculture of mouse vaginal tissues and *Candida* blastoconidia were evaluated for PMN chemotactic activity. Expressions of S100A8, S100A9 and a series of pattern recognition receptors (PRRs) were examined on vaginal epithelial cells from inoculated mice. **Results:** Similar to in vivo observations, supernatants from the coculture of estrogenized mouse vaginal explants and *Candida* showed increased PMN chemotactic activity. Epithelial cells from vaginal lavage fluid from inoculated mice with high PMN infiltration stained positive for S100A8 and S100A9 compared to epithelial cells with low or no PMNs, suggesting that the chemotactic calcium-binding proteins are produced by epithelial cells following interaction with *Candida*. Compared to epithelial cells from inoculated mice with low/no vaginal PMNs, those with high vaginal PMNs showed upregulation of mannose receptor and SIGNR1, but not TLR2, TLR4 or dectin-1. **Conclusion:** Together, we hypothesize that vaginal epithelial cells in susceptible hosts are sensitive to PRR activation by *Candida* and produce the calcium-binding proteins that recruit the PMNs responsible for the aberrant inflammatory response and symptoms associated with infection.

IM-01-4

Multiple roles of *Candida albicans*-derived cell wall components in human keratinocytes - Activation of immune response and induction of apoptosis

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Rapid immune response in *Candida* infections is mediated by a number of innate recognition molecules known as pattern recognition receptors (PRRs). PRRs recognize conserved motifs called pathogen-associated molecular patterns (PAMPs), which represent broad groups of microbial pathogens or components. The signalling pathways trigger subsequent inflammatory responses which are crucial for successful host defence against pathogens. Fungal cell wall components such as beta-glucan and mannoproteins have been shown to stimulate the innate immune response in myeloid cells in a toll-like receptor-dependent manner, particularly through TLR2 and TLR4. However, *Candida albicans* cell wall components that specifically induce TLR responses in keratinocytes have not yet been investigated in detail.

In our studies we first examined the effect of different cell wall extractions from *C. albicans* on TLR gene expression and found an increase of TLR4 and a slight increase of TLR10, accompanied with an induction of GM-CSF and IL-8 levels, analyzed by quantitative RT-PCR and ELISA. However, the different cell wall extractions showed no major differences in the TLR expression pattern and cytokine release.

Surprisingly, stimulated keratinocytes showed a strong growth inhibition after 24h of treatment with the cell wall components. Analysis by proliferation assays resulted in nearly 90% resting cells. This observed growth inhibition is caused by a strong accumulation of the cell cycle inhibitor p27Kip1 inside the nucleus. More detailed analysis showed that the cell cycle inhibition resulted in an increase of apoptotic cells up to 30% after 72h.

In conclusion, our results indicate that distinct pattern recognition receptors together trigger the innate immunity in human keratinocytes by recognizing different structures of *C. albicans*. Furthermore, our results demonstrate the diversity of signalling pathways mediated by fungal cell wall components. Triggering innate immune responses result in the secretion of pro-inflammatory mediators which is accompanied by growth inhibition and subsequent induction of apoptosis.

IM-01-5

TNF establish antifungal protection by epithelial TLR4 upregulation

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Immune responsiveness to many pathogens depends on innate recognition molecules known as pattern recognition receptors (PRRs) e.g. Toll-like receptors (TLRs). Infection of a three-dimensional organotypic epithelial model (RHE) with *C. albicans* suppressed TLR4 signalling despite clear evidence of mucosal injury and pro-inflammatory cytokine response. Integration of polymorphonuclear leukocytes (PMNs) mediated upregulation of epithelial TLR4 and concomitant protection against fungal infection which was independent of physical PMN/epithelial cell contact. *Candida* invasion and cell injury could be restored by the addition of TLR4 antibodies and TLR4 'knockdown' using RNA interference. The protective phenotype was associated with a pro-inflammatory cytokine release. Blocking of these cytokines with neutralizing antibodies showed the most significant impact on the TLR4 mediated protective effect for TNF. To confirm the important role of this cytokine in the host defence against *Candida* infections we investigated the role of exogenous TNF for TLR4 expression and protection from fungal invasion in the absence of PMNs. We observed a strong up-regulation of TLR4 gene and protein expression after addition of 1 or 10 ng/ml TNF 12 h after infection of the oral RHE. The increased TLR4 expression was associated with a reduced LDH release and protection from fungal invasion. In contrast, addition of TNF to the oral RHE 1h before inoculation with *Candida* led to increased fungal damage after 24 h compared to the control.

Our results point out that activation of cytokine release are crucial for the upregulation of epithelial TLR4 and the subsequent protection from fungal invasion. Among the cytokines tested, TNF seems to have the most significant impact, which confirms the important role of this cytokine in the host defence against systemic *Candida* infections.

IM-02-1

Renal responses during experimental disseminated *Candida albicans* infection

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Candida albicans bloodstream infections remain a problem in the clinic. A greater understanding of disease development is required to allow faster, more accurate diagnostics to be developed.

The mouse IV challenge model of *C. albicans* disseminated infection is a reproducible, well-characterized model, where the major organ targeted is the kidney, with burdens increasing during disease progression. A similar situation occurs in the human host, making this a good model to investigate host responses.

Renal responses during the early stages of *C. albicans* infection were studied using a combination of transcript profiling, histological analyses and measurement of cytokine/chemokine levels. Responses to both attenuated and virulent *C. albicans* strains were measured. Transcriptionally, the kidney showed only a minimal response to attenuated strain infection, but a massive induction of innate immune response gene expression occurred in response to the virulent strain. Differences in cytokine/chemokine gene expression levels were reflected in protein levels measured in the kidney, with higher levels associated with infections initiated by virulent strains. Histological analyses demonstrated that differences in cytokine/chemokine levels were reflected in lesion numbers and associated immune cell infiltrates found within the kidney. These results demonstrate that early host immune responses influence the pathological course of the infection.

IM-02-2

The influence of β-glucan on the growth and cell wall structure of *Aspergillus*

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β-glucan is one of the fungal cell wall main component polysaccharide. Also, it was detected in the culture supernatant of fungi such as *Aspergillus* and *Candida* so on. Furthermore, it was reported that β-glucan showed various biological activities such as the inflammatory mediator production in vivo and vitro. However, there are few reports to have examined the influence on fungal cell itself of β-glucan. In this study, it examined how the influence of β-glucan on the growth and cell wall structure of fungal cell. *Aspergillus fumigatus* and *Aspergillus oryzae* was cultured with the synthetic medium, C-limiting medium added β-glucan (curdlan and laminarin). In β-glucan adding group on 1day, the promotion of the growth, such as the rise of the turbidity was observed compared with normal culture group. Next, we compared morphological change of *Aspergillus* among these medium by the microscope. In the culture medium added β-glucan, the long hyphae where there is little ramification was observed. The NaClO oxidized cells of the fungus body in BG addition or not cultivation were prepared and their structure analyzed by C^{13}-NMR. In the normal cultivation, β-1,3-glucan was the main component but in the BG addition group, the peak ratio of β-1,3-glucan was rising. In this study, it was suggested that the presence of β-glucan in culture medium changed the growth of the fungi and induced qualitative change such as the cell wall structure. Because β-glucan are detected in the mycology-culture supernatant, the concerning with these phenomena and the pathogenicity has an interest.

IM-02-3

Invasive aspergillosis in hematological and transplant patients: Comparisons between pediatric and adult populations

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Invasive aspergillosis is an important cause of morbidity and mortality in immunocompromised children with hematological malignancies, hematopoietic stem cell transplantation, and solid organ transplantation. Pediatric patients with these conditions have several distinctive host conditions and therapeutic factors. The most common form of childhood leukemia (acute lymphoblastic leukemia) is managed with corticosteroids, which is an added burden of immunosuppression superimposed on chemotherapy-induced neutropenia. These children also receive vinca alkaloids, which may cause life-threatening drug interactions with antifungal triazoles. Patients undergoing lung transplant for cystic fibrosis may have residual tracheobronchial involvement by *Aspergillus* spp. Diagnosis of invasive pulmonary aspergillosis is difficult; however, recent advances in the use of serum galactomannan may improve early recognition of this infection. Although previous reports questioned the utility of serum galactomannan in the management of aspergillosis in children, more recent studies in pediatric oncology patients demonstrate that its sensitivity and specificity are comparable to those of adults. While chest CT scans are an important diagnostic tool in the management of invasive pulmonary aspergillosis, the characteristics of the pulmonary infiltrates may be less specific in pediatric oncology patients. A major body of work conducted during the last fifteen years has demonstrated that systemically administered antifungal agents may have different pharmacokinetic characteristics and dosing requirements in comparison to those of adults. As higher risk pediatric and adult patients are treated with an ever-expanding range of immunosuppressive modalities, diagnosis, treatment and prevention of invasive aspergillosis will present new challenges. Understanding the different immunologic, metabolic, and pharmacological differences in these populations will contribute greatly to successfully meeting the challenge of invasive aspergillosis in both pediatric and adult patients.

IM-02-4

Host susceptibility in mycetoma: The role of sex-hormone synthesis

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Madurella mycetomatis is the main agent of mycetoma, a chronic, subcutaneous infection characterized by discharge of grains and purulent material through sinuses. This infection is more common in men than women. It was therefore hypothesised that sexhormones were important factors in the development of mycetoma. To test this hypothesis, single nucleotide polymorphisms (SNP) in genes involved in sex hormone synthesis were studied in a population of Sudanese mycetoma patients versus geographically and ethnically matched controls. Sexhormones are synthesised from cholesterol by the following genes: CYP17, HSD3beta;, HSD17beta, CYP19, CYP1B1 and COMT. Single nucleotide polymorphisms (SNPs) for each of these genes, which influence sexhormone synthesis, are described. Polymorphisms in CYP19 and COMT were differentially distributed between patients and healthy controls. The CYP19 polymorphism was associated with a higher 17beta-estradiol (E2) production, while the COMT polymorphism was associated with a higher conversion from E2 to 4-methoxy estradiol. Furthermore, the COMT polymorphism was also associated with lesion size. The higher estradiol levels in male patients were confirmed by enzyme amplified sensitivity immunoassay. In women no significant difference in E2 levels was found, which could be due to the high variation of E2 concentrations during the menstrual cycle. Furthermore, for both males and females lower levels of dehydroepiandoresterone (DHEA) were present. No differences in testosterone levels were found. Furthermore, E2, testosterone and DHEA had no influence on the growth rate of *M. mycetomatis*. Therefore, the influence of the sex hormones on *M. mycetomatis* infection is probably not mediated by a direct effect on the fungal cells but more likely by the sex-hormonal stimulation of the immune system. Low DHEA levels and high conversion of estradiol to 4-hydroxyestradiol and methoxy-estradiol are considered a pro-inflammatory event. In conclusion, individuals with certain sexhormone biosynthesis polymorphisms are predisposed to the development of mycetoma.

IM-03-1

Immunoregulation by fungi through IDO

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Infectious agents can induce autoimmune diseases in several experimental settings, some of which have clinical counterparts. A variety of mechanisms have been invoked to explain these observations, including molecular mimicry and an increase in the immunogenicity of autoantigens caused by inflammation in the target organ. Paradoxically, infectious agents can also suppress allergic and autoimmune disorders. A central question is to determine whether immune dysregulation precedes, if not promotes, infection or alternatively, but not mutually exclusive, the extent to which microbial exposure /colonization contributes to the burst of pathogenic autoimmunity. Here we discussed recent evidence that help to accommodate fungi, either commensals or ubiquitous, within the immune homeostasis and its dysregulation. We will discuss how *Candida albicans* and Aspergilus fumigatus exploits multiple, functionally distinct, receptor/signaling pathways in dendritic cells ultimately affecting IDO expression and the local Th17: Treg cell balance. Despite the recognized importance of Tregs in the homeostatic regulation of immune responses, our understanding of their significance and interplay with other pathways of immunity and autoimmunity is limited. We have evidence that the IDO/Treg axis activated in fungal infections could be exploited for the control of inflammation and dysregulated immunity in experimental models of inflammatory diseases.

IM-03-2

Aspergillus fumigatus cell wall associated molecules and immune response in mice

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IM-03-3

IL-22 and IL-17 in anti-fungal immunity: What's new?

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It has been shown that resistance and tolerance are two types of host defense mechanisms to increase fitness in response to fungi. In experimental candidiasis and aspergillosis, both defense mechanisms are activated through the delicate equilibrium between Th1 cells, providing antifungal resistance mechanisms and regulatory T cells (Tregs) limiting the consequences of the associated inflammatory pathology. IDO and kynurenines pivotally contribute to this delicate balance by providing the host with immune mechanisms adequate for protection without necessarily eliminating fungal pathogens or causing an unacceptable level of tissue damage. In their capacity to induce Tregs and inhibit Th17, IDO and kynurenines pivotally contribute to cell lineage decision in experimental fungal infections and revealed an unexpected potential in the control of inflammation, allergy and Th17-driven inflammation in these infections. In this context, the Th17 pathway, which down-regulates tryptophan catabolism, may instead favor pathology and serves to accommodate the seemingly paradoxical association of chronic inflammation with fungal disease. Further tweaking the Th17 model, through production of IL-22, Th17 may exert a protective role in fungal infections. In the relative absence of Th1/Treg cell responses, IL-22Th17 cells may fulfill the role of a protective response that exploits primitive antifungal effector defense mechanisms. This finding suggests that functionally distinct 'modules' of immunity evolved to provide resistance, i.e, ability to limit fungal burden, or tolerance, i.e., the ability to limit the host damage in response to fungi.

IM-03-4

Clinical and experimental evidence for a relation between *Candida albicans* and Crohn's disease

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Crohn disease (CD) is a chronic inflammatory bowel disease. Its incidence has increased these last decades in developed countries. Compelling evidence suggest that uncontrolled inflammation of CD is based on genetic susceptibility to microbial antigens. Altough *C. albicans* (CA) is a commensal of the gut, its role has never been investigated. Development of CD is related with appearance of antibodies against microbial antigens. We showed that anti-S. cerevisiae mannan- antibodies (ASCA) were serological markers present in 60% of CD patients and 20% of their healthy relatives (HR) vs 7% in controls. Evidence was then gained that ASCA epitopes were expressed by CA in human tissues suggesting that CA was the immunogen for ASCA. This was reinforced by the recent demonstration that novel markers of CD consisting in antibodies against synthetic disaccharide fragments of chitin and glucan were also generated during a CA infection. Mycological exploration of CD families showed that CD patients and their HR were more colonized by CA than control families. In HR, CA colonization correlated with ASCA levels whereas disease outset was associated with ASCA stability and independence from CA intestinal load.
We showed that chemically induced colitis promotes stable CA colonization in mice. In turn CA colonization was shown to increase colon inflammation as assessed by histological scores and cytokines expression. This model confirmed that ASCA were generated by CA in an inflammatory background. CA was also shown to modulate pathogen recognition receptors expression. The use of mice KO for galectin-3, a lectin involved in both CA sensing and inflammation, confirmed that its presence and cooperation with TLR2 was important for modulation of CA induced inflammation.
Altogether these data suggest that intestinal diseases represent a quite unexplored research field to unravel yet unknown aspects of CA biology in its natural niche and possible medical impact.

IM-03-5

Natural killer cells exhibit direct activity against *Aspergillus fumigatus*

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Although animal models demonstrated that the recruitment of Natural Killer (NK) cells to the lungs plays a critical role in the host defense against invasive aspergillosis, little is known about the antifungal activity of NK cells. We therefore incubated purified unstimulated human CD56+CD3-NK cells ("fresh NK cells") and IL-2 (1000 units/ml, 7-10 days) stimulated human NK cells ("stimulated NK cells") with $1.5x10^4$ *Aspergillus fumigatus* conidia cultivated for 17 hours for germination to hyphae. Increasing E:T ratios (10:1, 20:1 and 50:1) resulted in increasing hyphal damage at 2, 4, and 6 hours of co-incubation, respectively, as demonstrated by means of the XTT assay. Notably, antifungal activity lasted longer in stimulated NK cells as compared to fresh NK cells. The direct activity of NK cells against the hyphae was also microscopically demonstrated in the viability staining with 5-carboxy-fluorescein diacetate (CFDA)/propidium iodide. The extent of the hyphal damage by both fresh and stimulated NK cells incubated with *Aspergillus* correlated with the concentration of perforin and granzyme B in the supernatant, as assessed by ELISA. Whereas no significant perforin and granzyme B concentration was measured in the supernatant of fresh NK cells without co-incubation with *Aspergillus*, high concentrations of both molecules were seen in the supernatant of IL-2 stimulated NK cells alone. Blocking experiments performed with antibodies against the Toll-like receptors TLR 2 and 4 and against the Natural Cytotoxicity Receptors (NCR) NKp30, NKp44, and NKp46 suggest that these receptors are also involved in the direct activity of human NK cells against *Aspergillus*. In conclusion, our results demonstrate that NK cells are directly involved in the host defense against *Aspergillus*, and further insight into these mechanisms might help in the development of immunotherapeutic antifungal strategies.

IM-04-1

Malassezia colonization and the IgE antibody response in atopic dermatitis

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Malassezia yeasts are part of the cutaneous microflora, also associated with a number of skin diseases including atopic dermatitis (AD). The contribution of *Malassezia* colonization to the pathogenesis of AD has been proposed on the basis of the observation that most AD patients have a high titer of *Malassezia*-specific serum IgE antibodies, and that organisms of the *Malassezia* species, particularly *M. globosa* and *M. restricta*, are identified at high frequencies in AD patients. However, the precise mechanisms by which *Malassezia* colonization induces the IgE antibody production and inflammatory cascades remain unclear.

Numerous *Malassezia* allergens have been identified and characterized previously. In a current approach, the proteomics analysis was adopted to identify major allergens from *M. globosa*. The IgE-reactive component of *M. globosa* with a molecular mass of 42 kDa, designated MGp42, was identified by two-dimensional immunoblotting, and sequenced partially by the matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) with post source decay (PSD) of the peptide digest. The full-length cDNA encoding MGp42 was cloned and sequenced by using the rapid amplification of cDNA ends (RACE) method. Comparison of sequences with known protein sequences revealed that MGp42 showed similarity to the heat shock protein (hsp) family.

The production of IgE antibodies is implicated in Th2 type immune response. Keratinocytes play a critical role in the pathogenesis of AD by secreting a variety of cytokines. The cytokine secretion profiles using antibody array analysis revealed that *M. globosa* and *M. restricta* induced the secretion of distinct Th2-type cytokines from human keratinocytes; *M. globosa* induced IL-5, IL-10, and IL-13 secretion, while *M. restricta* induced IL-4 secretion. These findings were confirmed by cDNA microarray analysis. It is possible that *M. globosa* and *M. restricta* play a synergistic role in triggering Th2-shifted humoral immune response in AD.

IM-04-2

Use of monoclonal and human domain antibodies against antigens of *Candida albicans* on passive protection against vaginal candidiasis

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Vulvovaginal candidiasis is a mucosal infection affecting a large proportion of women. Antifungal therapy does not prevent recurrences, in fact, there is clinical evidence of its important role in the pathogenesis of recurrent vulvovaginal candidiasis (RVVC).

To investigate RVVC pathogenesis and to optimize preventive and therapeutic antifungal strategies, we employed a rat model of vaginal infection in which an initial self-healing infection confers a high degree of protection against subsequent re-infection by *C. albicans*. The protection is associated with the presence of protective antibodies against *Candida* constituents in the vaginal fluids and the increased number of activated lymphocytes in the vaginal mucosa. Thus, we investigated the potential protective effect of specific Abs against an immunogenic cell-wall antigen (mannoprotein) and against a virulence factor of *Candida* such as aspartyl proteinase (Sap). Animals receiving vaginal fluids from *C. albicans*-infected rats and containing anti-mannan (MP) and anti- aspartyl proteinase (Sap) Abs were significantly protected against vaginitis compared to animals given Ab-free vaginal fluid from noninfected rats. A degree of protection against *Candida* vaginitis was also conferred by postinfectious administration of anti-Sap and anti-MP monoclonal antibodies.

We evaluated the activity of human domains antibodies (DAbs) generated against recombinant 65-kDa mannoprotein (rMP65) or aspartyl proteinase (r-Sap), which strongly inhibited *Candida* adherence to endothelial and epithelial cells. Domains antibodies which specifically bind Mp65 or Sap2 exerted both a marked preventive and curative effect on experimental vaginal candidiasis. In fact, both DAb families strongly accelerated clearance of *C. albicans* from rat vagina to a greater extent when they were intravaginally administered before or after *Candida* challenge and were equally effective against both azole-susceptible and azole-resistant isolates of *C. albicans*.

These results evidence a potential therapeutic use of some Abs or their engineered derivatives in the treatment of *Candida* vaginal infections.

IM-04-3

Immunomodulatory effects of monoclonal antibodies to the dimorphic pathogenic fungus *Paracoccidioides brasiliensis*

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Paracoccidioides brasiliensis, the etiological agent of paracoccidioidomycosis (PCM), is a thermally dimorphic fungus prevalent in Latin America. Historically, protection against paracoccidioidomycosis has been attributed to a vigorous cellular immune response whereas high levels of specific antibodies have been associated with disease severity. The major diagnostic antigen of P. brasiliensis known as gp43 has been the focus of several immunological studies since it is immunodominant. There is evidence that monoclonal antibodies to gp43 can modify the course of disease in mice infected with this fungus. Using a panel of monoclonal antibodies against gp43 we identified protective (mAb3E) and nonprotective (mAb32H) antibodies. The epitope of the protective mAb3E was located to the sequence NHVRIPIGYWAV that is shared with internal sequences of β-1,3-glucanases from *Aspergillus fumigatus*, *A. oryzae* and *Brunnea graminis*. The protective antibodies have no direct effect on yeasts and their activity is rather associated with capacity of opsonization of fungal cells. The ingestion of opsonized yeast cells led to an increase in NO production by macrophages. Passive administration of mAb3E during experimental infections led to reduced fungal burden, decreased pulmonary inflammation and enhanced levels of IFN-γ in the lungs of infected mice. On the other hand, animals immunized with mAb32H showed intense infiltration of macrophages, lymphocytes and epithelioid cells similar to untreated control animals.

Presently we show that protective and nonprotective monoclonal antibodies could be induced against the same P. brasiliensis antigen, the gp43. Only the protective mAb was able to opsonize yeast cells suggesting that the epitope recognized by the nonprotective mAb was less accessible at the yeast cell surface for reactivity. A potential therapeutic use of monoclonal antibodies is also envisaged. Protective mAbs can be associated to formulations based on peptide P10 that have progressed towards the design of a vaccine to be used adjuvant to chemotherapy.

IM-04-4

Antifungal cryptic activity of antibody peptides

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Paradigmatic approaches have revealed different functions for antifungal Abs, including direct fungicidal activity and ability to modify inflammatory response. Protective yeast killer toxin (KT)-like Abs have been produced by idiotypic vaccination with a KT-neutralizing mAb and revealed in the vaginal fluids of women infected with *Candida albicans*. A killer decapeptide (KP) has been engineered as functional mimotope from a fragment (P6) of a recombinant KT-like Ab. KP has proven to display fungicidal activity and exert therapeutic effects against experimental vaginal and systemic candidiasis, disseminated cryptococcosis and paracoccidioidomycosis. KP has shown to modulate the expression of costimulatory and MHC molecules on murine dendritic cells improving their capacity to induce lymphocyte proliferation. P6 has been found in sequences of Abs directed to different antigens. As a proof of concept of the antifungal potential of Ab peptides, synthetic CDRs of mAbs directed to a protein epitope of a *C. albicans* cell wall stress mannoprotein (C7), a peptide containing B-cell and T-cell epitopes (pc42) and difucosyl human blood group A substance (HuA) have been tested. Irrespective of the specificity of the native Ab, CDRs proved to exert fungicidal activity against *C. albicans*, Cryptococcus neoformans, *Aspergillus fumigatus*, Scedosporium prolificans. Engineered peptides, obtained by alanine substitution and used as surrogates of natural point mutations, showed differential antifungal properties. A CDR of a mouse IgM mAb (MoA) sharing no homology with the cross-reactive human IgM mAb (HuA) and devoid of Candidacidal activity, has shown immunomodulatory properties on macrophages thus exerting a therapeutic effect against experimental systemic candidiasis. Ab CDRs have shown differential antiviral (HIV-1, Influenza A) and antitumor (human cervix epitheloid carcinoma, human leukemia, human and murine melanoma) activities, conceivably involving different mechanisms of action. Bioactive CDRs are reminiscent of molecules of early innate immunity and expected to give rise to a new generation of chemotherapeutic agents.

IM-05-1

Neutrophil-*Candida* biofilm interactions

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Soft tissue biofilm infections are characterized by focal infiltration with neutrophils. It has been suggested that biofilm invasion by neutrophils is necessary for resolution of these infections. Oral pseudomembranous candidiasis is a mucosal biofilm infection prevalent in immunocompromised patients. To begin to understand the role of neutrophils in this infection we developed a model of pseudomembranous candidiasis in C57BL/6 mice and characterized the cellular composition of biofilms on the surface of the tongues of animals infected with *C. albicans* strain SC5314. Using immunofluorescence combined with confocal microscopy, we found that oral biofilms consist of yeast and hyphal organisms with abundant keratin in the intercellular spaces. Using a universal eubacterial FISH probe in conjunction with an anti-*Candida* antibody we found mixed biofilms of bacteria and *C. albicans* in all samples. Bacteria mostly consisted of cocci, and were more abundant in the apical aspect of biofilms, physically interacting with *Candida*. Neutrophil foci were detected within the biofilms and the adjacent infected tissues. In order to test whether neutrophils can kill *Candida* in a biofilm environment we first tested anti-*Candida* neutrophil function using a modified XTT assay in a 96-well plate biofilm model. Although neutrophils inflicted severe damage to early biofilms, their activity was compromised in late biofilms, compared to amphotericin B. There was no soluble inhibitory factor in supernatants from late biofilms, but high fungal cell density had a negative impact on neutrophil function. Finally, we used a three dimensional culture system of the oral mucosa where neutrophils were added apically after infection, in order to simulate the oral biofilm environment in vivo. Neutrophils inflicted a significant damage to early biofilms in this model system, which was enhanced by the presence of the oral epithelial component. We conclude that oral epithelium may augment neutrophil function which is compromised in late biofilms.

IM-05-2

Host response to *C. albicans* vaginal biofilm: The role of chemotactic calcium-binding proteins in susceptibility to vulvovaginitis

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Vulvovaginal candidiasis (VVC) and recurrent vulvovaginal candidiasis (RVVC) are both caused predominantly by the yeast *Candida albicans*. VVC occurs in up to 75% of otherwise healthy women of child-bearing age, while RVVC occurs in a separate, but similar population of 5-8% of healthy women. Both conditions cost the health care system millions of dollars annually as current antifungal therapies are effective against an individual infection, but do not protect against repeat infections. Following recent reports that 80% of infectious diseases are caused by biofilm formation on abiotic or biotic surfaces by the etiological agent, we now have evidence that *Candida albicans* forms a biofilm on the vaginal mucosa in vivo during experimental vaginitis in mice. Hence, the host response to the biofilm may play key roles in the immunopathogenesis of disease. Although healthy persons with *Candida*-specific acquired immunity are normally protected against *Candida* infections at mucosal sites, results from both clinical studies and an experimental mouse model have shown little to no role for induced systemic or local adaptive immunity in protection against VVC/RVVC. This is due to immunoregulatory mechanisms that seemingly prohibit the action of acquired immunity to avoid chronic inflammation against *Candida* at a reproductive site. Instead, symptomatic infections appear to result from a response to a *C. albicans* biofilm by vaginal epithelial cells that promotes an aggressive migration of polymorphonuclear neutrophils (PMNs) into the vaginal cavity. The ensuing inflammatory response ultimately causes the symptoms associated with vaginitis without any effects on *Candida*. Recent data suggest that chemotactic calcium-binding proteins (CBPs; S100A8/A9) that are present in vaginal secretions of both infected women and mice (murine model), are the trigger for the PMN migration. Once confirmed and fully characterized immunotherapeutic strategies can be developed to neutralize the PMN chemotactic factor that will ultimately reduce the symptoms associated with VVC/RVVC.

IM-05-3

Host and fungal prostaglandins influence dendritic cell interactions with *Candida albicans*

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Candida albicans is an opportunistic fungal pathogen and is a common inhabitant of mucosal surfaces of healthy individuals. When environmental conditions permit the outgrowth of *Candida*, colonization can lead to infection and disease. We are interested in understanding the immunological effects of persistence and the mechanisms that *Candida* uses to influence adaptive responses. The immune system regulates responses in part through production eicosanoids, which derived from arachidonic acid and include the prostaglandins and leukotrienes. Prostaglandin E2 (PGE2) is a potent regulator of host immune responses, with the ability to elicit both pro- and anti-inflammatory responses, depending on the target cell. *Candida* produces both endogenous immunomodulatory oxylipins that cross-react functionally with host eicosanoids and authentic host eicosanoids from arachidonic acid.

At the mucosal surface, dendritic cells (DC) direct the type of T-cell response after interacting with pathogen. Yeast forms induce protective DC1/Th1 responses, while hyphal forms induce non-protective DC2/Th2 responses. Our objective is to characterize the role of prostaglandins produced by the host and this fungus during pathogenesis both in vivo and during *Candida*-dendritic cell interactions. We hypothesize that production of eicosanoids by both *Candida* and host are required for persistent infection. We are testing this hypothesis by examining effects of host and fungal prostaglandins on murine bone marrow derived DC differentiation and function. Cytokine analysis indicates that both fungal PGEx and host PGE2 suppress DC1 cytokine production. Vaccination of mice with yeast-pulsed DCs was protective against systemic infection, resulting in a reduction in fungal burden and induction of Th1 cytokines. However, after vaccination with DCs pulsed with yeast in the presence of PGEx or PGE2, protection was abrogated. This was accompanied by decreased Th1 and increased Th2 cytokine production. This indicates that fungal prostaglandin production is a potential virulence mechanism that works by promoting non-protective type II adaptive immune responses.

IM-05-4

Mechanism of IL-12 synthesis by dendritic cells during cryptococcal infection

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Host defense to cryptococcal infection is largely mediated by cellular immune responses, in which IL-12 plays a central role. The mechanism of host cell recognition of *Cryptococcus neoformans* remains poorly understood. Previous study revealed that MyD88 played a critical role while TLR2 played a relatively limited role in the response to *C. neoformans* (Yauch et al. Infect. Immun. 72: 5373-5382, 2004). In another study (Biondo et al. Eur. J. Immunol. 35: 870-878, 2005), TLR2-deficient mice succumbed to the infection, which was associated with reduced production of TNF-α, IL-12 and IFN-γ. In contrast, in our recent study (Nakamura et al. FEMS Immunol. Med. Microbiol. 47: 148-154, 2006), both TLR2 and TLR4 did not seem to be involved in the host protective response to this infection.

In the present study, we asked if the DNA of this yeast activates mouse bone marrow-derived myeloid dendritic cells (BM-DCs). BM-DCs released IL-12 upon stimulation with cryptococcal DNA. IL-12 production was attenuated by chloroquin and bafilomycin A that suppressed the responses caused by CpG-ODN. Activation of BM-DCs by cryptococcal DNA was almost completely abrogated in $TLR9^{-/-}$ mice, similar to that by CpG-ODN. In addition, TLR9KO mice were more susceptible to pulmonary infection with this fungal pathogen than WT mice, as shown by increased number of live colonies in lungs. Treatment of cryptococcal DNA with methylase resulted in partial reduction of IL-12p40 synthesis by BM-DCs. Using a luciferase reporter assay, cryptococcal DNA activated NF-κB in HEK293 cells transfected with TLR9 gene. Finally, confocal microscopy showed colocalization of fluorescence-labeled cryptococcal DNA with the distribution of TLR9 in BM-DCs.

Thus, our results demonstrate that cryptococcal DNA causes IL-12 synthesis by BM-DCs in a TLR9-dependent manner and suggest that this mechanism may contribute to the host defense responses after infection with *C. neoformans*.

IM-05-5

Th17 cytokines in aspergillosis

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Infectious agents can induce autoimmune diseases in several experimental settings, some of which have clinical counterparts. A variety of mechanisms have been invoked to explain these observations, including molecular mimicry and an increase in the immunogenicity of autoantigens caused by inflammation in the target organ. Paradoxically, infectious agents can also suppress allergic and autoimmune disorders. A central question is to determine whether immune dysregulation precedes, if not promotes, infection or alternatively, but not mutually exclusive, the extent to which microbial exposure /colonization contributes to the burst of pathogenic autoimmunity. Here we discussed recent evidence that help to accommodate fungi, either commensals or ubiquitous, within the immune homeostasis and its dysregulation. We will discuss how *Candida albicans* and *Aspergilus fumigatus* exploits multiple, functionally distinct, receptor/signaling pathways in dendritic cells ultimately affecting IDO expression and the local Th17: Treg cell balance. Despite the recognized importance of Tregs in the homeostatic regulation of immune responses, our understanding of their significance and interplay with other pathways of immunity and autoimmunity is limited. We have evidence that the IDO/Treg axis activated in fungal infections could be exploited for the control of inflammation and dysregulated immunity in experimental models of inflammatory diseases.

IM-06-1

Recognition of fungal cell wall polysaccharides by innate immune system especially C-type lectins on macrophages

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Fungal cell wall polysaccharides serve as target molecules for phagocytes, including macrophages, and neutrophils. These polysaccharides are specifically recognized by C-type lectins on the leukocytes. Dectin-1, a glycoprotein C-type lectin receptor for 1,3-β-glucans, is assumed to be involved in recognizing fungi such as *Candida albicans*. We have examined the specificity of dectin-1 to various type of 1,3-β-glucans from pathogenic fungi including *Candida* and also Basidiomycetes. The higher binding affinity was observed in *Candida*-derived 1,3-β-glucan with long 1,6-glucosidic linkages. The mushroom-derived 1,3-β-glucans with 1,6-monoglucosyl branch was lower than pathogenic fungal glucans, suggesting important role of dectin-1 in infection. In spite of the significance of dectin-1 in the innate immunity, the staining of *Candida* cells with recombinant soluble dectin-1was only limited area on the cell surface assumed as bud scar after the cell proliferation. The most cell surface of *Candida* are not accessible for dectin-1. The recognition of *Candida* cells by dectin-1 was increased by treatment removing the mannans from the cell wall. The dectin-1-mediated *Candida* cell recognition by macrophages was enhanced by stimulation with GM-CSF. The macrophages produced more TNF-α and reactive oxygen species (ROS) in response to *Candida* by GM-CSF treatment. In addition to dectin-1-mediated recognition, dectin-2 expression on the macrophage was also enhanced. The macrophages from dectin-$1^{-/-}$ mice was able to bind to *Candida* cells in lower extent. The residual binding ability of dectin-$1^{-/-}$ macrophages to *Candida* was significantly decreased by treatment with dectin-2 neutralizing antibody or *Candida*-derived mannan preparation. In conclusion, dectin-1 and dectin-2 are indispensable receptors for recognizing 1,3-β-glucan and mannan on *Candida albicans* and for activating macrophage function.

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IM-06-2

Modulation of innate immune reponses to fungi

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Pneumocystis species are opportunistic fungal pathogens that cause severe pneumonia in immune compromised pateints, inclduign those with AIDS, malignancy and organ transplantation. Respiratory impairment during *Pneumocystis* Pneumonia (PcP) is closely related to exuberant pulmonary inflammation in response to the organism cell wall. Anti-inflammatory corticosteroids (in addition to antibiotics) improves outcome during PcP, but is associated with further immune suppression and increased incidence of co-infections. Our recent studies demonstrate that *Pneumocystis* cell wall components including beta-glucans of (PCBG) interact with alveolar macrophages and epithelial cells to stimulate the release of cytokines and chemokines (TNF-alpha and MIP-2) that promote inflammatory cell recruitment in the lungs. We further demonstated that host cell membrane lactosylceramide mediates host inflammatory activation in response to *Pneumocystis* organisms and purified PCBG compnents. Furthermore, glycosphingolipid (GSL) synthesis inhibitors, including PDMP, which potently reduce lactosylceramide levels, not only strongly suppress lung inflammation during PcP as expected, but were also shown to significantly suppress the numbers of *Pneumocystis* organisms present in treated mice. PDMP also strongly suppressed lung inflammatory responses in mice challenged with intratracheal instillation of isolated beta-glucans, in the absence of intact *Pneumocystis* organisms. Our data further support that Pc itself possesses GSL synthetic molecules necessary under the control of a glucosylceramide synthesis (PCGCS1) gene which is necessary for maintaining organism viability. Thus, GSL inhibitors represent a potential new class of anti-*Pneumocystis* agents with both beneficial immune modulating activity, as well as direct suppressive effects on *Pneumocystis* organism numbers and viability.

IM-06-3

LacCer-enriched membrane microdomain-mediated neutrophil innate immune responses

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The innate immune system is the first line of defense against invading microorganisms, including bacteria, fungi, and viruses. Phagocytes, such as neutrophils and macrophages, play important roles in the innate immune system by recognizing pathogen-associated molecular patterns (PAMPs) via pattern recognition receptors (PRRs) expressed on the cell surface, and then engulfing and eliminating pathogens. Over the last 30 years, many studies have indicated that glycosphingolipids expressed on the cell surface act as binding sites for microorganisms. Based on their physicochemical characteristics, glycosphingolipids form membrane microdomains (lipid rafts) with cholesterol and various signaling molecules. Among the glycosphingolipids, lactosylceramide (LacCer, CDw17) can bind to various microorganisms, including Mycobacteria, Helicobacter pylori, and *Candida albicans*. LacCer is expressed at high levels on the plasma membranes of human neutrophils, and forms membrane microdomains associated with the Src family tyrosine kinase Lyn. LacCer-enriched membrane microdomains mediate superoxide generation, chemotaxis, and non-opsonic phagocytosis. Therefore, LacCer-enriched membrane microdomains are thought to function as pattern recognition receptors (PRRs) to recognize pathogen-associated molecular patterns (PAMPs) expressed on microorganisms.

Polysaccharide ß-1,3-D-glucans (ß-glucans) are components of the cell walls of various fungi, and show immunomodulatory activities. ß-Glucans have been reported to enhance neutrophil accumulation in lung inflammation induced by pathogenic fungi. Among the several types of ß-glucan, ß-1,6 long glucosyl side chain-branched ß-glucan isolated from *C. albicans* (*Candida* soluble -D-glucan, CSBG) induced neutrophil migration in a dose-dependent manner. In contrast, 1,6-monoglucosyl-branched ß-glucans such as Sparassis crispa-derived β-glucan (SCG) and grifolan (GRN), which were derived from non-pathogenic fungi, hardly induced neutrophil migration. These results suggest that LacCer specifically recognizes the specific conformation of pathogenic fungus-derived ß-glucans. Here, we will introduce the membrane microdomain-associated immune functions of neutrophils, focusing on the molecular mechanisms of ß-glucan-induced neutrophil functions.

IM-06-4

CD4+ T cell-independent vaccination against opportunistic infections

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Host defenses are profoundly compromised in HIV-infected hosts due to progressive depletion of CD4+ T lymphocytes. Moreover, deficient CD4+ T lymphocytes impair vaccination approaches to prevent opportunistic infection. Therefore, we investigated a CD4+ T cell-independent vaccine approach to a prototypic AIDS-defining infection, Pneumocystis carinii (PC) pneumonia (PCP). We demonstrate that bone marrow-derived dendritic cells (DCs) expressing the murine CD40 ligand (CD40L), a molecule expressed on activated CD4+ T lymphocytes and critical for T cell helper function, when pulsed ex vivo by PC antigen, elicited significant titers of anti-PC IgG in CD4-deficient mice. Vaccinated animals demonstrated significant protection from PC infection, and this protection was the result of an effective humoral response. DC-vaccinated, CD4-deficient mice predominantly reacted to a 55-kDa PC antigen. After analysis this antigen by MS-MS, kexin was identified by this approach. We used a plasmid DNA vaccination with a cassette encoding kexin and a second cassette encoding full length CD40L. To investigate whether this approach leads to CD4+ T cell-independent vaccine protection against PCP, this plasmid DNA was used in a DNA vaccine strategy with or without CD40L. CD4-deficient mice receiving DNA vaccines encoding Kexin and CD40L showed significantly higher anti-PC IgG titers as well as opsonic killing of PC compared with those vaccinated with Kexin alone. Moreover, CD4-depleted, Kexin-vaccinated mice showed a greater protection in a PC challenge model. Adoptive transfer of CD19+ cells or IgG to SCID mice conferred protection against PC challenge, indicating a role of humoral immunity in the protection. The results of these studies show promise for CD4-independent vaccination against HIV-related or other opportunistic pathogens.

IM-06-5

Dendritic cell cytokine responses to fungal beta-glucans

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Dendritic cells (DCs) are the most potent antigen presenting cells present in the lung. They are located near the portals of entry of the microorganisms, in contact with either alveolar or airway epithelial cells. This location allows them to be one of the first cells to contact many inhaled pathogens. Fungal beta-glucans are polymers of glucose that provide fungi with structural support. In addition to this mechanical role, our previous data have shown that beta-glucans are also responsible for initiation of lung inflammation during *Pneumocystis* infection. In patients with *Pneumocystis* pneumonia, respiratory failure is one of the principal causes of death, mainly due to an exaggerated inflammatory response to the organism. DCs are key to the regulation of innate and adaptive immune responses. However, their participation in the inflammatory response directed against *Pneumocystis* infection is not well understood. We studied the role of *Pneumocystis carinii* cell wall-derived beta-glucans, in DC activation and subsequent T cell activation. Because cytokine secretion by DCs has been shown to be regulated by Fas ligand (FasL), its role in beta-glucan activation of DCs was also investigated. We demonstrated that DC activation by beta-glucans elicits T cell activation and polarization into a Th1 response in the absence of IL-12. These observations differed from LPS-driven T cell polarization, suggesting that beta-glucans and LPS signal DC activation through different mechanisms. We additionally determined that IL-1beta and TNF-alpha secretion by beta-glucan-stimulated DCs was partially regulated by Fas-FasL interaction. This suggests that dysregulation of FasL could further enhance exuberant and prolonged cytokine production by DCs following DC-T cell interactions, further promoting lung inflammation typical of *Pneumocystis* pneumonia.

IM-07-1

Animal models as a tool in medical mycology - Overview

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Animal models, as surrogates of human disease, have been used historically in studies of fungal pathogenesis and host response. Critical are the choice of animal, route of infection, parameters for evaluation and how closely a model mimics disease in humans. Mice are the most commonly used species, and have numerous advantages. We can modulate host response, severity of infection and genetically manipulate the host or the infecting organism to study specific aspects. However, no single model, or even strain of animal, will be useful to answer all questions. For example, mucosal candidiasis in SCID mice mimics infection in AIDS patients where the mucosal surfaces of the gastrointestinal tract are heavily colonized, but dissemination does not occur. In contrast, pretreating normal mice with 5-fluorouracil results in dissemination, which is similar to patients receiving cancer chemotherapy. Virulence potential can change depending on the strain of animal used in the model and pathogenesis is greatly influenced by the host response. The use of gene-knockout mice has aided our study of the importance of various host proteins such as collectins and cytokines. Animal models also allow us to examine host events associated with fungal infection. We developed a rabbit model of coccidioidal meningitis that results in vasculitis of the large arterial vessels of the CNS, similar to that occurring in human disease. A major role for animal models has been in therapeutic studies, where they have been very predictive of clinical results. More recently, nonmammalian models of fungal infection using Drosophila, nematodes, moth larvae and amoeba have been developed for virulence studies, as well as therapeutic studies. Overall, animal models of fungal infection are valuable tools in addressing questions concerning the infectious process and contribute to our deeper understanding of occurrence, evolution and how they might be controlled or better, prevented and eliminated.

IM-07-2

Mucosal model of *Candida* colonisation: Commensal vs pathogen and host innate immunity

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We have established a low-dose oestrogen murine *C. albicans* model that permits concurrent colonisation at oral and vaginal sites in the same mouse, thereby reducing the numbers of experimental mice by half. Weekly oestrogen administration of 5 ug intramuscular and subcutaneously was optimal for enhancement of oral colonisation and was essential for vaginal colonisation. In BALB/c mice, certain *C. albicans* clinical isolates (529L) consistently and stably colonised both oral and/or vaginal sites over 5 weeks, whereas other strains (SC5314 and NCPF 3153) did not colonise the model and were rapidly cleared by 2 weeks. Given that *C. albicans* can act as a commensal or pathogen, we performed in depth investigations using human oral epithelial cells (EC's) to identify innate mechanisms that might discriminate between the two strains and explain why 529L, but not SC5314, colonises the model. *C. albicans* activated both the NF-kB and MAPK pathways, including the MAPK-related MKP-1 phosphatase. Whilst NF-kB activation was linear, MAPK activation was bi-phasic (early and late response). Only hyphal forms of *C. albicans* activated the late phase of the MAPK bi-phasic response, which constituted MKP-1 phosphorylation, activation of the transcription factor c-Fos, and induction of pro-inflammatory responses. Unlike SC5314, strain 529L was found to be deficient in hypha-formation in the presence of oral EC's and consequently did not induce the bi-phasic MAPK/MKP-1/c-Fos response or pro-inflammatory cytokines. Therefore, we propose that, in vivo, production of hyphal forms activate EC innate immunity, ultimately resulting in the clearance of *C. albicans*, whereas the yeast/pseudohyphal form subverts EC innate immunity, thus permitting the fungus to colonise the mucosa without host challenge.

IM-07-3

In vivo role of myleloperoxidase for the host defense against fungi

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Neutrophils are believed to be the first line of defense against invading microorganisms, but *in vivo* roles of reactive oxygens produced by neutrophils are not well known. Myeloperoxidase (MPO) catalyzes reaction of hydrogen peroxide with chloride ion to produce hypochlorous acid that is used for microbial killing by phagocytic cells. To define the *in vivo* contribution of MPO to early host defense against pulmonary infections, MPO-deficient (MPO-KO) and control mice were infected with various fungi. MPO-KO mice showed severely reduced cytotoxicity to several fungi such as *Candida albicans*, *Aspergillus fumigatus*, and *Cryptococcus neoformans*. These results suggest that MPO-dependent oxidative system is important for host defense against fungi, although the effect varies from species to species of pathogens. The importance of two major oxidant-producing enzymes, MPO and NADPH-oxidase, in *in vivo* fungicidal action was directly compared. The NADPH oxidase-deficient (CGD) mice exhibited increased mortality and tissue fungal burden in a dose-dependent manner, whereas normal mice showed no symptoms. Interestingly, at the highest dose, the mortality of MPO-KO mice was comparable to CGD mice, but was the same as normal mice at the lowest dose. These results suggest that MPO and NADPH oxidase are equally important for early host defense against a large inocula of *Candida*. Hereditary MPO deficiency is a common neutrophil defect with estimated incidence of 1 in 2,000 in the United States, and of 1 in 58,000 in Japan. Our present results suggest that MPO-deficient individuals could exhibit similar problems as CGD patients if exposed to a large amount of microorganisms.

IM-07-4

Use of in vitro models to study the *Candida albicans* infection process

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In vitro infection models can help to elucidate the complexity of a disease process by dissecting certain aspects of infection. These models can mimic a defined step or a distinct situation of host pathogen interactions, for example *Candida albicans* invasion into or through various epithelial barriers in order to disseminate within the host. We used oral epithelial monolayers to dissect the processes by which *C. albicans* is capable of adhering to, entering and damaging epithelial cells during infections.

After initial attachment, two principle mechanisms have been proposed for cellular invasion. One process is induced endocytosis. Binding of cell surface components of *C. albicans*, such as the protein Als3, to host cell surface receptors, such as E-cadherin, leads to host actin rearrangements and subsequent uptake of the fungus. The second mechanism is active penetration, which is mediated by the physical forces of hyphae and the production of lytic enzymes, such as secreted aspartyl proteases (Saps). Although filamentation seems to be necessary for *C. albicans* to invade oral epithelial cells, activity from the fungus is dispensable for induced endocytosis as killed fungi are still endocytosed. However, damage of epithelial cells by killed hyphae is dramatically reduced. Overall, the relative roles of active penetration versus induced endocytosis to the invasion of epithelial cells remain unclear.

To elucidate which fungal factors contribute to the infection process, we have quantified the ability of mutants lacking selected factors to adhere to, invade and damage epithelial cells. To elucidate the relative contribution of active penetration versus induced endocytosis, we either blocked induced endocytosis or killed the fungus to quantify the remaining invasion potential.

Our data show that the invasion process of *C. albicans* can be dissected into adhesion, invasion and dissemination/damage and that certain fungal factors are important for distinct stages.

IM-07-5

The activation of host transcription factor, AP-1, triggered by *Aspergillus fumigatus*

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Aspergillus fumigatus is one of the most prevalent pathogenic fungi. Its conidia are common in the environment and are naturally inhaled into the lung, but are promptly removed by host innate immunity. In host cells, *A. fumigatus* has been shown to trigger the activation of NF-κB, but other transcription factors are also activated following the infection by this organism. We have reported that activation protein (AP)-1, a group of the host transcription factors that plays an important role in the production of cytokine and chemokine, is activated in dendritic cells during the infection by *A. fumigatus*. Swollen conidia but not resting conidia strongly induced the activation of AP-1 and were stained well with anti-1,3-β-glucan antibody, which suggests that the activation is related to β-glucan exposed on the surface of swollen conidia. Dectin-1 is a well-known host receptor to recognize 1,3-β-glucan. When the HEK293T cells with exogenous dectin-1 expression were treated by resting conidia or swollen conidia, the activation of AP-1 was induced only by swollen conidia. This suggests that the AP-1 activation is induced via dectin-1 through the recognition of 1,3-β-glucan. The activation of AP-1 was inhibited by the overexpression of dominant-negative form of Syk protein kinase, which is indicative that the activation of AP-1 depends on the activity of Syk kinase. In this talk, I will present an overview of our data collected from in vitro experiments for further understanding of the innate immune system against aspergillosis.

AF-01-1

Azole resistance in *Candida* species

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Azole antifungals are commonly used to treat patients with serious mucosal mycoses or with invasive fungal infections. Azoles possess good pharmacokinetic properties and low toxicity, but some fungi show reduced susceptibility to certain azoles. Fungal azole resistance mechanisms include point mutations in the drug target Erg11p, overexpression of Erg11p or overexpression of ATP-binding cassette (ABC) or major facilitator superfamily (MFS) efflux pumps. A substantial proportion of clinical *Candida glabrata* isolates show moderate innate resistance to azoles and they can also acquire increased azole resistance during patient therapy with azole drugs. This resistance often correlates with overexpression of ABC pumps CgCdr1p and CgPdh1p. *Candida krusei* is generally considered innately resistant to fluconazole with about 80% of strains being susceptible dose-dependent to itraconazole. We have cloned, and functionally characterized, CkErg11p and ABC pump CkAbc1p by heterologous expression in *Saccharomyces cerevisiae*. Although some *C. krusei* strains were trisomic for CkErg11p, azole resistance appeared predominantly due to the low affinity of CkErg11p for azoles combined with constitutive expression of CkAbc1p. *Candida albicans* is normally susceptible to azoles, but can acquire resistance through CaErg11p mutation and overexpression. High-level azole resistance in *C. albicans* clinical isolates, however, most often correlates with overexpression of ABC pump genes *CaCDR1* and *CaCDR2*. We have shown that CaCdr1p contributes considerably more than CaCdr2p to clinical azole resistance and we have used *S. cerevisiae* cells expressing CaCdr1p to screen for pump inhibitors that chemosensitize resistant clinical *C. albicans* isolates to azoles.

AF-01-2

Domain-shuffled chimeras of *Candida albicans* Cdr1p and Cdr2p reveal structural determinants affecting substrate and inhibitor specificities

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Full-sized (~170 kDa) ABC transporters in the fungal pathogen *Candida albicans* mediate the efflux of clinically important azole antifungals plus a variety of structurally unrelated compounds. The transporters consist of two homologous but functionally complementary halves that each contains a nucleotide binding domain (NBD) and a transmembrane domain (TMD). The NBDs energize the transport process by binding and hydrolysing ATP. The TMDs are thought to determine specificity for individual efflux substrates but the molecular basis of both substrate recognition and efflux are poorly understood. Molecular features determining efflux substrate and pump inhibitor specificity have been identified by using heterologous overexpression in *Saccharomyces cerevisiae* of functional and correctly localized domain-shuffled chimeric constructs of *C. albicans* CaCdr1p and CaCdr2p. Retention of transport-related activities and substrate specificities in chimeric constructs in which each NBD was replaced with its heterologous partner demonstrated the mutual interchangeability of the CaCdr1p and CaCdr2p NBDs. Resistance to the CaCdr1p-specific substrates nigericin, monensin and bafilomycin A1 required cells expressing chimeric proteins with the amino-terminal TMD (TMD1) from CaCdr1p. Oligomycin-sensitive ATPase activity in these chimeric proteins was obtained, provided TMD1 was from CaCdr1p but not CaCdr2p. The CaCdr1p-specific inhibitor FK506 affected only constructs in which both TMDs were from CaCdr1p. Functional chimeric proteins in which TMD1 and TMD2 were from CaCdr1p and CaCdr2p, respectively, transported rhodamines poorly. Their counterparts in which TMD1 and TMD2 were from CaCdr2p and CaCdr1p, respectively, transported itraconazole or ketoconazole poorly. The attenuated fluconazole transport found with chimeras containing heterologous TMD pairs was complemented substantially when both NBDs were from CaCdr2p. These results suggest that most of the transport inhibitors and substrates tested interact with specific regions located within one or both TMDs and that interactions between the TMDs are important for efficient transport of some substrates.

AF-01-3

Structure and function analysis of *Candida albicans* secondary multidrug transporter

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In pathogenic *Candida albicans*, an up-regulation of multidrug transporter genes belonging to either ATP Binding Cassette (ABC) or Major Facilitator Superfamily (MFS) is frequently observed in cells exposed to drugs leading to the phenomenon of multidrug resistance (MDR). Among the 28 putative ABC and 95 MFS transporter genes identified in the *C. albicans* genome, there is overwhelming clinical and experimental evidence showing that only ABC transporters like CaCdr1p and CaCdr2p and MFS transporter, CaMdr1p are major determinants of azole resistance.
CaMdr1p is a secondary active transporter - a 564 amino acid protein with 12 transmembrane segments (TMS). It has a much conserved proton antiporter motif within TMS 5. Our previous work has shown the importance of the conserved proton antiport motif of CaMdr1p in drug specificity and transport. In this study, we have rationalized our mutational strategy by improving methods for calculating Relative Entropy (RE) for multiple sequence alignments of MFS proteins to identify those sites which have amino acid distributions very different from the background distribution. As MFS is a class of membrane proteins, the relative entropy scoring scheme was improved by treating TMS and inter-TMS separately which drastically increased the credibility over the existing methods. With this approach, we could accurately predict functionally important residues of CaMdr1p which was confirmed by functional drug susceptibility and transport assays. Additionally, we could corroborate the functional relevance of each residue by predicting their location in a deduced 3D model of CaMdr1p and their role in maintaining inter-helical interactions of this protein. We further show that the predictions based on RE can be extended to other members of the MFS class.

AF-01-4

Update on echinocandin resistance in *Candida albicans* and *Candida glabrata*

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Echinocandin resistance is still relatively uncommon, although an increasing number of clinical failures have been noted. To better understand the underlying molecular mechanisms, 75 strains of *Candida albicans* and *Candida glabrata* isolated from patients following echinocandin failure were studied. Mutations in FKS1 and FKS2 hot spot regions were linked to the echinocandin resistant phenotype, which exhibited high echinocandin MIC values. A detailed kinetic characterization was performed on 1,3-beta-D-glucan synthase enzymes isolated from these resistant strains and compared with those from wild type strains. Amino acid substitutions in Fks1p and/or Fks2p reduce biochemical sensitivity (IC50) to echinocandins by 2-3 log orders. Specific mutations can alter enzyme kinetics by reducing Vmax, which in turn influences expression of FKS genes. As a consequence of the association of FKS mutations with clinical resistance, the new CLSI echinocandin susceptibility breakpoint of MIC $\geq$2 ug/ml was assessed. The *C. albicans* and *C. glabrata* breakpoint is appropriate for caspofungin, but it is sub-optimal for identifying isolates resistant to micafungin and anidulafungin. This discrepancy is lees pronounced when MICs were obtained in the presence of serum. Given the restricted nature of fks mutations, resistance may be most easily defined by molecular methods.

AF-01-5

Mechanisms of clinical antifungal resistance in *Aspergillus*

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Background: Itraconazole, voriconazole and posaconazole form the backbone of oral therapy for aspergillosis. Although recognised, azole resistance has been reported relatively infrequently; the frequency of resistance (approximately 3%) differs greatly between studies.

Aims and methods: The aims were to calculate the frequency of azole resistance in *Aspergillus fumigatus* in a clinical culture collection (Regional Mycology Laboratory, Manchester), determine susceptibility profiles for azole resistant isolates, and investigate epidemiological links between resistant isolates. Susceptibilities were determined by modified EUCAST microtitre method. Putative breakpoints used were >2mg/L for itraconazole and voriconazole, and >1mg/L for posaconazole.

Results: A high frequency (9%, 57/611) of itraconazole resistance was revealed between 1992 - 2008. The first case of resistance was in 1999, and since 2004 there has been a significant increase (18% of isolates received and 17% of patients sampled in 2007). Of the itraconazole resistant isolates, 78% and 81% had reduced susceptibility to voriconazole and posaconazole respectively. Clinical data was available for 14 patients, of whom 13 had had prior azole exposure, six had low drug levels. Eight patients failed therapy and 5 failed to improve (1 was not treated). The referral base for these isolates includes a specialist service for the management of aspergillosis, and this may partly explain the high frequency of resistance. Many of the resistant isolates came from this group; cases of allergic, invasive and chronic pulmonary aspergillosis, with the highest frequency in those with aspergillomas. Eighteen Cyp51A amino acid alterations were found, some novel. Evidence of emergence of resistance during infection was shown by; mutants arising from originally susceptible strains, different *cyp51A* mutations in the same strain, and alterations in microsatellite repeat number.

Conclusions: The increasing frequency of itraconazole resistance, and high probability of cross-resistance with other azoles is of concern. Routine azole susceptibility testing of *A. fumigatus* is now required.

AF-02-1

Transcriptional regulation of multidrug resistance genes

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Development of resistance to antifungal agents commonly involves upregulation of the expression of genes encoding ATP-binding cassette transporter proteins and other resistance determinants. We have used Saccharomyces cerevisiae to explore the molecular basis of control of gene expression of multidrug resistance loci. In this organism, the Sc PDR5 gene is the key ABC transporter-encoding locus that exhibits elevated transcription in drug resistant strains. Control of Sc PDR5 expression is provided by two homologous zinc cluster-containing proteins called ScPdr1 and ScPdr3. These transcriptional regulators bind to similar DNA elements present in target gene promoters but are regulated in response to different signals. Loss of the mitochondrial genome strongly induces ScPdr3 expression while ScPdr1 levels do not change. Interestingly, this retrograde (mitochondria to nucleus) circuit is well-conserved in the pathogenic fungus *Candida glabrata*. *C. glabrata* also expresses a single zinc cluster-containing protein called CgPdr1 that participates in this common regulatory pathway. Recent experiments have provided new insight into the extensive conservation of the retrograde pathways defined by CgPdr1 in *C. glabrata* and ScPdr3 in S. cerevisiae. A mitochondrial enzyme involved in biosynthesis of phosphatidylethanolamine called ScPsd1 has been found to participate in retrograde signaling in S. cerevisiae and more recent experiments have demonstrated this signaling in conserved in *C. glabrata*. Additionally, transcriptional mediator subunits have been found that are key in permitting the normal increase in mRNA to be seen in both fungal species. Progress in understanding the common mechanisms of transcriptional control of multidrug resistance genes in these two organisms will be discussed.

AF-02-2

Genome-wide gene expression profiles of individual *CgPDR1* hyperactive alleles and identification of CgPdr1p-dependent virulence factor(s) in *Candida glabrata*

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CgPdr1p is a *C. glabrata* Zn(2)-Cys(6) transcription factor involved in the regulation of ABC-transporter genes (*CgCDR1*, *CgCDR2* and *CgSNQ2*) mediating azole resistance. By comparison of *CgPDR1* alleles from azole-susceptible and azole-resistant clinical isolates, we observed a high diversity among *CgPDR1* alleles and identified 57 distinct single aa substitutions conferring hyperactivity to CgPdr1p. Although *CgCDR1, CgCDR2* and *CgSNQ2* are all regulated by CgPdr1p, they are not always co-ordinately expressed in azole-resistant isolates indicating that ABC transporter genes were differentially regulated depending on the mutation present on individual *CgPDR1* alleles. Moreover, the aa substitutions in CgPdr1p enhance virulence. Taken together these data demonstrate a high variability in *CgPDR1* mutations, which themselves have differentiated effects on target genes including ABC-transporters and probably on yet unidentified virulence factors.

In this study, we aimed to determine changes in gene expression driven by seven *CgPDR1* hyperactive alleles as compared to wild-type allele to identify i) the CgPdr1p target genes differentially expressed in presence of *CgPDR1* hyperactive alleles and ii) potential virulence factor(s) regulated by *CgPDR1* hyperactive alleles. Microarray experiments revealed a high number of genes (ranging from 80 to 400 genes) differentially regulated by individual *CgPDR1* hyperactive alleles. Enrichment of specific biological processes (stress response, resistance to DNA damage and cell wall biogenesis) was observed upon expression of specific *CgPDR1* alleles. These processes may contribute individually or in combination to modulate virulence of *C. glabrata*. Consistent with previous observations, we observed a poor overlap in the number of co-ordinately expressed genes from all hyperactive alleles. Only two genes were commonly upregulated by all hyperactive alleles. Since the *CgPDR1* hyperactive alleles used in this study were shown to enhance *C. glabrata* virulence in animal models, our current studies are addressing the involvement of these two genes in azole resistance and virulence.

AF-02-3

Transcriptional regulation of azole resistance in *Candida albicans*

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Azole antifungal drugs, especially fluconazole, are widely used to treat infections caused by *Candida albicans*, the most common human fungal pathogen. Azoles block the biosynthesis of ergosterol, the main sterol in the fungal cell membrane, by inhibiting sterol 14alpha-demethylase, which results in ergosterol depletion and production of toxic sterols. *C. albicans* can develop resistance to azoles by various mechanisms. In addition to mutations in the target enzyme, which reduce its affinity for the drug, and alterations in the sterol biosynthesis pathway that bypass the accumulation of toxic sterols, changes in gene expression play a major role in azole resistance. Mutations in three zinc cluster transcription factors are responsible for the constitutive upregulation of genes mediating azole resistance in clinical *C. albicans* isolates. Hyperactive alleles of UPC2, encoding the transcriptional regulator of ergosterol biosynthesis genes, confer increased resistance to azoles and other ergosterol biosynthesis inhibitors. Mutations in TAC1, which encodes a transcription factor that controls the expression of the highly related ABC transporters CDR1 and CDR2 as well as other genes that contribute to drug resistance, result in overexpression of the efflux pumps and multidrug resistance. Similarly, the transcription factor Mrr1 regulates the expression of another multidrug efflux pump encoded by the MDR1 gene, a member of the major facilitator superfamily. Mutations in the MRR1 gene are responsible for the constitutive MDR1 upregulation in all fluconazole-resistant *C. albicans* isolates tested so far. Loss of heterozygosity for mutated transcription factors further increases the drug resistance of the strains, and the various mechanisms can also be combined to generate strains that exhibit high levels of resistance.

AF-02-4

Rep1p involved in drug resistance by negatively regulating efflux pump MDR1 in *Candida albicans*

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In the past decade, the prevalence of yeast infections has increased dramatically. Among them, *Candida albicans* is the most frequently isolated fungal pathogen in humans and has caused morbidity in immunocompromised hosts. The increased use of antifungal agents has led to an increase in incidences of drug resistance. Overexpression of efflux pumps, including CDR1 and MDR1, is a major mechanism contributing to drug resistance in *C. albicans*. Recently, two transcription factors, CaNdt80p (3,19) and CaTac1p (5), have been identified as positive regulators of CDR1. In this study, we have found that overexpression of REP1, identified by library screening, in Saccharomyces cerevisiae increased the expression of both CDR1 promoter-lacZ (CDR1p-lacZ) and MDR1 promoter-lacZ (MDR1p-lacZ) reporter constructs. Surprisingly, overexpression of REP1 in S. cerevisiae increased susceptibility to certain antifungal drugs. In contrast, mutations on REP1 decreased the susceptibility to antifungal drugs in *C. albicans*. Our results further indicate that the expression of MDR1 is higher in rep1/rep1 cells than that in wild-type cells. Hence, Rep1p is involved in drug resistance by negatively regulating MDR1 in *C. albicans*.

AF-02-5

Functional genomic analysis of the *Candida albicans* Fcr1p regulon

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Transcription factors of the zinc cluster family are characterized by a fungal-specific DNA-binding domain (Zn2Cys6). They regulate a wide variety of cellular processes such as primary and secondary metabolism, meiosis and multidrug resistance. In *Candida albicans*, gain-of-function mutations in the Tac1p, Mrr1p and Upc2p factors are responsible for azole resistance by causing the overexpression of the genes CDR1 and CDR2 (ABC transporters), MDR1 (major facilitator) and ERG11 (lanosterol demethylase, the target of azole drugs), respectively. The *C. albicans* FCR1 gene codes for a putative transcription factor of the zinc cluster family. A *C. albicans* fcr1/fcr1 null mutant displays decreased susceptibility to fluconazole and constitutively upregulates CDR1 as compared to wild-type cells, indicating that FCR1 behaves as a negative regulator of azole resistance. We used genome-wide location profiling (ChIP-on-chip) to identify the transcriptional targets of Fcr1p in vivo. Fcr1p was tagged at its C-terminus with a hemaglutinin (HA) epitope and used to probe whole-genome oligonucleotide tiling microarrays. This identified 126 genes bound by Fcr1p-HA (binding ratio higher than 1.5-fold, p-value smaller than 0.0001), including several genes involved in ammonium, amino acid and oligopeptide transport (MEP1, CAN1, CAN2, CAN3, GAP2, GAP6, OPT1, OPT4, OPT9), nitrogen metabolism (GLT1, GLN1, GDH3, DUR1) and transcriptional regulation of nitrogen utilization (GAT1, STP3), suggesting that Fcr1p plays an important role in controlling nitrogen assimilation. Fcr1p-HA was also bound to the UPC2 and ERG11 genes, identifying additional mechanisms by which it may regulate azole resistance, but not to the CDR1 gene, suggesting that it indirectly regulates this gene. Interestingly, Fcr1p-HA was found to bind predominantly within the open reading frame of its targets, suggesting that it may bind indirectly to DNA, through an association with the chromatin machinery. Expression analyses are underway to determine whether Fcr1p functions as a transcriptional activator or as a repressor.

AF-03-1

Integration of functional genomics in pathogenic fungus *Candida glabrata* and development of antifungal drug targets

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With the completion of genome sequences of the major fungal pathogens, numerous scientists have focused on molecular biological studies of fungal pathogenesis for development of antifungal drugs, yet many problems haven't been resolved due to technical difficulties in studying specific fungi. With the completion of genome sequences of the major fungal pathogens, knowledge gained from study of a specific fungus can immediately be applied to other fungi, using genomics as a platform. Of several dozen fungal species causing disease in humans, *Candida glabrata* is the most amenable species for molecular biology. For this reason, *C. glabrata* is a good resource for basic studies that may be applicable to many pathogenic fungi. The *C. glabrata* phenome project consists of an effort to construct a strain set in which each strain includes a single manipulated gene. All essential genes will be inserted a promoter by which gene expression is repressed by tetracycline, whereas all dispensable genes will be knocked out.

The first aim of this project is integration of functional genomics and prioritization of drug targets amongst the 5,300 *C. glabrata* genes as a prelude to developing new antifungal drugs. Prioritization has been determined by the following criteria: genes representing targets must be essential for growth in vivo, and highly conserved in other pathogenic fungi to ensure drugs will have a broad spectrum, but should not be conserved in the human genome. Consequently, a few dozen genes have been identified as high priority Candidates.

AF-03-2

Candida albicans genomes and genomics

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The *Candida albicans* genome sequence has been publicly available for almost 10 years, with the full diploid assembly first published 5 years ago. This talk will highlight the types of studies that have exploited the genome sequence information to understand the organism, its growth and development, the requirements for virulence, as well as the diversity and responsiveness of its genome.

AF-03-3

Abrogation of iron acquisition as a novel therapeutic strategy for mucormycosis

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Mucormycosis is an increasingly common fungal infection with an unacceptably high mortality despite first-line antifungal therapy. Clinical and animal model data clearly demonstrate that the presence of elevated available serum iron (e.g. as seen in diabetic ketoacidotic [DKA] host) predisposes the host to develop mucormycosis which is commonly caused by Rhizopus oryzae. Therefore, abrogation of fungal iron acquisition is a promising therapeutic strategy to impact clinical outcomes for this deadly disease. The high affinity iron permease gene (rFTR1) is required for R. oryzae iron transport in iron-limited environments, such as those found in the host during infection. Our recent data demonstrated the expression of this gene in DKA mouse model during active infection with R. oryzae. Additionally, abrogation of the rFTR1 function by RNA-i technology resulted in reduced virulence in DKA mice infected intravenously or intranasally with R. oryzae. Finally, antibodies raised against a recombinant synthetic rFtr1p protected DKA mice from R. oryzae infection. These results further confirm the unique importance of iron in the pathogenesis of mucormycosis and demonstrate that rFtr1p can be utilized for developing immunotherapeutic strategies to prevent and/or treat mucormycosis.

AF-03-4

Human pharmacogenomic models for antifungal efficacy and toxicity

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Recent advances in the field of pharmacogenomics have elucidated the potential impact of heritable traits on the pharmacology and toxicology of antifungal agents. Candidate genes with potential pharmacogenomic importance include those encoding proteins involved in [1] drug transport (absorption and excretion), [2] metabolism (phase I enzymes, such as cytochrome P450-dependent mixed-function oxidases, and phase II enzymes, such as glucuronosyltransferases), and [3] distribution of antifungal compounds, such as albumin, A1-acid glycoprotein, and lipoproteins. Pharmacogenomic models for genetic variations in antifungal pharmacokinetics can be developed by using the tools of population genetics to define inter-individual differences in drug absorption, distribution, metabolism, and excretion. Variations in drug distribution, metabolism and excretion also may contribute to adverse drug reactions of antifungal agents. Genetic variations in critical target genes may alter the structure and expression of genes encoding phase I and II drug-metabolizing enzymes, such as CYP2C19 and N-acetyltransferase and drug transporters, such as P-glycoprotein and multidrug resistance proteins, may affect the disposition of antifungal agents and leading to dose-dependent (type A) toxicity. Genes encoding different lipoproteins may also affect the distribution of antifungal compounds. Considerably less is known about the possible role of genetic polymorphisms and gene products in contributing to idiosyncratic (type B or non-dose-dependent) toxicities of antifungal agents. There are several possible candidate genes that may affect antifungal agents: low-density lipoproteins and cholesteryl ester transfer protein in amphotericin B renal toxicity; toll-like receptor 1 and 2 in amphotericin B infusion-related adverse drug reactions; phosphodiesterase 6 in voriconazole visual adverse events; flavin-containing monooxygenase, glutathione transferases and multidrug resistance proteins 1 and 2 in ketoconazole and terbinafine hepatotoxicity; and multidrug resistance proteins 8 and 9 on 5-flucytosine bone marrow toxicity. Pharmacogenomic factors may become especially important in the treatment of immunocompromised patients or those with persistent or refractory mycoses that cannot be explained by elevated MICs and where rational dosage optimization of the antifungal agent may be particularly critical. Pharmacogenomics has the potential to shift the paradigm of therapy and to improve the selection of antifungal compounds through adjustment of dosages based upon individual variations in drug disposition.

AF-04-1

Bridging antifungal pharmacology between experimental models and humans

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Invasive fungal infections remain a significant cause of morbidity and mortality for immunocompromised patients. There are relatively few effective antifungal agents. Clinical trials are a relatively inefficient mechanism to identify optimal dosages and schedules of antifungal drug administration. Pharmacokinetics (PK) and pharmacodynamics (PD) are increasingly used to identify optimal antimicrobial regimens. There has been a progressive understanding of the PK-PD relationships of antifungal agents. Experimental models can be used to identify the magnitude of antifungal drug exposure that is associated with a high probability of a successful therapeutic outcome. If drug exposure is quantified with respect to the invading fungal pathogen rather than the host, results from experimental models can be bridged to humans. Population pharmacokinetics and Monte Carlo simulation can be used to explore the probability of attaining a desired drug exposure target in humans following the administration of various dosages and schedules of administration. Candidate regimens that are likely to be associated with a high probability of success and a low probability of toxicity for humans can be identified and expedited for further study in clinical trials. This approach represents an efficient and cost effective utilization of resources and a way that effective regimens can be identified as rapidly as possible.

AF-04-2

Pharmacodynamics of echinocandins in experimental candidiasis

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The role of pharmacokinetics and pharmacodynamics (PK/PD) has gained increasing recognition as critical for selection and dosing of antimicrobial therapeutics, including antifungal agents. The study of antimicrobial PD provides insight into the link between drug pharmacokinetics, in vitro susceptibility, and treatment efficacy. PK/PD investigations have been valuable for defining antifungal dosing regimens to optimize therapy, guiding therapeutic drug monitoring, developing in vitro susceptibility breakpoints and defining clinical resistance. PD observations from animal model studies have proven useful for outcome predictions in triazole treatment of human infections. Similar investigations have been undertaken with compounds from the echinocandin class. Results from studies in rodent invasive candidiasis models have demonstrated concentration dependent killing and prolonged persistent growth suppression. Dose fractionation studies show optimal outcome with the echinocandins is achieved by providing large exposures and efficacy is maintained with very widely spaced dosing intervals. Investigations have also begun to define the PD target or dose level needed for efficacy against *Candida* spp. Results from these experiments demonstrate the impact of MIC, *Candida* spp., and protein binding. Clinical trial results have become available that now allow exploration of the predictive value of these experimental models. Analysis thus far suggests concordance between the animal model and clinical trials. These data should be used to refine susceptibility breakpoints and guide dosing regimen design for this class of antifungal compounds.

AF-04-3

Nystatin - intralipid a novel formulation of nystatin

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The overall goal of our studies is to develop a formulation of Nystatin (NYT) that could be delivered systemically and hence suitable for treatment of invasive mycoses.

We developed a stable, standardized, Nystatin-Intralipid (NYT-IL) formulation which was characterized physically and chemically, that included determination of particle size, association of NYT with IL and stability of these characteristics at different temperatures.

The antifungal activity of NYT-IL was assessed in vitro against 5 major pathogenic *Candida*, 4 *Aspergillus* and 2 *Fusarium species* (35 strains). NYT-IL minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) were in most cases lower than those of NYT, indicating a better or at least comparable antifungal activity as NYT. It was also found that the activity of NYT-IL was maintained during storage at different temperatures, indicating the stability of the antifungal activity.

In order to examine the putative mechanism underlying the antifungal activity of NYT-IL we explored the ultra structural changes undergoing in *C. albicans* treated with NYT-IL in comparison to those following treatment with NYT and to non-treated yeasts. The studies included scanning and transmission electron microscopy (EM) analysis of treated and non-treated fungi at different time intervals post treatment and at different drug concentrations. The EM observations revealed that both preparations of Nystatin had significant destructive effect on the fungal cells with distinct ultra-structural changes in comparison to the non-treated microorganisms. NYT-IL was more effective even at lower concentrations and after shorter exposure time than NYT.

Toxicity of NYT-IL in comparison to NYT was evaluated by hemolysis of RBC and leakage of K^+ ions. Significantly higher concentrations of NYT-IL than of NYT were required for hemolysis of RBC, indicating a lower toxicity of the NYT-IL formulation.

In vivo experiments to assess activity and toxicity in a mice model are currently in progress.

AF-04-4

Therapeutic drug monitoring of posaconazole (PSZ) in adults

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We assessed the prevalence of low PSZ plasma levels (PPL) in case of prophylactic or curative treatment and host factors associated with low PPL by retrospectively reviewing all adult patients who underwent measurement of PPL after at least 5 days of treatment between 2006 and 2008 at Necker-Enfants malades university hospital. Therapeutic drug monitoring (TDM) was performed by high-performance chromatography and ultra-violet detection. Clinical and biological data were assessed at the initiation of PSZ. Low PPL was defined by a concentration lower than 500 ng/ml (Andes et al., AAC January 2009).

54 patients were included in this study: 36 receiving prophylaxis (200 mg t.i.d.) [allogeneic bone marrow transplantation (75%), hematological malignancy with prolonged neutropenia (19%) or constitutive immunodeficiency (6%)] and 18 curative posaconazole therapy (400 mg b.i.d.). Prevalence of low PPL was 16/36 (44%) in the prophylactic group and 22% (4/18) in the curative treatment group. In the prophylactic group, low PPL tended to be more frequent in case of any digestive disease (62% versus 30%, p=0.051), significantly more frequent in patients with diarrhea (71% versus 24%, p=0.009) or with mucositis (100% versus 33%, p=0.004). In the prophylactic group, only 2 patients experienced IFI and both had a low PPL. The only adverse event was hepatotoxicity in 2/54 patients (3,7%).

Low PPL is common, significantly more frequent in case of diarrhea or mucositis and potentially associated with the subsequent occurrence of IFI. PSZ TDM is therefore mandatory in immunosuppressed adults.

AF-04-5

Pharmacokinetics of antifungal agents in pediatric patients

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Invasive fungal infections have evolved into important causes of morbidity and mortality in children with severe underlying illnesses. Over the past decade, several new antifungal agents have entered the clinical arena, including less toxic lipid amphotericin B formulations, more versatile antifungal triazoles and the novel class of echinocandin lipopeptides. Although the final pediatric dosages of some of these agents remain to be established, their clinical development is moving forward at steady pace. Children, in particular neonates and young infants, represent a unique patient population, in particular with regard to the disposition of antifungal agents and safety issues. This presentation therefore reviews the pharmacokinetics, safety and dosing of antifungal agents in pediatric patients and the current status of their regulatory approval.

AF-05-1

Symposium introductory lecture: New developments in antifungal susceptibility testing

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Antifungal susceptibility testing has been greatly advanced by CLSI and EUCAST methodologies. Broth microdilution and agar diffusion methods are available for testing *Candida* spp. (CLSI documents M27-A3 and M44-A, respectively), *Aspergillus* spp. (CLSI documents M38-A2 and M51-P [under development], respectively) and other pathogens. In vitro results by these methods may play an important role in patient management of *Candida* infections, because interpretive breakpoints have been established by the CLSI for most antifungal agents for yeast testing. Furthermore, the incubation time to read results has been reduced to 24h for some of these agents versus *Candida* spp. (e.g., fluconazole and echinocandins) by the microdilution methodology. In addition, available commercial microdilution methods have incorporated some of the new triazoles and echinocandins. The EUCAST has also proposed a similar method for testing *Candida* spp. as well as interpretive breakpoints for fluconazole and voriconazole. In addition, EUCAST has proposed similar guidelines for testing *Aspergillus* spp. and other moulds and both EUCAST and CLSI have established the same epidemiological cutoffs for *Aspergillus fumigatus* versus the triazoles. Further efforts are being made by CLSI and EUCAST to harmonize their methodologies for testing *Candida* spp. These issues will be addressed in more detail by the speakers and during the discussion/question session after the lectures.

AF-05-2

Clinical applicability of interpretive breakpoints and methodologies for in vitro antifungal susceptibility testing

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During the past fifteen years, the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) have achieved major advances in development of standardized and clinically applicable in vitro susceptibility testing methods for systemically administered antifungal agents against yeasts and filamentous fungi. Validated interpretive breakpoints have been determined by data-driven methods for susceptibility testing of *Candida* species to fluconazole, itraconazole, voriconazole, flucytosine, and echinocandins. Recently, CLSI Antifungal Subcommittee followed the M23-A2 "blueprint" to develop interpretive MIC breakpoints for anidulafungin, caspofungin, and micafungin against *Candida* species (Pfaller et al 2008). In vitro susceptibility determinations of non-*albicans Candida* is used in algorithms for management of candidemia and invasive candidiasis. For example, for infection caused by *Candida glabrata,* transition from an echinocandin to fluconazole or voriconazole therapy is not recommended without confirmation of isolate susceptibility (IDSA Guidelines 2009). In vitro susceptibility testing is recommended for isolates of *C. glabrata* from blood and sterile sites as well as for other *Candida* species that have not responded to antifungal therapy or in which azole resistance is suspected. Increasing recognition of triazole-resistant and polyene-resistant isolates of Aspergillus spp. underscore the increasing importance of in vitro susceptibility determinations as an important laboratory adjunct in management of life-threatening infections in immunocompromised and critically ill patients.

AF-05-3

Usefulness of the EUCAST method for the analysis of antifungal susceptibility profiles and trends

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Several microbroth dilution methods have been developed by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) to test the susceptibility of fermentative yeasts and moulds to several antifungal drugs and to determine breakpoints. Several parameters differ between the methods including a higher inoculum size with the EUCAST method. They have been shown to generate concordant data. Both methods have allowed determining the spectrum of activity of commercialized and non commercialized antifungal drugs on a wide variety of yeasts and moulds. Breakpoints have only been defined for fluconazole and voriconazole on fermentative yeasts using the EUCAST method. Probably because the microbroth dilution EUCAST method has been implemented more recently than CLSI one, publications of correlations between in vivo and in vitro results are scare using the former.

Based on the antifungal susceptibility testing results generated at the French National Reference Center for Mycoses and Antifungals using the EUCAST methods and thanks to the clinical and epidemiological data associated with each isolate tested, we have been able to show (1) cross-reduced susceptibility between azoles, and between caspofungin and micafungin against yeasts (2) demonstrate that caspofungin MICs >=0.5 µg/ml in AM3 medium correlate with FKS mutation for *Candida* spp. (3) show that prior exposure to fluconazole or caspofungin lead to fungemia caused by isolates with a significantly higher fluconazole or caspofungin MIC, respectively, or to emergence of species with decreased susceptibility to the respective drugs; and (4) demonstrate the in vivo/in vitro correlation for caspofungin after exploring several episodes of treatment failure

These results suggests that antifungal susceptibility testing using the EUCAST method together with species identification are useful for the management of invasive fungal infections especially in the case where prior exposure to antifungal drugs is known .

AF-05-4

Commercial methods of antifungal susceptibility testing and their utility in the clinical laboratory

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Since the development and publication of an approved reference method for broth microdilution antifungal susceptibility testing from the Clinical and Laboratory Standards Institute (CLSI), several commercial test kits have been introduced. Studies have conducted and revealed high intra- and inter-laboratory accordance with CLSI standard method. ASTY (Kyokuto Pharmaceutical Industrial Co., Ltd) and SensititreYeastOne (Trek Diagnostic Systems, Inc.) have become available in clinical laboratories, both of which adopted CLSI method. There dry-form 96-well panels use oxidation-reduction colorimetric growth indicator (resazurin in ASTY and Alamar Blue in YeastOne). These colorimetric microdilution panels make judgement of end-points reading easy. In these systems, MICs were interpreted as the lowest concentration of antifungal solutions changing from red (growth) or purple (growth inhibition) to blue (no growth). Etest (AB BIODISK) is another commercial system, an agar-based predefined concentration gradient method for determining the MICs. Trailing growth (TG) is one of the phenomenon seen in susceptibility testing in some *Candida* strains that complicates the judgement of end-point of triazoles by turbidimetric methods, leading to misjudgement (false resistance). In Etest, TG arises microcolonies within a discernable ellipse, of which border is sometimes unclear. Relative ease of discrimination of TG from true resistance is one of the most significant advantage of colorimetric methods. Long shelf life of these panels or Etest strips (> 6 month in refrigerator or at ambient temperature) is another advantage in clinical laboratories. Summary of evaluation studies, advantages and matters that require attention in the use of these commercial methods will be discussed.

PT-01-1

The history of *Aspergillus* PCR

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The application of PCR to aid in the diagnosis of invasive aspergillosis (IA) is far from a novel concept. In the early 1990's the first manuscripts describing the PCR testing of BALs were published and since then interest in the topic has gathered pace annually.

To date BAL, serum, plasma, whole blood, CSF, and a variety of fluid, ocular and tissue specimens have been analysed by molecular procedures. However, within different specimen types the basic fungal target will vary from viable organism to free circulating DNA and this will affect the optimal extraction protocol. Fortunately, providing the PCR amplification system has been accurately designed and optimised then a single PCR system can be combined with various extraction protocols.

While methodological variation is beneficial by proving the concept using different specimens and molecular systems it limits the impact of PCR diagnosis as no single method has received extensive multi-centre evaluation and as a result will not be included in consensus defining criteria. Following-on from the successful collaborations of the UK Fungal PCR consensus group the ISHAM working group "The European *Aspergillus* PCR initiative" (EAPCRI) have completed studies into determining optimal molecular methodology for involvement in a large scale clinical assessment. This will determine the true performance of PCR aided diagnosis of IA, hopefully, for inclusion in future consensus criteria.

PT-01-2

A proposed standard for *Aspergillus* PCR

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The implementation of polymerase chain reaction (PCR) as a recognized tool for the diagnosis of invasive aspergillosis has so far been hampered by a lack of standardization and the subsequent variability of results. Therefore, it is mandatory that a consensus is reached on defined key issues, such as the type of specimens to be analyzed, the DNA extraction procedure, and the molecular targets and PCR techniques used.

In September 2006, under the auspices of the International Society for Human and Animal Mycology, the Laboratory Working Party of the European *Aspergillus* PCR Initiative (EAPCRI) was founded, involving 23 centres across Europe and one centre in Australia. The main focus of the initiative is the standardization of *Aspergillus* PCR methodology, including DNA extraction protocols, PCR assays and the required controls. Up until now, five panels of previously extracted *Aspergillus*-DNA and / or EDTA whole blood specimens spiked with defined numbers of *A. fumigatus* conidia were distributed among the participating centres. Individual results were analyzed based on previously set criteria and were statistically evaluated. The Laboratory Working Party has defined recommendations, covering blood volume, release of *Aspergillus*-DNA, PCR assays, internal controls and whole blood anticoagulants. In addition, we have been described footnotes, addressing the handling with inhibitory specimens and contamination of samples.

PT-01-3

A standard for *Aspergillus* PCR - how to validate the standard

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Validation of the standard should take the form of a clinical trial Consideration must be given to identification of the relevant study population, benchmarking and diagnostic accuracy against a gold standard reference.

EORTC/MSG II consensus diagnostic definitions should be used. It must be recognized that these are insufficient to establish diagnostic accuracy and an independent blinded review committee is also vital Preliminary studies suggest that optimal use of PCR is as a screening tool to utilise the high negative predictive value rather than as a purely diagnostic tool. Since diagnostic utility is heavily influenced by the prevalence of disease in different populations, risk stratification is needed to identify high risk groups for inclusion in the clinical trial. This would restrict inclusion to adult patients and allogeneic stem cell transplant, acute leukaemia patients. In addition, STARD criteria must be adhered to.

A blinded randomized controlled trial evaluating diagnostic screening versus empirical or prophylactic antifungal strategies would be optimal but raises ethical issues over withholding antifungal agents. It would also be expensive, difficult to set up across all centres within the EAPCRI and influenced by variations in clinical practice. A multicentre proof of concept study evaluating the standard against other emerging diagnostic technologies is an alternative approach.

PT-02-1

Strain identification of *Penicillium marneffei* by AFLP

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Penicillium marneffei is an emerging pathogenic fungus that can cause a fatal systemic mycosis in patients infected with human immunodeficiency virus (HIV). P. marneffei infection is endemic in tropical Asia, especially Thailand, northeastern India, China, Hong Kong, Vietnam, and Taiwan. The majority of infections by P. marneffei were diagnosed in AIDS patients in Thailand; however, infections were also observed in Cambodia, China, Hong Kong, India, Malaysia, Taiwan, and Vietnam. Cases from outside the region of endemicity were observed in HIV-infected patients from Australia, Belgium, France, Germany, Japan, Sweden, Switzerland, The Netherlands, the United Kingdom, and the United States.
The mitochondrial (mt) cytochrome b (cyt b) gene of species of the genus *Penicillium* was sequenced to determine the phylogenetic relationship and to design specific primers that could be used in real time PCR for the identification of *P. marneffei*. The sequence of *P. marneffei* is same in this parts of cyt b domain. We can not find DNA type or local specificity. One of the newest and most promising methods is amplified-fragment length polymorphism (AFLP) analysis. It was using for plant and animal genetic mapping, medical diagnostics, phylogenetic studies, and microbial typing. We were used AFLP for epidemiological study of *P. marneffei*.
Digestion of DNA with EcoRI and MseI and ligation to a single adapter generated useful fingerprints for *P. marneffei*. Amplified-fragments were analyzed by ABI Prism 3130 Genetic Analyzer. Phylogenetic analysis was processed by computer program (Infor Bio V5.26).
P. marneffei 42 strains showed inherent fragment length pattern. The results of phylogenetic analysis indicate tow big groups, one is isolated from China, and another group is Thailand. Three isolates from Italy were included in Thailand group. AFLP analysis has established itself as a broadly applicable genotyping method with high degrees of discriminatory power. AFLP analysis is also useful for epidemiology of pathogenic fungi.

PT-02-2

Evolution of Cyt*b*, rDNA & morphology of *Aspergillus* section *Nigri*

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Aspergillus sction *Nigri* is included in very important species because they are used in fermentation industries and they also are encountered as human and plant pathogens and produced mycotoxins. The concept of black aspergilli has been classified as the *Aspergillus niger* group by Raper and Fennel and *Aspergillus* sction *Nigri* by Games et al. In the past, the identification, classification and taxonomy of this group had mainly been based on morphological characteristics.

D1/D2 region of ribosomal DNA was broadly used for identification of fungi and other organisms. However, it did not sufficient for identification of species on fungi. The observation of conidiospore surface by scanning electromicroscope (SEM) is useful methods for identification of section *Nigri*. The typical morphology of conidiospore surface of strain is ease however, some strains show intermediated morphology.

Although some of the species can be readily distinguished morphologically, results obtained in several attempts at classifying this section are debatable and identification of some species is still difficult.

The partial mitochondrial cytochrome *b* gene (Cyt *b*) was analyzed for identification, classification and phylogeny of pathogenic fungi by L. Wang et al., S.K. Biswass et al. and K. Yokoyama et al.. DNA type of section *Nigri* were divided to 14 types and amino acid type were divided to 5 types. A. japonicus were divided into 4 DNA type, include in A. aculeatus. A. niger were divided into 4 DNA type.

We compared among D1/D2, Cyt *b* and SEM, The results of SEM observation show continuous variation of conidiospore surface. Phylogenetic tree of D1/D2 and Cyto *b* sequences were difference. These were different evolution of nucleus, cytoscopic and total genetic expression (morphology).

We discuss how to evolve the morphology, cytoplasmic inheritance and nuclear gene.

PT-02-3

Identification challenges for selected mould pathogens

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Although comparative sequence-based identification methods are now considered the "gold-standard" for fungal species identification and classification in research/reference laboratories, mould identification in the clinical laboratory is still based primarily on key morphologic characteristics. We will examine three selected genera/species and discuss the utility of employing both morphologic features and molecular relatedness to arrive at an accurate identification.

Geosmithia argillacea, an organism recently reported to cause disseminated disease in a German shepherd dog, and one that closely resembles members of the genus *Penicillium,* can provide an identification challenge. Key cultural features include buff-colored colonies, its thermotolerant nature, roughened stipes, metulae, and phialides, and its cuniform, catenulate conidia. A BLASTn search with ITS and D1/D2 sequences provides >99% identity with *G. argillacea* and its teleomorph, *Talaromyces eburneus*. This voriconazole-resistant organism also appears to be emerging in cystic fibrosis patients and lung transplant recipients.

Molecular sequencing of the ITS rDNA region of 188 U.S. clinical isolates of *Exophiala* has more clearly delineated the heterogeneity of the species referred to as *E. jeanselmei*. Information gained regarding the distribution of clinical species provides useful guidelines for laboratories lacking molecular facilities. The most clinically-significant U.S. species include *E. dermatitidis* (29.3%), *E. xenobiotica* (19.7%), *E. oligosperma*, and *E. lecanii-corni* (6.9%). *E. jeanselmei*, represented only 3.7% of the isolates.

Aspergillus granulosus, in the Section *Usti*, is morphologically similar and should be distinguished from the more antifungal-resistant species, *Aspergillus calidoustus* (formerly *A. ustus*), in the same section. Key morphologic features include striking clusters of colorless, variably-shaped Hülle cells, growth at 37°C, and frequently diminished conidiation. ITS sequencing places *A. granulosus* in the *A. ustus* species complex while other regions such as ß-tubulin, calmodulin, and actin may be used to confirm the species. This organism is being seen in organ transplant recipients.

PT-02-4

Uncommon and emerging fungal pathogens: Clinical manifestations and therapeutic options

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Candida spp. constitute the third to fourth most common causes of nosocomial blood stream infection. *Aspergillus* spp. is the most common cause of infectious pneumonic mortality in hematopoietic transplant recipients. *Cryptococcus neoformans* is the most common cause of fungal-related mortality in HIV-infected patients. Although these organisms are important pathogens, less common but emerging fungal pathogens also cause morbidity and mortality in an increasingly expanding immunocompromised patient population. Among these uncommon and emerging pathogens are the Mucorales, as well as septate hyaline moulds, such as *Fusarium* spp., *Scedosporium* spp., *Trichoderma* spp., and dematiaceous moulds, including *Cladophialophora bantiana, Alternaria* spp., and *Ochroconis gallopava)*. The endemic dimorphic pathogen *Penicillium marneffei* in HIV-infected patients and non-dimorphic yeasts such as *Trichosporon* species in non-HIV-infected immunocompromised patients are also increasingly recognized in these populations. Successful management of the life threatening invasive fungal infections caused by these organisms in immunocompromised hosts requires an understanding of early clinical manifestations, as well as familiarity with their distinctive microbiological, epidemiological, and therapeutic characteristics. The ratiuonal use of triazoles, lipid formulations of amphotericin B, and echinocandins in management of thses infections may improve outcome in complicated immunocompromised patients.

PT-03-1

Taxonomy and identification of *Malassezia*

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The taxonomy of *Malassezia* has changed considerably over the last 20 years. From the 2 species recognised in 1989, there are now 13 formally acknowledged species, with other species having considerable variation within them. This taxonomic revision is as a result of molecular studies, using a range of methods and future work may well result in the recognition of new species from within the species showing considerable genetic variability.

Identification of *Malassezia* species has also changed considerably over this same time period. When only 2 species were recognised, *Malassezia* pachydermatis which can grow without supplemental lipid could be identified by its growth on Sabouraud's agar, whilst the other species, M. furfur, required lipid supplementation to grow - a simple fact that could be used to identify the species. The recognition of M. sympodialis in 1990 and 4 new species (M. globosa, M. restricta, M. obtusa and M.slooffiae) in 1996, led to the use of Tween assimilation patterns as the main method of identification, with different species assimilating different Tweens. The micromorphology of the yeast cells was also useful as a contributory feature for identifying the species.
With the subsequent descriptions of more new species, these simple biochemical methods are no longer sufficient to identify all the species in the genus, although a recent revised culture-based system is able to identify 9 clinical significant species. To obtain an identification of the other species in the genus, molecular analysis is required.

PT-03-2

Malassezia pachydermatis on the skin of dogs: Distribution and population structure in the genomic era

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The yeast M. pachydermatis has been isolated from the skin and/or mucosa from different birds and mammals, including dogs in which it may be responsible for erythematous and pruritic dermatitis and otitis externa. M. pachydermatis has also been reported as a causative agent of nosocomial infection in humans or as an occasional commensal on the skin of dog owners. The presence of high population densities of the yeast in lesional skin in dogs with dermatitis refractory to antibacterial and anti-inflammatory therapy, and the clinical response following antifungal treatments provides good evidence for a pathogenic role. However it is not known whether there is a threshold population density above which infection occurs. The skin of dogs constitutes a complex ecosystem that may greatly differ according to the age, the gender and the breed of the animals. *Malassezia* populations in healthy basset hounds, cockers, dachshunds or West Highland terriers were found to exceed those of healthy dogs of other breeds. Strain differences may also be of importance, and quorum sensing events described in bacteria may modify the expression of virulence characteristics. Genotyping of M. pachydermatis is required to identify sources of infection and to discover possible connections between genotypes and particular cutaneous diseases in dogs. Direct sequencing of 28S rRNA resulted in the differentiation of 7 different sequence types (Ia-Ig). All M. pachydermatis isolated from dogs belong to types Ia, Id and Ie. Genotype Ia was found for isolates from both animals and humans, while Id included M. pachydermatis with small colonies and poor growth on Sabouraud agar. One single dog could be the carrier of two or more *Malassezia* genotypes. A recent investigation including additional loci (chitin synthase 2 gene and ITS1) confirmed that 3 main genotypes could be detected among M. pachydermatis isolates from dogs.

PT-03-3

Malassezia and atopic eczema

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Atopic eczema (AE) is a chronic relapsing inflammatory skin disease considered to be caused by the combination of a disturbed skin barrier which enables allergens to enter into the skin and inappropriate immune responses with contributions from both genetic and environmental factors. In approximately 50%of adult patients with AE, specific IgE- and/or positive skin prick test and atopy patch test (APT) to *Malassezia* have been found, as well as specific T-cell reactivity but rarely in other allergic diseases indicating a specific link between AE and *Malassezia*. The global transcriptional response in positive APT reactions to M. sympodialis is very similar to the gene-signature identified in lesional AE skin supporting the association of *Malassezia* with AE pathogenesis. The disturbed skin barrier and elevated pH of AE skin can also induce an enhanced allergen release from M. sympodialis, leading to increased host-microbe interactions. Several IgE binding components in the 10-100 kDa molecular weight range have been identified in *Malassezia*. Thirteen allergens from *Malassezia* species have been cloned. Interestingly, four of the M. sympodialis allergens, Mala s 1 and Mala s 7-9, encode proteins of unknown function without sequence homology to known allergens or to other known proteins, whereas others reveal significant homology with human endogenous proteins. The crystal structure of Mala s 1 shows a 6-fold beta-propeller structure representing a new fold among allergens. The dominating symptom in AE is severe itch which provokes scratching and increased inflammation. Mast cells most likely play a central role in this vicious circle. Fungal products can activate mast cells through TLR2 and cross-linking of the high-affinity IgE receptor leading to the release of potent inflammatory mediators. It was recently found that M. sympodialis can activate IgE-sensitized mast cells, a novel mechanism for the contribution of *Malassezia* to the inflammation and itch in AE.

PT-03-4

The *Malassezia* yeasts and diseases in humans

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The *Malassezia* yeasts are members of the normal human cutaneous flora in adults but also associated with several diseases. In pityriasis versicolor, under the influence of predisposing factors, *Malassezia* changes from the round blastospore form to the mycelial form. A great problem in pityriasis versicolor is the high rate of recurrence and to avoid this prophylactic treatment is mandatory. *Malassezia* folliculitis is a chronic disease characterised by pruritic follicular papules and pustules located primarily on the upper trunk, neck and upper arms. In direct microscopy clusters of round budding yeast cells are found. The disease responds rapidly to antimycotic therapy. There are now many studies indicating that the *Malassezia* yeasts play an important role in seborrhoeic dermatitis. Many of these are treatment studies showing a good effect of antimycotics paralleled by a reduction in number of organisms. Severe seborrhoeic dermatitis often difficult to treat is associated with AIDS. In skin biopsies from patients with seborrhoeic dermatitis we have found an increase in NK1+ and CD16+ cells in combination with complement activation indicating that an irritant non-immunogenic stimulation of the immune system is important. However, we also found an increase in the production of inflammatory interleukins as well as regulatory interleukins for both TH1 and TH2 cells indicating a complex activation of the immune system in the skin. The majority of adult patients with atopic dermatitis localised to the head, neck and scalp react with a positive reaction in skin prick test to a *Malassezia* extract as well as to recombinant *Malassezia* allergens. The majority also have specific IgE antibodies and react with a positive reaction in atopic patch test with a *Malassezia* extract. There are also treatment studies indicating that antifungal treatment may be beneficial in these patients. Even systemic diseases causes by *Malassezia* have been reported.

PT-04-1

Basidiomycetous yeasts as emerging pathogens

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Basidiomycetous yeasts are phylogenetically a diverse group of organisms that occur in many different habitats. In recent years the number of recognized species is rapidly increasing due to the application of molecular systematics and the availability of a large database of ribosomal DNA (rDNA) sequences, most notable the D1/D2 domains of the LSU rDNA and the ITS 1 and 2 regions. Only a few species are recognized as important pathogens for humans and animals, such as Cryptococcus neoformans and C. gattii, several Trichosporon species and some *Malassezia* spp. In recent years it became clear, however, that a considerable number of basidiomycetous yeast species may cause infection, or otherwise may cause health problems, such as hypersensitivity pneumonitis (HP) due to exposure to fungal antigens. In this presentation we will give an overview of emerging data on the role of the various species of basidiomycetous yeasts in human disease. Cases caused by Cryptococcus diffluens, a new species of Trichosporon, and a case of HP caused by Pseudozyma spp will be presented. Furthermore, an overview will be given of published reports on the clinical occurrence of non-neoformans Cryptococcus species, such as C. albidus, C. adeliensis, C. flavescens, C. curvatus and C. laurentii.

PT-04-2

Molecular typing of *Malassezia* yeasts: Clues to epidemiology and pathobiology

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The recent sequencing of the whole genome of *Malassezia globosa* and *M. restricta* has instigated interest on possible gene targets for future investigations into *Malassezia* virulence factors and molecular epidemiology studies. Currently the available *Malassezia* molecular typing methods highlight the potential scarcity of epidemiological data regarding this ubiquitous fungal commensal and pathogen. However, interesting clues to pathobiology have emerged through molecular typing of *Malassezia* isolates from healthy or diseased skin of from comparison of *Malassezia* sequences selectively amplified from DNA extracted directly from clinical material (skin scales).

Methods that have been employed for *Malassezia* molecular typing can be categorized into those detecting sequence variations of strains and those that selectively amplify polymorphic DNA markers for discriminating *Malassezia* species subtypes. The former exploit mostly rRNA gene sequence variations in order to trace *M. globosa*, *M. restricta* and *M. pachydermatis* subtypes associated with specific skin diseases, or detect *M. furfur* geographical variations.

Polymorphic DNA amplification methods, such as amplified fragment length polymorphism analysis, demonstrated association of *M. furfur* subtypes with the origin of the strain (skin or systemic isolate), whereas PCR-fingerprinting of the mini-satellite DNA clustered *M. furfur* strains according to their geographic origin and disease origin. Moreover, much typing work has already been performed regarding the zoophilic species *M. pachydermatis* and the relevant methods can be adapted for studying the anthropophilic *Malassezia* species.

In the near future, molecular typing will be a powerful tool in epidemiological studies that could be employed for the elucidation of the pathobiology of *Malassezia* species in associated skin diseases. In culture isolated strains it will be possible to complement typing results with additional testing for virulence factors, while PCR amplified gene sequence comparisons from diseased or healthy skin will demonstrate Candidate pathobiology traits.

PT-04-3

Recent progress in the taxonomy, identification, and epidemiology of the basidiomycetous pathogen *Trichosporon*

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Currently, the genus *Trichosporon* includes approximately 40 species, and the number of species in the genus will increase as more than ten Candidate species remain to be described a new species. Several of these species are associated with infection and allergy. Regarding disseminated trichosporonosis, the major causative agent is *T.asahii*, while *T. ovoides* and *T. inkin* are rarely isolated from clinical specimens. Although *Trichosporon* species are considered rare pathogens, trichosporonosis can occur as a breakthrough infection after patients are treated with candin derivative antifungal agents. *Trichosporon* species are also responsible for types III and IV allergies, the so-called "summer-type hypersensitivity pneumonitis (SHP)". These microorganisms are distributed widely in the environment, and SHP develops after inhalation of their spores. *T. asahii* and *T. dermatis* are the two major causative antigens of SHP. The IGS 1 region located between the 18S and 5S subunits of the rRNA gene shows remarkable diversity in *T. asahii*. The genotypes of strains isolated from infectious patients and from the homes of patients with SHP differ significantly. The genus *Trichosporon* is monophyletic and is subdivided into four clades (Cutaneum, Ovoides, Brassicae, and Gracile). Some *Cryptococcus* species such as *Cryptococcus humicola* and *C. curvatus* also belong to the genus *Trichosporon* phylogenetically. Analyses of IGS and ITS sequences should be used for species identification, since several species are very close phylogenetically.

This session will discuss recent progress in the taxonomy, identification, and molecular epidemiology of the genus *Trichosporon* from the perspective of medical mycology.

PT-04-4

Molecular genotyping of *Cryptococcus neoformans* var. *grubii* (serotype A)

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Cryptococcus neoformans var. *grubii* (serotype A) is the most common agent of fungal meningoencephalitis and responsible for more than 90% of all cases of cryptococcosis worldwide. In recent years, molecular epidemiology of *C. neoformans* serotype A has been studied extensively, and several methods of molecular genotyping have been developed. Three most commonly used genotyping methods are (i) PCR fingerprinting, (ii) scoring amplified fragment length polymorphisms (AFLP), and (iii) multilocus sequence typing (MLST). The applications, advantages and disadvantages of each method will be discussed in this presentation. While PCR fingerprinting allows rapid and economical assessment of genetic diversity of the population, MLST is more appropriate for the detailed analyses of the population structures and evolution. However, the ultimate goal of any molecular genotyping project is to determine correlations between molecular genotypes and clinically relevant phenotypes, which can lead to the identification of virulent and avirulent strains. How close are we to fulfilling this goal? Our recent analysis of the murine virulence of 21 clinical and environmental isolates demonstrated that strains with identical molecular genotypes often manifest vastly different virulence phenotypes. While most clinical isolates tested caused lethal infections in mice, environmental strains with identical or closely related genotypes are not lethal. In follow-up studies, we developed a new genotyping technique based on hybridization with TCN2 and TCN4 retrotransposon-specific probes. Although the retrotransposon banding patterns were unstable after prolonged subculturing in the laboratory, this method was able to differentiate clinical and environmental strains that had the same AFLP/MLST genotype.

PT-04-5

Molecular epidemiology divides *Cryptococcus gattii* into four major molecular groups and identifies VGII as the ancestral genotype

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Cryptococcus gattii is a close sibling taxa of *Cryptococcus neoformans*. It is a primary pathogen, causing life-threatening disease mainly in immunocompetent patients. Using PCR-fingerprinting, AFLP, URA5 RFLP, MLST & MLMT analysis) 8 major genotypes have been identified for the *C. neoformans*/*C. gattii* species complex - 4 within *C. gattii*: VGI/AFLP4, VGII/AFLP6, VGIII/AFLP5 & VGIV/AFLP7, all corresponding to serotypes B and C. In addition several hybrid strains between *C. neoformans* and *C. gattii* exist (serotypes A & D, A & B, D & B), making the taxonomic placement of both species controversial. To investigate the phylogenetic relationships among these haploid genotypes, we analysed globally selected representative strains of all previously identified major genotypes and carried out multigene sequence analysis using four genetically unlinked nuclear loci ACT1, IDE, PLB1, URA5. Both parsimony and likelihood analysis yielded high support for four the clades within *C. gattii*. Separate or combined sequence analyses of all four loci showed significant support for each of the four major genotypes of *C. gattii*. The topology of the separate gene trees was congruent for the 3 monophyletic groups in *C. neoformans* but was incongruent for the *C. gattii* clades, indicating recent recombination events within *C. gattii*. Applying the molecular clock *C. gattii* diverged from *C. neoformans* 49 million years ago. The major genotypes VGIII and VGI 8.5 < VGIV 11.7 < VGII 12.5 million years ago. As basal clade VGII represents the ancestral population of *C. gattii*. Recombination for this genotype was detected in the global population and South America and Australia. The highest genetic variation was found in South America placing the origine of *C. gattii* in this region from where it spread globally. The genetic variation found among all of these haploid monophyletic lineages indicates that they warrant at least variety if not species status.

PT-05-1

Opening remarks: Birth of medical phycology

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1. Protothecosis and infections caused by morphologically similar green algae are localized or disseminated diseases of humans and lower animals. The disease is sporadic and occurs worldwide. Although this group of disease is still uncommon, increasing numbers of cases are being diagnosed, the disease is viewed with increased interest in the current medical and veterinary circumstances.

2. These infections can occur in both immunocompetent and immunosupressed patients, although more severe and disseminated infections tend to occur in an immunocompromised individuals. So far, *P. wickerhamii* and *P. zopfii* have been reported to cause infections in humans, with *P. wickerhamii* being more common of the two.

3. In the past protothecosis and infections caused by algae has been studied in the field of medical mycology probably because of the history that Kruger, in 1984, described *Prototheca moriformis* and *P. zopfii* in the generic diagnosis of fungi and of the apparent yeast-like macro- and micro-morphologies of Prototheca species.

4. Although Casal in 1978 used the term "Ficología Médica" in Spanish, it has received neither recognition nor acceptance.

5. *Prototheca wickerhamii*, the most common human-pathogenic species, was isolated from the sap and described as a new species by Japanese scientists, Drs. Tubaki and Soneda, in 1959, just 50 years before. This 50-year commemoration is merely a coincidence, however, the ISHAM Congress-17 in Tokyo in 2009 would be the most appropriate place and year to propose the birth of "medical phycology" as a new realm of ever-changing microbiology.

PT-05-2

Protothecosis: Current assessment of five topics

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1. The phylogenetic relationship of Chlorella spp. and Prototheca spp. Based on their shared morphological, biochemical, and phylogenetic similarities, C. protothecoides is the closest extant progenitor of P. wickerhamii. On the other hand, such a close progenitor for P. zopfii has not been found.

2. Biofilm and Prototheca. Despite the fact that P. wickerhamii is the predominant Prototheca spp. in wastewater systems biofilm, capsulated P. zopfii variates may actually have biofilm forming capacities.

3. Sterols, storage carbohydrates, and sporopollenin. Since Prototheca spp. are sensitive to polyenes and azoles it is generally assumed that Prototheca spp. contain ergosterol. In fact, Prototheca spp. have a singular sterol, also found in C. protothecoides, that is not ergosterol.

Similarly, the storage carbohydrate in Prototheca spp. is frequently referred to as starch because all algae were assumed to be plants. In fact, Prototheca spp. store glycogen, as does C. protothecoides.

A trilaminar outer cell wall polymer of all Prototheca spp. that is highly refractive to both acid and alkaline hydrolysis was formerly called sporopollenin. This unique structure is likely necessary for the passage of Prototheca spp., undigested, through the mammalian GI tract.

4. Tropical sprue and enteritis. Is there any reason to believe that Prototheca spp. cause tropical sprue or enteritis? The case is very weak for a human cause and effect disease relationship, but for hemorrhagic canine diarrhea the evidence is convincing.

5. Experimental animal infections. Despite early discouraging results, several experimental models for lab animal infections with Prototheca spp. are available. Likewise, a model for naturally acquired chronic bovine mastitis fulfills Kochs postulates.

PT-05-3

Basic biology of *Prototheca*

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Prototheca species are achlorophyllic algae found in a wide range of habitats such as soil, water, and sewage. Their classification is based on ultrastructure and the mode of reproduction. Some species are also known to infect animals. *P. wickerhamii*, a pathogen to humans, varies in its size from 3 to 15 micrometers in diameter and grows easily on a variety of media at 30-37C forming creamy, white and yeast-like colonies.

It is believed that *Prototheca* has evolved from *Chlorella* and lost chloroplasts in the process. Therefore, it is heterotrophic and requires external sources of carbon and nitrogen. Reproduction is asexual and its cytoplasm forms 2-20 endospores every 5-6 hours under the adequate growth conditions. Spores are released when the sporangial cell wall ruptures and grow in size into sporangia, repeating the reproductive cycle.

Since early 90's we have witnessed the advancement in molecular phylogenetic studies of *Prototheca*. Today compete sequence of mitochondrial DNA (55kb) and partial sequence of leucoplast (plastid) DNA (28 of 54kb) for *P. wickerhamii* are available. RNA sequences of both small and large subunits of nuclear, mitochondrial, and leucoplast ribosomes are used in phylogenetic characterization of *Prototheca*.

Prototheca has retained some features of green algae. Analyses of expressed sequence tags (ESTs) revealed that *P. wickerhamii* lacks adenine- and uridine-rich elements at 3' end of mRNA transcripts. Instead, they use pentanucleotide UGUAA motif as polyadenylation signal. Another study on full-length ESTs has identified and predicted that 36 unique sequences correspond to plastid-targeted polypeptides. Some of these are chaperones and membrane proteins, while majority are enzymes involved in various metabolic pathways. Finally, leucoplast DNA encodes six subtypes of plastid-type ATP synthase in which each subunit shows greater than 60% homology to various algal species.

PT-05-4

Phylogenic analysis and molecular detection and identification of *Prototheca*

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Prototheca spp. exists in the environment as ubiquitous detritus inhabitants and contaminants of various substrates, however, their phylogenic or taxonomic analysis are still under developing. In this paper, characterization of a pathogenic strain isolated from dermatitis of the patient in a Japanese hospital will be presented. The isolate was first identified as *P. wickerhamii* using conventional biochemical method but the molecular based data suggested that the isolate represent a novel species. We need to have more isolates and information on the genus.
P. zopfii and *P. wickerhamii* has been reported as a pathogen involved in refractory subcutaneous disease and systemic infection in humans and in animals. Especially, *P. zopfii* causes bovine mastitis and canine fatal systemic infection, which have recently become major worldwide problems. Recently, the incidences of *P. zopfii* infection have been increasing rapidly in both cattle and humans. Therefore, a system to effectively and rapidly identify the pathogen in infected cattle or humans is necessary. Here, the specific nested PCR and quantitative PCR systems to detect *P. zopfii* were developed and evaluated with reference, clinical and environmental strains. Based on the high specificity and sensitivity, the system reported here is able to directly detect *P. zopfii* in milk or clinical specimens.

PT-05-5

Clinical, pathological, and microbiological features of Japanese cases of protothecosis

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1. Protothecosis is a relatively rare infection of human and lower animals caused by the species of the achlorophyllous genus *Prototheca*, morphologically similar to the genus *Chlorella*. Human protothecosis is classified into three categories: 1) cutaneous/subcutaneous protothecosis, 2) synovial/fibrous tissue protothecosis, and 3) systemic/disseminated protothecosis.
2. Most cutaneous/subcutaneous protothecosis occur in exposed areas such as upper extremities and face. Irregularly shaped erythematous plaque is the key to the diagnosis. Most patients have underlying diseases suggesting local or systemic immunosuppression.
3. Pathological features of protothecosis are usually nonspecific, chronic and granulomatous inflammation with variable combination of lymphocytes and plasma cells. The *Prototheca* cells in tissue are observed as small individual cells and/or wheel-like multicellular structures.
4. Based on our criteria, we confirmed 25 of over 30 reported human cases from Japan. Most of the cases were Cutaneous/subcutaneous infection. Three cases were systemic/disseminated protothecosis. No synovial/fibrous tissue infections were reported. *Prototheca wickerhamii* was the predominant etiologic species. Several reports lacked identification. There were no reports identified as *P. zopfii*. Itraconazole was the most successfully used antimicrobial agent.

PT-05-6

Closing remarks: Increasing importance of protothecosis in clinical medicine

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1. In 1989, a 29-year-old female was referred to us for chronic pain in the right wrist. She had previously had glucocorticosteroid injections and later surgery for tendonitis. After surgery, and prior to her wounds healing, she had worked in her garden. We diagnosed infection with Prototheca wickerhamii. First treatment with ketoconazole failed. Second treatment with amphotericin-B cured her.

2. We found 137 other published cases of human protothecosis, for a total of 138 cases.

3. The incidence of reported cases of human protothecosis has been rising: from 20 cases during 1964-78, to 71 cases during 1994-present.

4. Most of the increase has been among patients either immunocompromised or treated with glucocorticosteroids before protothecosis diagnosis. Of the 20 cases reported 1964-1978, 6 were either immunocompromised or steroid-treated, and 14 were not. Of the 71 cases reported 1994-present, 55 were either immunocompromised or steroid-treated, and 16 were not.

5. Sites of infection have included skin 82 cases, olecranon bursa 12, wound 10, disseminated 8, fingernail 4, peritonitis 4, septicemia 4 and miscellaneous other sites 14 cases.

6. Total surgical excision has cured some cases of small skin lesions and of olecranon bursitis.

7. The most common medical treatments have been intravenous amphotericin-B and oral azoles: itraconazole, fluconazole, and ketoconazole. Success rates have been 83% for amphotericin-B, 69% for itraconazole, 64% for fluconazole, and 55% for ketoconazole.

8. We recommend itraconazole or fluconazole for initial treatment of mild cases, and amphotericin-B for initial treatment of severe cases and re-treatment of azole failures.

9. We propose "medical phycology" as a new branch of medical microbiology dealing with algal infections.

PT-06-1

Application of *in situ* hybridization procedure on tissue sections to identification of molds causing invasive fungal infections

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Early, rapid, and accurate diagnosis of invasive fungal infections (IFIs) is essential for appropriate antifungal therapy, whereas the morphological similarities of many molds in tissue make their specific diagnosis difficult. Hence it is required to have a rapid and accurate method of diagnosis of IFIs in surgical pathology specimens. This study was carried out in order to find the usefulness of *in situ* hybridization (ISH) to identify various kinds of molds observed in tissue section and/or cytological specimen from the patients with invasive fungal infections. To establish the precise procedure for ISH in formalin-fixed and paraffin-embedded sections, various methods of pretreatment were tested. An excellent outcome was found in staining intensity and specificity on molds observed in the tissue sections, when specimens were treated with both heat and proteinase K, and were heating solutions were adjusted to higher pH value. In addition, it was examined that intensity and specificity of two each DNA and peptide nucleic acid (PNA) probes, using experimentally infected lung of mice and lung of autopsies with invasive mold infection confirmed by culture. As the result, DNA probes targeting the alkaline proteinase (*ALP*) gene and retrotransposon *Afut*-1 gene of *Aspergillus fumigatus* showed specificity for the *Aspergillus* species and *Aspergillus fumigatus*, respectively. PNA probes for *C. albicans* and *Fusarium* species also showed satisfactory specificity. We wish to emphasize that ISH is significant to be a valuable tool to identify medically important molds on formalin-fixed and paraffin-embedded sections or cytological preparations. The application of PNA probe is especially attractive as a choice for clinical diagnosis due to decreased test turnaround.

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PT-06-2

In situ immunodiagnosis of mycoses

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As a consequence of the difficulties in even suspecting the presence of deep-seated mycoses clinically, many cases are not diagnosed until tissue specimens are examined histologically. However, it must be appreciated that although special histochemical stains for fungi, like e.g. periodic acid-Schiff (PAS) and methenamine-silver techniques (Grocott), are useful for revealing the presence of fungal elements in tissues, they seldom permit the exact fungal genus involved to be identified. Histologically, distinctive morphological details may provide a tentative identification, but the appearance of fungi in sections is affected by steric orientation, age of the fungus, etc. Moreover, the elements of some of the most emerging fungal pathogens, i.e. species of Aspergillus, Fusarium, and Scedosporium cannot be differentiated in tissues due to morphological similarities. Also the presence of sparse and/or atypical fungal elements will hamper a clear-cut diagnosis and may result in confusion of e.g. aspergillosis, fusariosis, and scedosporidiosis with zygomycosis and candidosis, respectively.

As the therapy of deep-seated mycoses is becoming more and more specific and is directed by the fungal genus or even the species involved, there is an increasing demand for specific and reliable in situ diagnoses.

Highly sensitive and specific, indirect immunohistochemical techniques have been developed for the identification of the most prominent causes of mycoses. Moreover, as a range of different forms of fungal elements frequently is disclosed both in isolated lesions and/or in different organs of individuals, dual immunostaining techniques are often mandatory for obtaining a reliable and discriminative diagnosis.

An important limitation of the widespread application of immunohistochemical techniques for the diagnosis of deep-seated mycoses is due to the fact that sensitive and specific reagents are obtained through multiple heterogeneously absorbed polyclonal antibodies, which are not commercially available. However, in recent years more specific monoclonal antibodies have been commercialized though companies offering immunodiagnostic reagents.

PT-06-3

Serodiagnosis of aspergillosis and endemic mycoses

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The Platelia™ *Aspergillus* EIA was approved in the US for testing serum specimens in 2003, and its performance characteristics have been excellent. Positive results between 0.5-0.9 GMI are reproducible in 75% of cases, and those ≥1.0 or higher in 95%; and CVs for the positive and cut off controls are <10%. However, when both serum and BAL are tested from patients with suspected pulmonary aspergillosis, the sensitivity is 2-3 fold higher in BAL than in serum. Precision and reproducibility in BAL are similar to serum. Appropriate use of the test includes twice-weekly monitoring for antigenemia in patients at high risk for aspergillosis, and testing both BAL and serum in suspected pulmonary aspergillosis. The Fungitell™ beta glucan assay was approved in the US in 2004. While results are positive in most patients with aspergillosis, it is not specific, positive also in candidiasis, endemic mycoses, *Pneumocystis jiroveci* pneumonia, and in some patients without fungal infection. Serologic test for antibodies are useful in histoplasmosis and coccidioidomycosis, but maybe negative early, when antigen may be detected. While EIA methods are available for antibody detection, they may not be as accurate as immunodiffusion and complement fixation. The role of serology remains unclear in blastomycosis.

PT-06-4

Serological diagnosis of invasive *Candida* infections

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Although no serological tests for detection of circulating *Candida* antigens or antibodies to *Candida* spp. has achieved the status of an approved diagnostic test for invasive *Candida* infection, research into serodiagnosis has progressed, benefiting from cutting-edge biomedical technology. The problem to be solved for *Candida* infections has always been to find the most appropriate antigen for diagnostic purposes. Approaches such as proteomic analyses of antigen-antibody interactions with material from patients and characterization of molecular interactions between the fungus and its host in vitro and in vivo offer intriguing possibilities for novel biomarkers of clinically significant disease.

PT-07-1

Overview of sequence based identification for fungi

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The correct identification of fungal clinical isolates is an important challenge for clinical laboratories, especially those dealing with immunocompromised patients. Traditionally, phenotypic methods have been used for this purpose. However, these methods alone have proven to be insufficient for resolving all the cases. This is due, in part, to the increasing number of opportunistic fungi being reported, a significant number of them being cryptic species that can only be identified using molecular methods. Hence, more than 70 additional species able to cause human infections will be included in the next edition of the Atlas of Clinical Fungi. The DNA sequence-based methods have been considered the gold standard of molecular methods for fungal identification. Multilocus sequences analyses have proven to be a powerful tool for species delineation and for detecting phylogenetic species within important pathogenic moulds such as *Fusarium, Aspergillus, Mucorales, Pseudallescheria, Acremonium,* black yeasts or *Sporothrix,* among others. The choice of the loci for sequencing is crucial for successful identification of clinical isolates. The ITS region is the most often used marker, but is not very informative in some pathogenic groups. Other loci, mainly protein coding regions, have been used successfully in important pathogenic species. To assist clinical laboratories in molecular identification of fungi, the Clinical and Laboratory Standards Institute and the ISHAM working group have published some guidelines and recommendations. However, in spite of the progress made so far in this field, there are still numerous nonsporulating clinical isolates that show no significant percentage similarity with the sequences deposited in the databases. Further effort is needed in standardizing the molecular methods for fungal identification, determining appropriate breakpoints, and in extending the DNA sequence databases, including more validated clinical isolates.

PT-07-2

Sequence based fungal identification, databases, intra-species variation and molecular cut-off points

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Invasive fungal infections (IFIs) are on the rise due to an ever-increasing number of immunocompromised and otherwise debilitated patients and the emergence of new fungal pathogens. Management of IFIs is problematic since current identification techniques are insufficient, therapies are limited in efficacy and/or safety, and resistant fungi are emerging. Targeted intervention strategies that hinge on accurate and early identification are required to improve patient outcomes. Classical identification (morphology, physiology) is slow and often incorrect. Sequence based identification strategies are the new "gold standard" for species ID. However, there are major problems with sequence based ID: (A) lack of a universally accepted appropriate genetic locus, (B) lack of quality controlled sequence databases, and (C) arbitrary defined cut-off points for species ID. The Internal Transcribed Spacer (ITS) regions of the ribosomal DNA gene cluster together with BLASTn searches in GenBank are now widely used as an alternative to classical identification. Sequenced based ID is currently based on cut-off of 98-99% similarity with the type culture of the species in question. Population based studies showed that the sequences variation in clinical samples is much higher as those type culture dependent cut-off values. To overcome this problem we investigated 480 strains representing 182 human fungal pathogens. Our results demonstrate that fungi have species dependent variable rates of polymorphisms in their ITS1/2 regions. Intra-species variation varied from 0 to 8.35%, with C. parapsilosis showing 0% and C. tropicalis having as much as 8.35%. The recommended cut-off value was redefinition to as low as 92% sequence similarity for the ITS1/2 region depending on the fungal species to identify fungal agents isolated from clinical specimens. A quality controlled ITS database was established and can be access for comparative sequence based fungal ID at: http://www.mycologylab.org/BioloMICSID.aspx.

PT-07-3

The fungal barcoding initiative and sequenced-based identification of medical fungi

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The International barcode of life (iBOL) project aims to generate species-specific signature sequences and develop technologies for rapid and inexpensive species identification and for discovery of potentially new species. Since its inception in 2003, over 500,000 barcode sequences representing about 50,000 species have been curated in the barcode of life database (BOLD). Over 2600 scientists from over 150 organizations in 45 countries are now actively involved in this endeavour. Due to the heterogeneity in the rates of evolution and patterns of genome organization among the major groups of organisms, different genes have been selected as target for barcoding the different groups of organisms. In this presentation, I will briefly introduce the barcode of life project, including the fungal barcoding initiative. I will pay special attention to the current quality of data for human fungal pathogens with regard to the barcode locus, the internal transcribed spacer (ITS) regions. I will close by discussing some of our recent work in using the ITS sequences to study species and genotype distributions of fungal pathogens in human populations.

PT-07-4

Non-culture identification paradigms for diagnosis and epidemiology of nosocomial fungal infections

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Invasive fungal diseases are serious and occasionally life-threatening complications in immunocompromised patients. The recognition of selective susceptibility of certain yeast and mould species to antifungal agents made reliable identification of the causative fungus to species level a useful guide for targeted antifungal therapy, thus having an impact on outcome.

Currently, non-culture identification methods, essentially employing conventional and real-time simplex or high-throughput multiplex PCR, are used for detection and identification of fungal pathogens directly from body fluids and from fresh or paraffin embedded tissue. Further evaluation and standardization of the PCR methods, which are underway, will enhance the value of non-culture molecular approaches for fungal detection and identification in the clinic. Also, amplification and sequencing of target regions within the rDNA gene complex has become a useful detection tool for fungal pathogens in clinical specimens with generally good specificity and sensitivity.

DNA sequencing of single or multiple loci is used to differentiate species of clinical fungi. This approach can assist in the timely recognition of fungal isolates with increased virulence and can give clinically useful epidemiological information on the distribution and frequency of occurrence of virulent and drug-resistant isolates in the nosocomial setting.

Occasionally, the morphological characters of a clinical isolate, for instance *Aspergillus* or *Fusarium*, do not clearly fit within a given species whereas phylogenetic lineages, based on multiple gene genealogies, can provide a more accurate identification scheme. Even worse, the clinical isolate may be a non-sporulating fungus. In such cases PCR-based sequencing of the internal transcribed spacer region has been reported useful for rapid and reliable identification of clinical isolates.

Molecular identification techniques are not always available in each clinical laboratory. Therefore, rapid sequence-based confirmation of a conventionally identified fungal isolate from a normally sterile body site by a specialist laboratory is warranted.

PT-07-5

Specific detection and identification of fungal DNA using quantitative PCR and loop-mediated isothermal amplification; their advantages and limitations

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Based on their high sensitivity, specificity and quantification, real-time PCR (qPCR) is widely used as means of molecular detection and identification for pathogenic fungi. Recently, a novel rapid nucleic acid amplification method, loop-mediated isothermal amplification (LAMP) is developed and applied to pathogenic fungi in clinical specimens. We have developed several qPCR and LAMP assay for diagnosing *Pneumocystis* pneumonia (PCP) and some other mycoses. In our PCP-specific LAMP method, twenty-one of 24 clinical specimens (sputum and bronchoalveolar lavage fluid) from patients with suspected PCP tested positive using the LAMP assay by real-time fluorescence detection. The results of LAMP reaction were also observed by real-time turbidity detection and end-point visual turbidity or fluorescence detection. With real-time fluorescence detection, melting curves of the products were effective to distinguish the specific amplification from non-specific or self-amplification. Visual detection was also possible as a rapid and easy assay with only a heat block and a black light. These new molecular methods are reliable and the results will help clinicians to make correct diagnosis and treatment, however, fundamental process for molecular diagnosis, pre-treatment or DNA purification techniques have not been developed in these twenty years. Perspectives and limitations on molecular diagnosis of fungal infection will be discussed.

EP-01-1

A contemporary overview of emerging and re-emerging fungal pathogens

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Fungi that are ubiquitous in the environment are playing an increasing role in human disease. Patients suffering from cancer, diabetes, HIV/AIDS, amongst other chronic diseases, are at risk. Improved medical therapies or aggressive chemotherapy, steroids, antibiotics and other chronic drug treatments, enhances susceptibility of patients to fungal infections. Fungi including *Cryptococcus* spp., *Fusarium* spp., *Paecilomyces* spp., *Aspergillus* spp., *Pseudallescheria boydii*, *Scedosporium* spp., *Trichosporon beigelii*, *Geotrichum* spp., *Rhodotorula* spp., *Saccharomyces cerevisiae*, *Candida* and *Rhizopus* spp., amongst others, are involved in these opportunistic infections. Black yeast infections due to, eg., *Exophiala dermatitidis*, causing brain abscesses, meningitis or lesions due to dissemination from other organs, often result in a fatal outcome. Dematiaceous fungi, including *Alternaria alternata*, *Curvularia geniculata* and *Cladophialophora bantiana*, are increasingly reported as a cause of paranasal sinusitis. Peritonitis due to *Fusarium oxysporum* and *Paecilomyces variotii*, is often a complication of continuous peritoneal dialysis. *Fusarium* species are also most frequently recovered from keratomycosis, while *Exserohilum*, *Acremonium*, *Curvularia*, *Aspergillus* and *Candida* species have also been described, and are equally difficult to treat. Several underlying diseases may also play a role in the susceptibility and final outcome of keratomycosis. Fungal skin lesions due to trauma, direct inoculation or dissemination from a systemic infection, are being reported. An increased incidence of non-dermatophytic fungi causing nail infections is becoming important. *Candida* species such as *C. tropicalis*, *C. dubliniensis*, *C. krusei*, *C. glabrata*, *C. parapsilosis* amongst others and especially *Cryptococcus* species, have emerged as significant pathogens in the last ten years, raising unique and important issues in pathogenesis and antifungal drug resistance. Fortunately, several new triazole compounds, such as voriconazole and posaconazole, and other new antifungals, seem to have activity in resistant and other infections. Accurate mycological confirmations of fungal infections are therefore necessary, particularly in immunocompromised patients, due to the need for alternative antifungal therapy.

EP-01-2

Eumycetoma in Africa

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Eumycetoma is a chronic devastating infection affecting many patients worldwide. Africa seems to be a continent of the highest prevalence. In Africa, poor young patients are suffering from both actinomycetoma and eumycetoma, resulting in difficult social and economical consequences. Most of patients present late with advance lesions, due to the lack of basic health facilities and specialized clinics with well trained health staff. Differentiation of actinomycetoma and eumycetoma in many centers is still primitive and based on color and morphology of grains, which may sometimes result in inadequate management. Madurella mycetomatis is the most common cause of eumycetoma and responsible of more than 70 % of all causes. Other important causes of eumycetoma in African include: Leptosphaeria senegalensis (Senegal and Mauritania), Neotestudina rosatii (Cameroon, Guinea, Senegal and Somalia) and Pyrenochaeta romeroi (Somalia). Although Pseudallescheria boydii is usually associated with mycetoma cases in temperate countries, this fungus was isolated from the environment and from few documented eumycetoma cases in Sudan (unpublished data). M. mycetomatis eumycetoma is seen more in East Africa, an area from where all type strains were described. Generous support and long-term research collaboration between the Erasmus Medical Center (Rotterdam) and CBS (Utrecht), The Netherlands and the Mycetoma Research Center, University of Khartoum, Sudan resulted in better understanding of the mycology, pathology, immunology and molecular basis of infection due to Madurella mycetomatis. Such model of collaboration is badly needed to help many centers in providing better care for patients. The management of eumycetoma is challenging and response to currently used antifungals is poor. Relatively newer azoles such as voriconazole and posaconazole need to be introduced and tested in the African setting. Eumycetoma in Africa needs more attention from health authorities, more support from pharmaceutical industry and more research collaboration.

EP-01-3

Cryptococcosis in Sub-Saharan Africa

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Cryptococcosis is one of the most important AIDS-related, opportunistic infections, especially in sub-Saharan Africa. As part of a global burden of disease estimation using incidence data from studies published during or after 1996, we have previously shown that sub-Saharan Africa has the highest annual burden estimate (median incidence 3.2%, 720,000 cases, range, 144,000 - 1.3 million), with an estimated 504,000 deaths (range, 100,800 - 907,200), 3 months after diagnosis. In comparison to other diseases excluding HIV, the number of deaths associated with cryptococcosis was higher than that related to tuberculosis (350,000), and approached the number of deaths related to childhood-cluster diseases (530,000), diarrhoeal diseases (708,000), and malaria (1.1M), as estimated by the World Health Organization. In sub-Saharan Africa, expanded and early access to antiretroviral treatment (ART) for HIV-infected persons is a priority to reduce the number of people at risk for cryptococcosis. In South Africa, incidence rates for laboratory-confirmed disease in the HIV-infected population have not yet declined [8]7100,000, [1]17100,000, [1]33100,000 and [1]46100,000 in 2005, 2006, 2007 and 2008 respectively), despite rapid expansion of the government-driven, ART programme, launched in 2004. It is evident that ART programmes will ultimately impact on cryptococcal incidence rates in sub-Saharan Africa, as has been demonstrated in developed countries. However, it is important that public health and research efforts also focus on areas like improved laboratory capacity to diagnose cryptococcal infection, as well as expansion of the treatment armamentarium available to physicians in resource-poor settings (e.g. flucytosine, high-dose fluconazole), to mitigate the current effects of this devastating disease.

EP-01-4

Keratomycosis in Egypt

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One hundred and fifteen patients suffering from corneal ulcers were clinically examined in the Inpatient Clinic of the Ophthalmology Department, Faculty of Medicine, Assiut University, Egypt. Thin specimens from the affected corneas were asceptically taken for microbial analysis. Direct microscopic examination (DME) and culturing of corneal specimens revealed the isolation of bacteria and fungi from 55 and 35 cases respectively. Cases of mycotic keratitis produced 15 fungal species of which *Aspergillus flavus*, *A. terreus* and *A. niger* were relatively more frequent. Other fungal species belonging to *Candida*, *Cladosporium*, *Cochliobolus*, *Fusarium*, *Penicillium*, *Stemphylium* and *Trichoderma* were also identified. The most affected persons were adults of 31-70 years of age (72.2 % of total cases). Males outnumbered females (71.3 % versus 28.7 % of cases). Farmers represented 40 % of total cases. Corneal trauma was the main risk factor of keratitis (63.4 % of total cases). Most cases of trauma were of plant origin (78 % of trauma cases and 49.6% of total cases). To the best of our knowledge, *Fusarium proliferatum, Trichoderma hamatum* and *Stemphylium botryosum* are new etiologic fungal agents of human mycotic keratitis. Twenty five fungal isolates were tested for their capabilities to produce extracellular enzymes (catalase, lipase, protease, phosphatase, urease, and hemolysins). Mycotoxins were detected in 50% of fungal cultures. In vitro sensitivity test showed terbinafine to be the most active antifungal agent followed by ketoconazole, citrimide and amphotericin-B.

EP-02-1

Public health and mycology: The role of epidemiology in helping to combat fungal diseases

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The emergence of new fungal pathogens and the resurgence of mycotic diseases that had previously been uncommon is a serious and growing public health problem. Defining public health's role in mycology is a unique challenge. Fungal Diseases are generally not notifiable to public health; therefore very few hard data are available on incidence and prevalence. Data that exist are fragmentary and there are serious deficiencies in surveillance systems and our ability to detect infections. Given these challenges, it is difficult to understand the true burden of disease which leads to a lack of awareness by the general public.

There many other competing public health priorities to consider and in order to get the resources we need to address fungal diseases; we need to initiate global efforts to estimate the burden of these diseases. We must start by performing appropriate epidemiology and developing and improving surveillance of these infections and then work to provide cost effective intervention strategies.

EP-02-2

Prospective surveillance of invasive aspergillosis in France: 2005-2007

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An active prospective collection of invasive aspergillosis (IA) cases was implemented in 2005 in 12 French academic hospitals from 4 geographical regions, to describe IA epidemiology, diagnostic modalities and therapeutic practices. Each notification was reported by hospital microbiologists through a secured website and missing data were checked at the national reference centre for mycoses and antifungals, Institut Pasteur, Paris. Only proven and probable IA according to 2002 EORTC/MSG criteria were considered. Numbers of patient admissions per hospital were obtained from national health statistics.
From 2005 to 2007, 442 case-patients (male gender 62%, median age 55 yrs) were included, 71 of whom (16%) had proven IA. Overall, the median incidence of IA was 0.223 per 1000 admissions (range 0.095 to 1.078). Among the 442 case-patients, 354 (80%) had hematological malignancies, with acute leukemia and lymphoid malignancies including myeloma representing 60% and 32% of cases, respectively. Cancer and solid organ transplantation patients represented 8 and 9 %, respectively. IA involved the lungs (94%), brain (12%), sinus (10%), or was disseminated (6%). CT scan was performed for 373 (84%) patients and showed major signs in 201 (halo sign in 56; cavitation in 145). Galactomannan serum detection was performed for 433 (98%) patients, and 362 (82%) had samples processed for mycological culture, mainly through BAL. When positive (n=268), culture yielded *A. fumigatus* (85%), *A. flavus* (4.5%), or few other spp. (<3% each).
This network will expand to other regions in the near future, and data will help assessing the impact of new management strategies such as prophylaxis with posaconazole and modification of new diagnostic criteria (de Pauw, Clin Infect Dis, 2008), identifying new populations at risk for IA and assess the potential emergence of recently indentified *Aspergillus* species.

EP-02-3

Epideminology of visceral mycoses in autopsy cases in Japan

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To discern the relationship between the changes of visceral mycoses and recently advanced diagnosis or treatment techniques, the data on visceral mycoses in cases with leukemia and myelodysplastic syndrome (MDS) reported in the "Annual of the Pathological Autopsy Cases in Japan" published in 1990 to 2006 by the Japanese Society of Pathology were analyzed epidemiologically. The frequency rate of visceral mycoses among the annual total number of pathological autopsy cases were 4.45% (1990), 4.28% (1998) and 4.6% (2006), and the frequency rate of visceral mycoses in the cases with leukemia and MDS were 27.94% (1990), 22.26% (1998) and 20.99% (2006).

The predominant causative agents were *Candida* and *Aspergillus*, followed in order by *zygomycetes* and *Cryptococcus*. Although the rate of candidosis decreased gradually, the rate of aspergillosis increased and then surpassed that of candidosis in 1994. The annual frequency of visceral mycoses including complicated infections showed little change, but severe mycotic infection clearly showed a tendency of conspicuous increase from 58.85% in 1990 to 75.61% in 1998.
Among a total of 1,000 cases with mycotic infections, acute lymphatic leukemia (ALL) and acute myeloide leukemia (AML) were the major diseases by histological type (40.55% and 34.81%, respectively) followed by MDS (26.1%).

The reasons for decrease of candidosis combined with an increase of aspergillosis or of severe mycotic infection might be that 1) candidosis had become controllable by prophylaxis and/or empiric therapy with antifungal drugs such as Fluconazole, 2) the launched antifungal drugs were not efficacious against severe infections by *Aspergillus*, or 3) the number of patients living longer in an immunocompromised state had increased because of developments in chemotherapy and progress in medical care. From this, we emphasize that a greater interest in mycoses should be taken by clinicians and that hospitalized patients should be protected from opportunistic invasive fungal infections.

EP-02-4

Epidemiology of candidemia in Latin America

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Candida spp, have been recognized worldwide as common agents of invasive fungal infections in immunosuppressed patients. Nosocomial bloodstream infections due to *Candida* spp. have been progressively documented among critical patients admitted in tertiary care medical centers. The epidemiology of candidemia has been extensively studied in the USA and Europe, but it is still not largely investigated in Latin America. Fungal burden in Latin America seem to be higher than in North Hemisphere countries, where different authors have documented incidence rates of candidemia varying from 2 to 5 per 1000 admissions, contradictory to northern hemisphere studies, where incidence rates range from 0.5 to 1 per 1000 admissions. Prevalence of pediatric patients appears to be higher in Latin American casuistics of candidemia. Mortality rates are also high, especially considering the high percentage of children and *C. parapsilosis* in our community. The frequency of isolation of specific non-*Candida albicans Candida* species may vary by region. *C. parapsilosis* and *C. tropicalis* remain the two most common aetiological agents of candidemia in Latin America, whereas *C. glabrata* starts to emerge in particular medical centers where fluconazole is highly used. In our series of 166 bloodstream isolates belonging to the *C. parapsilosis* complex, *C. orthopsilosis* and *C. metapsilosis* accounted for 8% and 3%, respectively. *C. rugosa* frequency of isolation has also been increasing. ARTEMIS DISK Antifungal Surveillance Program database documented an increase in the frequency of isolation from 0.03% to 0.4% of this species during a period of 6.5 years of study. Regarding to antifungal susceptibility, resistance remains uncommonly found. In conclusion, given the high prevalence of candidemia in our region, clinicians are required to be aware of susceptible populations and clinical manifestations of this mycosis in order to provide early diagnosis and consistent approach to treatment.

EP-02-5

Trends in antifungal drug susceptibility of *Cryptococcus* species in South Africa, 2002-2008

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Cryptococcosis, most commonly caused by the fungal pathogen *Cryptococcus neoformans*, is a common, laboratory-confirmed, AIDS-defining, opportunistic infection in South Africa. Fluconazole, which has been widely available at no cost through a philanthropic programme since 2000, is frequently used for treatment of HIV-infected patients with newly-diagnosed cryptococcal disease, as well as severe or refractory candidiasis. In order to monitor antifungal drug susceptibility trends, testing was performed on cryptococcal isolates obtained from patients through ongoing, active, population-based, laboratory-based surveillance. Susceptibility to fluconazole for randomly-selected, incident-episode isolates from patients at four Gauteng Province hospitals was compared for two periods (2002-2003 and 2007-2008) with no change, demonstrated to date, in fluconazole minimum inhibitory concentration (MIC) range, MIC_{50} and MIC_{90}, as determined by the Etest® (AB bioMérieux, Solna, Sweden). These Etest® MIC results will be confirmed by a reference broth microdilution method. In addition, susceptibility testing will be expanded to include a wider panel of antifungal drugs (amphotericin B, flucytosine, voriconazole and posaconazole). Analysis of fluconazole MICs determined by reference methodology, from isolates obtained serially from 92 patients with consecutive cryptococcal episodes, showed that a fourfold-increase in MIC occurred between the incident-episode and recurrent-episode isolate in a very small proportion of cases ($^{5}/_{92}$, 5%). In the absence of interpretive breakpoints, a four-fold increase in fluconazole MIC may indicate in-vitro resistance. Despite reports to the contrary, preliminary data suggest that in-vitro fluconazole ″resistance″ amongst South African, clinical isolates has not changed substantially over 6 years, and remains relatively uncommon even amongst isolates from patients with recurrent disease.

EP-03-1

Malassezia in Asia

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Malassezia yeasts are part of the normal microflora recovered in 75-80% of healthy adults. Previously, it was classified into seven species - *Malassezia*(M.) furfur, M. pachydermatis, M. sympodialis, M. globosa, M. obtusa, M. restricta and M. slooffiae - in 1996 by Guého et al. Additional four new species had been found through molecular biology: M. dermatis, M. japonica, M. yamatoensis (Sugita et al. 2002, 2003, 2004) and M. nana (Hirai et al. 2004).
Although it is associated with number of diseases affecting the human skin, the link between specific species and dermatologic disorder is yet unclear. Recently a large scale study was conducted in Korea on the distribution of the *Malassezia* flora in body sites and diseases. In this presentation, based on these findings, I will consider the relationship between specific *Malassezia* species and various dermatologic disorders.

EP-03-2

Skin and mycoses in Indonesia

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Fungal skin infection continues to be an important public health problem in Indonesia and ranks as one of the top infectious skin diseases. Humid and warm climate contributes to the high incidence and high recurrence of superficial mycoses.

Data from various hospitals in big cities across Indonesia showed that the most frequent cases were dermatophytoses, followed by pytiriasis versicolor. However, field surveys in rural area indicated that pytiriasis versicolor was the highest. A different pattern of superficial mycoses was found in immunocompromised cases, where candidosis and *Malassezia* folliculitis were more common. Among the various types of dermatophytoses, tinea curis was the most prevalent. However, in special population group, such as among military personnel, tinea pedis was more frequently found. The most frequent isolated agents in tinea cruris and corporis were *Trichophyton rubrum*, followed by *Trichophyton mentagrophytes* and *Epidermophyton floccosum*. *Trichophyton concentricum* infection was endemic in certain remote areas. In tinea capitis, the main causative agent was *Microsporum canis*.

Subcutaneous mycoses was rarely reported and consisted of mycetoma (eumycetoma and actinomycetoma), chromoblastomycoses, subcutaneous zygomycoses and sporotrichosis, with different pattern of prevalence in different areas.

In recent years, due to the increase in HIV/AIDS cases, systemic mycoses with skin manifestations were more frequently found. Consecutively, from the more frequent causes were histoplasmosis, criptococcosis and candidosis. Eventhough bamboo rats which were considered to be the carrier of *Penicillium marneffei* were found in Indonesia, up until now only one AIDS case had been diagnosed with Penicilliosis.

EP-03-3

Dematiaceous fungus infections in East Asia - molecular biological aspects -

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The most frequently isolated dematiaceous fungus from cutaneous mycoses in Japan is *Fonsecaea pedrosoi*, which is morphologically well-defined and proven based on typing results using mitochondrial DNA (mtDNA) and ribosomal RNA (rRNA) gene. Strains from several collections worldwide were investigated for phylogeny and epidemiology. *F. pedrosoi* isolates were divided into 6 rRNA gene types of which types 1 ~ 4 were phylogenetically closely related while types 5 and 6 were distantly related. In East Asia and Australia, strains of type 2 occurred exclusively and in Madagascar type 3 occurred exclusively whereas in South America all types were prevalent except type 3. Recently type 2 isolates have been newly described as *F. monophora* species. Phylogenetic relations between *F. pedrosoi* and *F. monophora* will be discussed.

Exophiala jeanselmei, incidence of which has been recently increasing in Japan as the causative black fungi of cutaneous mycoses, was revealed to be a highly complex species in contrast with *F. pedrosoi*. Morphologically identified *E. jeanselmei* isolates showed big variation in ITS sequences, many of which could not be identified by BLAST search. Among 27 isolates morphologically identified in our laboratory as *E. jeanselmei* over twenty years (1989 to 2008), 15 were molecularly identified as *E. jeanselmei* var. *jeanselmei*, 1 was *E. jeanselmei* var. *lecanii-corni*, 6 were of an unknown species genetically distant from other *Exophiala* species, 3 were also of another unknown species and 2 isolates were each of additional unknown species.

EP-03-4

Recent developments in epidemiology of histoplasmosis in humans and animals in Asia

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Histoplasmosis, a globally distributed systemic mycosis of man and animals, comprises three distinctive entities: (a) histoplasmosis capsulati caused by *Histoplasma capsulatum* var *capsulatum*. (b) histoplasmosis duboisii caused by *H. capsulatum* var *duboisii* and hitoplasmosis farciminosi caused by *H. capsulatum* var *farciminosum*. Histoplasmosis capsulati is primarily a respiratory disease acquired by inhalation of air-borne micro-conidia of the capsulatum variety originating from soils, especially around bat or avian habitats where the pathogen grows saprobically. In Asia, authentic human cases of histoplasmosis capsulati occur sporadically in many countries, and their number has increased notably in recent decades. The disease is unknown in animals except Japan where canine cases occur, and Israel where a naturally infected bat, *Myotis myotis*, was reported. The etiologic agent has to-date been isolated from soil or bat guano only in India, Israel and Malaysia.

Histoplasmosis duboisii has a marked tropism for bones and skin but pulmonary lesions also occur. Authentic cases of human and animal histoplasmosis duboisii are largely restricted to Africa. The only Asian autochthonous human case has been reported from Japan. The natural habitat of *H. capsulatum* var *duboisii* was unknown until its isolation in 1994 from soil in a bat cave in Nigeria. Histoplasmosis farciminosi is restricted to equines, occurring endemically in many Asian countries including India and Japan. It involves subcutaneous lymphatics, especially of limbs and neck, developing into discharging abscesses, leading to conjunctivitis or pneumonia. Interestingly, *H. capsulatum* var *farciminosum*, identified by gene sequencing, has been implicated as etiologic agent in a Japanese autochthonous case of human histoplasmosis by Sano and Miyaji (2003). Unlike its sibling clinical entities, histoplasmosis farciminosi is contagious. In short, the epidemiology of various forms of histoplasmosis in Asia remains virtually unexplored which underlines an urgent need for comprehensive interdisciplinary studies to delineate the endemic areas and devise control measures.

EP-03-5

Pathogenicity and epidemiology of *Penicillium marneffei* infection in Southeast Asia

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Penicillium marneffei infection is an endemic mycosis, especially among AIDS patients in Southeast Asia. The natural reservoirs and the molecular epidemiology of *Penicillium marneffei* have been studied. Using a highly discriminatory molecular technique, MLMT, isolates from bamboo rats and humans were shown to share identical multilocus genotypes, showing that bamboo rats are a possible zoonotic source. By quantitative PCR and nucleotide sequencing, it has been shown that this fungus is present in soil, and is able to grow in soil under certain conditions. Further study is required to ascertain the specific conditions that regulate their growth in natural environments.

After *Penicillium marneffei* conidia are inhaled, they undergo transformation into yeast within the infected tissues. The factors that affect the pathogenicity of this fungus remain unclear. A number of *Penicillium marneffei* putative virulence genes have been isolated. The characterized genes include those involved in stress response, such as Cu, Zn superoxide dismutase (*sodA*), catalase-peroxidase (*cpeA*), and heat shock protein 70 (*hsp70*). Another group of genes consists of those responsible for cell adhesion and cell adaptation, such as glutaraldehyde-3-phosphate dehydrogenase (*gapdh*), and isocitrate lyase (*acuD*). We investigated the expression of these genes in different fungal forms and during macrophage infection. The results revealed that *sodA* and *cpeA* expressions were upregulated in the yeast phase and during macrophage infection. The expression of *hsp70* and *acuD* were induced in conidia after prolonged co-incubation with macrophages. In contrast, the expression of *gapdh* was repressed during macrophage infection, presumbably due to nutritional deprivation and the glucose-poor intracellular environment. In addition, the following genes possibly associated with pigmentation and morphology of *Penicillium marneffei* have been isolated by using an *Agrobacterium*-mediated transformation strategy: *stuA*, S-adenosylmethionine decarboxylase, and U1 snRNP encoding genes. Further characterization of these genes will provide the key knowledge to understanding the pathogenicity of *Penicillium marneffei*.

EP-03-6

Invasive fungal infections: Diagnosis and treatment in China

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The clinician always wants to know what the fungus is and how to treat the mycosis. Fungal pathogens are often stealthy and difficult to detect in infected patients during the early stages of the diseases, and this is when therapies would be the most effective. Routine techniques commonly employed in the detection of fungal diseases including microscopic examination, culturing and serology are seriously hampered by lengthy wait times for results and low accuracy. The clinician may want to take prophylaxis or to use empirical antifungal treatment to see if they are effective or not. The problem is that some of the patients do not respond to the antifungal treatment, because the doctor lacked sufficient evidence of fungal infection to give the doctor confidence to continue treatment.

Accurate and early diagnosis of fungal diseases is critical for managing mycotic diseases. This is usually done by direct microscopic examination of KOH preparations. Good specimens are the key point that directly affects the quality of microscopic evidence and culture. It is of utmost importance to culture samples on different media with or without chloramphenicol and cycloheximide and incubate them at both room temperature and 37°C. Early treatment could save a patient's life. We start treatment when we have the proof of fungal infection, i.e., KOH positive. Itraconazole, fluconazole, terbinafine, amphotericin B or its liposome form, can be used alone or in combination based on the fungal species involved and the site of infection.

Based on our experiences in west China, we have encountered the following pathogenic or opportunistic fungal species: *Fusarium spp., Rhizomucor variabilis, Malassezia spp., Sporothrix schenckii, Aspergillus fumigatus, Trichophyton mentagrophytes, Cryptococcus neoformans, Candida spp., Penicillium marneffei* and mixed infections. These involved eye, nose, middle ear, mouth, vocal cord, face, scalp, subcutaneous tissue, bone, lymph nodes, or, disseminated to numerous internal organs.

EP-04-1

Genetic variability among animal and human strains of *Microsporum canis* using microsatellite markers

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The zoophilic dermatophyte species *Microsporum canis* is the causative agent of Tinea capitis et corporis in prepubescent children. *M. canis* is distributed worldwide and transmitted by contact with a range of mammals, e.g. cats, dogs and horses where it occurs asymptomatically or causes lesions on the furred skin. Phylogenetically closely related species are the anthropophilic dermatophytes M. audouinii and M. ferrugineum.

We have developed 8 microsatellite markers to analyse the epidemiology and population structure of *M. canis*. A collection of about 120 isolates from human and animal origin have been analysed which were from geographically distant locations (Austria, Germany, Mexico, Egypt, Italy, Turkey, Korea, Netherlands, Dominican Republic and USA). The analysis of the multilocus genotypes of the markers containing dinucleotide-repeats detected 50 alleles (5-18 per locus) among the *M. canis* strains and a total of three among six M. audouinii and M. ferrugineum which were used as outgroup. Mean repeat lengths of the microsatellite markers were between 11 and 34.

Using the software STRUCTURE (V2.2) genetic distances were calculated based on allele frequencies under the no-admixture model revealing seven major populations of which six were recombining. Population separation was due to allopatric as well as sympatric (horses) speciation.

EP-04-2

Out-of-Africa origin of *Cryptococcus neoformans* var. *grubii* (serotype A)

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Cryptococcus neoformans var. *grubii* (serotype A) is the most common cause of fungal meningoencephalitis, one of the most prevalent and deadliest fungal infections in humans, which has a particularly devastating effect on AIDS patients in sub-Saharan Africa. Although this pathogen is ubiquitous around the world, yeast population in southern sub-Saharan Africa is genetically different from the global population. Here we present evidence that African population of the pathogen has a unique ecological niche in endemic African trees. We demonstrated that this niche harbors the ancestral yeast population, which represents the evolutionary hotbed and center of speciation of serotype A. We also demonstrate that global population of this fungus originated from a single expansion of two strains from the ancestral population in Africa, which became associated with the pigeon guano and were spread around the world by migration of humans and pigeons.

EP-04-3

Multi-locus sequence typing (MLST) and antifungal susceptibility analysis of *Candida glabrata*: Results from previous and current population-based surveillance studies

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As part of an ongoing population-based surveillance study, we have collected *Candida glabrata* incident isolates from two metropolitan areas (Atlanta and Baltimore) and have concurrently analyzed, by MIC testing and MLST typing, strains from two previous surveillance studies conducted in 1992-1993 and 1998-2000. For all strains collectively, we observed a total of 8 new Sequence Types (STs), of which 6 were new alleles and 3 were new mutations. For each population, defined as separate metropolitan areas for each surveillance study, we observed that ~85% of strains were of four to five sequence types (termed major STs), with the remainder consisting of a variety of minor STs with a frequency of one or two each. With the exception of one major ST (ST-16), the frequency of all other major STs were similar between populations. The frequency of ST-16 for both Atlanta and Baltimore was significantly higher in 2008 vs. 1992-1993 and 1998-2000, where it was observed as a minor ST (Fishers exact test P <.01). ST-16 has been previously shown to be exclusively North American and does not fit into any of the previously defined five major clades. Isolates which are non-susceptible to fluconazole or resistant to itraconazole have increased in frequency between the earlier surveys and the present survey. We observed that this was not solely attributable to an increase in ST-16; resistance was found in all major STs as well as some minor STs. We are currently conducting a multivariate analysis of antifungal resistance and the specific alleles that are components of a given ST.

EP-04-4

Genotyping study of *Trichophyton schoenleinii* and *Microsporum canis* isolated from tinea capitis in Xinjiang province, west China

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Xinjiang is the largest and most westerly province in China. It is reported that tinea capitis has been epidemic in Uyghur (a minority of Chinese living in Xinjiang) school children in this province for recent 40 years, and caused by *Trichophyton violaceum*, *Microsporum ferrugineum*, *T. schoenleinii* and *T. verrucosum*, but rarely by *T. tonsurans* and *M. canis*. This time, we surveyed the tinea capitis of Uyghur school children in Hotan area (located at the extreme south west corner of the Taklamakan Desert). Their causative agents were *M. ferrugineum*, *T. violaceum*, *T. schoenleinii*, *T. verrucosum*, *M. canis*, and *T. tonsurans*. *M. canis* is one of common cause of tinea capitis in Central and Eastern China, but not previously reported from the Xinjiang. But recent two years cases of tinea capitis caused by *M. canis* are increasing. *M. canis* was isolated from 11 cases of tinea capitis in this survey. Genotyping study was performed for 26 strains of *T. schoenleinii*, and *M. canis* (including 5 Japanese strains used for reference) respectively by the inter-single-sequence-repeat (ISSR) PCR method reported by Jose Cno et al. (2005). We decided genotypes according to the electrophoresis by normal agarose, not by GeneScan™ polymer. Twenty-six *T. schoenleinii* strains showed 15 genotypes with primer ACA and 11 types with the primer CCA. Meanwhile, our previously study for genotyping of *T. schoenleinii* from Xinjiang showed this species had high genetic homogeneity. Twenty-six *M. canis* strains were divided into 17 genotypes with primer CCA and 5 types with ACA. Our results suggest that ISSR-PCR method have high reliability and reproducibility and it is available method for molecular epidemiological study of *T. schoenleini* and *M. canis*, to be able to determine the detail genotypes.

VM-01-1

Phenotypic and genotypic comparison of an equine and four human clinical isolates of *Madurella mycetomatis*

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Madurella mycetomatis is one of the most prevalent etiological agents of human black grain eumycetoma. Among the few cases of the fungus' involvement in animal eumycetomas, was that of a horse. The isolate was, however, not described (Van Amstel SR. et al., 1984. J. South. Afr. Vet. Assoc. 55:81-3).

Recently, the fungus was isolated from a case of equine eumycetoma in Israel. The lesion was above the hoof of the left hindleg, showing the typical characteristics of this mycosis: chronic tumefaction, draining sinuses and black grains. The phenotypic and genotypic of the isolate were compared to those of 4 human isolates acquired from the CBS collection in the Netherlands.

The strains were cultured on a variety of media, including Sabouraud's Dextrose Agar, Trypticase Soy Agar (TSA) with horse or human serum, Potato Dextrose Agar, Potato Carrot Agar, Corn Meal Agar with and without Tween 80, Water Agar and Hay Agar and compared the results Optimal growth temperatures (37°C or 30°C) were assessed. In addition, a genetic comparison (Desnos-Ollivier M. et al., 2006. J. Clin. Microbiol. 44:3517-23) between the strains was performed.

The culture media significant influenced the morphology of the *M. mycetomatis* strains. Moreover, such differences were observed between the strains when grown on the same medium, as well. Two of 5 strains examined were morphologically similar: the equine and a human strain including soluble pigment and sclerotia production. Except for one human strain, the isolates grew best a 37°C.The fastest growth rate was observed on TSA, regardless of type of serum added to this medium (human or equine). Genetically, 4 strains were identical and one human strain differed from the others in 2 bases.

VM-01-2

Aspergillosis in breeding turkeys: From experimental infections to field investigations

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Aspergillosis has been described worldwide in a very large number of avian species. Turkey poults in large confinement houses, marine birds that are brought into rehabilitation, captive raptors or penguins commonly die from aspergillosis. In turkey poults, aspergillosis is responsible for consequential economic losses related to mortality and carcass condemnations at slaughter inspection. Acute aspergillosis leads to severe outbreaks in very young birds whereas the chronic form of aspergillosis most commonly occurs later in the growing cycle. The development of specific avian models is critical to our current understanding of pathogenesis of avian aspergillosis and to the advancement of prevention and therapy. Models have been described in chickens, turkeys, quails, starlings, pigeons and ostriches, with ages ranging from hatchlings to adult birds. We recently had the opportunity to test different routes of inoculation and challenge dosages. Initially we inoculated birds by transcutaneous injection of A. fumigatus conidia within one of the thoracic air sacs. Experimentally-infected animals were sacrificed from day 1 to day 7 post-inoculation and a subsequent histological analysis was performed in order to describe the first steps of Aspergillus development and concomitant immune response in tissues. We currently use an inhalational chamber to reproduce chronic aspergillosis and sequential infection with mixture of isolates. Field investigations include the detection and characterization of Aspergillus isolates in hatchling or breeding facilities. To improve our knowledge of the genetic and epidemiological relationships between environmental and clinical isolates, we developped a new method to type Aspergillus isolates. The use of this discriminant MLVA method can also give a deeper understanding of the colonization pattern of birds.

VM-01-3

Aspergillosis in wild and captive birds in Japan

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As in the US and other countries, a wide variety of both wild and captive birds in Japan have died of aspergillosis; however, more frequent cases were found among captive birds and those in zoos and aquariums. The most frequent aspergillosis cases were seen in flocks of various sub-species of penguins maintained in zoos and aquariums. The incidence rate varied from 5% to 28% in our laboratory. The prevalence rate appears to have decreased recently because of application of a barrier system with air filtration and maintenance of a cold room environment. There were also occasional cases of aspergillosis in raptors kept for falconry. Additionally, sporadic cases of aspergillosis have been found in cormorants captured for cormorant fishing in Gifu. In companion birds, there have been sporadic cases of aspergillosis in close association with other infectious agents such as *mycobacterium genavence*, which has been spreading in companion birds. The red-billed Toucan (*Ramphastos tucanus*) seems more sensitive to these infections. In wild birds, aspergillosis has sometimes been seen in waterfowl such as swans which migrated from Siberia to the north of Japan. A Whooper swan captured and rehabilitated in the far south of Japan had severe aspergillosis. Clinically, most affected birds showed no apparent signs or external abnormalities except for frequent emaciation. Grossly, the most affected birds had varying degrees of multi-focal and coalescent caseated nodules, whitish green in color, on the thoracic and abdominal air sacs and the lungs, and occasional serosal nodules in the spleen or liver. Usually, the inner side of the air sacs contained hyphal growths, dark greenish in color. Histopathologically, thickened air sacs and pulmonary nodules showed granulomatous lesions with central necrotic areas consisting of necrotic cell debris and hyphae of *Aspergillus (A). A. fumigatus* was identified in all cases examined PCR.

VM-01-4

Aspergillosis of the dog and cat

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Aspergillus ordinally causes either nasal or pulmonary infection and sometimes disseminated infection in dogs and cats.

In this session, we introduce two animal cases of aspergillosis, describing molecular identification of *Aspergillus* species and susceptibility testing to antifungal drugs for the appropriate treatment of animal aspergillosis.

Case 1

We reported the first case of aspergillosis due to *A. udagawae* in a cat. Identification of this isolate from orbital lesion was secured by comparative sequence based analyses of the ITS and the β*tubulin* regions. Antifungal susceptibility testing on the isolate revealed that its *in vitro* MIC (minimum inhibitory concentration) to amphotericin B (AMB) was high, relating to clinical failure of therapy with AMB. Low susceptibility of *A. udagawae* to AMB *in vitro* should be consider on therapeutic decision making, though AMB is currently the antifungal drug of choice for canine and feline aspergillosis.

A. udagawae is quite similar to *A. fumigatus* which is frequently isolated from animalsand is implicated as a causative agent of invasive aspergillosis in humans and they are required clearly to discriminate from *A. fumigatus* by molecular methods.

Case 2

A. terreus has been reported as an etiological argent of disseminated aspergillosis in dogs and cats. Human and animal aspergillosis due to *A. terreus* is poor to response against AMB therapy. We isolated and identified *A. terreus* by the molecular analysis from a canine case of renal aspergillosis. The case had been treated with itraconazole, however, *A. terreus* was still isolated from urine sample. Since antifungal susceptibility testing revealed that this isolate had higher *in vitro* MIC to itraconazole rather than voriconazole, voriconazole was administered to the case.

In conclusion, *Aspergillus* isolates should be identified molecularly and their susceptibility to antifungal drugs should be determined by MIC testing for appropriate therapy on animal aspergillosis.

CL-01-1

Do fungi cause asthma?

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The evidence on the associations between home dampness, indoor mould and respiratory symptoms such as wheezing, coughing and asthma, both in children and adults is remarkably consistent. However, methodological weaknesses in most of these studies do not allow firm conclusions on whether fungi cause asthma or contribute to persistence of asthma. Lack of objective assessment of mould exposure in some studies has raised the question whether moulds are merely markers of dampness or are causally related to the symptoms associated with dampness. Even with objective markers of mould exposure, cross sectional nature of some studies has begged the question on the temporality of the association. However, some longitudinal studies with objective markers of mould exposure have shown indoor mould to be associated with development and persistence of asthma in children but the controversy around the ability to detect asthma in early childhood precludes drawing firm conclusions out of these studies. On the other hand, the evidence arising from longitudinal studies in adults on the association between mould exposure and asthma persistence suggest that mould exposure is related to persistence of adult asthma. If mould exposure is a risk factor for asthma, what are the likely mechanisms? Several possible mechanisms have been suggested for the observed associations between home dampness or indoor mould and asthma including both IgE mediated and non IgE mediated mechanisms as well as unknown immunological responses.

This presentation will review the current evidence on the association between fungi and asthma including the work conducted by the presenting author and will provide recommendations on how to move forward with research in this area.

CL-01-2

T cell response to *Candida albicans* acid protease is associated with the isolated late asthmatic response

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To delineate the mechanisms of nonatopic asthma, peripheral blood mononuclear cells (PBMC) obtained from atopic asthmatics, nonatopic asthmatics, and healthy controls were incubated with various allergen molecules. IL-2, IL-4, IL-5, IL-13, and IFN-gamma productions were measured by specific ELISAs. T cell proliferation was assessed by ^{3}H-thymidine uptake. IgE and IgG antibody were assayed by RAST and ELISA, respectively. Intradermal and bronchial inhalation challenges of the antigens were performed according to the standard procedures. Histamine releasing tests (HRT) were performed using peripheral blood leucocytes. Proliferative response to crude *Candida albicans* (CA) extract was not statistically different among the three groups, indicating a common sensitization against CA antigen. Significant amount of lL-5 was produced by PBMC obtained from several nonatopic asthmatics upon incubation with crude CA extract and a purified antigen, secretory aspartic proteinase 2 (SAP2). IL-5 production was undetectable for the PBMC obtained from healthy control subjects in response to SAP2. Upon intradermal and bronchial challenge of SAP2, late but not immediate skin and bronchial responses were observed for the IL-5-producing asthmatics, respectively. Neither IAR nor LAR was detectable for the IL-5-nonproducing asthmatics, indicating the specificity of the responses. LAR was not induced for the IL-5-nonproducing, IL-13-producing asthmatics. IgE-dependent mechanism was ruled out by negative RAST, HRT, or immediate skin reaction. Anti-SAP2 IgG antibody (precipitin) was not detectable in the serum of either the asthmatics or the control subjects. Nonatopic asthma may be caused by an IgE-independent, T cell-dependent immune-recognition, and *in vitro* cytokine synthesis become a reliable diagnostic test for "T cell allergens".

CL-01-3

Are fungi responsible for chronic sinusitis?

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Although bacteria have long been implicated as the cause of most forms of chronic rhinosinusitis (CRS), fungi are considered in few specific forms of the disease. However the interest in this subject has intensified since 1999, when it was claimed that fungus might be an important cause of most cases of CRS. With improved techniques fungi were detected or isolated from nearly 100% patients with CRS, though fungi could be isolated from similar proportion of healthy hosts. Still it is believed that the CRS is due to an enhanced immune response to common airborne fungi, particularly Alternaria, which attracts eosinophil to the sinus. The major basic protein released from eosinophil cause damage to the nasal epithelium. This hypothesis remains controversial, and there are counter-claims of multifactorial nature of CRS, which may include fungus. Many believe that CRS is Th2 like inflammatory disease. Once initiated by allergens (may be derived from bacteria or fungi), cellular differentiation pathways may lead to the development of antigen-independent permanent phase. The case of causative agent can be proved only with definitive evidence that T-cells are actively responding to the derived antigen in the sinus. In a distinct group of CRS (allergic fungal rhinosinusitis), it was claimed that IgE-mediated systemic fungal allergy drive the pathologic process. Proteomic blood testing further substantiated this discrete subtype. Another interesting claim of microorganisms living as biofilms in the sinuses leading to CRS pathology is intriguing. Further claim of aberrant innate immune response to fungi or surfactant protein D in development of CRS is not clear. In the treatment aspect, the results of the use of amphotericin B in the form of topical application or nasal spray or nasal lavage, and antifungal immunotherapy are conflicting. Therefore more questions than answers exist concerning the cause of CRS and the role of fungi.

CL-01-4

What is the role of antifungals in allergic fungal disease

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Background: Millions of patients with asthma and allergic fungal sinusitis are sensitized to fungi. Exacerbations of asthma are precipitated by fungal exposure, and asthma severity is also linked to fungal sensitization. Does antifungal therapy help these patients?

Published data and clinical experience: There is clear evidence from uncontrolled observations and 2 RCTs that itraconazole is helpful for patients with ABPA. About 60% of patients respond. Some data suggest better response rates at higher itraconazole concentrations. Adverse events rates are moderately high. Relapse is common after discontinuation. Two studies have examined antifungal therapy for severe asthma; on using fluconazole for *Trichophyton*-sensitized patient, the other for patients sensitized to at least 1 of several fungi (SAFS). Both studies showed marked benefit. With relapse to baseline in the second study, 4 months after discontinuation. In contrast to these positive results, 5 of 6 RCTs for chronic rhinosinusitis were negative rhinitis symptoms improved in the SAFS study with itraconazole, and 12 of 32 (37.5%) refractory patients with allergic fungal rhinosinusitis responded to oral itraconazole over at least 3 months. In my experience, patients who fail or develop toxicity to itraconazole, may respond to voriconazole or posaconazole, although data are few. Drug interactions between itraconazole and inhaled steroids are problematic in ~50% patients, increasing steroid exposure.

Conclusions: All patients with ABPA and SAFS should be offered oral antifungal therapy. Itraconazole has many drug interactions. Optimisation of exposure with blood level monitoring is preferable. At least 3 months of therapy are required to evaluate response. It is possible itraconazole will be helpful for some patients with chronic fugal rhinosinusitis, but this has yet to be demonstrated in an RCT. Relapse on discontinuation of therapy is usual, but not universal. Azole resistance could supervene, reducing the utility of oral therapy in the future.

CL-02-1

Invasive aspergillosis in the intensive care unit

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The incidence of invasive aspergillosis (IA) in the intensive care unit (ICU) varies and is for various reasons difficult to determine. Autopsy studies however have revealed the emergence of *Aspergillus* species as major pathogens in ICU also in patients without haematological diseases. New risk groups for IA are increasingly recognized, especially patients with chronic obstructive pulmonary disease and patients with cirrhosis. To diagnose IA in the critically ill patient is even more challenging than in patients with a haematological malignancy. Underlying lung pathologies interfere with the detection of signs of IA by high resolution CT scans. Direct examination of (respiratory tract) samples may provide the clinician valuable information in a rapid way but has a limited sensitivity. Also the diagnostic value of culture of respiratory tract specimens for the diagnosis of IA is limited due to its low sensitivity (± 50%) and the fact that the positive predictive value of culture depends on the underlying disease or condition of the patient. Because of the limitations of conventional diagnostic tools research is directed to the identification of non-invasive markers that allow a timely diagnosis of IA. The sensitivity of serum galactomannan testing in non-neutropenic patients is rather low (,50%) but recent data indicate that sensitivity can be improved significantly by performing the test on bronchoalveolar lavage fluid. Unfortunately many ICU patients do receive β-lactam antibiotics which may cause false positive test results. Few data are available on the value of β-D-glucan testing in ICU, but specificity of this test seems to be the difficult point. Standardized PCR tests for IA are becoming commercially available but many questions are still unsolved among them the optimal sample type and extraction protocol. Further studies are needed to answer the question if improved diagnosis of IA in ICU patients can improve the poor outcome.

CL-02-2

Chronic pulmonary aspergillosis

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Aspergillus species develop wide range of invasive, chronic (and saprophytic), and allergic forms of pulmonary disease. Chronic pulmonary aspergillosis (CPA) is characterized as slowly progressive inflammatory pulmonary syndrome due to *Aspergillus* spp., however, the apparent world-wide consensus of this form of disease has not been established. Though several names of this chronic form of diseases; semi invasive aspergillosis, chronic necrotizing pulmonary aspergillosis (CNPA), simple or complex aspergilloma, chronic cavitary and fibrosing pulmonary aspergillosis have been proposed, the clear distinct entities do not exist for this syndrome and these forms usually overlapped. The common characters of these forms of disease are consisted of (1) an underlying pulmonary disorders (eg, tuberculosis sequelae, bronchiectasis, COPD, cystic lesions and pulmonary fibrosis), (2) the status of low grade immunosupression (eg, low-dose steroid administration, diabetes, collagen diseases, renal disorders or alcohol) which is related to the reduced host immunity (not non-neutropenic), and (3) not highly severe findings of angioinvasion in histopathology. Establishing a definite diagnosis of CPA requires a combination of clinical characteristic features above, serological, mycological and radiological features. However, other bacterial ornamentation in the pre-existing lesions of the lungs, low reliability of serological tests and low detecting rate of Aspergillus in culture influence the accurate diagnosis of CPA. Another major concern is that the efficacy of antifungals and evidences of appropriate antifungal treatment options have not been established either. Due to the large and increasing number of patients with tuberculosis sequelae, COPD and other chronic pulmonary diseases in Japan, increasing number of CPA patients will be problem. I would like to present the clinical aspects of CPA patients in Japan and also introduce our challenge to establish the new reliable diagnosing tools and treatment strategies.

CL-02-3

Antigen detection for diagnosis of the endemic mycoses in the immunocompromised host

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Endemic mycoses may present as opportunistic infections in immunocompromised patients. In the US, histoplasmosis is more common than tuberculosis in patients treated with TNF blockers, and coccidioidomycosis and blastomycosis often cause fatal infection in patients with AIDS or who have undergone transplantation. Prompt diagnosis is essential successful outcome of treatment, and is usually achieved by direct examination of respiratory secretions, or lesions, and antigen detection. In histoplasmosis, antigenuria can be detected in over 95% of patients, and antigenemia in over 90% following EDTA heat treatment of serum using the MVista® Histoplasma EIA. Antigenuria was detected in about 71% of patients with moderate to severe coccidioidomycosis in the MVista®*Coccidioides* EIA, most of whom were immunocompromised. Antigenuria can be detected in the MVista® *Blastomyces* EIA in about 90% of patients with more severe forms of blastomycosis. As most patients have both pulmonary and extrapulmonary involvement, diagnosis they also be established by testing bronchoscopy specimens for antigen. Cross reactions occur in nearly all patients with histoplasmosis and blastomycosis, and some with coccidioidomycosis. The highest sensitivity for diagnosis can be achieved by direct examination of respiratory specimens and lesions, and testing for antigen in serum, urine, and bronchoscopy specimens.

CL-02-4

Current status on invasive candidiasis in surgical fields

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Invasive candidiasis can occur in immunosuppressed patients as well as in critically ill, non-immunosuppressed patients and is associated with high mortality rate of 20-40 %. Intestinal *Candida* species colonization is an important source for invasive candidiasis. The following would be risk factors for invasive candidiasis; presence of an intravascular device, organ dysfunction, impaired mucosal or skin barrier function, therapy with antacids or corticosteroids, prolonged stay at the ICU, total parenteral nutrition, prolonged antibiotic therapy and also stepwise elevation of ß-D-glucan below cut off value in patients with risk factors for deep-seated fungal infections. Among invasive candidiasis in surgical fields in Japan, the isolation frequency of non-*albicans Candida* have reached to about 60%. The mortality rate of fungal peritonitis caused by *Candida tropicalis* was the highest in *Candida* species in our hospital. Among patients with invasive candidiasis, antifungal treatment should be started without delay. Over 72 hours delay of antifungal therapy after the diagnosis of fungal peritonitis led to high mortality rate. Antifungal prophylaxis would not be currently recommended in critically ill, non-immunosuppressed patients in surgical fields in Japan.

CL-02-5

Individual differences in voriconazole N- and C-Oxidation in vivo independent on cytochrome P450 2C19 genotypes

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Trough blood levels of voriconazole and its principal metabolites were analyzed during therapy of 60 patients. Genotyping found 15 were heterozygous for the slow metabolizer phenotype (CYP2C19*2 or *3), one was homozygous at that locus, while 15 were heterozygous and two were homozygous for the rapid metabolizer phenotype (CYP2C19*17). No difference was found in serum levels of voriconazole, the N-oxide or the 4 hydroxymetabolite based on genotype with the exception that N-oxide levels were slightly slower (1.9 vs 2.4 mcg/ml, p=0.03) in the slow metabolizer group.

Concern about altered metabolism in patients with CYP2C19 genotypes other than the wild type does not appear appropriate for the largely Caucasian and almost entirely heterozygotic population we studied.

CL-03-1

Paracoccidioidomycosis: A permanent challenge for clinicians and epidemiologists

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Paracoccidioidomycosis(PCM) is a systemic endemic mycoses caused by P.brasiliensis, a thermodimorphic fungus geographically restricted to Latin America. Recent molecular findings showed that P.brasiliensis is not a single species, but is a species complex. The clinical impact of this genotypic diversity is unknown. Non-autochthonous cases were observed but only in individuals who visit the endemic area. According to data from intradermal sensitivity surveys, the prevalence rate of P.brasiliensis infection in endemic areas may range from 50 to 75% with a mortality rate of 1.45 deaths per million inhabitants. The infection is more prevalent in rural dwellers but recent epidemiologic data suggest that changes in agricultural practices may result in a reduction in the incidence of infection in some specific areas. The primary pulmonary infection is usually unapparent or olygosymptomatic and infected individuals may never develop the disease. Less frequently, infection may progress to the acute (juvenile) or chronic (adult) clinical forms. In both cases, the disease may disseminate, involving many sites (lungs, mucous membrane, skin, lymph nodes, adrenals, CNS, etc). PCM natural evolution is usually to death. Despite cell-mediated immunity plays a major role against P.brasiliensis, the disease is unusually associated with AIDS or other T-cell mediated immunodeficiency
such as cancer and organ transplantation. The diagnosis is mainly made by direct exam or culture of clinical specimens but serologic tests may be of help, especially antigen detection. Oral itraconazole or amphotericin B are indicated for mild to moderate or severe forms of PCM respectively. Recent data shows that posaconazole and voriconazole are both effective in this disease. Voriconazole may be a good alternative for the therapy for neuroPCM. Although prolonged antifungal therapy is the basis of the treatment of PCM, in experimental models, a gp43 derived peptide combined to conventional therapy, seems to reduce the time of treatment and prevents relapses.

CL-03-2

Cryptococcus gattii infections in adult and children populations (emphasis on clinical features, epidemiology and outcome)

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Cryptococcosis is a life threatening infection affecting animals and humans and it is caused by two species of *Cryptococcus, C. neoformans* (serotypes. A, D and hybrid AD) and *C. gatti* (serotypes B and C). Disease caused by *C. gattii* is distinguished from that due to *C. neoformans* by an increased incidence of cryptococcomas in lung and brain, increased neurological morbidity and a slower response to antifungal therapy. The difference in clinical presentation is predominantly due to the effect of underlying immunocompromise in patients infected with *C. neoformans. C. gatti* has a worldwide distribution predominantly associated with tropical and subtropical climates. Some parts of the globe have higher incidence of *C. gatti*, such as Australia and New Zealand, however an outbreak has been well documented in the temperate area of Vancouver Island.

In South and Southeast regions of Brazil, human cryptococcosis is caused mainly by *C. neoformans* serotype A, VNI and is associated with AIDS. Clinical and epidemiological studies performed in the North and Northeast regions showed the occurrence of severe cases of disseminated form of the disease including pulmonary and meningitis due to *C. gatti* serotype B in adults and in immunocompetent children. Cryptococcosis *gatti* in children living in these areas resulted in high mortality rates and sequels such as blindness and mental retard. The studies suggested an endemic primary mycosis in HIV-negative individuals including children. Molecular typing of *C. gatti* isolates obtained from these patients, using URA5-RFLP and PCR fingerprinting identified genotypes VGI, and VGII.

C. gatti has been recognized as an emerging endemic mycosis requiring the implementation of an active surveillance system in high risk area to monitor the mycosis.

CL-03-3

Development and evaluation of an assay to detect *Histoplasma capsulatum* antigenuria: A diagnostic test needed in resource-limited settings

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Histoplasma capsulatum infection causes significant morbidity and mortality in HIV-infected individuals, particularly those in countries without access to diagnostic tests or antiretroviral therapies. The fungus grows unchecked in persons with AIDS, resulting in progressive, disseminated histoplasmosis (PDH), which can be fatal within weeks if left untreated. The availability of a simple, rapid method to detect *H. capsulatum* infection would dramatically decrease time to diagnosis and treatment of PDH in resource-limited countries. We have developed an antigen-capture ELISA to detect antigenuria in infected patients. The assay uses polyclonal antibodies against *H. capsulatum* as both capture and detection reagents and a standard reference curve is included to quantify antigenuria and ensure reproducibility. Urine specimens were collected prospectively from patients at a Guatemalan HIV clinic (n=101), and from healthy residents of the USA (n=33) and Guatemala (n=50). Additionally, we evaluated urines from patients in prior studies who had been confirmed by culture to have non-histoplasmosis fungal infections (n=61), for a total of 245 patients tested. The H. capsulatum antigen-capture ELISA showed a sensitivity of 81% (39/48) in detecting antigenuria in patients with culture-proven PDH and an overall specificity of 95% (187/197) against the negative control urine cohorts. Longitudinal analysis of serial urine specimens from 14 PDH patients with good follow-up showed that there was a marked decrease in detectable *H. capsulatum* antigenuria during antifungal therapy. Use of this simple, rapid ELISA in endemic resource-limited countries may lead to reduced PDH-related morbidity and mortality. The test may also prove useful to clinicians wishing to monitor PDH patient recovery.

CL-03-4

A fatal case of blastomycotic meningoencephalitis with neutrophilic pleocytosis in an immunocompetent patient

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INTRODUCTION: Blastomycosis is endemic to the Ohio and Mississippi River valleys. Blastomycotic central nervous system (CNS) infection is generally characterized by lymphocytic pleocytosis. We report a fatal case of blastomycotic meningoencephalitis with neutrophilic pleocytosis from a non-endemic area.

CASE: A 59-year-old immunocompetent male from North Dakota developed headache, confusion, hearing loss and dizziness during June 2005. He was prescribed 10 days of amoxicillin but demonstrated no clinical improvement and was admitted to the hospital during July 2005.
Lumbar puncture revealed a white blood cell count of 525 cells/mm^3 (70% neutrophils and 29% lymphocytes), glucose 24 mg/dL, and protein 180 mg/dL in the cerebrospinal fluid (CSF). CSF was sent for Gram stain, culture, cryptococcal antigen and herpes simplex DNA. All tests were negative. Blood was sent for culture and was tested for Lyme antigen, West Nile virus, and *Blastomyces dermatitidis* serology, which were all negative. Chest radiograph and head CT findings were unremarkable.
Partially treated bacterial meningoencephalitis was suspected because of prior exposure to amoxicillin. Intravenous vancomycin, ceftriaxone, and ampicillin were started empirically. Despite treatment with three antibiotics, the patient's neurologic symptoms gradually deteriorated. Repeated head CT remained unremarkable and repeated CSF analysis demonstrated no improvement. Therapy was changed to intravenous gatifloxacin, cotrimoxazole, and fluconazole, however, the patient expired during August 2005 despite this change. Autopsy revealed *B. dermatitidis* in the lungs, brain, and meninges. Fungal cultures of the blood and CSF were negative.

DISCUSSION: This unusual case presentation of *B. dermatitidis* infection highlights the importance of including this organism in differential diagnosis in the case of CNS infection with neutrophilic pleocytosis, in the absence of travel to or residence in an endemic area. Fungal culture and *B. dermatitidis* serology were insufficiently sensitive to diagnose CNS or pulmonary blastomycosis. Furthermore, CT and chest radiographic findings may be misleading.

CL-03-5

Diagnosis of endemic systemic mycoses in non-endemic areas - a challenge

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In non-endemic areas clinicians are rarely aware of the possibility of imported systemic mycoses such as histoplasmosis or coccidioidomycosis, especially not in an immunocompetent host. So 3/8 disseminated coccidioidomycoses in Germany have been misdiagnosed for up to 7 years. A recent survey on histoplasmosis in Europe by the ECMM revealed that the cases reported might have been only the tip of the iceberg and that there is a need to improve the diagnostic facilities. Regarding the possibility of reactivated infections after decades, our aim should be to confirm any endemic systemic mycosis, even in a self-limited course of infection.

The optimal diagnostic strategy for patients with suspected endemic systemic mycosis depends on the immunological status, the clinical manifestation of the suspected fungal disease, the residency of the patient and the diagnostic facilities available.
The diagnostic value of antibody (AB) detection in patients living in endemic areas is limited. In contrast, AB detection against primary pathogens in immunocompetent residents of non-endemic areas has a high diagnostic value and even more sensitive AB-detection methods such as Western blot can be used for serodiagnosis.

Direct detection of the pathogen by microscopy and culture are still the aim of laboratory diagnostics. Molecular methods - although still under evaluation - can be highly beneficial for detection of systemic infections by dimorphic fungi and have an increasing value for diagnosis. In patients with suspected disseminated histoplasmosis or coccidioidomycosis PCR and sequencing may substitute the antigen detection, a test, which is commercially not available outside of the USA.

Central reference laboratories in non-endemic areas with a broad spectrum of diagnostic tests available to detect infections due to primary pathogens and Penicillium marneffei would be desirable.

The ECMM has started a survey on coccidioidomycoses to improve our knowledge on these important infections also in non-endemic areas.

CL-04-1

Diagnosis and treatment of *Fusarium* infections

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Among hyaline hyphomycoses, *Fusarium* spp. represent an emerging threat, with an increasing number of reported infections, particularly among immunocompromised patients. These agents demonstrate a high rate of resistance to currently available anti-fungal agents, and disseminated infections are associated with poor clinical outcomes and low rates of survival. The discussion will broadly divided into three sections to provide an overview of the diagnosis and treatment of invasive fusariosis. The initial segment will briefly cover taxonomy and epidemiology, together with an overview of the clinical spectrum of disease attributable to these agents. The second portion will center on diagnostics, with a focus on laboratory methods. Traditional techniques, such as direct morphologic examination and culture will be covered, as well as a description of newer techniques, with particular emphasis on molecular diagnostics. Finally, susceptibilty to anti-fungal agents and current opinions on treatment will be discussed.

CL-04-2

Recent developments in the epidemiology of infections caused by *Scedosporium* species

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Scedosporium spp. cause a wide spectrum of conditions that may be classified as colonization, saprobic involvement, mycetoma, sinopulmonary disease, and disseminated infections. *Scedosporium apiospermum* causes a characteristic infection of sinopulmonary and central nervous infection following near-drowning. *Scedosporium* species vary in the geographic distribution of reported cases. *Scedosporium aurantiacum* is a newly recognized pathogen reported in Australia. Clusters of nosocomial infection caused by *Scedosporium* spp. have been described and the organism may be identified in the hospital environment using amphotericin B selective medium. Molecular epidemiological methods, including random amplification of polymorphic DNA (RAPD), intergenic spacer region PCR (IGS-PCR), ITS sequencing, PCR fingerprinting, and restriction fragment length polymorphism (RFLP) analysis, ITS-RFLP, and multilocus sequence typing (MLST), demonstrate a relatively high degree of inter-strain genotypic variability. *Scedosporium apiospermum* causes the majority of eumycotic mycetomas in temperate zones, while non-*Scedosporium* genera dominate in tropical regions. Patients with acquired or primary immunodeficiencies, hematological malignancies, and solid organ or hematopoietic stem cell transplant recipients are the most common host populations suffering pulmonary and disseminated *Scedosporium* infections. *Scedosporium prolificans* greatly exceeds *S. apiospermum* as a cause of fungemia. The outcome from infections caused by *Scedosporium* species varies as a function of underlying host factors and organism with *S. prolificans* being refractory to available antifungal agents.

CL-04-3

Host defenses related with hyalohyphomycoses

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The innate host defense system (IHDS) against filamentous fungi causing hyalohyphomycoses, such as *Aspergillus* spp., *Fusarium* spp. and *Scedosporium* spp., includes dedicated phagocytic cells (peripheral blood monocytes, monocyte derived macrophages, pulmonary alveolar macrophages, neutrophils, myeloid dendritic cells and natural killer cells), cytokines, chemokines, toll-like receptors, and antimicrobial peptides. During the past decade, the advances in the field of the IHDS have been enormous, allowing a better understanding of the immunopharmacological control, immunoregulation, and expression of innate host defense molecules against *Aspergillus fumigatus*, other aspergilli and other fungi causing hyalohyphomycoses. *Fusarium* spp. are emerging as important causes of invasive fungal infections. They tend to have decreased susceptibility to antifungal agents, making host defences very important. The ability of human phagocytes to cause damage to hyphae of *Fusarium solani, F. oxysporum* and *Verticillium nigrescens*, a mould with very low pathogenicity, was assessed using the XTT assay. Phagocytes respond to the test fungi differentially, with *F. solani* being the least susceptible to damage by MNC. This may correlate with the observation that, compared to the other fungi studied, it causes a relatively high incidence of infections in neutropenic patients. Innate and adaptive immune responses to *Scedosporium* spp. have been studied much less than those to *A. fumigatus* in the past. The increasing importance of these emerging fungal pathogens has made the thorough study of the host defense against them necessary. *In vitro* studies have shown that *S. apiospermum* and *S. prolificans* conidia and hyphae are susceptible to phagocytes in a manner comparable to *A. fumigatus* with minor differences. Other studies have demonstrated that phagocytes are capable of exhibiting sufficient oxidative burst to control *S. prolificans* strains.

CL-04-4

Antifungal Susceptibility Trends for *Fusarium* spp. and Other Agents of Hyalohyphomycosis

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The hyalohyphomycetes have emerged as significant pathogens in both the immunocompetent and severely immunocompromised host. In addition to *Aspergillus* spp., *Fusarium* spp. have emerged as significant pathogens. Although not as commonly encountered, *Paecilomyces, Acremonium*, and *Penicillium* species are also being increasingly implicated in disease. Infections caused by the hyalohyphomycetes may be cutaneous or disseminated but either disease state is difficult to treat. Antifungal susceptibility testing has gained recognition as a valuable tool for physicians faced with making difficult decisions regarding treatment of patients with serious fungal infections. The Clinical Laboratory and Standards Institute (CLSI, formerly NCCLS) released the latest version for testing filamentous fungi in 2008. This document, M38-A2 has expanded methods for the dermatophytes and includes information regarding the newer antifungal agents. Although this method has been available for several years, it is difficult to find reliable information regarding susceptibility trends for various species, especially against the newest antifungal agents released on the market. During this session, the latest revisions to M38-A2 will be discussed in addition to antifungal susceptibility trends with an emphasis on reviewing several years of data to determine if acquired resistance might be a factor to be considered when evaluating therapy options.

CL-05-1

Should we monitor plasma levels of antifungal agents?

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Successful management of invasive fungal infections continues to pose a difficult challenge to clinicians. Despite recent advances in antifungal pharmacology, the morbidity and mortality due to invasive fungal infections remains unacceptably high. Numerous factors determine the outcome of these infections. The choice of the antifungal drug and dosing regimen are among the factors the clinician can dictate. However, even if the appropriate drug and regimen is initiated, drug exposure at the site of infection may be inadequate due to pharmacokinetic variability and contribute to treatment failure. Conversely, antifungal exposures may exceed those anticipated and result in toxicity. Many antifungal drugs exhibit marked variability in drug blood concentrations due to inconsistent absorption, metabolism, elimination, or interaction with concomitant medications. One tool to detect drug exposures outside of the therapeutic window is drug blood concentrations monitoring. Therapeutic drug monitoring of any pharmacologic agent should be considered when there is both significant pharmacokinetic variability and strong, clinically relevant exposure-effect relationships. Several clinical trials suggest that therapeutic drug monitoring of 5-FC and azoles antifungals can be useful to both reduce drug toxicity and optimize efficacy. The utility of this tool to inform drug choice and dosing decisions is being increasingly explored. While larger investigations are needed to rigorously define the optimal timing and concentration needed for specific clinical scenarios, preliminary results provide a useful guide for clinicians.

CL-05-2

Impact of susceptibility testing in antifungal therapy

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The incidence of infections caused by fungi is increasing in selected populations of patients as those with haematologic cancer, chronic granulomatous disease, chronic obstructive pulmonary disease, etc. Also patients receiving haematologic stem cell transplant or solid organ transplantation are at high risk of developing a fungal infection. The mortality and morbidity of this kind of patients is very high and thus many strategies trying to lower this trend have been investigated. Among them, the identification of what species or isolate are resistant to antifungals is a mandatory area. Thus, the subcommittee for antifungal susceptibility testing of EUCAST has developed a standardized methodology able to distinguish between susceptible and resistant strains. This standard is available at www. EUCAST.org. Although, the development of a standard is key in the complex process devised to generate break points, there are other issues that have to be accomplished to define what is a clinically resistant isolate. Those issues include, dosage, wild type MIC distributions in order to define epidemiological cut-offs, pharmacokinetics, pharmacodynamics, Montecarlo simulations, and clinical data. The compilation of this kind of information allows us to recommend break points of susceptibility and resistance. Nowadays, breakpoints for fluconazole and voriconazole are available for *Candida* spp. However, for moulds we are starting the process. In this session we will revise the way to obtain breakpoints and the clinical relevance of those moulds that having high MICs respond poorly to antifungal treatment.

CL-05-3

Interpretation of serodiagnostic tests in chronic pulmonary aspergillosis

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The diagnosis of the chronic form of pulmonary aspergillosis including pulmonary aspergilloma or chronic nerotizing pulmonary aspergillosis (CNPA) are able to be made almost clinicaly by a patient's symptoms, in inflammatory tests such as C-reactive protein and by chest X-ray or CT in the Japanese field of respiratory medicine. Serodiagnostic tests like detection of serum galactomannan antigen, anti-*Aspergillus* antibody and (1 → 3)-β-D-glucan are also available for making a diagnosis of chronic pulmonaly aspergillosis as surrogate makers. The usefulness of these surrogate makers have been evaluated in several studies in patients with invasive pulmonary aspergillosis in hematological or organ transplantation fields. However, the clinical evaluations of them in chronic type of pulmonary aspergillosis in the field of respiratory medicine are limited.

Recently, the cut off index of detection of serum galactomannan antigen using enzyme-linked immunosorbent assay (ELISA) was changed from 1.5 to 0.5 through some large clinical studies in hematological fields. Despite this fact, the new cut off index of galactmannan 0.5 has been adapted to every forms of pulmonary aspergillosis including CNPA, diagnosed and treated by respirologists. I speculate that a lot of Japanese respirologists consider the new cut off index 0.5 is too low for diagnosis and treatment for CNPA.

I will show some interpretative data about the use of ELISA galactomannan detection kit for diagnosis of CNPA in the field of respiratory medicine, and the differences in correct usage of galactomannan detection kit between invasive pulmonary aspergillosis in patients with hematological malignancy and CNPA in patients with chronic destructive pulmonary underlying diseases. I will make a small lecture about the appropriate use and problems of detection of galactmannan antigen using ELISA for diagnosis and treatment of CNPA.

CL-05-4

(1→3)-β-D-Glucan assay for the diagnosis of invasive fungal infections: Review of the literature

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Background: The measurement of plasma (1→3)-β-D-glucan (BDG) was developed for the diagnosis of invasive fungal infections (IFI) in 1992. Since then, BDG assay has been widely used in Japan especially in patients with hematological diseases. To evaluate its usefulness, we have analyzed the sensitivity of BDG in patients with IFI by review of the literature.

Methods: Related English and Japanese papers were searched for with terms of BDG and any of IFI due to *Candida*, *Aspergillus*, *Cryptococcus*, *Pneumocystis*, and other fungi from PubMed (English) and JMEDICINE (Japanese).

Results: Sixty-five pieces of literature were identified and BDG was measured by 5 different methods. The earliest method estimated plasma BDG as Fungal Index from the difference between measurements by a conventional limulus test, which reacts with both endotoxin and BDG, and an endotoxin-specific test. In 1995, test kits for direct measurement of plasma BDG was developed (Fungitec G-test and G-test MK). In the next year, a turbidimetric assay (β-glucan Wako) was introduced and in 2001, another colorimetric assay (β-glucan Maruha). Recently, yet another colorimetry, Fungitell, became available in USA/EU. IFIs were identified in 1136 patients, and 888 (78.2%) were positive. Fungemias were found in 375, and 329 (87.7%) were positive: 305 of 350 (87.1%) in candidemia and 24 of 25 (96.0%) in other fungemias including cryptococcemia. As for pulmonary infections, 15 of 22 (68.2%) were positive in *Candida* pneumonia, 182 of 240 (75.8%) in invasive pulmonary aspergillosis and 143 of 149 (96.0%) in *Pneumocystis* pneumonia. In contrast, the positive rate of cryptococcal pneumonia was 10.9% (5 of 46).

Conclusions: The high incidence of positive plasma (1→3)-β-D-glucan in IFI was reconfirmed.

CL-06-1

Rat models of invasive pulmonary aspergillosis

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There is a large body of research with the human pathogenic fungus *Aspergillus fumigatus*. The aim of much of this research is to either determine the host-pathogen interactions early and late in colonization and infection, to assess the efficacy of antifungal agents or develop improved diagnostics. In each of these research areas appropriate animal models are required.

There are many options available when selecting an appropriate host, immunosuppression regimen (if required), fungal isolate and route of infection. In addition it is essential to model the different immune states *Aspergillus fumigatus*. might encounter in human hosts.

In order to fulfill these varying requirements we developed novel rat models of invasive pulmonary aspergillosis in non-neutropenic and neutropenic hosts following exposure to aerosolized *Aspergillus fumigatus*. spores. In the non-neutropenic host rats are immunocompromised by repeat high dose cortisone and develop a disease that is predominantly restricted to the airways. In the neutropenic model rats are immunocompromised with both cytotoxic drugs and prednisolone and develop an aggressive invasive disease. In both models rats remain healthy for 4 days but rapidly deteriorate between days 6-8 resulting in 100% mortality.

The model is highly amenable to therapeutic studies however treatment is much less effective in the non-neutropenic rat model. Benefits of the models include simple collection of sequential blood samples through tail vein bleeds allowing the progression of disease to be monitored using surrogate markers. The neutropenic model demonstrates that a positive PCR signal and/or galactomannan from blood can be detected 3 days post infection (before disease is apparent) which correlates with the infectious burden invading the lung. In the non-neutropenic model markers in the blood remain negative but very high burdens are detected in bronchoalveolar lavage.

The rat provides a flexible host to model human pulmonary diseases caused by *Aspergillus fumigatus*.

CL-06-2

Clinical trial evaluation of new antifungals

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CL-06-3

Efficacy and safety of micafungin for the treatment of invasive fungal infections in patients with hematological malignancies

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Two prospective multi-center studies were conducted consecutively to clarify the efficacy and safety of MCFG for the treatment of invasive fungal infections (IFIs) in patients (pts) with hematological malignancies from April 2003 to September 2006 in Japan. For Study 1, pts were registered if they were diagnosed with one of the following: proven IFI, probable IFI, possible IFI, or a fever refractive to antibiotic treatment. For Study 2, neutropenia was added to the above inclusion criteria.

In Study 1, 277 pts were registered, of which 197 (mean age: 56.6 years) were evaluable for clinical efficacy. The mean dose of MCFG and duration of treatment were 170.7 ±71.4 mg/day and 22.0±15.2 days, respectively. The main underlying disorders were acute leukemia (49.7%), non-Hodgkin's lymphoma (18.8%), multiple myeloma (10.2%), and myelodysplastic syndrome (10.2%). The clinical response rate (CRR) was 68.0% (134/197): 87.5% (7/8) with proven IFI, 44.7% (17/38) with probable IFI, 61.9% (39/63) with possible IFI, 80.7% (71/88) with refractory fever, respectively. Drug-related adverse events (DAEs) were observed in 14.1% (39/277).

In study 2, 534 pts were registered, of which 425 (mean age: 58.6 years) were evaluable for clinical efficacy. The mean dose of MCFG and duration of treatment were 156.9±56.8 mg/day and 14.0±6.9 days, respectively. The main underlying disorders were acute leukemia (60.7%), non-Hodgkin's lymphoma (18.6%), and myelodysplastic syndrome (11.3%). The CRR was 60.6% (255/421): 33.3% (2/6) with proven IFI, 35.5% (11/31) with probable IFI, 60.1% (89/148) with possible IFI, 64.8% (153/236) with refractory fever, respectively. Pts with refractory fever whose neutrophil counts were < 500/μl throughout the treatment had a CRR of 49.4% (38/77). DAEs were observed in 18.5% (99/534).

MCFG was confirmed to have a high clinical efficacy and be safe for the treatment of IFIs in pts with hematological malignancies.

CL-07-1

Epidemiology and outcomes of invasive aspergillosis in hematopoietic stem cell transplant recipients: Impact of changing transplant practice

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Invasive aspergillosis (IA) is an important cause of life-threatening infection in hematopoietic stem cell transplant (HSCT) recipients, and the incidence of IA varies among institutions. In recent years, the number of HSCT recipients has increased as more elderly patients undergo allogeneic HSCT using nonmyeloablative or reduced-intensity conditioning. The incidence of IA in patients who underwent HSCT with nonmyeloablative or reduced-intensity conditioning was similar to that in patients who underwent conventional myeloablative HSCT. Although outcomes of HSCT recipients who developed IA are generally poor, mortality in patients with IA may have decreased in recent years with more prompt diagnosis of IA and the use of newer anti-fungal agents. In this symposium, we will discuss recent trends of epidemiology and outcomes of IA among HSCT recipients.

CL-07-2

Fungal infections in patients with hematological malignancies: Advances in diagnosis and prevention

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Fungal infection is one of the most serious complications in patients with hematological malignancies, especially in those who undergo allogeneic stem cell transplantation. The prognosis of overt invasive fungal infection, particularly that of invasive aspergillosis, in such patients is extremely poor. Therefore, prophylactic treatment, empiric treatment and early presumptive treatment based on novel diagnostic tools have been developed. We have shown that the Aspergillus galactomannan assay was better as an adjunct for the diagnosis of invasive aspergillosis than the beta-D-glucan assay or the real-time polymerase chain reaction to detect Aspergillus DNA. However, the positive predictive value of galactomannan assay was significantly lower early after transplantation and in patients with gastrointestinal graft-versus-host disease, probably due to the translocation of galactomannan contained in foods. We also showed that an early presumptive treatment strategy, in which antifungal agents were changed from fluconazole prophylaxis to anti-mold agents when patients developed positive serum-test or an positive chest-X-ray/CT-scan associated with persistent febrile neutropenia, was feasible in the neutropenic period early after transplantation. However, the risk of invasive aspergillosis strongly depends on the degree of immunosuppression of the patients and also on the environment where the patients are treated. For example, patients receiving high-dose steroids for graft-versus-host disease or those who were treated in a hospital near the construction area may require prophylactic anti-mold agents. Therefore, we have to monitor the immune status of the patients throughout the treatment course and modify strategies to prevent overt invasive fungal infection.

CL-07-3

Fungal infections in patients with hematological malignancies: Current treatment strategies

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CL-08-1

Epidemiology, diagnosis and management of *T. tonsurans* infection in Japan

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[Epidemiology] Since 2000, *T. tonsurans* infection has become a serious problem in Japan. To determine the incidence of infection, we undertook a series of screening tests of athletes using the questionnaire and hairbrush method. In 2008, 11% of study participants were positive for *T. tonsurans* infection, indicating that the incidence had not decreased since 2005. We found an increase in the numbers of asymptomatic carriers and infected family members, and we noted the infection's emergence in school children. **[Diagnosis]** In a further study to determine which body sites are most commonly infected in contact-sports athletes, we observed tinea corporis on the forehead, auricles, neck, shoulders, upper chest, elbows, wrist, and knees. Tinea capitis was most common in the occipitonuchal region at the hairline and in the temporal and frontal regions, at both auricles. Initial screening of these sites along with routine use of the hairbrush method might facilitate the identification of the infection in individuals at risk, especially judo practitioners. **[Management]** Subjects with tinea corporis and a negative hairbrush culture can be treated with topical anti-fungals for 1-2 months. Oral terbinafine or itraconazole is the recommended treatment for subjects with a positive hairbrush culture; a complete treatment course almost eradicates the infection. Hairbrush-positive subjects with only 1 or 2 colonies can be treated with antifungal shampoo alone. **[Conclusion]** The spread of *T. tonsurans* infection in sports clubs can be controlled by regular mass screening examination, therapy, and routine measures to prevent infection. Medical mycology research centers should work in collaboration with managers of individual sports clubs to perform hairbrush examinations in order to identify infected individuals for referral to clinics. Improvements in treatment and management guidelines, publication of educational materials, such as color atlas and DVD, and development of a clinical network must be promoted.

CL-08-2

Onychomycosis 2009

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Onychomycosis is infection of the nail unit caused by predominantly by *Trichophyton rubrum*; other dermatophytes, yeasts and/or nondermatophyte molds may also be pathogenic. Some infections are mixed combinations of fungal organisms, but the etiology is often difficult to precisely establish by conventional microscopy and culture. Newer diagnostic techniques including molecular detection and PCR analysis could improve future treatment outcomes by rapidly establishing diagnosis and pathogenesis thereby permitting targeted therapy selection. Oral antifungal therapy is generally considered standard of care for most patients with moderate to severe onychomycosis. The oral antifungal agents that became available almost 20 years ago- terbinafine, itraconazole and fluconazole- are still useful today. Of these, terbinafine has the best MICs against dermatophytes, but has limited activity against yeasts and nondermatophyte molds. Itraconazole and fluconazole have broad spectrum activity. Newer antifungal agents including the triazoles, albaconazole and posaconazole are under development for onychomycosis therapy. Posaconzole is currently available for select invasive mycoses, but not yet indicated for cutaneous mycoses. Both have excellent in vitro activity against dermatophytes, molds and yeasts, as well as unique pharmacokinetic profiles favorable to nail therapy. Although several topical antifungal agents have become available to treat onychomycosis, they are not comparable to the efficacy of oral agents in patients with moderate to severe disease. There is significant ongoing research in the area of topical delivery of antifungal agents to the nail bed, which may result in the availability of a new generation of topical onychomycosis therapy for future usage.

CL-08-3

Nondermatophyte infections of the skin and nails: Implications for therapy

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The overall prevalence of onychomycosis is between 3-7% but somewhat higher in certain groups of workers and patients over 40 years of age. European data have shown that about two-thirds of cases were caused by dermatophytes, with similar findings in tinea pedis. Moulds infection, predominantly *Aspergillus* spp. and *Scopulariopsis brevicaulis,* were shown to be the causes in about 11% of onychomycosis and 7% of tinea pedis cases. Thailand data revealed that 37.1% of randomly selected individuals attending dermatology outpatient clinic had fungal foot infections. Of note, the percentage of isolated non-dermatophyte pathogens was much higher than previously reported. Specifically, we found that more than half of patients with clinically diagnosis of tinea pedis or onychomycosis had nondermatophyte infection. *Scytalidium dimidiatum* was the leading cause, in contrast to similar studies in which dermatophytes were identified as leading pathogen. By contrast the prevalence of *Candida albicans* in our study was only 2.6-3%

Scytalidium spp. is now considered a pathogen for fungal foot infection. It is clinically indistinguishable from that caused by dermatophytes. There is no treatment of choice since the infection is quite resistant to presently available antifungal drugs. Mycological diagnosis requires inoculation of specimens in Sabouraud's dextrose agar without cycloheximide based on strict criteria to avoid misinterpretation. *Scytalidium* infection should be included as a possible cause of treatment failure. Without proper culture identification, clinically diagnosed patients were treated with standard antifungal regimen without satisfactory response and interpreted as drug resistance resulting in switching drugs and more aggressive management procedures. Ciclopirox olamine and amorolfine topical nail lacquers, oral itraconazole and terbinafine had been reported successful in exceptional cases. *Fusarium* spp. has been reported to increase morbidity in immunocompromised hosts.

Nondermatophyte should be suspected in difficult-to-treat fungal foot infections. Definite mycological examinations would prevent delayed diagnosis and ineffective treatment.

CL-08-4

Candidiasis

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CL-09-1

Epidemiology of IFI in children

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There are increasing data to suggest that pediatric fungal infections differ from their adult counterparts in terms of epidemiology, risk factors, and outcomes. Furthermore, even among pediatric patients with fungal infections there are differences. For example, fungal infections in neonates have a unique epidemiology. This session will provide an overview of the unique epidemiologic features of invasive fungal infections in children. The overview will include presenation of data regarding the incidence, risk factors, and outcomes of invasive fungal infections in children with an emphasis on candidiasis and invasive aspergillosis. Newer data on risk factors for candidiasis in the pediatric intensive care unit will also be presented.

CL-09-2

Invasive fungal infections (IFIs) in pediatric ICU

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IFIs are serious complications in the PICU. The most common IFIs are those due to *Candida* spp. such as *C. albicans, C. parapsilosis*. Invasive aspergillosis, zygomycosis, scedosporiosis and other IFIs due to filamentous fungi are rare and occur in immunocompromised hosts. The PICU may have a number of risk factors, the most important of which are immunocompromise, central catheters and administration of multiple antibiotics. In addition, major abdominal surgery and liver transplantation are high risks for IFIs. The critically ill patients may be admitted colonized with *Candida* spp. (about 50%) or acquire the fungi in the unit. The most frequent fungal infections are candidemias but additional forms of infections are various abscessess in different loci. Novel antifungal triazoles and echinocandins appear to exhibit good activity as first-line or salvage therapy, whereas amphotericin B formulations are valuable. In cases of non-*albicans Candida* spp., such as *C. krusei* and *C. glabrata* either amphotericin B or echinocandins can be used, whereas the treatment of choice in a patient with an infection caused by a fluconazole-sensitive *Candida* spp. is fluconazole. Prompt initiation of antifungal therapy is considered essential for a favourable outcome; however, empiric antifungal therapy is still being defined and investigated, whereas guidance based on sensitive diagnostic non-culture methods needs further validation before becoming a standard. Removal of central catheters and other foreign bodies is essential and it is associated with better outcome. Salvage therapy for invasive candidiasis in children remains a therapeutic challenge that is often complicated by the lack of definitive pediatric studies for new agents. Although response in invasive candidiasis is still not satisfactory, improved understanding of risk factors, rapid advances in immunosuppressive therapies, diagnostic mycological tests and new antifungal therapies have transformed its management into a fast-moving field promising a better future outcome.

CL-09-3

Diagnosis of IFI in children

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The accurate and early diagnosis of invasive fungal infections is crucial to improving outcomes. The advent of newer molecular tools has ushered in opportunities for prompt and non-invasive diagnosis, but there are few dedicated studies in children studying these new strategies. Here we will focus on the newer molecular diagnostic tools available to the clinician to diagnosis invasive fungal infections in immunocompromised children. We will highlight the use of the galactomannan to diagnose invasive aspergillosis, including the many special populations and concerns with this assay. Additionally, we will focus on the use of beta-glucan testing to diagnose *Candida* infections both in the neonatal as well as pediatric population. We will mention newer research tools such as PCR and other assays, but focus largely on the assays currently available to the clinician.

CL-09-4

Antifungal therapy for children

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Invasive fungal infections are important causes of morbidity and mortality in immunocompromised children and adolescents. These infections remain difficult to diagnose and outcome depends critically on the prompt initiation of appropriate antifungal chemotherapy, expert supportive care and restoration of host defenses.

The past two decades have seen a considerable increase in both number and overall relevance of invasive fungal infections in pediatric hospitals. At the same time, however, improved microbiological and imaging techniques and an increased awareness among physicians have shifted the diagnosis from the autopsy theatre to the bedside. Major advances have been made in the definition of fungal diseases, the algorithms of antifungal interventions, the design and implementation of clinical trials and the development of standardized in vitro susceptibility testing. Most importantly, a number of new antifungal agents has entered the clinical arena with the general perception that antifungal therapy has become safer, more effective, but also more complicated. However, as most of the scientific evidence has been generated in adults, there is an urgent need for clinical investigation in the pediatric setting.

This presentation reviews the current approaches to the treatment and prevention of invasive fungal infections in immunocompromised pediatric patients beyond the neonatal period.

CL-10-1

Chromoblastomycosis in the panorama of the neglected diseases

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The subcutaneous mycoses include a heterogeneous group of fungal infections that develop at the site of transcutaneous trauma. Together with sporotrichosis and mycetoma, chromoblastomycosis (CBM) is one of the most prevalent subcutaneous mycoses. It is caused by several species of dimorphic melanized fungi. It is usually an occupational disease, mainly affecting rural dwellers living in tropical and temperate regions. Although several species are related to its etiology, F.pedrosoi and C.carrionii are prevalent in the endemic areas.CBM lesions are polymorphic and must be differentiated of many clinical conditions. Diagnosis is confirmed by the observation of muriform (sclerotic) cells in tissue and the identification of the causal agent in culture. CBM still is a therapeutic challenge for clinicians due to the recalcitrant nature of the disease especially in the severe clinical forms. Treatment options include physical methods, chemotherapy and combination of both. Therapeutic success depends on the etiologic agent (C.carrionii is more sensitive than F.pedrosoi), the severity of the disease (edema and dermal fibrosis can reduce the antifungal tissue levels), and the choice of antifungal drug. As in other endemic mycoses, comparative clinical trials are lacking in CBM. According to open non comparative trials, most of the patients can be treated with itraconazole, terbinafine or the combination of both. Among the second generation triazole derivatives, voriconazole and posaconazole have a potential for use in CBM, despite high costs in long-term therapy. Voriconazole has not yet been evaluated in CBM, but posaconazole proved to be effective in a few F.pedrosoi cases that were previously refractory to various treatment regimens. In general, therapy should be guided according to clinical, mycological and histopathological criteria. It is also important to evaluate the patient's individual tolerability, absorption and if the antifungal will be provided for free or purchased, since antifungal therapy must be maintained in long-term regimens.

CL-10-2

Phaeohyphomycosis

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The dematiaceous (brown pigmented) moulds are a very large group of fungi that can cause a wide range of diseases. These organisms are ubiquitous and found particularly in soil, wood and plant debris. They are also more common in tropical and sub-tropical climates.

Phaeohyphomycosis is one of the infections caused by these organisms and the cutaneous and subcutaneous infection may result from traumatic implantation. They may affect both immuno-competent and immuno-compromised individuals with increased incidence in the latter group. Clinical presentation of cutaneous and sub-cutaneous lesions are varied and include erythematous papules or plaques, verrucous lesions and deep sub-cutaneous abscesses.

Whilst experience with various modalities of treatment has been varied, most forms of the disease require surgical and/ or systemic treatment. In extensive disease a combination of surgical debridement with Amphotericin B has been recommended. For localised disease, surgical treatment alone has been successful in some instances. Some serious cutaneous infections may require long term treatment and relapses have been reported.

Combination therapy has been used successfully in some cases. There is a need for the development of new anti fungal agents or combination therapies that may result in better outcome for patients in need of management of phaeohyphomycosis in the future.

CL-10-3

Sporotrichosis in Japan

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Sporotrichosis is a profund dermatomycosis caused by *Sporothrix schenckii*. This organism spread wide and isolated from nature soil and vegetation. Contact with nature is a major factor for the onset of the disease. In Japan, the patients of the disease distribute on the warm district, especially Kanto region where Tokyo is located, and Chikugo region of Kyushu Island. These regions are rich plains formed by big river, and where agriculture or gardening businesses are prosperous. I show the statistical survey of sporotrichosis seen at the Dermatological Clinic of Kurume University Hospital, which is in Chikugo region, and I review Japanese cases of sporotrichosis. Fall and winter are main onset season in the frequent occurrence area. But the number of patients is decreasing gradually after 1980's, because of changing of living environment and the industrial structure. Isolation of *Sporothrix schenckii* from the cutaneous lesion is indispensable for diagnosis. Sporotrichin skin test is a reliable serological examination, but it does not used very much in Japan for the reasons of the antigen solution supply. Almost Japanese cases are classified in localized (fixed) type or lymphangitic cutaneous type, but disseminated type is extremely rare. For treatment, oral potassium iodide, itraconazole and terbinafin are usually used in Japan, especially potassium iodide should be chosen first. Even the lower dose less than 1g of potassium iodide is effective for some Japanese patients. Local thermotherapy is also useful and easy treatment. It is performed by using the disposable pocket warmer, and recommended as a concurrent treatment of medication.

CL-10-4

Mycetoma due to *Cladophialophora* spp.

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Eumycetoma is a subcutaneous infection which is caused by two typeS: hyaline fungi and dematiaceous fungi. This disease tends to be more frequent among farmers as a result of local trauma and exposure to the potential etiologic agents. *Cladophialophora* spp. are exceptional and its clinical and therapeutic behavior is very similar to others mycetomas caused by black fungi.

Case 1.- A 57-year-old male presented with a dermatosis in the foot, consisting of tumefaction, deformity and sinus tract formation. Direct examination of the exudates and biopsy tissue demonstrated the presence of black granules. A dematiaceous fungus, *Cladophialophora* bantiana, was isolated from the lesions and identified by ribosomal DNA sequence. Clinical and mycologic cure was achieved after 20 months of treatment with itraconazole. The patient's isolate had an in vitro MIC of 0.012mg/ml

Case 2.- A 49-year-old male farmer, from Jicaltepec Oaxaca (semi-arid zone), presents with a dermatosis localized to the left leg at the dorsum of the foot; the lesion consisted of tumor area, conformed by nodules, with draining sinuses with thread-like material (black granules). The dermatoses had begun one and half year previously, after a traumatism with a thorn of cactaceousl (Opuntia sp). Direct examination showed black granules composed of septate, branching, dematiaceous hyphae. We isolated a fungus which was morphologically classified like *Cladophialophora* sp and identified by amplification and sequence analysis of ribosomal DNA like *Cladophialophora* mycetomatis.sp nov. Treatment with itraconazole, 200 mg/day was instituted, with important clinical improvement at 8 months.

Comment: Eumycetoma caused by *Cladophialophora* spp. are exceptional. The first case is a second human report, being the most important causative agent of phaeohyphomycosis. The second case due to *Cladophialophora* mycetomatis is new specie reported causing mycetoma. We emphasized the importance of the study of the etiological agents, especially the molecular biology (sequence analysis of ribosomal DNA).

CL-10-5

Diagnostic and therapeutic aspects of subcutaneous zygomycosis: An update

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Subcutaneous zygomycoses (entomophthoromycosis) is a chronic inflammatory or granulomatous disease that is generally restricted to the subcutaneous, nasal and sinus tissue of immunocompetent hosts, caused by fungi of the order Entomophthorales in the class Zygomycetes. Contrary to systemic zygomycosis, there is no invasion and occlusion of blood vessels. The disease is seldom life-threatening but very disfiguring. It encompasses two clinically and mycologically distinct entities, i.e., basidiobolomycosis (entomophthoromycosis basidiobolae) caused by Basidiobolus ranarum and conidiobolomycosis (entomophthoromycosis conidiobolae) caused by Conidiobolus coronatus and rarely C. incongruus. The former manifests typically as large, palpable, non ulcerating, subcutaneous masses adherent to overlying skin of the limbs, buttocks, back or chest whereas the latter involves the nasal submucosa, formation of polyps or palpable restricted subcutaneous masses, with extension to contiguous areas. The disease is acquired by traumatic implantation of infectious propagules of the etiologic agent, growing saprobically in soil, decaying vegetable matter, reptile or amphibian dung, etc. Human cases of basidiobolomycosis occur primarily in children whereas those of conidiobolomycosis in the adults. The infected persons in both of the clinical entities are, however, predominantly males. Gastrointestinal infection due to B. ranarum has recently, emerged as a new clinical entity of basidiobolomycosis.

Regardless of the infecting species, demonstration of wide, sparsely septate hyphae in biopsy material is quite diagnostic of zygomycosis. However, angioinvasion is predominantly seen in infections caused by Mucorales, whereas, presence of Splendore-Hoeppli phenomenon around hyphae in tissues typically characterizes entomophthoromycocsis. Although characteristic colony and microscopic morphology are helpful in the identification of causative species in most of the instances, the application of molecular methods provides new tools for the identification of zygomycetes even in tissues which fail to yield positive cultures. The management of subcutaneous zygomycosis in the light of new therapeutic options will be discussed.

CL-11-1

Emerging invasive fungal infections - an epidemiological update

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The epidemiology of invasive fungal infections (IFIs) is increasingly better understood due to increased awareness of emerging fungal infections and intensified surveillance and diagnostic efforts within the mycology community. Recent large surveys have highlighted the geographic variability of moulds such as *Scedosporium, Fusarium* and Zygomycetes as well as yeasts such as non - *albicans Candida* species and *Cryptococcus gatti*. The relevance of cryptic species of *Aspergillus* such as *A. lentulus* and newly described *Scedosporium spp.* such as *S.aurantiacum* have also been determined due to the application of molecular identification techniques to clinical isolates from epidemiologic studies.

Important factors influencing the changing epidemiology of invasive fungal infections are the identification of new risk groups for IFI, antifungal prescribing patterns and the availability of non-culture based diagnostics.

New risk groups for IFI have recently been described. In particular, patients with COPD and ventilated ICU patients have been identified as at risk for invasive mould infections. While patients receiving TNF blockers for rheumatological conditions have been recently identified as at risk for histoplasmosis, blastomycosis, coccidiomycosis and other IFIs.

Antifungal agents used as prophylactic and empiric therapy have been linked to the emergence of Trichosporonosis, Scedopsoriosis, Zygomycosis and Phaeohyphomycosis.

The application of fungal PCR to biopsy samples taken from patients with haematological malignancy and IFI in one European study revealed that in many cases *A. fumigatus* was not the predominant isolate and *A. terreus* and Zygomycetes were more common than initially suspected.

On a global basis Cryptococcus remains an important IFI, with *C. neoformans* one of the most important opportunistic infections in people living with HIV world-wide and *C. gatti* emerging in North America.

Understanding local epidemiology and what drives it is critical when choosing antifungal prophylaxis and therapy and our knowledge of this area continues to advance.

CL-11-2

Diagnosing rare fungal infections

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In the settings of an expanding population of immunocompromised hosts, selective antifungal pressures and shifting conditions in hospitals and the environment, the spectrum of fungal pathogens is evolving.

Fungi of the mucorales order (zygomycetes), previously uncommon hyaline filamentous fungi such as Fusarium species, Pseudallescheria boydii and Scedosporium, as well as dematiaceous filamentous fungi such as Bipolaris species, Cladophialophora bantiana, Exophiala species and Alternaria, are increasingly encountered as causing disease. They can cause a vast spectrum of infections from cutaneous to life threatening invasive fungal infections (IFIs), depending on the immunity status of the host and underlying disease.

Patients with hematologic malignancies, prolonged neutropenia, those receiving high dose corticosteroid therapy, or have diabetic ketoacidosis are at high risk. As disease usually has a rapid progress, timely diagnosis is of paramount importance. Early signs such as persisting fever after broad spectrum antibiotics, sinusitis, pulmonary infiltration, should alert for prompt action implementing a diagnostic work up and initiate therapy. Diagnostic tools include imaging, endoscopy, biopsies, cultures, and molecular techniques. Identification at species level of emerging fungal pathogens is getting more useful for treating IFIs and epidemiological surveys are compulsory in order to know prevalence of emerging fungi in any given geographical area.

Imaging techniques may be suggestive but are rarely diagnostic. Histopathology of infected sites provides evidence but is not specific. Direct microscopic examination is very useful but it requires proper sampling and laboratory expertise.

Isolation of the fungus is desirable but is not always possible, is time-consuming and identification at species level is often a challenge. Molecular techniques have been developed to detect fungal DNA but as yet lack proper standardization and validation. Moreover they need an extended and reliable data base. International collaboration is essential for expanding such data bases for a place of these techniques in the clinical laboratory in the near future.

CL-11-3

Treating rare fungal infections - Current evidence

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Emerging rare fungal infections have become a significant cause of morbidity and mortality in immunosuppressed patients. An increasing spectrum of yeasts and moulds that have previously been considered having no or limited pathogenic potential has been reported to cause invasive fungal infections in susceptible hosts. These organisms are often resistant to typical antifungal regimens and require atypical approaches to therapy. Newer antifungals offer increased activity against many of these pathogens. Voriconazole is active against many emerging yeasts, like Trichosporon spp., as well as many rare moulds, including agents of hyalophyphomycosis and dematiaceous fungi. However, voriconazole is inactive against agents of zygomycosis. Posaconazole offers increased activity that includes agents of zygomycosis but may be limited in the bioactivity achieved due to the oral formulation of the drug. Lipid formulations of amphotericin B offer increased a broad spectrum of activity, but some species like Fusarum spp. and Scedosporium spp. have less intrinsic susceptibility to polyene therapy so that a mycological diagnosis is key. Combinations of agents, some with atypical fungal activity, may be used in an attempt to control these infections. Surgical debridement may also improve outcomes in some patients. In patients with these rare infections, therapy is more likely to be successful with early, effective therapy, but recovery of abnormal host defects is critical to a favorable outcome of therapy.

CL-11-4

Fungiscope - A global database for rare fungal infections

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Introduction
The incidence and clinical relevance of rare invasive fungal infections is increasing worldwide, but reliable information on their clinical course, diagnosis and treatment is scarce. To determine the clinical pattern of disease, to describe and improve diagnostic procedures, and therapeutic regimens, as well as to facilitate the exchange of clinical isolates, we are coordinating a global registry for rare invasive fungal infections.

Methods
Patients with cultural, histopathological, antigen, or molecular biologic evidence of invasive fungal infection may be included into the study. Those with infections due to Aspergillus spp., *Candida* spp., Cryptococcus neoformans, Pneumocystis jiroveci or any endemic fungal infection, such as coccidioidomycosis or histoplasmosis, as well as colonization or other non - invasive infections are excluded. Data entry is accomplished via a web-based electronic case report form.

Results
By now, 65 patients with rare invasive fungal infections from a wide variety of pathogens have been included. The most common underlying conditions were chemotherapy for a hematologic malignancy (13 %, n=15), hematopoietic stem cell transplantation (11 %, n=13), Diabetes mellitus (13 %, n=11) and/or stay at an intensive care unit (11 %, n=13). The lungs were the most common site of infection (38 %, n=26), followed by soft tissues (16 %, n=11) and the paranasal sinuses (13 %, n=3). 20 patients displayed disseminated disease. At the latest assessment, complete response to antifungal therapy was observed in 44 % (n=28). The crude mortality rate was 39 % (n=26). 5 patients (8 %) were lost to follow up and in 2 patients (3 %), final evaluation of clinical evolution is still pending.

Discussion
The clinical relevance of rare invasive fungal infections is increasing steadily. In a short period of time, current cases from Europe, Asia and South America could be documented. Further investigators and coordinators are cordially invited to contribute to Fungiscope.

CL-12-1

PCP 2009. Clinical features, advances, and future directions

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Pneumocystis jiroveci pneumonia (PCP) is a major cause of morbidity and mortality among immunocompromised persons, especially those with the acquired immune deficiency syndrome (AIDS). Numerous epidemiological surveys indicate that the incidence of PCP has declined in patients with AIDS and other at risk populations in countries with resources for prophylaxis and antiretroviral therapy. In resource limited regions, however, PCP remains an important opportunistic condition. Advances in understanding the pathogenesis of PCP and the development of new treatments have been slow due to inherent difficulties working with the organism, for example the inability to sustain Pneumocystis in a culture system outside of the infected lung. Nonetheless investigators have applied advanced molecular techniques to study the biology of this difficult fungal pathogen. The use of heterologous expression models, genomics, and proteomics promises to advance the understanding of this intractable fungal pathogen. The molecular detection of the organism in persons without pneumonia suggests colonization and transmission with links to the development of chronic lung disease. This talk will review the clinical features of PCP in patients with and without AIDS and focus on recent advances in the biology and treatment of PCP.

CL-12-2

Pneumocystis jirovecii diagnosis by polymerase chain reaction technique

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Pneumocystis jirovecii pneumonia (PCP) is a severe respiratory infection, considered as one of the most common complications in immunocompromised patients. There are few researches on PCP in Venezuela, all of them carried out using direct immunofluorescence technique (DIF). Currently, it is necessary to have another detection method that increases the sensibility and specificity of PCP diagnosis, additionally to the use of diagnostic conventional methods, in order to provide an early diagnosis of this disease. The aim of this work was to implement the polymerase chain reaction technique (PCR) for the diagnosis of Pneumocystis jirovecii. Sixty two (62) clinical samples (spontaneous and induced sputa, bronchioalveolar lavage, and tracheal aspirates) collected from patients with AIDS, cancer and non-AIDS-non cancer low respiratory tract infections, were processed by DIF and nested PCR, using external (pAZ102-E and pAZ102-H) and internal (pAZ102-X and pAZ102-H) primers, targeting to the mitochondrial Large Subunit RNA region (mtLSUrRNA) of P. jirovecii genome, proposed by Wakefield et al. The PCR results were compared with DIF results (as a reference technique), using X2 test. Values of sensibility (S), specificity (E), positive and negative predictive values (PPV and NPV), positive and negative verisimilitude reasons (PVR and NVR), errors and agreement for the PCR technique were also calculated. P. jirovecii was detected by DIF in 14 patients and by PCR in 24 patients. PCR had values of S=100%, E=79.2%, PPV=58.3%, NPV=100%, PVR=4.8, NVR=0.3, and an agreement of 84%. PCR is a high diagnostic value technique that successfully predicts the absence of PCP with a negative result. A positive result does not discriminate among infection and colonization; therefore, it should be interpreted with caution taking into account the signs and symptoms of the patient.

CL-12-3

New notions on *Pneumocystis* transmission

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The genus *Pneumocystis* is comprised of multiple species that attach specifically to type-I alveolar epithelial cells in the lungs of mammalian hosts, including humans. These fungi can cause severe pneumonitis, particularly in hosts with marked impairment of the immune system. The strong host species specificity in *Pneumocystis* strains suggests that *Pneumocystis* infection in humans is an anthroponosis, and that humans serve as reservoir of *Pneumocystis jirovecii*, the sole species found in humans. Airborne transmission of *Pneumocystis* sp. from host to host has been demonstrated in rodent models and several observations suggest that inter-individual transmission occurs in humans in both hospital and community. In mice, a one day-exposure is enough for airborne transmission of the infection. An airborne transmission mouse model of *Pneumocystis*, which mimics the route and intensity of natural *Pneumocystis* infection, revealed that healthy hosts can serve as transient *Pneumocystis* carriers being able to transmit the infection to either immunocompromised or immunocompetent hosts. Consequently, *Pneumocystis* infection of healthy hosts is increasingly becoming a recognized public health issue. Finally, vertical *Pneumocystis* transmission by transplacental route has been proved in rabbits, but it seems not to occur in rats and SCID mice. In humans, congenital transmission was suspected for long-time, and a recent report documented the presence of *Pneumocystis jirovecii* DNA in foetal lung and placenta samples, recovered from nonimmunodepressed pregnant women with miscarriage.

CL-12-4

Pneumocystosis in Venezuelan patients: Epidemiology and diagnosis (2001-2008)

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The objective of this work was to investigate the epidemiology of pneumocystosis in Venezuelan patients using a retrospective study during an eight year period. Three hundred and seven (307) clinical samples collected from patients with AIDS (40.1%), cancer (20.2%), and non-AIDS-non-cancer (39.7%) low respiratory tract infections patients were processed by direct immunofluorescence technique. Pneumocystosis was diagnosed in 81 patients with a general frequency of 26.4%, which varied according to the patient's group: 35.8% in AIDS patients, 30.6% in cancer patients, and 14.8% in non-AIDS-non-cancer low respiratory tract infection patients. This study demonstrated the existence of differences in pneumocystosis frequency related to the patient's underlying disease, and that the illness is an important health problem in immunocompromised patients in Venezuela. Pneumocystosis must be suspected in non-immunocompromised patients with signs and symptoms of low respiratory tract infection, and the study of this illness must include patients with cancer and chronic obstructive pulmonary disease. Direct immunofluorescence is a usefull technique for pneumocystosis diagnosis; however, it requires an optimal sample and skilled personnel in the laboratory.

CL-12-5

Pneumocystis spp.: Proxies for mammalian host phylogeny and ecology?

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Several investigations recently suggested that, just as Dr Watson's records enable the reader to follow the progress of Holmes's investigation, parasite species might help to resolve the evolutionary and ecological history of their hosts. This approach was developed using different types of organisms (mainly viruses, bacteria, trematodes or tapeworms) to gain insight into the phylogeny and migrations of humans or wild animals. To be a good "proxy", a parasite should be highly likely to have shared a common history with its host, and to display a more resolved genetic pattern. We recently demonstrated that *Pneumocystis* organisms fit to this definition. *Pneumocystis* are uncultured, highly diversified fungal organisms able to attach specifically to type - I alveolar cells and to proliferate in pulmonary alveoli of many mammalian species. The outstanding narrow host-parasite specificity of *Pneumocystis* species suggested that they resulted from a long cospeciation process. A first study about primate - related *Pneumocystis* showed that more than 60% of the homologous nodes of the respective host and parasite cladograms may be interpreted as resulting from codivergence events. Similar observations were made for *Pneumocystis* from different populations of single mammalian species: the common woodmouse Apodemus sylvaticus or the bat Tadarina brasiliensis. In these cases, *Pneumocystis* were used as proxies at the phylogeographic scale and we may imagine that *Pneumocystis* populations were more diversified than were host populations. However, by contrast to the retention of too much ancestral polymorphism, too much divergence among *Pneumocystis* populations can also preclude the reconstruction of the evolutionary history of the corresponding mammalian host species. This situation was recently observed in pig-derived Pneumocystis from Brazil.

OT-01-1

Impact of impact factor on mycology journals

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Impact factor has evolved into the generally accepted method of evaluating the importance/quality of scholarly publications. Impact factor assumes that (a) articles most frequently cited are the most significant and (b) journals which include a high percentage of these articles are the premier publications. However, close examination of the criteria used in establishing this comparative quality model raises serious questions, especially with respect to mycological journals.

A journal' s impact factor is calculated by dividing the total number of cited articles in a two year period by the total number of articles published in the journal during the same timeframe. Consequently, publications dealing with rapidly changing areas of scientific investigation earn high impact factors. However, since many highly citable papers appearing in mycology journals have longer "shelf-lives", they are not included in the calculations of these journals' impact factors. Furthermore, impact factor of mycology journals is negatively affected by the comparatively small size of the research community; fewer numbers of citable works are generated. In addition, while case reports form an integral part of many mycology publications, they're among the least cited papers, and the journals which publish them as an educational service have lower impact factors. Since impact factor is applied as a comparative evaluation tool, journals are ranked within a group of similar publications. However, the primary factor that unites mycology publication is that they all, in some way, are concerned with fungi. Yet they significantly differ in their individual focus and content.

These and other issues to be discussed raise questions as to the accuracy of impact factor as a tool in evaluating mycology journals. Unfortunately, it has been adopted as the sole criterion to quantify what is essentially a qualitative issue, the importance/quality of scientific journals.

OT-01-2

Open Access publication and its consequences for medical mycology

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The last decade has seen a progressive rise in the volume of free “Open Access” availability of research papers. The activities of many scientific societies, including ISHAM, are largely dependent upon the income derived from the publication of society journals. A collateral consequence of Open Access publication is that payments for publications of scientific literature migrate from the point of journal subscription by libraries, personal subscriptions etc to the point of publication of individual research papers. What will be the effect of this on societies that support grass root science through the profits made on their journals? Also what is the result of Open Access publication on measurable parameters such as numbers of article downloads and citations? This talk will attempt to evaluate the effects of the Open Access culture on scientific reporting and upon the scientific community, with particular emphasis on the field of medical mycology.

OT-01-3

Ethics of scientific publishing: A growing concern?

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Excellence in scientific publication is the shared responsibility of the authors, editors, and publishers. Publishing of research results in peer-reviewed journals is important to scientists because it permits; (1) independent assessment of the quality of their studies, (2) dissemination of the data to workers in their fields, (3) determination of those responsible for the work, (4) justification for future outside funding and (5) contribution to the broad scientific archive. Due to all of these factors, scientists are under increasing pressure to publish their results as soon as is possible in journals with the highest impact. The Instructions to Authors in almost all journals include ethics requirements, e.g., authorship standards, prohibition of image modification that must be met by authors submitting manuscripts through current peer-review electronic programs. Despite these and other safe guards, misconduct in scientific publishing continues to be a serious problem. In my own experience as an Editor-in-Chief of FEMS Yeast Research, I have encountered data manipulation, authorship issues, and plagiarism. Some of these were noted by alert editors, but in other cases I received complaints from the authors' co-workers and colleagues during the revision of manuscripts, or, in one case, when the manuscript had been published on-line. It seems clear, based on past and present editorial practices, that not all cases of misconduct will be detected. For example, a recent survey by the NIH Office of Research Integrity estimated that there are greater than 2,300 cases of misconduct/year in research funded by the institutes. It remains a challenge to minimize the extent of fraud in scientific publishing which will require the concerted actions of authors, editors and publishers.

OT-01-4

Standards for publication of case reports

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The essential characteristic of a publishable case report is its educational value. Some case reports are published because they support the findings in previously published cases or because they are useful reminders of an important point in diagnosis or treatment. They are by their nature little mysteries that hold readers' interest. Most case reports fall into one of these five topics: (1) An unexpected association between diseases or symptoms; (2) An unexpected event in the course of observing or treating a patient; (3) Findings that shed new light on the possible pathogenesis of a disease or an adverse effect; (4) Unique or rare features of a disease; and (5) Unique therapeutic approaches or extensions of existing therapeutic practice. It should be recognized that collecting good evidence can be extremely difficult, emphasizing the value of case reports. Case reports should include an up-to-date review of all previous cases in the field, and should include relevant positive and negative findings from the history, examination and investigation, and can include clinical images and photomicrographs. Substantive case reports are where the authors believe that it is the first report of this kind in the literature, and where the case will significantly advance our understanding of a particular disease etiology or drug mechanism. They should not be based merely on the first incidence of a known cosmopolitan or widely distributed etiologic agent in the nations of the authors' residence. An introduction about why the case is important and needs to be reported is required followed by details of the patient history. A full mycological description and taxonomic verification is essential. Finally, include a brief conclusion of what the reader should learn from the case report and what the clinical impact will be.

OT-02-1

Ochratoxin A - producing species

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Ochratoxin A (OTA) is a nephrotoxic mycotoxin naturally occurring in a wide range of food commodities. It has been classified as a possible human renal carcinogen (IARC group 2B) and among other toxic effects, it is teratogenic, immunotoxic, genotoxic, mutagenic and carcinogenic. The main source of OTA in the diet are cereals and cereal products, but other food products can also contribute to the dietary intake, such as coffee, wine, beer, spices, grape juice and also products of animal origin. Currently, about twenty species which are circumscribed within the genera *Aspergillus* and *Penicillium* are considered OTA producers. Traditionally, *Aspergillus ochraceus* (currently *A. westerdijkiae*) and *Penicillium verrucosum* were considered the main OTA-producing species. Despite some reports on OTA production by other species in the genus *Penicillium*, OTA is only produced by two close species: *P. verrucosum* and *P. nordicum*. However, recent surveys have clearly shown that *Aspergillus niger* and *Aspergillus carbonarius* are sources of OTA in food commodities such as wine, grapes and dried vine fruits and probably in coffee beans. Within these black aspergilli, taxa included in the *A. niger* aggregate are difficult to distinguish by morphological means. Some of these species have a long history of use in biotechnology (production of hydrolytic enzymes, citric acid) and also in traditional japanese fermented beverages (awamori,kusu), as black-koji mold. In our laboratory, a method that differentiates the *A. niger* aggregate isolates into two ITS-5.8S rDNA RFLP patterns, type N and type T, corresponding to the type species of *A. niger* and *A. tubingensis* respectively was described. The ITS-5.8S rDNA and 28S rDNA (D1/D2) sequencing, microsatellite, RAPD and AFLP analyses have been also used to study genetic diversity in the *A. niger* aggregate, and they also confirmed the separation of N and T *A. niger* aggregate strains.

OT-02-2

Sirodesmin and gliotoxin: Secondary metabolite toxins in fungal pathogens of plants and animals

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Filamentous fungi produce a diverse array of secondary metabolites with a range of activities. We have discovered a cluster of genes involved in biosynthesis of a class of secondary metabolite toxins, epipolythiodioxopiperazines (ETPs). The best known ETP is gliotoxin, which causes apoptosis and is implicated in aspergillosis, a disease caused by *Aspergillus fumigatus*. Another ETP, sirodesmin, is a virulence factor in the plant pathogenic fungus, Leptosphaeria maculans, which causes blackleg of oilseed rape. I will discuss the biosynthesis, regulation and proposed functions of these toxins in these fungi. I will also present evidence for horizontal gene transfer in the evolution of these biosynthetic gene clusters.

OT-02-3

Effect of deoxynivalenol on Toll-like receptor signaling

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It is known that Deoxynivalenol (DON) causes immune dysfunction leading to affect various infections. Toll-like receptor (TLR) signaling plays important roles in the induction and establishment of innate and adaptive immunity. Lipopolysaccharide (LPS) which is a component of cell wall of gram-negative bacteria is recognized by TLR4. Therefore, the effect of DON on lipopolysaccharide (LPS) - induced macrophage activation was explored. It is found that DON significantly inhibited LPS-induced nitric oxide (NO) production by a mouse macrophage cell line, RAW264. In addition, it is revealed that the expression and transcription of inducible NO synthase (iNOS) mRNA were also repressed. Since IFN-β produced in response to LPS is involved in the expression of iNOS, we examined the effect of DON on LPS-induced IFN-β promoter reporter activity, and found that the promoter activity was repressed in a concentration-dependent manner. IFN-β expression can be induced by TLR4-mediated MyD88-independent signaling pathway. Thus, this symposium will also describe the effect of DON on MyD88-dependent and MyD88-independent signaling pathway.

OT-02-4

Pulmonary hypertension caused by inhalation of fungal spores - a new mycotoxic disease? -

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Pulmonary arterial hypertension (PAH), a kind of pulmonary hypertension, is known as an intractable lung disease. We have previously shown that the inhalation of spores of *Stachybotrys chartarum* causes PAH in mice resulting in the symmetrical thickening of pulmonary arterial wall, elevation of right ventricular systolic pressure and right ventricular hypertrophy. This finding may be helpful in understanding the mechanism of PAH. When environmental fungi other than *S. chartarum* were used, thickening of pulmonary arterial wall was also seen in some isolates of *Cladosporium cladosporioides* and *Aspergillus fumigatus*, but not in *Penicillium citrinum* or *P. chrysogenum* isolates. There were strain differences in the rate of the pulmonary arterial lesion: among *S. chartarum* isolates trichothecenes-producing strains had a significantly higher rate of forming pulmonary arterial lesions ($p < 0.05$). This suggests that trichothecene, which is a kind of mycotoxin, may have some effect on the formation of the lesion. However, *C. cladosporioides* and *A. fumigatus*, which did not produce a detectable amount of trichothecenes, also formed the pulmonary arterial lesion to some extent. These results are indicative that multiple fungal substances, including trichothecenes, are related to the arterial changes, and PAH may be the result of the combination effect of these substances produced by inhaled fungi. Further investigation is now on the way focusing on the relationship between fungal substances and formation of the pulmonary arterial lesions. In this presentation, the potential role of fungal substances in PAH will be discussed.

OT-02-5

Poisoning of dogs with tremorgenic *Penicillium* toxins

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Fungi in genus *Penicillium* are commonly found in mouldy food, feed and food waste. This genus includes known producers of a wide range of mycotoxins. Among *Penicillium* extrolites are tremorgenic mycotoxins, like penitrems, nephrotoxins such as ochratoxins and citrinin, and a range of other compounds including suspected tremorgens such as thomitrems and roquefortine *C. Penicillium crustosum* is among the known producers of extensively studied tremorgenic mycotoxins such as penitrem A and E as well as less studied toxins such as roquefortine C and thomitrems. Intoxications of dogs ascribed to ingestion of mouldy feed infected with *Penicillium crustosum* or restaurant food waste have been reported previously. The reported clinical signs include vomiting, tremors and ataxia.

Accidental intoxications of 6 dogs will be presented and discussed. The clinical signs included vomiting, convulsions, tremors, ataxia, and tachycardia - all classical signs of many intoxications affecting the nervous system. Poisonings with tremorgenic mycotoxins were suspected. One dog was euthanized in the acute phase. Three dogs recovered completely within a few days. In two dogs neurological symptoms were still observed four months after the poisoning. One of these dogs recovered completely within the next 2-3 months, while the other still suffers from ataxia three years later. Available samples of feed, stomach content and/or tissues from the intoxications were analysed by mycological and chemical analysis. Penitrem A was found in all reported poisonings and roquefortine C in cases, where it was included in the analysis. The producer of these toxins, *Penicillium crustosum*, was detected in all mycological examinations. This is the first report of poisoning of dogs with *Penicillium* toxins in Norway.

MO-01-1 ISHAM Working Group: Mycetoma

Eumycetoma: An overview

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Mycetoma is chronic disabling subcutaneous mycoses affecting many young active society members of endemic areas. The disease has a world-wide distribution, but it's more commonly seen in poor tropical countries where basic health education and health care facilities are badly needed. The infection is difficult to treat, and surgical intervention is malpractice in many centers, leading to more disabilities and increasing the negative social and economical consequences. Currently used antifungal agents are expensive and not affordable by many poor patients. In mycetoma endemic regions it's common to lose patient follow-up because of financial reasons. Many patients discontinue medical treatment or switch to traditional treatment either due to lack of money or because of the un-convincing outcome of antifungal treatments. Joint effort and collaboration between local authorities, academic institution and pharmaceutical industry are needed for the management and the control of this devastating infection.

MO-01-2 ISHAM Working Group: Mycetoma

Melanin biosynthesis in *Madurella mycetomatis*: Implications for rational therapy

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Mycetoma is a chronic, subcutaneous infection which is characterized by discharge of grains and purulent material through sinuses. Treatment of the disease still relies on surgery and prolonged antifungal treatment with either itraconazole or ketoconazole. Unfortunately failure of therapy is still common.

To estimate the success of antifungal treatment MICs should be determined for the causative agent, and breakpoints should be established. To develop an in vitro susceptibility test for *M. mycetomatis* is troublesome, since this fungus does not normally sporulate, therefore a hyphal inoculum is used. The first test introduced was a test based on the CLSI criteria. To facilitate endpoint reading MICs were determined after adding 2,3-Bis(2-methoxy-4-nitro-5- sulfophenyl)-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide (XTT). Later, a commercial assay, the Sensititre-assay, was adjusted to determine *M. mycetomatis* MICs for various antifungal agents.

From our in vitro susceptibility tests we learned that *M. mycetomatis* is highly susceptible to the antifungal agents itraconazole and ketoconazole. Unfortunately therapy success rates vary considerable.

Mycetoma caused by *M. mycetomatis* is characterized by the discharge of its black grains. The black colour of these grains was shown to be due to DHN-melanin. After isolating *M. mycetomatis* melanin and adding it to the culture medium, it appeared that MICs of ketoconazole and itraconazole were 16 to 32 times elevated in comparison to MICs determined in the absence of melanin.

Although *M. mycetomatis* appeared to be highly susceptible in vitro for itraconazole and ketoconazole under normal test conditions, caution should be taken when translating such results into clinical practice. Melanin, for instance, seemed to influence the in vitro susceptibility to these agents. To evaluate the full use of antifungal susceptibility testing for this fungus in vitro results should be coupled to either therapy success rates or results from therapy trials in animal models of mycetoma.

MO-01-3 ISHAM Working Group: Mycetoma

Molecular characterisation of the *Madurella grisea* complex reveals at least three new taxa associated with human mycetomas

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Dark-grain mycetomas are destructive infections of the skin and subcutaneous tissues, that progress to involve muscle and bone. Numerous different dematiaceous fungi are capable of provoking mycetoma following traumatic implantation. While some of these organisms are well characterised, many remain difficult to identify, at least in part due to delayed or absent sporulation in vitro. Current practise is to group these recalcitrant organisms under the generic umbrella of *Madurella grisea*, which thus potentially encompasses any species of dematiaceous fungus isolated from mycetomal lesions that fails to sporulate in vitro. Here, using isolates cultured from confirmed cases of dark grain mycetoma, and stored in the National Collection of Pathogenic Fungi and in the Institut Pasteur culture collection, we have attempted the molecular characterisation of members of the *M. grisea* complex. Over 50 isolates, collected worldwide from cases of dark grain mycetoma were subjected to sequencing of the ITS rDNA regions. LSU rDNA regions were also compared for a selection of these organisms. In agreement with previous reports, *Madurella mycetomatis* and Pyrenochaeta romeroi are homogenous species in the orders Sordariales and Pleosporales, respectively. Interestingly, over 50% of isolates comprising the *M. grisea* complex were shown genetically to be P. romeroi, and presumably represent strains that had been incorrectly identified. The remaining *M. grisea* complex isolates (none of which had sporulated even after over two years continuous culture) could be grouped in three genetically distinct clades. All three clades, which fall within the Dothidiales/Pleopsorales, comprise hitherto un-described taxa. Here we have begun the characterisation of these new taxa by using molecular phylogenetic analyses, and examining their geographic origins and antifungal susceptibility profiles.

MO-01-4 ISHAM Working Group: Mycetoma

Mycetoma due to a novel species of *Pleurostomophora* in an indigenous woman from the Kimberley region of Western Australia

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Mycetoma is a local, chronic, slowly progressive, often painless infection of subcutaneous tissues caused by fungi. We report a 73-year-old indigenous woman from the remote rural Kimberley region of Western Australia, who was admitted for management of left ankle mycetoma (neglected for at least 10 years). She had a large, non-tender indurated 10cm x 15cm mass with multiple draining sinus tracks near her left lateral malleolus. MRI showed changes consistent with extensive mycetoma.

The tumour was surgically removed; latissimus dorsi muscle flaps and skin grafts were required to cover the defect. Histopathology of the biopsy demonstrated multiple micro-abscesses with fungal grains surrounded by hyaline-like material (Splendori-Hoeppli reaction) consistent with fungal mycetoma. Operative tissue cultures yielded two fungi with distinct phenotypic features: a melanized isolate identified as a *Pleurostomophora* sp. (nov. species) by The CBS (Utrecht, The Netherlands) and another isolate identified as Phialamonium curvatum. The *Pleurostomophora* sp. isolate could only be identified by complete sequencing of the 18S rRNA gene. Using CLSI M38A methodology, the isolates had matching antifungal susceptibility test (AFST) results (Women's and Children's Hospital, North Adelaide, Australia), testing voriconazole 0.5 mg/L (S) and itraconazole 1.0 mg/L (R). She was initially treated with voriconazole, but had intolerable nausea and vomiting. Itraconazole was therefore tried empirically, despite the in vitro AFST results, with complete wound healing. Treatment in the Kimberley was given for approximately 18-months with reasonable adherence, and was associated with good clinical efficacy. Currently, at 5-years follow-up, she remains well with a good functional outcome and without any signs of relapse.

We report a novel species of *Pleurostomophora* sp. associated with mycetoma. This case also illustrates the challenges in identification of the phylogenetically diverse dematiaceous agents of mycetoma, and the imperfect correlation between AFST results and the clinical efficacy of antifungals used to treat mycetoma.

MO-02-1 ISHAM Working Group: Chromoblastomycosis

The clinical polymorphism of chromoblastomycosis lesions

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Chromoblastomycosis (CBM) is a member of the heterogeneous group of subcutaneous (implantation) mycoses presenting typical features: lesion beginning at the site of a transcutaneous trauma; chronic evolution associated with survival of the fungal agent and fibrotic reaction; and non protective humoral immune reaction. In tissues all etiologic agents form thick-walled, dark multiseptate structures, the muriform (sclerotic) cells. CBM lesions are considered a biologic adaptation enabling the agents to survive in the hostile
host tissue environment. In relation to the site of infection, evolution time, and individual host response, and perhaps the etiologic agent, the primary lesion can evolve to polymorphic skin lesions, including nodular, tumoral, verrucous, cicatricial, and plaque lesions. Nodular, tumoral and verrucous types are more frequent then the cicatricial and plaque-type. In the advanced cases, more than one type of lesion can be observed in the same patient. In addition, lesions can be graded according to their severity. To date there is no data explaining the clinical polymorphism of CBM lesions. Additional studies to further define a probable relation between CBM type of lesion and the causative agent are needed. In the future, protocols on this disease should include clinical, epidemiological, histopathological and respective isolates from patients recruited in different endemic areas.

MO-02-2 ISHAM Working Group: Chromoblastomycosis

Genetic diversity and species delimitation in the opportunistic genus *Fonsecaea*

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Genetic diversity and species delimitation were investigated among 39 isolates recovered from clinical and environmental sources in Central and South America, Africa, East Asia and Europe. All had been morphologically identified as *Fonsecaea* spp. Molecular analyses were based on sequences of the ribosomal internal transcribed spacers (ITS), b-tubulin (TUB1) and actin (ACT1) regions. A phylogenetic approach using haplotype networks was used to evaluate species delimitation and genetic diversity. The presence and the modes of reproductive isolation were tested by measuring the index of differentiation (ID) and the index of association (IA). Based on the sequence data, 39 *Fonsecaea* strains were classified into three major entities: (i) a group representing *Fonsecaea* pedrosoi, (ii) a second composed of F. monophora, and (iii) a third group including mostly strains from South America. The two major, clinically relevant Fonsecaea species, F. monophora and F. pedrosoi, also differed in the pathological symptoms found in patients. Moreover, F. pedrosoi is mostly recovered in clinical settings, whereas F. monophora is commonly isolated from the environment. One environmental strain with *Fonsecaea*-like appearance was shown to belong to a different species, only distantly related to the core-group of *Fonsecaea*.

MO-02-3 ISHAM Working Group: Chromoblastomycosis

Overview of the recent work in antifungals with strains isolated from patients with chormoblastomycoses

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Chromoblastomycosis is a subcutaneous fungal infection, usually an occupational related disease, mainly affecting individuals in tropical and temperate regions. The most common etiologic agents described are Fonsecaea pedrosoi and Cladophialophora carrionii. However new species has been described especially in cases recently reported in China. There is no treatment of choice for this mycosis, being the conventional drugs used itraconazole and terbinafine. The new azole, posaconazole could be a promising agent, due its activity against black fungi. Alternatively, drug combinations could helps. In vitro tests have been performed, and the MICs of agents such those described above show good activity in vitro. Thus, it is important to search for cases, obtain the clinical data and collection of strains to test their susceptibility and follow up the patient during treatment to establish the outcome. New strategies and new tools are needed such correct identification, virulence studies, immunology findings, routes of infections in order to study more deeply this neglected disease.

MO-02-4 ISHAM Working Group: Chromoblastomycosis

A chronic chromoblastomycosis model by *Fonsecaea monophora* in Wistar rat

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Chromoblastomycosis is a chronic, cutaneous and subcutaneous infection characterized by verrucose lesions, mainly caused by Fonsecaea monophora in southern China, and poorly responds to available therapies. For investigating the pathogenicity of Fonsecaea monophora, we established a chronic chromoblastomycosis model with *Fonsecaea monophora* in Wistar rats. The suspensions of 2×10^6cfu conidia and fragment hyphae were injected by intracutaneous (ic) and subcutaneous (sc) routes at either side back of Wistar rats. Small nodules were formed at the inoculation sites in the first week after inoculation. In the second week, the nodules enlarged and became soft, and pus could be aspirated from the nodules. In the forth week, the nodules in ic group ulcerated and sclerotic bodies were observed in pus smear by both inoculation routes. In the 3rd month, the nodules by ic route became flat with thin black crust on the surface. For ic group, sequential biopsy revealed the extensive necrosis with neutrophil infiltration and sclerotic bodies and some debris of fungi around in the 1st month; sclerotic bodies inside multinucleated giant cells in the 2nd month and widespread granulomatous inflammations in the 3rd month.

This study presents a promising animal model that can be used to investigate the pathogenicity of the different etiologic agents, the immune response of the host involved in the pathogenic process and to explore the effective antifungal agents for chromoblastomycosis therapy in vivo.

MO-03-1 ISHAM Working Group: Rhinosinusitis

Categories of fungal rhinosinusitis including the problem of AFRS/EFRS/EMRS

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Though rhinosinusitis is a common disorder and acute rhinosinusitis is well categorized, controversies surround categorization of chronic rhinosinusitis (CRS) and the role of fungus in CRS. American Academy of Otolaryngology Head & Neck Surgery and other related societies through a workshop in 2004 attempted a consensus and divided rhinosinusitis into four categories: acute rhinosinusitis, CRS without polyps, CRS with polyps, and allergic fungal rhinosinusitis (AFRS). However the definition of AFRS has faced great challenge in recent years with the description of fungi in eosinophilic mucin independently from Type I hypersensitivity in most cases of CRS. This condition is named as Eosinophilic fungal rhinosinusitis (EFRS). Confusion in this classification is further increased when Ferguson in 2000 claimed that eosinophilic mucin could be present and cause rhinosinusitis without the presence of fungi and named the entity as eosinophilic mucin rhinosinusitis (EMRS). She speculated that EMRS is a systemic disease with dysregulation of immunological control where eosinophilic mucin could be present without the presence of fungi. Though much confusion exists regarding classification, the most commonly accepted system divides fungal rhinosinusitis (FRS) into invasive and non-invasive diseases based on histopathological evidence of tissue invasion by fungi. The invasive disease include a) acute invasive, b) granulomatous invasive, c) chronic invasive. The non-invasive disease include d) localized fungal colonization, e) fungal ball, and f) fungus-related eosinophilic FRS that include AFRS/EFRS. Extending the hypersensitivity process in causation of AFRS some workers demonstrated consistent presence of AFRS and ABPA in the same patients and named the process as Sino-bronchial allergic mycosis (SAM syndrome). All these controversies in the definition of the categories of FRS have emphasized the need of collaborative work and exchange of findings among people working in this field. ISHAM has convened a working group on 'Fungal Rhinosinusitis' to attempt consensus on terminology and disease classification.

MO-03-2 ISHAM Working Group: Rhinosinusitis

Special staining techniques to identify fungi in fungal rhinosinusitis

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Fungi as cause of diseases of the nose and the nasal sinuses are known since more than a century. These comprise saprophytic colonisation, fungal ball, hypersensitivity reactions and invasive fungal sinusitis.
Some 25 years ago, the form of allergic fungal rhinosinusitis was first described, and since a decade the role of fungi in triggering chronic rhinosinusitis (CRS) or eosinophilic fungal rhinosinusitis (EFRS) is discussed intensively.
Whereas molecular tools and culturing techniques are the most reliable methods to show the presence of fungi with the possibility to identify them, staining techniques offer the opportunity to study these fungi in situ. Only histological preparations show the presence of mucus invasion in invasive fungal rhinusinusitis or the interaction of fungal hyphae and eosinophilic granulocytes in EFRS.
To comply with these requirements novel fungal staining techniques based on immunological reactions were developed recently. These will be presented and discussed in the working group meeting.

MO-03-3 ISHAM Working Group: Rhinosinusitis

Chronic rhinosinusitis: In immune response to fungi

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Chronic rhinosinusitis (CRS) presents histologically as an underlying, damage inflicting eosinophilic inflammation. Eosinophils are understood to play a role in the host defense against parasites, and one of the key questions was whether eosinophils play a similar role against certain airborne fungi.

After demonstrating through novel methods the presence of fungal organisms in virtually every upper respiratory track, it was found that CRS patients' immune system produces the cytokines which are crucial for the eosinophilic migration (IL-13) and activation (IL-5) when it recognizes certain common airborne molds, especially Alternaria spp. This immune response was in striking contrast to its absence in healthy controls, and independent from an IgE mediated allergy.

On CRS histology, we found that eosinophils actually migrate from the tissue into the sinus mucus and attack extramucosal fungi through the release of their toxic Major Basic Protein (MBP), which destroys the fungi but also severely damages the sinus mucosa, leading to CRS. MBP is now used for diagnostic purposes, since its presence is in the sinus lumen indicates that fungi are being attacked by eosinophils, and antifungals are used to downregulate this inflammatory response.

When multiple fungi were tested, only Alternaria spp. demonstrated an ability to induce degranulation of eosinophils. The fraction from Alternaria alternata, which induced the degranulation, had a molecular weight of ≈ 60 kDa, was highly heat labile, and worked protease dependant through a G protein-coupled receptor. Other fungal antigens, including Aspergillus, Cladosporium, and Candida, did not induce eosinophil degranulation, suggesting the presence of a fungal species and cell type specific novel innate immune response to certain fungi in human. Thus, both innate and acquired immune responses to environmental fungi, such as Alternaria may increase production of the cytokines and provide cellular activation signals necessary for the robust eosinophilic inflammation in CRS patients.

MO-03-4 ISHAM Working Group: Rhinosinusitis

Fungal rhinosinusitis - a categorization and definitional schema

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Background: Fungal (rhino-) sinusitis encompasses a wide spectrum of immune and pathological responses, including invasive, chronic, granulomatous and allergic disease. The recent descriptions of eosinophilic fungal rhinosinusitis (EFRS) in patients with severe bilateral disease with nasal polyps and fungal hyphae visualized in mucin from the sinuses, has illuminated the pathogenesis and thrown up major challenges to disease classification. However, consensus on terminology, pathogenesis and optimal management is lacking. The International Society for Human and Animal Mycology (ISHAM) convened a working group to attempt consensus on terminology and disease classification.

Working Party conclusions: **'rhinosinusitis'** is preferred to 'sinusitis'; **'acute invasive fungal rhinosinusitis'** is preferred to 'fulminant' or 'necrotizing' and should refer to disease of <4 weeks duration in immunocompromised patients; both **'chronic invasive rhinosinusitis'** and **'granulomatousr hinosinusitis'** were useful terms encompassing locally invasive disease over at least 3 months duration, with differing pathology and clinical settings; **'fungal ball of the sinus'** is preferred to either 'mycetoma' or 'aspergilloma' of the sinuses; that **'localized fungal colonization of nasal or paranasal mucosa'** should be introduced to refer to localized infection visualized endoscopically, which is not (yet) invasive or a fungus ball; that the term **'eosinophilic mucin'** is preferred to 'allergic mucin'; that the terms 'allergic fungal rhinosinusitis' (AFRS), 'eosinophilic fungal rhinosinusitis' and 'eosinophilic mucin rhinosinusitis' (EMRS) are imprecise and require better definition. In particular to implicate fungi (as in AFRS and EFRS), hyphae must be visualized in eosinophilic mucin, but this is often not processed or examined carefully enough by histologists, reducing the universality of the disease classification. A schema for sub-classifying these entities, including aspirin-exacerbated rhinosinusitis, is proposed allowing an overlap in histopathological features and with granulomatous, chronic invasive and other forms of rhinosinusitis. Recommendations for future research avenues were also identified.

MO-04-1 *Lacazia loboi* infections in humans and dolphins

Human *Lacazia loboi* infection

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Jorge Lobo's disease (Lacaziosis), is caused by *Lacazia loboi* an uncultivated fungus inflicting cutaneous and subcutaneous infections and rarely visceral involvement in humans and dolphins. *Lacazia loboi* is restricted to Mexico, Central America, and South America. However, three imported cases diagnosed in Europe and North America has been also recorded. Little is known about the pathogenesis of the disease. Based on experimental human animal inoculation it is believe that the disease could be acquired after skin trauma and exposure to *L. loboi* propagules. Environmental characteristics such as places with large forest and river and host features such as age, sex, ethnicity, occupation, immune status may also play a role. The disease in humans is characterized by the development of cutaneous and subcutaneous parakeloidal lesions. There are more frequent on the head and limbs, although some cases could also develop lesions on the chest, back and the abdominal region. Clinical manifestations of the disease include the typical para-keloidal lesion with a hyperergic stage, which includes the macular, gummatous, and nodular forms and a hypoergic stage, which includes the para-keloidal and the verrucous forms. The differential diagnosis includes chromoblastomycosis, leishmaniais, leprosy, various neoplasias, paraccocidioidomycosis, and other skin diseases. The diagnosis of the disease is made by collecting scrapings of the lesions and by biopsied tissue. The finding of uniform in side yeast-like cells forming chains inside focal granulomas is diagnostic of the disease. Although several authors had claimed successful isolation of this pathogen in culture, these strains were later identified as contaminants of isolates of Paracoccidioides brasiliensis. Thus, culture of *L. loboi* has been so far unsuccessful.

MO-04-2 *Lacazia loboi* infections in humans and dolphins

Evaluation of humoral immune response to *Lacazia loboi* antigens in sera from patients with lobomycosis

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Jorge Lobo's disease is a mycosis of the skin and subcutaneous tissue caused by Lacazia loboi, a fungus that presents phenotypic similarities to Paracoccidioides brasiliensis. Because it resists culture, most previous serological studies have used antigens from P. brasiliensis. The objective of the present study is to evaluate the host humoral immune response to L. loboi yeast-like cells extracted from mice experimentally infected with the fungus. BALB/c mice were inoculated with yeast-like cells extracts obtained from fragments of skin lesions of patients with lobomycosis. Six months after inoculation, the mice developed typical lesions in both foot pads. Mice were sacrificed and the lesions from the foot pads were excised, then macerated in a glass tissue grinder and the fungal suspension was filtered to eliminate debris. The extracted antigens were maintained at - 20°C P. brasiliensis gp43 glycoprotein was isolated from the Pb18 strain and purified from the concentrated crude exoantigen. Western blotting analyses were carried out using sera from 32 patients with lobomycosis, one patient with paracoccidioidomycosis and sera from 5 healthy controls from a non endemic area. The same procedure was performed with sera from 6 infected and 9 non infected mice. IgG antibodies from all patients and mice with lacaziosis detected a ~193kDa antigen. The purified gp43 glycoprotein of P. brasiliensis was detected by the IgG of all evaluated sera, but the IgG in these sera failed to detect the same molecular antigen in the extracts from L. loboi yeast-like cells. Sera from healthy volunteers and control mice did not react with the antigens used. The molecular characterization of the detected antigens, particularly the ~193kDa protein, may be important for the development of new treatments, immunotherapy, vaccine and diagnostic tests for lobomycosis.

MO-04-3 *Lacazia loboi* infections in humans and dolphins

Lacazia loboi in dolphins: A South American origin?

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Jorge Lobo's disease (lacaziosis) caused by Lacazia loboi was first reported in 1930 developing cutaneous and subcutaneous lesions in Brazilian humans. For more than 40 years it was believed that humans were the only affected species. However, 1971 Migaki and co-workers captured a female bottle-nosed dolphin (Tursiops truncatus) in coast of Saratosa, Florida, USA with crusty verrucoid plaques on the tail-fluke skin. Histopathology showed the typical yeast-like cells observed in humans with the disease. After this firs report other species of dolphins in the Atlantic coasts of USA (Florida, Georgia, North Caroline and South Caroline (T. truncates), the Gulf of Mexico (Florida, Texas USA, T. truncatus), France (T. truncates), Surinam (Surinam river Sotalia guianensis fresh water dolphin) and the coast of Brazil (S. guianensis and T. truncatus) have been also reported. In dolphins cutaneous ulcerated verrucouse, and plaque-like that slowly evolve into large lesions are observed. The epidemiology of Lacaziosis in dolphins is not clear, but it is believe to be acquired from water sources. Dolphin to dolphin transmission and the immune status of the infected dolphins has been proposed. Because *L. loboi* is restricted to humans in Central and South America, lacaziosis in dolphins diagnosed outside this area (USA and France) has puzzle investigators. The hypothesis of infected dolphins traveling across de Atlantic Ocean from South America has been challenged by investigators arguing that so far all study dolphins are permanent residents of the studied areas. We have extracted genomic DNA from yeast-Like cells and amplified L. loboi rDNA and protein coding sequences from humans and dolphins with the disease. Our phylogenetic data showed *L. loboi* strains from dolphins clustered together with the DNA sequences from Brazilian infected humans. This data indicate that the *L. loboi* cells collected from dolphins originated from a L. loboi ancestor in South America.

MO-05-1 EORTC/MSG definitions - changes and challenges

EORTC/MSG definitions - changes and challenges

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The Revised Definitions of Invasive Fungal Disease have recently been published by the Consensus Group of the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) (De Pauw et al 2008 Clinical Infectious Diseases 46:1813-21). The original EORTC/MSG definitions have been used extensively in major trials of antifungal drug efficacy, strategy trials, clinical practice guidelines, epidemiological studies and to validate diagnostic tests but were not without their shortcomings. For instance, the original category of possible invasive fungal infection was too permissive labelling cases as such without there being any evidence of invasive fungal infection. The area of diagnostics - high-resolution CT of chest, the detection of galactomannan, beta-D-glucan and fungal DNA had also matured. The original definitions were also restricted to patients with cancer and to recipients of hematopoietic stem cell transplants and there were no criteria for infections caused by less common fungal pathogens. How the revised definitions addressed these issues will be explored in this session. Clearly defining invasive fungal diseases is an evolving process and the challenges that are still unmet will be highlighted and discussed.

MO-05-2 EORTC/MSG definitions - changes and challenges

Challenges of the EORTC/MSG definitions

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The yield of clinical efficacy trials is influenced by the definitions used to characterize the disease under investigation. Eligibility and response criteria need to be carefully chosen. This is particularly relevant when the diagnosis of the disease in question is difficult to establish. Diagnosing invasive aspergillosis (IA) is challenging. We rely on a scoring system that weighs indirect results from tests by their presumed strength of evidence, and consensus criteria for IA are an "area under construction". During the initial development of the criteria in the 1990s results of microbiologic tests were not crucial for establishing a diagnosis, but gained major importance in the revisions of 2002 and 2008. In IA, higher certainty comes with a worsening of prognosis: 2 large trials showed global response rates of ~50%. Patients with proven infection had the worst response rate, followed by those with positive microbiology. Patients without microbiologic evidence had the most favourable response rates. A first trial including only patients with microbiologic evidence yielded low response rates ~35%. The evidence of invasive aspergillosis may become more and more reliable in the absence of treatment, while the physician in favor of early therapy may rarely prove the nature of the disease. There may be a sequence of events from halo sign to positive microbiology to proof through histology. Scientifically, it appears reasonable to treat only those with proven infection, medically one would not wait that long. How can we still run clinical trials? Most recent trials allow inclusion of possible IA, who are then "upgraded" to probable cases through further test results. Using this approach a sufficiently rapid recruitment is still possible. "Upgrading" as a trial strategy is the current compromise between a purist trial design and our clinical reality.

MO-06-1 ISHAM Working Group: Black yeasts

Evolution of *CDC42*, a putative virulence factor triggering meristematic growth in black yeasts

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The cell division cycle gene "CDC42" controlling cellular polarization was studied in members of *Chaetothyriales*. Based on ribosomal genes, ancestral members of the order exhibit meristematic growth in view of their colonization of inert surfaces such as rock, whereas in derived members of the order the gene is a putative virulence factor involved in expression of the muriform cell, the invasive phase in human chromoblastomycosis. Specific primers were developed to amplify a portion of the gene of 32 members of the order with known position according to ribosomal phylogeny. Phylogeny of *CDC42* proved to be very different. In all members of *Chaetohyriales* the protein sequence is highly conserved. In most species, distributed all over the phylogenetic tree, introns and 3rd codon positions are also invariant. However, a number of species had paralogues with considerable deviation in non-coding exon positions, and synchronous variation in introns, although non-synonomous variation had remained very limited. In some strains both orthologues and paralogues were present. It is concluded that *CDC42* does not show any orthologous evolution, and that its paralogues haves the same function but are structurally relaxed. The variation or absence thereof could not be linked to ecological changes, from rock-inhabiting to pathogenic life style. It is concluded that eventual pathogenicity in *Chaetothyriales* is not expressed at the DNA level in *CDC42* evolution.

MO-06-2 ISHAM Working Group: Black yeasts

Molecular diversity of the black yeast *Exophiala dermatitidis*, a neurotropic opportunist in humans

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Exophiala dermatitidis is a fungus causing brain infection in immunocompetent patients in Asia. In CF patients, frequencies of pulmonary colonization vary between 2 and 8%, and have mainly been reported from western Europe. The species has consisted of two preponderant genotypes (***A*** and ***B***) on the basis of rDNA ITS sequencing data. The separaration of genotypes eventually acting as units of evolution was investigated in the potentially pathogenic fungus *Exophiala dermatitidis* using a multilocus analysis of ITS, TUB1 and EF1-α. This differentiation, ultimately leading to ecological speciation processes, are likely to mark the species' transition from its natural habitat as an asymptomatic associate of frugivorous animals in the tropical rain forest to the human-dominated environment, where novel environments are public bathing facilities, railway ties, and eventually humans themselves. Despite phenetic similarities with adjacent species, *E. dermatitidis* has a marked molecular distance from any other member of the clade to which it belongs. Intraspecific variation was moderate, but phylogenetically informative sites were found in ITS and TUB1. Comparing different partitions, standardized index of association (I_A^S) demonstrated recombination between main ITS genotypes ***A*** and ***B***, but there was also a significant degree of clonality. Recombination networks based on concatenated sequences also showed moderate evidence of recombination. Phenetically the genotypes responded differentially on incubation at 37°C We conclude that in *E. dermatitidis* ribosomal genotypes are subject to drift and selection, eventually leading to increased virulence of the human-associated genotype ***A***.

MO-06-3 ISHAM Working Group: Black yeasts

Analyses of the putative secondary structure of the ITS2 RNA of *Herpotrichiellaceae*

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Members of the herpotrichiellaceous black yeasts i.e. Cladophialophora spp., Exophiala Fonsecaea spp., Phialophora spp., Ramichloridium spp., and Rhinocladiella spp., are of medical importance because they can cause a variety of different mycoses whereas some of them could be life threatening. The sequence of nuclear internal transcribed spacer (ITS2) turned out as a useful genetic marker for discrimination of members of the *Herpotrichiellaceae*. Nevertheless, inference of phylogeny using this gene is hampered by difficulties in obtaining reliable alignments was mainly due to lengths variation. Recently numerous algorithms had been proposed to derive putative secondary structures of non-coding RNAs.
We have analyzed the derived putative secondary structures of the ITS2 RNA molecule by applying such programs e.g. Mfold, FOLDALIGN in case of sequences of ex type strains of medically important *Herpotrichiellaceae* (8 genera, 120 species). Thereby it could be shown that the transcribed ITS2 RNA could be folded accordingly to universal 4 domain model recently proposed for Eukarya. Comparative analyses revealed that the observed length differences of the sequences observed were mainly due the length variation of the 4th domain. The highest degree of nucleotide variation was found in the external loop regions. Thereby the distal part of the second domain turned out to represent a species-specific region of about 30 nucleotides which can be used for reliable identification of fungal isolates. This finding is in contrast to *Candida* species where the 3rd domain showed the highest degree of nucleotide variation.

Analyses of the putative secondary structure of the ITS2 enable a reliable alignment and thereby an inference of the phylogeny of this diverse group of medically important fungi. Furthermore it provides inside in the evolution of this molecule and hopefully gives clues for unrevealing structure / function relationships.

MO-06-4 ISHAM Working Group: Black yeasts

Cerebral phaeohyphomycosis due to *Rhinocladiella mackenziei* (formerly *Ramichloridium mackenziei*)

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Cerebral phaeohyphomycosis caused by *Rhinocladiella mackenziei* (C.K. Campb. & Al-Hedaithy) Arzanlou & Crous, comb. Nov. (formerly *Ramichloridium mackenziei*) is extremely rare, and geographically limited to the Middle East. The fungus targets the brain exclusively with a grave prognosis. Eighteen cases have been reported in the literature from the period 1983 to 2004 with almost 100% mortality. Our patient, case nineteen, 2008, presented with a brain abscess while receiving chemotherapy for carcinoma of the breast. Diagnosis was by craniotomy and aspiration of the brain abscess. Direct microscopy showed dematiaceous fungal hyphae. Cultures grew *Rh. mackenziei* and this was confirmed by molecular analyses. Histopathological sections of brain biopsy manifested moniliform hyphae characteristic for phaeohyphomycosis. The patient failed to respond to antifungal therapy with amphotericin B and voriconazole or amphotericin B and posaconazole and finally expired in 64 days after diagnosis. In vitro antifungal susceptibility testing showed this strain to be resistant to amphotericin B while susceptible to itraconazole, voriconazole, and posaconazole. Previously published antifungal susceptibility data indicate that although strains show variable susceptibility to amphotericin B the organism is generally refractory to treatment with this agent. Similar outcomes are seen with the azole agents used alone or in combination with other drugs. Although no specific risk factors have been identified, the majority of cases have occurred in immune compromised individuals. *Rh. mackenziei* is highly virulent agent of serious cerebral phaeohyphomycosis, and should be considered in the differential diagnosis of central nervous system disease in the Middle East.

MO-06-5 ISHAM Working Group: Black yeasts

In vitro activities of conventional and new antifungal drugs against *Rhinocladiella mackenziei* an agent of cerebral phaeohyphomycosis

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Background: *Rhinocladiella mackenziei* is exclusively a central nervous system pathogen with 100% mortality and restricted to the Middle East. Limited in vitro and animal studies suggested that *R. mackenziei* is resistance to amphotericin B and presumably new antifungal drugs with broad spectrum efficacy might be more effective.
Methods: 10 clinical isolates of *R. mackenziei* were obtained from the CBS reference collection. MICs were determined for amphotericin B (AmB), fluconazole (FLU), itraconazole (ITC), voriconazole (VOR), posaconazole (POS), isavuconazole (ISA) or MECs for caspofungin (CAS) and anidulafungin (ANI). Microdilution testing was done in accordance with CLSI M38-A2 guidelines adjusted spectrophotometrically at 530 nm wavelength to optical densities that ranged from 0.17- 0.15 in RPMI 1640 MOPS broth with L-glutamine without bicarbonate. Plates were incubated at 35°C for 96 h. The MIC was determined visually as the lowest concentration of drug showing absence of growth or &≥ 50% reduction of growth (for fluconazole) compared with that of the growth control. For the echinocandins the MEC was microscopically determined as the lowest concentration of drug that leads to the growth of small, rounded, compact hyphal forms as compared with the long, unbranched hyphal clusters that were seen in the growth control well (drug free). Quality control was ensured by including *Paecilomyces variotii* (ATCC 22319), *Candida parapsilosis* (ATCC 22019), and *C. krusei* ATCC 6258.
Results: The MIC_{90} (mg/L) are as follows: AmB 16, FLU 64, ITC 0.25, VOR 2, POS 0.125, ISA 1, CAS 8, ANI 8. AmB, fluconazole and the 2 echinocandins had no activity. In contrast, POS, ITC, ISA and to a lesser extend VOR demonstrated activity against *R. mackenziei*.
Conclusions: Our results are in line with animal data, demonstrating that ITC and POS had the highest *in vitro* antifungal activity against *R. mackenziei*. Isavuconazole seems to have also significant in vitro activity.

MO-07-1 *Pythium insidiosum*

Human pythiosis

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Human pythiosis, caused by *Pythium insidiosum*, was first reported in the world literature from Thailand : subcutaneous infection successfully treated with SSKI. Subsequently, we reported other forms of pythiosis : vascular form, ocular, and disseminated form. In vascular form, patients presented with symptoms and signs of arterial insufficiency with or without prior history of soft tissue infection. Almost all patients affected have thalassemia/hemoglobinopathy syndrome. In contrast, corneal ulcer caused by *Pythium insidiosum* occurs in normal hosts. Farmers who work in the tropical area, exposing to the common habitat of this organisms, are at risk of this infection Practically, fresh KOH preparation revealing large nonseptate, thin wall, right-angle branching hyphae in the above clinical settings is suggestive of this organisms. Regarding treatment, amphotericinB and azole agents have been tried but unsuccessfully in our patients. However, combination of itraconazole and terbinafine was reported to be effective. We have tried this regimen in 1 case of pythium aortitis in which all diseased part cannot be removed and he remains well for more than a year now which is quite promising regarding the historical control of fatal aortic rupture. Immunotherapy with *Pythium insidiosum* vaccine (PIV) has been used to treat our human cases with arteritis with 50% success. In our experience, removal of infected tissue is the key to successful treatment. Almost 20 years have passed but we still know very little about Pythium insidiosum. Many unanswered questions remain to be explored.

MO-07-2 *Pythium insidiosum*

Pythium insidiosum from environmental samples. Epidemiological consideration

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Pythium insidiosum is the causative agent of the life-threatening disease, pythiosis. Human and animal pythiosis occur primarily in tropical and subtropical regions of the world. *Pythium insidiosum* infection is acquired from aquatic areas that contain the infectious pathogen. The epidemiology of *Pythium insidiosum* has been extensively studied in Thailand, an endemic area of human pythiosis. Although the occurrence of human pythiosis has been reported in many regions of the country, the dispersal potential of the organisms, including the natural factors involving the spreading, have never been examined. We described here the natural habitat and epidemiological aspects of *Pythium insidiosum*.

Water and soil samples collected from known endemic areas were examined for the presence of *Pythium insidiosum*. The existence of *Pythium insidiosum* was confirmed by sequencing the ITS region of rRNA gene and constructing a phylogenetic tree. Aquatic cultures of *Pythium insidiosum* and clinical isolates collected from pythiosis patients in the northern and central regions of Thailand were further processed for epidemiological study and genetic structure investigation by using multi-locus microsatellite typing (MLMT) based on the multiplex PCR technique. Nine appropriate microsatellite loci were used.

The results showed that irrigation water, especially water from reservoirs may be an important natural habitat of *Pythium insidiosum*. Six geographic origin-based populations of clinical and environmental isolates showed low to moderate levels of genetic differentiation between samples and little correlation between pathogens' genotype and geography. These data indicate substantial gene flow between the northern and central regions of Thailand. *Pythium insidiosum* probably spreads mostly via thick-walled, resistant oospores (sexual spores). Outcrossing may occur in nature, as no genotypes common to particular regions were detected and there is evidence of recombination within populations. It could be hypothesized that transportation, flood and irrigation systems may be involved in the dispersal of *Pythium insidiosum* across Thailand.

MO-07-3 *Pythium insidiosum*

Diagnosis and treatment of *Pythium insidiosum* infection in animals

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Early and accurate diagnosis of pythiosis is essential for successful treatment of this disease in veterinary patients. Several methodologies have been used for pythiosis diagnosis: microscopic analysis of wet mount preparations, culture, histopathology, and various serologic assays. In 1994 Pan American Veterinary Labs developed a serologic test which delivers accurate results in 1 hour utilizing ELISA technology.

The *Pythium* ELISA detects patient IgG class antibody which binds to a soluble protein antigen in a 96 well plate format. In validation studies the assay has demonstrated >98% sensitivity and specificity. Cross reactivity studies utilizing serum containing antibodies to one or more of a group of common fungi showed less than 0.5% positive reactions in the *Pythium* ELISA. *Pythium* ELISA detected 100% of samples positive by immunodiffusion and 100% of ELISA positive sample were positive by Western Blot analysis.

The *Pythium* ELISA has proven to be a valuable diagnostic tool which has been utilized by > 400 veterinarians to diagnosis some 3000 patients with suspected *Pythium* infection. The *Pythium* ELISA has also proven useful in diagnosing equine respiratory allergic disease due to *Pythium*. Additionally the *Pythium* ELISA has been used to survey canine populations at high risk for *Pythium* exposure.

When *Pythium* ELISA is used to secure an early and accurate diagnosis initiation of PAVL Immunotherapy treatment has demonstrated 90% efficacy in infected equines, 50% efficacy in infected canines and 80% efficacy in equines with *Pythium* allergy.

MO-08-1 ISHAM Working Group: Fungiscope

Principles of collaboration: Authorship and local groups

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Fungiscope is a global database collecting clinical specimens and data on rare invasive fungal infections. Research on rare diseases usually requires the involvement of a diverse group of contributors from different countries and institutions to pool a sufficiently large amount of clinical cases. Once the time of publishing results draws near, assignment of authorship positions can be problematic and often results in disappointment for many contributors, particularly if transparent agreements have not been established in advance. In order to guarantee adequate and maximum distribution of authorship positions for all Fungiscope publications, we have worked out a set of rules, including a point system.

In general, it is intended to publish each subset of the Fungiscope cohort at a time, preferably by pathogen. Authorship will be restricted to those centers contributing patients or translational work to the subset published. If the journal chosen for publication should have a limit on maximum authorship positions available, the point system will be used to select the most active contributors. Points are distributed for each clinical case entered and each isolate or biopsy added to our specimen collection. Local coordinators can gain additional points by raising the participation in their countries.

Apart from making publication of our collected data more transparent, we would also like to encourage ideas of local groups on potential publications. All groups are invited to use the Fungiscope specimen collection for their research ideas. Particularly projects involving molecular methods are welcome.

MO-08-2 ISHAM Working Group: Fungiscope

ClinicalSurveys.net - the technology behind Fungiscope

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Fungiscope is the first project to use the technology of ClinicalSurveys.net, a new research portal created by researchers of the University Hospital of Cologne to allow infectious disease specialists from around the world to contribute to a growing number of case registries. The ClinicalSurveys.net technology allows easy and secure access to the database and quick registration of new cases. In this talk, we give a brief introduction to the technology, demonstrate the registration and data-entry process and discuss input and experience of the audience.

MO-08-3 ISHAM Working Group: Fungiscope

Under the Fungiscope - Zygomycetes

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Introduction
Within Fungiscope, our global database for rare invasive fungal infections, infections with a Zygomycete account for a major proportion of all registered cases. In this analysis, we would like to present data from this particular subgroup.

Methods
Patients with cultural, histopathological, antigen, or molecular biologic evidence of invasive fungal infection may be included into the study. Those with infections due to Aspergillus spp., *Candida* spp., Cryptococcus neoformans, Pneumocystis jiroveci or any endemic fungal infection, such as coccidioidomycosis or histoplasmosis, as well as colonization or other non-invasive infections are excluded. Data entry is accomplished via a web-based electronic case report form.

Results
By now, 35 patients with a zygomycosis have been documented. The most common underlying conditions were chemotherapy for a hematologic malignancy (34 %, n=12), hematopoietic stem cell transplantation (31 %, n=11), major surgery (23 %, n=8) and/or immunosuppressive therapy (23 %, n=8). The lungs were the most common site of infection (66 %, n=23), followed by the paranasal sinuses (14 %, n=5) and soft tissues (11 %, n=4). 2 patients displayed disseminated disease. At the latest assessment, complete response to antifungal therapy was observed in 40 % (n=14). The crude mortality rate was 43 % (n=15). 2 patients (6 %) were lost to follow up and in 2 patients (6 %), final evaluation of clinical evolution is still pending.

Discussion
The Zygomycetes account for a large fraction of the so-called emerging fungal infections. Results from a preliminary analysis of this subgroup may help to improve diagnostics and treatment of this patient group.

MO-09-1 ISHAM Working Group: *Pseudallescheria / Scedosporium* infections

Keeping an eye on environmental sources for *Scedosporium* species

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Scedosporium and *Pseudallescheria* species became more and more important as an opportunistic fungal pathogen for patients infected during a near drowning event, by other traumata or those being predisposed by a hematological disorder. The risk of colonization in cystic fibrosis patients is still unclear. Nevertheless environmental sources for exposure of *Scedosporium* spec. are not sufficiently studied. Most of these isolates have not been identified according to the new taxonomy.

Based on the ScedoSelect agar, recently published by Rainer et al., environmental samples from Thailand, Germany, Italy and Israel have been cultivated.

Samples have been taken from wet areas like borders from ditches, streams, puddles und rain water barrels and from cow dung.

So far *P. boydii, S. apiospermum, P. minutispora* and *S. dehoogii* have been found in the environmental samples. The isolation of *S. apiospermum* from salty water in a wellness facility on Ischia / Italy was one of the most spectacular findings.

Ref.: Rainer J, Kaltseis J, de Hoog SG, Summerbell RC (2008) Efficacy of a selective isolation procedure for members of the *Pseudallescheria boydii* complex. Antonie van Leeuwenhoek 93(3):315-322.

MO-09-2 ISHAM Working Group: *Pseudallescheria / Scedosporium* infections

Scedosporium aurantiacum: An emerging pathogen in Australia and New Zealand?

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Scedosporium aurantiacum is a new Scedosporium species. Reports of *S. aurantiacum* infection are as yet, uncommon. To understand the potential of this species to cause disease and to identify predisposing factors for its isolation, we analysed 52 episodes of *S. aurantiacum* isolations in 42 patients in Australian and New Zealand hospitals from 2001-08. Speciation of *S. aurantiacum* was performed by DNA sequencing.

The mean age of patients was 44.4 y (range 5-93); 55% patients were female. The major predisposing factors for isolation were chronic suppurative/obstructive lung disease (58% episodes), diabetes (17%), corticosteroids (12%) and trauma (6%). Eleven (21%) episodes occurred in non-transplanted cystic fibrosis patients and 7 (14%) in lung transplant recipients. The colonization (25 patients): invasive disease (17 patients) ratio was 1.5: 1. All cystic fibrosis patients were colonized. Fifty-four percent of isolates were recovered from sputum/BAL. Among 17 patients with invasive disease, the main sites of infection were lung (n=4), eye (n=4). Three patients each had sinus, inner ear and skin/subcutaneous abscess involvement, 2 had osteomyelitis and one had *S. aurantiacum* recovered from resected cardiac tissue. MIC90 results were: amphotericn, 16 mg/L, itraconazole, 1 mg/L, voriconazole, 0.25 mg/L, posaconazole, 0.5 mg/L. Thirteen patients (invasive disease) received antifungal drugs (all with voriconazole), 9 underwent surgery - 3 had surgery alone. All patients were alive at 90 days following isolation of *S. aurantiacum*. *S. aurantiacum* caused a range of serious infections although the outcome was apparently benign. Clinical variables associated with its isolation include chronic lung disease and cystic fibrosis.

MO-09-5 ISHAM Working Group: *Pseudallescheria / Scedosporium* infections

Osteomyelitis caused by *Scedosporium apiospermum* in an immunocompetent Patient

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Fungal osteomyelitis caused by *Scedosporium apiospermum* is extremely rare. Osteomyelitis of the lower extremities usually occurs only after the soft tissue is extensively involved. Our patient had no cutaneous or subcutaneous infection and no draining sinus was observed. This unusual clinical presentation suggests that deep fungal infection can occur without cutaneous manifestation. This patient's only injury was a left ankle laceration from falling two years earlier. The case was diagnosed by bone biopsy. Cultures grew *S. apiospermum*, which was confirmed by molecular identification. Histopathology sections manifest fungal balls in the ankle bone an unusual setting of the disease, while synovial fluid was negative. *Scedosporium* is a potent pathogenic agent of serious invasive diseases of bone, and should be considered in the clinical diagnosis of fungal osteomyelitis.

MO-10-1 ISHAM Working Group: Zygomycosis, a global registry

Zygomycosis in tropical areas: Experience from India

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Since the first report of zygomycosis in 1963 from India, the incidence of the disease is increasing alarmingly. The increase is largely associated with the rise in number of patients with uncontrolled diabetes in this country. In a recent review of 461 cases of zygomycosis from India, majority (70%) of the cases were reported from a single tertiary care center in north India. This peculiarity may be attributed to better awareness, expertise, and infrastructural facilities for mycological diagnosis in that Institute rather than to a particular regional preponderance in this country. Rhino-orbito-cerebral manifestations were the most common (>50%) presentations in those patients and isolated renal zygomycosis in immunocompetent host is an interesting recognized entity from India. The improvement in awareness of the disease has led to significant improvement in the antemortem diagnosis of those cases. Still a large number of cases with gastrointestinal zygomycosis are diagnosed only on postmortem. The etiological agents encountered from patients with zygomycosis are *Rhizopus oryzae, Absidia corymbifera, Apophysomyces elegans, Saksenaea vasiformis, Cunninghamella bertholletiae, Basidiobolus ranarum, and Conidiobolus coronatus*. Though *R. oryzae* is the commonest agent isolated, *A. elegans* is an emerging fungus in India. *A. elegans* not only produced cutaneous and sub-cutaneous infection, but also caused rhino-orbito-cerebral, renal, and disseminated zygomycosis. The crude mortality rate in patients with zygomycosis remained at ~40% in spite of aggressive management and considerable early diagnosis in recent years.

MO-10-4 ISHAM Working Group: Zygomycosis, a global registry

Molecular methods for the identification and detection of zygomycetes

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Organisms of the class Zygomyctes cause invasive infections in patients with underlying illnesses that include diabetes, bone marrow or solid organ transplantation and renal failure and may have an overall mortality of 76% in patients with pulmonary zygomycosis. Molecular taxonomy of Zygomycetes is in a state of flux but several recent studies are demonstrating the utility of comparative sequence analyses methods in identification of these organisms to genus and species level. Little is known about the genomic diversity of isolates within a species in Zygomycetes. Advances are being made in detection of these organisms directly from body fluids and tissue samples using molecular methods. This presentation will offer an overview of available molecular methods of identification of Zygomycetes isolates both from culture and serum and tissue.

MO-10-5 ISHAM Working Group: Zygomycosis, a global registry

A global registry for Zygomycosis: Results from the first ECMM study and plans for the future

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Zygomycosis has emerged as an increasingly important infection with a high mortality. In 2005, a Working Group was formed under the auspices of ECMM and started a study. The aim of this study was to analyze the epidemiology of this disease in Europe.

Initially, thirteen European countries collaborated in the study. In each country, a national co-ordinator prospectively collected zygomycosis cases, recorded them on specially provided case report forms, which were then sent to the study co-ordinator.

During the period 2005-2007, 212 cases were collected. The mean age of the patients was 50 yrs (range 1-87) and 61% were male. Underlying conditions were hematological malignancies (including bone marrow transplantation) (53%), non-haematological malignancies (5%), solid organ transplantation (5%), diabetes mellitus (10%), trauma (15%), burn (3%), AIDS (1%) and others (8%). The most frequent clinical presentations were rhinocerebral (29%), pulmonary (27%), soft tissue (24%) and disseminated (17%). The isolated fungi were *Rhizopus* spp. (47 cases), *Mucor* spp. (45), *Absidia* sp. (29), and others (24). 51% of patients received only amphotericin B, 6.3% only posaconazole and 31.6% received both.

Mortality was 47.8%. Trauma was related to a significantly lower rate of mortality, compared to other causes ($p=0.003$). Previous administration of voriconazole or caspofungin was related to increased mortality ($p=0.013$ and 0.006, respectively), while on multivariate analysis, factors related to outcome were surgery ($p=0.000$), age ($p=0.037$), site of infection ($p=0.022$) and previous use of corticosteroids ($p=0.035$).

The study is continuing. We have currently collected more than 245 cases. Furthermore, the working group has expanded and is now under the auspices of both ECMM and ISHAM. Our aim is to compare the epidemiology of the disease in Europe to this in other parts of the world.

Poster Forum PF-09

PP-01-1

Phosphorylation regulates polarised chitin synthesis in *Candida albicans*

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Chitin synthesis is essential for growth, viability and rescue form cell wall stress (Walker et al., 2008). In *Candida albicans*, chitin synthesis is achieved by four isoenzymes, Chs1, Chs2, Chs3 and Chs8, each with individual but sometimes overlapping functions. Chs3 is a class IV chitin synthase enzyme and synthesises the majority of the chitin found in the cell wall, as well as the chitin ring at bud sites. It is required for the formation of short, chitin rodlets found in the cell walls of both yeast and hyphal cells (Lenardon et al., 2007). Chs3-YFP is localised to the tip of growing buds and hyphae, and relocates to the site of septum formation just before cytokinesis (Lenardon et al., 2007). An analysis of the *C. albicans* phosphoproteome revealed that Chs3 could be phosphorylated on a specific serine residue. Mutation of this site showed that both phosphorylation and de-phosphorylation are required for the correct localisation and function of Chs3. The kinase Pkc1 was required to target to Chs3 to zones of polarised growth.

References:
Walker LA et al (2008). Stimulation of chitin synthesis rescues *Candida albicans* from echinocandins. *PLoS Pathog* **4(4):**e1000040.

Lenardon MD et al (2007). Individual chitin synthase enzymes synthesize microfibrils of differing structure at specific locations in the *Candida albicans* cell wall. *Mol Microbiol* **66(5):**1164-1173.

Poster Forum PF-08

PP-01-2

Fungal cell wall glycobiology and interaction with the host innate immune system

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The outer layer of *Candida albicans* cell wall is heavily enriched with mannosylated proteins and is the immediate point of contact and interaction with the human host, while the inner cell wall layer contains the structural polysaccharides chitin and β-glucans that have to be unmasked before they can be recognised by immune cells. We have previously demonstrated that *C. albicans MNT1* gene family is composed of five members and that *MNT1* and *MNT2* encode partially redundant α1,2-mannosyltransferases that participate in the *O*-linked mannan elaboration. In order to establish the role of the other Mnt family members, we constructed a series of single and multiple mutant strains and used these to explore their function. Our data indicate that Mnt3, Mnt4 and Mnt5 do not participate in the synthesis of *O*-linked mannans, but that Mnt3 and Mnt5 have redundant phosphomannosy ltransferase activity, and are required for the synthesis of 50% of the cell wall phosphomannan. We also found that *MNT4* and *MNT5* encode proteins involved in the branching of *N*-linked mannan. Cytokine production by human mononuclear cells was markedly reduced when stimulated by *C. albicans mnt3*delta/*mnt5*delta double null mutant. This mutant had a normal β-glucan and mannan content, but had increased chitin levels, suggesting that chitin may attenuate the immune recognition of *C. albicans*. To investigate this, we purified chitin from the *C. albicans* cell wall and found that this polymer blocked proper recognition of *C. albicans* by human mononuclear cells and murine macrophages. Our data indicated that TLR2, TLR4, nor Mincle are involved in the chitin recognition, but its blocking effect is dectin-1 dependent.

PP-01-3

Chemical structure and antigenicity of the cell wall galactomannan from *Malassezia furfur* and *Malassezia pachydermatis*

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Lipophilic yeasts of the genus *Malassezia* are associated with several skin diseases, such as pityriasis versicolor, *Malassezia* folliculitis, seborrheic dermatitis, and atopic dermatitis and are also increasingly associated with catheter-related fungemia. The cell wall components of pathogenic microorganisms behave as an antigen and/or ligand of the innate immune response. Live cells of *Malassezia furfur* and *Malassezia pachydermatis* did not react with the anti-α-1,2-mannoside antibody. However, they showed a strong hydrophobicity and reactivity with the anti-β-1,3-glucan antibody compared with those of *C. albicans*. The cell wall polysaccharides of *M. furfur* and *M. pachydermatis* were isolated and their structures were analyzed by 1D and 2D ^{1}H and ^{13}C NMR experiments. Both polysaccharides were β-1,6-linked linear galactofuranosyl polymers with a small amount of mannan. The presence of galactomannan on the *Malassezia* cells has previously not been described. The galactomannan did not react with the anti-*Aspergillus fumigatus* monoclonal antibody of Platelia *Aspergillus* which has a specificity to β-1,5-linked galactofuranosyl residues. The anti-*M. furfur* antibody strongly reacted with the galactomannans of *M. furfur* and *M. pachydermatis*, but did not react with the galactomannans of *Trichophyton rubrum*, *A. fumigatus*, and *Fonsecaea pedrosoi*. The characteristics of the anti-*M. furfur* antibody suggest that there is a potential for the diagnostic application to *Malassezia* infections by antigen detection in sera or tissues.

PP-01-4

Structural changes in the cell wall mannans of pathogenic *Candida albicans* and other *Candida* species cultured under various stress conditions

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To obtain information about the pathogenicity and the accurate diagnosis of candidiasis, we have been investigating changes in the antigenicity and chemical structure of the cell wall mannans of the pathogenic *Candida albicans* and other *Candida* species cultured under various stress conditions. The obtained changes are summarized as follows.

1. Temperature and pH stresses The mannans prepared from *C. albicans* and *C. stellatoidea* strain cells (yeast and hyphal forms) cultured at high temperature (37°C, body temperature) under acidic (pH 2.0) conditions in various liquid media clearely decreased their reactivity against the factor sera 4, 5, and 6 in the kit 'Candida Check' and contained neither a phosphate group nor a β1,2-linked mannopyranose unit, although the mannan increased the amount of the nonreducing terminal α1,3-linked mannopyranose unit compared to that from cells cultured under conventional conditions (27°C, pH 5.9) in the medium. The mannan of the *C. tropicalis* IFO 0589 strain-formed hyphae at 37°C had significantly lost the β1,2-linked mannopyranose units. The mannan obtained from the *C. glabrata* IFO 0622 strain cells at 37°C had completely lost the non-reducing β1,2-linked mannopyranose unit.

2. Oxidative and osmotic stresses The proportion of the terminal β1,2-linked mannose side chain unit in the mannan increased during the oxidative stress (3.5 mM H_2O_2) condition. The osmotic stress (1.5 M NaCl) induced a slight decrease in the proportion of the β1,2-linked mannose unit in the acid-labile fractions of the mannan.

Based on these results, we propose that the *Candida* species cells significantly changes the antigenicity, namely the cell wall mannan structures under various stress conditions and that sufficient attention to the cultural conditions is needed to perform an accurate diagnosis of the candidias. We also discuss the mechanisms of the changes and a common relation to the pathogenicity and the structures.

PP-01-5

Role and localization of Scw4p in Saccharomyces cerevisiae cell wall

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In the Saccharomyces cerevisiae cell wall more than 20 different mannoproteins were evidenced. They are considered to play different roles in building, maintaining and modifying the wall itself during growth and different cell cycle events and they are important for interactions of cells with their surrounding. Yeasts have evolved three different ways of attaching proteins to cell wall glucan. Some proteins are bound to beta-1,3-glucan noncovalently (Scw ; soluble cell wall proteins; extracted by hot SDS), while others are attached covalently (Ccw; covalently linked cell wall proteins; extracted by glucanases) through GPI-anchor and beta-1,6-glucan, or directly to beta-1,3-glucan by alkali labile ester linkage between the gamma-carboxyl groups of glutamic acid and hydroxyl groups of glucoses (Pir; proteins with internal repeats; extracted by NaOH).
Disruption of genes coding for the Pir-proteins was performed to investigate their potential role. After disruption of all PIR genes, 67kDa protein still remained in NaOH extract. SCW4 disruption resulted in disappearance of a 67kDa band, indicating that Scw4p could also be covalently linked to the cell wall similar to Pir-proteins. Since it was reported previously that Scw4p was a noncovalently attached protein, this finding could be relevant for the role of Scw4p.
In order to get insight in binding to glucan Scw4p was tagged and its localization in different mutants was studied. Besides, effects of different levels of synthesis of Scw4p, as well as its homologs Scw10p and Scw11p on yeast phenotype was studied to investigate their physiological roles.

PP-01-6

Chemical structure difference in yeast and hyphal forms of cell wall mannan of *Candida albicans*

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Candida albicans is a polymorphic fungal pathogen, which is the most common cause of invasive fungal infections in immunocompromised hosts. Hyphae are thought to play an important role in *Candida* tissue invasion. Cell wall mannan could enhance adhesion of *Candida albicans* and activate a wide range of innate immune response. In this study, we sought to analysis the structures of mannan from cell wall of yeast and hyphal forms of *C. albicans* and compare the differences between the structures of the mannan s in the two cell form. Yeast form of *Candida albicans* (ATCC32354) was collected after incubation in YPD at 37 degree for 18 hours, while hyphal form was induced after incubation in RPMI1640 at 37 degree. Alkali-extracted mannan was deproteinated, dialyzed, concentrated, centrifuged and lyophilized, then was further purified by using a DEAE-cellulose column. The eluate was pooled according to the detection by modified phenol-sulfuric acid analysis. Mannan from cell wall of yeast and hyphal forms both showed a single symmetrical peak on high-performance gel-permeation chromatography (HPGPC). Structures were elucidated using monosaccharide composition analysis by gas chromatography (GC), methylation analysis by gas chromatography-mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR) spectroscopy. The results showed that both of mannan from yeast and hyphal form contained a-1,6-linked backbone with a side chain mainly containing a-1,2, a-1,3 linked mannans residues, whereas yeast form contained a longer a-1,2 -linked mannose branch. These data suggested that the structure differences of mannan between yeast and hyphal form may contribute to the virulence differences between these two forms of *C. albicans*.

PP-01-7

Characterization of the *Afu3g08990* gene encoding a GPI-anchored, tandem repeat-rich cell wall protein (CWP) in *A. fumigatus*

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Aspergillus fumigatus is a ubiquitous soil-dwelling fungus and the most common mold pathogen in humans. Therefore, an understanding of the molecular details of *A. fumigatus* virulence and host-pathogen dynamics is urgently needed.
We recently performed a genome-wide analysis of all 9,926 *A. fumigatus* open reading frames to identify those containing internal coding repeats. One of the genes identified in a scan for all GPI-anchored *A. fumigatus* CWPs containing tandem repeats and exhibiting repeat-number variation between clinical isolates was *Afu3g08990* (Levdansky et al. Euk. Cell 6(8):1380-91, 2007). The crucial importance of this gene was pointed out by Balajee et al. (Euk. Cell 6(8):1392-9, 2007) who showed that variations in the *Afu3g08990* nucleotide repeat sequence can be used to type and identify closely related pathogenic isolates of *A. fumigatus* and identify outbreak clusters occurring in hospitals.
Recently, we showed that Afu3g08990p is located on the outer cell wall of the fungus. Deletion of *Afu3g08990* resulted in major morphological changes to the structure of the conidial cell wall. The *Afu3g08990*-deleted strain is highly sensitive to the activity of cell wall degrading enzymes and to mechanical agitation in the presence of glass beads, suggesting that it is significantly weakened. Interestingly, over-expression of Afu3g08990p resulted in delayed germination, slow growth and increased resistance to cell wall degrading enzymes, suggesting that over-expression may harden the cell wall. Suggesting a possible link to pathogenesis, conidia from the *Afu3g08990*-deleted strain were internalized more rapidly and were more sensitive to killing by cultured human macrophages, when compared to *A. fumigatus* and *Afu3g08990* over-expression strain. We are currently examining the role of *Afu3g08990* in pathogenesis. In addition, we plan to analyze the genetic interactions between the *Afu3g08990* gene and additional genes encoding *A. fumigatus* CWPs.

PP-01-8

The influence of β-glucan on the growth and cell wall structure of Aspergillus

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β-glucan is one of the fungal cell wall main component polysaccharide. Also, it was detected in the culture supernatant of fungi such as *Aspergillus* and *Candida* so on. Furthermore, it was reported that β-glucan showed various biological activities such as the inflammatory mediator production in vivo and vitro. However, there are few reports to have examined the influence on fungal cell itself of β-glucan. In this study, it examined how the influence of β-glucan on the growth and cell wall structure of fungal cell.
Aspergillus fumigatus and *Aspergillus oryzae* was cultured with the synthetic medium, C-limiting medium added β-glucan (curdlan and laminarin). In β-glucan adding group on 1day, the promotion of the growth, such as the rise of the turbidity was observed compared with normal culture group. Next, we compared morphological change of *Aspergillus* among these medium by the microscope. In the culture medium added β-glucan, the long hyphae where there is little ramification was observed. The NaClO oxidized cells of the fungus body in BG addition or not cultivation were prepared and their structure analyzed by C^{13}-NMR. In the normal cultivation, β-1,3-glucan was the main component but in the BG addition group, the peak ratio of β-1,3-glucan was rising.
In this study, it was suggested that the presence of β-glucan in culture medium changed the growth of the fungi and induced qualitative change such as the cell wall structure. Because β-glucan are detected in the mycology-culture supernatant, the concerning with these phenomena and the pathogenicity has an interest.

PP-01-9

Genome-wide analysis of *Candida albicans* cell wall remodelling

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Fungal cell walls are dynamic organelles that can alter their structures in response to environmental conditions and cell wall perturbing agents. We were interested in identifying the proteins that are important for cell wall remodelling in response to cell wall defects. A non-gel proteomics approach was used to analyse the proteins that are localised to the *C. albicans* cell wall. Comparisons were made between untreated wild type cells and cells treated with agents that interfere with cell wall integrity including Calcofluor White, Congo Red, SDS and the echinocandin class of antifungal drugs. In addition, the cell wall proteome of signalling pathway mutants and mutants with defective cell walls was analysed. The predicted GPI-anchored proteins Phr1, Pga31 and Sap9 were notable in appearing under cell wall stress conditions but were not detected in untreated wild type cells. Phr1 is a member of the Gas family of transglycosidases that modify cell wall beta-(1,3)-glucan. Pga31 is a novel protein that may play a role in chitin assembly as a *pga31* mutant has significantly reduced chitin levels. Sap9 is a member of the Sap family of secreted aspartyl proteinases but is predicted to be GPI-anchored, analogous to the *Saccharomyces cerevisiae* yapsins, which act as sheddases. Transcript profiling by DNA microarray and Northern analysis confirmed that the expression of *PHR1, SAP9* and *PGA31* as well as genes encoding other predicted GPI-proteins was increased in cell wall stress conditions. One cell wall stress-activated, novel, predicted GPI-protein Pga54 was selected for further analysis by generating null mutant and reintegrant strains and by expression in *S. cerevisiae*.

PP-01-10

Isolation of *Candida glabrata* regulatory elements that affect the sterol transporter *AUS1* -regulated azole susceptibility of cells grown in serum-containing medium

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Sterol uptake in *Saccharomyces cerevisiae* generally occurs only under anaerobic conditions due to aerobic sterol exclusion. In contrast, the pathogenic fungus *Candida glabrata* imports exogenous sterols under aerobic conditions, and thus compromises the antifungal potency of sterol biosynthesis inhibitors, with implications for the clinical management of *C. glabrata* infections. We have determined that *AUS1*, a member of the ATP-binding cassette (ABC) family, is responsible for sterol uptake in this fungus. *C. glabrata* cells grown in a medium containing serum, expressed *AUS1* mRNA and became less susceptible to azoles, whereas cells from a related strain in which *AUS1* was deleted cells were azole-susceptible. In order to understand the molecular mechanism(s) of this sterol uptake-related serum response, orthologues of the *S. cerevisiae* genes which are responsible for exogenous uptake were identified by a search of the *C. glabrata* genome database. We identified Candidate genes that may act as transcription factors of *AUS1*, which were similar to *S. cerevisiae SUT1* and *UPC2*, respectively, as well as closely related genes encoding cell wall mannoproteins harboring serine-alanine-rich proteins (Srp1p/Tip1p family). Deletion mutants of each gene were constructed and assessed for their effect on sterol uptake and azole susceptibility. Phenotypic studies of their null mutants revealed that a putative *C. glabrata* transcription factor *UPC2*, that contains a zinc-finger motif, could promote *AUS1* gene expression. An *UPC* deletant showed a similar phenotype to the *AUS1* deletant for sterol uptake and azole susceptibility. Furthermore, expression of both *UPC2* and *AUS1* were up-regulated in the presence of serum. We conclude that *C. glabrata UPC2* may participate in the serum response pathway underlying sterol uptake.

PP-01-11

Identification of non-coding RNAs in *Candida albicans* using DNA tiling microarrays

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Systemic infections by *Candida albicans* are an important cause of mortality in individuals with impaired immunity. The development of new antifungal compounds is currently limited by the availability of safe targets, a large proportion of the *C. albicans* proteome being conserved in humans. The publication of the *C. albicans* genome has considerably increased the possibilities of genomic and transcriptomic studies, including the discovery of non-coding RNAs. Non-coding RNAs (ncRNAs) are functional RNA transcripts that do not contain an open reading frame and are not translated into proteins. In other eukaryotes, ncRNAs have been shown to regulate important cellular functions such as translation, splicing and silencing. To investigate the role of ncRNAs in the biology of *C. albicans* and possibly uncover new targets, different computational approaches were used to design a focused tiling microarray of 72,000 60-mer probes tiling 1,979 predictions, including 135 positive and 200 negative controls, every 20 nucleotides in both directions (representing 5% of the *C. albicans* genome). To validate the expression of the predicted ncRNAs, we used direct RNA labelling of small RNA-enriched fractions compared to total RNA prepared from strain SC5314 grown at 30°C. This experiment identified the expression of 167 small RNAs shorter than 250 nucleotides, including 105 tRNAs annotated in the *Candida* Genome Database. The remaining ones (62), tentatively assigned as new ncRNAs, were classified in three different groups: i) 60% (37) transcripts found inside a coding region including 2 snoRNAs in intronic regions (62% in antisense); ii) 13% (8) found in the 5'- or 3'-UTR of potential coding regions (87.5% in antisense); and iii) 27% (17) found in intergenic regions. Our results argue in favour of the presence of a significant number of ncRNAs in *C. albicans* and open the possibility of uncovering novel mechanisms that could be exploited to develop antifungal therapies.

PP-01-12

Both transcriptomic and proteomic analysis of the *Cryptococcus neoformans* phospholipase C1 mutant indicates a pleiotropic role for PI-PLC

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Background and objectives: Cryptococcal phospholipase C1 (*PLC1*), encoding a phosphatidylinositol-specific phospholipase C (PI-PLC), is essential for growth at 37°C, cell wall integrity, melanin production (via laccase transcription), secretion of the invasin, phospholipase B1 (Plb1) and virulence. *PLC1* regulates at least some of these phenotypes via activation of the PKC/MAPK signalling pathway. The objective of this study is to further investigate the molecular mechanism of *PLC1* in cryptococcal pathogenesis using comparative transcriptomics (microarray) and proteomics.

Methods: Spotted long oligonucleotide microarray and 2D differential in-gel electrophoresis (DIGE) were performed to compare gene and protein expression profiles of the *PLC1* knockout mutant (*d-plc1*) and wild type (WT) *C. neoformans* strain H99. Overrepresented gene ontologies (GO) were used as a basis for determining correlative phenotypic analysis.

Results: In *d-plc1*, 61% of the 491 differentially expressed (DE) proteins identified showed increased expression, compared to WT (301 up-regulated and 190 down-regulated). Similarly, 68% of the 219 DE genes identified in *d-plc1* were up-regulated (149 up-regulated and 70 down-regulated), indicative of a strong correlation between transcription and translation. Many of the DE genes had roles in secretory processes, cell wall homeostasis and nutrient uptake, supportive of previously published phenotypes including reduced secretion of Plb1, a cell wall integrity defect and compromised growth in *d-plc1*, respectively. Other DE genes have roles in fatty acid biosynthesis, transcription regulation, protease and β-glucanase enzyme activities and α-pheromone production, supportive of *d-plc1* phenotypes now presented.

Conclusion: *PLC1*, encoding a PI-PLC, exerts a pleiotropic effect in *C. neoformans* and its molecular mechanisms are complex. Molecular and phenotypic analyses confirm that *PLC1* has an essential role in secretion which is required for host invasion, cell wall homeostasis and integrity, and nutrient uptake and energy utilization. Further epigenetic study in this secretory pathway is warranted.

PP-01-13

Study of hypoxia response in Cryptococcus neoformans

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Cryptococcus neoformans is an obligate aerobic pathogenic yeast for which oxygen is a growth-limiting nutrient. In order to cause an infection it must be able to adapt to very low oxygen concentrations in the host tissue. Therefore such sensitivity to oxygen must rely on a delicate oxygen sensing system which among others also controls cell proliferation.
Recently, a link between sterol synthesis and oxygen sensing has been established. But the newest findings imply that multiple pathways are involved in oxygen sensing in C.neoformans including an intact respiratory system and iron homeostasis.
Also a unique hypoxia-response has been described in C. neoformans, represented by cell cycle arrest partly in unbudded G2 as well as G1. It is not clear yet whether the pathways already linked to hypoxia response are also responsible for the hypoxia-induced unbudded G2-arrest and what are the molecular mechanisms linking this sensing system to the cell cycle control machinery.
Therefore, we have performed random insertional mutagenesis of C.neoformans by co-cultivation with Agrobacterium tumefaciens harboring kanamycin resistance plasmid and transformants were screened according to hypoxia response with regard to cell cycle progress in order to identify components of hypoxia-induced G2 arrest on molecular level. As the next step, it should be clarified whether there is any reduction in virulence in the strains unable to arrest in G2 in response to oxygen limitation.

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PP-01-14

Conservation of SREBP processing pathway in *Cryptococcus neoformans*

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C. neoformans is an opportunistic fungal pathogen that causes life-threatening meningoencephalitis primarily in immunocompromised patients. *C. neoformans* is an obligate aerobic fungus and its growth is markedly reduced when the fungus is cultured in an environment with oxygen concentrations lower than atmospheric levels. In order to understand how *C. neoformans* responds and adapts to low oxygen conditions, we screened numerous insertional mutants for their growth defects in low oxygen conditions. We have previously established a link between sterol biosynthesis and oxygen sensing in *C. neoformans*. Under growth conditions of low sterol or oxygen, *C. neoformans* homologs of the mammalian SREBP (sterol regulatory element-binding protein) transcription factor and its binding partner SCAP (SREBP cleavage-activating protein) which are designated Sre1 and Scp1 respectively, up-regulate the expression of several genes involved in ergosterol biosynthesis and iron homeostasis. Mutations in *SRE1* and *SCP1* genes resulted in reduced growth at low oxygen conditions in vitro and these strains were unable to cause fulminating CNS infection in mice. In this study, we have characterized six additional genes that are involved in Sre1 processing in *C. neoformans*. Mutations in two of these six genes resulted in a temperature sensitive phenotype. The remaining four genes were found to be involved in oxygen sensing and all but one also contributed to virulence of the fungus. Our findings suggested that the ability of *C. neoformans* to grow at low oxygen conditions is an important factor contributing to the pathogenicity of the fungus and the conserved SREBP processing pathway plays a definitive role in enabling the fungus to adapt and grow in such an environment.

PP-01-15

Functional analysis of genes involved in drug resistance in *Cryptococcus neoformans*

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C. neoformans is a pathogenic fungus that causes cryptococcosis in AIDS patients, people with cancer, and healthy individuals. This disease is generally treated by chemotherapy, and therefore, appearance of the antifungal drug resistant strains has been reported. However, few studies on genes involved in drug resistance in *C. neoformans* have been reported. In *Saccharomyces cerevisiae*, a number of pleiotropic drug resistant (*PDR*) genes, encoding ATP binding cassette transporter, transcription factor, heat shock protein (Hsp) and phosphatidylinositol transfer protein (PITP), have been identified. *PDR13* codes for Hsp70 which elevates Pdr1p activity, a pleiotropic drug resistant regulation factor. *PDR16* codes for PITP, which is regulated by Pdr1p.
In *C. neoformans* genome database, 6 homologues of Pdr13p (designated as *CnPDR131*, *CnPDR132*, *CnPDR133*, *CnPDR134*, *CnPDR135* and *CnPDR136*) and 1 homologue of Pdr16p (*CnPDR16*) were found. The functions of *CnPDR131*, *CnPDR132*, *CnPDR133* and *CnPDR16* were analyzed by gene disruption. The *CnPDR132* deletion strain showed elevated sensitivity against flucytosin, but showed no difference in response to other azole drugs, fluconazole, miconazole and itraconazole, and a polyene antibiotic, amphotericin B. The growth rate, at 20°C, 30°C and 37°C, of the *CnPDR133* deletion strain was slower compared to the wild type.

PP-01-16

Oxylipin studies expose antifungals with dual action in *Candida albicans* and *Cryptococcus neoformans*: A Review

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Cyclo-oxygenases-1/2 (COX-1/2) catalyse the oxygenation of arachidonic acid (AA) and related polyunsaturated fatty acids to endoperoxide precursors of prostanoids. The fungus *Dipodascopsis uninucleata* has been shown by us to convert exogenous AA into 3(*R*)-hydroxy-5Z,8Z,11Z,14Z-eicosatetraenoic acid [3(*R*)-HETE]. 3(*R*)-HETE is stereochemically identical to AA, except that a hydroxy group is attached at its C-3 position. Molecular modeling studies with 3-HETE and COX-1/2 revealed a similar enzyme-substrate structure as reported for AA and COX-1/2. 3-HETE is an appropriate substrate for COX-1 and -2, with a lower activity of oxygenation than AA. Oxygenation of 3(*R*)-HETE by COX-2 produced a novel cascade of 3-hydroxyeicosanoids, as identified with mass spectrometry. Analogous to interaction of AA and aspirin-treated COX-2, 3-HETE was transformed by acetylated COX-2 to 3,15-dihydroxy-HETE (3,15-di-HETE), where C-15 showed the (*R*)-stereochemistry. Evidence for *in vitro* production of 3-hydroxy-prostaglandin E_2 (3-OH PGE_2) was obtained upon infection of HeLa cells with *Candida albicans*. 3-Hydroxy-PGs are potent biologically active compounds. Thus 3-OH-PGE_2 induced interleukin-6 gene expression via the EP_3 receptor (PGE_2 receptor 3) and raised cAMP levels via the EP_4 receptor. Moreover, 3*R*,15*S*-di-HETE triggered the opening of the K+ channel in HTM (human trabecular meshwork) cells, as measured by the patch-clamp technique. Since 3-HETE and 3-OH PG production is inhibited by low concentrations of asetylsalicylic acid (ASA), this NSAID may serve as effective agent to combat *Candida* infection. Similarly, other 3-hydroxy oxylipins, but not 3-HETE, were demonstrated in *Cryptococcus neoformans*. Gas chromatography mass spectrometry analysis confirmed that ASA inhibits mitochondrially produced 3-hydroxy oxylipins in a dose-dependant manner. Transmission electron microscopy in combination with immuno-gold labeling and confocal laser scanning microscopy reveal a novel 3-hydroxy oxylipin release mechanism which is facilitated by capsule associated protuberances.

Ciccoli et al. (2005). Biochem. J. 390: 737-747.
Sebolai et al. (2008). Can. J. Microbiol. 54 (2): 91-96.

PP-01-17

Detection and prevalence of ERG11 gene mutations in clinical *Candida albicans* isolates with reduced susceptibility to fluconazole by rolling circle amplification and DNA sequencing

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Amino acid substitutions in the target enzyme Erg11p of azole antifungal agents contribute to clinically-relevant azole resistance in *Candida albicans*. We developed a novel padlock probe and rolling circle amplification (RCA)-based method to detect point mutations in the *C. albicans* ERG11 gene in eight previously-characterized azole-resistant reference isolates. RCA interrogation of 25 clinical isolates with reduced fluconazole susceptibility, and 23 fluconazole-susceptible isolates was then performed. The results were compared with ERG11 sequence analysis. RCA accurately identified ERG11 mutations in all study isolates and showed good agreement with DNA sequencing. Missense mutations resulting in 20 amino acid substitutions were detected in 24 of 25 isolates with reduced fluconazole susceptibility (prevalence 96%) compared with five amino acid substitutions in 18 of 23 fluconazole-susceptible isolates (prevalence 78%). Substitutions common to both sets of isolates were D116E, E226D, K128T, V437I and V448I. Among strains with reduced fluconazole susceptibility, additional amino acid substitutions included G464S (n=4 isolates), G448E (n=3), G307S (n=3), K143R (n=3) and Y123H, S405F and R467K (each n=1); the novel substitution, G450V was detected in one isolate. There was no clear correlation between the number or character of substitutions and azole MICs but the nature of mutations varied with geographic region. ERG11 mutations in fluconazole-susceptible isolates did not appear to be influenced by recent fluconazole exposure. The sensitive, specific RCA assay showed potential as a simple method for the rapid detection of ERG11 polymorphisms. The estimated prevalence and character of ERG11 mutations in hitherto uncharacterized Australian isolates with reduced fluconazole susceptibility are presented.

PP-01-18

Mutations of CaENO1 affect cell growth, virulence, susceptibilities to drug, and the resistance to sodium chloride in *Candida albicans*

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CaENO1 encodes Enolase, an enzymatic component of the glycolytic pathway in *Candida albicans*, which is the most frequently isolated human fungal pathogen. The protein product was also found to present in the cell wall and to bind host plasminogen. In order to understand its cellular roles other than that in glycolytic pathway, we have performed mutagenesis analysis on CaENO1 by gene-replacement with the SAT1 flipping cassette. Strains lacking CaENO1 were not able to grow on glucose or fructose and they also failed to grow on Bacto-yeast nitrogen base media without amino acid. It has been suggested that CaENO1 may be involved in the susceptibility to fluconazole. Hence, the mutant strains were also subjected to susceptibility tests of various drugs and compounds. It was observed that null mutations affected the susceptibility to amphotericin B and miconazole, in addition to the xx M of NaCl. Hence, CaENO1 were involved in drug susceptibility in addition to its role in carbon utilization. CaENO1 may also be involved in regulation of cell osmolarity or the activities of ion channels. Furthermore, CaENO1 null mutants were avirulent when tested in a mouse model for systemic infection. Together with the observation that CaENO1 is involved in drug resistance, our findings may help to design new and more effective antifungal agents for preventing and treating bloodstream fungal infection since sera also contain glucose.

Poster Forum PF-09

PP-01-19

Interaction of mannooligosaccharides from *Cryptococcus neoformans* and triosephosphate isomerase on *Staphylococcus aureus*

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We found that *C. neoformans* died as a consequence of the adherence of *S. aureus*, and that the death appeared similar to apoptosis via mitochondrial pathways accompanied by decreased actin turnover, enhanced reactive oxygen species (ROS) accumulation, and DNA fragmentation. The glycolytic enzyme triosephosphate isomerase (TPI) on *S. aureus* was identified as the adhesion molecule that recognized mannose residues of equal or greater size than triose in the mannan backbone of glucuronoxylomannan (GXM) on *C. neoformans*. To investigate the binding of TPI and mannooligosaccharides, we purified TPI from extracts of *S. aureus*. After several chromatography purification steps, purified TPI was obtained as a single band on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with silver. The immobilized TPI was seen to interact with GXM in a dose-dependent manner using surface plasmon resonance analysis (SPR). Then, the interaction between staphylococcal TPI and α-(1,3)-mannooligosaccharides derived from GXM was examined. The α-(1,3)-mannooligosaccharides bound to TPI, while monomeric mannose did not. The slopes and shapes of the sensorgrams differed between α-(1,3)-mannooligosaccharides with even (mannotetraose and mannobiose) and odd (mannopentaose and mannotriose) numbers of residues. A kinetic analysis of this interaction revealed that the heterogeneous ligand-parallel reaction model fit, suggesting the existence of at least two binding sites on TPI. α-(1,3)-Mannooligosaccharides larger than triose inhibited the enzymatic activity of TPI in a dose-dependent manner. The binding of staphylococcal TPI and cryptococcal α-(1,3)-mannotriose near the substrate binding site was predicted *in silico*. These results imply that TPI binds mannooligosaccharides with its binding pockets resulting in the apoptosis-like death of *C. neoformans*.

PP-01-20

The antimicrobial peptide LL-37 induces cell death of *Candida albicans* by evoking oxidative stress

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Candida albicans and its related species represent the major fungal pathogen in humans. In the past decades, these fungal pathogens have emerged as the leading cause of the hospital-acquired infections with a high mortality rate. The colonized sites of *C. albicans* are mainly on skin and mucocutaneous surfaces of oral cavity, gastrointestinal tract and vagina. In immunocompromised individuals, *Candida* spp. can cause life-threatening systemic infections. Antimicrobial peptides (AMPs) play important roles in host immunity to against pathogens. Among them, LL-37, a human cathelicidin-derived AMP, is reported to exert its activity to against *C. albicans*. Recent studies have been focused on the damage of *C. albicans* cell surface by LL-37. However, other influence of LL-37 on *C. albicans* cell death is mostly unknown. In this study, the effective dosages and the time of LL-37 treatment led to *C. albicans* cell death were first determined. Using this information, the effects of LL-37 treatment led to cell death were further investigated. Our results indicated that LL-37 triggered intracellular oxidative stress by regulating stress-related genes. Furthermore, a mitogen-activated protein kinase pathway was suggested to be involved in this process. Together, these findings help us to understand the mechanisms of cell death induced by antimicrobial peptides, and may provide important insights for future drug development.

PP-01-21

Does *Candida albicans* sterol composition influence biofilm formation?

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Candida albicans has a high capacity to adhere and grow on different substrates as well as to develop biofilm. This yeast also displays the ability to adapt to various environmental conditions. In a previous study, we reported that different growth conditions (media composition, glucose - 0.9 and 2 %, pH - 5.6 and 7.0, and fluconazole, FLC) could directly influence *in vitro* biofilm formation. Interestingly, variations in pH (5.6 and 7.0) resulted in the most striking differences on biofilm efficiency. We took advantage of these observations to identify some specific genes displaying differential regulations under both conditions and that could therefore impact biofilm development. Earliest microarrays from Garcia-Sanchez *et al.* (2004) and Rossignol *et al.* (2009) had revealed that genes involved in fatty acid metabolism, ergosterol biosynthesis and glycolysis were upregulated in biofilms. Relevantly, our results from microarray screen showed a high variability in expression of more than 200 genes, and confirmed an induction of several *ERG* genes at pH 5.6 with reverse effect at pH 7. From these data, it was tempting to speculate that sterols could play a crucial role in biofilm formation and that pH may be important for ergosterol synthesis. To evaluate this hypothesis, sterol composition from planktonic cells and biofilm was determined as a function of pH. Accordingly, we found that cells grown at pH 5.6 produce less biofilms than at pH 7.0, and displayed lower ergosterol amounts. The role of ergosterol on biofilm formation was confirmed with the use of FLC subinhibitory concentrations. Indeed, cells grown in the presence of FLC could hardly elaborate biofilms at both conditions. Our results demonstrate that sterol composition of biofilm varies depending on the growth conditions considered. Moreover, low ergosterol amounts, which correlate with the accumulation of early intermediates, directly impact biofilm viability and structure.

PP-01-22

Monoclonal antibodies against components of the cell wall of *C. albicans* can mimic the inhibition of adhesion of the fungus to human epithelial cells mediated by human saliva

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Introduction.
Inhibition of adhesion of *Candida albicans* to oral surfaces is considered to be an important strategy in preventing oral candidiasis. *C. albicans* and *Candida dubliniensis* adhere to oral surfaces to form biofilms (Ramaje et al. Rev Iberoam Micol 2008; 25:37-40). The adherence of *C. albicans* to oral surfaces is modulated by different salivary components, including secretory IgA (sIgA), mucins, lysozyme, lactoferrin, peroxidase and histatins.

Objectives.
To assess the role of whole saliva, four saliva-derived preparations and six monoclonal antibodies (mAbs): four IgM (C7, 26G7, 3D9 and 21E6) and two IgG (16B1-F10 and 14-8) directed against components of the cell wall of *C. albicans*, on adhesion of *C. albicans* and *C. dubliniensis* to human epithelial cells (HEC).

Results.
Data obtained by indirect immunofluorescence and Western blotting showed that the saliva samples used in this study contained specific sIgA antibodies against cell wall mannoproteins of *C. albicans* serotype A (NCPF 3153) and, to a lesser extent, against *C. albicans* serotype B (ATCC 90028) and *C. dubliniensis* (NCPF 3949).
C. albicans serotype A and serotype B showed higher adhesion to HEC than *C. dubliniensis*.
Whole saliva was more efficient than salivary secretory IgA partially purified by chromatography, in inhibiting the adhesion of *C. albicans* serotype A to HEC.
MAbs C7, 14-8 and 26G7 were the most potent inhibitors of adhesion.
Our results show that mAbs can mimic the inhibition of adhesion of *C. albicans* to HEC mediated by human saliva.

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PP-01-23

Assessing *Candida* biofilm formation in a new in vivo non vascular model

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More than half of all human microbial infections can be associated to biofilms, highlighting the impact of biofilm on public health. *Candida albicans* can form biofilms on a wide variety of medical devices such as urinary and vascular catheters, but also dental, joint and voice prostheses, pacemakers, ocular lenses. We have optimized an in vivo biofilm model for *Candida* based on a subcutaneous rat model previously described by Van Wijngaerden et al. (1999). Pieces of three-lumen catheters treated with serum are implanted under the skin of rat after an adhesion phase with *Candida* cells. Biofilms, assessed by colony forming units and scanning electron microscopy, reach an optimal size after two days, but they can remain in the host for up to 9 days. Immunosuppression treatment of the animal host increases the reproducibility of the biofilm generated inside the lumens. A critical step in biofilm production is the adhesion phase: incubating cells in RPMI medium instead of Spider or YNB glucose resulted in an increased biofilm formation, not only in wild type but also in strains defectuous in biofilm development in other models, such as als3. Both wild type and als3 can form biofilm in vitro on 96-well plate polystyrene substrate and on polyurethane disk when grown in RPMI, but the mutant does not when grown in Spider. We also observed biofilm formation in vivo with als3, this mutant being able to adhere and grow as hyphae in RPMI. In contrast, cells of the cph1 efg1 strain are not able to form biofilm in in vitro assays in RPMI, and also do not generate biofilm in vivo. Even in absence of constant flow, adherence is crucial to the in vivo subcutaneous biofilm model.

Van Wijngaerden, E., et al. (1999). J Antimicrob Chemoth 44: 669-674.

PP-01-24

Overexpression of the *Candida albicans MSI3* encoding a novel member of the HSP70 family effects on the germination regulated by farnesol

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The pathogenic fungus *Candida albicans* is polymorphic and forms biofilms composed of yeast, pseudohypae and hyphal forms on tissues and catheters. The hyphal development is mediated via cAMP protein-kinase A (cAMP-PKA) pathway and MAP kinase pathway. Farnesol, a quorum sensing (QS) molecule in *C. albicans* inhibits morphogenesis. However, it is still remains to be elucidated how farnesol regulates the yeast-hyphal transition. We have previously isolated *MSI3* as a gene related to the yeast-hyphal transition which belongs to a putative novel member of the heat shock protein 70 family (Yeast. 20:149, 2003). The homologue in *Saccharomyces cerevisiae* is suggested to negatively regulate the cAMP-PKA pathway. Recently, other labs have shown that the expression of *MSI3* is down-regulated in *C. albicans* biofilm that was treated with farnesol.

In the present study, we generated mutant strains in which *MSI3* was controlled under the tetracycline-regulatable promoter to investigate the function of this gene in the QS in *C. albicans*. All mutants showed a growth defect in the presence of doxycycline (DOX), indicating that *MSI3* was essential for cell growth. The expression of *MSI3* in the absence of DOX was 90-fold higher than that in the presence of DOX. No change in heat-shock sensitivity by overexpression of *MSI3* was observed. We further analyzed the effect of the overexpression on germination in a minimum germination-medium containing *N*-acetyl-$_D$-glucosamine (GlcNAc) or glucose plus ammonium chloride. The results showed that high percentages of germination were observed in both media. However, inhibitory effect of germination by farnesol was significantly enhanced in GlcNAc medium, whereas high percentages of germination were observed in glucose medium w/wo farnesol. These results suggest two observations: (i) farnesol targets the hyphal development induced by GlcNAc, (ii) *MSI3* negatively regulates the signaling pathway with farnesol.

PP-01-25

Regulation of Rac1 in *Candida albicans* invasive filamentous growth

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In the human opportunistic pathogen *Candida albicans*, morphological changes are critical for the invasion and infection of host tissues. Rho G-proteins are key regulators of the cytoskeleton and cell morphology in all eukaryotic cells. In *C. albicans* it has been shown that distinct Rho G proteins are required for filamentous growth in response to specific stimuli. Cdc42 is essential for filamentous growth in response to serum, while Rac1 is essential for filamentous growth in an agar matrix. We have identified Dck1, a protein with homology to the Ced-5, Dock180, myoblast city (CDM) family of guanine nucleotide exchange factors. Proteins of this family have been shown to directly activate Rac1 in different organisms. In *C. albicans*, Dck1 is required for invasive filamentous growth, similar to Rac1. Results from in vitro binding and genetic suppression studies suggest that the *C. albicans* Dock180 homologue, Dck1, activates Rac1 during this process. The CDM family of exchange factors typically function in association with an additional protein, a member of the Elmo family. Elmo family members function as scaffold proteins. Here we report the identification of Lmo1, a protein with homology to the Elmo family. We have analysed the role of *LMO1* in *C. albicans* and show that it is also required for invasive filamentous growth. The phenotype of *lmo1* deletion mutant is similar to that of *rac1* and *dck1* deletion mutants. Furthermore, we show an interaction between Lmo1 and Rac1, using a protein pull down assay. Together, these results show that Lmo1 is required for invasive filamentous growth and indicate that Lmo1, Rac1 and Dck1 function as a complex in the same pathway.

PP-01-26

Cell surface expression of adhesins for fibronectin correlates with virulence in *Sporothrix schenckii*

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The virulence of four *Sporothrix schenckii* isolates was compared in a murine model of sporotrichosis, together with the protein pattern of the yeast cell surface and their capacity to bind the extracellular matrix protein fibronectin. Virulence was determined by the mortality rate, fungal burden and histopathology. Two clinical isolates were more virulent for C57BL/6 mice, but no direct correlation was seen between virulence and the clinical or environmental origin of the isolates. Although all isolates could effectively disseminate, the dissemination patterns were not similar. Previous results of our group had shown that S. schenckii uses a paracellular route to transpose the endothelial barrier, a process mediated by extracellular matrix proteins. Consequently, using flow cytometry analysis, we have investigated the interaction of all strains with fibronectin, showing that the binding capacity correlated with virulence. Western blot analysis of S. schenckii cell wall extracts revealed positive bands for fibronectin in the range of 44 to 70 kDa. The 70 kDa protein was identified by MS/MS. This protein was also recognized by a protective monoclonal antibody raised against a gp70 antigen of S. schenckii (mAb P6E7). Confocal microscopy confirmed the co-localization of fibronectin and the mAb P6E7 on the yeast cell surface. To our knowledge, this is the first report identifying adhesins for fibronectin on the surface of this human pathogen. Supported by CNPq, Faperj and Brazilian Healthy Ministry.

Poster Forum PF-08

PP-01-27

Heat shock response in fungi of medical interest

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Heat shock proteins (Hsp) fulfill fundamental functions for the cellular homeostasis and their production is inducible by numerous stressors. They play an important role in inflammatory and immunomodulating processes and may contribute as essential triggers in immunogenic fungi related diseases.

Nine fungi of medical interest including Alternaria alternata, *Aspergillus fumigatus*, Aspergillus terreus, *Candida albicans*, Cladosporium cladosporioides, Penicillium chrysogenum, Saccharomyces cerevisiae, Scedosporium apiospermum and Trichophyton mentagrophytes were investigated regarding their temperature- and heavy metal exposure-dependent heat shock response. Expression of fungal Hsp60 and Hsp70 mRNA was quantified by real-time RT-PCR followed by sequencing and subsequent translation of nucleotide sequences into corresponding amino acids.

Both, Hsp60 and Hsp70 mRNAs were detected consistently in all fungi analyzed. Relative quantification analyses showed a temperature dependent induction of Hsp60 in eight, and of Hsp70 mRNA in six fungi whereas one fungus showed a strong CdCl2 dependent induction of Hsp60 mRNA, 2 fungi responded with an induction of Hsp70 mRNA. Sequencing and translation into amino acids yielded in a similarity of almost 100% with Hsps of related fungi.

The induction of fungal Hsps under stress conditions may support the immunomodulationg role of fungal Hsps, contributing to a better understanding of host-pathogen relationship.

PP-01-28

A method for mating clinical *Candida albicans* isolates

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Candida albicans can be induced to mate in the laboratory. This raises the question of why mating has never been observed in human patients. It also allows, in principle, strains to be crossed for genetic analysis, although the absence of meiosis complicates the interpretation of these crosses. Both the study of *in vivo* mating and the genetic analysis of strains would benefit from efficient methods for mating clinical isolates. Existing mating techniques involve selection by the use of auxotrophic markers, requiring time-consuming sequential disruption of two copies of biosynthetic genes if wild-type isolates are to be crossed. Furthermore, auxotrophy reduces fitness in animal models, and could potentially interfere with assessing the fitness of recombinants in such models. We have developed a method for mating clinical isolates marked with two drug resistance markers, the mycophenolic acid (MPA) resistance-conferring allele of *IMH3* and the nourseothricin (NAT) resistance gene *CaNAT1*, allowing the selection of recombinants on the basis of resistance to both agents. We could obtain, from 6 pairwise combinations of 7 clinical isolates, recombinants, as verified by PCR amplification of mating type loci and drug resistance cassettes and by FACS analysis of DNA content.

PP-01-29

A, B, C genotyping and virulence factors of C albicans strains isolated from patients during episodes of colonization versus infection

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Candida albicans is a common member of the human microbiota and may cause invasive disease in susceptible populations. Besides host immunity, pathogen virulence factors may play an important role in establishing infection. The aims of the present study were to determine A, B, C genotyping (based on the presence of a intron in the 26 S region of rDNA) and to investigate virulence factors (maximum growth rates, adhesion to human buccal epithelial cells, proteinase and phospholipase activity, yeast-to-hypha transition and biofilm formation) of *C. albicans* strains isolated during 2 specific clinical scenarios: a) Group I: patients only colonized by *C. albicans* (17 isolates) and b) Group II: patients colonized by *C. albicans* who developed candidemia (35 isolates). *C. albicans* SC5314 was used as a control strain. Patients belonging to Group I were colonized by a mix of A and C, B and C or A and B genotypes, whereas patients belonging to Group II were exclusively colonized by either A or B genotypes. When analyzing virulence factors of strains which only colonized the patients versus the strains which colonized and caused candidemia, we could not find differences in a regular manner. However, genotype A strains obtained from patients who developed candidemia expressed significantly higher virulence factors when compared to genotype B strains ($p<0,05$). Our findings suggest that: 1) Persistent colonization by a single A or B genotype was more predictive of invasive infection when compared to colonization by more then one genotypes. 2) There was an association between genotype A and higher expression of virulence factors in patients who developed candidemia. Further studies are necessary to clarify if these findings represent a true trend in larger populations.

PP-01-30

Phenotypic relationship between environmental and clinical isolates of human pathogenic *Pythium insidiosum*

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Pythium insidiosum is an aquatic fungus-like organism, classified in the kingdom Stramenopila. It is the causative agent of the life-threatening human and animal disease, pythiosis. Prolonged work in agricultural areas is an important risk factor for *Pythium insidiosum* infection. It has been proposed that irrigation water is a natural source of this organism. Recently, we reported the isolation with molecular identification of *Pythium insidiosum* from irrigation water in northern Thailand. We also hypothesized that the environmental isolates collected from water may be potentially infective propagules. To prove this hypothesis, the phenotypic relationship between clinical and environmental isolates of *Pythium insidiosum* was determined by using (i) temperature growth-assays, (ii) protein and (iii) immunoblot profiling. The profile analyses were based on SDS-PAGE patterns of mycelium-extract and culture filtrate proteins and immunoblot patterns of culture-filtrate protein antigens. The phenotypic analyses enabled us to determine that pathogenic and natural-living *Pythium insidiosum* could not be distinguished by phenotypic criteria. Overall, clinical and environmental isolates were able to grow at 30, 37 and 40°C. The growth of both groups was significantly inhibited at 40°C. For protein and immunoblot profiling, most isolates showed unique patterns; only the patterns of closely related isolates and clonal individuals were similar. Moreover, the study demonstrated that the 68-kDa band of mycelium-extract protein and the 95, 90, 55 and 25-kDa antigens were dominant for clinical strains tested in this study. Several bands of mycelium-extracted proteins and culture filtrate proteins were shared in both groups. These findings showed that the methods contained high discriminatory power and variability for distinguishing heterogeneous isolates. In conclusion, the study revealed (i) high levels of phenotypic variation of *Pythium insidiosum* isolates, both clinically and in nature and that (ii) most environmental isolates are potentially infectious.

PP-01-31

Development of multilocus microsatellite typing (MLMT) system for Rhizopus arrhizus

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Zygomycosis is a rapidly fatal infection caused by fungi belonging to the class Zygomycetes. The emergence of zygomycosis in India is alarming. Rhizopus arrhizus is the most frequent causative agent. Since the molecular genetics of R. arrhizus is very poorly defined and there is no standard molecular strain typing method available for this pathogenic fungus, we attempted to develop Multilocus Microsatellite Typing (MLMT) for R. arrhizus. R. arrhizus genome sequences were downloaded from Fungal Genome Initiative - Broad institute website (www.broad.mit.edu/annotation). All available ORFs and few Intergenic regions were screened for the repeat regions with help of an online bioinformatics tool Repeat Masker (www.repeatmasker.org). We could found twenty microsatellite loci (14 intragenic and 6 intergenic). The primers were designed for all the twenty microsatellite loci and each microsatellite locus was screened for their presence and size variation on polyacryalmide gels in 8 clinical isolates of R. arrhizus. The Intergenic region microsatellites could not be amplified, in most of the isolates tested, possibly due to high sequence variation even in primer binding sites. Finally out of 20 microsatellite loci, the best three intragenic microsatellite loci RA3 (CCT)10, RA8 (GGA)12, and RA11(AG)29 which amplified all strains tested, were selected for analysis of 30 clinical isolates. Sequencing of the three selected loci was done from clinical isolates of R. arrhizus to confirm their presence and to demonstrate whether the size variation is due to variation in repeat numbers. The sequence information demonstrated polymorphism in microsatellites repeat number. Further we assessed the applicability of theses microsatellites loci in strain differentiation, based on the fragment analysis in capillary electrophoresis. Thus we describe the first report of characterization of microsatellites for MLMT of R. arrhizus. This developed MLMT would help us to understand the epidemiology of the disease caused by R. arrhizus.

PP-01-32

Morphological and pigmentation mutants of *Penicillium marneffei* generated by *Agrobacterium*-mediated transformation

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The dimorphic fungus *Penicillium marneffei* causes fatal infections in immune compromised humans. At 25C, this fungus secretes a soluble red pigment and develops mycelia and conidia typical of other *Penicillium* species. At 37C, or upon invasion of host tissue, this fungus undergoes phase transition to form fission yeast-like cells that do not produce the red pigment. Although development of the yeast phase is associated with pathogenesis, molecular analyses have yet to identify the molecular mechanism(s) responsible for the dimorphism of *P. marneffei*.

To better understand the molecular basis of phase transition in *P. marneffei*, we employed an *Agrobacterium*-mediated transformation strategy to randomly mutate genes in this fungus via T-DNA integration. From approximately 7,000 transformants selected at 37C, 35 mutants harboring morphological or pigmentation differences from the wild-type strain were further studied. The T-DNA insertion sites in several of these isolates were identified by PCR-tailing and sequencing methods. BLAST analysis of the integration site in an albino mutant indicated that it was located in *stuA*, a gene previously shown to affect pigmentation in this fungus. A second mutant initially selected as a yeast colony at 37C, but defective in conidiation at 25C, possessed a defect in the gene encoding S-adenosylmethionine decarboxylase. This enzyme is critical to polyamine biosynthesis. Polyamines have been reported to be important in cell differentiation processes including conidiogenesis in *Aspergillus nidulans*. A third mutant did not form true mycelia, but grew as yeast-like colonies at 25C. This isolate possessed a T-DNA insertion in the 3' UTR of the gene encoding U1 snRNP. Collectively, these results demonstrate a useful strategy to search for the genetic basis of morphogenesis in *P. marneffei* as well as its potential use to determine the underlying basis of pathogenicity in this fungus.

PP-01-33

Cloning and Characterization of the Phospholipase B gene from *Malassezia pachydermatis*

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Phospholipase B (PLB) is present in many microorganisms, including pathogenic fungi, and is thought to be an important factor for fungal pathogenicity. For example, PLBs are thought to be a virulence factor associated with the diseases caused by *Candida albicans*. The opportunistic fungus species *Malassezia* is a lipophilic cutaneous microflora commonly found on animal and human skin. *M. pachydermatis* is mainly associated with canine skin diseases and does not show lipid-dependency. Our previous analysis of lipid degradation by the *Malassezia* species revealed that *M. pachydermatis* produces one of the highest extracellular PLB activities among this species. Our work has focused on clarifying the detailed molecular mechanism of *M. pachydermatis* PLB. To date, we have attempted to clone the *PLB1* gene from *M. pachydermatis* genomic DNA (*MpPLB1*). Using degenerate primers based on other PLB sequences from several fungi, we successfully PCR amplified a DNA fragment including part of the *MpPLB1* ORF. This gene consisted of 2124 bp (707 amino acids) and contained a consensus motif G-X-S-X-G that is highly conserved among lipolytic enzymes. It was also deduced that the N-terminal region of *Mp*PLB1 has a 20-amino acid signal peptide for secretion. Compared to other phospholipases, *Mp*PLB1 has high identity with other pathogenic fungal PLBs (34-45% identity).

PP-01-34

Molecular analysis of *Malassezia* microflora in seborrheic dermatitis patients: Comparison with other diseases and healthy subjects

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Malassezia species colonize the skin of normal and various pathological conditions including pityriasis versicolor (PV), seborrheic dermatitis (SD) and atopic dermatitis (AD). To elucidate the pathogenic role of *Malassezia* species in SD, *Malassezia* microflora of SD patients was analyzed using a PCR-based, culture-independent method. Samples were collected by means of tape stripping using Opsite from both the lesional and non-lesional skin of 31 SD patients in the outpatient clinic of the dermatology department. Nested PCR assay using the primers in the rRNA gene indicated that the major *Malassezia* species in SD were *M. globosa* and *M. restricta*, found in 93 and 74% of the patients, respectively. The detection rate and number of each species varied similarly in SD, PV and healthy subjects, whereas AD showed higher values. Real-time PCR assay showed that the lesional skin harbored approximately three times the population of genus *Malassezia* found in non-lesional skin, and that *M. restricta* is a significantly more common species than *M. globosa* in SD. Genotypic analysis of the rRNA gene showed that the *M. globosa* and *M. restricta* from SD patients fell into specific clusters, and could be distinguished from those collected from healthy subjects, but not from those colleted from AD patients. Our results indicate that certain strains of *M. restricta* occur in the lesional skin of SD patients.

PP-01-35

The transcription factor AfPrtT regulates the expression of key secreted proteases in *Aspergillus fumigatus*

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Aspergillus fumigatus is the most common opportunistic mold pathogen of humans, causing invasive diseases in immunocompromised patients. In these patients, the fungus can invade the lung and any other organ, causing severe damage. Penetration into the pulmonary epithelium is a key step in the infectious process. *A. fumigatus* uses extracellular proteases it produces to degrade the structural barriers of the host. To date, studies conducted on single or double mutants of major secreted proteases did not attenuate virulence. Here, we take a comprehensive approach in which the secretion of numerous proteases is impaired due to the deletion of a putative transcription activator *AfPrtT*. *AFPrtT* shows similarity to the fungal Gal4-type Zn(2)-Cys(6) DNA-binding domain of several transcription factors. An *AfprtT* disruption mutant was generated in our lab. Azocasein and azocollagen degradation assays show that *AfprtT* mutant culture filtrate is completely devoid of proteolytic activity. Nitrogen /Phosphate starvation increased proteolytic activity of the wild type but not the *AfprtT* mutant, suggesting that PrtTp is essential for protease activity under those conditions. XTT and hemacolor assays show that culture filtrate of the *AfprtT* deficient strain has greatly reduced killing activity against A549 lung pneumocytes. Furthermore, culture filtrate of the *AfprtT* mutant exhibited 10-fold less hemolytic activity on sheep red blood cells than the wild type.

We are currently in the process of assessing the expression profile of some of the most important *A. fumigatus* proteases in this mutant using RT PCR and northern blotting, as well as assessing the virulence of the *AfprtT* mutant strain using several mouse models of invasive pulmonary aspergillosis.

PP-01-36

Targeting the oligopeptide transporter (OPT) family of *Aspergillus fumigatus*

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Pathogenicity of the opportunistic human pathogen *Aspergillus fumigatus* appears to be a multifactorial trait, and several fungal attributes have to be taken into account to support aspergillosis. Upon pulmonary infection, which may eventually result in invasive and disseminated disease forms, fitness and growth of the pathogen is crucial, which relies on accurate nutritional support. During the infection process A. fumigatus must mobilise and utilise nutrients that are present at the site of infection. Previous research has indicated that inside the lung the primary nutrient source might be host proteins.

This study concentrates on the uptake of host protein degradation products, in particular of oligopeptides. In fungi, these are translocated into the cell by conserved oligopeptide transporters (OPT), which are encoded in A. fumigatus by an eight-member gene family (optA-H).

Here we describe a first functional characterization of the A. fumigatus OPT family. Transcript profiles of each member were deduced on various media to assess gene-specific expression patterns on varying nutritional sources. Single knock-out mutants for each individual opt gene were created, as well as multiple deletions by repetitively opt gene targeting via the loxP-Cre system. Eventually, this resulted in an octuple deletion mutant lacking every member of the opt gene family. Additionally, both genes encoding dipeptidyl-peptidase activities (dppD and dppE) were targeted, as these might play a role in further breakdown of extracellular oligopeptides, and combinatorial deletants were included in the comprehensive phenotype characterisation studies.

Our data will shed light on the role of A. fumigatus oligopeptide transport in host colonisation, which may prove valuable in the development of novel strategies for antifungal therapy.

PP-01-37

Microtube-like projections of *Cryptococcus gattii* - Unique virulence attributes or structural anomalies?

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Cryptococcus gattii infects healthy people and immunocompromised patients worldwide. *C. gattii* differs from *C. neoformans* in phenotypes, natural habitat, epidemiology, clinical manifestations, and possible response to therapy. We have investigated *C. gattii* genes involved in oxidative stress, mating, and secretion, which revealed unique differences between *C. gattii* and *C. neoformans*. Recently, we hypothesized that *C. gattii* has distinct environmental attributes. We adapted model plant (*Arabidopsis thaliana*) to create reproducible scratch wound *C. gattii* growth/infection in plants grown in a customized chamber at 20^0 -23^0C with 12 hour light/dark cycles. We created a gene knockout *C. gattii* acapsular mutant (cap59) to compare role of capsule. *C. gattii* cells were also grown on media with hard woods to simulate likely natural growth in tree hollows. Parallel comparisons of virulence were carried out using a BALB/c murine model of cryptococcosis and fungal- human phagocyte assays. *C. gattii* parent strain colonized and spread over plant leaves in an organized cellular community. The cells were connected to each other and to plant surfaces via unique projections. These 'microtubes-like projections' measured 0.5 &mum - 2.0 &mum in length and 0.1 7mum- 0.4 &mum in diameter, and originated in the cytoplasm. Their assembly was variously inhibited by prior treatment with drugs and chemicals that disrupt cytoskeletal elements. Similar 'microtube-like projections were also formed on agar media containing various hard woods. Acapsular mutant of *C. gattii* did not exhibit robust growth and failed to produce projections. *C. neoformans* cells survived and grew in scratch-wounded *A. thaliana* plants, but they did not make 'microtube-like projections'. *C. gattii* cells with 'microtube-like projections' exhibited significantly more virulence in mammalian models. In summary, a unique of *C. gattii* structural attribute was observed in growth on plants and plant-derived materials, which might yield clues about distinct virulence mechanisms in this pathogen.

PP-01-38

Multilocus sequence typing of Cryptococcus neoformans var. grubii from Thailand

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Multilocus Sequencing Typing (MLST) was applied to investigate the genotype variation of human, animal, and environmental Cryptococcus neoformans isolates from Thailand collected between 2003 - 2005. Nine major subtypes (A to I) were observed when 481 C. neoformans var. grubii isolates were initially typed using M13-fingerprinting, 31 isolates representing the nine subtype were selected for MLST analysis, using 7 loci, including the following housekeeping genes: ACT1, SOD1, TEF1, IGS1 and URA5 and virulence genes: CAP59, LAC1 and PLB1. The genes were amplified by PCR using locus specific primers. We found 13 STs among the 31 representative isolates. Comparing the obtained sequences of the 7 loci with the MLST database at www.mlst.net, 15 new alleles were identified. Phylogenetic analysis using the combined data from www.mlst.net and the herein obtained data clustered all Thai isolates in the same clade. In addition, genetic relationships with global isolates were demonstrated. The results of M13-fingerprinting were in concordance with the one obtained by MLST analysis. The discriminatory power for M13-fingerprinting was 0.75 and for MLST was 0.86. In conclusion, 13 STs. were found amongst C. neoformans var. grubii isolates from Thailand.

PP-01-39

Evaluation of phospholipase activity of Cryptococcus neoformans and Cryptococcus gattii and its purification

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Infections caused by the fungus Cryptococcus neoformans (Cn) is potentially fatal. Phospholipase activity (PLA) is considered a virulence factor related to pathogenicity of *Candida albicans*,*Aspergillus fumigatus* and Cryptococcus neoformans. Out of the twenty clinical isolates tested, four belonged to variety neoformans (Cnvn), twelve grubii (Cnvg) and four to C. gattii (Cg). All the isolates showed PLA and Pz determined. The optimum pH for extracellular phospholipase of Cn and Cg was between 4.0 to 5.0 (acidic) and optimum temperature for Cn was 37 degree C and 40 degree C for Cg. PLA was inhibited by Fe3+ions, palmitoyl carnitine and Triton X-100 and it could hydrolyse major membrane phospholipids. The secreted extracellular phospholipases of Cn and Cg were characterized as complex enzymes made up of phospholipaseB (PLB), lysophospholipase (LPL) and lysophospholipase transacyclase (LPTA).Purification of phospholipase was successfully achieved by Ion exchange and gel filtration chromatography for Cnvg yielding a single band of 70KDa on SDS-PAGE analysis,whereas Cnvn showed two bands of 43KDa and 70KDa. Attempts to purify the phospholipase of Cg was not successful using the same protocol. The study describes the purification and properties of phospholipase,which may be involved in virulence from pathogenic fungi.

PP-01-40

Phylogenetic relationships of Thai Pythium insidiosum isolates using cytochrome oxidase II sequences

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Pythium insidiosum is the causative agent of pythiosis in humans and both domestic and pet animals. The vascular and ocular forms in the human are common whereas local subcutaneous and cutaneous manifestations are mostly reported in animals. Recently the evidence of this aquatic parafungus from irrigation areas in Thailand was unconcealed. The objective of this study is to investigate the phylogenetic relationship of P. insidiosum isolated from human and environment sources in Thailand based on cytochrome oxidase II (cox II) gene. Nineteen isolates from these two sources were used. The genomic DNAs from these strains were used as template for PCR. The PCR product of partial cox II gene, 613 base pairs, was analyzed by Program Bioedit (V7.0.9.0). The Phylogenetic tree inferred from genetic distance using Neighbor joining (NJ) analysis was performed by program MEGA (V4.1). The strengths of internal branches of this tree were statistically tested by bootstrap analysis of 10,000 replications. The other species, Pythium aphanidermatum, Pythium catenulatum, Pythium deliense including Basidiobolus meristosporus were fetched from the data base to construct the phylogenetic tree. Three clusters, A, B, and C, similar to those encountered in previous studies were found. Only the isolate from Equine, Costa Rica is in Cluster A whereas other isolates regardless sources are classified in Cluster B & C. Comparing to the those reports based on the ITS analysis, Thai isolates from clinical cases and environmental sources in Thailand were classified in both cluster B and C. Our study suggests the cox II gene is a good gene to analyze the genetic relationship of this emerging aquatic pathogen. This is a preliminary study that explores the use of the coding gene cox II to investigate phylogenetic relationships in P. insidiosum.

PP-01-41

Study on the molecular characteristics of Trichosporon inkin

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To study the molecular characteristics and intraspecific genotyping of Trichosporon inkin strains which isolated from different sources, internal transcribed spacer (ITS) and the intergenic spacer 1 (IGS1) regions of rRNA gene sequencing, PCR-restriction fragment length polymorphisms (PCR-RFLPs), and random amplified polymorphic DNA (RAPD) of genomic DNA were performed. The ITS and IGS1 sequence homologies of different strains of T. inkin were reach up to 100%, while macroretriction maps of PCR-RFLP were concordance intraspecies. The map of RAPD displayed intraspecific variability in some degree. Our researches demonstrated that the sequencing of IGS1 and PCR-RFLP of rRNA gene are suitable to identify the species of T. inkin, while RAPD of genomic DNA is more suitable to intraspecific genotyping.

PP-01-42

Fungal glucosylceramide plays an important role in the hyphal growth of the pathogenic yeast *Candida albicans*

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Glucosylceramide (GluCer) is present in most eukaryotic organisms. Several pathogenic fungi share a canonical GluCer structure with (*E*, *E*)-9-methylsphinga-4,8-dienine as a sphingoid base, carrying (*E*)-double bonds at the delta4- and delta8-positions and a methyl branch at the C9-position. A prime structural feature that distinguishes the fungal glucosylceramides from those of plants and animals is a methyl group at the C9-position of the sphingoid base. Previously, it has been reported that antibodies against this fungal GluCer inhibit the cell differentiation of *C. albicans* and *Pseudallescheria boydii*. Since the budding yeast *Saccharomyces cerevisiae*, which is often used as a fungal model, is not capable of synthesizing GluCer, the functional and physiological roles of this sphingolipid in fungal kingdom are still unknown. In order to investigate the necessity of fungal-specific GluCer for *C. albicans* growth and cell differentiation, we constructed full mutants of four genes encoding the following glucosylceramide synthesis enzymes: sphingolipid delta4-desaturase (DES), sphingolipid delta8-desaturase (SLD), sphingolipid C9-methyltransferase (SLM) and glucosylceramide synthase (GCS). A sphingolipid analysis of these mutants indicated that the *des*, *sld* and *slm* mutants accumulated sphinganine, (*E*)-sphing-4-enine and (*E*, *E*)-sphinga-4,8-dienine as a shingoid base in GluCer, respectively, whereas the *gcs* mutant was incapable of synthesizing GluCer. Moreover, all of these mutants showed a decreased hyphal growth rate compared to the wild-type strain and only the slm mutant did not show reduced resistance to SDS because of the altered membrane structure. These results suggest that the introduction of a methyl branch at the C9-position of the sphingoid base included in GluCer has an important role in the hyphal growth of *C. albicans*.

PP-01-43

Effects of single amino acid on the morphogenesis of *Candida albicans*

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Candida albicans is termed a dimorphic fungus because it proliferates in either a yeast form or a hyphal form. Long, narrow hyphae develop from yeast cells grown at 37 degree and neutral pH, and in response to external stimuli such as serum. *Candida albicans* hyphae could be induced in vitro in Lee medium, which comprises 8 kinds of amino acids. Single amino acid on the morphogenesis of *Candida albicans* was studied. *Candida albicans* ATCC32354 and clinical isolations were used in this study. A basal synthetic culture medium was established with references to synthetic medium SD. Synthetic medium SD consists of yeast nitrogen base without amino acids and 2% glucose. One of the 17 amino acids at 10mmol/L concentration was added to synthetic medium SD as a single component of amino acid, and the morphology of colony and cell of *Candida albicans* was examined. L-arginine induced perfect filament. L-cysteine, L-threonine, L-valine and L-tryptophan induced perfect yeast cells. Mixture of filament and yeast could be detected when L-alanine, L-aspartic acid, L-phenylalanine, L-glycine, L-histidine, L-isoleucine, L-lysine, L-leucine, L-methionine, L-asparagine, L-proline, and L-tyrosine were used as a single amino acid. All the colonies were smooth on SD plate with or without single amino acid, and L-arginine resulted in colonies with small spinules. *Candida albicans* growth was inhibited and only yeast could be detected after incubation with or without glucose under anaerobic condition. Filament of *Candida albicans* could be induced by L-arginine in synthetic medium SD. No filament of *Candida albicans* could be induced by L-arginine under anaerobic condition or without glucose.

PP-01-44

Effect of electron transfer system on the hyphal formation of *Candida albicans*

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Candida albicans is a polymorphic fungal pathogen which could exist in the oral cavity, vagina and intestinal organs. *Candida albicans* was incubated in Muller-Hinton broth at 37 degree for induction of filamentation in vitro. Hyphae were completely inhibited and only yeast form could be detected while *Candida albicans* were incubated under anaerobic condition. *Candida albicans* can thrive at a wide range of extracellular pH from 3 to 9. Acidic conditions favor yeast growth, while alkaline conditions favor hyphal growth. *Candida albicans* mitochondria contains three respiratory chains: the classical respiratory chain (CRC), a secondary parallel chain and a cyanide-insensitive alternative oxidative pathway (AOX). Hyphae were completely inhibited and only yeast form could be detected while *Candida albicans* were incubated under anaerobic condition. Hyphal formation of *Candida albicans* might be controlled by respiratory chain. Inhibitors or activator of electron transfer system were used, which include rotenone, antimycin A (AA), oligomycin, sodium azide, thenoyltrifluoroacetone (TTFA), malonic acid, benzhydroxamic acid (BHAM) and guanosine 5'-monophosphate disodium (GMP). *Candida albicans* was incubated in RMPI1640 supplemented with 10% ovum serum at 5%CO2 37 degree for induction of hyphal formation in vitro. Growth curve, doubling time and percent of filamentation of *Candida albicans* were observed with or without inhibitors or activator of electron transfer system at intervals. MTT assay was used to assess the viability of *Candida albicans*. Growth inhibition of *Candida albicans* was found in the log phase but not in the lag phase. Percent of filamentation was significantly inhibited by oligomycin, AA, TTFA and BHAM. Viability of *Candida albicans* was significantly inhibited by TTFA and BHAM, while was enhanced by GMP. Hyphal formation of *Candida albicans* is suppressed by inhibitors of both electron transfer systems. Hyphal formation of *Candida albicans* is controlled by AOX pathway.

PP-01-45

Characterization of the *SKN7* homologue in *Candida glabrata*

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[Background]
Candida glabrata is an opportunistic fungal pathogen that causes invasive mycosis, and the increase of morbidity becomes serious. To cause invasive fungal diseases, pathological fungi must overcome host defense system including phagocytosis in macrophages and neutrophils. Reactive oxygen species (ROS) has a key role in killing pathogens by phagocytes, and in response, microorganisms develop oxidative stress response (OSR) to defense themselves from ROS. In *Saccharomyces cerevisiae*, Skn7p is known as a transcriptional factor participates in the OSR. In the present study, we investigated the role of *C. glabrata SKN7* to the OSR and to the pathogenicity with consideration of its epistatic regulation to some antioxidative defense genes.
[Material & Method]
C. glabrata skn7 deletant strain was generated with urablaster technique, and *skn7* mutant strain and *SKN7* intact strain (parental strain) were used. Phenotypic analysis to oxidative agents such as Hydrogen peroxide (H_2O_2) and tert-butyl hydroperoxide (t-BOOH), was performed with spot dilution test. Epistatic regulation of *SKN7* to some antioxidative defense genes such as *TRX2*, *TRR1*, *TSA1*, *CTA1*, *AHP1*, *GSH1* and *GLR1* was investigated with quantitative real-time RT-PCR method. Alteration of virulence was examined with the murine model of disseminated candidiasis.
[Result & Conclusion]
The *skn7* disruptant mutant was sensitive to H_2O_2 and t-BOOH. Quantitative real-time RT-PCR showed that the induction of *GLR1* was higher, but the induction of *TRX2*, *TRR1*, *TSA1* and *CTA1* was lower in the *skn7* disrupant relative to *SKN7* intact strain with H_2O_2 treatment. No difference was seen in the expression level of *AHP1* and *GSH1* between these two strains with H_2O_2 treatment. In vivo study showed an attenuation of virulence in the *skn7* disruptant strain. In conclusion, *C. glabrata* Skn7p engages in resistance to certain oxidants and to virulence. *C. glabrata* Skn7p may have negative effect on the induction of *GLR1* with H_2O_2 treatment.

PP-01-46

Evolution of *CDC42*, a putative virulence factor triggering meristematic growth in black yeasts

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The cell division cycle gene "CDC42" controlling cellular polarization was studied in members of *Chaetothyriales*. Based on ribosomal genes, ancestral members of the order exhibit meristematic growth in view of their colonization of inert surfaces such as rock, whereas in derived members of the order the gene is a putative virulence factor involved in expression of the muriform cell, the invasive phase in human chromoblastomycosis. Specific primers were developed to amplify a portion of the gene of 32 members of the order with known position according to ribosomal phylogeny. Phylogeny of *CDC42* proved to be very different. In all members of *Chaetohyriales* the protein sequence is highly conserved. In most species, distributed all over the phylogenetic tree, introns and 3rd codon positions are also invariant. However, a number of species had paralogues with considerable deviation in non-coding exon positions, and synchronous variation in introns, although non-synonomous variation had remained very limited. In some strains both orthologues and paralogues were present. It is concluded that *CDC42* does not show any orthologous evolution, and that its paralogues haves the same function but are structurally relaxed. The variation or absence thereof could not be linked to ecological changes, from rock-inhabiting to pathogenic life style. It is concluded that eventual pathogenicity in *Chaetothyriales* is not expressed at the DNA level in *CDC42* evolution.

PP-01-47

Analysis of the role of the single G1 cyclin, CnCln1, in *Cryptococcus neoformans* cell cycle

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Cryptococcus neoformans is a basidiomycetous yeast which is an opportunistic pathogen responsible for life-threatening infections among immunocompromised persons. Understanding the biological properties of *C. neoformans* is essential for the management of cryptococcosis. Our group has found *C. neoformans* to exhibit distinctive cytological changes during infection, and that it possesses a unique cell cycle pattern, different from that of the model budding yeast *Saccharomyces cerevisiae*. To clarify the cell cycle mechanisms in this pathogen, homologues of cell cycle control genes were cloned. We have previously reported the cloning of the CDC28/Cdc2 homologue, *CnCDK1* and three Cdk1 cyclin Candidates: two B-type G2/M cyclin genes and a single G1 cyclin gene, *CnCLN1*. Extensive search of the completed *C. neoformans* genome database however did not yield additional sequences with G1 cyclin similarities. Sequence analysis and comparison with other cyclin sequences showed that CnCln1 possesses the typical amino acid residues conserved among G1 cyclins. One of the features that were observed in *CnCLN1* is the occurrence of two short upstream ORFs in the 5' leader of its mRNA which are known to affect translational efficiency of many eukaryotic genes. Complementation tests showed that CnCln1 can perform G1 functions in *S. cerevisiae* but not in the fission yeast, *Schizosaccharomyces pombe*. We also succeeded in obtaining a deletion mutant of *CnCLN1* by biolistic transformation. G1 cyclin deletion in *C. neoformans* resulted to abnormally enlarged cells that have impaired budding and that do not readily separate after nuclear division, delayed growth at 30°C which is aggravated at 37°C, and subsequent hyphal formation under conditions that normally do not cause formation of hyphae. These results indicate an important role for CnCln1 in morphogenesis of *C. neoformans* and that it is essential for proper cell growth and division.

PP-01-48

Eicosanoids of *Candida dubliniensis*

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The oxygenated metabolites of twenty carbon polyunsaturated fatty acids are known as eicosanoids. These compounds include prostaglandins and hydroxy fatty acids. It is speculated that eicosanoids play important roles in the pathogenesis of yeasts such as *Candida albicans*, where they may serve as morphogenesis factors and result in immune modulation of the host. *Candida albicans* has the ability to produce prostaglandin E_2 either *de novo* or from exogenous arachidonic acid (20:4). In addition, *C. albicans* is able to produce 3,18-dihydroxy-5,8,11,14-eicosatetraenoic acid (3,18-diHETE). Interestingly, this compound was localised in hyphae but not in yeast cells. This study examined the ability of the closely related pathogenic species, *Candida dubliniensis*, to produce similar eicosanoids. Biofilms of *C. dubliniensis* were grown in the presence of 20:4 and the eicosanoids extracted and analysed using gas chromatography-mass spectrometry as well as ELISA. Results indicate that *C. dubliniensis* is able to produce prostaglandin E_2 as well as 3,18-diHETE from 20:4. Interestingly, *C. dubliniensis* was also capable of producing another 3-hydroxy fatty acid in the presence of 20:4. However, this compound still needs to be identified. This is the first report of the production of eicosanoids by *C. dubliniensis*.

PP-01-49

CaHap43 acts as a potential regulator of iron homeostasis in *Candida albicans*

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C. albicans is a dimorphic saprophyte which can be found in human bodies as normal flora, but it can cause life-threatening infections particularly in immunocompromised persons, such as elder or babies, AIDS patients, and people undergo chemotherapies. Beside native innate and adaptive immunity, a protective system, named natural resistance can help human hosts against pathogen infection by maintaining an extremely low level of free iron (E-18 M)in tissue fluids. To support the growth during the process of pathogenesis, *Candida albicans* must overcome such a low iron environment. Three-types of iron-responsive regulators among fungi have been identified, including Aft1/Aft2 activators in *S. cerevisiae*, GATA-type repressors in many fungi, and HapX in *Aspergillus* spp. For example, Sfu1, a GATA factor, has been identified in *C. albicans* to repress iron-responsive genes. In this study, we further identified a potential iron-responsive regulator, Hap43, from the microarray study and BLAST analysis. Hap43 is a homolog of *Aspergillus* HapX. We provided evidence that Hap43 functions as a transcription activator by one-hybrid analysis and this regulatory activity possibly depends on iron-level of external environment. In addition, Hap43 controls several genes within iron-regulon. Hap43 deletion strain also loses its ability to grow under low-iron condition while it seems to have no effect on the iron-uptake under iron-rich condition. Finally, Hap43 may contribute to the *C. albicans* virulence in the mouse model of systemic infection.

PP-01-50

Transcription regulation of an iron-responsive gene CaSIT1 in *Candida albicans*

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Iron is essential for the growth of almost every organism. There is no free iron available in the mammalian host. As a consequence, iron-withdolding is an important mechanism for the host to against microbial infection. Microbial pathogens have developed different systems to acquire iron from the host and their surrounding environments. In addition, iron availability can serve as a signal to induce the expression of genes involved in iron-uptake and virulence traits of microorganisms. *Candida albicans*, an opportunistic fungal pathogen, usually presents as a commensal in the host. However, when the host becomes immunocompromised, it can cause severe system infections. For survival, *C. albicans* have the ability to efficiently acquire iron as a nutrient. Previous studies have indicated that *C. albicans* harbors four different strategies for iron uptake, including a siderophore uptake system mediated by a ferrichrome siderophore transporter CaSit1p. Transcription of the CaSIT1 gene is highly expressed at the low iron condition, while is repressed in high iron environment. In this study, we examine the transcriptional regulation of CaSIT1 by analyzing the 5'UTR of CaSIT1 with a lacZ reporter gene. Serial deletion of the promoter region of CaSIT1 exposed a 51-bp region as a cis-element responsible for gene repression at high-iron condition, while a 68-bp region shows as a cis-element involved in gene activation under a low-iron condition. In addition, electrophoretic mobility shift assays were performed to examine protein interactions with these elements. Together, these results suggest that there is a DNA binding protein activates CaSIT1 in low iron condition, while another protein seems to repress CaSIT1 at high iron condition.

PP-01-51

A small G protein Rhb1 and a GTPase-activating protein Tsc2 involved in nitrogen starvation-induced morphogenesis and cell wall integrity of *Candida albicans*

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Rheb is a member of the Ras small G protein superfamily in eukaryotic organisms and controls various physiological processes. Activity of Rheb is regulated by Tsc2, a GTPase-activating protein (GAP). The active form of Rheb is a positive regulator of downstream TOR kinase. In this study, we have identified *Candida albicans* homologs of Rheb (named as Rhb1) and Tsc2. Deletion of the RHB1 gene showed enhanced sensitivity to rapamycin (an inhibitor of TOR kinase), suggesting that Rhb1 is associated with the TOR signaling pathway in *C. albicans*. Further analysis indicated Rhb1 and Tsc2 are involved in nitrogen starvation-induced filamentation, likely by controlling the expression of MEP2 whose gene product is an ammonium permease and a sensor for the nitrogen signal. The MEP2 gene expression is also enhanced by rapamycin treatment. The expression of nitrogen catabolite repression (NCR)-related gene indicated that the inactivation of TOR kinase mimics the nitrogen starvation in *C. albicans*, as shown in Saccharomyces cerevisiae. Moreover, we have demonstrated that Rhb1 is also involved in maintaining cell wall integrity, by transferring signals through the TOR kinase and the Mkc1 MAP kinase pathway. Together, this study brings new insights into the complex interplay of signaling and regulatory pathways in *C. albicans*.

PP-01-52

Structure based *de novo* peptide design for development of antifungal drug

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Expectation for new antifungal drug targets and seed compounds has been increasing due to the emergence of the drug resistant strains. In the recent drug development, Computer-Aided Drug Design (CADD) based on the 3D structure of target protein has been adopted to design seed compounds. Peptides are adequate seed compounds because of easier synthesis than other small compounds and can be transformed into the alternative micro-antibody such as an avimer or the pseudo-peptide compounds based on the peptidomimetics.

In this study, we focused on the fungal profilin, a small actin elongation-controlling protein, with the following reasons; (i) a little homology to human profilins, (ii) essential for the proliferation of *Saccharomyces cerevisiae*, (iii) 3D structure has been available from the protein data bank. To validate suitability as a drug target, a conditional profilin mutant of *Candida glabrata* was constructed with the Tet-OFF promoter, and its growth was monitored in vitro. When the expression was suppressed by doxycyclin, severe growth defect was observed, indicating the profilin was a suitable drug target. Next, the 3D structure prediction of *C. glabrata*'s profilin was performed CADD with the PS(2) server. The seed peptides were designed so as to bind to critical surface of the profilin to inhibit its interaction with actin with the computer program, Peptidesign. It performs *de novo* peptide design based on the target structure. To evaluate the physical interaction between the peptides and profilins, ELISA analysis was carried out. It showed that those peptides bound to the wild type profilin with higher affinity than that with amino acid substitutions predicted to be critical for the peptide binding, and they didn't bound to GST-tag protein.

These results demonstrated that useful seed compounds could be designed by this approach, and applied to other targets and organisms.

PP-01-53

Essential genes identified in the pathogenic yeast *Candida* as the potential antifungal targets

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Candida albicans is the major fungal pathogen for the immunocompromised host, which generally grows as a diploid. On the other hand, *C. glabrata*, which is also well known to be a pathogen of opportunistic infection but less virulent than *C. albicans*, always grows as a haploid cell and does not form hyphae, which makes genetic manipulation amenable. Among medically important *Candida* species, both of these two species have great, common advantages for studies at the molecular level; i) possessing abundant sources of information coming from total genomic DNA sequences, ii) establishment of the methodology, called "Tet system" (Nakayama et al, 1998, 2000), useful for analyzing the essentiality of the various genes. Against a background of these facts, we have focused attention to essential genes from these yeast species as Candidates for antifungal targets. One of them, to which we have focused attention in this presentation, is one of the genes for cyclin-dependent protein kinases (CDKs) identified in *C. albicans, PHO85 (CaPHO85)*. The *Saccharomyces cerevisiae* Pho85 (ScPho85) has been well known to be a negative regulator of the PHO system, reported to be a non-essential CDK with 51% identity to Cdc28. It should be emphasized that Pho85 has emerged as an important model for the role of CDKs in processes beyond cell-cycle regulation. Here we show the results of our recent studies on the essentiality of CaPho85, together with that of its *C. glabrata* homologue, using the in vitro-Tet system. We also show the importance of CaPho85 in the pathogenicity of *C. albicans* by using the in vivo-Tet system. Moreover, we would like to introduce our recent attempts to establish a useful system for screening and identification of essential genes from *C. glabrata* using various temperature-sensitive mutants.

PP-01-54

Establishment of a useful system for screening and identification of the essential genes from the pathogenic haploid yeast *Candida glabrata* by the complementation of the temperature-sensitive mutations

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The incidence of opportunistic fungal infections has been increasing popular in the past few decades, especially in immunocompromised hosts. The major fungal infection occurring in patients undergoing prolonged hospitalization is candidiasis. The survival of these patients depends on early diagnosis and prompt initiation of antifungal therapy. Therefore, in an effort to develop new types of agents with chemotherapeutic usefulness, we attempted to establish a system for wide scope of screening potential antifungal targets in *Candida* spp. For this purpose, we focused attention on cellular constituents that are indispensable for growth of this organism, i.e., essential genes (and their gene products). Our strategy for effective screening of these essential genes has started from isolation of a number of temperature-sensitive (ts) mutants of a haploid yeast *C. glabrata*, which are defective in colony-forming ability at the non-permissive temperature. Using these ts mutants, we were able to establish a useful system for screening and identification of the essential genes by the complementation of ts mutations to search antifungal targets. This system was constructed according to the following concepts : i) ts mutations are due to point mutation generated within essential genes in the genome, ii) therefore, some compounds inhibiting the function or the expression of the genes that complement the point mutations are Candidates for antifungal agents. Using this system, we have successfully identified a variety of essential genes that complement ts point mutations. These genes have been shown to confer important role in various cellular events, such as cell cycle progression, cell wall integrity, secretion and protein/RNA processing. The essential genes identified by using the present system would be expected to be potential targets for antifungal agents.

PP-01-55

Molecular types of *Cryptococcus spp.* isolated from captive bird excreta in Uberaba, MG, Brazil

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In order to evaluate some molecular aspects of *Cryptococcus spp*. isolated from captives Columbine, Psittacine and Passerine excreta, an epidemiological survey was carried out from December 2006 to September 2008. A total of 253 samples of bird excreta (120 fresh and 133 dry) were collected with swabs from cages in seven pet shops and three houses located in different districts of Uberaba, Minas Gerais, Brazil. The samples were inoculated into a tube with a 0,9% sterile saline solution and afterward, spread on birdseed agar plates (Guizotia abyssinica) and observed during 10 days. The smooth, beige to dark brown colonies, were subcultured and their morphological and biochemical profile were characterized. *C. neoformans* was isolated in 19 (14.28%) samples of dry feces, while C. laurentii in 7 (5.26%) of them. *C. neoformans* was isolated only in 1 (0.84%) of 120 samples of fresh feces. The CGB test was negative in all *C. neoformans*, but positive in all but one C. laurentii isolates. *C. neoformans* genotypes were characterized by PCR-RFLP of URA5 gene and by PCR of the locus mating type. Nineteen (95.0%) of them presented VNI genotype and one (5%) VNII. All isolates of *C. neoformans* presented the mating type alfa. When the former technique was tested for C. laurentii isolates, a fragment close to 1600pb was amplified, and with HhaI and Sau96I digestion, all isolates presented the same profile. Thus, the molecular typing of *C. neoformans* isolated from captive bird faces are similar to others already described and they can contribute to define their geographical distribution around the country. Otherwise, the first attempt to characterize C. laurentii isolates suggests that it is very important to test other molecular tools aiming to define its molecular types.

Keywords: *Cryptococcus neoformans*. *Cryptococcus* laurentii. PCR-RFLP. Mating Type

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PP-01-56

Genotype and mating type analysis of 81 clinical isolates of *Cryptococcus neoformans* and *Cryptococcus gattii* from patients with *cryptococcal* meningitis in Uberaba, MG, Brazil

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Cryptococcus species complex have been divided in eight major molecular types which show biological, epidemiological and host preferences differences between them. The aim of this work was to identify the genotypes and mating types of *Cryptococcus* spp. clinical isolates by PCR-restriction fragment length polymorphism of URA5 gene analysis and PCR, respectively. A total of 81 isolates from cerebrospinal fluid (CSF) of 77 patients admitted at the teaching hospital with cryptococcal meningitis between 1998 and 2007 were analyzed. Seventy two, (88.9%) isolates were obtained from AIDS patients and the remaining nine of patients non AIDS. *Cryptococcus neoformans* were identify in 76 (93.83%) cases and *Cryptococcus gattii* in 5 (6.17%) by Canavanine Glycine Bromothimol Blue (CGB) test. The 76 isolates of *C. neoformans* presented the molecular type VNI whereas five isolates of *C. gattii* presented genotype VGII. Among these, two had been recovered from AIDS patients. All isolates presented mating type alfa;. Of nine isolates from non AIDS patients, six were VNI genotype and three presented VGII. Relapsed of cryptococal meningitis occurred in three out 72 AIDS patients after two years of the first event, but their isolates presented molecular profile similar to the former. The molecular type and CGB test were accurated to diferenciate the two species. The prevalence of 100% of VNI, mating type alfa; among *C. neoformans* clinical isolates is similar to others results already elsewhere. The genotype VGII is more prevalent in the North East region of Brazil and the two isolates of this report were recovered from patients in South East region where its prevalence is very low as described by others.

Keywords - *Cryptococcus neoformans*, *Cryptococcus gattii*, mating type, genotype, URA5-RFLP, PCR

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PP-01-57

Molecular characterization of environmental isolates of Cryptococcus spp. in Uberaba, MG, Brazil

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The objectives of this study were to isolate Cryptococcus spp. from peri- and intra-hospital environments in Uberaba, Minas Gerais, Brazil, to characterize genotypes by the URA5-RFLP technique and to determine the presence of mating type locus by the PCR. Seventy-three different samples were collected, being 62 from bird excreta and 11 from the tree debris. The samples were spread on Niger seed agar (Guizotia abyssinica) and incubated for 15 days at 35oC. Cryptococcus neoformans was recovered in 32 (43.8%) of the samples, Cryptococcus laurentii in 17 (23.3%) and both in 8 (10.9%). The number of colonies isolated was 98, being divided in 39 (39,7%) colonies of C. laurentii and 59 (60.3%) of C. neoformans. Five C. laurentii isolates were obtained from the trees (Anacardium occidentale, Guazuma ulmifolia, Mangifera indica and Ficus benjamina). Of 51 isolates of C. neoformans, 47 (92.2%) were VNI molecular type and four (7.8%) VNII. All C. neoformans isolates presented the mating type alfa. The concordance between the CGB agar and PCR-RFLP to identify C. neoformans was 100%. Of the 21 isolates of C. laurentii, 11 (52.4%) amplified URA5 gene with primers SJO1 and URA5, providing a DNA segment closed to 1.600pb. The digestion of this segment with Sau96 and HhaI produced a similar profile in ten isolates, and preliminary was named CLI, different from another, named CLII. The genotypes and mating types of C. neoformans obtained from environmental sources are similar to others already reported elsewhere. Furthermore, the attempt to characterize C. laurentii isolates by PCR-RFLP, amplified a segment different from that obtained from pathogenic species and suggests that other molecular tools or strategies may be used in order to define both genotypes and mating types of this specie.

Keywords: Cryptococcus spp., Genotypes, PCR-RFLP, Mating type
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Poster Forum PF-09

PP-01-58

Bloodstream infections due to *Trichosporon*: Phenotypic and genotypic identification, species distribution and *T. asahii* genotypes based on rDNA IGS1 sequencing

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The genus *Trichosporon* has been completely reevaluated and the old taxon Trichosporon beigelli was replaced by 6 new species. Here we describe a comparison between phenotypic and genotypic methods current used for *Trichosporon* identification. We also investigated Trichosporon species distribution and prevalence of *T. asahii* genotypes based on rDNA IGS1 sequencing of 22 isolates recovered from blood cultures. All strains able to produce arthroconidia as revealed by their micromorphology on corn meal-Tween 80 agar and to hydrolyze urea were considered as belonging to the genus Trichosporon and further identified by assimilation profiles of the following carbohydrates: L-arabnose, galactitol, inositol, melibiose, rafinose, rhamnose and xylitol. To double check for the correct genus identification, we amplified a fragment of the 18 S rDNA conserved region. For the accurate identification of Trichosporon strains to the species level, the IGS1 region was amplified and sequenced by using the dideoxy-nucleotide method. Only 14 out of 22 strains were accurately identified to the species level when phenotypic methods were used (64%). The clinical isolates were identified by molecular methods as follows: 15 *T. asahii* isolates, 5 T. asteroides isolates, 1 T. coremiiforme isolate and 1 T. dermatis isolate. We found a great diversity of different species causing trichosporonemia, including a high frequency of isolation of T. asteroides from blood cultures, only behind *T. asahii*. Regarding to *T. asahii* genotyping, the majority of our isolates belonged to genotype 1 (86.7%). We report the first *T. asahii* isolate belonging to genotype 4 in South America. This study reinforces the relevance of correctly identifying Trichosporon isolates by using molecular methods. We documented a great diversity of different species causing fungemia and a high frequency of T. asteroides isolation from blood, only behind *T. asahii*.

PP-01-59

Partial characterization of extracellular membranous vesicular structures from *Paracoccidioides brasiliensis*

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Exosomes have been recognized as important structures related with virulence of microorganisms and modulation of the host's immunity. Exosome-like vesicles have recently been characterized in fungal pathogens such as Cryptococcus neoformans and Histoplasma capsulatum. Here we describe the isolation and partial characterization of membranous extracellular vesicles in *Paracoccidioides brasiliensis*, a dimorphic fungus that causes human paracoccidioidomycosis (PCM). We compared two *P. brasiliensis* isolates: Pb3, which represents phylogenetic group PS2, and Pb18, from the main species S1 and widely used in experimental PCM. We have previously shown that the progression of disease and pattern of immune responses in B10.A mice differ when comparing infection with S1 (more virulent) to PS2 isolates. Cell-free supernatant fluids from *P. brasiliensis* yeast cells cultivated at 36oC in F-12 defined medium (Gibco)/glucose were concentrated in Amicon and ultracentrifuged (100,000g). Pellets analyzed by electron microscopy showed the presence of 2-layered membranous vesicles sizing 20 to over 200 nm. Intense immunogold labeling with MOA lectin was observed on the surface and inside the vesicles. Sterols have been detected in 100,000g extracellular pellets derived from live, but not dead cells, suggesting that the membranous structures do not result from cell debris. Specific *P. brasiliensis* antigens were present in 100,000g extracellular preparations, as revealed in immunoblots with sera from PCM patients, but not from healthy individuals. Similar reactivity patterns between Pb3 and Pb18 were observed. Enzymatic activities of laccase, phosphatases, and urease were detected in dose-response experiments with extracellular pellets from both Pb18 and Pb3, however the levels of laccase and phosphatase activities were comparatively higher in Pb18. Preliminary data using 100,000g pellets further fractionated in sucrose gradient suggested the induction of at least nitric oxide by J774 cultured macrophages. Proteomic and lipomic analyses of the vesicle fractions are being performed.

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PP-01-60

Structural and stability properties among *Paracoccidioides brasiliensis* gp43 isoforms

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Gp43 is a largely studied secreted glycoprotein from Paracoccidioides brasiliensis. It is the main antigen for diagnosis and prognosis of paracoccidioidomycosis (PCM), but it also has a protective Th1 epitope and adhesive sites for proteins associated with the extracellular matrix. Gp43 has one high-mannose oligosaccharide chain and is secreted as isoforms according to the PbGP43 genotypes. Three recombinant gp43 isoforms (r3gp43, r10gp43, and r14gp43) were expressed as soluble glycoproteins in the culture supernatants of Pichia pastoris. The expressed isoform from genotype A (r3gp43) has a calculated pI of 8.3, while the translated sequences from genotypes D (r10gp43) and E (r14gp43) have pIs of 6.8 and 7.1, respectively. Genotype A gp43 is characteristic of phylogenetic cryptic species PS2, while genotypes D and E are found in the main species S1. Presently, recombinant P. pastoris were induced with methanol and rgp43 was purified from the culture supernatant by affinity chromatography in columns containing anti-gp43 monoclonal antibodies. Enzymatic deglycosylation was achieved using Endo H. Secondary structures of rgp43 (deglycosylated or not) were compared by circular dichroism. To examine the molecule stability properties, thermal denaturation at 50oC was monitored at different pHs by intrinsic fluorescence emission analysis. Secondary structures were similar in r10gp43 and r14gp43 even after deglycosylation. However, differences in secondary structures were observed in r3gp43 after treatment with Endo H. Fluorescence emission analysis detected differences among isoforms: r10gp43 and r14gp43 were more stable than r3gp43 in acidic pH. R3gp43 was more stable in basic and neutral pHs. However, deglycosylated r3gp43 completely lost stability in basic pH, although it increased at acidic pH. Isoforms r10gp43 and r14gp43 were overall more labile when glycosylated. It will be interesting to figure out how structural and stability differences among gp43 isoforms may possibly modulate the host-parasite relationship.

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PP-01-61

Purification and recombinant expression of the polyphenoloxidase from *Agaricus brasiliensis*

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Polyphenoloxidases (PPOs), including tyrosinases and laccases, are ubiquitous metalloenzymes that have several copper-binding sites. The enzymes cause undesirable browning in vegetables, mushrooms and fruits, and melanin-production of animals. *Agaricus brasiliensis* is an edible mushroom whose water-extract shows biological response modifier effects. Since the cold-water extract of the mushroom fruiting body turns brown upon incubation, it has been speculated that the mushroom contains significant amounts of PPOs.

The *A. brasiliensis* fruiting body was subjected to extraction with phosphate buffer containing ascorbic acid at 4°C for overnight. The resulting cold water extract was subjected to two-phase partitioning system using Triton X-114 and then the detergent poor phase was further subjected to ammonium sulfate fractionation. The precipitated PPO was resolved in 0.1 M phosphate buffer (pH 7.0) and then dialyzed against the same buffer. The partially purified protein solution was analyzed by semi-denatured SDS-PAGE. After the gel was stained with 4-tert-butylcatechol, two bands corresponding to 45 kDa and 66 kDa appeared. The partially purified PPO was subjected to the enzymatic activity measurement. In its latent state, PPO uses monophenols as the substrates. Its monophenolase and diphenolase activities were enhanced by sodium dodecyl sulfate (SDS). Next, we tried the recombinant expression of PPO using *Escherichia coli* as a host. The cDNA coding for *A. brasiliensis* polyphenoloxidase was cloned into the expression vector, pET15b, and then used to transform *E. coli* Rosetta2 (DE3). The transformant was cultured to over-produce recombinant PPO. The cells were disrupted by sonication and PPO was partially purified by a column chromatography. The recombinant protein solution exhibited PPO-like activity. However, a SDS-PAGE analysis showed several smaller bands than expected, suggesting that the recombinant protein has tendency to degrade.

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PP-01-62

Molecular cloning of polyphenoloxidase genes from *Agaricus brasiliensis*

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Mushrooms contain a large amount of biological response modifiers, such as beta-glucans. *Agaricus brasiliensis* (*A. blazei*) is an edible mushroom and its hot-water extract shows anti-tumor effects. Recently, it has been revealed that the cold-water extract of the mushroom also shows several immunological responses. The color of the extract turns to brown time-dependently after incubation at room temperature. It has been suggested that the extract contains polyphenols and polyphenoloxidases (PPOs), which are general enzymes including tyrosinase and laccase. They are metalloenzymes, containing copper binding domains and catalyze not only the hydroxylation of mono-phenols to di-phenols but also the oxidation of di-phenols to di-quinones. In this study, we carried out the molecular cloning of the PPO genes from the cDNA library of *A. brasiliensis*.

First, a partial cDNA fragment of tyrosinase-type PPO was obtained by an RT-PCR using degenerate primers designed based on the amino acid sequenses of the copper-binding sites from several fungi and the total RNA isolated from *A. brasiliensis* fruiting bodies. Several PCR primers were further designed based on the partial cDNA sequence determined and then used to amplify the entire length of the PPO gene by PCR from the cDNA library. DNA sequencing of the entire PPO gene showed that its deduced amino acid sequence is significantly similar to the known PPO sequences from other fungi. For laccase, the partial cDNA fragment was obtained by PCR using the laccase-specific primers that had been designed for amplification of another fungus laccase and then its nucleotide sequence was determined. The partial cDNA sequence was labeled and then used as a probe for plaque hybridization experiments to clone full-length laccase cDNAs from the *A. brasiliensis* cDNA library. The amino acid sequence deduced from the sequence of the cloned cDNA is significantly similar to the known laccase sequences.

PP-01-63

Nested PCR for histoplasmosis vs routine diagnosis

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The polymerase chain reaction (PCR), is an important tool for the diagnosis of certain diseases due to its sensibility and specificity. We used the double polymerase chain reaction or Nested PCR, as diagnostic test for histoplasmosis, a very frequent deep mycosis in the immunocompromised host produced by the infection *Histoplasma capsulatum*. The test was compared with conventional mycology studies. Objective: to standardize and compare routine diagnostic tests for the mycosis: direct exam, culture and serology with Nested PCR. Materials and methods: we used serum samples and organs (liver, spleen, lung) from Balb/c mice, inoculated with different concentrations of the mycelial phase of the fungus, and also slices of tissues included in paraffin from patients with diagnosis of histoplasmosis. Samples from mice were evaluated by direct exam, culture, counterimmunoele ctrophoresis, double immunodiffusion and Nested PCR and tissue slices were processed by Nested PCR. Results: culture was the best diagnostic tool with greater sensitivity and specificity. When comparing the different serological tests, suitable for diagnostic presumption, the CIE test had greater sensitivity and specificity when compared with IDD. The Nested PCR trial, using the specific primers (HcI : 5' GCG TTC CGA GCC TTC CAC CTC AAC 3' y Hc II : 5' ATG TCC CAT CGG GCG CCG TGT AGT 3', for the fist chain reaction and HcIII : 5' GAG ATC TAG TCG CGG CCA GGT TCA 3' and HcIV: 5' AGG AGA GAA CTG TAT CGG TGG CTT 3' (1,2,3) showed high specificity for the diagnosis, but a lower sensitivity when compared with conventional mycology diagnostic tests. However, the sensitivity could be increased with the use of a greater amount of specimen for the fungus DNA extraction, considering the tissue to be processed, as we found that liver and spleen tissue where more efficient for diagnostic purposes that lung tissue.

PP-01-64

Immunohistochemistry and PCR on formalin-fixed paraffin-embedded tissue for detection of fungal etiology in a tertiary healthcare setting

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Background: While biopsy is very useful for establishing diagnosis of invasive fungal infections, cultures are usually negative hence there is a need to make accurate mycological diagnosis at the earliest possible time so that timely and exact treatment can be instituted to save lives of patients. Objectives: To establish diagnosis of fungal infections by using immunohistochemistry and DNA extraction from formalin-fixed paraffin-embedded tissue followed by PCR. Patients: In this study, 39 patients with hyalohyphomycosis and 1 with disseminated candidiasis on histopathology were studied. The fungal etiology was established on histopathology. Methods: The formalin-fixed paraffin-embedded blocks of patients having diagnoses of fungal infections were retrieved. Sections were cut from each block for immunohistochemistry and DNA extraction for PCR. Immunohistochemistry was performed by using a polyclonal Aspergillus genus-specific antibody antibodies from Abcam and a polyclonal Aspergillus genus-specific antibody from Genway. The DNA extraction was done using xylene and methanol, followed by phenol chloroform isoamyl alcohol method. It was further followed by PCR using ITS1 and ITS4 primers and sequencing the ITS region. Results: Hyphae consistent with Aspergillus species were seen. The specimens were positive with both types of antibodies by immunohistochemistry. In addition both antibodies were positive with yeast cells (n=1). PCR was found to be positive in 26 specimens out of 39. Sequencing was done in 3 cases which showed 2 cases positive for *Aspergillus fumigatus* and one as Anethum graveolens Conclusions: The immunohistochemistry and simultaneous DNA extraction and PCR followed by sequencing establishes final diagnosis which may have been very difficult merely on the basis of histopathology as Fusrium, Pseudoalleschria and other fungal species may be confused with Aspergillus.

PP-01-65

The titer of anti-β-glucan antibody in human

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Glucan is one of the major cell wall components of fungi. Many pattern recognition receptors were cited as Candidates for a β-glucan receptor. On the other hand, there are few reports of an antibody to β-glucan. We have recently detected an anti-β-glucan (BG) antibody highly reactive to *Candida* solubilized cell wall β-glucan (CSBG) in normal human sera. Anti-BG antibody may be able to interact with the fungal cell wall or extracellular glucan. Thus, we examined anti-BG antibody titer, reactivity of anti-BG antibody in human volunteer and patients.

To understand the clinical role of anti-BG antibody, we measured anti-BG antibody titer in mycosis patients, collagen diseases (rheumatoid arthritis (RA), ANCA associated vasculitis (AAV)) and cancer patients. Mycosis patients (aspergillosis : N=2, carinii pneumonia : N=3), whose sera is β-1,3-glucan positive showed a significantly low titer (414 ±355 U/mL) compared with normal volunteers (2371±1107 U/mL). This change correlated with clinical symptoms and other parameters such as C-reactive protein and β-1,3-glucan. RA, AAV and cancer patients showed decreased titer of anti-BG antibody.

It has previously been reported that plasma levels of 1,3-β-glucan are elevated in dialysis patients We compared the anti-BG antibody titer of dialysis patients and healthy volunteers. In dialysis patients, the titer was lower than in healthy volunteers. Long-term (15-40 years) dialysis patients had lower titers than short-term (<5 years) dialysis patients.

In conclusion available data suggest that anti-BG antibody could play a role for β-glucan recognition and induce clearance of pathogenic fungi and biological activity through collaboration with other recognition molecules such as β-glucan receptor or complement in human. Anti-BG antibody interacted with pathogenic fungal cell wall glucan in vivo and was eliminated from the blood as an antigen-antibody complex. Measurements of anti-BG antibody could be useful as a response index of pathogenic fungal cell wall β-glucan.

PP-01-66

Standardized PCR technique for diagnosis of Candidosis

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During the last decade, deep mycosis have shown an increased frequency and a more complex presentation of the infection. Among these we find candidosis, infectious disease with different clinical presentation such as the opportunistic form, acute, subacute or chronic disease produced mainly by *Candida albicans*. We felt the necessity to have the availability of rapid and specific methods for the early diagnosis and identification of the causal agent of this infection. Objective: to standardize the PCR technique for the diagnosis of the infection by *C. albicans* from tissues from mice infected by *C. albicans*. Materials and methods: male mice Balb/C were inoculated with *C. albicans* at concentrations from 1 x 10^2; to 1 x 10^7, to produce the infection and the disease. Posteriorly, the mice were sacrificed between 15 and 30 days post inoculation. Slices of tissues from liver, spleen and lung were processed for conventional mycology diagnosis and molecular tests with specific primers for *C. albicans*. Results: we managed to standardize the yeast DNA isolation conditions from murine infected tissues, the procedure and amplification of PCR, getting a final product of 310 pb from specimens corresponding to an inoculum greater than 1x10^3;. Conclusion: in this study of the experimental candidosis, we did not find a significant difference between the conventional mycological diagnosis and the PCR trial, both showed a high percentage of positive results, but the PCR assays were more rapid and the time consumed were just hours.

PP-01-67

Candida albicans antigens for serology diagnosis of Candidosis

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Candidosis is an opportunistic disease, caused by a fungus of the *Candida* gender, and the more frequent infecting species is *Candida albicans*. Invasive candidosis affects frequently those patients in critical care units and those immunocompromised, with high mortality. Conventional diagnostic methods such as clinical data, culture and histopathology have limitations and for this reason, at present serology is being considered of utility. Objectives: To obtain a *Candida albicans* antigen for serological diagnostic of candidosis. Materials and methods: three metabolic antigens of *C. albicans* B-385 CDC were prepared from the culture supernatant, two from the mycelial phase, from germ tubes AgmTGCA-HB and AgmTGCA-Lee, and one from the yeast phase AgmLevCA-PYG, where submitted against serum from subjects with diagnosis of superficial and invasive candidosis and *Candida* colonization, to detect antibodies by means of the double immunodiffusion technique (IDD) and counter-immunoelectrophoresis (CIE). Results: The AgmTGCA-HB for diagnostic purposes of invasive candidosis showed a high specificity with IDD 92.7% (CI95%:83.38-97.65) and with CIE 87.2% (CI95%:76.44-94.26), greater than antigens AgmTG-Lee and AgmLevCA-PYG, with 60% (CI95%:18.24-92.65) sensitivity with both techniques. When comparing conventional diagnostic methods (clinical data and culture) and immunodiagnostic methods with AgmTGCA-HB by means of IDD and CIE, results were similar with a $p>0.05$ value. The other two antigens presented $p<0.05$ with both techniques. The AgmTGCA-HB showed bigger values than the other two antigens with a positive verosimilitude ratio of 8.2 with IDD and 4.6 with CIE. Conclusion: the AgmTGCA-HB could be considered useful as a diagnostic tool for suspected cases of invasive candidosis.

PP-01-68

Application of PCR on detection of aflatoxinogenic molds

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Aflatoxins are carcinogenic metabolites produced by several strains of Aspergillus flavus group in food and feed. To differentiate between aflatoxinogenic and non-aflatoxinogenic A.flavus strains a PCR with four primer sets for nor-1, ver-1 and omt-1 genes coding for key enzymes and aflR gene a regulatory factor in aflatoxin biosynthesis was used. The obtained result was compared with thin layer chromatography, as a conventional method. DNA of fourteen A.flavus strains was extracted by phenol-chloroform procedure and subjected to each primer pair. DNA of four strains was amplified by all four primer pairs and three of them were positive by chromatography. The results indicate that PCR is a rapid method for detecting aflatoxinogenic A.flavus strains, but does not differentiation between them and non-aflatoxinogenic strains completely and further studies should be carried out to develop a suitable screening technique.

PP-01-69

Assessment and comparison of two SCAR markers for the detection of Histoplasma capsulatum

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Histoplasma capsulatum, the pathological agent of histoplasmosis is a dimorphic fungus of worldwide distribution. In Mexico, it has been seen throughout the country representing a possible health problem. We have developed for diagnostic and epidemiologic screening two SCAR (Sequence Characterized Amplified Region) markers from a repetitive band in the RAPD-PCR profile of H. capsulatum isolates from different American countries. These markers have been named 1253220(Hc) and 1253230(Hc). To propose SCAR markers as new tools to reveal H. capsulatum from different types of sources, their specificity and sensitivity parameters were assessed in the detection of this pathogen in human and wild mammal samples a well as in other samples from the environment (bat guano), another H. capsulatum marker (antigen M-probe) was used to compare results. All three markers detected H. capsulatum strains from different geographical origins in America. The marker 1253220(Hc) was absolutely specific and detected only H. capsulatum in both clinical and guano samples. In contrast, the SCAR 1253230(Hc) and the antigen M-probe markers amplified DNA from Aspergillus niger and Cryptococcus neoformans, respectively. The two SCAR markers detected 1 pg of the H. capsulatum DNA, while the antigen M-probe only amplified 500 pg of DNA from each strain tested. In environmental samples, the two SCAR markers detected H. capsulatum up to 20 CFU/g of guano, whereas the antigen M-probe detected 640 CFU/g. Based on the present results, the 1253220(Hc) marker could be proposed as a tool to reveal H. capsulatum in clinical and environmental samples, which would support its application in diagnostic and epidemiologic studies.

PP-01-70

Molecular evidence of Histoplasma capsulatum infection in organs of the migratory bat Tadarida brasiliensis

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Histoplasma capsulatum infection was monitored in organ samples from 90 migratory wild bats, Tadarida brasiliensis, captured in Mexico (n = 69) and Argentina (n = 21). Six fungal isolates were cultured from all organs tested. Infection was screened by nested-PCR of the Hcp100 gene, which amplified a fragment considered unique for H. capsulatum. This fungal gene was detected in different bat organs, and evidence for the presence of the pathogen was found in several Mexican and Argentinean sampled bats. High homology was observed among all aligned Hcp100 sequences, while a few mutation sites were found in every sequence when compared with the GenBank sequence of the H. capsulatum reference strain, G-217B, from the United States. Genetic diversity based on the Neighbor-Joining sequence analysis highlights the homology among some Mexican and all the Argentinean bat samples, whereas sequences from other Mexican samples revealed genetic diversity probably related to the migratory behavior of T. brasiliensis. (This work was supported by grants: SEP-CONACYT-ANUIES-MEXICO/ECOS-NORD-France, ref. M05-A03 and DGAPA/UNAM, ref. IN-203407-3).

PP-01-71

Rapid direct colony PCR from fungi by ampdirect plus

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The need to perform PCR from filamentous fungi has increased over the past years. DNA extraction for preparation of DNA template is time consuming and labor intensive. We used Ampdirect Plus to reduce the time for performing PCR from filamentous fungi. Using this kit, we tested 63 strains (27 yeast and 36 molds) by PCR targeting ITS region of rDNA and after rapid treatment by special lysis buffer. A variety of that lysis buffer was prepared and employed for testing 4 strains by Ampdirect PCR. Moreover, direct colony PCR (DCPCR) was performed by standard PCR reagents and Ampdirect PCR and compared in 35 mold strains without any lysis buffer. The Ampdirect PCRs using the special lysis buffer and its varieties were positive in all cases. From the other hand, 97.14% of the strains used in DCPCR showed positive results with Ampdirect PCR while only 31.43% of strains tested by standard PCR reagents gave positive results. Nonetheless, PCR products were generated from both hyphae and spores of 4 fungal strains tested by Ampdirect DCPCR. In conclusion, Ampdirect DCPCR is a useful approach to facilitate performing of PCR from fungi.

PP-01-72

Real-time PCR quantitation of DNA contaminants in recent β-glucanase products used for fungal preparations

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The molecular diagnosis of environmental or invasive pathogenic fungi based on polymerase chain reaction (PCR) of fungal DNA has high sensitivity and specificity, though false-positive results may occur due to small amounts of contaminations in these sensitive detection systems. A decade earlier, several investigators (Rimek et al., 1999, Loeffler et al., 1999) mentioned about these contaminants appearing in Zymolyase-20T amplified by their broad-range detection systems for fungi based on conventional PCR and agarose gel electrophoresis analyses or DNA sequencing. Another type of β-glucanase product with higher specific activity is available from same manufacturers: Zymolyase-100T, which has been produced by an extra affinity chromatography purification step derived from Zymolyase-20T. To analyze the current contaminations and understand the usability of of these enzymes, we have estimated the rDNA contamination in Zymolyase-100T (Seikagaku Biobusiness Corp.) and Lyticase (Sigma-Aldrich Corp.) by real-time PCR which is more sensitive and quantitative than conventional PCR methods. The Zymolyase-100T contained 7.5 times more DNA than Lyticase on total DNA quantitation. The estimated amount of ribosomal DNA contamination by real-time PCR was 9,210 copy/U for Zymolyase-100T and 0.0323 copy/U for Lyticase. From aspects between these enzyme products, we deserve careful and thoughtful consideration on contaminating DNA included in the reagents used for molecular diagnostics.

PP-01-73

Development of *Prototheca zopfii* detecting system with TaqMan®MGB probe and Resolight Dye

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Prototheca, the unicellular algae with atrophic chlorophyll has been reported as the pathogens responsible for refractory subcutaneous diseases and systemic infection in human and animals. *Prototheca zopfii* has been known as agent of human and animal protothecosis. In addition, bovine mastitis and canine fatal systemic infection are large problems around the world. In treatment of this disease, although early diagnosis and selection of effective antifungal agent, etc., are important, the conventional identification techniques have problems with regard to reliability and rapidity.

In this study, the detection system using specific TaqMan® MGB probe and Resolight Dye was developed for the identification of *P. zopfii* isolates and identification of yeast like colonies isolated from bovine mastitis. The *P. zopfii*-specific primers 18PZF1 and 18PZR1 were generated based on the alignment of the SSU domain base sequences of the genera *Chlorella* and *Prototheca* obtained from DDBJ/EMBL/GenBank, and the TaqMan®MGB probe PZP1 was made corresponding to this amplification region. Analysis of melting curves using Resolight Dye was also performed for identification of *P. zopfii* type 1 and type 2. The specificity of this detection system was examined using clinical isolates (n=50 strains), as well as strains of culture collection; *P. zopfii* (n=9 strains), *Prototheca wickerhamii* (n=4), *Prototheca stagnora* (n=1), *Prototheca blaschkeae* (n=2), *Chlorella vulgaris* (n=1), *Candida* spp. (n=4), etc., as standard strains, as well as clinical isolates (n=50). On TaqMan®MGB probe assays, the target amplicon product was detected only in samples from *P. zopfii* (n=9) and clinical strains (n=50), and Resolight Dye analysis showed two types of melting curve. This system was proved to be useful for the differentiation of pathogenic *P. zopfii* type 2 from other yeast-like organisms.

PP-01-74

Heat resistance of *Cladosporium cladosporioides*

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Cladosporium cladosporioides is a widely distributed saprophyte that is reported to occasionally infect the lung, skin, eye and brain of humans[1], and *C. cladosporium* is an important allergenic fungus worldwide.[2] Heat resistance test for *C. cladosporioides* has been carried out by kawai et al[3]. In this report, it can be seen that *C. cladosporioides* was definitely sensitive to heat. However, the cause of the heat sensitivity was not clarified. Thus, we tried to investigate the mechanism of the heat sensitivity. In this study, we used *C. cladosporioides* NRBC4457 strain. *C. cladosporioides* NRBC4457 grew in Sabouroud's medium at 27°C, however, the growth was stopped in the cultivation at 37°C. *C. cladosporioides* was sterilized by culturing at 37°C for 48 hours. Because *C. cladosporioides* is an aerobic fungus, we expected that ATP was mainly produced by the respiration. Alamar blue is an indicator of oxidation-reduction reaction in a cell, and analyses by alamar blue become one index to measure the respiration activity. As metabolism of alamar blue at 37° was higher than that of the cells cultured at 27°, we expected the oxidation-reduction activity of respiratory chain components was not obstructed at 37°. However, the amount of ATP in *C. cladosporioides* cultured at 37° was significantly decreased compared with that of the cells cultured at 27°. These results suggested that the inhibition of ATP synthetase was a cause of the growth inhibition at 37°.

1) Gugnani H. G., Sood N., Singh B., Makkar R., Mycoces 2000. 43, 85-87.
2) Hong C., Ming F. T., Lin-Hau L., Chi-Huei C., Hsiao-Yun T., Raphael C. P., Horng-Der S., Allergy Immunology. 2008, 146(4), 277-286
3) Kawai S., Takatori K., Ohtaki T., Jikken Dobutsu. 1990. 39(3), 319-323.

PP-01-75

Characterization of RAD51 and RAD59 from *Candida albicans*

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We have cloned and characterized the RAD51 and RAD59 orthologs of the pathogenic fungus *Candida albicans*. CaRad51 exhibited more than 50% identity with several other eukaryotes, and has a conserved catalytic domain of bacterial RecA involved in binding and hydrolysis of ATP. CaRad59p also showed similarity with orthologs of other fungi. Null strains of rad51 exhibited a filamentous morphology and had a grow rate about 80% of wild type. As compared to the parental strain, the rad51 null also exhibited a moderate sensitivity to UV light and oxidizing agents, and a higher sensitivity to compounds that cause DSBs in DNA, including the radiomimetic compound MMS and camptothecin, indicating a role in DNA repair. By comparison, the rad52 null had a higher percentage of filaments, a more severe growth defect, and a greater sensitivity to compounds hat caused DSBs (MMS and camptothecin), but a similar sensitivity to UV light and oxidizing agents. The rad59 null behaved as the parental strain in all these assays. Double mutants rad51rad59 and rad52rad59 were indistinguishable from rad51 and rad52 respectively in the same assays, except that the filaments were wider. These results may be explained by the existence of at least two homologous recombination pathways, one dependent and one independent of Rad51. However, in contrast to the situation reported for S. cerevisiae, the Rad51-independent pathway of DNA repair seems to be also independent of Rad59. The absence of Rad51, Rad59, or both did not affect the length of the telomeres, which was increased in the rad52 null.

PP-02-1

Suppression of anti-*Candida* activity of macrophages by farnesol

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[Purpose]
Farnesol is well known as a quorum sensing molecule of *Candida albicans*. We have already reported that farnesol inhibited the formation of white patches on the tongue surface in murine oral Candidasis through the suppression of mycelial growth of *C.albicans*. However, roles of this molecule in physiological or pathological interactions between *Candida* and hosts have been only partially elucidated. Here, we examined the effects of farnesol on macrophage viability and functions including growth inhibitory activities against *C.albicans in vitro*.

[Methods]
1. After preincubation of murine macrophages with or without farnesol, *C.albicans* was added to the plate, which was further incubated for 16 hours. The macrophage-mediated inhibition of the mycelial growth of *C. albicans* was determined using a crystal violet staining assay.
2. Cytotoxic action of farnesol against murine macrophages was examined by L-lactate dehydrogenase (LDH) release assay. Intracellular production of reactive oxygen species (ROS) in the farnesol-treated macrophages was measured by PeroxiDetect™KIT.

[Results and Discussions]
1. Murine macrophages, when cultured in the presence of 56-112 μM of farnesol, decreased their activity inhibiting the mycelial growth of *C. albicans*. This suppression of macrophage function by farnesol was neutralized by the coexistence of the antioxidant probucol (50 μM).
2. Farnesol (>56 μM) caused the release of LDH from macrophages. This release was dose-dependently blocked by the antioxidant probucol. Microscopically, the macrophages cultured with farnesol lost their spindle shape and displayed blebbing on the cell surface. Farnesol significantly increased intracellular peroxide production. This increase of peroxides was also inhibited significantly by the addition of probucol.

These results indicate that farnesol lowered viability of the murine macrophages and suppressed their anti-*Candida* activity through induction of ROS.

PP-02-2

Immunological aspects of Chitin, the legand of toll-like receotor-2

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Recently, Da Silva *et al.* confirmed the correspondence of toll-like receptor (TLR)-2 to chitin, and stated that chitin therefore participates in an integral part of the innate immunity. Other recent findings by van Eijk *et al.* (2007) on chitin-degrading defense factor, chitotriosidase, well agree with those of Da Silva *et al*. In 2004 and 2005, Poporatto *et al.* published 2 papers dealing with a water-soluble low molecular weight chitosan, per os 1 to 3 mg per rat. These workers reported release of anti-inflammatory cytokines, and increase of CD3+ cells in mesenteric lymph nodes and spleen. The above findings indicate that both chitin and chitosan serve as the ligand of TLR-2. Since 1982, Suzuki S, and Suzuki M, and their co-workers conducted a series of immunodefense studies on chitin, chitosan and their oligosaccharides as follows: 1. Growth-inhibitory effect of chitin and chitosan against Ehrlich and sarcoma 180 ascites tumors in mice, and killing effect of the mouse peritoneal exudate cells to *Staphylococcus aureus* (Suzuki S *et al.* 1982). 2. Enhancing effect of active oxygens in mouse peritoneal exudate cells and Candidacidal activities by chitin- and chit osan- oligosaccharides (Suzuki K *et al.* 1985); 3. Growth-inhibitory effect of chitin- and chitosan- oligosaccharides to Meth A solid tumor (Suzuki K *et al.* 1988); 4. Growth-inhibitory effect of chitin- and chitosan-oligosaccharides to MM46 solid tumor (Tokoro A *et al.* 1988). From these findings, N-acetyl chitohexaose (NACOS-6) was found to be the most potent inhibitor of transplantable mouse tumors.

Last year, Kan presented a paper on treatment of patients of stomach, bladder, and lung cancers with "Chitin-oligosaccharide mixture 6", per os, 2,5 to 110g/day for different periods, up to 3 years.
Improvement of the results can be done by use of pure NACOS-6 via intravenous route.

PP-02-3

Sporotrichosis of the face by autoinoculation associated with tacrolimus treatment

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A 73-year-old woman presented with a 4-month history of a rapidly growing eruption on her face. Five months previously, she had been diagnosed with lupus nephritis and had been treated with tacrolimus 2mg daily. Two weeks after the dose of tacrolimus was increased to 3mg daily, she recognized an erythematous nodule on her left cheek. The number of eruptions on her face then rapidly increased. Physical examination revealed a number of erythematous nodules on bilateral cheeks and anterior neck with crusty or erosive surfaces; some were adhesive. Sporotrichin reaction was positive. An incision biopsy showed spores distributed into the deep dermis surrounded by a granulomatous inflammatory response with neutrophils and lymphocytes. Plated on Saburaud agar at room temperature, biopsy specimens grew a white carpet-like colony with central wrinkles. Microscopic examination revealed fungus that had narrow hyphae and conidia with a flower-like formation at the tips of the conidiophores. These examinations together suggested a diagnosis of Sporotrichosis caused by *Sporothrix schenckii* spread by autoinoculation. Restriction fragment length polymorphism analysis was performed in mitochondrial DNA and internal transcribed spacer regions of ribosomal RNA. The molecular analysis showed the present strain belonged to mt-DNA type5 and rDNA groupB that were predominant in Chiba prefecture, where the patient is resident. The lesion resolved clinically after 5 months of treatment with itraconazole 200mg daily with local hyperthermia and withdrawal of tacrolimus. Our results indicated that tacrolimus can cause rapid growth of a sporotrichosis eruption with autoinoculation by inhibiting the TCR-mediated transcription of IL-2.

PP-02-4

Differences in sensitization between allergic bronchopulmonary mycosis and fungus sensitized bronchial asthma

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BACKGROUND:Allergic bronchopulmonary mycosis (ABPM) is characterized by type I, III, and IV allergic reactions to fungal antigens. We investigated whether sensitization to fungal antigens differed between patients with ABPM and fungus sensitized bronchial asthma (FSBA).
METHODS:Our study population comprised 58 patients with ABPM and 68 patients with FSBA.We examined serum precipitins which was reflected IgG and IgM antibody to 17 kinds of fungal antigens:*Thermoactinomyces vulgaris*, *Micropolyspora faeni*, *Trichoderma viride*, *Cepharosporium acremonium*, *Aureobasidium pullulans*, *Candida albicans*, *Penicillium* mix, *Trichosporon cutaneum*, *Trichosporon asahi*,*Cryptococcus neoformans*, and 7 *Aspergillus* spp. In addition, we evaluated the levels of total serum IgE and specific IgE antibodies to 13 antigens including fungi, house-dust mites, pollen, and cat.
RESULTS:Allergic rhinitis affected 66.2% of the patients with FSBA compared with 38.5% of the patients with ABPM ($p< 0.01$), but the incidence of allergic dermatitis did not differ between the two groups. Patients with ABPM had higher titers of serum total IgE and specific IgE antibodies to not only *Aspergillus*, but also *Penicillium*, *Cladosporium*, *Alternaria*, and *Mucor* than did those with FSBA ($p< 0.01$). Whereas 34.5% of patients with ABPM had specific IgE antibodies to all 8 fungi tested, 7% of patients with FSBA had specific IgE antibody to *Aspergillus* only and at lower levels than those in patients with ABPM. However, patients with FSBA had specific IgE antibodies to more antigens, including pollen and cat, than did those with ABPM. Patients with FSBA lacked serum precipitins to most fungal antigens except *Candida albicans*.
CONCLUSIONS:Patients with ABPM were sensitized to diverse fungi and produced high IgE and IgG antibody titers to fungal antigens in serum. In contrast, patients with FSBA mounted only low-level IgE responses to fungal antigen but also reacted to pollen and cat antigens.

PP-02-5

Genetic typing of *Aspergillus flavus* isolates from allergic fungal rhino sinusitis (AFRS) cases in Northern India

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Allergic fungal rhino sinusitis (AFRS) is an emerging disease in India and majority of the cases are due to *Aspergillus flavus*. Investigation of clonality of the isolates is important to understand the epidemiology of the disease. Multi Locus Sequence Typing (MLST) is a versatile method that provides definite information on clonality. Using MLST, we investigated the genetic relatedness of 22 *A. flavus* strains isolated from AFRS patients belonging to Punjab and neighbouring states, as well as 5 isolates from body sites other than the nasal sinus. Five environmental isolates were also included in this study. We analysed the nucleotide sequence of segments of eight house keeping genes in all the isolates. The total number of alleles ranged from 2-8 for the various genes and combinations of these alleles resulted in 25 sequence types among the 32 *A. flavus* strains analysed. The resulting dendrogram revealed at least 6 genetic lineages. All the environmental isolates belonged to a single genetic lineage. Included in the same lineage were 5 AFRS isolates, indicating the environmental origin of the fungal pathogen in these cases. Four *A. flavus* strains of fungal sinusitis and two strains isolated from other body sites were present in one cluster, implying absence of body site preference by any specific strain type. The results further show that sequence analysis of six of the eight genes used in this study may be sufficient to infer levels of genetic relatedness among strains, and to reconstruct the evolutionary events.

PP-02-6

Pentraxin 3 protects from *Aspergillus* infection and inflammation in chronic granulomatous diseases

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Chronic granulomatous disease (CGD) is a primary immunodeficiency characterized by life threatening bacterial and fungal infections. However, CGD patients not only suffer from recurrent infections, but also present with inflammatory, non infectious conditions. The susceptibility of CGD mice to aspergillosis is associated with the failure to control the inherent inflammatory response to the fungus and to restrict the activation of inflammatory Th17 cells, a finding suggesting that unrestricted Th17 cell activation is a general mechanism underlying hyperinflammation in condition of NADPH deficiency. We found that exogenous administration of PTX3 inhibited the local fungal growth and dissemination to distal organs and restrained the inflammatory response to *A. fumigatus* in CGD mice. This occurred through down regulation of IL23 production by dendritic cells and epithelial cells which resulted in limited expansion of IL23R+ gamma delta+ T cells producing IL17A and the emergency of Th1/ Treg responses to the fungus with minimum pathology. PTX3 worked synergistically with voriconazole to restrict the fungal growth and decrease the lung inflammatory response. This study suggests that the NADPH independent mechanism(s) of antifungal immune protection are amenable to manipulation in CGD and that PTX3 could be successfully exploited as a novel therapeutic agent with anti inflammatory properties.

PP-02-7

Host susceptibility in mycetoma: The role of sex-hormone synthesis

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Madurella mycetomatis is the main agent of mycetoma, a chronic, subcutaneous infection characterized by discharge of grains and purulent material through sinuses. This infection is more common in men than women. It was therefore hypothesised that sexhormones were important factors in the development of mycetoma. To test this hypothesis, single nucleotide polymorphisms (SNP) in genes involved in sex hormone synthesis were studied in a population of Sudanese mycetoma patients versus geographically and ethnically matched controls. Sexhormones are synthesised from cholesterol by the following genes: CYP17, HSD3beta;, HSD17beta, CYP19, CYP1B1 and COMT. Single nucleotide polymorphisms (SNPs) for each of these genes, which influence sexhormone synthesis, are described. Polymorphisms in CYP19 and COMT were differentially distributed between patients and healthy controls. The CYP19 polymorphism was associated with a higher 17beta-estradiol (E2) production, while the COMT polymorphism was associated with a higher conversion from E2 to 4-methoxy estradiol. Furthermore, the COMT polymorphism was also associated with lesion size. The higher estradiol levels in male patients were confirmed by enzyme amplified sensitivity immunoassay. In women no significant difference in E2 levels was found, which could be due to the high variation of E2 concentrations during the menstrual cycle. Furthermore, for both males and females lower levels of dehydroepiandoresterone (DHEA) were present. No differences in testosterone levels were found. Furthermore, E2, testosterone and DHEA had no influence on the growth rate of *M. mycetomatis.* Therefore, the influence of the sex hormones on *M. mycetomatis* infection is probably not mediated by a direct effect on the fungal cells but more likely by the sex-hormonal stimulation of the immune system. Low DHEA levels and high conversion of estradiol to 4-hydroxyestradiol and methoxy-estradiol are considered a pro-inflammatory event. In conclusion, individuals with certain sexhormone biosynthesis polymorphisms are predisposed to the development of mycetoma.

PP-02-8

Natural killer cells exhibit direct activity against *Aspergillus fumigatus*

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Although animal models demonstrated that the recruitment of Natural Killer (NK) cells to the lungs plays a critical role in the host defense against invasive aspergillosis, little is known about the antifungal activity of NK cells. We therefore incubated purified unstimulated human CD56+CD3-NK cells ("fresh NK cells") and IL-2 (1000 units/ml, 7-10 days) stimulated human NK cells ("stimulated NK cells") with $1.5x10^4$ *Aspergillus fumigatus* conidia cultivated for 17 hours for germination to hyphae. Increasing E:T ratios (10:1, 20:1 and 50:1) resulted in increasing hyphal damage at 2, 4, and 6 hours of co-incubation, respectively, as demonstrated by means of the XTT assay. Notably, antifungal activity lasted longer in stimulated NK cells as compared to fresh NK cells. The direct activity of NK cells against the hyphae was also microscopically demonstrated in the viability staining with 5-carboxy-fluorescein diacetate (CFDA)/propidium iodide. The extent of the hyphal damage by both fresh and stimulated NK cells incubated with *Aspergillus* correlated with the concentration of perforin and granzyme B in the supernatant, as assessed by ELISA. Whereas no significant perforin and granzyme B concentration was measured in the supernatant of fresh NK cells without co-incubation with *Aspergillus*, high concentrations of both molecules were seen in the supernatant of IL-2 stimulated NK cells alone. Blocking experiments performed with antibodies against the Toll-like receptors TLR 2 and 4 and against the Natural Cytotoxicity Receptors (NCR) NKp30, NKp44, and NKp46 suggest that these receptors are also involved in the direct activity of human NK cells against *Aspergillus*. In conclusion, our results demonstrate that NK cells are directly involved in the host defense against *Aspergillus*, and further insight into these mechanisms might help in the development of immunotherapeutic antifungal strategies.

PP-02-9

Interferences between seric level of Zn and immunity status in pregnant women with oral and vaginal mycoses

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It is well known that the zinc plays an important role in the harmonious growing up of human fetus. The importance of this trace element for fetal development is based on its involvement in genome expression, nucleic acids metabolism, and proteins biosynthesis. On the other side, this element is mandatory for an efficient immune system. In zinc deficiency, a thymus atrophy may occur and functional thymocytes are no longer forming, the macrophages and T lymphocytes activity is impaired, and therefore the organism become unable to fight against infections.
Aim: To determinate a possible correlation between seric level of zinc and occurrence of oral / vaginal mycoses in pregnant women.
Material and method: The study has been performed on a 35 patients group with clinical signs of oral/vaginal mycosis, hospitalized in Elena Doamna Obstetrics Clinic from Iasi. The Zn dosing was performed using atomic absorption spectrophotometry on serum samples stored at -20&deg C. Qualitative and quantitative evaluation of immune response was done through lymphocytes reactivity assay to PHA and by CD4+/CD8+ lymphocyte clones study using a flow-cytometric test.
Results and discussions: The zinc seric concentration has varied between 54-656 &mug/100mL, being smaller in patients with proved fungal infections. The CD4+/CD8+ ratio decrease in the same time with seric Zn reduction. This decrease is significant in pregnant women diagnosed with fungal infection (r=-0.84, p=0.00) and insignificant in those without this illness (r=-0.14, p=0.517). The diminution of CD4+/CD8+ ratio may indicate either an increase of suppressor T lymphocytes number or a reduction in T helper lymphocytes and this fact may explain a higher incidence of fungal infections.
Keywords: zinc, immune status, mycoses, pregnancy, T lymphocytes

PP-02-10

Non-lethal *Candida albicans cph1/cph1 efg1/efg1* mutant partially protects mice from systemic infections by lethal wild-type cells

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The prevalence of fungal infections has increased significantly. Among fungal pathogens, *Candida albicans* is the most frequent cause of diseases, ranging from minor infections in immunocompetent individuals to lethal systemic infections in immunocompromised individuals. *Candida albicans* can switch from a unicellular yeast form into filamentous forms, pseudohyphae and hyphae. Under laboratory culture conditions, such as in media containing sera, where the wild-type cells are highly induced to form hyphae, the *cph1/cph1 efg1/efg1* mutant cells fail to form either pseudohyphae or hyphae. In a mouse model of systemic infections, the wild-type cells cause mortality of the injected mice, most likely due to tubular necrosis leading to renal failure. Recently, we have investigated the in vivo proliferation and invasion of *C. albicans* cells in infected mouse kidneys in order to shed light on why the wild-type cells but not the *cph1/cph1 efg1/efg1* mutant cells are lethal to the mice. Our results indicated that although the *cph1/cph1 efg1/efg1* mutant cells were not lethal, they were capable of establishing zones of infection in restricted areas and colonizing near the renal pelvis instead of simply being cleared by the mouse immune system. Interestingly, we have found that the *cph1/cph1 efg1/efg1* mutant partially protects mice from systemic infections by the lethal wild-type *C. albicans* cells. Our results further indicate that a second dose of the *cph1/cph1 efg1/efg1* mutant did not boost the degree of protection.

PP-02-11

The antimicrobial peptide LL-37 inhibits the adherence of *Candida albicans* via interaction with glycans and glycoproteins

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Antimicrobial peptides (AMPs) play an important role in the first line of mucosal immunity to defense against microbial invasion. Cathelicidins are a main family of AMPs in mammals and are expressed in neutrophils and myeloid bone marrow cells, as well as at epithelial surfaces. *Candida* species, particularly *C. albicans*, are the major human fungal pathogens which are the fourth leading cause of nosocomial infections and with a mortality rate of 40%. *C. albicans* commensally exists on the skins, nails and mucosal surfaces, and commonly causes the infections in immunocompromised patients. In this study, we demonstrated that the synthetic mature bioactive cathelicidin LL-37 decreased the adherence of *C. albicans* in a dosage-dependent manner and induced the aggregation of the floating pathogen. LL-37 was also characterized as a carbohydrate-binding peptide possessing inhibition activity of cell adherence, preferentially bound to mannan, and partially chitin on the cell surface of *C. albicans*. Moreover, we identified several cell wall proteins on *C. albicans* were also identified to involve in this process of LL-37 binding. The binding of LL-37 changed several biological behaviors in *C. albicans*, including reduction of adherence and induction of cell aggregation. Together, these results suggest that LL-37 serves as an antimicrobial barrier protecting mucosal surfaces against invasive *C. albicans* attachment and infection via its interaction with glycans and glycoproteins on the surface of this pathogen. In the future, LL-37 can be developed as an useful therapeutic peptide to prevent or against *C. albicans* colonization and infection.

PP-02-12

The effects of *Candida* cell wall glycosylation status on neutrophil activity

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The cell wall of the human pathogenic fungus *Candida albicans* is a complex structure comprising networks of discrete biochemical elements contributing to its overall stability and functionality. The outer cell wall is populated mainly by covalently linked proteins, modified by various degrees of glycosylation, generally mannose residues. This outer layer is the point of first contact between the immune system and the fungal cell, and previous studies from our group have shown that modification of the glycosylation status of the fungal cell wall has a significant impact upon recognition and cytokine stimulation by peripheral blood mononuclear cells. Neutrophils are the most common leukocyte present in the circulatory system and thus are likely to be among the first immune cell encountered by the fungus inside the host. We have used flow cytometry to monitor the adhesion and phagocytosis of *Candida* cell wall glycosylation mutants by primary neutrophils in vitro. In addition, we characterise neutrophil activation by quantification of secreted IL-8 and myeloperoxidase. Using live and unmasked heat-killed fungal strains has allowed the dissection of neutrophil stimulation into glycosylation-dependent and -independent signalling events.

PP-02-13

Cytokine responses and histology analysis in mouse tissues infected with *Candida albicans* mannosylation mutants

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Glycosylated mannoproteins in *Candida albicans* play an important role in adhesion, modulation of the host immune responses and virulence. To determine the role of glycosylation components in stimulating the innate immune response in mice, we infected BALB/c mice IV with pmr1 and mnn4 *C. albicans* mannosylation mutants. The mnn4 mutant lacks cell wall phosphomannan, but it not affected markedly in its ability to colonize and kill mice in a systemic disease model. The pmr1 mutant, however is characterized by the absence of phosphomannan, reduced O and N Linked glycans and is severely attenuated in virulence. We studied the histology in the kidneys and gross sequence of production of cytokines and chemokines in the kidneys and spleen. In the histopathology analysis, we did not find differences between CAI-4, mnn4 mutants and MNN4 reintegrant strains. However, the percentage of *Candida* by lesion and lesion size for the pmr1 mutant were lower than CAI-4 and the PMR1 reintegrant strains. Additionally, a range of cytokines and chemokines were measured in supernatants from homogenized kidneys and spleens at intervals up to 48 h post challenge. The production of cytokines or chemokines (IL-1b, KC, MIG, TNF, MCP-1, RANTES, G-CSF, IL-6, and MIP-1b) in mouse kidneys infected with the pmr1 mutant, 48h post challenge were lower than in CAI-4 and the PMR1 reintegrant. Only seven of 20 cytokines or chemokines increased in levels in spleen from mice infected with the different strains. Infection in the spleen is steadily cleared, whereas it progresses in the kidney. We demonstrated that a global defect in the mannosylation of cell wall mannoproteins due to in the absence of the pmr1, resulted a significant reduced cytokine induction. Meanwhile a mannosylphosphate-deficient mnn4 mutant induced almost normal cytokine production.

PP-02-14

Rho-kinase inhibitor suppresses pulmonary artery remodeling induced in mice by repeated inhalation of *Stachybotrys chartarum*

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[Background]
We have previously demonstrated that repeated inhalation of *Stachybotrys chartarum*, a dematiaceous fungus, induces pulmonary hypertension (PH) in mice. However, the pathogenesis of this model is yet to be understood. Recent reports suggest that the activation of the small GTPase RhoA and its downstream effector Rho-kinase (ROCK) play an important role in the pathogenesis of PH. RhoA/ROCK pathway contributes to vasoconstriction and vascular remodeling in various experimental animal models of PH. In this study, we investigated whether RhoA/ROCK pathway is involved in the pulmonary artery remodeling caused by *S.chartarum*.
[Methods]
S.chartarum isolated from house dust (IFM 53637) was used. 6-week old male ddY mice were assigned to treatment (n=6) or control (n=6) groups. All mice received repeated intratracheal injection of *S.chartarum* conidia (1×10^4 spores/mouse) totally 18 times over 12 weeks. Treatment group received fasudil hydrochloride hydrate, Rho-kinase inhibitor (100 mg/kg per day) orally in drinking water. All mice were sacrificed and examined after 84 days.
[Results]
Histopathological examination revealed pulmonary artery stenosis due to symmetric fibrocellular thickening of the intima and media in 50% (3/6) of the fasudil-treated group, and in 83% (5/6) of the control group. No significant difference was observed in the weight ratio of right ventricle to left ventricle plus septum [RV/(LV+S)] between the two groups. Significant decrease of stenotic index [1-(lumen area of artery)/(total area of artery)] of small pulmonary arteries (50-100 μm in diameter) was observed in the fasudil-treated group (0.47±0.20) compared with the control group (0.62 ±0.26) (mean±SD, $P<0.01$), indicating that Rho-kinase inhibitor suppressed pulmonary artery remodeling.
[Conclusion]
These results suggest that RhoA/ROCK pathway is involved in the pulmonary artery remodeling caused by *S.chartarum*. Further study is necessary to understand the role of RhoA/ROCK pathway in the pathogenesis of this model of PH.

PP-02-15

Role of *Candida albicans* surface antigen in adherence in *in vitro* biofilm model

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Candida albicans expresses many immunodominat antigens participating in adherence. The CR3-RP (*C. albicans* CR3-related protein) is related to human adhesion glycoprotein, also known as Mac-l, the iC3b receptor. This research studied a contribution of this protein to adherence using *in vitro* biofilm model. For experiments, standard strain *C. albicans* CCY 29-3-162 and some *C. albicans* clinical catheter isolates were selected. Biofilm was formed in polystyrene 96-well plates according to Li *et al.*, 2003. The expression of the CR3 RP was determined after 1.5-h (period of adhesion) or after 48-h (mature) biofilm and compared with that estimated in planktonic yeast cells employing two polyclonal antibodies (AB): anti CR3-RP Ab or anti CR3-RP-M Ab (immunization of rabbits with synthetically prepared peptide DINGGATLPQ and peptide conjugated with *Candida* mannan). The effective peptide and peptide-mannan conjugate vaccination was demonstrated via spectrum of specific Ig-isotype antibody response. The expression of the CR3-RP was very week in planktonic cells using both antibodies. After period of adhesion, yeasts have started to express this protein detected exclusively after interaction with anti CR3-PR Ab and significant increasing of the CR3-RP expression was observed in 48-h biofilm. It is of interest, that CR3-RP-M antibody did not react with CR3-RP expressed by biofilm. We suppose some inhibitory effect of mannan on CR-RP peptide in conjugate. The CR3-RP Ab was also tested for ability to reduce 48-h biofilm development due to binding to CR3-RP receptor. Experiment was conducted using pre-incubation of the *C. albicans* with anti CR3-PR Ab. In standard strain as well as in selected *C. albicans* catheter isolate, pre-incubation with anti-CR3-RP Ab significantly decreased biofilm. Similar results were observed employing CLSM. In summary, biofilm proved to be a suitable model for adhesion study. Moreover, *C. albicans* CR3-RP seems to be an antigen of interest in design of novel *Candida* vaccine.

PP-02-16

Multiple roles of *Candida albicans*-derived cell wall components in human keratinocytes - Activation of immune response and induction of apoptosis

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Rapid immune response in *Candida* infections is mediated by a number of innate recognition molecules known as pattern recognition receptors (PRRs). PRRs recognize conserved motifs called pathogen-associated molecular patterns (PAMPs), which represent broad groups of microbial pathogens or components. The signalling pathways trigger subsequent inflammatory responses which are crucial for successful host defence against pathogens. Fungal cell wall components such as beta-glucan and mannoproteins have been shown to stimulate the innate immune response in myeloid cells in a toll-like receptor-dependent manner, particularly through TLR2 and TLR4. However, *Candida albicans* cell wall components that specifically induce TLR responses in keratinocytes have not yet been investigated in detail.

In our studies we first examined the effect of different cell wall extractions from *C. albicans* on TLR gene expression and found an increase of TLR4 and a slight increase of TLR10, accompanied with an induction of GM-CSF and IL-8 levels, analyzed by quantitative RT-PCR and ELISA. However, the different cell wall extractions showed no major differences in the TLR expression pattern and cytokine release.

Surprisingly, stimulated keratinocytes showed a strong growth inhibition after 24h of treatment with the cell wall components. Analysis by proliferation assays resulted in nearly 90% resting cells. This observed growth inhibition is caused by a strong accumulation of the cell cycle inhibitor p27Kip1 inside the nucleus. More detailed analysis showed that the cell cycle inhibition resulted in an increase of apoptotic cells up to 30% after 72h.

In conclusion, our results indicate that distinct pattern recognition receptors together trigger the innate immunity in human keratinocytes by recognizing different structures of *C. albicans*. Furthermore, our results demonstrate the diversity of signalling pathways mediated by fungal cell wall components. Triggering innate immune responses result in the secretion of pro-inflammatory mediators which is accompanied by growth inhibition and subsequent induction of apoptosis.

PP-02-17

Characterization of PMN chemotactic factors involved in susceptibility to vaginal candidiasis

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Background: Vulvovaginal candidiasis (VVC) caused by *Candida* species is a common mucosal infection affecting significant numbers of women during their reproductive years. While adaptive immunity and innate resistance by polymorphonuclear neutrophils (PMNs) have no protective role against VVC, an aggressive PMN migration into the vagina occurs in susceptible women resulting in an aberrant inflammatory reaction associated with symptomatic infection. The migration of PMNs is strongly correlated to the vaginal presence of calcium-binding proteins, S100A8 and S100A9, during symptomatic vaginal infection. The purpose of this study was to characterize the role of the calcium-binding proteins in the immunopathogenesis of VVC using the established experimental mouse model. **Methods:** Supernatants from coculture of mouse vaginal tissues and *Candida* blastoconidia were evaluated for PMN chemotactic activity. Expressions of S100A8, S100A9 and a series of pattern recognition receptors (PRRs) were examined on vaginal epithelial cells from inoculated mice. **Results:** Similar to in vivo observations, supernatants from the coculture of estrogenized mouse vaginal explants and *Candida* showed increased PMN chemotactic activity. Epithelial cells from vaginal lavage fluid from inoculated mice with high PMN infiltration stained positive for S100A8 and S100A9 compared to epithelial cells with low or no PMNs, suggesting that the chemotactic calcium-binding proteins are produced by epithelial cells following interaction with *Candida*. Compared to epithelial cells from inoculated mice with low/no vaginal PMNs, those with high vaginal PMNs showed upregulation of mannose receptor and SIGNR1, but not TLR2, TLR4 or dectin-1. **Conclusion:** Together, we hypothesize that vaginal epithelial cells in susceptible hosts are sensitive to PRR activation by *Candida* and produce the calcium-binding proteins that recruit the PMNs responsible for the aberrant inflammatory response and symptoms associated with infection.

PP-02-18

Impact of Lactobacillus species on localised *Candida albicans* infection and the mucosal innate immune response

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Objectives: The commensal yeast *Candida albicans* is present in about 50% of the oral cavity of healthy humans and the main-agent of fungi-caused diseases in humans. In general *C. albicans* is part of the normal microflora and can become pathogen when the host immune system is weak. Several probiotic Lactobacillus species are known, that exert inhibiting and/or protective effects on *C. albicans* and other infections in vivo and in vitro. Therefore we choose L. rhamnosus GG to investigate the role of this species on localised *C. albicans* infections.
Methods: Using a model system of localised candidiasis based on reconstituted human oral epithelium (RHE) we investigate a number of different aspects of host/*Candida* interactions.
Results: Preliminary results indicate a protective role for L. rhamnosus GG in our model. RHEs treated with *Candida* and Lactobacilli showed significantly lower levels of lactate dehydrogenase (LDH), used as a marker of cell damage, compared to LDH-levels of RHEs treated only with *Candida*. This can also be confirmed by light microscopy where epithelium with co- cultured *Candida* and Lactobacilli resemble untreated controls whereas epithelium cultured with *Candida* alone is strongly damaged. Furthermore, L. rhamnosus GG seems to reduce the proinflammatory cytokine response of the RHEs towards *Candida* infection. These effects can also be obtained by the use of heat inactivated L. rhamnosus GG. Additionally expression of TLRs seems to be affected by treatment with L. rhamnosus GG.
Conclusions: L. rhamnosus GG exert protective effects in a model of localized oral candidiasis that are possibly mediated via TLRs.

PP-02-19

Renal responses during experimental disseminated *Candida albicans* infection

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Candida albicans bloodstream infections remain a problem in the clinic. A greater understanding of disease development is required to allow faster, more accurate diagnostics to be developed.
The mouse IV challenge model of *C. albicans* disseminated infection is a reproducible, well-characterized model, where the major organ targeted is the kidney, with burdens increasing during disease progression. A similar situation occurs in the human host, making this a good model to investigate host responses.
Renal responses during the early stages of *C. albicans* infection were studied using a combination of transcript profiling, histological analyses and measurement of cytokine/chemokine levels. Responses to both attenuated and virulent *C. albicans* strains were measured. Transcriptionally, the kidney showed only a minimal response to attenuated strain infection, but a massive induction of innate immune response gene expression occurred in response to the virulent strain. Differences in cytokine/chemokine gene expression levels were reflected in protein levels measured in the kidney, with higher levels associated with infections initiated by virulent strains. Histological analyses demonstrated that differences in cytokine/chemokine levels were reflected in lesion numbers and associated immune cell infiltrates found within the kidney. These results demonstrate that early host immune responses influence the pathological course of the infection.

PP-02-20

Clinical and experimental evidence for a relation between *Candida albicans* and Crohn's disease

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Crohn disease (CD) is a chronic inflammatory bowel disease. Its incidence has increased these last decades in developed countries. Compelling evidence suggest that uncontrolled inflammation of CD is based on genetic susceptibility to microbial antigens. Altough *C. albicans* (CA) is a commensal of the gut, its role has never been investigated. Development of CD is related with appearance of antibodies against microbial antigens. We showed that anti-S. cerevisiae mannan- antibodies (ASCA) were serological markers present in 60% of CD patients and 20% of their healthy relatives (HR) vs 7% in controls. Evidence was then gained that ASCA epitopes were expressed by CA in human tissues suggesting that CA was the immunogen for ASCA. This was reinforced by the recent demonstration that novel markers of CD consisting in antibodies against synthetic disaccharide fragments of chitin and glucan were also generated during a CA infection. Mycological exploration of CD families showed that CD patients and their HR were more colonized by CA than control families. In HR, CA colonization correlated with ASCA levels whereas disease outset was associated with ASCA stability and independence from CA intestinal load.
We showed that chemically induced colitis promotes stable CA colonization in mice. In turn CA colonization was shown to increase colon inflammation as assessed by histological scores and cytokines expression. This model confirmed that ASCA were generated by CA in an inflammatory background. CA was also shown to modulate pathogen recognition receptors expression. The use of mice KO for galectin-3, a lectin involved in both CA sensing and inflammation, confirmed that its presence and cooperation with TLR2 was important for modulation of CA induced inflammation.
Altogether these data suggest that intestinal diseases represent a quite unexplored research field to unravel yet unknown aspects of CA biology in its natural niche and possible medical impact.

PP-02-21

Characterization of mycological features of putative α -type mannosyltransferase deleted *Candida albicans*

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Background: *Candida* spp. has a common structure of N-linked glycochain on their outer layer of cell wall. However, the glycochain structure differs depending on *Candida* species, and seems unique. This might lead the differences on early immune response of host human, or pathogenesis. **Methods**: A total of 46 putative mannosyltransferase genes of *C. albicans* were listed from *C. albicans* genome data base. Among them, a total of 20 genes, of which gene disruptant had not been reported before, were selected and each gene disruptant *C. albicans* strain was constructed by consecutive allele replacement using the *URA3* and *ARG4* marker gene. Mycological studies, such as analysis of doubling time at exponential growth phase, temperature sensitivity, colony forming on agar plate, were evaluated on each disruptant. In addition, a mannoprotein of each disruptant was purified, and co-cultured with human PBMC, followed by evaluation of inflammatory cytokine productions as early immune response. **Results and Conclusions**: A total of 20 mutant strains, which were deleted in putative mannosyltransferase genes related to α -1,2, α -1,3, α -1,6 linkage mannosides, were obtained. Some mutant showed clearly decreasing of hyphae growth on agar medium, however the sensitivity to high temperature at 42C did not change. In addition, proinflammatory cytokine production, such as IL-6 production, was enhanced when human PBMC was cultured with a mannoprotein purified from putative α -1,2-, α -1,3- and α -1,6-mannosyltranferase gene deleted strain, respectively. These results suggest that the manipulation of cell wall surface structure of *C. albicans* induce clearly change of hyphae growth. Additionary, these changes influence immune response such as proinflammatory cytokine production.

PP-02-22

The effect of fungal species and murine strains on the development of pulmonary arterial hypertension

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Purpose: We previously showed that the inhalation of *Stachybotrys chartarum* spores causes pulmonary arterial hypertension in mice. Here we examined to know if ubiquitous environmental fungi such as *Cladosporium* spp. and *Penicillium* spp. cause similar effect on murine pulmonary arteries. Furthermore, the effect of murine strains was examined.

Methods: <Experiment 1> Isolates of *Cladosporium cladosporioides* (Cc), *Penicillium citrinum* (Pci), *P. chrysogenum* (Pch) and *Aspergillus fumigatus* (Af) were used. Each fungus was cultured on potato dextrose agar slants and spores were collected. Ddy mice were anesthetized and the spore suspension was injected into trachea repeatedly, i.e. 1×10^4 spores into each mouse at 4-5 day intervals for 4 weeks. Some animals received injections of 1×10^5 or 1×10^6 spores of Cc or Pci. <Experiment 2> *Stachybotrys chartarum* (Sc) was used. The spore suspensions (1×10^4 spores) were injected intratracheally into ddY, ICR, C57BL/6J and BALB/c mice.

Results: <Experiment 1> Histopathological examination showed that Cc and Af injection caused symmetrical thickening of pulmonary arterial wall, indicating the development of pulmonary arterial hypertension. Cc has a tendency to require more spores to cause similar effects than Af. There were some differences in the severity/frequency of the lesion among fungal strains. In contrast, spores of Pci or Pch caused no vascular changes. In the other organs no arterial changes were seen. Germination of spores was not observed throughout the experiment. <Experiment 2> DdY, ICR and C57BL/6J mice all showed pulmonary arterial wall thickening. In BALB/c no histopathological changes were present.

Conclusions: Pulmonary arterial changes in mice after spore inhalations were not limited to Sc but also seen in ubiquitous indoor airborne fungi such as Cc and Af. The difference of mouse strains has significant effect on pulmonary arterial lesion. Further investigation using the other fungal species and animal strains is warranted.

PP-02-23

The model of aortitis-induced heart failure in DBA/2 mice developed by fungal PAMPs, CAWS, water-soluble polymer complex obtained from *Candida albicans*

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Introduction - CAWS is a water-soluble polymer complex of mannoprotein-beta-glucan obtained from culture fluid of *Candida albicans* NRBC 1385. It was reported previously that CAWS has strong potency in inducing fatal necrotizing arteritis in DBA/2 mice. In this study, histopathological changes and cardiac function were investigated and an effect to control fatality by the anti-inflammatory agents was examined in this system.

Methods and Results - One mg/day of CAWS was given to DBA/2 mice for five days. All CAWS-treated DBA/2 mice died within several weeks. Histological findings included severe stenosis in the left ventricular outflow tract and inflammatory changes of the aortic valve. The ostial stenosis of coronary arteries with fibrinoid necrosis was found. Cardiomegaly was observed and heart weight increased 1.62-fold ($P<0.01$). Echocardiography revealed a severe reduction in contractility and dilatation of the cavity in the left ventricle (LV): LV Fractional shortening (LVFS) decreased from 71% to 38% ($P<0.01$), and the LV end-diastolic diameterdimension (LVDd) increased from 2.21 mm to 3.26 mm ($P<0.01$). The titer of BNP mRNA increased in the CAWS-treated group. Furthermore, the fatal toxicity of the CAWS arteritis was reduced by administration of prednisolone or sirolimus.

Conclusion - From these findings, it is thought that the progress of CAWS-arteritis is classified for three following durations, the first period to develop the vasculitis, the second period to complicate myocardial remodeling by an aortic valvular disease and the left ventricular volume overload with ischemic stress, and the last period to be given fatal influence by severe heart failure. The curative effects for fatal toxicity of CAWS-arteritis were found to the anti-inflammatory agents. This system is proposed as an easy and useful experimental model of a vasculitis and heart failure because CAWS-arteritis can be induced by CAWS injection alone.

PP-02-24

Studies on immunotoxicity of water soluble polysaccharide fraction from culture supernatant of *Candida* spp

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CAWS is a mannoprotein-beta-glucan complex obtained from the culture supernatant of *C. albicans* NBRC 1385. CAWS has two major biological activity, lethal toxicity and arteritis in mice. CAWS is performed diversified analysis, however water soluble fraction isolate from other *Candida* species is not investigated. In the present study, immunotoxicity of water soluble fractions (WS) isolated from other *Candida* species, *C. parapsilosis* and *C.metapsilosis*, were examined and compared with CAWS.

Seven strains of *Candida* spp, were cultured and prepared WS fractions using similar protocol with CAWS preparation. In the large scale production two strains produced enough quantity of WS (*C. metapsilosis* NBRC 0640 and 1068) but others are not. Using these two WS fractions, acute anaphylactoid reaction in ICR mice and induction of arteritis in DBA/2 mice were tested. Both WS showed comparative acute anaphylactoid reaction with CAWS. When administered WS to DBA/2 mice, groups of mice administered WS 1068 were all died with arteritis, however, mice administered WS 0640 were survived. These facts suggested that immunotoxicity of CAWS could be induced by other *Candida* spp, but not all of *Candida* show such toxicity.

PP-02-25

Interleukin-10 gene transfer inhibits the induction of CAWS vasculitis in mouse

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CAWS (*Candida albicans* water soluble fraction) vasculitis is induced by polysaccharide fraction isolated from *Candida albicans* NBRC 1385. CAWS arteritis is induces prominent vasculitis of the aortic valve and the coronary arteries in mouse and similar to KD. CAWS vasculitis is significantly strain dependent, i.e., sensitive strain; DBA/2, Balb/c, C57BL/6, resistant strain; C3H/He, CBA/J. From the data of cytokine profile between sensitive and resistant strains of mice, IL-10 is a key negative regulator for CAWS vasculitis, and CBA/J is a high inducer of IL-10. CBA/N mice, a mutant strain of CBA/J, deficient to produce IL-10, induced CAWS vasculitis. In this study, effect of IL-10 on CAWS vasculitis in CBA/N was studied using hydrodynamic-based IL-10 gene therapy.

We performed the gene therapy to CBA/N mice with plasmid DNA; pCAGGS-mouseIL-10 (mIL-10) and pCAGGS-ratIL-10 (rIL-10) administered by hydrodynamic-based gene delivery. As a result of the gene therapy, CAWS vasculitis of CBA/N mice was significantly inhibited compared with the control group. And, CBA/N mice administered pCAGGA-mIL-10 was decreased the production of MCP-1 and IL-6 induced CAWS. It suggested that gene delivery of pCAGGS-mIL-10 was decreased production of inflammatory cytokine; therefore, CAWS vasculitis was inhibited in CBA/N mice.

PP-02-26

Interleukin-10 is a negative regulatory factor of CAWS-vasculitis in CBA/J mice assessed by comparison with Bruton's tyrosine kinase deficient CBA/N mice

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Candida albicans water-soluble fraction (CAWS), a mannoprotein-beta glucan complex obtained from the culture supernatant of *Candida albicans* NBRC 1385, exhibits vasculitis-inducing activity (CAWS vasculitis) in mouse administered intraperitoneally. The sensitivity to CAWS vasculitis greatly varies among mouse strains. In this study, we examined the factors contributing to or inhibiting CAWS vasculitis, using CAWS-vasculitis-resistant CBA/J mice and Bruton's tyrosine kinase (Btk)-deficient CBA/N mice, which have the same origin as CBA/J mice. Btk is believed to be essential for B cell differentiation and maturation. In particular, this enzyme is reported to participate in the rearrangement of light chain gene during pre-B cell differentiation to immature B cell. Consequently, few mature B cells exist in CBA/N mouse and antibody-producing ability is decreased. After stimulation with various kinds of pathogen-associated molecular patterns (PAMPs), the production of inflammatory cytokines IL-6 and IFN-gamma was induced in the spleen cells of CBA/N mice, whereas that of immunosuppressive IL-10 was induced in splenocyte of CAWS-vasculitis-resistant CBA/J mice. Furthermore, the production of TIMP1, an endogenous matrix metalloproteinase (MMP) inhibitor, was observed in CBA/J mice. The results strongly suggest that the difference in the production of these cytokines is closely linked to the development of CAWS vasculitis.

PP-02-27

Measurement of (1,3)-β-D-glucan concentration in several drugs for injection

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(1, 3)-β-D-glucans (BG) are present in a variety of organisms including fungi, yeast, algae, bacteria, and higher plants. Compared with lipopolysaccharides (LPS), their structures differ significantly, depending upon molecular weight, degree of branching, and ultrastructure. BG exhibit a variety of biological activities including antitumor activity, macrophage activation, complement activation, and others. In addition to these biological activities, we reported that BG induced septic shock in mice administered the nonsteroidal anti-inflammatory drug, indomethacin. In addition, BG induced autoimmune arthritis in SKG mice and also induced allergy. We measured BG concentrations in several branded and generic drugs, using the BG detection kit, Glucatell®. *Limulus*- or *Tachypleus*-based reagents are used for the highly sensitive detection of blood-borne fungal cell wall-derived BG in the clinical diagnosis of invasive mycosis. Parenteral drug-based contamination of patients can lead to diagnostically false positive results. Accordingly, we measured the BG concentrations in 94 drugs for injection. These included 8 antifungals, 4 heparins, 16 nafamostat mesilates, 8 physiological salines, 15 monoammonium glycyrrhizinates, 5 concentrated glycerins, 14 elcatonins, 11 sodium ozagrels, and 13 procaine hydrochlorides. The majority of these drugs contained less than detectable concentrations of BG. Some of the monoammonium glycrrhizinates, elcatonins, and sodium ozagrels contained BG at greater than 10 pg/mL. It is generally accepted that BG is difficult to break down and accumulates in reticuloendothelial organs such as liver and spleen over the long term in mice. Considering the fact that BG affects biological activities in human tissues, it is reasonable to measure and regulate BG concentrations in drugs for injection.

PP-02-28

Analysis of cytokine production from PBMC by stimulation with solid phased intravenous immune globulin

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Application of Intravenous immunoglobulin (IVIg) has still growing to various intractable diseases. The two major clinical indications for IVIg use are IgG replacement therapy and anti-inflammation therapy in variety of acute and chronic autoimmune diseases, such as idiopathic thrombocytopenic purpura (ITP), Guilain-Barre syndrome, and Kawasaki disease. One of the proposed mechanisms for IVIg activity is the modulation of cytokine expression and function. Thus we analyzed the cytokine production by Peripheral Blood Mononuclear Cell (PBMC) in vitro stimulated with solid phase IVIg. Assessed by Multi-Plex multiple cytokine measurement system, production of various cytokines/chemokines, such as IL-1beta, IL-6, and MIP-1beta, were increased significantly. Productions of cytokine/chemokines were increased more by solid phase IVIg in the presence of Lipopolysaccharide (LPS), except for IL-10 and MCP-1. These results suggest that cytokine/chemokine dynamics were significantly modulated by the solid phase IVIg.

PP-02-29

Involvement of branched units at position 6 in the reactivity of a unique variety of β-D-glucan from *Aureobasidium pullulans* to antibodies in human sera

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[Objective]
We determined the structure of a unique type of 1,3-β-D-glucan obtained from *Aureobasidium pullulans* (AP-FBG) and found that it reacted with the antibodies in human sera. The reactivity of AP-FBG to the antibodies differed from other β-D-glucans. However, the difference between these β-D-glucans that react strongly and weakly with human antibodies is not completely understood. Therefore, we postulated that the difference in the reactivity of β-D-glucans to antibodies in human sera depends on the presence of branching chains at position 6 of the β-D-glucan molecule. This is because the reactivities of human sera to β-D-glucans seem to differ depending on the frequency and length of the branched chains at position 6. Here, we report the reactivity and specificity of AP-FBG to human sera and discuss the relationship between its reactivity and primary structure.

[Methods]
The structure of AP-FBG was determined by NMR spectroscopy. The reactivities and specificities of β-D-glucans to antibodies in human sera were determined by ELISA experiments.

[Results]
AP-FBG has a mixed structure comprising a 1,3-β-D-glucan backbone with single 1,6-β-D-glucopyranosyl-branching units at every 2nd or 3rd residues (approximately 7:3). From the results of the competitive ELISA experiments, it was apparent that 1,6-β-D-glucans such as pustulan inhibit the binding of anti-AP-FBG IgGs to AP-FBG to a greater extent than the 1,3-β-D-glucans such as curdlan do.

[Conclusion]
Our results strongly suggest that the reactivity of β-D-glucans to IgG in human sera depends on the branched chains at position 6. It is well known that the 1,6-β-D-glucopyranosl side chains extend from the outer side of the 1,3-β-D-glucopyranosl backbone. This may explain the strong influence of the 1,6-β-D-glucopyranosl side chains on the reactivity of β-D-glucans to IgG in human sera. This characteristic highly branched structure of AP-FBG could be an advantage for its use in medicine, e.g., as an immunostimulatory agent.

PP-02-30

Effect of anti-β-glucan monoclonal antibody to biological activity of fungal cell wall β-glucan

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We showed that the antibody to β-glucan which is one of the main fungal cell wall components existed and this antibody titer decreased in patients with a deep mycosis whose plasma was β-1,3-glucan-positive. Furthermore, to demonstrate a role of anti-β-glucan (BG) antibody, we developed mouse anti-BG monoclonal antibody (mAb). In this study, we examined reactivity of anti-BG mAb to fungal cell wall β-glucan and pathogenic fungi, and modification of anti-BG mAb to biological activity of BG.

Hybridoma (1A5; IgM, 2B8; IgG_3) secreting the anti-β-glucan antibody was generated after fusion of spleen cells of CSBG immunized DBA/2 mice and myeloma cells of the murine line p3X63. We examined reactivity of 1A5 and 2B8 by ELISA plate coated various BG. These antibodies showed high reactivity to CSBG. Moreover, 1A5 showed high titer to β-1,3-glucan and 1A5 showed high titer to β-1,6-glucan. Furthermore, we examined the binding activity of these antibodies to fungal body by confocal microscopy. Both antibody bound *C. albicans* cells. Next, we prepared the complex of *Candida* particulate BG (OX-CA) and 1A5 at a various ratios, and stimulated DBA/2 mice splenocyte with these complexs for 48h. The IFN-γ production of culture supernatant was enhanced dose dependently of 1A5.

We developed anti-β-glucan monoclonal antibody which showed reactivity to pathogenic fungal cell wall β-glucan. Also, these antibody reacted pathogenic fungal cell, and modified immune response to fungal cell wall β-glucan. It was suggested that anti-β-glucan antibody play a role of one of recognition molecules to fungal cell wall β-glucan in host.

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PP-02-31

Preparation of *Trichophyton* cell wall β-glucan by NaClO-DMSO methods

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β-glucan is one of the main fungal cell wall components and shows variety in structural and physical properties such as degree of branching, molecular weight, solubility in water, and conformation: the triple helix, single helix and random coil. We have successfully developed *Candida albicans* and *Aspergillus niger* solubilized cell wall glucan (CSBG and ASBG) by sodium hypochlorite (NaClO) oxidation and subsequent dimethyl sulfoxide (DMSO) extraction. In this study, we tried to prepare of *Trichophyton* cell wall β-glucan by application of this method to *Trichophyton* spp. Furthermore, we examined the reactivity of antibody in human sera to these β-glucan.

The acetone-dried mycelia of *Trichophyton* spp. (2g) was suspended in 200mL of 0.1M NaOH with NaClO of various available chlorine concentrations for 1d at 4° C. After the reaction was completed, the reaction mixture was centrifuged to collect the insoluble fraction (NaClO-treated *Trichophyton*, OX-TR). OX-TR suspended in DMSO was ultrasonically disrupted and the resulting suspernatant was designated as TSBG. Analysis of the C^{13}-NMR spectra revealed the preparations to be composed of β-1,6-glucan and β-1,3-glucan. Next, we examined the reactivity of human immunoglobulin and sera to TSBG by ELISA plate coated TSBG.

We could prepare *Trichophyton* cell wall β-glucan by NaClO-DMSO method. It was showed that the anti-BG antibody in human sera bound to various fungi including *Trichophyton*. It was suggested that TSBG became one factor that influences the pathological condition of trichophytosis.

This work was supported by the Program for Promotion of Basic and Applied Researches for Innovations in Bio-oriented Industry (BRAIN)

PP-02-32

Immunomodulation by *Agaricus brasiliensis* extracts in mice

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We previously reported that the *Agaricus blazei* [*Agaricus brasiliensis*] extracts show various immunopharmalogical activities. We have also determined that the major carbohydrate component of the fruit body of *A. brasiliensis* is β-1,6-glucan. In order to demonstrate the molecular mechanisms mechanisms of these immunopharmalogical activities, we examined the cytokine production in vitro and the antibody titer of *A. brasiliensis* extracts.

Hot water extract (AgHWE) and cold alkali extract (AgCA) were prepared from the fruit bodies of *A. brasiliensis*. Splenocytes of DBA/2, Balb/c or C57BL/6 mice were stimulated with AgHWE or AgCA (1, 10, 100 μg/mL) for 48h. GM-CSF and IFN-γ production were induced dose dependently by the AgCA stimulation. DBA/2 mouse showed the higher production in both cytokines than in the other strain. To neutralize GM-CSF, splenocyte was stimulated with AgCA in the presence of anti-GM-CSF, and found that IFN-γ production by AgCA was decreased. These facts strongly suggested GM-CSF is a key cytokine for immunopharmacological activity of AgCA.

Anti-β-glucan antibody specific for β-1,3-glucan and β-1,6-glucan chains was detected in human sera We examined the reactivity of the anti-BG antibody to *A. brasiliensis* extracts in human sera. A significant titer to AgHWE and AgCA was detected.

This study demonstrates that *A. brasiliensis* extracts induced GM-CSF and IFN-γ production in vitro. The existence of the antibody to each extract was confirmed and that it participates in the immune response to *A. brasiliensis*. The DBA/2 mouse is the strain which shows high response to β-glucan. The cytokine induction in this mouse was potently related with the GM-CSF and cell to cell contact. There is possibility that *A. brasiliensis* extracts induce a cytokine production through similar mechanisms.

PP-02-33

Comparison of response of PBMC stimulated with β-glucan in human volunteers

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β-1,3-glucan is fungal cell wall component, and shows various immunological activities. Responses of murine lymphocytes to such β-glucans are significantly different among strains of mice. Similarly anti-β-glucan titer in human volonteers was significantly different in each other. In this study, comparison of response to β-glucan was examined in human volunteers.

A peripheral blood mononuclear cell (PBMCs) is isolated peripheral venous blood in 45 healthy volunteers. PBMCs were cultured in RPMI-1640 supplemented with 10% inactivated autologous plasma and were stimulated with β-glucan (100μg/mL; BBG, OX-CA, SPG, CSBG). Cytokine production (TNF-α, IL-8) was compared after 12 or 24 h of culture. LPS was used as control to examine whether β-glucan response was peculiar.

Volunteers that responded high or low responded were appeared in TNF-α and IL-8 production. Those responses were different from those shown by stimulation with LPS. When the responses between various β-glucans were compared, the difference was seen between the healthy volunteers.

There are some reasons to show such differences in humans, such as the heterogeneity of β-glucan structure, the expression of β-glucan receptor as well as the signal transduction of such receptor in PBMC, and so on.

PP-02-34

Comparison of biological activity between soluble and particle β-glucan

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β-Glucans are major cell wall structural components in fungi. We are focusing on analyzing the biological activities of soluble β-glucan: SCG, which is a 6-branched 1,3-β-D-glucan in *Sparassis crispa Fr.*. We recently found that the leukocytes from DBA/1 and DBA/2 mice are highly sensitive to SCG, producing cytokines, and that GM-CSF regulates this reaction. The pattern of the response of the leukocytes differed between soluble glucan and particulate glucan. In this study, we examined the activity of particulate glucan: OX-CA from *Candida albicans* on leukocytes from DBA/2 mice, and compared it with that of SCG.

Splenocytes were cultured in the presence of SCG or OX-CA in vitro for 48hr, and then the supernatant was collected to measure cytokines, such as TNF-α, IL-6, IFN-γ, GM-CSF. OX-CA stimulated splenocytes in DBA/2 mice to produce these cytokines. The amounts of cytokines induced were large compared with those produced in response to SCG. In C57BL/6 mice, cytokine production was also induced by OX-CA, but not by SCG. Neutralizing GM-CSF in splenocyte cultures significantly inhibited the production of these cytokines elicited by SCG in DBA/2 mice. On the other hand, IFN-γ induction of OX-CA was partially inhibited by neutralizing GM-CSF. Cell to cell contact was an essential step for the induction of IFN-γ and GM-CSF by SCG. On the other hand, OX-CA directly induced adherent splenocytes to produce IFN-γ and GM-CSF. The induction of TNF-α by OX-CA was inhibited by SCG.
Taken together, the manner of the effect of OX-CA on leukocytes would be similar to that of SCG. However, the pathways of cytokine induction in the spleen by OX-CA and SCG include specific steps in each other in addition to the common pathways.
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PP-02-35

Effect of GM-CSF on cytokine induction by β-glucan: SCG *in vitro* in β-glucan-treated mice

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β-Glucans are major cell wall structural components in fungi. SCG is a 6-branched 1,3-β-D-glucan in *Sparassis crispa Fr.* showing antitumor activity. *S. crispa* is an edible / medicinal mushroom. We recently found that the leukocytes from DBA/1 and DBA/2 mice are highly sensitive to SCG, producing cytokines, such as GM-CSF, IFN-γ, TNF-α and IL-12p70, but not IL-6. The finding that the induction of cytokines by SCG was inhibited by neutralization of GM-CSF indicated that GM-CSF regulates this reaction. In this study, we examined the effect of the i.p. administration of SCG to DBA/2 mice on cytokine production *in vitro*.
SCG was administered i.p. to DBA/2 mice on day 0. Splenocytes were prepared on day 7, and cultured in the presence of SCG (100 μg/ml) *in vitro*. The levels of cytokine production induced by SCG *in vitro* were significantly lower in the cells from SCG-treated mice than control mice. This reduction was observed from day 3 to day 28. Augmentation of GM-CSF consumption was observed in the culture medium of splenocytes from the SCG-treated mice on day 7. The addition of rmGM-CSF to the culture medium, resulted in producing larger amounts of TNF-α and IL-6 in splenocytes of the SCG-treated mice than normal mice. These results indicated that the mobilization to the spleen of cells which consumed GM-CSF on the i.p. administration of SCG changed the profile of cytokine production induced by SCG *in vitro*.
Taken together, these findings indicated that GM-CSF was closely related with the reactivity of β-glucan, and that the regulation of GM-CSF would be effective for controlling the reactivity of β-glucan.
This work was supported by the Program for Promotion of Basic and Applied Researches for Innovations in Bio-oriented Industry (BRAIN).

PP-02-36

Effect of a linear 1,3-glucan, Curdlan-oligo on cytokine induction of SCG: 6-branched 1,3-glucan *in vitro*

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β-Glucans are major cell wall structural components in fungi. SCG is a 6-branched 1,3-β-D-glucan in *Sparassis crispa Fr.* showing antitumor activity. We recently found that the leukocytes from DBA/1 and DBA/2 mice are highly sensitive to SCG, producing cytokines, such as GM-CSF, IFN-γ, TNF-α and IL-12p70. The structural features of β-1,3-glucans, including the primary structure, solubility, degree of branching, conformation and molecular weight, could play an important role in various kinds of glucan-associated biological activities. Curdlan, an extracellular bacterial polysaccharide, is a linear β-1,3-glucan. Previously, we developed Curdlan-oligo (CRDO, MW: 340-4000). In this study, we investigated its effect on the production of cytokines in leukocytes from mice, and compared its activity with that of SCG.

Splenocytes from DBA/2 mice were cultured with CRDO or SCG (0, 1, 10 or 100 μg/ml) *in vitro*, and then the supernatant was collected to measure cytokines. SCG stimulated the splenocytes to produce GM-CSF, IFN-γ and TNF-α. CRDO induced production of GM-CSF and IFN-γ, but not TNF-α. The amounts of GM-CSF and IFN-γ were small compared with those produced in response to SCG. The effect of SCG on TNF-α production was partially inhibited by CRDO. CRDO also inhibited cytokine induction of particulate β-glucan: OX-CA from *Candida albicans*. High amount of Laminariheptaose inhibited cytokine induction of SCG.

Taken together, these results showed that CRDO inhibited the cytokine induction of SCG and OX-CA. These results suggested that β-glucan oligosaccharide composed of seven glucose units including in CRDO could be related to its activity.

PP-02-37

Influence of *Candida* β-glucan on the hapten-induced contact dermatitis in mice

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β-glucan (BG) is a major component in fungal cell wall and induces various biological responses such as activation of leukocyte, promotion of phagocytosis and production of pro-inflammatory cytokine. Moreover, the fungi have been known as a factor in which allergic symptoms such as asthma is deteriorated. We have shown that fungal BG enhances IgE-mediated allergic reaction in NC/Nga mice. However, the influence in other allergic reactions by BG has not been elucidated. Then, to examine the possibility of BG as the deteriorating factor in the delayed type allergic skin reaction, we investigated the influence of *Candida* BG on a mouse model of the contact dermatitis caused by repeated application of 2,4,6-trinitro-1-chlorobenzene (TNCB) as a hapten.

C57BL/6J mice were sensitized with TNCB and *Candida albicans*-derived soluble BG (CSBG) on the skins of their abdomens "initial sensitization". After 5 days from the initial sensitization, they were challenged every 4 days by painting TNCB and/or CSBG on the ear robe after removing the corneum. Ear thickness was measured at regular time intervals. The IgE level and cytokines in the mice plasma and in the ear tissues were measured by ELISA.

The increased ear swelling by application with TNCB/CSBG was observed on day 16 after the first ear challenge. The swelling was severer than TNCB-treatment alone. Especially, the swelling by TNCB/CSBG was sustained in 48h after the last painting. The cytokines, IFN-γ and IL-17A, in the ear tissue and plasma IgE level on day 24 were also increased by the TNCB/CSBG-application. These results demonstrate that CSBG acts as worsening factor by enhancing allergic immune response in the dermatitis. Taken together, it was suggested that *Candida* cell wall in the dermal tissue might deteriorate the allergic reaction.

PP-02-38

Dectin-1-mediated innate immune response to *Candida albicans* cultured in various conditions

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The cell wall of *Candida albicans* (CA) is recognized by immune receptors and subsequently induces various immune responses. Because the components of the cell wall might vary in culture conditions, the compositional changes seemed to affect usage of the immune receptors and outcome of the immune responses. Here we focused on the major cell wall polysaccharides, beta-glucans, and the C-type lectin receptors to examine the co-relation between culture conditions of CA and innate immune responses by macrophages.
C. albicans NBRC1385 was cultured in natural source mediums or chemically defined artificial medium, and then treated with formaldehyde- or heating to fix the cell wall state. To examine the dectin-1 binding to CA prepared, CA were treated with soluble dectin-1-Fc. The binding to CA cultured at 37degree was higher than 27degree. The CA obtained by artificial medium showed higher binding to the dectin-1-Fc than the CA cultured with Potato dextrose broth. The heat-treatment showed much higher binding to the dectin-1-Fc than formaldehyde-treatment. The CA significantly activates the transcription factors, NF-kappaB and NF-AT in dectin-1-transduced cells. Macrophages from wild type mice showed significant CA binding and increased TNF-alpha production. In contrast, dectin-1-/- mice showed decreased binding and no TNF-alpha production in response to any preparations of CA. The residual CA binding to dectin-1-/- macrophages was significantly decreased with dectin-2 mAb, implying dectin-2 on the macrophages might recognize CA in addition to dectin-1. The CA with higher binding to dectin-1-Fc induced higher level of TNF-alpha production, suggesting dectin-1 is important for recognizing CA and inducing TNF-alpha. Above results demonstrate that the culture condition of CA influences the beta-glucan exposure on CA surface, and affects the dectin-1-mediated innate immune response.
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PP-02-39

Complement receptors modulate macrophage activation in response to particulate *Candida* β-glucan

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<Objective> *Candida*-derived particulate β-glucan (OX-CA) activates macrophage (MP) function such as proinflammatory cytokines and nitric oxide production to augment host defense system. Although Dectin-1, a major functional receptor in the β-glucan-mediated biological response, has been well documented, the function of other glucan receptors including the complement receptors, CR3 (CD11b/CD18) or CR4 (CD11c/CD18), are still vague. To examine the effect of complement receptors on the MP activation, we compared between $CD11b^{-/-}$ and wild type mouse peritoneal MPs in the stimulation with OX-CA.
<Results and Discussion> Peritoneal MPs from C57BL/6J (WT) or $CD11b^{-/-}$(KO) mice were cultured with or without OX-CA or TLRs ligands. Nitric oxide (NO) production in the culture supernatants were measured with Griess reagent. The OX-CA-induced NO production by KO MP was significantly higher than WT, whereas LPS or lipopeptide induced equal level of NO in WT and KO. KO showed enhanced iNOS mRNA and phospho-syk, JNK, and p38 MAP kinase than WT. The effect of OX-CA on the NO production was abrogated in $dectin\text{-}1^{-/-}$ mouse-derived MP, suggesting dectin-1 is essential for the signaling. CD11b KO MP showed higher surface expression of CD11c than WT. CR4/CHO transfectant but not CR3/CHO effectively bound to OX-CA, suggesting CR4 functions as recognizing unit for *Candida*-derived β-glucan particles. The MPs were cultured with monoclonal antibodies against CD11c or dectin-1, then cross-linked with the 2nd Ab. The receptor cross-linking of dectin-1 alone significantly activate the NO production. CD11c-ligation alone did not. However, simultaneous ligation by the antibodies for dectin-1 and CD11c significantly enhanced the NO production than dectin-1-ligation alone. These results suggest that up-regulated CR4 preferentially binds to OX-CA to enhance the dectin-1-mediated signal transduction in macrophages. CR4 might augment the dectin-1-mediated cell signaling by co-ligation with β-glucan moiety of *Candida*.

PP-02-40

Comparative study of receptor functions of dectin-1 in rat, mouse and human

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Dectin-1 is a small C-type lectin receptor that recognizes fungal cell wall β-glucan, and is responsible for host defense against fungal infection by producing ROS and proinflammatory cytokines. Dectin-1 homologues in some species including mouse and human have been characterized and their importance in anti-fungal immunity has also been clarified. However, its homologue in the rat has not been identified. In this study, we analyzed DNA/amino acid sequence of rat dectin-1 by rapid amplification of cDNA ends. The aminoacid sequences including YXXL, DED, and WIH of rat dectin-1 is highly conserved in rodents and human. It possesses essential motifs for the signaling and recognition of β-glucan. However, the position of the start codon of rat dectin-1 was different from mouse and human. As with mouse dectin-1, rat has two major isoforms generated by alternative splicing. The one is a full-length isoform and the other is a short isoform that racks stalk domain. We also demonstrated that rat dectin-1 is capable of binding fungal β-glucan and activating NF-κB via Syk and CARD9-Bcl10 mediated pathway. However, the expression level on the cell surface is varied in rodents and human. The expression level and the abilities of ligand binding and signaling are altered by mutating N-glycosylation sites, implying cell surface expression is important for the receptor function. These results suggest that basic function as a β-glucan receptor is common in the animal species tested, but the physiological roles of the isoforms with or without glycosylation may have unique roles on the immune response.

PP-02-41

Insoluble β-glucan from the cell wall of *Candida albicans* induces immune responses of human keratinocytes through Dectin-1

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Backgroud: β-glucan is the major structure component of *Candida albicans* cell wall. It has been demonstrated that Dectin-1 as the principal C-type lectin pattern-recognition receptor (PRR) can recognize fungal β-glucan and induce immune responses. In this study, we sought to clarify whether insoluble β-glucan from the cell wall of *C. albicans* (CaIG) could induce an inflammation response in human keratinocytes and to determine the underlying mechanisms.

Methods: The structure of insoluble β-glucan from the cell wall of *C. albicans* were elucidated using monosaccharide composition analysis by gas chromatography (GC), methylation analysis by gas chromatography-mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR) spectroscopy. Human primary keratinocytes were challenged with CaIG in vitro. The mRNA expression of Dectin-1, TLR2, proinflammatory cytokine (TNF-alha) and chemokine (IL-8) was assayed by real time RT-PCR. The secretion of TNF-alpha and IL-8 were measured by ELISA. Western blotting was used to analyze IkappaB-alpha phosphorylation and degradation.

Results: The insoluble β-glucan contained beta-1, 3-linked backbone with a side chain mainly containing beta-1, 6 linked glucose residue. Exposure of keratinocytes to CaIG led to increased gene expression and secretion of TNF-alpha and IL-8. CaIG up-regulated the mRNA of Dectin-1, whereas the mRNA level of TLR2 was not altered. Human keratinocytes challenged with CaIG resulted in the activation of NF-kappB in a time dependent manner. Dectin-1 inhibitor laminarin blocked the CaIG-induced -production of TNF-alha in keratinocytes, but no such effect was observed in pretreatment with anti-TLR2 neutralizing antibody and the LPS inhibitor (polymyxin B).

Conclusions: These data suggest *Candida albicans* native insoluble β-glucan may play a role in activation of inflammatory responses in human keratinocytes through Dectin-1, not TLR2.

PP-02-42

Effect of dietary oils on host resistance to *Paracoccidioides brasiliensis* infection in mice

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The effect of dietary oils on host resistance to *Paracoccidioides brasiliensis* infection was investigated. Mice fed palm oil, which is rich in saturated and monounsaturated fatty acids, supplemented with docosahexaenoic acid (DHA, n-3 polyunsaturated fatty acid, 22:6) showed reduced antifungal activity in the spleen and liver, as compared with mice fed palm oil or soybean oil, which is rich in n-6 unsaturated fatty acids, without supplementation with DHA. Mice fed DHA-supplemented soybean oil also showed reduced antifungal activity in the liver, but the extent of reduction was less profound. This reduction in antifungal activity was not observed with eicosapentaenoic acid (EPA, n-3 polyunsaturated fatty acid, 22:5)-supplemented palm or EPA-supplemented soybean oil. Thses results suggest that two factors, DHA and palm oil in combination, are involved in reducing the host resistance. DHA-enriched palm oil was also responsible for an increase in DHA concentration and a marked decrease in arachidonic acid content in the spleen and liver. However, this group did not show elevated spleen and liver phospholipid hydroperoxide levels compared with the other groups, excluding the possibility that the reduction in antifungal activity observed with DHA-enriched palm oil is due to acceleration of in vivo lipid peroxidation. Greater infection-induced increases in spleen and serum interferon-gamma concentrations were observed in mice fed DHA-enriched palm oil compared with the other groups.

PP-02-43

Paracoccidioidomycosis and patients dendritic cells: Influence of *Paracoccidioides brasiliensis* antigen on surface costimulatory molecules expression and cytokines release

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Introduction. Paracoccidioidomycosis (PCM) is a systemic disease caused by *Paracoccidioides brasiliensis (P. brasiliensis)*, a dimorphic pathogenic fungal agent and is characterized by a cellular Th1 protective immune response or a Th2 type associated with susceptibility. In resistant mice, dendritic cells (DCs) were able to activate Th1 response during infection with *P. brasiliensis*. The role human dendritic cells have not yet been clarified. Objective. To investigate the effects of 43kDa glycoprotein of *P. brasiliensis* (gp43) on surface molecules expression and secretion of interleukin-10 (IL-10) and interleukin-12p40 (IL-12p40) by DCs from PCM patients. Materials and Methods. Monocyte-derived DCs were generated from peripheral blood of ten cured PCM patients (CP), ten with active PCM (AP) and five healthy controls (CO). Then DCs were pulsed with gp43 and/or activated with inflammatory recombinant cytokine TNF-alfa, after differentiation (CD11c+, CD1a+, CD14-). Costimulatory molecules analysis was expressed as MFI (mean of fluorescence intensity) by flow citometry. Supernatants from cultures of DCs were assayed for IL-10 and IL-12p40 by ELISA. Results. Gp43 and TNF-alfa stimuli on DCs resulted on activation and up regulation of costimulatory molecules. Higher MFI of CD86 was observed when DCs from CP ($p<0,05$) and AP groups ($p<0,05$) were pulsed with gp43 and activated with TNF-alfa Gp43 pulsing also up regulated IL-12p40 production by DCs from CP while the opposite effect was observed on the IL-10 release by patients cells. Conclusions. DCs from patients with PCM can be activated in vitro with TNF-alfa and with gp43 of *P. brasiliensis* as shown by high secretion of IL12p40 and up regulation of CD86.
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PP-02-44

Paracoccidioidomycosis infection in the families of patients: Lymphoproliferation to 43 kDa glycoprotein (gp43) of *Paracoccidioides brasiliensis* and epidemiological data

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Introduction. Paracoccidioidomycosis (PCM) is a systemic mycosis affecting mainly agricultural workers. As the mechanisms involved in the evolution of host-parasite are not well known, analysis of epidemiological data and cytokines pattern presented by relatives of patients with PCM can contribute to understand the events occurring at the initial phase of the infection. Objectives. Descriptive study of epidemiological data and lymphoproliferative response to gp43 of *Paracoccidioides brasiliensis (P. brasiliensis)* from relatives of patients with PCM. Material and methods. Twenty-three patients with treated PCM (PA) and 63 relatives of patients with PCM (RE) and 20 healthy individuals (CO) were included. Peripheral blood mononuclear cells (PBMC) were stimulated with gp43. The cut-off was calculated by ROC curve. Statistical analysis: Kruskall-Wallis test followed by Dunn post-test. Epidemiological and clinical data related to the relatives (migration, age, sex, place of birth, and clinical manifestations of PCM) were registered. Results. Statistical differences between PA-CO, PA-RE and CO-RE groups ($p<0.001$) were observed in lymphoproliferation levels. Sensitivity of lymphoproliferation to gp43 was 84,2% and specificity, 81.8%. In RE group, 62.9% were considered infected (INF). The frequency of male in RE, INF and PA groups was 56.5%, 61.1% and 98.4%, respectively. The age distribution was similar between PA and FA. Conclusions. The age distribution and migration of PA and FA groups suggested the occurrence of simultaneous exposition to fungus in these groups. The frequency of male in INF group is similar to the literature, in contrast to the male predominance in PA group. The high frequency of infection in the relatives of patients compared to low frequency of disease in the same group is a challenge for studies on the mechanisms responsible for the evolution of host-parasite interaction.

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PP-02-45

Cytokines profile in the relatives of the patients with paracoccidioidomycosis

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Introduction: Paracoccidioidomycosis (PCM) is a systemic and endemic mycosis prevalent in rural areas of Latin America. The mechanisms involved in fungus-host interaction are not well understood. Individuals with PCM infection are reactive to *Paracoccidioides brasiliensis (P. brasiliensis)* antigens and secrete gamma IFN; but have no detectable serum specific antibodies. Peripheral blood mononuclear cells from patients with acute form secrete IL-4, IL-5 e IL-10 and lower levels of gamma IFN ; than patients with chronic form. Objective: To describe the levels and profile of cytokines among relatives of patients with PCM secreted after stimulation with 43kDa glycoprotein (gp43) of *P. brasiliensis*. Material and Methods: One hundred blood samples were analyzed: 63 relatives without clinical signs and symptoms (RE), 14 from treated patients with chronic PCM (PA) and 20 from healthy individuals (CO). Lymphoproliferation: peripheral blood mononuclear cells were stimulated with gp43 and cut off was defined as 1,000 cpm. Cytokines levels: supernatants from cells cultures were assayed for IL-10 and gamma IFN by ELISA. Statistical analysis: Kruskall-Wallis followed by Dunn pos test. Results: There was no difference on the levels of IL-10 in the supernatants from culture of cells stimulated with gp43 among all groups. The comparison between RE and PA groups showed statistical difference ($p<0.005$) on gamma IFN; levels. In the RE group, 62.9% were considered infected (cpm>1,000) and the comparison between infected and non-infected showed difference on gamma IFN-γ levels ($p<0.003$). Conclusions: The results showed that some infected individuals of RE group produced high levels of gamma IFN, suggesting that they are able to control the fungus replication after exposition and sensitization in comparison with treated chronic cases.

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PP-02-46

Recognition of peptides from *Paracoccidioides brasiliensis* 43 kDa glycoprotein by blood mononuclear cells from patients with different clinical forms of paracoccidioidomycosis

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Background: Paracoccidioidomycosis (PCM) is a systemic granulomatous mycosis, endemic in Latin America, caused by *Paracoccidioides brasiliensis*. Immunization with peptides from *P. brasiliensis* gp43 has an additive effect on chemotherapy in experimental models. Considering the importance of identifying immunoprotective peptides in the PCM, the present work investigated recognition of overlapping gp43 synthetic peptides by mononuclear cells from patients presenting different clinical forms of the mycosis. Methods: Cells from 44 PCM patients and 19 healthy subjects were tested in vitro with 41 synthetic peptides overlapping the immunodominant gp43 antigen of *P. brasiliensis.*
Results: In the present work, we analyzed the profile of peptides recognized by mononuclear cells from cured Paracoccidioidomycosis patients and observed a higher lymphoproliferative response of the mononuclear cells from patients suffering from an acute form of the disease to selected gp43 peptides in comparison to those with other clinical forms of the disease. In addition, the lymphoproliferative response to gp43 peptides of the cells of patients suffering from the unifocal form of the disease was low. We also demonstrated that ten immunoreactive gp43 peptides were recognized by the cells of 77.1% of the patients and by 26.3% of the controls.
Conclusions: Our results suggest that patients with an acute form of PCM may regain cellular reactivity quickly after treatment. We also identified novel gp43 epitopes, that could be used in the future as adjuvant immunotherapy in addition to antifungal drugs in PCM patients, by improving the immune response of PCM patients.

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PP-02-47

Production and analysis of polyclonal antibodies to *Arthrographis kalrae* soluble antigens with hemolytic activity

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This study aimed to obtain and analyze polyclonal antibodies more specific to soluble antigens in order to investigate the hemolytic activity of *Arthrographis kalrae* a dimorphic fungus considered as an opportunistic pathogen. Soluble antigens (CFA) in its native form and heated (56°C, 30 min), and fractions of CFA obtained by chromatography in Sephadex G-200 were tested in relation to hemolytic activity. Mice were immunized with isogenic red blood cells (RBC) sensitized with CFA (anti-RBC-CFA) and the immune sera was analyzed by western blotting and by their ability to interact with CFA fractions by ELISA. Additionally, anti-RBC-CFA antibodies were used for hemolysis inhibition test. The results showed hemolytic activity of CFA, both in its native form as heated and the CFA samples treated with anti-RBC-CFA demonstrated decreased hemolytic activity, indicating specificity of the antibodies to the factors responsible for hemolysis. The chromatography fractions analysis resulted in hemolysis of two fractions also positive by ELISA. The western blotting demonstrated reactivity of two main bands with anti-RBC-CFA with ~252 kDa and ~79 kDa MM. In conclusion, the fungus *A. kalrae* releases thermostable hemolytic factors consisting at least which two fractions probably with ~252 kDa and ~79 kDa MM.
This work was supported by Fundacaoo Araucaria/SETI/PR and FAEP/UEL.

PP-02-48

Characterization of dendritic cells from bronchoalveolar lavage after experimental infection with *Paracoccidioides brasiliensis*

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Paracoccidioidomycosis (PCM) is a systemic mycosis, caused by the *Paracoccidioides brasiliensis* (Pb), that it commits the lung preferentially. The lung is one of the few organs in the body where there is a continuous and extensive interaction between the environment and our immune system. The big challenge for the pulmonary immune system is to discriminate the good from the bad and to react accordingly. Unnecessary action against harmless particles (e.g. innocent antigens, self proteins) should be avoided, while a rapid and strong immune response is needed against potentially dangerous microorganisms. Pulmonary dendritic cells (DCs) are ideally suited to maintain this delicate balance between tolerance and active immune responses. Seen the importance of these cells in the immune system and knowing that the infection for Pb attacks the lung primarily, we analyzed the DCs from bronchoalveolar lavage (BAL) after experimental infection with Pb. We observed a significant increase of DCs in the BAL after 24 hrs of intrachaqueal infection with 106 yeast form of Pb. The characterization of DCs showed that these cells expressed high levels of CD11c, MHC-II and DEC205. The results demonstrated that the infection with Pb induced the recruitment of mature DC to BAL, suggesting that these cells could initiate the immune response and consequently the polarization of T-cell.
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PP-02-49

IgG-IgE and IgG-gp43 immune complexes in acute and chronic Paracoccidioidomycosis

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Paracoccidioidomycosis (PCM), caused by the dimorphic fungus *Paracoccidioides brasiliensis*, is characterized by two clinically distinguished forms: a more severe and acute (AF) characterized by and a less severe chronic (CF) form. Circulating IgG-IgE and IgG-gp43 immune complexes (IC) were analyzed in 22 CF, 12 AF PCM and 29 normal donors (NHS) by capture immunoassay (ELISAc). Additionally, total IgG and IgE and specific IgG and IgE to P. brasiliensis antigens was analyzed. The increased level of IgG-IgE IC was observed in CF but not in the AF PCM. On the other hand, IgG-gp43 IC, total and specific IgG and IgE levels were higher in AF than CF ($p<0.05$). The results of this study reaffirm the association of increased IgG-gp43 IC, IgE and IgG anti-*P. brasiliensis* and total IgG and IgE levels to PCM severity and introduce for the first time the presence of IgG-IgE IC distinguishing chronic and acute PCM, which could provide a new differential marker between both forms of this disease.
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PP-02-50

Effects of drugs on the extracellular matrix in Paracoccidoiodomycotic granulomas

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Paracoccidioidomycosis (PCM), the most important mycosis in Latin America, is a granulomatous disease. The formation of granulomas can be understood as a mechanism of the body to block and limit *Paracoccidioides brasiliensis* (Pb), once unable to kill them.

In the present study we sought to interfere in the fibrotic process, which worsens the quality of life of PCM patients. Susceptible mice were infected with Pb, and treated with IFN-gamma (antifibrotic activity), Tetracycline (extracellular matrix-ECM synthesis inhibition) or Lumiracoxib (ECM components inhibition). Control groups were only infected.

After 15 days, we collected the omentum (target organ in experimental PCM) studied the presence of Pb, the deposit of collagen fibers by histological and immunohistochemical analyses and determined nitric oxide (NO) and hydroxyproline (product of collagen synthesis and degradation) concentrations. We found in infected, non-treated mice, few, loose granulomas, dissemination of Pb with typical morphology, and low NO levels. IFN-gamma-treated mice had few, compact granulomas, circumscribing scattered Pb with preserved or altered morphology and increased NO production. Tetracycline treatment caused absence of granuloma formation, presence of rare collagen fibers, few heterogeneous morphology Pb and increased NO levels. Lumiracoxib treatment elicited numerous loose granulomas, increased dissemination of Pb with preserved morphology, and decrease of NO. Only Tetracycline-treated mice showed negative staining for collagen type I. The deposition of total collagen fibers in the tissue and hydroxyproline concentration were similar in infected and INF-gamma-treated mice. These parameters were markedly decreased in Tetracycline-treated mice and extremely high in Lumiracoxib-treated mice.

Our results suggest that in susceptible animals to PCM, treatment with IFN-gamma or Tetracycline restrained the early infection; whereas treatment with Lumiracoxib facilitated fungal dissemination to the entire tissue examined, and that hydroxyproline concentration in omentum extracts correlated to collagen fibers deposits and with impaired control of Pb dissemination.

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PP-02-51

Passive immunization with monoclonal antibody against a 70-kDa putative adhesin of *Sporothrix schenckii* induces protection in murine sporotrichosis

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Cell-mediated and innate immunity are considered the most important mechanisms of host defense against fungus infections. However, recent studies demonstrated that specific antibodies show different degrees of protection against mycosis. In a previous study, antigens secreted by *Sporothrix schenckii* induced a specific humoral response in infected animals, mainly against the 70-kDa molecule, indicating a possible participation of antibodies to this antigen in infection control. In the present study, an IgG1 mAb was produced against a 70-kDa glycoprotein of *S. schenckii* in order to better understand the effect of passive immunization of mice infected with *S. schenckii*. Results showed a significant reduction in the number of CFU in organs of mice when the mAb was injected before and during *S. schenckii* infection. Similar results were observed when T-cell-deficient mice were used. Moreover, in a second schedule treatment, the mAb was injected after infection was established, and again we observed a significant reduction in CFU associated with an increase of gamma-IFN production. Also, the 70-kDa antigen is shown to be a putative adhesin present on the surface of this fungus. In conclusion, we report for the first time the protective effect of a specific antibody against *S. schenckii.*

PP-02-52

Immunogenic cell wall and exopolysaccharides of *Exophiala spinifera*

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Species of *Exophiala* belong to chaetothyrialean fungi (teleomorph family Herpotrichiellaceae), which are known for causing diseases and neurotropic dissemination in immunocompetent individuals. Among the diseases caused by this group of fungi, chromoblastomycosis and other traumatic skin disorders are the most frequent. *E. spinifera* is considered one of the most aggressive species to human beings well known for its morphological plasticity and difficulty to be identified. The present work aimed to characterize a strain of *Exophiala spinifera* (HCEML), using morphology and ITS sequencing. Evaluation of the pathogenic potential of this strain was determined by isolated the cell wall polysaccharides and expolysaccharides (EPS). The strain was cultivated under constant agitation for seven days at 36°C in minimum medium (MM), and Czapeck-Dox medium. Exopolysaccharides were recovered from the culture media through centrifugation and ethanolic precipitation (3:1, v/v). The monosaccharide composition gave Man, Gal, Glc, in a 5.3-6.1:2.3:1.6-2.4 molar ratio. ^{13}C-NMR experiments, suggesting that EPS should be galactomanans containing b-D-Galf, b-D-Galp, b- and a-D-Manp units. A strain of *E. spinifera* inactivated by heat was used for the retrieval of antibodies for conducting immunological tests. Immunization was conducted intraperitonially using isogenic balbC mice. The produced antibodies were tested against polysaccharides obtained from the cell wall and EPS, in which the strongest immunogenic activity was elicited by the EPS extracted from cultivation in MM medium followed by the Czapeck-Dox. HCEML strain produced manose, galactose, and glucose in poor-Carbon source medium. The polysaccharides wall presented a low immunogenic activity.

PP-02-53

Immune answer of animals vaccinated against dermatophytosis

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The purpose of the present researches was finding-out of the immunobiological changes in the animal organisms after vaccination against dermatomycosis with inactivated vaccine Polivac-TM.

Immune answer in the animal organism was determined by the levels of interleukins (IL-1β, TNF-α, IL-12, INF-γ, IL-4, IL-10) in plasma of vaccinated mice (*in vivo*) and in blood cells culture with stimulation by vaccine Polivac-TM (*ex-in-vivo*), by the release of antibodies (IgA/IgM/IgG) in blood after vaccine application, by dynamic of development the Delay Type of Hypersensitivity (DTH) in vaccinated guinea pigs, by protection of rabbits and guinea pigs against superficial skin infection with dermatophytes in challenge experiments.

Results of researches have shown that vaccination stimulates release of IL-12, and also a plenty pro-inflammatory IL-1β TNF-α and IL-10. Presence IL-10 in plasma of blood also was unexpected as IL-12 is the core interleukin in Th1 cellular immune answer. Reduction of the vaccine dose in 10, 100 and 1000 times led to significant decrease in release IL-1β И TNF-α in blood.

The highest titers of antibodies were revealed in ELISA tests in 10 - 14 days after vaccination. Also it was established, that at 7 and 15 day after application of the vaccine increase IgA and IgM at vaccinated animals were observed whereas at 40 day the titer of IgA remained at the same level and titer of IgM noticeably was decreased.

Occurrence of DTH was observed at 6 day after vaccination.

Thus, immunization of animals by vaccine Polivac-TM stimulates the release of interleukins (IL-1β, TNF-α, IL-12, INF-γ, IL-10) in blood and blood cells culture, which lead to shift of the immune answer aside Th1. The cellular-mediated immune answer proves to be true positive results of skin tests and positive dynamics of recover of animals after superficial skin infections.

PP-03-1

Species distribution and in vitro susceptibility of *Candida* bloodstream isolates to six new and current antifungal agents in a Turkish tertiary care military hospital, recovered through 2001 and 2006

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It was aimed to determine the species distribution and antifungal susceptibility of *Candida* bloodstream isolates recovered from patients admitted to the largest tertiary-care military hospital between 2001 and 2006 in Ankara, Turkey. Of 173 *Candida* species recovered, *C. albicans* was the most common yeast (48.0%) and it was followed by *C. parapsilosis* (31.8%), *C. tropicalis* (9.8%), *C. glabrata* (4.6%), *C. krusei* (3.5%), and *C. kefyr* (2.3%). Although the mostly isolated strain was *C. albicans* in the study period, prevalence of *C. albicans* was replaced with *C. parapsilosis* in 2006. The antifungal susceptibility testing was performed only to a total of 95 *Candida* species due to maintenance problem of stock cultures. The susceptibility pattern of the isolates, including 45 *C. parapsilosis*, 35 *C. albicans*, 7 *C. tropicalis*, 4 *C. krusei*, 3 *C. glabrata*, and 1 *C. kefyr* was determined against fluconazole (FLU), itraconazole (ITC), voriconazole (VOR), posaconazole (POS), caspofungin (CAS), and amphotericin B (AMB). Almost all strains showed low MIC values to all six antifungals tested. Only 2 of 45 *C. parapsilosis* isolates were resistant to FLU, one was susceptible in dose-dependent manner (SDD) to ITC, and 14 were nonsusceptible to CAS.
In conclusion, a possible trend for increasing prevalence of *C. parapsilosis* emphasizes a need for a better catheter-care. And also continuous surveillance programs are needed in order to identify possible future changes in the species distribution and antifungal susceptibility pattern.

PP-03-2

In vitro susceptibility testing of the polyene pentamycin and comparison with fluconazole and nystatin

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Pentamycin is a polyene macrolide with a broad range of activity against fungi, bacteria and protozoa. Pentamycin is approved in Switzerland for the treatment of vaginitis caused by fungal, protozoal, or mixed infections. Additional regulatory submissions are pending in many countries around the world and a dose-optimised vaginal tablet with pentamycin is in phase II development.
To compare the antifungal activity of pentamycin with flucytosine and nystatin, in vitro susceptibility testing according to the protocol NCCLS (CLSI) M27-A2 was performed in parallel sets for twenty clinical strains each of the following yeasts: *Candida albicans*, *C. glabrata*, C. parapsilosis, C. krusei, C. tropicalis and Saccharomyces cerevisiae.
All tested strains were susceptible against pentamycin and nystatin, whereas single strains of *C. albicans* and S. cerevisiae, many strains of *C. glabrata* and all C. krusei strains were resistant against fluconazole. The mean minimum inhibitory concentration of pentamycin compared to nystatin both after 24 and 48 hours reading was lower in all six investigated yeast species by a factor of two.
These results show that pentamycin is a potent antibiotic also active against a wide spectrum of clinically relevant yeasts, including fluconazole-resistant strains.

PP-03-3

In vitro susceptibility of some essential oils against aspergillius and fusarium isolates

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In the past few years, the incidence of invasive mycoses has increased considerably.Though *candida* is the most common, *Aspergillus and Fusarium* species have also been reported to cause opportunistic infections especially in immunocompromised patients such as in HIV/AIDS. Currently, no satisfactory conventional antifungal treatment exists,thus making the search for other alternatives necessary. This study investigated the effect of three essential oils of *Cinnamomum cassia,Ocium gratissium* and *Syzygium aromaticum* on ten isolates each of *Aspergillus* and *Fusarium* species using the disc diffusion method. All the three essential oils showed promising activities on majority of the isolates. *S.aromaticum* showed the best activity among the three of them with inhibition zone diameter (IZD) varying from 20 - 45mm for 80% of the *Aspergillus* isolates and 22-43mm for 70% of the *Fusarium* isolates .*C. cassia* inhibited 70% of *aspergillus* species with inhibition zone diameter ranging from 25-39mm but had no activity on three of the isolates. Similarly it inhibited *Fusarium* spp with IZD of 18-29mm. Though *O.gratisium* showed the least activity on all the isolates tested, it had good activities on 30 and 40% of *Aspergillus* and *Fusarium* isolates respectively.
This study supports our earlier proposal that essential oils can be used as aromatherapeutic agent for the remedy of fungal diseases in man especially those resistance to conventional antifungal agents.

PP-03-4

Strain distribution and antifungal susceptibility of Aspergillus at four hospital indoor air in afyon region

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PURPOSE: Aspergillus are patogen and opportunistic patogen species. The aim of the study was to investigate Aspergillus spor density in four hospital indoor air and to detect antifungal susceptibility (itraconazol, voriconazol, fluconazol, amfoterisin B).

METHODS: Air samples were collected from ten different units by using AIR/IDEAL 90 mm biocollector (BioMerieux, France). These samples were inoculated in PDA and Czapex-Dox agar. The identification of the fungi was based on their macroscopic and microscopic features. Antifungal susceptibility was performed by CLSI 38-A microdilution method.

RESULTS: Totally 118 Aspergillus strain [59% A. fumigatus, 12% A. niger, 16% A. versicolor, 4% A. terricola, 1% A. flavus, 1%A. parvulus, 1% A. ochraceus, 1% A. sydowi, 1% A. fishcheri, 2% A. aureolatus, 2% A. carneus] were isolated. Total of 72% Aspergillus strains were isolated from University Hospital and Chest Diseases Hospital. While 48% of all Aspergillus was isolated in the autumn and 15% in the summer, 64% of A. fumigatus were detected in the autumn and 53% of A. versicolor was detected in the summer periods. In the studies carried out in the hospital units, 38% of all isolates were detected inside the hospital, 14% of the isolates from chest diseases ward room, 24% from the intensive care unit and 6% from the dialysis units. All of the strains were detected resistant to fluconazol. It was found that itraconazol, voriconazol and amfoterisin B are the most effective antifungals.

CONCLUSIONS: It is important to be aware of the fungal flora in places where patients live. Reducing these indoor fungi is necessary to improve the health of individuals with fungal induced diseases.

PP-03-5

Antifungal resistance of *Candida* tropicalis isolated in the Ceara - Brazil

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Candida tropicalis has been reported to be one of the *Candida* species which is most likely to cause bloodstream and urinary tract infections in the hospital. C. tropicalis is the second most common *Candida* spp. A total of 51 C. tropicalis clinical isolates collected in Fortaleza-Ceara; were used in this study. The purpose of this study was to evaluate the antifungal resistance of C. tropicalis. The 51 collection and stock strains were obtained from the collection of the Laboratory of Medical Microbiology, School of Pharmacy, Federal University of Ceara-Fortaleza, Ceara-Brazil. Frozen yeast isolates were sub cultured onto Potato dextrose agar. Prior to testing, each isolate was passaged at least twice on potato dextrose agar and chromogenic media agar to ensure identification, purity and viability. Susceptibility testing was evaluated in medium Mueller-Hinton agar supplemented with glucose and methylene blue was used for disk diffusion testing. Paper disks containing: fluconazole, itraconazole and amphotericin B were used. A standardized 0.5 McFarland suspension of yeast taken from potato dextrose agar was made in saline. Plates were inverted and incubated for 24 h at 35C. Plates were read at 24h. Zones of inhibition were read and zone diameters was as described in CLSI M44-A. *Candida* parapsilosis ATCC 22019, *Candida albicans* ATCC 14053 and *Candida albicans* ATCC 10231 were used as the quality controls and tested in each run of the experiments. All C. tropicalis in our investigation were susceptible to Amphotericin B. 4(5.9%) of isolates of C. tropicalis were considered resistant to fluconazole and itraconazole.

PP-03-6

Antifungal susceptibility and virulence factors of strains *Candida* spp isolated in Ceará - Brazil

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Candida albicans is the *Candida* species most frequently isolated from patients with candiduria. However, other species with more reduced susceptibility to antifungal agents, such as C. parapsilosis, C. tropicalis, *C. glabrata*, C. krusei, and C. guilliermondii, are teadily increasing their isolation frequency. The pathogenesis of candiduria involves several factors, among which may be counted germ tube and hypha formation, adhesion factors, phenotypic switching, and slime production, as well as the production of different enzymes. The purpose of this study was to evaluate the susceptibility and virulence factors of strains *Candida* spp isolated of urine in Ceará - Brazil. The strains were streaked onto Potato glucose agar plates (Mumbai, India) at 37C for 24 to 48 h. After this period, the strains were streaked onto chromogenic media agar plates and incubated at 37C for 24 to 48 h. Twelve yeasts isolates have been evaluated (8 *C. albicans*, 2 C. tropicalis, 1 *C. glabrata*, 1 C. parapsilosis). Slime and exoenzymes (proteinase, coagulase, and phospholipase) production tests and determination of their levels were performed. Susceptibility testing was evaluated in medium Mueller-Hinton agar supplemented with glucose and methylene blue was used for disk diffusion testing. Paper disks containing fluconazole, itraconazole and amphotericin B were used. Plates were inverted and incubated for 24 h at 35C. Zones of inhibition were read and interpretation of zone diameters was as described in CLSI M44-A. The following reference strains were also included in the studies: *C. albicans* ATCC 10231, *C. albicans* ATCC 14053, C. parapsilosis ATCC 22019. In the present study, a higher phospholipase and coagulase activity was also observed for *C. albicans*. All strains were susceptible to amphotericin B. Candiduria is even more common in the setting of indwelling catheters. Of concern is that candiduria is associated with higher mortality, especially in patients with comorbidities.

Poster Forum PF-03

PP-03-7

Decreased susceptibility to miconazole and ketoconazole against *Candida albicans* from APECED patients

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Most patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED, APS-I) suffer from chronic oral candidosis since childhood and receive repeated courses of antifungals throughout their life. Eleven of our patients (31.4%) have become colonized with *Candida albicans* with decreased sensitivity to fluconazole. This has been found to be mainly due to the usage of other azoles than fluconazole, namely miconazole and ketoconazole. We have now analysed the susceptibility of 43 isolates of *C. albicans* from 23 APECED patients isolated during the years 1994-2004 to miconazole and ketoconazole using the CLSI M27-A2 methodology. Of the isolates 18 were of decreased susceptibility to fluconazole (MIC 16-32 mg/l) and 25 were susceptible to fluconazole (MIC =<8 mg/l) and all were susceptible to voriconazole and posaconazole. Of all the isolates, 16.3% had a decreased susceptibility to miconazole. All isolates with decreased susceptibility to miconazole had also decreased susceptibility to fluconazole. Of all the fluconazole dose-dependent isolates (n=18), 38.9% were cross-resistant to miconazole (n=7). All strains were susceptible to ketoconazole. Correlations between fluconazole and miconazole MICs (P=0.0044); fluconazole and ketoconazole MICs (P= 0.0010); miconazole and posaconazole MICs (P=0.0014); and miconazole and voriconazole MICs (P=0.0041) were significant. These results highlight that topical compounds, in particular miconazole, may influence azole susceptibility and lead to cross-resistance.

Poster Forum PF-04

PP-03-8

Antifungal susceptibility of *Candida glabrata* isolates collected during population-based candidemia surveillance in metropolitan Atlanta, GA and Baltimore City and County, MD, 2008

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Introduction: *Candida glabrata* is the second most common cause of invasive candidiasis in the U.S. Reduced susceptibility or resistance to azole antifungals, which may emerge during therapy, makes *C. glabrata* infections challenging to manage. Previous studies have shown the prevalence of reduced fluconazole susceptibility and resistance to be approximately 25% and 10%, respectively. We sought to determine the antifungal susceptibility profiles of *C. glabrata* isolates collected during an ongoing, population-based candidemia surveillance project.

Methods: *Candida* isolates causing bloodstream infections were submitted to CDC from ongoing surveillance in the 8-county Atlanta, GA metropolitan area and Baltimore City and County, MD for species determination and antifungal susceptibility testing. Species determination was performed using a DNA-based Luminex assay. Susceptibility testing was performed using TREK Diagnostic microbroth dilution panels following the recommendations of CLSI M27-A3.

Results: A total of 103 *C. glabrata* isolates were evaluated. Thirty - five of 103 (34%) were fluconazole-susceptible, with minimum inhibitory concentrations (MIC) > = 8 ug/ml; 52 (50%) were susceptible dose-dependent, with MIC 16-32 ug/ml, and 16 (16%) were resistant, with MIC > = 64 ug/ml. Among the 16 fluconazole-resistant isolates, 14 (88%) had itraconazole MICs > = 16 ug/ml, 12 (75%) had voriconazole MICs > = 4 ug/ml, and 10 (63%) had posaconazole MICs > = 4 ug/ml. In addition, 5 isolates (31%) were non-susceptible to one or more echinocandins, with MICs > 2 ug/ml. We did not detect any echinocandin non-susceptibility among the fluconazole-susceptible isolates.

Conclusions: Early results from ongoing, population-based candidemia surveillance suggest that a high proportion of *C. glabrata* isolates show reduced fluconazole susceptibility or resistance, and that most fluconazole-resistant isolates are cross - resistant to the broad - spectrum azoles voriconazole, itraconazole and posaconazole. Although our numbers are small, almost a third of fluconazole - resistant isolates also had high echinocandin MICs. Further work is needed to elucidate the relationship between azole and echinocandin resistance in these isolates.

Poster Forum PF-08

PP-03-9

The effects of caspofungin and voriconazole in experimental *Candida* otitis media

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PURPOSE: To evaluate the effectiveness of caspofungin and voriconazole in the treatment of experimental *Candida* otitis media in an experimental rabbit model.

METHODS: Thirty New Zealand white rabbits were divided into four treatment groups and one control group. Rabbits were immunosuppressed by cyclophosphamide and triamcinolone
acetonide. Right ear of each rabbit was infected by injection of the inoculum of 0.1 ml (10000/0.1ml) of *C. albicans* into the middle ear cavity. Seventy hours after the inoculation, Amphotericin B 1 mg/kg/day (n: 6), itraconazole 10 mg/kg/day (n: 6), voriconazole 10 mg/kg/day (n: 6) and caspofungin 5 mg/kg/day (n: 6) were injected to the each treatment group. No antifungal drug was administered to the control group (n: 6). Clinical and histopathologic examination scores and microbiological analysis of middle ear mucosa were compared.

RESULTS: There was statistically significant difference in the clinical scores, histopathologic scores, and mean CFU/g between the treatment and control groups ($p< 0.05$). There was no statistically significant difference among the treatment groups in the point of clinical and histopathological scores, whereas there was statistically significant difference in the point of mean CFU/g ($p< 0.05$). The mean CFU/g of Amphotericin B and caspofungin groups were similar and both were lower than the itraconazole and voriconazole groups. Also, the mean CFU/g of voriconazole group was lower than itraconazole group ($p< 0.05$).

CONCLUSIONS: Caspofungin and voriconazole were demonstrated at least as effective as amphotericin B and itraconazole. We suggest that caspofungin and voriconazole may be considered in the treatment of fungal infection of the ear.

Poster Forum PF-07

PP-03-10

A head-to-head comparison of analytical grade powders against pharmacy preparations for antifungal susceptibility testing

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Antifungal susceptibility testing has become an important tool for physicians faced with making difficult treatment decisions regarding appropriate therapy for patients with various mycoses. The Clinical Laboratory and Standards Institute (CLSI) has published approved methods for the testing of both yeasts and moulds. These guidelines stress the strict use of only pharmaceutical grade powders due to concerns regarding purity, potency, and inert substances used in the preparation of patient medications. Frequently, it is difficult to impossible to obtain these analytical powders making the use of pharmacy preparations an attractive option.
We compared analytical grade powders of amphotericin B (AMB), anidulafungin (ANID), caspofungin (CAS), micafungin (MICA), fluconazole (FLU), itraconazole (ITRA), and voriconazole (VORI) with their pharmacy counterparts Fungizone® IV (AMB), Eraxis® IV (ANID), Cancidas® IV (CAS), Mycamine® IV (MICA), Diflucan® IV (FLU), Sporonox® IV (ITRA), and Vfend® IV (VORI). A panel of 92 *Candida* species including *C. albicans* (47), *C. glabrata* (20), C. parapsilosis (15), C. krusei (6), and C. tropicalis (4) was tested. In addition, Trichosporon asahii (4), and Cryptococcus neoformans (4) was tested for a total of 100 yeast fungi.
Overall, identical minimum inhibitory concentration (MIC) values were obtained at a rate of 58%. A difference between the MIC of both preparations of only one dilution occurred at a rate of 39%. A difference of two dilutions was observed at a rate of 3% and no differences greater than two dilutions were observed. An analysis of errors revealed the rate of very major errors to be 0.17%, major errors to be 0.17%, and minor errors to be 0.67%. It appears that pharmacy preparations may indeed be a viable source for antifungals required in antifungal susceptibility testing panels.

PP-03-11

In vitro activity of isavuconazole against Trichosporon

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Background: The emergence of less common, but clinically important, fungal pathogens including Trichosporon has contributed to the substantial morbidity and mortality observed in immunocompromised patients. This genus, which has recently undergone extensive taxonomic revisions, can be resistant or refractory to existing antifungal agents, particularly the echinocandins. We sought to evaluate the activity of the new triazole isavuconazole against different Trichosporon species.

Methods: Trichosporon species were identified using the API 20CAUX yeast ID system, microscopic morphology, temperature studies, and cycloheximide susceptibility. Minimum inhibitory concentrations (MICs) were measured for isavuconazole, voriconazole, posaconazole, fluconazole, amphotericin B, and flucytosine against 54 Trichosporon species (40 T. asahii, 10 T. mucoides, and 4 T. inkin) in accordance with the M27 - A2 reference method. Minimum fungicidal concentrations were also measured for each agent.

Results: Isavuconazole demonstrated excellent in vitro activity against all tested isolates. MIC50 values ranged from 0.06 to 0.125 mg/L, and MIC90 values ranged between and 0.125 to 0.25 mg/L. No MICs greater than 0.25 mg/L were observed. The geometric mean MICs of isavuconazole were similar to that of voriconazole and similar or less than those of posaconazole, fluconazole, amphotericin B, and flucytosine for all species tested. The MFC50 and MFC90 values for the extended spectrum triazoles including isavuconazole were lower than those of fluconazole, amphotericin B, and flucytosine. However MFC90 values often exceeded the highest concentration tested and exhibited wide variability.

Conclusions: Isavuconazole is a welcome addition to the growing antifungal armamentarium with potent in vitro activity against Trichosporon spp. Although this agent may be useful in the treatment of trichosporonosis clinical data are needed to verify these results.

PP-03-12

Successful treatment of sporotrichosis with voriconazole

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Voriconazole (VCZ) has a broad antifungal spectrum against the most common fungal pathogens. Recently we experienced two cases of sporotrichosis which were successfully treated with VCZ, although previous reviews have revealed that in vitro susceptibility of *Sporothrix schenckii* to VCZ is lower than that to potassium iodide (KI) and itraconazole (ITZ). Case 1 is a 75-year-old male with localized osteoarticular sporotrichosis of his right elbow. Treatments with ITZ, intra-articular injection of Amphotericin B and local heat therapy weren't effective. He was treated with VCZ and surgical debridement. Unfortunately medication was stopped after twelve weeks because of side effects, but he has remained well without any relapse of the infection. Case 2 is a 73-year- old male with lymphocutaneous sporotrichosis of his right forearm. He was treated with KI, but cutaneous lesions increased in number. After treatment with VZC was introduced, there was progressive improvement, and all the cutaneous lesions healed for six weeks. Treatment was continued for two more weeks. Based on mycological findings, each pathogen of two cases was identified as *S. schenckii*. In addition isolates from case 1 and case 2 were classified as type 2 and type 5 of *S. schenckii* respectively according to restriction fragment length polymorphism analysis of the mitochondrial DNA (mtDNA-RFLP). Our results indicate that VCZ has great potential usefulness to sporotrichosis in vivo.

PP-03-13

Susceptibility to anidulafungin and other systemic antifungal drugs of 637 invasive yeast isolates: The GISIA3 study

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During a study (January 2007 - December 2008) on the aetiology of invasive yeast infections, we isolated 637 yeasts from blood (74.4%), endovascular catheters (17.6%) and other sterile samples including peritoneal and cerebrospinal fluids (8%) of patients hospitalized in intensive care units and surgical wards of 13 Italian hospitals.

The isolates (325 *Candida albicans*, 143 *C. parapsilosis*, 78 *C. glabrata*, 38 *C. tropicalis*, 15 *C. krusei*, 10 *Cryptococcus neoformans*, 7 *C. lusitaniae*, 5 *C. guilliermondii*, 5 *C. famata*, 3 *C. lipolytica*, 2 *C. utilis*, 2 *C. sake*, and 1 each of *Geotrichum capitatum*, and *Saccharomyces cerevisiae*) were tested in vitro with amphotericin B, anidulafungin, caspofungin, fluconazole, itraconazole, posaconazole, and voriconazole using Etest and experimental Sensititre panels.

The majority of yeasts were susceptible with both methods to all the antifungal drugs. The MIC_{90} (ranges) in mcg/ml for Etest - Sensititre were respectively: amphotericin B 0.5 (0.002-1.5) - 1 (0.015-2); anidulafungin 2 (<0.002->32) - 1 (<0.008->16); caspofungin 1 (<0.002->32) - 0.5 (0.008->16); fluconazole 12 (0.023->256) - 16 (<0.12->256); itraconazole 1 (<0.002->32) - 0.5 (<0.008->16); posaconazole 0.5 (<0.002->32) - 0.5 (<0.008->8); voriconazole 0.25 (<0.002->32) - 0.25 (<0.008->16).

With the expected exception of *C. parapsilosis* with MIC_{90} (ranges) in mcg/ml of 4 (0.004 - >32) - 2 (0.03 - 4) for Etest and Sensititre respectively, and *C. neoformans* MIC_{90} over the highest concentration with both methods, anidulafungin was very active against all other species; for azoles the highest MIC values were observed in *C. glabrata* (MIC_{90} values in Etest/Sensititre of >256/64 for fluconazole; >32/>16 for itraconazole and posaconazole; 1.5/1 for voriconazole). No cross-resistance for echinocandins and azoles was detected among the 637 yeast isolates tested.

PP-03-14

Combination therapy of micafungin with voriconazole and amphotericin B against *Candida* biofilms

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Background: To date, combination treatment remains an empirical strategy in patients with difficult-to-treat infections including biofilm-associated infections, and basic evidence about the efficacy of combination therapy against biofilms is urgently demanded. Here we compared the effect of the combination treatment of micafungin (MCFG) with voriconazole (VRC) and amphotericin B (AMB) against planktonic cells and biofilms of *Candida albicans*.

Methods: *C. albicans*, SC5314, was used with MCFG, VRC and AMB. Susceptibility testing was performed by broth microdilution (two-fold dilution) in several combination. To make biofilms, silicone elastomer (SE) disks (4mm diameter) were immersed in a *Candida* cell suspension and incubated for 90 min at 37°C, and the disks were then incubated in yeast nitrogen base (YNB) medium with 2% dextrose for 24 hours. After planktonic cells or biofilms were treated with several combination, XTT reduction assay was performed for viability test and MICs and FIC indexes were calculated.

Results: FIC indexes against planktonic cells of MCFG+VRC and MCFG+AMB were both 1. FIC indexes against biofilms of MCFG + AMPH and MCFG + VCZ were 1 and >2, respectively. Since combination of MCFG and VRC showed antagonism on biofilms in contrast to planktonic cells, we performed time-lag treatment to examine if the attenuation effect of VRC continues after removal of the drug. Biofilms were treated with VRC or MCFG alone, or without any drugs for the first 24 hours and then were treated with another drug alone or combination of VRC and MCFG for the next 24 hours. Simultaneuos or serial combinations of VRC followed by MCFG were less effective than MCFG alone or MCFG followed by VRC.

Conclusions: We should beware of the antagonisms within practicable combinations antifungal agents on biofilms. Studies of the mechanism of the antagonism revealed in the present work should provide clues for new antifungal strategies.

PP-03-15

In vitro synergistic effects of metergoline and antifungal agents against *Candida* krusei

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Candidiasis is one of the most common fungal infections in humans caused by *Candida* species, most notably by *C. albicans*. However, extensive uses of antifungal agents and rise in compromised host population have increased the non-albicans species related infections, particularly C. krusei, C. tropicalis and *C. glabrata*. Among these strains, C. krusei related infections have dramatically increased due to its intrinsic resistance to fluconazole and decreased susceptibility to amphotericin B. As a result, highest mortality rate (30-60%) has been observed in Candidal infections in association with C. krusei because of the chemotherapy failure. To search for novel antifungal agents, recent studies have been focused on developing novel antifungal agents from other sources. In this regard, serotonin and its reuptake inhibitors have been suggested as potential novel antifungal Candidates due to their efficacy against *Candida* and Aspergillus species. In the present study, metergoline, a serotonin receptor antagonist, was found having in vitro synergistic effect with amphotericin B (fractional inhibitory concentration index: 0.375) by a checkerboard assay. Metergoline also inhibited extracellular phospholipase production in a dose-dependent manner (>20% at 8 μg/ml), which may be a possible action mechanism of metergoline on C. krusei. Further genome-wide experiments are needed to investigate the molecular mechanism of metergoline and to harness its antifungal potential on C. krusei.

PP-03-16

Testing antifungal combinations in diagnostic laboratories - relevance, tool kits and interpretations

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A number of new antifungal drugs have been tested against a wide variety of pathogenic fungi even though breakpoints have been published for a few pathogenic yeasts. There is a recent trend to use the newer drugs in combination or in sequence with more established therapy. The potential benefits include better antifungal spectrum, reduced toxicity and no acquired resistance. However, there are no standardized methods for testing antifungal combinations routinely in clinical laboratories. Over the years, we have used in house studies and multi-laboratory testing to identify rapid and reproducible methods for antifungal combination testing. QC strains of *Candida* krusei, C. parapsilosis, azole resistant strains *C. albicans*, and, *C. glabrata* and echinocandin resistant strain C. parapsilosis were tested by CLSI M-27A2 protocol using 96-well custom-made plates containing checkerboard pairwise combinations of amphotericin B (AMB), anidulafungin (AND), caspofungin (CSP), micafungin (MFG), posaconazole (PSC), and voriconazole (VRC). Similar combinations of antifungals were also tested using a flow cytometry (FC) method developed in our laboratory. The drug combinations were scored visually for MIC50.and by FC for mean channel fluorescence (MCF). MIC antifungal activities in combinations were evaluated by calculating FICIs (fractional inhibitory concentration indices) using the Lowe additivity formula. All combination MICs were lower than the MIC of either of the two test drugs tested alone ($p < 0.05$). This determination was independent of the test method used. FC results were more rapid and accurate than visual scores. FICi median range was 0.36 - 2.0 for all drug combinations tested. AMB-AND, AMB-CSP, AMB-MFG combinations were synergistic for azole-resistant *C. albicans* and echinocandin-resistant C. parapsilosis. Combinations of AMB-azoles or echinocandin-azoles were indifferent (additive). In conclusion, checkerboard dilutions of antifungal combinations can be easily tested in diagnostic laboratories and results evaluated using FICi.

PP-03-17

Clinical isolates of *Aspergillus fumigatus* and *A. niger* from transplant recipients in Japan

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Invasive aspergillosis (IA) is common in transplant recipients. Although *A. fumigatus* accounts for the majority of IA cases, non- *fumigatus* species are emerging as causes of IA in Japan. However, antifungal susceptibility of clinical isolates of *Aspergillus* species in Japan has not well investigated against new azoles. We investigated *Aspergillus* isolates from transplant recipients in Japan by a detailed molecular analysis. A total of 23 *Aspergillus* isolates were examined by phenotyping and subjected to detailed sequence analyses using the ITS regions and β-tubulin (benA) to identify the species of these isolates. Gene sequences derived from the ITS regions and benA of all *Aspergillus* isolates were compared with sequences in the GenBank database to identify the species. Nineteen isolates were identified as *A. fumigatus* and 4 isolates were *A. niger*. Antifungal susceptibilities of the isolates against itraconazole (ITZ), voriconazole (VCZ) and posaconazole (POS) were performed using E-test by 48 hour of incubation. MICs of 19 *A. fumigatus* isolates were 0.54 (0.032-4) μg/ml against ITZ, 0.13 (0.023-1.5) μg/ml against VCZ and 0.1 (0.032-0.38) μg/ml against POS. MICs of 4 *A. niger* isolates were 0.27 (0.006-1) μg/ml against ITZ, 0.28 (0.008-0.064) μg/ml against VCZ and 0.04 (0.008-0.125) μg/ml against POS. These results suggested that clinical isolates of *Aspergillus* species are more susceptible against POS than ITZ. MICs for azoles of Japanese isolates correlated well with previous reports for E-test and NCCLS M-38 for *A. fumigatus* and *A. niger* in other countries. The E-test are currently available and methodologically easier antifungal susceptibility testing.

Poster Forum PF-07

PP-03-18

Phenotypic and molecular characterisation of drug sensitive and resistant fungal isolates in mycotic keratitis

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Purpose - To study the susceptibility of clinical isolates of Aspergillus species to Amphotericin B and Natamycin in infectious keratitis and to find out if drug resistance patterns could have any association with other phenotypic and molecular characteristics.
Methods-Two hundred isolates of Aspergilli from cases of infectious keratitis at our Centre were tested for susceptibility to Amphotericin B and Natamycin by broth microdilution method following CLSI guidelines.The isolates comprised of 74 strains of Aspergillus flavus,68 of *Aspergillus fumigatus* and 58 Aspergillus niger.Fungal DNA was extracted by glass bead pulverization technique.PCR was standardised and assay was conducted to amplify the 28S rRNA gene.Single Stranded Conformational Polymorphism (SSCP)of the PCR product was performed by the standard protocol.Proteinase activities of all the isolates were studied using Yeast Carbon Base,Yeast Extract and Bovine Serum Albumin agar.
Results - The MIC values ranged between0.2 ug/ml to 6.25ug/ml both for A fumigatus and A flavus and between 0.2 to 25 ug/ml for A niger.Incubation at 37 degrees celsius yielded better reproducibility than that at 25 degrees celsius. Of the 200 isolates studied,125(62.5%)were proteinase producers.Ninety eight(78.4%)of these 125 proteinase producing fungi showed high MIC values as compared to only 27(21.6%)nonproteinase producing fungi($p<0.001$). SSCP patterns could well diffentiate between drug resistant and drug sensitive isolates.
Conclusion-Such phenotypic and molecular characterisation will not only help managing the cases effectively, but will also elucidate the pathogenesis of fungal keratitis of which very little is known till today.

PP-03-19

Pramiconazole short dose-regimen in the treatment of pityriasis versicolor

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Pramiconazole, a novel antifungal, with a higher selectivity for fungal cytochrome P450 suggesting a potential lower risk for drug-drug interactions and a terminal half-life 3 times longer than itraconazole's, could bring new therapeutic options.
Objective: Evaluate the safety and efficacy of oral pramiconazole in patients with Pityriasis versicolor.
Subjects and Methods:
MICs values for pramiconazole and ketoconazole of 7 *Malassezia* spp. were determined with the agar dilution method.

In a POP study, 19 patients given a single dose of 200 mg pramiconazole were evaluated on erythema, itching, desquamation, hypo- and hyperpigmentation (0=absent, 4=severe) and a global investigator clinical evaluation vs. baseline (0=deterioration, 4=cured) before inclusion and after days 4, 10 and 30. Mycological evaluation was also performed.
In a dose finding study, 147 patients were randomized to placebo or one of five dosage regimens of pramiconazole. Efficacy was based on mycological response, severity of clinical signs and symptoms, and the IGA of lesion clearance.
Results:
M. sympodialis, M. restricta, M. obtusa, M. globosa and M. pachydermatis were the most sensitive against pramiconazole and *M. furfur and M. slooffiae* less sensitive in comparison with ketoconazole.
Significant reduction of signs and symptoms were observed from day 4 on ($p < 0.001$). Further reductions were observed at day 10 and 30 ($p < 0.001$). Clinical improvement was seen at all follow-up visits ($p < 0.001$) and mycological cure was seen in all patients at day 30.
Statistically significant ($p < 0.001$) dose-dependent effect was observed. When compared to placebo, the most effective treatment regimen included 200 or 400mg taken once, and 200mg taken OD for 2 or 3 days.
There were no serious, treatment-related adverse events or other safety concerns.
Conclusion: Pramiconazole shows an excellent in vitro activity against Malasezzia spp. In both clinical studies, pramiconazole was well-tolerated and an apparently effective short dose-regimen for the treatment of Pityriasis versicolor.

PP-03-20

Antifungal activity of cerumen from normal patients

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Background and introduction: Ear wax is the combination of fat producing glands and cerumen producing glands and also cellular debris. There are controversy ideas about the antibacterial and antifungal effects of cerumen glands. Some of them believes that cerumen has anti-microorganism characteristics .Some also believes that this can act as medium and enhance the growth of microorganism. So the present research has been performed to reveal the antifungal effect of cerumen.
Materials and Methods: This experimental study was carried out on the 30 sample of healthy peopleõs ear who came to the E.N.T Clinic by use of micro dilution method. The experiment was conducted on 4 fungi: *Aspergillus fumigatus*, Aspergillus niger, *Candida albicans* (clinical) and standard *Candida albicans* (PTCC5027) by using of 10% cerumen solution in glycerol buffer (30%glycerol¡ 5% sodium bicarbonate and 70% water). Two time dilutions of cerumen (2.5% - 0.02%) were used accompanying with positive and negative control in sterile ELISA micro plate. The antifungal activities of cerumen (MIC, MFC) were obtained with culture of transparent well on plate.
Results: The average ages of the subjects were between 2-85 years. The results, showed the most antifungal effect (MIC50) observed on Aspergillus niger (27 sample) and least effect observed on the species of *Candida albicans* (16 sample).
Conclusion: Regarding of the results, cerumen has antifungal effect on fungi ¡but it is related to fungi.
Key words: Cerumen, Antifungal activity, *Aspergillus fumigatus*, Aspergillus niger, *Candida albicans*, Micro dilution.

PP-03-21

The multi trial of generic terbinafine-sandoz in toenail onychomycosis

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The Generic Drugs use by administrative guidance is recommended strongly.
In present, there are a little clinical trial reports concerning clinical effectiveness of the Generics product. Examination of clinical and mycology study and density measurement was administered in toenail onychomycosis.
Object and method
Anti-fungal Generic-Terbinafine was administered for 12 weeks per 26 patients in toenail onychomycosis. The advanced improvement after end administering 12W in clinical effect was 4 patients and fingernail becoming turbid ratio has improved from 6.8 to 4.6. In the result of a mycological examination, the gloomy making the bacterium rate in which the 12 weeks in the administering 12 week group can be put is 27%.
And also was administered a similar patient for 24 weeks per 21 patients with taking a picture of the diseased part is executed when every consulting a physician, the thickness of fingernail part is measured, the part is executed and end administering. The advanced improvement in clinical effect was 18 patients and fingernail becoming turbid ratio has improved from 8 to 5.5 (after 24W) and from 9 to 1(after 36-48W). In the result of a mycological examination, it was all 100% gloomy making rate in the administering group on the week 24 for 24 weeks.
The method of measuring the density when the foot-fingernail is measured by extracting the in case of 23 patients of Terbinafine from the fingernail organization using the high-speed liquid chromatography. Four weeks after administering of Terbinafine and treating the effective density was maintained after 12 weeks after administering had ended. Five times or more the density were detected from 0.004ng of treating the effective density of Terbinafine-Sandoz125mg tab.
In conclusion, examination of clinical and mycology of Generic-Terbinafine in toenail onychomycosis and efficacious concentration measurement result were equal with original drug.

PP-03-22

Screening the antifungal activity of some endemic plants used in traditional medicine against dermatophytes by sylinder plate and bio-autography methods

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Dermatophytoses which are caused by keratinophylic fungi named darmatophytes, have been considered to be a major public health problem in Iran and many parts of the world. Considering the limited diversity of antifungalagents, recent fungal resistance to the antifungal compounds and several known side effects of such synthetic drugs, development of novel of antifungal compounds especially from natural sources is still needed. The aim of this study was to evaluate the antifungal activity of methanolic and petroleum-ether crude extracts of seven plants which have been used traditionally in Iran as a cleanser and anti-inflammatory compounds against dermatophyte spp.
Seven plants species (*Ferula gummosa Boiss*, *Iris spp*, *Dorema ammoniacum*, *Zataria multiflora*, *Myrtus communis* L.,*Achillea eriophora*, *Ferula assa-foetida* L.), which used in folk medicine were collected from southern part of Fars Province from May to September 2007. The plant powders were extracted with methanol and petroleum ether by maceration method at room temperature for 48 h. The filtrate was concentrated under vacuum to give crude extract. The antifungal effects of the crude extracts were investigated by sylinder plate and bioautography methods against *Trichophyton mentagrophytes*, *T. rubrum*, *Epidermophyton flucosum* and *Microsporum canis* .
Among tested materials the strong antifungal activity was shown by the extracts of *Zataria multiflora*, *Ferula assa-foetida* and *Myrtus communis*, respectively. In the bioassay method, clear inhibition zones were observed for both methanolic and petroleum ether extracts of all studied plants against dermatophytes. The separation of compounds and metabolites, as in the cases of *Ferula gummosa* and *Dorema ammoniacum*, enhanced their antifungal activity against *E. flocossum*.
The results seem to indicate that petroleum ether extracts may be more effective than methanolic ones. The obtained results show a spectrum of antifungal activities which provide support to some traditional uses of these plants.

PP-03-23

In vitro activities of conventional and new antifungal drugs against *Rhinocladiella mackenziei* an agent of cerebral phaeohyphomycosis

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Background: The prevalence of cerebral phaeohyphomycosis caused by melanized fungi is rare but increasingly recognized in human disease. Rhinocladiella mackenziei is exclusively a central nervous system pathogen with 100% mortality and restricted to the Middle East and the Persian Gulf region. Limited in vitro and animal studies suggested that R. mackenziei is resistance to amphotericin B and presumably new antifungal drugs with broad spectrum efficacy might be more effective. Therefore we have tested a total of 8 conventional and new antifungal drugs against 10 clinical isolates involved in this rare cerebral infection.

Methods: A collection of 10 clinical isolates of R. mackenziei were obtained from the CBS Fungal Biodiversity Centre in Utrecht, The Netherlands. MICs were determined for amphotericin B (AmB), fluconazole (FLU), itraconazole (ITC), voriconazole (VOR), posaconazole (POS), isavuconazole (ISA) or MECs for caspofungin (CAS) and anidulafungin (ANI). Microdilution testing was done in accordance with CLSI M38-A2 guidelines adjusted spectrophotometrically at 530 nm wavelength to optical densities that ranged from 0.17-0.15 in RPMI 1640 MOPS broth with L-glutamine without bicarbonate and incubated at 35°C for 96 h.

Results: R. mackenziei gave ranges, MI_{50} and MIC_{90} values for AmB, FLU, ITC, VOR, POS, ISA, CAS and ANI of 8, 32, 0.125, 1, 0.031, 0.5, 8, 2 and 16, 64, 0.25, 2, 0.063, 1, 8, 8 mg/L, respectively. AmB, fluconazole and the two echinocandins had no activity against R. mackenziei. In contrast, POS, ITC, ISA and to a lesser extend VOR demonstrated in vitro activity against R. mackenziei.

Conclusions: Our results are in line with animal data, demonstrating that ITC and POS had the highest in vitro antifungal activity against R. mackenziei. Isavuconazole seems to have also significant *in vitro* activity. Clinical effectiveness in the treatment of cerebral infection remains to be determined for these promising drugs.

PP-03-24

In vitro activities of eight antifungal drugs against 70 clinical and environmental isolates of *Alternaria* species

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Background: There is an increase in the emergence of less common, but medically important fungal pathogens, especially in the expanding population of immunocompromized patients. Not all existing antifungal drugs are suited to cover these rare, mostly filamentous fungi. Little is known of the in vitro activity of antifungal drugs against *Alternaria* spp.

Purpose: To determine the in vitro activity of eight existing and new antifungal drugs against *Alternaria infectoria* and *A. alternata*, the main *Alternaria* species involved in human infection, and some related species.

Methods: A collection of 70 clinical and environmental *Alternaria* strains was obtained from the reference collection of the CBS Fungal Biodiversity Centre in Utrecht, The Netherlands. MICs were determined in accordance with CLSI M38-A2 guidelines and read visually as the lowest concentration of drug showing absence of growth or > 50% reduction of growth (for fluconazole) compared with that of the growth control. Drug and fungus free controls were included. Quality control was ensured by including *Paecilomyces variotii* (ATCC 22319), *Candida parapsilosis* (ATCC 22019), and *Candida krusei* ATCC 6258.

Results: All strains (n=70) taken together gave MIC90 values for amphotericin B, fluconazole, itraconazole, voriconazole, posaconazole, isavuconazole, caspofungin and anidulafungin of 0.5, 32, 1, 4, 0.25, 4, 2 and 0.031 mg/L, respectively. There was no significant difference in MIC90 between clinical strains of *A. infectoria*, *A. alternata*.Isolates of the environmental species. *malorum* A(n=13) yielded similar, low MIC90 for azoles, but demonstrated low activity for echinocandins (MIC90 16 mg/L)

Conclusions: Posaconazole, amphotericin B and anidulafungin seem to be the most active drugs for treating *A. infection*. Voriconazole and isavuconazole demonstrated low in vitro activity against *Alternaria* species. Although the results need to be correlated with clinical outcome.

Key word: *A. infectoria*, *A. alternata*, dematiaceous fungi, Antifungal drugs, in vitro susceptibility

PP-03-25

Rep1p involved in drug resistance by negatively regulating efflux pump MDR1 in *Candida albicans*

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In the past decade, the prevalence of yeast infections has increased dramatically. Among them, *Candida albicans* is the most frequently isolated fungal pathogen in humans and has caused morbidity in immunocompromised hosts. The increased use of antifungal agents has led to an increase in incidences of drug resistance. Overexpression of efflux pumps, including CDR1 and MDR1, is a major mechanism contributing to drug resistance in *C. albicans*. Recently, two transcription factors, CaNdt80p (3,19) and CaTac1p (5), have been identified as positive regulators of CDR1. In this study, we have found that overexpression of REP1, identified by library screening, in Saccharomyces cerevisiae increased the expression of both CDR1 promoter-lacZ (CDR1p-lacZ) and MDR1 promoter-lacZ (MDR1p-lacZ) reporter constructs. Surprisingly, overexpression of REP1 in S. cerevisiae increased susceptibility to certain antifungal drugs. In contrast, mutations on REP1 decreased the susceptibility to antifungal drugs in *C. albicans*. Our results further indicate that the expression of MDR1 is higher in rep1/rep1 cells than that in wild-type cells. Hence, Rep1p is involved in drug resistance by negatively regulating MDR1 in *C. albicans*.

PP-03-26

Phosphorylation analysis of the *Candida albicans* multidrug transporters Cdr1p and Cdr2p

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Candida albicans frequently develops resistance to treatment with azole drugs. An important mechanism of clinical azole resistance in *C. albicans* is the selection of gain-of-function mutations in the Tac1p transcription factor, leading to the constitutive overexpression of the *CDR1* and *CDR2* genes. Cdr1p and Cdr2p are two homologous multidrug transporters of the ATP-binding cassette family comprising two nucleotide binding domains (NBD) and two transmembrane domains (TMD), with a $[NBD\text{-}TMD]_2$ topology. We recently deleted *CDR1* and *CDR2*, individually and in combination, in an azole-resistant clinical isolate. We showed that both transporters are involved in the azole-resistant phenotype of this strain, Cdr1p playing a more important role than Cdr2p and the double *cdr1,cdr2*-deleted mutant being highly hypersusceptible to azole drugs. Using specific anti-Cdr1p and -Cdr2p polyclonal antibodies, we observed that Cdr1p and Cdr2p migrate as multiple bands in an immunoblot, suggesting that they are posttranslationally modified. Treatment of the protein extracts with λ-phosphatase reduced the abundance of the upper bands and this mobility shift was inhibited by the addition of phosphatase inhibitors, suggesting that the two transporters are phosphorylated. Mass spectrometry analysis (LC-MS/MS) of Cdr1p and Cdr2p from the azole-resistant strain identified phosphorylated residues in both transporters: T49, T51, S54 and S849 in Cdr1p and T52 and S847 in Cdr2p. Interestingly, these residues are clustered within two distinct locations, the N-terminal segment upstream of NBD1 and the linker region between TMD1 and NBD2. We are currently investigating whether the phosphorylation of these residues regulates Cdr1p and Cdr2p activity, using site-directed mutagenesis and expression of the mutant alleles from the endogenous *CDR1* or *CDR2* promoter in the *C. albicans cdr1,cdr2*-deleted strain, under the control of hyperactive Tac1p. This system allows high expression of the Cdr1p and Cdr2p transporters for their molecular analysis in an azole-resistant thus clinically relevant host.

PP-03-27

Chemical genetic screening to identify new ligand-like inhibitors and their targets in *Candida*

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In a first selective screening, we have used the chemical compound Diverset from Chembridge Corporation to identify specific inhibitors of *Candida albicans* trehalose-6-phosphate phosphatase Tps2 by a growth assay. Deletion of Tps2 (enzyme which has no homolog in mammalian systems) reduces the survival of *Candida* cells in a mouse model (Maidan et al., 2008; Van Dijck et al, 2002). This first screening was done at high temperature, at which the tps2 homozygote can not grow and resulted in the identification of less than 70 compounds (out of 10 000), most of them with fungistatic activities. The specificity towards Tps2 turned out to be very low as most compounds also inhibited the growth of a wild type laboratory *Candida* strain. The fungicidal compounds were further tested against other *Candida* species, such as *C. glabrata*, C. krusei and C. parapsilosis (clinical isolates). Biofilm formation in vitro was also assayed in a 96-well plate set up: fungicidal compounds inhibited biofilm when added at the adhesion stage, and only one retained a fungicidal activity on mature biofilm (from 24h biofilm to 6-days old biofilm) formed by *C. albicans* but not by *C. glabrata*. Clusters of active compounds permitted the identification of potential known inhibitors by similarity search, one of these clusters showing some similarity to Hsp90 inhibitors for instance. The most potent compound did not share any similarity to known inhibitors, and its target or mode of action remains to be identified.

Maidan, M., et al. (2008) Infect Immun 76: 1686-1694
Van Dijck, P., et al. (2002) Infect Immun 70: 1772-1782

PP-03-28

Hsp90 inhibitor preferentially attenuates postnadir resistance to micafungin and tolerance to voriconazole of *Candida albicans*

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Background: Since currently available antifungal agents are limited, avoiding the emergence of resistance is important. Understanding the mechanism of resistance is helpful for that purpose, and we investigated the relationship between resistance and stress response of *Candida albicans*.
Methods: Appr. $2x10^3$ cells of SC5314 strain were inoculated in each well of 96 well plates containing yeast nitrogen base media with 2% dextrose and with indicated concentrations of micafungin (MCFG) or voriconazole (VRC), and inhibitors of heat shock protein (Hsp) 90 or its effectors. After 24hour incubation, the viability was measured by XTT assay as previously described.
Results: Hsp90 inhibitor radicicol (Rad) tended to attenuate the postnadir resistance rather than to intensify the effect of MCFG at subMIC. In contrast to MCFG, VRC inhibited the viability in the presence of Rad at 0.008 to 0.063μg/ml by 90% and at 0.125 μg/ml to maximum by 99%. We also investigated if Hsp90-calcineurin-chitin synthesis pathway is associated with this phenomenon. Cyclosporine A (CsA) and Nikkomycin Z (NZ), which are calcineurin and chitin inhibitors respectively, attenuated the postnadir resistance to MCFG but also inhibited the viability at 0.016 μg/ml somehow more strongly than Rad. CsA effectively inhibited the tolerance to VRC but NZ only slightly attenuated the tolerance to VRC.
Conclusions: Our results suggest that Hsp90 may act on the emergence of resistance to echinocandins and azoles in different ways. From the viewpoint of basic medicine, the different action of Hsp90 inhibitor could provide a clue to know more about the role of Hsp90. It is unclear whether the paradoxical effect (PE) is clinically important but some studies in vivo suggested that the PE potentiates the threat of clinically relevant resistance. Therefore inhibiting Hsp90 or its effectors might be important for reducing the risk of the emergence of resistance.

PP-03-29

Morphological study on the antifungal action of voriconazole against *Aspergillus fumigatus*

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Voriconazole (VRCZ) is a new triazole antimycotic that is active against a wide spectrum of pathogenic fungi such as *Candida* spp. and Aspergillus spp. Recently, VRCZ has been shown to be effective as the primary therapy for invasive aspergillosis. It acts by inhibiting of ergosterol biosynthesis. The morphological consequences of the action of VRCZ on susceptible fungal pathogens have not yet been studied. Therefore, we conducted a morphological study on A. fumigatus in order to gain a better understanding of the mode of action of this drug.

A. fumigatus was grown at 30°C in the RPMI 1640 medium with or without VRCZ. For scanning electron microscopy (SEM), fungal cells were fixed with glutaraldehyde and osmium tetroxide, dehydrated in acetone, and freeze-dried in t-butyl alcohol. The dried samples were coated with osmium and examined under a JEOL JSM-6700F. For transmission electron microscopy, cells were fixed with glutaraldehyde and potassium permanganate, dehydrated in acetone, and embedded in Quetol 653. Next, thin sections were stained with uranyl acetate and lead citrate, and observed under a Hitachi H-7000.

VRCZ at concentrations of 0.1-1 μg/ml strongly inhibited the in vitro growth of A. fumigatus and induced striking changes in hyphal morphology depending on the drug concentration and the length of the incubation. SEM revealed swelling and deformation of the hyphal tips and formation of short branches from the lateral walls, and finally disruption of the hyphal tips and collapse of whole hyphae. In the thin sections, cell wall thickening, accumulation of electron-dense granules in the cell wall, and disruption of the cytoplasmic membrane and intracellular organelles were observed. These morphological findings of indicate that VRCZ first affects the cytoplasmic membrane and then inhibitis the formation of the cell wall and induces disintegration of cytoplasmic organelles, resulting in a lethal effect.

PP-03-30

Posaconazole-resistant Mucor circinelloides as a cause of invasive maxillofacial zygomycosis

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Objective: Zygomycosis is a life-threatening infection occurring mostly in immunocompromised patients. The aim of this study is to report a case of maxillofacial zygomycosis caused by Mucor circinelloides in a diabetic man who had undergone teeth extraction. Methods: The fungus was grown from the debrided tissue and was provisionally identified as a Mucor species on the basis of characteristic morphological features. Molecular identification was achieved by direct DNA sequencing of internally transcribed spacer (ITS) region of rDNA (ITS-1, 5.8S rRNA and ITS-2) and of D1/D2 region of 28S rRNA gene. Drug susceptibility testing was performed by E-test. Results: Lactophenol-cotton blue examination of the isolate showed recurved (circinate) lateral branches of sporangiophores, chains of thick-walled intercalary and terminal chlamydospore and its conversion into yeast forms (unipolar, bipolar and multipolar budding) when grown on brain-heart infusion agar at 370C. The ITS region amplicon of ~600 bp, amplified with panfungal ITS1 and ITS4 primers, was sequenced and BLAST search of DNA sequence data revealed complete identity (100%) in ITS-1 and ITS-2 regions with corresponding sequences available in the databank for M. circinelloides. Similarly, ~700 bp amplicon of D1/D2 region of 28S rRNA obtained with NL-1 and NL-4 primers was sequenced and the BLAST search revealed nearly complete identity (1 or 2 nucleotide differences) with the corresponding sequences available in the databank for two strains M. circinelloides. The isolate was found to be resistant to posaconazole, voriconazole, and caspofungin, but susceptible to amphotericin B. The patient was treated successfully with liposomal amphotericin B and surgical debridement. Conclusions: The report highlights the emerging role of M. circinelloides in invasive zygomycosis and reinforces the importance of prior susceptibility testing of zygomycetes for improved prognosis. The possibility of emergence of M. circinelloides as a cause of breakthrough zygomycosis among patients receiving posaconazole prophylaxis is also suggested.

PP-03-31

Mechanisms of azole-resistance in *Candida albicans* isolates from a uterine leiomyosarcoma patient

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Background: The azole-resisitant *Candida albicans* were isolated from the blood of Uterine Leiomyosarcoma patient. She had received therapy with Fos-fluconazole at 200mg/day for 20 days. We investigated the resistant mechanism to azole-antifungal agents.

Methods: 6 strains of *C. albicans* isolated from stool, urine (3 strains) and blood cultures (2 strains) with patient and reference strain were used. All strains were green on CHROMagar plates and were identified with *C. albicans* by VITEK YBC and api ID32C. Especially, 2 strains isolated from blood cultures analyzed ITS region with direct sequence. The MICs of antifungal agents for the *C. albicans* strains were determined by the CLSI M27-2A microdilution method. 8 oligonucleotide primers were used for Randomly Amplified Polymorphic DNA (RAPD) analysis. The level of mRNA for *ERG11*, *CDR1*, *CDR2*, *MDR1* and *ACT1* in *C. albicans* cells were measured quantitatively by real-time RT - PCR. *ERG11* genes were amplified in three overlapping regions of the gene and sequenced.

Results: (i) The 2 strains isolated from blood culture exhibited resistance to fluconazole, itraconazole and voriconazole with MICs of >64, >8 and >8 micro g/mL, respectively. The other strains were susceptible to azole - antifungal agents. (ii) The profiles of *C. albicans* strains analyzed by RAPD method showed several patterns from where the samples derived. Especially,strains from urine samples changed when they were taken. (iii) The expression of the *CDR1* and *MDR1* mRNA in the strains from blood culture were increased compared to that in the reference strain.

Conclusions: Though short antifungal therapy, it is very rare and important that isolated azole-resistant *C. albicans* from the blood culture of this case.

PP-03-32

Mechanism of echinocandin resistance in *Candida albicans*

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The mechanism of echinocandin resistance was investigated using a micafungin resistant clinical isolate and several laboratory generated mutants of *Candida albicans*. DNA sequences of open reading frames which may contribute to the β-1,3-glucan synthase of a micafungin sensitive strain (MIC = 0.031 μg/ml) and a clinical isolate showing high level resistance (MIC = 4 μg/ml) were compared. This approach identified a homozygous nucleotide change in *GSC1* that was predicted to cause an S645P amino acid change in the region of the echinocandin resistance region (hot spot). *GSL1* had two homozygous amino acid changes and five non-synonymous nucleotide polymorphisms due to allelic variation. *RHO1* showed no predicted amino acid changes between strains. Exposure of a micafungin-sensitive *C. albicans* laboratory strain to micafungin selected a spontaneous mutant with intermediate resistance to micafungin (MIC = 1 μg/ml) and a heterozygous S645F amino acid change in *GSC1* only. The hypothesis that clinically significant resistance to candins requires a homozygous mutation in both alleles of *GSC1* was tested using a panel of homozygous and heterozygous mutants that possessed combinations of the candin sensitive S645 and resistant F645 alleles, or mutants with individual *GSC1* alleles deleted. Candin susceptibility tests showed that a homozygous F645 mutant had high MICs for both micafungin and caspofungin while the heterozygous S645/F645 mutant showed intermediate resistance. Kinetic analysis of β-1,3-D-glucan synthase activity showed that the homozygous and heterozygous mutations gave candin susceptibility profiles that correlated with the MIC values. This study demonstrates that a functional homozygous hot spot mutation is required for high level candin resistance and implies that both alleles of *GSC1* encode equally active catalytic subunits of β-1,3-glucan synthase.

PP-03-33

Mechanism of echinocandin resistance in *Candida glabrata*

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The echinocandins inhibit the synthesis of β-1,3-glucan, an essential component of fungal cell walls. The incidence of candin-resistant pathogenic fungi is rare but has been associated with mutations in the echinocandin resistance region (the hot spot) of β-1,3-glucan synthase catalytic subunits encoded by *FKS* genes. We analysed the *FKS* genes in a micafungin-resistant *Candida glabrata* clinical isolate. Single nucleotide changes were detected in both *C. glabrata* genes that are syntenic orthologues of the *S. cerevisiae FKS1* and *FKS2* genes. One mutation was predicted to cause an amino acid change in the hot spot of *CgFKS1* and the other a premature stop codon in *CgFKS2*. We hypothesized that clinically significant candin resistance in *C. glabrata* may require single nucleotide changes in both *CgFKS1* and *CgFKS2*. Relationships between the observed mutations and candin resistance were assessed experimentally by reproducing the nucleotide changes in the *CgFKS* genes of a candin-susceptible *C. glabrata* strain (micafungin MIC = 0.031 μg/ml) using site-directed mutagenesis. Introduction of the hot spot mutation into the *CgFKS1* gene alone conferred intermediate resistance (MIC = 0.5 μg/ml) whereas the introduction of a premature stop codon in *CgFKS2* alone had no effect on susceptibility. However, the insertion of both mutations conferred high level resistance (micafungin MIC = 2 μg/ml) equivalent to that of the clinical isolate, and cross-resistance to caspofungin. The phenotypes and candin susceptibilities of *δfks1* deletant and *δfks2* deletant indicate that the two *CgFKS* genes are functionally redundant, with each encoding a β-1,3 glucan synthase catalytic subunit. Like *S. cerevisiae*, the deletion of both *CgFKS1* and *CgFKS2* was found to be lethal. Clinically significant micafungin resistance in *C. glabrata* appears to be rare because *CgFKS1* and *CgFKS2* are differentially expressed and mutations in both *CgFKS1* and *CgFKS2* are required for the acquisition of high level candin resistance.

PP-03-34

Gain of function mutations in *CgPDR1* of *Candida glabrata* not only mediate antifungal resistance but also enhance virulence

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C. glabrata develops azole resistance mostly via the upregulation of ABC transporter genes *CgCDR1*, *CgCDR2* and *CgSNQ2*. CgPdr1p is the major *C. glabrata* transcription factor involved in their regulation. Gain of function (GOF) mutations in *CgPDR1* are responsible for the increased expression of *CgCDR1, CgCDR2* and *CgSNQ2* and thus to contribute to azole resistance of clinical isolates.

In this study, we investigated the incidence of *CgPDR1* mutations in a large collection of clinical isolates and tested their relevance not only to azole resistance *in vitro* and *in vivo* but also to virulence. The comparison of *CgPDR1* alleles from azole-susceptible and azole-resistant matched isolates (n=122) enabled the identification of 57 amino acid substitutions present only in *CgPDR1* alleles from azole-resistant isolates. These mutations are GOF mutations since only alleles containing these mutations conferred ABC-transporter genes constitutive high expression. Interestingly, the major transporters involved in azole resistance (*CgCDR1, CgCDR2* and *CgSNQ2*) were not always co-ordinately expressed in presence of specific *CgPDR1* GOF mutations, suggesting that these are rather trans-acting elements (GOF in *CgPDR1*) than cis-acting elements (promoters) that lead to azole resistance by upregulating specific combinations of ABC-transporter genes. Moreover, *C. glabrata* isolates complemented with *CgPDR1* GOF alleles were not only more virulent in mice than those with wild type alleles, but they also gained fitness in the same animal model. The presence of *CgPDR1* hyperactive alleles also contributed to fluconazole treatment failure in the mouse model.

This study shows the high variability in *CgPDR1* GOF mutations having differentiated effects on target genes including the major ABC-transporters involved in azole resistance. Importantly, this study shows for the first time that *CgPDR1* mutations are not only responsible for *in vitro/in vivo* azole resistance but that they can also confer a selective advantage under host conditions.

PP-03-35

Functional dissection of Tac1p, a *Candida albicans* transcription factor involved in antifungal drug resistance

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For treating *C. albicans* infections, the use of antifungal agents including azoles can lead to drug resistance. One of the most frequent resisance mechanism is increased drug efflux through enhanced expression of the ABC-transporters *CDR1* and *CDR2*. The understanding of the regulation of these genes is critical for the control of azole resistance through efflux mechanisms. The regulation of *CDR1* and *CDR1* is mediated by *TAC1*, a transcription factor able to bind to the promoters of its target genes. We previously identified *TAC1* hyperactive alleles (*TAC1*-hyp) from clinical azole-resistant strains, which in contrast to wild-type alleles (*TAC1*-wt) conferred constitutive high *CDR1/CDR2* expression in a *tac1* deletion mutant. Hyperactivity of *TAC1* are due to gain-of-function mutations (GOF). A better knowledge of Tac1p and its partners will lead to a better understanding of the azole resistance phenomenon. In this work we functionally dissected this protein. For this purpose, tagged versions of Tac1p were constructed to perform immuno-precipitation of distinct Tac1p forms (wt or hyp). Our results demonstrate that Tac1p could form dimers. Chromatin immuno-precipitation (ChIP) assays allowed to establish that Tac1p binds intrinsically to target promoters. Finally, we established functional regions of Tac1p by deleting the putative DNA binding domain, the putative transcriptional inhibitory and activation domains. Functionality of the mutant proteins were analysed with two different systems. Our results showed that the last 60 aa of Tac1p were sufficient for transcriptional activity. Nevertheless, optimal activity was obtained using the last 160 aa. In contrast, a region including the last 240 aa of the protein led to complete loss of transcriptional activity suggesting the presence of a transcriptional inhibitory domain located upstream of the last 160 aa of the protein. Further constructions are currently designed and tested to determine the role of other Tac1p regions in the activation of target genes.

PP-03-36

Ketoconazole induced P450 enzyme (CYP1A1) in normal human keratinocytes

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There were many reports that ketoconazole (KCZ) had broad-spectrum activities including the potent effect against *Malassezia* spp. However, the mechanism of its activities was still unknown. CYP1A1 is one of P450 enzyme and plays important roles in detoxificating toxins and is induced via the aryl hydrocarbon receptor (AhR) signaling. The AhR had been recognized as a receptor for dioxin only, however, recent papers reported that not only dioxins but also other chemicals could bind with AhR and some AhR ligands (ex. resveratrol, curcumin) exerted anti-inflammatry effects by playing as an antagonist in activating AhR signalings. And it was reported that CYP1A1 was induced by KCZ via AhR signaling in human hepatoma cells, which indicated that KCZ was one of the AhR lignds. Thus, we examined whether KCZ could induce the CYP1A1 in normal human keratinocytes. KCZ (100nM-1uM) increased mRNA lavels and protein levels of AhR and CYP1A1. In con-focal microscopic study, we confirmed that the AhR translocated from cytoplasm to nuculei by administrating KCZ (100nM-1uM). This study provides the evidence for the ability of KTZ to induce the CYP1A1 through an AhR-dependent mechanism.

PP-03-37

Frequency of azole resistance phenotypes in the two most prevalent human pathogenic yeasts *C.albicans* and *C.glabrata*

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We have investigated the resistance phenotypes of a collection of over 200 clinical *C.albicans* and *C.glabrata* isolates with increased tolerance towards azole antifungal drugs. Although the range of MIC values for Voriconazole is generally 100-fold lower than the one for Fluconazole, MIC testing (EUCAST) of the strains revealed that there is a clear linear relation of Fluconazole and Voriconazole tolerance. Interestingly, we did not observe any Fluconazole resistant strains that did not have elevated Voriconazole tolerance. In contrast, there were several *C.albicans* strains which showed Voriconazole resistance, but were still susceptible to Fluconazole. To elucidate the mechanisms underlying these patterns, all strains were tested for unspecific drug efflux (Rhodamine6G accumulation) and for the membrane sterol composition (gas chromatography). For *C.glabrata* the analysis shows that resistance is mediated almost exclusively by drug efflux, only two isolates were found which showed the phenotype of an *ERG11* mutation. In contrast, *C.albicans* showed a large variety of different sterol compositions, indicating different mutations in the ergosterol biosynthesis pathway. The majority of cross-resistant strains showed drug efflux, many in combination with a sterol composition phenotype of an *ERG11* mutation. Strains which were exclusively Voriconazole resistant mediated this only by changes in sterol composition. Also, a series of mutants exhibiting altered sterol C5-desaturation (encoded by *ERG3*) with infinite azole resistance were identified. Additionally, these isolates showed a linear correlation of ergosterol content and Amphotericin B resistance. The molecular reasons for the phenotypes described here as well as the phylogenetic relationships of the strains are currently under investigation. In conclusion, we were able to identify phenotypical changes leading to increased azole drug tolerance for the majority of resistant isolates in our collection. However still a few strains remained for which no potential resistance mechanism could be postulated.

PP-03-38

The transcription activator AtrR is involved in azole drug resistance by regulating the expression of ABC transporter genes in *Aspergillus fumigatus*

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Infections by human pathogenic fungi such as *Aspergillus fumigatus* are treated with some antifungal agents, including azole fungicides that inhibit the ergosterol biosynthesis in the fungal cell membrane. During the long-term usage of the drug for therapy, azole resistant isolates of *A. fumigatus* have been recently documented. One of the possible mechanisms of azole resistance in *A. fumigatus* is the upregulation of genes encoding drug efflux pumps, mainly belonging to ABC transporters. However, the mechanism that regulates gene expression of ABC transporters has not been elucidated in *Aspergillus* species. Previously, overexpression of a transcriptional factor gene, designated as *atrR*, resulted in increased drug resistance and also induced the gene expression of ABC transporters in *A. oryzae*. In addition, deletion of the *atrR* led to downregulation of three ABC transporter genes and consequently resulted in significant increase in azole drug susceptibility. Orthologous genes of the *A. oryzae atrR* have been found widely in genomes of *Aspergilli*, including *A. fumigatus*, *A. nidulans*, and *A. niger*. In the present study, we constructed a deletion mutant of the *atrR* ortholog in *A. fumigatus* and investigated the role of the gene in drug resistance. The mutant was similarly hypersensitive to azole drugs, especially susceptible to fluconazole that is not effective to many *Aspergillus* species. The mutant also showed reduced expression level of several ABC transporter genes that would function as drug efflux pumps. These results indicate that the transcription factor AtrR also regulates gene expression of ABC transporters and contributes to azole resistance in *A. fumigatus*. In addition, this suggests that fluconazole resistance of *Aspergillus* species is attributed to expression of drug efflux ABC transporters regulated by AtrR. This transcriptional factor AtrR provides novel therapeutic targets for the treatment of azole drug resistant fungal infections.

PP-03-39

Correlation between mutations in the *Aspergillus fumigatus* cyp51 gene and their azole resistance profile

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Molecular studies have shown that triazole resistance in *Aspergillus fumigatus* is associated with amino acid substitutions in the cyp51A gene. Amino acid substitution in G54 to -E,-Q,-R or -W, M220 to -V,-K,-T,-I or -L and a change in codon 138 from G to C have all been correlated to different patterns of elevated minimum inhibitory concentrations (MICs) for the triazole drugs. A multi triazole resistant phenotype was also associated with a substitution at L98H combined with a 34bp tandem repeat in the promoter region.

In our collection the cyp51A gene of 220 resistant and susceptible *A. fumigatus* isolates has been sequenced. In 15 (7%) of the *A. fumigatus* isolates, similar combinations of two to eight mutations were found related to amino acids F46Y, G89G, M172V, N248T, D255E, L358L, E427K or C454C. Two mutated isolates showed a MTR phenotype, while 13 isolates showed a full azole susceptible profile. All of the substitutions but one (C454C-heme ligand) were located at the outside of the protein according to the model of the *A. fumigatus* cyp51A protein that was build using as a template the cyp51A crystal structure of M. tuberculosis. Molecular dynamics simulations of the wild-type protein show that there is no correlation between located positions of the mutations and either of the two substrate access channels. Mutations in the cyp51A gene not related to resistance have not been described in the literature before. In this study we found that the majority of the cyp51A gene mutations do not correlate to azole resistance in *A. fumigatus*. Cyp51A mutations need to be analyzed by recombinant analysis before a conclusive correlation to triazole resistance can be made.

PP-03-40

Overexpression of cyp51A gene and a transposition event in multi-azole resistant clinical isolates of *A. fumigatus*

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Azole resistance in *Aspergillus fumigatus* is well documented. Mutations in the target enzyme cyp51A appear to be the dominant mechanism. We investigated other mechanisms of resistance that could be operative in eight multi-azole resistant clinical isolates.

METHODS: We have investigated the mechanisms of resistance to azoles in eight isolates obtained serially from a patient with bilateral aspergillomas. Antifungal susceptibility of isolates was determined. Strain identity was investigated. Expression of genes, cyp51A, cyp51B and five efflux pumps was done. Cyp51A and cyp51B genes were sequenced. Morphological characteristics of isolates were investigated.

RESULTS: A resistance profile characterized by very high MICs of itraconazole (> 8.0 mg/l), voriconazole (8.0 mg/l), ravuconazole (8.0 mg/l) and posaconazole (4.0 mg/l) was observed in all 8 isolates. Sequencing of the cyp51A gene revealed 3 novel alterations leading to amino acid substitution. These mutations are G138C, Y431C and G434C. cyp51A expression was up-regulated 4-8 fold in the 8 isolates. Interestingly, by analyzing the promoter region, a type II transposon (1882bp) was found to be inserted upstream of the promoter of Cyp51A in one isolate with the highest level of expression which could be responsible for the up-regulation of gene. This is the first mobile transposon to be identified in *A. fumigatus*. Two isolates also exhibited reduced growth rate and decreased conidiation which could be as a result of transposition events.

CONCLUSION: Resistance to azoles in *A. fumigatus* is associated with overtranscription of cyp51A gene, sometimes in association with mutation. Resistance and up-regulation could be associated with the insertion of a transposon into the promoter of the cyp51A gene. These results indicate the complex nature of antifungal resistance in *A. fumigatus*.

PP-03-41

Factors influencing the permeability of amphotericin B in an *In vitro* blood brain barrier (BBB) model

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Background: Amphotericin B (AmB) exhibits low penetration into the central nervous system and little is known about the factors affecting its blood brain barrier (BBB) permeability. We studied the effects of cytokines, microbial factors and dexamethasone (DM) on BBB permeability.
Methods: The model consisted of co-cultured bovine brain endothelial cells on permeable polyester membranes and rat astrocytes on the bottom of the wells. The effect of the following variables on transendothelial electrical resistance (TEER) and permeability to deoxycholate amphotericin B (dAmB) and liposomal amphotericin B (LAmB) at 24h was studied: interleukin 1 (IL-1β), tumor necrosis factor alpha (TNF-α), lipopolysaccharide (LPS), lipoteichoic acid (LTA) and DM. Endovascular and CNS compartments were assayed for AmB by UPLC. All experimental conditions were conducted in 3 wells and experiments were performed in triplicate. Continuous variables were analyzed by Mann-Whitney U test.
Results: IL-1β (0.1 and 0.01 μg/ml) decreased TEER by 62.9% ($p<0.001$) and 70.3% ($p<0.001$), respectively but had no significant effect on AmB permeability. TNF-α and LPS (all at 0.1-1.0 μg/ml) decreased TEER by 75.5-98.3% ($p=0.004$) and 85.9-100% ($p<0.001$) and significantly increased AmB permeability up to 14.5 μg/ml ($p<0.003$). By comparison, LTA (all at 0.1-1.0 μg/ml) increased TEER by 40.7% ($p=0.001$) with no effect on AmB permeability. DM (0.1 and 1 μg /ml) increased TEER by 18.2% ($p=0.04$) and 26.4% ($p=0.003$), respectively, with no significant effect on AmB permeability.
Conclusions: Dexamethasone decreases BBB permeability to small ions but not to AmB. TNF-α and LPS increase permeability to small ions and AmB; whereas, Il-1β increases permeability to small ions but not to AmB.

PP-03-42

A morphological study of the antifungal action of amphotericin B against *Aspergillus fumigatus*

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The polyene antibiotic amphotericin B (AMPH) is the drug of choice in case of severe fungal infections, including invasive aspergillosis. It is generally accepted that AMPH binds to the ergosterol in cell membranes, increases membrane permeability, and eventually kills the fungal cells. However, the morphological changes in fungal cells like *A. fumigatus* that accompany the killing process remain unclear. Therefore, we investigated the effect of AMPH on the morphology of growing hyphae of *A. fumigatus*, which was cultured in a liquid medium, by performing light and electron microscopy(EM).
A. fumigatus was grown at 30°C in the RPMI 1640 medium with or without of AMPH. For scanning EM, the cells were fixed with glutaraldehyde and osmium tetroxide, dehydrated in acetone, and freeze-dried in t-butyl alcohol. The dried samples were finally coated with osmium and examined under a JEOL JSM-6700F. For Transmission EM, the cells were fixed with glutaraldehyde and potassium permanganate, dehydrated with acetone, and embedded in Quetol 653. Next, thin sections were stained with uranyl acetate and lead citrate and observed under a Hitachi H-7000.
Hyphal growth was inhibited at the drug concentration of 0.1 μg/ml (MIC, 1/10). Distortion or collapse of the hyphae was the most prominent morphological change observed in cultures treated with a relatively low concentration of the drug. In the ultrathin sections, abnormal formation of the septal walls was also observed. The extent of structural changes in the cells increased with an increase in the drug concentration in an exposure-time-dependent manner. After a 1-h treatment with 0.5 μg/ml (MIC, 1/2) almost all the hyphae had collapsed, the cytoplasmic membrane and intracellular organelles had degenerated, and ultimately almost all the cytoplasmic materials were released externally. The morphological findings of this study indicate that AMPH affects both normal cell wall formation and membrane structures of fungal cells.

PP-03-43

The post anti-fungal effect (PAFE) of itraconazole: PAFE is an important parameter in anti-fungal drug treatment

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The efficacy of anti-fungal drugs is only evaluated with the minimal inhibitory concentration (MIC). As azole anti-fungal drugs have few side effects, they are therefore frequently used to and sometimes have a high MIC for non-albicans *Candida* species, but a clinically good effect for candidiasis caused by non-albicans *Candida* species. However, the evaluation of such drugs does not consider the post anti-fungal effect (PAFE) but only the MIC. Purpose: The purpose of this study is to establish a clinical effective evaluation for anti-fungal drugs. Materials and Methods: The quantitated MIC and PAFE of Itraconazole (ITCZ) using a parameter T/C (T =time required for the drug-treated culture to reach a 5-fold increase in turbidity; C =time required for the drug free control culture to reach a 5-fold increase in turbidity) for *Candida albicans* (ATCC 18804 and Ka747) and *Candida* glabrata (ATCC 90030, Kg799 and Kg804). In addition, the adherence capability (AC) of *Candida* strains to a pharynx carcinoma cell line (FADU: ATCC HTB-43) was compared in both a drug free group and in a drug (ITCZ)-treated group. The AC of *Candida* strains to FADU were evaluated by colony formation units (CFU) on CHROM-agar. Results: *C.albicans* (Ka747) and *C.glabrata* (Kg799, Kg804) showed a high MIC (>8micro g/ml) for ITCZ. All *Candida* strains of T/C were high (>1) and the CFU was observed to decrease in the drug-treated group. This may be the reason why ITCZ is clinically effective for *Candida* species although it has been evaluated to be drug tolerant based on the MIC. Conclusions: ITCZ may have a PAFE and therefore have a good effect for *Candida* species even though it has been evaluated as being drug tolerant by the MIC. As a result, evaluating the PAFE (T/C and AC) may therefore be an important parameter in the anti-fungal drug treatment.

PP-03-44

The mechanism of amphotericin B nephrotoxicity and its neutralization by conjugation with arabinogalactan

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Background: Amphotericin B(AMB) remains the gold standard treatment of invasive fungal infections(IFI) due to its broad spectrum of activity and low resistance rates. However, its use is hampered by adverse effects, mainly nephrotoxicity. We previously described an injectable AMB-arabinogalactan(AG) conjugate that was safer and more therapeutically effective in murine models of various IFI than the conventional AMB.

Methods & Results: We demonstrated that while free-AMB was highly toxic to kidney epithelial cells, conjugation of AMB to AG significantly reduced the AMB cytotoxicity without impairing antifungal activity. Using cell biology methodologies, we showed that AMB treatment of kidney cell-lines that express caveolin-1, known to increase the amount of caveolae and cholesterol at the plasma membrane(PM), resulted in 2-3 fold increase in cell death, most likely due to an increase in AMB-insertion sites on the PM, whilst expression of a mutant which inhibits endocytosis (dynamin 1-K44A) resulted in reduced AMB-induced cell death. These results indicated that AMB toxicity to kidney cell-line is in part due to endocytosis of AMB based pores from the PM to endosomes.

Studding the effect of free and conjugated AMB in yeast cells revealed two mechanisms of action that contrary to the kidney-cell line, are not affected by conjugation of AMB with arabinogalactan: the arrest of endosomal trafficking (using the GFP-HXT2 dextrose transporter model), the formation of polar channels across the membrane (determined by leakage of potassium ions).

Conclusions: AMB toxicity is due in part to the leakage of metabolites through pores in the PM and in part to PM pores endocytosed and incorporated into endosome membranes where they abolish the pH gradient thereby disrupting the host cell post-endocytic and exocytic traffic machineries. Conjugation of AMB to AG abolishes these toxic effects in kidney cells but not in yeast cells.

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PP-03-45

Efficacy of combination treatment with liposomal amphotericin B (L-AMB) and caspofungin (CS) in murine models of pulmonary vs systemic aspergillosis

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Background: *Aspergillus fumigatus* infection begins in the lungs and disseminates to kidneys and spleen. We compared murine pulmonary (pul) and systemic aspergillosis (sys) infections to evaluate the efficacy of monotherapy vs combination therapy with L-AMB and CS.

Methods: Swiss Webster mice were immunosuppressed (6mg/kg triamcinolone IP -3d, 0d and +2d (pul) or 100mg/kg cyclophosphamide, IP, q3d beginning -3d (sys)) and challenged with 6 x 10ex7 *A. fumigatus* spores intranasally (pul), or 5.3 x 10ex4 A. fumigatus spores IV (sys). Six days of IV dosing (n=14/gp) included 5% dextrose(D5W), L-AMB(AmBisome®) at 5mg/kg(pul), 10mg/kg(pul/sys) or 15mg/kg(sys) or CS (Cancidas®) at 5mg/kg(pul/sys) as monotherapy or combination therapy (concomitant or sequential with 3dL-AMB/3dCS or 3dCS/3dL-AMB). Morbidity (n=7/gp) was followed for 14 days(pul) and 28 days(sys); 24h post-treatment, tissues (n = 7/gp) were collected for fungal burden (Log10 CFU) and drug concentration.

Results: In sys model, survival was 100% in all drug treated mice vs 0% in D5W mice; only L-AMB monotherapy, concomitant or sequential treatment with L-AMB given first, significantly reduced fungal burden in kidneys and spleen ($p \leq 0.002$). Drug concentrations in kidneys were the same for both L-AMB and CS monotherapy. In the pul model 100% survival was only observed with L-AMB monotherapy, concomitant or sequential treatment with L-AMB given first. In contrast, survival with CS alone or given first ranged from 0-29%. In the lung significant reduction in fungal burden vs D5W was demonstrated in all drug groups except for mice given CS monotherapy ($p \leq 0.05$). Lung drug concentration was significantly higher with L-AMB vs CS monotherapy ($p \leq 0.05$).

Conclusions: Differences between L-AMB and CS for treatment of pul vs sys murine aspergillosis suggest important interactions between the fungus and the drugs in different tissues. Overall, L-AMB outperformed CS for the treatment of pul and sys aspergillosis infections.

PP-03-46

Improved *in vitro* and *in vivo* efficacy of micafungin (MCFG) against *Aspergillus fumigatus* in combination with posaconazole (POCZ)

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Background: Invasive pulmonary aspergillosis (IPA) continues to be a serious threat to immunocompromised patients despite improved antifungal therapy. The limited efficacy of current agents has resulted in significant interest in combination therapy for these difficult-to-treat infections. In this study, we evaluated the efficacy of MCFG in combination with POCZ *in vitro* and *in vivo*.
Methods: *In vitro* activity against 16 clinical *A. fumigatus* isolates was assessed using CLSI M38-A2 and checkerboard microdilution. Mice (10/group) treated with cyclophosphamide were infected intranasally with *A. fumigatus* FP1305 conidia. Treatment was initiated 2 hr post-infection (PI) with 0.5 mg/kg of intravenous MCFG and/or 0.25 mg/kg of oral POCZ twice a day for 5 days. Survival was monitored for 14 days PI and analyzed using the log-rank test in comparison with corresponding monotherapy.
Results: While the *in vitro* FIC index of MCFG-POCZ combination was defined as marginally additive (FIC=0.52-2), microscopic morphological analyses detected a clear synergistic effect on hypha. Small, rounded, and compact hyphal forms remained, even at concentrations above the MIC of MCFG alone. However, the remaining hyphal forms were much smaller and more stunted in combination with POCZ at 1/4 MIC and 1/2 MIC. In the mouse IPA model, monotherapy with MCFG or POCZ resulted in poor survival rate (40% for both; all control mice died within 5 days PI). In contrast, all animals in the MCFG-POCZ combination group survived until the end of the experiment, which indicates that the MCFG-POCZ combination significantly improved the survival rate compared to the monotherapy groups ($p<0.01$).
Conclusions: This study demonstrated that the combination of MCFG with POCZ in a mouse IPA model was superior to either monotherapy, and is consistent with the *in vitro* results. The combination of MCFG with POCZ is a promising potential option for the treatment for IPA infection.

PP-03-47

Efficacy of combination antifungal therapy of micafungin and aerosolized liposomal amphotericin B in murine invasive pulmonary aspergillosis model

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[Background]
Invasive pulmonary aspergillosis (IPA) is an important cause of high mortality in patients with hematological malignancies or hematopoietic stem-cell transplantation. Liposomal amphotericin B (L-AMB) is recommended for the treatment of IPA as an alternative therapy in the clinical practice guidelines of the Infectious Diseases Society of America. L-AMB however, possesses serious adverse effects, even though remarkably reduced compared to conventional amphotericin B. Targeted intrapulmonary delivery of drugs may reduce systemic toxicity and improve treatment efficacy. Our goal in this study is to evaluate the effect of aerosolized L-AMB in murine IPA model and combination effect of intraperitoneal (i.p.) administration of micafungin (MCFG).
[Material & Method]
Female ICR mice were immunosuppressed with cortisone acetate and cyclophosphamide on 2 days prior to inoculation and inoculation day. A total of 5X106 conidia of A. fumigatus MF13 were inoculated intratracheally. IPA mice were divided into following four groups; controls, MCFG i.p., aerosolized L-AMB, and combination of MCFG i.p. with aerosolized L-AMB. Survival rates were observed until day 11 after challenge. All treatments were initiated 16 hours after inoculation and continued for 5 days. To investigate the pharmacokinetics of aerosolized L-AMB, amphotericin B concentration of serum and lung were measured by high-performance liquid chromatography.
[Result & Conclusion]
The survival rate of single administration of MCFG and aerosolized L-AMB arms were significantly better than that of control. Combination treatment arm indicated significantly superior survival rate compared to each of single drug administration ($P < 0.05$ by the log rank test). The concentrations of amphotericin B in lung tissue after inhalation were increased in proportion to inhaled drug concentrations, whereas, concentrations in the serum samples were not detected. In conclusion, the combination therapy of MCFG i.p. and aerosolized L-AMB may be effective and reduce systemic toxicity in treatment of invasive pulmonary aspergillosis.

PP-03-48

Evaluation of experimental invasisve pulmonary aspergillosis (IPA) in a non-neutropenic murine model utilizing aerosolized inoculation

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Background: Differences in the pathogenesis and response to therapy have been reported between neutropenic and non-neutropenic animal models of IPA. We evaluated a non-neutropenic murine model of IPA using an established and reproducible aerosol method for inoculation. **Methods:** ICR mice were immunosuppressed with cortisone acetate (10 mg/animal SC) administered on days -4, -2, 0, +2, and +4 of inoculation. Mice were challenged with *A. fumigatus* AF293 using an aerosol chamber. Untreated mice ($\geq$ 3 per time point) were euthanized on days +3, 4, & 5 post-inoculation and serum collected. For treatment, posaconazole (PSC 40 mg/kg/day PO), and caspofungin (CFG 2 mg/kg/day IP) were begun on day +1 and continued through day +8. On day +5 serum was collected from $\geq$ 4 mice per group. Remaining animals were followed until day +12 to assess survival and lung fungal burden by PCR. Beta glucan (BG) was assayed using the Fungitell assay. Conidial equivalents (CE) were assessed by real time PCR. **Results:** Serum BG in untreated mice was raised on day +3 (median 1423 pg/mL) and remained elevated on days +4 & 5 (1306 & 641 pg/mL). Median survival for untreated controls was 8 days, (range 3-12 days; 100% mortality); PSC 12 days (range 3-12 days; 40% mortality) and CFG 12 days (range 5-12 days; 40% mortality). Median BG on day +5 for untreated controls was 419 pg/mL (range 304-1056); PSC 345 pg/mL (range 46-994) and CFG 224 pg/mL (range 34-477). Median $\log_{10}$CE/g for untreated controls was 7.4 (range 6.3-8.5); PSC 6.8 (range 5.9-8.1) and CFG 8.1 (range 6.4-9.0).
Conclusions: In this non-neutropenic animal model serum BG was detectable early in the course of IPA. Both posaconazole and caspofungin prolonged survival however, no changes in serum BG or fungal burden were observed with antifungal therapy compared to untreated controls.

Poster Forum PF-03

PP-03-49

Establishment of novel model of onychomycosis in rabbits for evaluation of antifungal agents

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It has been reported that animal models of onychomycosis were used to evaluate many new drugs, the pathophysiology of experimental infection in rodent however has been obscure. In the study, we report an establishment of novel animal model using rabbit of which excellent reproducibility was confirmed. In addition, it was also confirmed that pathophysiology of the model accurately reflect that of a particular type of onychomycosis in human, subungal type. Finally we evaluated some antifungals using our model with mycological and histopathological exainations.

Trichophyton mentagrophytes(TIMM2789) were infected to the nail of hind limb in the Japanese white rabbit under the steroid treatment for 2 or 4 weeks as their nails were made moistened by latex glove. After treatment with infection, these nails in rabbits were observed for 0, 2, 4 and 6 weeks to check the change of condition. Then, these nails were taken from the feet, and they were evaluated the localization of fungus in the tissue sample after staining with periodic acid-Schiff stain.

In the result, the onychomycosis model using rabbit were established under the condition which microconidia was infected for 2 weeks. Organism was confirmed in the nail plate and nail bed side in the nail, and infection rate in this model was over 86.7%. Additionally, fungi increased into the nail and were confirmed to the localization into the nail. Moreover, Penlac, as an antifungal drug, was applied to the nail for 4 weeks, and was confirmed the efficacy.

In conclusion, we established the onychomycosis model which it was useful for the evaluation of the antifungal efficacy.

PP-03-50

Antifungal activity of luliconazole in a guinea pig seborrheic dermatitis model with *Malassezia restricta*

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Malassezia restricta is known to be involved in the pathogenesis of seborrheic dermatitis (SD). We previously developed a guinea pig SD model inoculated with *M. restricta* mimicking the cutaneous lesions of SD with erythema and scaling. The present experiment was conducted to assess the antifungal activity of luliconazole (LLCZ), a novel imidazole derivative, in a model using ketoconazole (KCZ) as a comparative reagent. As *M. restricta* did not grow easily in a conventional culture method, we used real time PCR for mycological evaluation.

Clinical isolates of *M. restricta* suspended in modified Leeming and Notman broth was inoculated to the clipped dorsal skin (40mg wet weight/φ 2 cm) of male SPF guinea pig by gentle rubbing. The inoculation was repeated once daily for seven consecutive days without occlusion. Starting on the day after the final inoculation, 1% LLCZ cream or 2% KCZ cream was applied topically once daily for three consecutive days. The cutaneous lesion was macroscopically observed at the indicated days, and the histological samples were collected for PCR analysis and histopathology (PAS stain). Real time PCR was performed using a *Taq-Man* probe designed from the ITS 2 region of the rRNA gene.

The PCR results and the yeast counts in the histopathology were closely correlated. Topical application of 1%LLCZ cream and 2%KCZ cream macroscopically improved the cutaneous lesions when compared with the non-treated guinea pigs. The number of organisms as measured by PCR had significantly decreased after treatment with 1%LLCZ cream or 2%KCZ cream. These results suggest the clinical utility of LLCZ for the treatment of SD.

PP-03-51

The durable effect of luliconazole in a guinea pig tinea pedis model

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Luliconazole is an optically active imidazole antifungal agent discovered by Nihon Nohyaku Co., Ltd. (Japan). It exerts a wide spectrum of potent antifungal activities against pathogenic fungi, especially dermatophytes. Luliconazole was developed for use as a topical antifungal drug and the 1% cream and solution are clinically available in Japan for the treatment of superficial mycoses such as dermatophytosis, candidiasis and pityriasis versicolor.

The present study was undertaken to compare the prophylactic efficacy of 1% luliconazole cream (LLCZ) with that of 1% terbinafine cream (TBF) against tinea pedis. These creams were applied topically to the planta of both hind paws of guinea pigs once 14 or 21 days before inoculation. A sterile adhesive bandage impregnated with the inoculum suspension of *Trichophyton mentagrophytes* TIMM2789 ($1.5x10^7$ conidia/site) was affixed to the pretreated planta using an occlusive dressing for 3 days, and histopathologic observation (PAS stain) was subsequently performed. The invasion of *T. mentagrophytes* into the stratum corneum of the infected skin was completely inhibited by the single prophylactic application of LLCZ at 14 days before inoculation compared to 50% inhibition by TBF. Similar results were obtained in the groups pretreated at 21 days before inoculation. These findings substantiate the excellent clinical efficacy of short-term luliconazole therapy against dermatophytosis.

PP-03-52

Evaluation of the rate and extent of anidulafungin and flucoanzole activity against *Candida albicans*

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Background: Invasive candidiasis is associated with significant morbidity and mortality. Clinically, the echinocandin anidulafungin has been reported to have greater activity against invasive infections caused by *C. albicans* compared to fluconazole. The exact mechanism for this difference is unknown. Our objective was to determine the in vitro and in vivo rate of activity of anidulafungin and fluconazole against *C. albicans* in vitro and in vivo.

Methods: In vitro time-kill studies were performed with anidulafungin (0.125 - 8 mcg/mL) and fluconazole (0.5 - 32 mcg/mL) against *C. albicans* ATCC 90028 in RPMI with 50% serum. In animal studies, ICR mice were inoculated with *C. albicans*, and anidulafungin (1 & 5 mg/kg), or fluconazole (5 & 10 mg/kg) begun 24 hours later. Treatment continued until day 7 and animals were followed off therapy until day 21 to assess survival. Kidney tissue and blood were collected on days 1, 4, 5, 6, and 7 post-inoculation. Fungal burden was assessed by colony-forming units and (1, 3)-β-D-glucan was measured in the serum using the Fungitell assay.

Results: Anidulafungin 2 and 8 mcg/mL demonstrated in vitro fungicidal activity (> 3 log reduction CFU/mL) at 24 hours. In contrast, fungistatic activity was observed for fluconazole at all concentrations tested. In vivo, percent survival was significantly greater with anidulafungin 1 and 5 mg/kg (90%) compared to control (10%, $p < 0.01$) and fluconazole 5 & 10 mg/kg (50% & 40%, $p < 0.05$). Significant reductions in tissue burden and (1, 3)-β-D-glucan were observed earlier for anidulafungin 5 mg/kg (day 6) compared to fluconazole (day 7).

Conclusions: Anidulafungin demonstrated more rapid activity against *C. albicans* compared to fluconazole. This was observed both in vitro and in vivo at the highest dose as measured by survival and reductions tissue burden and (1, 3)-β-D-glucan.

PP-03-53

A silkworm model of fungal infections for antifungal screening

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The efficacy of human antimicrobial drugs has been evaluated in several animal models. To date, vertebrate animal models such as mouse models have been frequently used to develop new drugs. However, the use of these infection models has recently raised both economic and ethical concerns. As a result, invertebrate infectious models using the roundworm, fruit fly and silkworm have been developed and are expected to be a primary tool for studying pathogenic microbial infections. The silkworm (*Bombyx mori*) has been used as a model to study pathogenic bacteria that also afflict humans. However, a fungal infection model, especially one that can be used to test antifungal drugs, has not been developed. Therefore, we established a silkworm model for screening antifungal drugs using the pathogenic fungus *Candida albicans*.

Injecting 1 x 10^6 *C. albicans* cells into 5th instar insects was optimal for the silkworm infection model, and most silkworms died within 48 hours after injection. To elucidate the effect of antifungal drugs on *C. albicans* in the silkworm model, the silkworms were inoculated with pathogenic fungal cells and then injected with amphotericin B or fluconazole. All of the silkworm larvae were cured with amphotericin B for 4 days. When fluconazole was injected, most of the larvae survived for 2 days, but thereafter most larvae died. Moreover, many *C. albicans* cells were observed in the silkworm blood 2 days after the cells and fluconazole had been injected, while no cells were observed in the blood of larvae injected with amphotericin B. These data revealed that amphotericin B has a potent fungicidal effect on *C. albicans* whereas fluconazole shows only a fungistatic effect on this fungus in the silkworm model. Thus, these data indicate that this silkworm model is a useful tool for screening new antifungal drugs.

PP-03-54

Antifungal activity of the WSP1267, an inhibitor of the squalene synthase, on *Candida* spp. isolates: Effects on growth, cell cycle and ultrastructure

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Sterol biosynthesis inhibitors targeting fungal sterol C14-alfa-demethylase (CYP51) are frequently used in the candidiasis treatment. However, the increase of resistance and pharmacological proprieties has become important issue for therapeutic failure. Therefore, other targets in the sterol biosynthesis pathway could be useful to inhibit fungal growth. In this work, we evaluated the antifungal activity of WSP1267, a squalene synthase (SQS) inhibitor, against *Candida* spp. The minimum inhibitory concentration (MIC) of WSP1267 was determined for 65 clinical isolates of *Candida* (21 *C. albicans*, 19 C. parapsilosis, 14 C. tropicalis, 3 C. guilliermondii, 2 *C. glabrata*, 1 C. krusei, 1 C. lusitaneae, 1 C. zeylanoides, 1 C. rugosa, 1 C. dubliniensis e 1 C. lipolytica) by broth microdilution method according to the CLSI (M27-A3, 2008). Fluconazole (a CYP51 inhibitor) was used as standard antifungal. Three of the isolates were resistant to fluconazole (2 C. tropicalis and 1 C. krusei). The MIC value for WSP1267 ranged from 0.5 to 8 microg/mL and 50% of isolates had MIC values below 2 microg/mL, including strains fluconazole-resistant. C. dubliniensis e C. lipolytica were the species less susceptible (MIC = 8 microg/mL). To evaluate morphological alterations, fluorescence microscopy by DAPI staining and transmission electron microscopy of the *C. albicans* treated with MIC value were performed. Treatment with WSP1267 leads to several ultrastructural alterations, including alteration in cell wall shape and thickness, a pronounced discontinuity of cytoplasm membrane and detachment from the cell wall, small vesicles budding from the cytoplasm membranes migrating through the periplasmatic region and the presence of electron-dense vacuoles. In addition, fluorescence microscopy analyses indicated alterations of the yeasts' cell cycle and abnormal chromatin condensation. Taken together, these results suggest that inhibition of SQS may be a novel approach to control *Candida* spp. infections, and could be considered as an alternative for the development new antifungals.

PP-03-55

Antifungal activity of saponin SC-2 from *Solanum chrysotrichum*, an integral study: Clinical vaginal candidiasis and in vitro ultraestructural changes on *C. albicans* and *C. glabrata*

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Herbal Mexican traditional medicine uses to *Solanum chrysotrichum* to treat fungi-associated dermal and mucosal illness since pre-Columbian age. Several studies support the antifungal activity of spirostanic saponins SC-2 from *S. chrysotrichum*. SC-2 showed high fungicidal effect against *Candida albicans* and non-albicans strains. The aim of this work is presenting an integral study of saponin SC-2 antifungal activity over clinical vaginal candidiasis and in vitro ultrastructural changes on *C. albicans* and *C. glabrata.*
The clinical study included 101 women with *Candida* vaginal infection; 49 experimental group (Sc-hmp, *S. chrysotrichum* herbal product) and 52 control group (ketoconazole). Treatments, vaginal suppositories: Sc-hmp (standardized in 1.89 mg of SC-2) and ketoconazole (400 mg) were administered by 7 nights. Clinical (elimination of signs and symptoms) and mycological effectiveness (negative mycological studies) between Sc-hmp against ketoconazole was assessment. At the end of the administration period, both treatments demonstrated 100% tolerability, and clinical cure in 57.14% of cases *S. chrysotrichum*-treated and in 72.5% of ketoconazole-treated (p =0.16), as well as 62.8% and 97.5% of mycological effectiveness, respectively (p=0.0001).
Antifungal activity of SC-2 was showed in clinical isolates of *C. albicans* and *C. glabrata*. Strains were grown on potato dextrose agar for 24 h at 35°C. Strains were growing in RPMI 1640 and SC-2 (800 µg/mL final concentration) was added. Fungi were harvested at 6, 12, 24, and 48 h; controls without SC-2 were included. They were processed and examined with transmission electron microscopy. Damage observed was: i) Cellular disorganization and severe damage in organelles; ii) Cell cytoplasm matrix degradation; iii) and fungus death. Conclusion: In vitro severe damage observed on both species of *Candida* caused by SC-2, can explain results observed on clinical study.

PP-03-56

In vitro activity of a novel propiconazole derivative (MXP 4509) against 110 clinical isolates of *Candida albicans*

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Background: MXP 4509 is a novel investigational antifungal drug derived from propiconazole. Previous studies have successfully demonstrated its high efficacy against *Candida* strains isolated from vulvo-vaginal infections using an experimental model of biomimetism.

Methods: A total of 110 clinical isolates of *Candida albicans* were tested using the EUCAST Def. 7.1 method. The antifungal drug MXP 4509 has been tested per se (using DMSO as solvent) and as nanoconjugate with beta-cyclodextrin (using water as solvent), at following concentrations (mg/L): 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, and 0.0625. To assure the quality of test results we used two control strains (*Candida* krusei ATCC 6258 and *Candida* parapsilosis ATCC 22019) each time when the susceptibility testing was performed. The minimum inhibitory concentration (MIC) for the tested drug was considered the lowest concentration giving rise to an inhibition of growth of more 50% comparing with that of the drug-free control.

Results: The MIC value was 0.0625 mg/L for 80 strains and smaller than 0.0625 mg/L for other 30 strains. The MIC50 and MIC90 were of less 0.0625 mg/L and 0.0625 mg/L respectively. Statistical comparison between the MICs obtained with the drug per se and with nanoparticles showed no significant differences ($p > 0.05$). The MICs for control strains were 0.5 mg/L and 0.25 mg/L respectively.

Conclusions: Both forms of the drug showed comparable potent in vitro activity against *Candida albicans* strains. This fact indicates a good disponibility of the drug in aqueous medium after the dissolution of nanoparticles. MXP 4509 is a promising antifungal agent and more studies are necessary to establish its activity spectrum and interpretative breakpoints.

Keywords: MXP 4509, propiconazole, nanoparticles, antifungal activity

PP-03-57

In vitro activity of a novel nystatin formulation against Aspergillus and fusarium species

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Nystatin is a non-soluble polyene, used topically, primarily for treatment of *Candida* superficial mycoses. The overall goal of our studies is to develop a formulation that could be delivered parenterally and hence suitable for treatment of invasive mycoses.

We developed a stable, standardized, Nystatin-Intralipid (NYT-IL) formulation which was characterized physically and chemically, that included determination of particle size, association of NYT with IL and stability at different temperatures. The antifungal activity of NYT-IL as assessed in vitro against the five major pathogenic *Candida* species was better than that of the free NYT and remained unchanged.

In the present study we focused on pathogenic molds involved in invasive mycoses. We assessed the in vitro activity of NYT-IL against Aspergillus and Fusarium species. We used a modification of the CLSI microbroth dilution method for susceptibility testing. Three strains of each species of the following molds were used: A. fumigatus, A. flavus, A. terreus, A. niger, F. solani and F. oxysporum. We determined the minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) of NYT-IL in comparison to free NYT.

The mean MIC and MFC values of NYT-IL for the Aspergillus species tested were lower than those of free NYT, with intra-species variability. A. flavus strains were the most susceptible, while A. terreus were the least. The susceptibility of Fusarium strains showed variability.

In conclusion, we report on the development of a novel formulation of Nystatin, that has in vitro activity against pathogenic molds as well as yeasts.

Poster Forum PF-08

PP-03-58

Antifungal activity of propolis from two valleys of the Basque Country (Spain)

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Propolis is a resinous substance collected by honeybees (*Apis mellifera*) from different trees and bushes. It shows antifungal, antibacterial, antiviral and antiparasitic properties, with variable activity depending on its geographical origin.
The aim of this work was to evaluate the antifungal activity of propolis ethanolic extracts from two different valleys -Urdaibai and Dima- in the Basque Country (Spain), on different species of *Candida* and some dermatophytes. Following the CLSI microdilution methods M-27-A and M-38-A, the Minimal Inhibitory Concentrations of propolis reducing control microbial growth by 50% (MIC-50) and 90% (MIC-90), and the Minimal Fungicidal Concentration (MFC) were determined.
We studied several reference strains of the genus *Candida* (*Candida albicans*, *Candida tropicalis*, *Candida glabrata* and *Candida parapsilosis*) and clinical isolates of dermatophytes (*Trichophyton rubrum*, *Trichophyton mentagrophytes* var *interdigitalis*, *Trichophyton mentagrophytes* var *granulosum*, *Paecilomyces* sp., and *Acremonium* sp.).
MIC-90 for *Candida* spp. ranged from 0.01-0.078 mg (d.w.)/ml, and MFC from 0.078-0.313 mg (d.w.)/ml; *C. glabrata* strains were the most susceptible, while CLSI reference *C. parapsilosis* ATCC 22019 was in the midst of the range (0.039 and 0.156, respectively).
The different strains of *Tricophyton* showed a rather homogeneous sensitivity to propolis with MIC-50 ranging from 0.001 to 0.002, MIC-90 of 0.01, and MFC between 0.02 and 0.04 mg (d.w.)/ml. On the contrary, strains of *Paecilomyces* and *Acremonium* showed similar results to those of the less sensitive species of *Candida* assayed.
Both propolis ethanolic extracts showed similar biological activity, being higher than that reported for other countries (Brazil[1], *C. albicans* (ATCC 28366) MIC 0.09 mg/ml; Mexico[2], *C. albicans* (ATCC 10231) MFC 0.6 mg/ml).
These propolis ethanolic extracts could be further investigated for its alternative use for the treatment of some *Candida* infections and dermatophytoses.

References:
1. Bruschi. Drug Development and Industrial Pharmacy, 2006; 32:229.
2. Quintero-Mora. Revista Iberoamericana de Micología, 2008; 25: 22.

Poster Forum **PF-07**

PP-03-59

Antifungal activity of alcoholic extracts of *Quercus semen*

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The increasing resistance of *Candida* towards antifungal compounds and the reduced number of available drugs has resulted in a search for new therapeutic alternatives. The objective of this study was to examine in vitro antifungal activity and susceptibility of *Candida* species to *Quercus semen* in 60% ethyl alcohol extracts, and to present the results of studies concerning antifungal efficacy. *Candida* species were isolated from different hospital patient samples. The Mueller Hinton agar was inoculated with *Candida* species isolates. After inoculation, 6 mm diameter wells were made in the agar. Plant extract was added directly into each well. 60% ethyl alcohol without plant extract was added to one well as a control. The plates were incubated at 37°C for 24 hour and the growth of *Candida* species was observed. The inhibition zone of antifungal susceptibility was measured in mm. The highest alcohol extract dilution added to the agar and showing no visible *Candida* species growth after incubation was regarded as the MIC. MFC is defined as the lowest concentration of alcohol extract which when added to an agar medium shows no *Candida* sp. growth after incubation. The 100% susceptibility results of *Candida* sp. are encouraging and indicate the potential use of *Quercus semen* in the control of selected phytopathogenic fungi. Other medical herbs have shown antifungal activity. The distinctive antifungal activities of commercial phytochemicals and their extracts provide some promising clues for anti-infective efficacy of the drug in clinical practice, which encourages us to elucidate the antifungal efficacy of phytochemical drugs.

PP-03-60

Antifungic activity and effects of extracts of medicinal Brazilian plants on *Candida albicans* isolated from oral cavity

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Yeasts of the genus *Candida* does part of the indigenous human microbiota. However, they can behave as opportunistic pathogens, depending of host and fungal factors. The study of virulence factors of the fungus becomes important to an understanding of its pathogenicity and development of antifungal drugs. Plants are known to produce a plethora of secondary metabolites which are recognized as a useful source of new drugs and leads. A study was conducted in order to evaluate the antifungal activity and effects of ethyl acetate fraction from leaves of Schinus terebinthifolius and Punica granatum, medicinal Brazilian plants, on *Candida albicans* isolates. The minimum inhibitory concentration for S. terebinthifolius was on average 33.40µg/ml, while for P. granatum was 59.77µg/ml. Both extracts had fungistatic activity upon *C. albicans*. The treatments with extracts of S. terebinthifolius and P. granatum increased yeasts hydrophobicity, respectively by 14.22% and 33.23%. However, this increase was not significant in relation to control. The extract of S. terebinthifolius inhibited the adherence of yeasts to buccal epithelial cells in 29.59%, and this inhibition was statistically significant in relation to the untreated control ($P < 0.05$). Despite the inhibition of 29.59% of adhesion, the treatment with extract of P. granatum was not more efficient than control in reducing the adhesion. Despite the different values obtained in two methods of biofilm quantification (crystal violet and XTT), in both it was possible to verify that plant extracts didn't inhibit significantly the pre-formed biofilms. The analysis of the morphology of the biofilms showed that the extract of S. terebinthifolius reduced pseudo-hyphae formation, while the extract of P. granatum didn't cause changes to it. Both extracts didn't cause DNA fragmentation.

PP-03-61

Ultrastructural changes on clinical isolated of *Trichophyton rubrum, T. mentagrophytes* and *Microsporum gypseum* caused by *Solanum chrysotrichum saponin* SC-2

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Solanum chrysotrichum is vegetal species utilized in herbal traditional Mexican medicine. Several clinical and mycological studies have provided evidence of its antifungal activity against dermatophyte and yeast infections. Methanolic extract from *S. chrysotrichum* have been achieved and identification of five spirostatic saponins; SC-2 has the higher antifungal activity. In this study was analyzed the ultrastructural changes caused by SC-2.on clinical isolates of three mainly dermatophytes species in Mexico.

The clinical isolates from *T. rubrum (Tinea ungium)*, *T. mentagrophytes* (interdigital *Tinea pedis*) and *M. gypseum* (microsporic *Tinea capitis*) pediatric patient, were identified in the Mycology Medical Laboratory, Escuela Nacional de Ciencias Biológicas, IPN, Mexico. Strains were grown on potato dextrose agar for 3 weeks at room temperature. Strains were growing in RPMI 1640 and SC-2 (1600 µg/mL final concentration) was added. Fungi were harvested at 6, 12, 24, and 48 h; controls without SC-2 were included. They were processed and examined with transmission electron microscopy (TEM). *T. mentagrophytes* was the most susceptible strain to saponin SC-2 effect (changes began at 6 h); *M. gypseum* showed moderate susceptibility; and *T. rubrum* was more resistant. The ultrastructural changes included: i) Severe damage on cytoplasmic membrane and organelles, loss of cellular organization; ii) Changes in cell wall morphology and density; iii) Total degradation of cellular components and cell death. Changes were showed since 6 h, reaching their maximum effect at 24 h.

In conclusion fungistatic and fungicide activity were observed by saponin SC-2 against all dermatophytes analyzed.

PP-03-62

Activity of novel bispyridinium compounds against a panel of pathogenic yeasts

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A novel bispyridinium compound with activity against the fungal virulence determinant phospholipase B and broad spectrum antifungal activity was used as a lead compound to examine structure-activity relationships. Ten analogues were synthesized and screened for cytotoxicity against mammalian erythrocytes and cell lines. Antifungal activity was measured in duplicate experiments by broth microdilution, against 26 clinical isolates (seven yeast and 12 filmentous fungal species). The MIC/MFCs of the lead compound, C1, were 1.5-12 mg/L for *Candida albicans, C. krusei, C. glabrata, C. tropicalis*, 0.7-47mg/L for *C. parapsilosis, C. neoformans* and *C. gattii* and 6-48mg/L for three *Aspergillus* spp. Amongst nine species of other filamentous fungi the lowest MICs (1.5-3mg/L) were found for *Scedosporium prolificans, S. apiospermum, Rhizopus oryzae* and two *Exophiala* spp. MICs and MFCs of the nine analogues varied with the compound and fungal species but were within one 2-fold dilution of each other. Based on the MIC/MFC data and cytotoxicity, 4 compounds (C1, C4, C5 and C8) were selected and tested against 10 isolates each of *C. albicans, C. dubliensis, C. glabrata, C. parapsilosis, C. tropicalis, C. krusei, C. guilliermondii, C. lusitaniae, C. neoformans* and *C. gattii*. MICs of amphotericin B, fluconazole, itraconazole, voriconazole, posaconazole and caspofungin were also determined. MIC_{90}s of all 4 compounds were <2mg/L for *C. albicans, C. dubliniensis, C. tropicalis, C. glabrata, C. guilliermondii* and <3.2mg/L for *C. lusitaneae* and *C. gattii*. MICs of C1, C2 against *C. neoformans* were 6mg/L and 3.2 mg/L for C3, C4. MICs of all compounds against *C. parapsilosis* were 48mg/L. No isolates were resistant to AMB. MIC_{90} of fluconazole against *C. glabrata* was 16mg/L. *C. neoformans* and *C. gattii* were equally sensitive to fluconazole. We conclude that these bispyridinium compounds have potentially useful activity against most pathogenic yeasts and that further investigation of their antifungal spectrum and toxicity is warranted.

PP-03-63

Efficacy of carbohydrate derived fulvic acid against *Aspergillus terreus* and *Candida albicans* in murine models of sepsis

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Objectives: Humic acid is a component of soil organic matter formed by degradation of plant & animal matter. The fraction of humic acid soluble in water at all pHs, is termed fulvic acid, acts as a colloid. Carbohydrate Derived Fulvic Acid (CHD-FA) is produced in industrial bioreactors from organic sugars and was well tolerated in phase I studies following oral administration and effective against fungi *in vitro*. We investigated the efficacy of CHD-FA against *Aspergillus terreus* and *Candida albicans* in murine sepsis models.

Methods: CHD-FA (Fulhold Ltd) was buffered to pH 5.0 before use. Male CD1 mice 22-25g were compromised with 1 dose of cyclophosphamide then infected IV (3 days later) with an LD90 (5-7 days post infection). For *Aspergillus terreus*mice were treated with 25 or 160 mg/kg CHD-FA BD oral, 0.5 or 2.5mg/kg IP amphotericin, CHD-FA +amphotericin, 40mg/kg posaconazole, 5mg/kg caspofungin or vehicle 5h later. Mice were euthanized 101hr post infection. For *Candida albicans*mice were treated with 25 or 100mg/kg CHD-FA BD oral, 10mg/kg fluconazole OD, CHD-FA +fluconazole, 0.5mg/kg amphotericin or vehicle 5h later. Mice were euthanized 53h post infection. Kidneys were quantitatively cultured to determine burden.

Results: CHD-FA at pH 5.0 was well tolerated. For *Aspergillus terreus*vehicle mice had high burdens (2x10^4cfu/gm). CHD-FA or amphotericin monotherapy had some effect on burden (~5x10^3cfu/gm). In contrast 25mg/kg CHD-FA + 2.5mg/kg amphotericin was more effective (3x10^3cfu/gm) and equivalent to caspofungin. For *Candida albicans*vehicle mice had high burdens (3.7x10^6cfu/gm). Monotherapy with CHD-FA had a modest effect ~1x10^6cfu/gm; fluconazole reduced this to ~4x10^4cfu/gm. The combination of CFH-FA +fluconazole was superior to monotherapy reducing the burden to ~1x10^4cfu/gm ($p<0.0001$ compared to vehicle) and equivalent to AMB ($p=0.11$).

Conclusions: CHD-FA was well tolerated. Combination treatment of *Aspergillus terreus* with CHD-FA +amphotericin and *Candida albicans* with CHD-FA +fluconazole were highly effective at reducing kidney burden.

PP-03-64

Antifungal activity of PHU-AgNO3 nanocomposites and nanostructured bioactive protein structures doped with silver nanoparticles

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Some biomaterials based on composite and nanocomposite materials, including silver nanoparticles (nanoAg), with antibacterial and antifungal characteristics, may have large application range both in making medical devices and leather products of daily use.

Objectives: To characterize the antifungal action for six aqueous solutions containing Polyhydroxyurethane (PHU) and AgNO3 in variable proportions and 10 leather samples doped with different concentrations of silver nanoparticles, synthesized in Romania.

Materials and methods: Silver nanoparticles were synthetized by methods based on reduction process of precursor's metal ions that includes chemical reduction. PHU was used as Ag stabilizer. The morphology of the compounds was investigated with Zetasizer Nano ZS and transmission electron microscopy. Functionalized biomaterials were obtained by hide tanning operations involving hide and fur collagen cross-linking by means of composite polymer matrices doped with metal nanoparticles in an aqueous medium. The antifungal activity against *Candida albicans* ATCC 90028, *Candida albicans* and *Candida* kefyr was examined with minimum inhibitory concentration (MIC), leather-addapted test method of specified requirements of antibacterial textiles for medical use (FTTS-FA-002).

Results: One of the 6 tested solutions showed antifungal activity against *Candida albicans* (MIC = 87.65 ppm nanoAg). This solution contained 0.3038 grams of PHU and 87.65 ppm nanoAg in 9.8114 grams of H2O. Two of the 10 leather samples, which were processed without Cr and with a nanoAg-concentration ranged between 480-650 ppm, presented antifungal activity against *Candida albicans* ATCC 90028 when tested by FTTS-FA-002. Other two samples containing nanoAg particles at a concentration between 56-67 ppm presented antifungal activity only against *Candida* kefyr.

Conclusions: Our results correlate the bacterial action of the tested solutions with naoparticles size, which are for all compound between 1- 10 nm. Regarding the leather samples, this study showed that the antifungal activity against each *Candida* strain is strongly related with the nanoAg particles concentration.

PP-03-65

Study the effects of monoterpenes and their related derivatives on the growth of pathogenic yeasts

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Pathogenic yeasts are associated with diseases ranging from simple dermatosis to systemic and life threatening infections. Recently, resistance to antifungal drugs, especially among *Candida* species, increased dramatically. Considering the limited diversity of antifungal agents, resistance of some *Candida*species to the antifungal compounds and several known side effects of such synthetic drugs, development of novel antifungal agents especially from natural sources is still needed. Since past centuries, essential oils have been used in preservation of food against microbial decayed. Monoterpenes are major constituents of the essential oils that are common in plants and contribute to their aroma. In the present study, eight monoterpens including carvacrole, carvone, alpha-pinene, beta-pinene, alha-terpinene, linalool, menthol and thymol were investigated for their antifungal activities against standard and clinical isolated of *Candida spp* and *Cryptococcus neoformance*. The antifungal testing was carried by using disc diffusion and broth microdilution methods.

Among examined monoterpens, carvacrole, carvone and alpha-pinene had highest inhibitory effects against standards species of *candida* and *cryptococcus* and exhibited strong inhibition of >37 mm in concentration of 10 mg per disc. Consistent with the disc diffusion test, carvacrol, carvone and alpha-pinene exhibited strong inhibition in borth microdilution method. As both carvacrol and thymol have phenolic residue in their molecules, carvone and linalool were selected as the best Candidates for further analysis based on their lower cytotoxicity and higher antifungal effects against clinicalisolates.

Determination of antifungal effects and MICs of monoterpens, may help ones to predict the inhibitory effects of essential oils against pathogenic yeasts just by determination of their integrants. On the other hand, as previous toxicological studies showed that carvone and linalool are safe at the examined concentrations, they might be considered as potential substances in prevention or even treatment of muco-cutaneous candidiasis.

PP-03-66

Quantitative image analysis of effects of antimycotic agents on the hyphal growth in *Trichophyton rubrum*

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Purpose: We performed image analysis with grids to evaluate the hyphal growth of *Trichophyton rubrum* under antimycotic agents.

Methods: Continuous video images of the hyphal growth were obtained for 48 hours from the start of the culture. Culture media contained 0.4 μg/ml of terbinafine (TBF) and itraconazole (ITCZ). No antimycotic agents were added to the media of the control group. Image analyses of morphological changes of hyphae were performed every 6 hours. A square lattice with a regular array of lines at a distance of 50 μm was superimposed on each image, and the number of cross points between hyphae was counted per 50 μm-square- grid. The mean density of cross points in each grid was used as a parameter of the hyphal growth. The number of cross points on dead hyphae was also counted in the TBF and ITCZ groups. The mean ratio of dead cross points to total cross points was used as a parameter of the antimycotic effects of TBF and ITCZ.

Results: Mean densities of total cross points in both the TBF and ITCZ groups were significantly lower than those in the control, and that in the TBF group was significantly lower than that in the ITCZ group. There was a significant difference between the TBF and ITCZ group in the mean ratio of dead hyphae. The ratio of dead hyphae in the TBF, but not the ITCZ group, significantly increased during the 48-hr time course.

Conclusion: Cross point counting provides a new parameter to assess hyphal growth and fungicidal activity quantitatively.

PP-03-67

Antifungal activity of Proxy Acetic Acid (PAA) compounds on a group of fungi (Dermatophyte, Saprophyte) with Invitro method

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Abstract: Nasocomial infections are too important problems in under treatment patients. They can endanger and increase duration of hospitalization. Fungal infections are dangerous in immunocompromised patient and transplant wards.

Objective: The aim of this study is evaluation of PAA compounds (persidin 1% and 513) on Microsporum gypseum, *Candida albicans*, Aspergillus niger with Invitro method.

Materials and methods: Tree times sub cultured standard strains (PTCC) on malt extract agar. Suspensions contain 4* 10 7 CFU/ml conidia and yeast cell were prepared and contact with 1%,3%,5%,10%,20% of persidin at 3,5,10,20,30 minutes and persidin 513 with 2% concentration at 3,5,15,30,45,60 minutes. After the end of time, number of conidia and yeast cells and colonies were counted.

Results: On the base of protocol 6986 of Institute of Standards and Industrial Research of IRAN (ISIR) 10000 reduce in vital conidia is suitable. The optimum effect on *C.albicans* and M.gypseum was started in 1% and 3% of persidin after 10 minute but, in 5% after 3 minute was seen.

On the other hand, optimum effect on A.niger was started in 3% after 20 minute and in 5%, 10% and 20% after 10 and 5 minutes respectively.

The suitable effect of persidin 513 with 0.2% concentration on M.gypseum after 15 minutes and *C.albicans* after 5 minute was seen. But optimum reductions of A.niger conidia after 30 minutes were started.

Conclusion: On the base of protocol No 6980 (ISIR) persidin as a high capability disinfectant against fungi can use.

Key words: Persidin, Proxy Acetic Acid (PAA), Saprophyte fungi, Dermatophyte

PP-03-68

Screening and identification of antifungal metabolites from soil organisms

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Objective: Identification of new antifungal agents are represented by screening of the vast biodiversity prevalent in natural resources such as soil samples.Methods: A total number of 140 soil samples from Tehran were studied for organisms with potential antifungal activity by agar plate technique.Selected organism with highly antifungal activity was cultured against pathogenic fungi such as *Candida albicans*, C. dubliniensis, *C. glabrata*, C.tropicalis, C. krusei, *Aspergillus fumigatus*, A. niger, Fusarium oxysporum and Trichophyton mentagrophytes. Results: The organism with potent growth inhibitory activity against fungi identified as Bacillus amyloliquefaciens by 16S rDNA sequence analysis.The culture filtrate of B. amyloliquefaciens was partially purified and examined against above fungi by broth mirodilution assay.The mean MIC 90 was determined as ≥ 8 µg/ml and ≥8-16 µg/ml for *Candida* species and mycelial fungi, respectively.Conclusion: On the basis of these results, partially purified culture filtrate of B. amyloliquefaciens strongly inhibited growth of such a different fungal species. It could be considered as an antifungal agent in the control of pathogenic and phytopathogenic fungi by more experiments.

Poster Forum PF-07

PP-03-69

Voriconazole serum dosage using a modified microbiologic method

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There is an increasing need for the measurement of voriconazole (VORI) in patient's serum to guide antifungal treatments. Accurate methods based on chromatography may not be quickly available for clinical management. The more accessible microbiologic assays may lack precision and be biased by the presence of other antifungal agents. We developed a microbiologic assay to accurately measure the VORI serum levels.

Since echinocandins are the most likely antifungal drug that may be used concomitantly with VORI, *Cryptococcus neoformans* ATCC 90112 was selected as the biologic indicator in this assay. This strain is simultaneously susceptible to VOR and highly resistant to all echinocandins.

We selected serums with known VORI amounts, as previously tested by a reference lab using a HPLC or a biologic method. We also tested serum containing caspofungin alone or in combination with various VORI concentrations. Furthermore, serum from patients treated with high doses of calcineurin inhibitor drugs (cyclosporine A, tacrolimus and sirolimus) were included because of their potential antifungal activity. Each serum was dispensed into 96-well microplates and incubated during 27h at 35°C after being inoculated with an adjusted *C. neoformans* suspension in RPMI 1640. The turbidity was measured with a spectrophotometer. Results were mathematically compared to the growth inhibition obtained from a known range of twofold VORI dilutions that served as a standard curve. Results from the modified microbiologic method correlated exactly with the reference biologic method (r^2: 0.98, two-tailed paired t-test) or HPLC (r^2: 0.89). The presence of caspofungin did not change VORI measurements. However, VORI values were significantly increased by the presence of calcineurin inhibitors.

The modified microbiologic method was easy to perform and could be used to accurately determine the level of VORI in serum samples, even with the presence of caspofungin. Calcineurin inhibitors may limit the method.

PP-03-70

Development of a high pressure liquid chromatography (HPLC) assay for quantitation of posaconazole in human serum

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Introduction: The blood concentrations of the azole antifungal agents may be affected by medications or conditions that induce or inhibit cytochrome P450, and can vary greatly from subject to subject. Furthermore, low concentrations may lead to treatment failure. Therapeutic Substance Monitoring (TSM) has been recommended when using itraconazole or voriconazole, and most experts believe that monitoring is also appropriate for posaconazole, based on pharmacokinetic studies.
Rationale: Posaconazole half-life is long (>24 hr), and levels vary little over a 12-hour dosing interval, supporting the feasibility of using random levels if the time of administration is not known. Even less information is available correlating posaconazole concentration with toxicity, but as with voriconazole and itraconazole, concentrations > 10 µg/mL would appear to be unnecessary.
Methodology: Samples containing posaconazole were extracted with 5 mL hexane-dichloromethane (70:30, v/v), evaporated to dryness, and reconstituted with mobile phase, ammonium phosphate buffer: acetonitrile: dichloromethane: triethylamine (1060:940:10:1, v/v/v/v) before injection over C18 column. UV absorbance at 255 nm was used for quantitation. The calibration range was 0 to 10 µg/mL and the assay was linear from 0.1 µg/mL to at least 8.6 µg/mL. Analytical limit of detection (LOD) and lower limit of quantitation (LLOQ) were 0.006 µg/mL and 0.0125 µg/mL respectively and clinical LLOQ was determined to be 0.045 µg/mL. Intra-assay and inter-assay precision was $\leq$ 6.4% and $\leq$10.2% respectively. Spike and recovery were within 10% of their expected values and a variety of disease states, anticoagulants and serum samples showed no interference.
Conclusions: Although therapeutic serum levels for Posaconazole have not been established, accurate and reliable quantitation of serum Posaconazole levels can be achieved using HPLC. HPLC assays instead of microbiological assays reduce the potential for technical error and eliminate the falsely high readings that can be created by co-administered antifungal medications.

PP-03-71

Intracellular concentrations of antifungals in different compartments of the peripheral blood

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Introduction: Peripheral blood mononuclear cells (PBMC) and polymorphonuclear leucocytes (PMN) are important components of host defense against invasive fungal infections (IFI). Information on penetration and concentration of antifungal drugs in these cells is limited. Prior laboratory-based experiments with fluconazole and voriconazole showed rapid intracellular uptake and elution for both agents. Experiments comparing the concentration of posaconazole in human plasma and alveolar cells yielded a 33fold increased intracellular concentration. To assess whether the prophylactic efficacy of antifungals is determined by their intracellular rather than plasma concentrations, we are establishing a liquid chromatography tandem mass spectroscopy (LC-MS/MS) method to determine intracellular concentrations of various antifungals, i.e. anidulafungin, caspofungin, micafungin, posaconazole, and voriconazole.
Methods: Patient samples are being collected as part of the Cologne biobank protocol on Improving Diagnosis of Severe Infections in Immunocompromised Patients (ISI). Whole blood, collected in EDTA treated tubes, was separated by double-discontinuous Ficoll-Hypaque density gradient centrifugation into PBMC, PMN and red blood cells (RBC). The washed cells were counted and extracted with methanol by sonication. Concentrations were determined by LC-MS/MS. The validation of this method is currently in progress.
Results: So far, the limit of quantification is sufficient for the expected concentrations. Accuracies of all concentrations above the limit of quantification were within +-15%. Correlation coefficients of these curves were 0.979 or better for the echinocandins and 0.99 or better for the azoles. Recently analyzed peripheral blood of a patient receiving posaconazole indicated the presence of posaconazole in PBMC. Conclusion: To our knowledge, we are establishing the first method to determine azole and echinocandin antifungals within one sample. The accuracy of the method is expected to be sufficient for determination of intracellular concentrations of azole antifungals. Whether the method is apt to determine intracellular concentrations of echinocandins depends on the extent of intracellular uptake of these antifungals.

PP-03-72

Comparative evaluation of ATB FUNGUS 3 procedure and CLSI M27-A2 broth microdilution method for antifungal susceptibility testing of pathogenic yeasts

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Objective To investigate whether ATB FUNGUS 3 method has the agreement in determining the susceptibility of pathogenic yeasts to common antifungal drugs with CLSI microdilution method M27-A2. Methods The in vitro susceptibilities of 172 strains of *C. albicans* and Cryptococcus neoformans to the common antifungal agents, includingamphtericin B(AMB),flucytosine(5-FC),fluconazole(FLC),voriconazole(VRC)and itraconazole(ITC),were assayed by using both ATB FUNGUS 3 method and CLSI M27-A2 method. Results Ninety-three percent agreements between the two methods for ITC, 95.9% for AMB, 98.8% for 5-FC,92.4% for FLC,and 93.6% for VRC. Conclusion ATB FUNGUS 3 method,a rapid and simple procedure, has good agreement with CLSI M27-A2 method in determining the antifungal susceptibility of pathogenic yeast to common antifungal drugs.

PP-03-73

Microwave irradiation for disinfecting shoe insoles?

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Objective:
As Tinea pedis is almost always transmitted indirectly from a patient to a non-infected person, disinfection of ″fungal reservoirs″is important. The objective of this study was to determine the influence of microwave radiation on different dermatophytes inoculated on shoe insoles.

Material and Methods:
Thirty-three-day-old cultures of T. rubrum (CBS 301.60), T. rubrum olexa and T. interdigitale were scraped off gently with a sterile scalpel and mixed in 300 ml cycloheximide-containing broth. As radiation source, a 2450 MHz microwave oven (SHARP, type R-24W, 800 Watt) was used.
Shoe insoles (polyethylene sponge and cork) were inoculated with 2x5 µl 5-day-old T. rubrum and irradiated for 20 and 30 s (240 Watt), respectively. After that, 3mm punch biopsies were taken and implanted in selective agar for pathogenic fungi (SPF, Merck, BRG). The same experiment with varying duration and radiation energy was repeated with T. rubrum olexa and T. interdigitale. As control, the same procedure without irradiation was done. During irradiation, temperature was measured with a strip thermometer. Each assay contained 10 inocula on 5 SPF plates. Daily growth was inspected for three weeks, each experiment was repeated three times.

Results:
Irradiation at 240 Watt completely eliminated T. rubrum after 20 s (polyethylene sponge) and 30 s (cork), while 40 s were required to eliminate T. rubrum olexa (both types of shoe insoles). In the case of T. interdigitale 50 s of irradiation (400 Watt) were needed for cork and 30 s (560 Watt) for polyethylene sponge. Maximal temperatures reached 35°C (T. rubrum), 50°C (T. rubrum olexa) and 60° C (T. interdigitale).

Conclusion:
Microwave irradiation is a simple alternative to disinfect shoe insoles from dermatophytes at relatively low temperatures, which may be advantageous in the case of heat-sensitive material.

PP-03-74

The life cycle of *Nadsonia*: A novel antifungal screen

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The yeast *Nadsonia fulvescens* is characterized by a unique life cycle which is automictic and homothallic. After heterogamic conjugation, between the parent cell and a bud, the contents of the zygote move into another bud formed at the opposite end of the parent cell. This second bud is then delimited by a septum and becomes the ascus. Usually one, rarely two spherical brownish spiny to warty ascospores are formed with a prominent lipid globule. Using mitochondrial trans-membrane potential (delta ψm) and β-oxidation probes as well as mitochondrial enzyme activity markers, we found increased mitochondrial activity in the mother cell during ascospore formation. This activity decreases in the presence of mitochondrial inhibitors. Similar results were found when mitochondrial products, i.e. 3-hydroxy oxylipins, were analysed using gas chromatography mass spectrometry. Strikingly, when mitochondrial inhibitors are added to this yeast, the sexual cycle is inhibited causing malformed colourless ascospores sometimes without spiny protuberances to be formed. This can easily be visualized when this yeast is used together with the agar plate diffusion method where sensitivity towards mitochondrial inhibitors is tested over a concentration gradient. At high concentrations, a white lawn of cells is formed while the lawn turns brown (amber) at lower mitochondrial inhibitor concentrations. This phenomenon may have value in the screening of mitochondrial inhibition drugs that may have various actions i.e. antifungal, anticancer and anti-inflammatory. Here, various known anti-mitochondrials with anti-inflammatory and antifungal activity were screened to define the bio-assay.

PP-03-75

Asci: Indicators of novel antifungals

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Various anti-inflammatory and anticancer drugs were tested using a bio-assay, with the mitochondrion-dependent sexual structure (ascus) of the yeast *Eremothecium ashbyi* as indicator. When the anti-inflammatory drugs (some also anticancer) aspirin, ibuprofen, indomethacin, salicylic acid and benzoic acid were tested, similar results were obtained as previously hypothesized i.e. development of the ascus with increased mitochondrion activity was more sensitive to these drugs than the less active asexual growth stage. Here ascospore release nano-mechanics, which are dependent on oxylipin production by β-oxidation, are the most sensitive. Consequently higher concentrations of these drugs are necessary to inhibit asexual growth. The same was found for the anticancer drug Lonidamine, antimalarial drug Artemisinin as well as traditional medicine such as pepperbark. The results suggest that certain antimicrobial, anti-inflammatory and anticancer drugs have anti-mitochondrion activity targeting the yeast sexual phase at low concentrations. This bio-assay may find application in the screening for novel drugs with multiple actions from various sources.

PP-03-76

Anti-inflammatory drugs selectively target sporangium development in *Mucor*

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It is known that aspirin, an anti-inflammatory and anti-mitochondrial drug, targets asci of yeast with elevated levels of mitochondrial activity i.e. β-oxidation and high trans-membrane potential. Similarly, using antibody probes prepared against chemically synthesised β-oxidation products, we found that sporangia of *Mucor* are also characterised by increased mitochondrial activity yielding high levels of 3-hydroxy oxylipins. Using confocal laser scanning microscopy and a mitochondrial function (delta ψm) probe, we found increased levels of mitochondrial activity in sporangia of *Mucor circinelloides* var. *circinelloides*. This is supported by enzymatic studies showing an increased mitochondrial metabolic status in sporangia. It is reported that aspirin also targets sporangium development while hyphae, with lower levels of mitochondrial activity, are more resistant. Similar results were obtained for the anti-inflammatory compounds benzoic acid, ibuprofen, indomethacin and salicylic acid. These anti-inflammatory drugs exert similar effects as found under limited oxygen conditions. These results prompt further research to assess the applicability of these anti-mitochondrial antifungals to protect plants and animals against *Mucor* infections.

PP-03-77

Impact of phylogenetic relationship on the outcome of yeast in vitro susceptibility testing

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Background:
Clinical important ascomycetous yeasts isolates (CAYI) are grouped traditionally according to their species identification in anamorphic form genera (ANA-G) which are polyphyletic (*Candida*, Geotrichum).
The aim of this study was to evaluate whether grouping of CAYI in corresponding teleomorphic genera (TELE-G) or in phylogenetic groups (PHYL-G) results in a more coherent susceptibility pattern when comparing with placement in ANA-G.

Methods:
In a recently performed German antifungal multicenter study the MIC of voriconazole (VOR), itraconazole (ITR), and fluconazole (FLC) for 7,976 CAYI had been determined in parallel (DIN 58940).

Epidemiological cut-off values and susceptibility pattern analysis were used for assessment of yeast susceptibility data when grouping anamorphic CYI in (i) corresponding TELE-G (when existing) or in (ii) PHYL-G. The latter were arranged according to a recently published study on inferring phylogenetic clades based upon multi-gene analyses.

Results:
The CAYI studied compromised 33 different species belonging to 2 ANA-G (81%) which alternatively could be classified into (i) 14 TELE-G (only for 19% of the isolates achievable), respectively (iii) 7 major PHYL-G.

CAYI of TELE-G showed significantly higher resistance rates than those which could not be regrouped in TELE-G: 43.0%, 26.1%, 6.0% versus 4.4%, 8.0%, 2.5% for FLC, ITR, and VOR, respectively. The susceptibility patterns of PHYL-G differed significantly.

CAYI of PHYL-G B and D (e.g. C. guilliermondii, C. intermedia, C. lusitaniae; C. inconspicua, C. krusei) showed consistently higher MICs for all triazoles tested as compared to those of clade A (*C. albicans* complex, C. parapsilosis complex, C. tropicalis), which encompasses the most frequently encountered clinical isolates, and frequently exhibits low MICs.

Conclusions:
Grouping of the ana-/telemorphic ascomycetous yeasts into phylogenetic clades thus reflecting their natural relationship, results reliably in a more coherent susceptibility pattern when compared to grouping into anamorphic or teleomorphic genera, which may be also sometimes polyphyletic (e.g. Pichia).

PP-03-78

Garlic extract effects on production of Aflatoxin and on aflR gene expression in *Aspergillus flavus*

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Aflatoxins are a group of hepato-carcinogenic teratogenic secondary metabolites are produced by *A.Flavi* section. These toxins contaminate a variety of foods and agricultural products under appropriate temperature and moisture conditions. Garlic *(Allium sativum)* has subsequently been reported to inhibit fungal growth and toxin production. To assess the inhibition effects of garlic extract on aflatoxin production in *A.flavus*, the spore suspension of this fungus was placed in petri dishes containing sterile potato dextrose broth (PDB) as well as different concentrations of garlic extracts and were incubated for 7 days at 30°C. RNA extraction from *A.flauvs* were performed by the standard methods and reverse transcriptase PCR (RT-PCR) were performed for all growth-conditions by using oligonucleotide primers designed and synthesized based on aflR gene. The results indicated decreasion of fungal growth after 4 days but this fungal growth was similar after 7 days in all concentrations including: 14 mg/ml,42 mg/ml and 56 mg/ml as well as normal condition (without garlic extract). In contrast, quantitative RT-PCR analysis revealed a down regulation in aflR gene expression in fungal growth with garlic extracts compare fungal growth in normal condition. HPLC analysis for detection the quantity of the produced toxin in culture media with normal condition and media containing 56 mg/ml garlic extract, revealed a considerable decrease of aflatoxin in treatment substance compare to the normal stock. The data showed the garlic extract can be used as a potential Candidate to prevent the production of toxin in *A.flavus* and may probably be used in prevention of aflatoxicosis in human and animals.

PP-04-1

Classification and distinction for pathogenic species of *Aspergillus* section *Fumigati*

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Aspergillosis is a clinically important mycosis, and encompasses a wide variety of bronchopulmonary infections. The most causative agents are *Aspergillus fumigatus* and relatives. Recently molecular phylogenetic analyses based on DNA sequences have promoted a great change in the taxonomy of the species of *Aspergillus* section *Fumigati*. Some cryptic species have been proposed as new phylogenetic species, *A. lentulus*, *A. fumigatiaffinis* and *A. novofumigatus*, based on a polyphasic approach that combines morphological, physiologic and molecular data. *A. lentulus* was isolated from clinical specimens in the USA, and there are many strains have since been isolated from clinical specimens and soil in various locations. The isolates of *A. lentulus* and *A. udagawae*, have ever been identified as *A. fumigatus* based on morphology, have low in vitro susceptibilities to multiple antifungal drugs, including amphotericin B, voriconazole and caspofungin.

We re-evaluated the identification of the strains identified as *A. fumigatus* based on morphology preserved at the MMRC, Chiba University, as causative agents of mycosis in human and animals. Most of the examined strains from clinical specimens in Japan were clustered together in the clade including *A. fumigatus. The other strains were identified as A. lentulus, A. viridinutans* and *A. udagawae*. We have found no strain included the clade, including *A. fumigatiaffinis* and *A. novofumigatus*. The maximal growth temperatures of are *A. fumigatus*, *A. lentulus* and *A. udagawae* above 50C, 45C and 42C, respectively. These data are useful for classification of those species. Clinical isolates of *A. fumigatus* are not necessarily morphologically uniform, and mistaken identifications of them by morphological characteristics have often happened. In order to develop rapid identification of *A. fumigatus*, *A. lentulus* and *A. udagawae*, respectively, using PCR and LAMP methods, we newly designed the primer sets.

PP-04-2

Classification of the pathogenic *Aspergillus* section *Fumigati* and *Neosartorya* based on phyogenetic analysis, and value based on the morophology

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Aspergillus section *Fumigati* (*A. fumigatus* group) with its teleomorph *Neosartorya* is an ubiquitous fungus in the environment and an important human pathogen. *A. fumigatiaffinis*, *A. fumigatus* (*N. fumigata*), *A. fumisynnematus*, *A. lentulus*, *A. viridinutans*, *N. fischeri*, *N. glabra*, *N. hirasukae*, *N. pseudofischeri* and *N. udagawae* (*A. udagawae*) have been reported to be the causative agents of mycoses. Those species have been also isolated from soil in Japan, China and other places. Recently Samson et al. (2007) revised species of this section as 10 *Aspergillus* species and 23 *Neosartorya* species based on phenotypic (morphology and extrolite profiles) and molecular (β-tubulin and calmodulin gene sequences). This time, the phylogenetic relationship of all species of *Aspergillus* section *Fumigati* and *Neosartorya* was analyzed based on sequences of β-tubulin, hydrophobin and calmodulin genes. As a result, topologies of three trees were almost similar such as those of Samson et al. *A. fumigatiaffinis*, *A. fumigatus*, *A. fumisynnematus*, *A. lentulus*, *A. novofumigatus*, *N. coreana*, *N. fischeri* and *N. laciniosa* were closely related on phylogeny and the topologies were supported by high bootstrap values. Five species of *Neosartorya* distinguished from known species by morphology and phylogeny were found. *A. neoellipticus* were included to *A. fumigatus* by sequence data, but its ellipsoidal conidia were completely different from those of *A. fumigatus*. *N. laciniosa* was identical to *N. paulistensis* by phyogenetic and morphological date. Likewise, *N. ferenczii* was identical to *N. sublevispora*. Meanwhile, *A. lentulus* was similar to *A. fumisynnematus* and *A. novofumigatus* also to *A. fumigatiaffinis* in spite of differences of sequences on several genes.

Poster Forum PF-05

PP-04-3

Development of rapid and specific molecular discrimination method in the pathogenic *Emericella* species

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The number of mycoses is increasing with progress of advanced medical care. Among them, aspergillosis is very important and the most causative agents are *Aspergillus fumigatus* and relatives. They have already been investigated well into the relationship among the molecular phylogenetics, morphological knowledge and physiological characters. However, other species of *Aspergillus* and related genera that are etiological agents of aspergillosis have never been investigated enough.

The genus *Emericella* is a teleomorph related to the *Aspergillus* section *Nidulantes*. Six species of this genus are reported to be etiological agents of chronic granulomatous disease (CGD), osteomyelitis, onychomycosis etc. In 2004, Dotis and Roilides compared osteomyelitis in CGD patients due to *A. nidulans* (*E. nidulans*) with those due to *A. fumigatus*. Half of the CGD patients with *A. nidulans* osteomyelitis died compared with none of those with *A. fumigatus* osteomyelitis. In addition, Verwejii et al. insisted on the importance of correct species identification by sequence-based analysis because they reported that *E. nidulans* and *E. quadrilineata* from clinical specimens exhibited different sensitivities against antifungal drugs.

In this study, we carried out phylogenetic analysis and attempted to clarify the relationship among the molecular phylogenetics, morphological knowledge and growth temperature regimens in the genus *Emericella*. We also tried to develop the rapid and specific molecular distinction method in the pathogenic *Emericella* species; especially, discrimination between *E. nidulans* and *E. quadrilineata*.

PP-04-4

Genetic diversity and species delimitation in the opportunistic genus *Fonsecaea*

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Genetic diversity and species delimitation were investigated among 39 isolates recovered from clinical and environmental sources in Central and South America, Africa, East Asia and Europe. All had been morphologically identified as *Fonsecaea* spp. Molecular analyses were based on sequences of the ribosomal
internal transcribed spacers (ITS), b-tubulin (TUB1) and actin (ACT1) regions. A phylogenetic approach using haplotype networks was used to evaluate species delimitation and genetic diversity. The presence and the modes of reproductive isolation were tested by measuring the index of differentiation (ID) and the index
of association (IA). Based on the sequence data, 39 *Fonsecaea* strains were classified into three major entities: (i) a group representing *Fonsecaea pedrosoi*, (ii) a second composed of *F. monophora*, and (iii) a third group including mostly strains from
South America. The two major, clinically relevant *Fonsecaea* species, *F. monophora* and *F. pedrosoi*, also differed in the pathological symptoms found in patients. Moreover, *F. pedrosoi* is mostly recovered in clinical settings, whereas *F. monophora*
is commonly isolated from the environment. One environmental strain with *Fonsecaea*-like appearance was shown to belong to a different species, only distantly related to the core-group of *Fonsecaea.*

PP-04-5

A case of chromoblastomycosis caused by *Fonsecaea pedrosoi* arising in a vietnamese patient living in Japan

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A 66-years-old Vietnamese man who has been living in Japan for 20 years, was referred to our outpatient clinic because of refractory ulcer on the right femur. One and a half years before the first consultation, he had his right thigh injured with the cutting wood which he picked up in Japan. He has been leaving the wound unhealed. At the first examination, we noted a well-defined erythematous and scaly plaque involving scar and ulcer with a diameter of 4cm on the right thigh.

A skin biopsy was obtained, which showed intense infiltrate of many epithelioid histiocytes with multinucleated giant cells containing sclerotic cells. Excision with 5 mm unaffected margins and skin graft were performed. Cultured tissue showed black-gray velvety colony, and its smears showed growth of *Fonsecaea pedrosoi*-like. According to the phylogenetic study based on sequence analysis of the internal transcribed spacer (ITS) region of ribosomal DNA (ITS1, 5.8S and ITS2), this isolate was identified as *F. pedrosoi* sensu stricto and is the first record from a clinical specimen in Japan.

No recurrent lesion has been noted during 4 months follow up after operation without any medication.

PP-04-6

Preliminary identification and typing of pathogenic and toxigenic fusarium species based on restriction digestion of ITS1-5.8S rDNA-ITS2 region

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Fusarium species are capable of causing a wide range of crop plants infections as well as uncommon human infections. Many species of the genus produce mycotoxins which are responsible for acute or chronic diseases in animals and humans. Identification to the species level is necessary for biological, epidemiological, and toxicological purposes. In the present study we undertook a computer based analysis of ITS1-5.8SrDNA-ITS2 of 192 Genbank sequences from 36 *Fusarium* species and 640 restriction enzymes, and subsequently designed and validated a PCR-restriction enzyme system for identification and typing of *Fusarium* isolates. Sequence data were analyzed for choosing Restriction Fragment Length Polymorphism (RFLP) profiles. DNA extracted from 32 reference strains of 16 species were amplified using ITS1 and ITS4 universal primers followed by sequencing and restriction enzyme digestion of PCR products. The following 3 restriction enzymes i.e. TasI, ItaI and CfoI provided the best discriminatory power. Using ITS1 and ITS4 primers a product of approximately 550 base pair (bp) was observed for all *Fusarium* strains, as expected regarding the sequence analyses. Some *Fusarium* strains had different RFLP patterns in same species; therefore our PCR-RE profile has potential not only for identification of species, but also for genotyping of strains. However, some *Fusarium* species were 100% identical in their ITS-5.8SrDNA-ITS2 sequences, therefore differentiation of these species is impossible regarding this target alone. The PCR-RFLP method reported in the study was useful for preliminary differentiation and typing of most common *Fusarium* species including *F. proliferatum*, *F. verticillioides*, *F. acuminata*, *F. thapsinum*, *F. compactum*, *F. avenaceum*, *F. poae*, *F. nygamai*, *F. pseudograminearum*, *F. subglutinans*, *F. sporotrichioides*, *F. graminearum*, *F. sachcari*, *F. oxysporom*, *F. napiforme* and *F. polyphialidicum*.

PP-04-7

Arthroderma vanbreuseghemii is a synonym of *A. simii*

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Trichophyton mentagrophytes include anamorphs of three sexual species, *Arthroderma simii*, *A. benhamiae* and *A. vanbreuseghemii*, differentiated based on their sexual abilities and more recently on their genotypes. However, based on genotypes, the conspecificity of *A. vanbreuseghemii* and *A. simii* remains presumptive. They were closely related, and the clade including *A. vanbreuseghemii* and *T. interdigitale* was placed on one of *A. simii* branches on a phylogenetic tree based on topoisomerase 2 genes[1)], therefore we tried to mate isolates of these two taxa. A clinical isolate of *A. simii* was successfully mated with *A. vanbreuseghemii* tester strain, RV27961, and produced many hybrid F1 progenies[2)] confirmed to be fertile by mating with parental strains or other species strains and production of hybrid F2 progenies.
In the case of *A. benhamiae*, genetic differences between two races are 28/596-28/601 in the ITS region of rRNA gene and 16/369 in topoisomerase 2 gene, in contrast, differences between *A. simii* and *A. vanbreuseghemii* are 27/592-27/597 and 8/369, respectively.
As mating tests cannot prove incompatibility but only prove compatibility *A. vanbreuseghemii* and *A. simii* should not be concluded as incompatible since they have been mated successfully.
We propose that *A. vanbreuseghemii* is a synonym of *A. simii* because of their genetically close relationship and their ability to produce fertile progenies.

1) Kawasaki M. et al: Different genes can result in different phylogenetic relationships in *Trichophyton* species. Jpn J Med Mycol 49: 311-318, 2008.
2) Kawasaki M. et al: Successful mating of a human isolate of *Arthroderma simii* with a tester strain of *A. vanbreuseghemii*. Jpn J Med Mycol 50: in press.

PP-04-8

Mating among three teleomorphs of *Trichophyton mentagrophytes*

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Trichophyton mentagrophytes include anamorphs of the three sexual species, *Arthroderma simii*, *A. benhamiae* and *A. vanbreuseghemii*, differentiated by their sexual behavior and genotypes. Nevertheless, based on sexual abilities[1)] and phylogenetic relationships[2)], conspecificity of *A. vanbreuseghemii* and *A. simii* was strongly suggested.
An F1 progeny (Asv11) produced between *A. simii* (KMU4810) and *A. vanbreuseghemii* (RV27961) mated with *A. benhamiae* (RV30001) and produced F2 progenies, one of which was a hybrid of three species. Furthermore *A. simii* (KMU4810) and *A. benhamiae* (RV26680) produced an F1 progeny (Asb57), which mated with *A. vanbreuseghemii* (RV27961). These matings were confirmed using three unlinked genes, rRNA, actin and topoisomerase2. Asb57 showed both rRNA and actin genes of *A. simii* as well as topoisomerase2 gene of *A. benhamiae*. Four of 12 F2 progenies produced between Asb57 and RV27961 showed genes from all three species.
Although *A. simii* and *A. benhamiae* seem to be distinct lineages in a phylogeny, *A. simii* might be conspecific not only with *A. vanbreuseghemii* but with *A. benhamiae*. As *A. benhamiae* still retains the ability to produce hybrids with *A. simii*, it implies that though *A. benhamiae* may have started to separate from the *A. simii* lineage it is still undergoing evolution and has not yet attained the full status of separate species.

1) Kawasaki M. et al: Successful mating of a human isolate of *Arthroderma simii* with a tester strain of *A. vanbreuseghemii*. Nippon Ishinkin Gakkai Zasshi 50: 15-18, 2009.
2) Kawasaki M. et al: Different genes can result in different phylogenetic relationships in *Trichophyton* species. Nippon Ishinkin Gakkai Zasshi 49: 311-318, 2008.

PP-04-9

Dermafinder: A new approach for fast and sensitive detection of dermatophyte skin infections

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Objective:
Superficial dermatophytosis is the most common fungal infection in humans. Dermatophytes are keratinophilic fungi which are able to infect keratinized tissue. Diagnosis of dermatophytosis is based on microscopic observation of fungal structures in KOH threated skin scales plus culturing and identification of the causative species. However, direct microscopy lacks specificity and culturing is slow because it requires generally 2-4 weeks. To address this we have developed a molecular test, the DermaFinder. The DermaFinder assay is able to detect the major 8 pathogenic dermatophytes in a single reaction.

Method:
The DermaFinder is based on the MultiFinder technology which enables simultaneous amplification of up to 40 fragments. Primers and probes were designed based upon unique AFLP markers. The assay includes two probes targeting 2 dermatophytes species; *Trichophyton rubrum* combined with T.soudanense and Microsporum canis combined with Microsporum audouinii. The DermaFinder can detect four single dermatophytes: T. mentagrophytes, T. violaceum, T. tonsurans and Epidermophyton floccosum. In addition, the assay includes also one probe which detects all members of the genus Trichophyton.

Results:
A set of skin samples (232) from sporters with athlete's foot were used to validate the DermaFinder assay. Results where compared with microscopy, KOH/blankophor and culture and showed a good correlation. A specific dermatophyte real-time assay was used to test the discrepancies. In most cases the real-time PCR confirmed the DermaFinder results. In total 38% (87) of the athlete's were suffering from a dermatophytosis. Moreover, the DermaFinder assay was able to detect an additional 25% (36) of pathogenic dermatophytes in culture negative samples.

Conclusion:
The DermaFinder is able to detect the major 8 pathogenic dermatophytes in clinical specimens and proved to be more sensitive and specific than culture and direct microscopy. Earlier therapy and information on the source of infection is possible with this test.

PP-04-10

Caves as potential habitats for pathogenic fungi in Nigeria

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Following the increasing incidence of opportunistic fungal infection globally, there is need to begin to identify the original habitats of some of these fungi in our enviroment.This becomes better appreciated, considering the level of human activities going on in most of these caves. For instance, some are used as tourist sites / attraction by all sorts of persons from within the country and abroad. In most developing countries such as ours where there is a growing number of immunocompromised patients due to HIV/AIDS and cancer, there is need to monitor and continue to follow the trend of opportunistic pathogens since the control of these pathogens is one of the best ways of managing immunocompromised patients. Following a previous preliminary investigation, the spectrum of pathogenic fungi in two popular caves located in Southern Nigeria was investigated. They are the Ogbunike cave located in Anambra state and the Nkpuruma cave located in Ebonyi state. Isolation was carried out using standard procedures and subcultures were made as appropriate to enable correct identification of the species. Several pathogenic fungi were isolated. Some of them include *Exophiala dermatitidis, Fusarium solani, Scedosporium apoispermum,Phialophora Spp,Histoplasma Spp,* etc.We observed the existence of several wild birds and bats in these caves. Plans are on going to characterize these isolated fungal strains by molecular methods and compare them with clinical strains in other collections for further epidemiological studies.

PP-04-11

A new species of genus *Ochroconis* closely related to O. gallopava isolated from a hot spring effluent

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The present study describes 2 fungal strains related to *Ochroconis gallopava* (IFM 54738 and 54739) isolated from a hot spring effluent along with one of the first environmental *O. gallopava* strains isolated in Japan.

The colonies of the 2 isolates were light olive-green in color. Further, colonies of IFM 54738 had a corrugated surface and those of IFM 54739 were crateriform. The colonies of the 2 isolates turned reddish dark brown after several passages on PDA at room temperature and became indistinguishable from *O. gallopava* strains. The conidia grown on oatmeal agar were slightly elongated, while those grown on PDA or cornmeal agar were identical to those of *O. gallopava*. The isolates showed thermophilic characteristics as well as *O. gallopava*. Their morphological and physiological data were within the variation of *O. gallopava* strains.

The concatenated sequences of partial small subunit to D1/D2 region of large subunit of the 2 isolates consisted of 1,696 base pairs and were 100% identical. Their homologies with *O. gallopava* at the ITS and D1/D2 regions were 79.2% and 95.9%, respectively.

The susceptibility of the 2 isolates to antifungal drugs was equivalent to that of *O. gallopava*, except in the case of micafungin, towards which the isolates showed lower susceptibility.

When mice were infected with the isolates, there was no dead mouse within 28 days in contrast to *O. gallopava*. Marked lesions caused by the 2 isolates were observed in the kidneys, whereas those caused by *O. gallopava* were observed in the brain.

Based on these results, the present isolates would be proposed as a new species of the genus *Ochroconis*.

PP-04-12

Molecular characterisation of the *Madurella grisea* complex reveals at least three new taxa associated with human mycetomas

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Dark-grain mycetomas are destructive infections of the skin and subcutaneous tissues, that progress to involve muscle and bone. Numerous different dematiaceous fungi are capable of provoking mycetoma following traumatic implantation. While some of these organisms are well characterised, many remain difficult to identify, at least in part due to delayed or absent sporulation in vitro. Current practise is to group these recalcitrant organisms under the generic umbrella of *Madurella grisea*, which thus potentially encompasses any species of dematiaceous fungus isolated from mycetomal lesions that fails to sporulate *in vitro*. Here, using isolates cultured from confirmed cases of dark grain mycetoma, and stored in the National Collection of Pathogenic Fungi and in the Institut Pasteur culture collection, we have attempted the molecular characterisation of members of the *M. grisea* complex. Over 50 isolates, collected worldwide from cases of dark grain mycetoma were subjected to sequencing of the ITS rDNA regions. LSU rDNA regions were also compared for a selection of these organisms.

In agreement with previous reports, *Madurella mycetomatis* and *Pyrenochaeta romeroi* are homogenous species in the orders Sordariales and Pleosporales, respectively. Interestingly, over 50% of isolates comprising the *M. grisea* complex were shown genetically to be *P. romeroi*, and presumably represent strains that had been incorrectly identified. The remaining *M. grisea* complex isolates (none of which had sporulated even after over two years continuous culture) could be grouped in three genetically distinct clades. All three clades, which fall within the Dothidiales/ Pleopsorales, comprise hitherto un-described taxa. Here we have begun the characterisation of these new taxa by using molecular phylogenetic analyses, and examining their geographic origins and antifungal susceptibility profiles.

PP-04-13

Phylogenetic position of human isolates of *Basidiobolus* analysed from rRNA gene sequences and from growth response to the elevated temperatures

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Basidiobolus ranarum is known as a causative agent of basidiobolomycosis in human beings. And some isolates identified as *B. haptosporus*, or *B. meristosoprus* were also reported as pathogens. Greer and Friedman (1964, 1966) reported that saprophytic *B. ranarum* would not grow at human body temperature whereas the pathogenic ones grew well. RFLP analysis of rDNA (Nelson et al. 1990) and studies on the isozyme variation (Cochrane et al. 1989) strongly suggested natural isolates were quite different from the laboratory human isolates. The results so far reported suggest that phylogenetic relationship between human and natural isolates are not yet settled.

In this study we analyzed the phylogenetic relationship among the *Basidiobolus* species based on sequences of the D1/D2 region of large subunit ribosomal RNA gene from our strains combined with the data from GenBank. Our analyses showed the *Basidiobolus* strains of human origin constituted a clade different from the one that included non-human isolates. The clade was related to the one including *B. meristosorus*. We confirmed the earlier reports that human isolates grew well at the temperatures above 37 C, at which temperatures the non-human isolates could not. In addition, we found the cessation of hyphal growth of natural isolates at the elevated temperatures brought about the morphological change of the filamentous hyphal tips into the sherical cells. And sherical cells grew again in the filamentous form when the culture returned to 25 C. This morphological transition from filamentous to spherical cell was not observed in the human isolates. We also present the nuclear behavior during this transition observed by microscopy.

PP-04-14

A putative new species in the *Sporothrix schenckii* complex and new records of Sporothrix species from Australia

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The *Sporothrix schenckii* complex is a species-rich group of morphologically very similar taxa which includes important human pathogens as well as soil and wood-inhabiting fungi. For many decades, *S. schenckii* was considered the only species of the genus pathogenic to humans. However, this taxon has proven to be genetically heterogeneous and containing several cryptic species. Currently, four *Sporothrix* species are known to cause human disease, i.e., *S. schenckii* sensu stricto, *Sporothrix brasiliensis*, *Sporothrix globosa*, and *Sporothrix luriei*, which can be distinguished on the basis of nucleotide sequence analysis of the calmodulin gene (CAL), growth rates at different temperatures, carbohydrate assimilation tests and morphology. In addition, similar apparently non-pathogenic species occur in nature, such as *Sporothrix mexicana* and *Sporothrix inflata*. Recently, we used partial CAL sequences to assess the phylogenetic relationships of 28 isolates (19 clinical and 9 environmental) morphologically identified as *S. schenckii*, collected from different regions of Australia. Parsimony analysis yielded 5000 most parsimonious trees with 420 steps in length. The best tree grouped the isolates as follows: 17 clinical and 4 environmental isolates within the *S. schenckii* clade, 2 clinical isolates within the *S. globosa* clade and one isolate from soil within the *S. mexicana* clade. Interestingly, four isolates from hay were grouped together constituting an undescribed lineage genetically related to *S. mexicana*. We are currently working in the phenotypic characterization of this putative new species. *S. globosa* and *S. mexicana* are new to Australia.

Poster Forum PF-05

PP-04-15

Barcoding of the therapy-refractory species of *Pseudallescheria* and *Scedosporium*

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Members of *Pseudallescheria*/*Scedosporium* are known as emerging opportunistic fungal pathogens. Infections may vary from subcutaneous to disseminated, with pronounced neurotropism; when cerebral, infections are fatal in 95 percent. To maximize the survival of patients, treatment should commence within the first day after appearance of symptoms. Given known differential susceptibilities of etiologic agents, fast and reliable identification of the latter is necessary. In view of molecular identification of *Pseudallescheria*/*Scedosporium* isolates, a database containing all haplotypes of these species is required. The aim of this study is the creation of a database validated by type strains to design species-specific ITS primers and RLFP-based identification for rapid and economic diagnostics. Sequences of the internationally agreed fungal barcoding gene ITS of 608 strains belonging to *Pseudallescheria*/*Scedosporium* species and relatives were used to generate the phylogenetic trees of this genus. More than 200 of the isolates were also sequenced for beta-tubuline (TUB) and more than 50 strains were sequenced for large subunit (LSU) and trees were calculated. Inter- and intraspecific variabilities were calculated and compared for ITS and TUB. Alignments of the ITS regions were screened for species-specific primers and species-specific endonucleases cutting sides. All clinically important members of *Pseudallescheria*/*Scedosporium* can be identified using routinely applied ITS sequences, although differences between species may be small.

We designed (i) a set of species-specific ITS primers, (ii) a species-specific ITS-RLFP, and (iii) an ITS database for blasting ITS sequences. The following species can be distinguished: *P. angusta*, *P. aurantiacum*, *S. apiospermum*, *S. dehoogii*, *S. fimeti*, *P. minutispora*, *S. prolificans*, and *P. boydii.*

These economic and fast molecular identification techniques for *Pseudallescheria*/*Scedosporium* offer reliable identification tools for epidemiologist and physicians.

Poster Forum PF-05

PP-04-16

Phylogeny of *Ochroconis* and *Scolecobasidium*

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Ochroconis and *Scolecobasidium* are closely related melanized hyphomycetes which are occasionally involved in human and animal infections. Some species are repeatedly found causing infections in fish. *Ochroconis gallopava* is the most virulent species of the group, since it is able to cause cerebral infections in immunocompromised humans. Most remaining species are saprobes. To clarify the taxonomic status and relationship among their species, sixty eight strains were cultured and DNA was extracted, and partial ribosomal and household genes were sequenced. Sequences, including some downloaded from GenBank and all presently available type isolates characterizing individual species, were analyzed using BioNumeric version 4.61, BioEdit, MrBayes version 3.1.2 and TreeFinder version 2007. The ITS of *Ochroconis* species was remarkably long compared to most known hyphomycetes, and had higher %GC content. With all algorithms used, a very robust grouping was found, with all 22 analyzed isolates of *O. gallopava* being nearly identical, and remaining species located at very large distances. Most classical, morphologically defined taxa were split up into separate species which hardly could be aligned with any of the genes analyzed. Phylogenetic distances between taxa appeared to be very large, despite the morphological and ecological unity of the group. Interestingly, *S. terreum* species seemed to be more closely related to *Ochoconis* than to remaining species of the genus. The species of *O. constricta* and *O. humicola* fell apart into three distinct clusters, several of which might represent novel species. In conclusion, multilocus analyses established the taxonomic status of species and revealed relationships between species of *Ochroconis* and *Scolecobasidium*. The large gaps between species makes ITS an excellent tool for identification and laboratory diagnosis.

PP-04-17

Intraspecies variability in Greek clinical *Scedosporium* isolates, molecularly typed by multilocus PCR-fingerprinting

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Pseudallescheria boydii/*Scedosporium apiospermum* species complex remains a worldwide emergent pathogen. Besides the new species lately separated from the complex, the species is characterised by large genomic variability. In the present study, the Greek isolates of the last 10 years were reidentified according to the modern species concepts and molecularly typed by PCR-fingerprinting.

Thirteen clinical isolates (7 derived from invasive disease cases and 6 from cystic fibrosis colonised patients) were collected in the past 10 years from paediatric and adult patients. All isolates were identified conventionally and by ITS and TUB sequencing and typed by M13 PCR-fingerprinting. Twenty-nine reference strains from the CBS culture Collection, of global geographic origin, were used in parallel. Cluster analysis was performed by Bionumerics 4, (Bio-Maths, Kortrijk, Belgium) using the Dice Coefficient of similarity and cluster analysis with the unweighted pair-group method with arithmetic averages (UPGMA).

All isolates were identified as either *Pseudallscheria boydii* or *Scedosporium apiospermum* (sensu Gilgado et al). Two epidemiologically unrelated clinical *Scedosporium apiospermum* isolates produced the same genotype while each one of the other clinical strains produced a unique genotype, different from the genotypes of the reference strains. No positive association of a particular genotype with male gender, trauma and site of infection was observed.

The intraspecies variability observed so far in the organism was also seen with the Greek isolates. As before, no geographically specific clusters were confirmed, although more isolates have to be studied before drawing definite conclusions.

PP-04-18

Benefit and difficulties of ITS barcoding in medical fungi - a comparison of datasets belonging to three fungal classes: Zygomycota (Mucorales), Ascomycota (Onygenales, Arthrodermataceae - dermatophytes), and Basidiomycota (Agaricales, Psathyrellaceae - *Hormographiella*)

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Identification to the species level is prerequisite for an appropriate antifungal therapy, and essential to address epidemiological questions. DNA barcoding aims to provide rapid, accurate and inexpensive species identification based on a short marker sequence that is known to differ between selected groups of organisms. For fungi, the internal transcribed spacer region (ITS) is currently the region of choice.

During the past three years we generated ITS barcodes for three medically important fungal groups belonging to different classes: Mucorales (Zygomycetes), dermatophytes (Ascomycetes), and Psathyrellaceae (Basidiomycetes). The current datasets comprise about 700 barcodes belonging to circa 180 species of the Mucorales, about 200 barcodes belonging to 47 species of dermatophytes, and about 220 barcodes belonging to 75 species of the Psathyrellaceae. The sets include all species and type strains deposited in the CBS reference collection.

In all groups, the ITS is sufficiently variable to discriminate between species. Depending on the evolutionary age of the group, inter- and intraspecific variation shows very large differences. While anthropophilic dermatophytes species may differ only by a single basepair, in Mucorales the ITS varies strongly between and sometimes also within species. In the genus *Lichtheimia*, for example, differences between sister species may reach up to 18%. This high polymorphism in zygomycete ITS makes it even possible to distinguish taxa at lower levels, like varieties or forma. In the Psathyrellaceae, the variation between sister species ranges from 1 to 3%. Although in all datasets rare groups such as geophilic dermatophytes or strictly coprophilous Mucorales are underrepresented, the sister species of the clinically relevant taxa are thoroughly sampled ensuring reliable diagnostics. However, in many groups species delimitation is still problematic, either because of a large share of clonal species (dermatophytes) or because the taxonomy is still unresolved (Mucorales, Psathyrellaceae).

PP-04-19

Prevalence of pathogenic zygomycetes in the United States

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Several members of the order Mucorales (subphylum Mucoromycotina) are important agents of human infections, particularly in diabetics and other immunocompromised individuals, and carry a high mortality. The identification of these fungi, not only to the species level, but also to the genus, is often difficult and time-consuming using standard mycological methods. Frequently, the etiological agent in clinical cases is either reported as a Mucor sp., which is one of the less-frequently seen genera of zygomycetes, or only as a member of the Mucorales. For this reason, the actual spectrum of species of zygomycetes and their incidence in the clinical setting is poorly known. We reidentified 190 United States clinical isolates morphologically identified as zygomycetes by sequencing of the internal transcribed spacer (ITS) region of the rDNA. Molecular identification revealed that *Rhizopus oryzae* represented approximately half (44.7%) of these isolates. The remainder were identified as *Rhizopus microsporus* (22.1%), *Mucor circinelloides* (9.5%), *Mycocladus* (*Absidia*) *corymbifer* (5.3%), *Rhizomucor pusillus* (3.7%), *Cunninghamella bertholletiae* (3.2%), *Mucor indicus* (2.6%), *Cunninghamella echinulata* (1%), and *Apophysomyces elegans* (0.5%). The percentage of isolates from deep tissue and cutaneous sites was 67.4% and 32.6%, respectively. The most frequent anatomic sites from which zygomycetes were isolated were the respiratory tract which included the lung, sputum, and pleural fluid (26.8%), and the nasal sinus including the hard palate, sinus, and sino-orbital areas (25.8%). A high level of correlation (92.6 %) between morphological and molecular identification was found.

PP-04-20

Molecular identification and antifungal susceptibility of the *Stephanoascus ciferrii* complex

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Background: *Stephanoascus ciferrii* complex, a teleomorph of *Candida ciferrii* is an ascomycetous yeast-like fungus that mainly caused otitis externa in humans and animals. In this study we carried out the phylogenetic analysis of the isolates of *S. ciferrii* complex obtained from the otorrhea in ear canal of patients. Methods: Eighteen clinical isolates of *S. ciferrii* complex identified by biochemical characterization were used. The D1/D2 region of 26S rDNA and ITS region of rDNA of these isolates were sequenced and compared with those of type strains of *S. ciferrii*, *S. allociferrii*, *S. mucifera, S. farinosus* and *S. smithiae*. MICs of various antifungal drugs against these isolates were determined by microdilution methods followed the CLSI M27-A3. Results: Sequence of 26S rDNA of 10 isolates of *S. ciferrii* complex revealed 100% similarity to that of type strain of *S. ciferrii*. The remaining 8 isolates showed 100% similarity to type strain of *S. allociferrii*. ITS1-5.8S-ITS2 nucleotide lengths of *S. ciferrii* were about 950 base pairs. Sequence of ITS1-5.8S-ITS2 of *S. ciferrii* showed 95% similarity to *S. mucifera*. The nucleotide length of *S. allociferrii* was about 550 base pairs. The ITS sequencing demonstrated that high genetic variation exists within *S. ciferrii* complex. All of the isolates of *S. ciferrii* complex were susceptible to MCFG and intermediate or resistant to AMPH-B, FLCZ, ITCZ, MCZ and VRCZ. Although the isolates of *S. ciferrii* were resistant to flucytosine with MIC of >64μg/mL, the isolates of *S. allociferrii* were susceptible to the drug with MICs of 1-2μg/mL. Conclusions: *S. ciferrii* and *S. allociferrii*, which had previously been considered synonyms could be differentiated genetically by the ITS analysis described in this study. Further studies are needed to demonstrate whether the species included in the *S. ciferrii* complex have differentiated by the clinical symptom and antifungal susceptibility.

PP-04-21

Molecular phylogeny of *Hormographiella*-like fungi from clinical and environmental sources, and associated teleomorphic basidiomycete fungi

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Background: Opportunistic fungal infections caused by *Hormographiella* spp. are increased in recent years. The aim of this study is to examine morphological and molecular properties of *Hormographiella*-like fungi and relatedness of these anamorphic fungi and *Coprinus*, *Coprinopsis* or *Coprinellus*. Methods: Twenty isolates of *Hormographiella*-like fungi including environmental isolates as well as the clinical ones in Japan were investigated. The D1/D2 region of 28S rDNA and ITS region as well as IGS 1 region of the 20 isolates of *Hormographiella*-like fungi were sequenced and compared with those of type strains of *H. aspergillata, H. candelabrata* and *H. verticillata*. Results: Sequence analysis of 28S rDNA and ITS region revealed that *Hormographiella* was a polyphyletic group and was separated into two distinct clades (Clade I and II). Clade I was composed with conidiophores differentiated species and encompassed *H. aspergillata* and two new anamorphic species (*Hormographiella* sp. I and II). Cultures of two new anamorphs were compared with type strains of *Coprinopsis rhizophorus* and *C. kimurae*, it was demonstrated that *Hormographiella* sp. I and *Hormographiella* sp. II were anamorphic stages of *Coprinopsis rhizophorus* and *C. kimurae*, respectively. Clade II represented conidiophores undifferentiated group with *H. verticillata*, as the prototype organism within the group. *H. candelabrata* also fell within this clade. In the analysis of IGS 1, *H. verticillata* were split into three types. IGS 1 revealed intraspecific variability in *H. verticillata*, which allowed differentiation of clinical and environmental isolates. Conclusions: Our investigation based on morphological and molecular characterization of the 28S rDNA and ITS regions supported the establishment of 2 new species of *Hormographiella* sp. I nov.(teleom.:*Coprinopsis rhizophorus*) and *Hormographiella* sp. II nov. (teleom.: *C.kimurae*). The anamorph-teleomorph connections suggest that the anamorphic species included in clade II (related to *Coprinellus*) should be segregated from *Hormographiella* and placed in a new genus.

PP-04-22

Multilocus microsatellite analysis in *Cryptococcus neoformans* var. *grubii* from 12 different countries

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Fifteen randomly selected microsatellites (simple sequence repeats (SSRs)), from the H99 *Cryptococcus neoformans* var. *grubii* (serotype A) genome, were sequenced, characterized and applied to sequencing based Multilocus Microsatellite Typing (MLMT) of 87 clinical and environmental *C. neoformans* var. *grubii* islates from 12 different countries. Among the 15 SSR loci, three (designated CNG1, CNG2 and CNG3) were polymorphic among the isolates studied. The remaining 12 SSR loci showed no variation. Thirty different MLMT types were globally found by combining polymorphisms of CNG1, CNG2 and CMG3 loci. The highest genetic variation was found amoug strains isolated from South and Latin American, which had 27 MLMT types. One MLMT type (type 22) was found globally with the exception of Asia (Japan, China, Taiwan, and Thailand). Another type (type 17) was found in Asia and Latin America, yet some other types (type 5, 7 and 10) were specific to Thailand. A unique MLMT type (type 29) was found only in Japan and China. The results show the three polymorphic microsatellites are useful markers for strain genotyping, population genetic analysis, and epidemiological studies.
The specific PCR primers of the polymorphic microsatellites: CNG1, CNG2 and CNG3, amplified those loci only from strains of *C. neoformans* (*C. neoformans* var. *grubii*, *C. neoformans* var. *neoformans* and the AD hybrid), but not from *C. gattii*, suggesting a species-specific association, and may be helpful for the diagnosis of cryptococcosis due to *C. neoformans*.

Poster Forum PF-05

PP-04-23

Phylogenetic relationship of *Pythium insidiosum* isolates from Thailand and around the world

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Oomycetes are aquatic, fungus-like, eukaryotic microorganisms. Many oomycetes are capable to infect plants and some animals. Among pathogenic oomycetes, *Pythium insidiosum* is the only species that infect humans and animals, leading to the fatal infectious disease called pythiosis. Pythiosis in animals has been increasingly reported worldwide, while the disease in humans has been reported mostly from Thailand. Differences in clinical features and disease severity of pythiosis are noted. Here, we explored a relationship between genetic variation, geographic origins, infective capability, and host specificities of *P. insidiosum*. The unweighted pair group method with arithmetic averages and maximum parsimony methods were applied to construct phylogenetic trees, using ribosomal DNA internal transcribed spacers rDNA ITS-1, 5.8S rDNA and ITS-2 (rDNA ITSs) sequences from 32 clinical and 59 environmental isolates from Thailand, and 22 clinical isolates from around the world. This study contained the largest set of rDNA ITSs sequences of *P. insidiosum* clinical isolates from Thailand. The rDNA ITSs sequence from the causative agent of canine pythiosis in Africa was also included. *P. insidiosum* existed in 3 clades, which associated with geographic distribution: clade-I contained American isolates, clade-II contained Asian and Australian isolates, and clade-III contained mainly Thai isolates. It seemed that the isolates in clade-III geographically related to Thailand, and associated with human host. The African isolate located far distant from other *P. insidiosum* isolates, suggesting existence of a new mammalian-pathogenic oomycete species. All clinical and environmental Thai isolates existed in clade-II and -III, but not in clade-I, suggesting there were 2 major subpopulations of *P. insidiosum* in Thailand. There was no correlation between genetic variation, form of infection, severity of the disease, and regional geographic distribution of *P. insidiosum* Thai isolates.

PP-04-24

CHROM-Pal medium for primary isolation and identification of *Candida dubliniensis* in oral samples from HIV positive patients

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Candida dubliniensis oral isolates are increasing, but they are difficult to discriminate from *Candida albicans*. CHROM-Pal (CH-P) medium is used in our laboratory to differentiate the two species on the basis of colony colour and morphology, and chlamydospore production (Sahand. J Clin Microbiol 2005; 43:5768-70).

Objectives: Comparison of CH-P and CHROMAgar *Candida* (CAC) media for primary isolation and presumptive identification of yeasts from oral specimens, and a crude estimation of prevalence of *C. dubliniensis* in the oral cavity of HIV-infected individuals.

Results: Oral swabs from 54 HIV-positive patients attending the Department of Infectious Diseases in Hospital de Cruces (Spain) were processed. The 48 positive specimens (88.9%) resulted in growth of 53 isolates of *Candida* species on both media, while ten more isolates were detected only on CH-P medium (4 *C. parapsilosis*, 3 *C. albicans*, 1 *C. dubliniensis*, 1. *C. tropicalis*, 1 *C. famata*). Several samples gave rise to mixed cultures of two different species.

C. albicans was the most frequently isolated species (33 isolates; 52.4%) followed by *C. dubliniensis* (10; 15.9%), *C. parapsilosis* (7; 11.1%), *C. glabrata* (4; 6.3%), *C. tropicalis* (4; 6.3%), *C. famata* (2; 3.2%), *C. guilliermondii* (2; 3.2%), and *C. krusei* (1; 1.6%).

The sensitivity and specificity values for identifying *C. albicans*, *C. krusei*, *C. tropicalis* and *C. dubliniensis* in CH-P were over 98.5%, always higher than those obtained for CAC.

Conclusion: CH-P is a simple reliable medium for primary isolation and presumptive identification of yeast isolates from the oral cavity and is particularly suitable to differentiate *C. dubliniensis* and *C. albicans*. CH-P was superior to CAC in both isolating a larger number of strains and in correctly differentiating *C. dubliniensis* and *C. albicans*.

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PP-04-25

Candida dubliniensis identification in Venezuela

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Candida dubliniensis, was described in 1995 as a new species of *Candida*. As a *Candida* species they share morphological and physiological characteristics with *Candida albicans* and for the adequate identification of the species it is required to perform molecular biology trials such as the polymerase chain reaction (PCR). Objective: we desired to identify *C. dubliniensis* from clinical isolates of yeasts previously identified by conventional procedures as *C. albicans*, by means of the PCR technique. Materials and methods: we proceeded to extract the DNA of thirty isolates of *C. albicans*, recovered from clinical specimens of patients with candidosis from different clinical centers coming from several towns of Venezuela (Barquisimeto, Caracas, Ciudad Bolívar, Coro, Cumaná). These DNA's were examined by the PCR method using the specific primers DUBR/DUBF for *C. dubliniensis*. Results: from the thirty DNA's studied, one (3.3%) gave a product of 288 pb, specific for *C.dubliniensis*. We conclude that *C. dubliniensis* was identified in Caracas from a patient with oral candidosis. The data above reported are partial results from a nation wide project.

PP-04-26

Prevalence of *Candida dubliniensis* and *C. dubliniensis* screening using the germ tube test in clinical yeast isolates in Korea

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Objectives: Although *Candida dubliniensis* has been reported to exist in various geographic locations, the prevalence of *C. dubliniensis* has not yet been reported in Korea. The aim of the present study was to investigate the prevalence of *C. dubliniensis* in Korea and to screen for *C. dubliniensis* using the germ tube test with human pooled serum (HPS) in clinical isolates.

Methods: Among 1,854 yeast strains isolated, 1,404 strains of *C. albicans* (on the basis of positive results of the germ tube test) and 192 germ tube-negative yeast strains were examined. All 1,596 clinical isolates were examined using the germ tube test with HPS, the differential temperature and NaCl tolerance test. Only 81 isolates that did not grow at 45˚C nor on Sabouraud 6.5% NaCl broth were selected and tested using the VITEK 2 ID-YST system and the multiplex-PCR assay for the study.

Results: The 2 strains, *C. dubliniensis* ATCC MYA-646 and KCTC 17427 failed to produce germ tubes in HPS but produced them in fresh rabbit serum (FRS) and fetal bovine serum (FBS). Among 81 isolates, all germ tube-positive isolates in HPS (16 isolates) were confirmed as *C. albicans*. No *C. dubliniensis* was found in the present study population.

Conclusions: The results of the present study suggest that the prevalence of *C. dubliniensis* appears to be extremely low in Korea and that the germ tube test with HPS in combination with FRS or FBS can be used for discriminating between *C. albicans* and *C. dubliniensis* strains.

Key words: *Candida dubliniensis*, Germ tube test, Multiplex-PCR, Korea

Poster Forum PF-05

PP-04-27

Prevalence, phenotypic identification, and antimycotic susceptibility of *Candida dubliniensis* from fecal samples of outpatients in Thuringia/Germany

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Objectives. Epidemiological data for *Candida dubliniensis* are sparse due to similarity to *Candida albicans.* In this study we determined (1) the prevalence of *C. dubliniensis* in stool samples of outpatients in Thuringia, (2) the performance of phenotypic methods for the identification of *C. dubliniensis,* and (3) the susceptibility against seven antimycotic agents.
Methods. From 2005 to 2007, yeasts were isolated from stool samples from patients with bacterial or viral diarrhea and from healthy people. They were specified by seven phenotypic methods. Susceptibility testing was done by Etest.
Results. Yeasts were grown from 1.162 of 1.913 stool samples (60.7%). A total of 1.452 isolates included 870 strains of *C. albicans* (59.9%), 24 *C. dubliniensis* (1.7%), and 558 strains of 20 other yeast species. Each of the following three methods had a sensitivity of 100% and a specificity of 100% for the identification of *C. dubliniensis*: (1) rough colonies with pseudomycelium on Staib agar, (2) agglutination with the Bichro-Dubli latex test, and (3) the ID32C system. All 24 *C. dubliniensis* strains produced chlamydospores on rice agar, 6 strains (25%) showed a dark green color on CHROM agar *Candida*, the germ tube test on Mueller Hinton agar was positive in 10 of 24 strains (42%), and 21 isolates (87.5%) did not grow at 42°C. The MIC_{90} data [mg/L] (range) for 24 C. dubliniensis isolates were: amphotericin B 0.064 (0.008-0.094), fluconazole 0.75 (0.125-2), itraconazole 0.094 (0.002-0.25), voriconazole 0.016 (0.003-0.023), pos aconazole 0.032 (0.002-0.064), caspofungin 0.25 (0.047-0.38), and anidulafungin 0.008 (0.002-0.012).
Conclusions. The prevalence of *C. dubliniensis* in stool samples from outpatients in Thuringia was 1.3%. Colony morphology on Staib agar and latex agglutination with the Bichro-Dubli test were simple, sensitive (100%), and specific (100%) tests for *C. dubliniensis*. All 24 *C. dubliniensis* strains showed low MICs against seven antimycotic agents.

PP-04-28

CandIDazol 2008 - 2011: Innovative diagnostics for the rapid identification of *Candida* yeasts

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Candida yeasts are the fourth most common source of nosocomial bloodstream infections and are a growing concern in modern European society. Especially during the last decade, the prevalence of opportunistic *Candida* infections has increased and has become a serious threat to high risk patients, often causing high rates of morbidity and mortality (Pfaller, 2007).
To date, most diagnostic methods have difficulties discriminating closely related species. Therefore, polymicrobial infections can be under diagnosed. Furthermore, most molecular diagnostic tools are based on ribosomal regions and housekeeping genes. This results in an urgent need of high discriminatory and accurate detection methods for clinical purposes using novel probes.
The CandIDazol project was initiated in 2008. The aim of this project is to improve molecular diagnostics of yeast-related infections. Comparative genomics analysis of available yeast genomes resulted in the identification of unique target sequences. A novel DNA based diagnostic system using Multiplex Ligation-dependent Probe Amplification (MLPA) technology (Schouten et al., 2002; Reijans et al., 2008) serves as our platform for molecular diagnostics. Preliminary results show the potency of the selected target sequences. Several new Candidate probes for the detection of *C. albicans*, *C. dubliniensis*, *C. tropicalis*, *C. parapsilosis*, *C. glabrata*, *C. guilliermondii*, and *C. lusitaniae* have been defined. Validation on clinical samples of these probes in a diagnostic MLPA kit will be demonstrated.

References:
Pfaller, M. A. and D. J. Diekema. "Epidemiology of Invasive Candidiasis: a Persistent Public Health Problem." Clinical Microbiology Reviews 20.1 (2007): 133-63.
Reijans, Martin, et al. "RespiFinder: a New Multiparameter Test To Differentially Identify Fifteen Respiratory Viruses." Journal of Clinical Microbiology 46.4 (2008): 1232-40.
Schouten, Jan P., et al. "Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification." Nucleic Acids Research 30.12 (2002): e57.

PP-04-29

Evaluation of pyrosequencing to identify *Candida* to the species level

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Background: In patients with *Candida* infections, identification to the species level can help to guide empiric drug therapy. DNA pyrosequencing is a rapid molecular tool that can be used for species-level identification of various microbes. Our objective was to evaluate the utility of this molecular tool for the identification of *Candida* to the species level.
Methods: Thirty-three banked *Candida* isolates collected from HIV/AIDS patients with oropharyngeal candidiasis were used in this study, and were subcultured twice on Sabouraud dextrose agar prior to phenotypic and genetic analysis. Isolates were identified to the species level phenotypically by plating onto CHROMagar *Candida* plates (CHROMagar Company, Paris, France) or by use of API 20C identification assay (bioMerieux, Marcy-l'Etoile, France). Prior to genetic identification, DNA was isolated using the MasterPure DNA purification kit (EpiCentre, Madison, WI). Extracted DNA was sequenced by pyrosequencing using a commercially available kit (PyroMark Fungal ASR, Biotage, Kungsgatan, Sweden). In addition, dideoxy sequencing was used to sequence the *ITS1* and *ITS2* regions of each isolate independent of pyrosequencing.
Results: Species-level identification by DNA pyrosequencing was in concordance with the those obtained by phenotypic analysis and dideoxysequencing for 29 of the 33 isolates, including each *C. albicans* and *C. dublinensis* strain tested. In addition, the sequence obtained by pyrosequencing was > 97% identical to those reported in GeneBank for 25 of the isolates tested. However, for *C. glabrata* isolates, DNA pyrosequencing was in agreement with the phenotypic and dideoxy sequencing identification for only 2 of 5 isolates.
Conclusions: DNA pyrosequencing was effective in identifying *Candida* isolates to the species level, including the genetically similar species *C. albicans* and *C. dublinensis*. Further evaluation is needed to assess the utility of this assay for other non-*Candida albicans* species.

PP-04-30

Prevalence, identification of *Candida* species, and risk factors of vulvovaginal candidosis among female sex workers in Yogyakarta, Indonesia

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Background: *Candida albicans* was isolated from vagina in 85-90 %. Recently, there is an increase of occurrence of vulvovaginal candidosis (VVC) caused by C.non albicans which is more resistant to azol. Previous study showed that *Candida* can be transmitted trough sexual intercourse. Female sex workers (FSW) are in high risk to get sexual transmitted infection and human immunodeficiency virus infection.
Aim: To determine the prevalence of VVC, identify of *Candida* species and risk factors of VVC among FSW.
Methode: Descriptive cross sectional study of 95 FSW were conducted with simple random sampling. Interview about risk factors, physical examination, measurement of vaginal pH, Sabouraud dextrose agar (SDA), and CHROMagar™culture were performed.
Results: As 45,3% FSW were identified VVC positive. Mostly (86.1%) were caused by *C. albicans* and 13.9% by C.non albicans, which were included *C.dubliniensis*, *C.tropicalis*, dan *C.kefyr*. Risk factor of recurrent VVC history increased the risk 4.37 times to have VVC. No statistically significant found between contraception use, condom, vaginal douching, antibiotic, sexual intercourse frequency, orogenital sex, and type of underwear with VVC.
Conclusion: Prevalence of VVC in Yogyakarta with SDA culture was 45.3%. In this study, *Candida albicans* was the main cause of VVC besides C.non albicans. One of the type known as *C.dubliniensis* was first reported in Indonesia.

Key words: vulvovaginal candidosis, prevalence, risk factors, species identification

PP-04-31

Application of CHROMagar *Candida* with blood agar for presumptive identification of five major medically important *Candida* species

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Disseminated candidiasis is one of the major systemic mycoses in immunocompromised patients. Rapid, simple and reliable identification of major causative *Candida* species is required for diagnosis and treatment of this disease because of different antifungal susceptibility patterns of each species. CHROMagar *Candida* is differential culture medium that allows selective isolation of fungi and simultaneously identifies colonies of *C. albicans*, *C. tropicalis*, and *C. krusei*. However, this medium cannot differentiate two major *Candida* species such as *C. glabrata* and *C. parapsilosis* because of similar colony appearance with pale pink to violet color. We would propose a novel method to distinguish these two species using growth characteristics of blood agar and Sabouraud dextrose agar. A total of 101 *Candida* strains including 46 *C. glabrata*, 34 *C. parapsilosis*, 5 *C. metapsilosis*, 2 *C. orthopsilosis*, 4 *C. lusitaniae*, 3 *C. guilliermondii*, 5 *L. elongisporus*, 1 *C. pelliculosa*, and 1 *C. utilis* with a similar colony color on CHROMagar *Candida*. No or very tiny growth of *C. glabrata* strains was shown on blood agar compared with Sabouraud agar. While, strains other than *C. glabrata* could grow well equally on both agar plates. We presumptively identified 39 strains showed pale pink to violet colonies on CHROMagar *Candida* by growth characteristics on blood agar. Direct sequencing of 26S rRNA D1/D2 region confirmed species identification of *C. glabrata* for all the 27 strains that could not grow on blood agar. In conclusion, application of CHROMagar *Candida* in combination with growth characteristics on blood agar and Sabouraud dextrose agar can be easily, simply, and inexpensively identify five medically important *Candida* species presumptively such as *C. albicans*, *C. tropicalis*, *C. krusei*, *C. glabrata* and *C. parapsilosis*.

PP-04-32

Genotypic heterogeneity within *Candida orthopsilosis* strains identified among clinical *Candida parapsilosis*-complex isolates in Kuwait

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Objective: Recent molecular studies have shown that phenotypically identified *Candida* parapsilosis isolates represent a complex of three species, namely, *C. parapsilosis*, *C. orthopsilosis* and *C. metapsilosis*. This study determined genotypic heterogeneity and species spectrum among 114 phenotypically documented *C. parapsilosis* complex strains. Materials and Methods: The presence of *C. parapsilosis*, *C. orthopsilosis* and *C. metapsilosis* species within *C. parapsilosis*-complex strains was detected by PCR amplification of internally transcribed spacer (ITS) region of rDNA using species-specific primers. The results were confirmed by direct DNA sequencing of ITS region and D1-D2 regions of 28S rRNA gene for selected isolates. Genotypic heterogeneity among *C. parapsilosis* and *C. orthopsilosis* strains was further determined by PCR amplification of intergenic region sequences between 28S rRNA gene and 5S rRNA gene (IGS1), followed by restriction digestion of amplicons to generate RFLPs. Results: Species-specific amplification of ITS region of rDNA showed that only 109 of 114 (96%) isolates were *C. parapsilosis* strains while 5 of 114 (4%) phenotypically identified *C. parapsilosis*-complex isolates in Kuwait were in fact *C. orthopsilosis* strains. The latter included three strains isolated from blood. Two distinct genotypes among five *C. orthopsilosis* strains were identified based on DNA sequences of ITS region and PCR-RFLP of IGS1 region. Genotype I included all three strains isolated from blood while genotype II included the two strains isolated from other body sites. Only two genotypes were apparent among 81 of 109 *C. parapsilosis* strains tested. Conclusions: This is the first report on the isolation of *C. orthopsilosis* from clinical specimens including blood from Arabian Gulf and Middle Eastern region. The association of a distinct genotype of *C. orthopsilosis* strains with candidemia cases is also noteworthy. Supported by KURA grant YM04-06

PP-04-33

A rapid pigmentation test for identifcation of *Cryptococcus neoformans*

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A non-medium-based test was developed for identification of *Cryptococcus neoformans* isolates. Identification is based on pigment produced by the organism's phenoloxidase activity. Paper strips containing a buffered extract of sunflower seeds-ferric citrate solution were inoculated with isolates and incubated at 37°C. The test was conducted to the 50 clinical isolates of *C. neoformans*. Brown pigment production occurred with all isolates of *C. neoformans* within 4 hours. *Cryptococcus* species and common clinically isolated yeasts failed to produce pigment on the test strip.

PP-04-34

Mating-, sero- and genotype diversity of clinical *Cryptococcus neoformans* strains in a tertiary hospital in Madrid, Spain

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Background: The *C.neoformans* - *C.gattii*-complex currently includes two different species: *C.gattii* and *C.neoformans*, the latter with the varieties *grubii* and *neoformans*, clustered in nine different Amplified Fragment Length Polymorphism (AFLP) genotypes. We studied the diversity of serotypes and genotypes of *C.neoformans* from patients with cryptococcosis in a Spanish tertiary hospital.

Methods: From 1989 to 2007, we collected 96 clinical isolates of *C.neoformans* from 57 infected patients. The underlying conditions of the patients were HIV infection (n=48), other debilitating conditions (n=7), none (n=1), and unknown (n=1). All strains were genotyped using AFLP analysis and *C. neoformans* isolates were analyzed for mating-type and serotype with four different PCRs that specifically amplify the *STE20***a** and *STE20*α locus of serotype A and D isolates.

Results: The distribution by serotypes was A (n=56; 58.3%), AD (n=24; 25%), D (n=13; 13.54%), B (n=1; 1.1%), and non-typeable (n=2; 2.1%). The distribution of genotypes was AFLP1 (n=28; 29.2%), AFLP1B (n=16; 16.6%), AFLP2 (n=14; 14.6%), AFLP3 (n=35; 36.5%), AFLP4 (n=1; 1.1%), and a new AFLP type (n=2; 2.1%). A total of 46 strains were *C.neoformans* variety *grubii* (AFLP1 and 1B), 49 were *C.neoformans* variety *neoformans* (AFLP2 and 3), and 1 was *C.gattii* (AFLP4). The etiological agents of the 58 episodes were *C.neoformans* variety *neoformans* (n=31), *C.neoformans* variety *grubii* (n=24), a mixture of both varieties (n=2), and *C.gattii* (n=1). The patient infected with *C.gatti* was immunocompetent and lived in Australia. Although we did not find a specific genotype which was more prevalent in HIV-infected patients, *C.neoformans* variety *neoformans* was linked more to HIV patients and *C.neoformans* variety *grubii* was linked more to patients with other immunodeficiencies.

Conclusions: In patients attending our hospital, cryptococcosis was caused almost exclusively by *C.neoformans* variety *neoformans* and variety *grubii* in similar proportions. *C.neoformans* variety *neoformans* was more likely to infect patients with HIV infection.

PP-04-35

Genotypic and phenotypic characterization of serotype B, molecular type VGII, clinical *Cryptococcus gattii* isolates from Cúcuta, Colombia, and their comparison to the Vancouver Island outbreak isolates

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Cryptococcus gattii serotype B, molecular type VGII emerged recently as primary pathogen on Vancouver Island, Canada, causing a cryptococcosis outbreak. In Cúcuta, Colombia *C. gattii* isolates of the same serotype and molecular type have been reported. To identifying similarities and differences in their virulence profiles we compared 13 clinical and 2 environmental isolates of *C. gattii* serotype B from Cúcuta with 20 Vancouver Island outbreak isolates. *URA5* RFLP analysis was used to identify their molecular type. Factors associated with virulence were evaluated: mating type (mating type PCR and *in vitro* mating), colony characteristics, phenotypic switching capability, cell and capsular size, enzyme activity (phenoloxidase, phospholipase and protease), and growth kinetics at 25°C and 37°C. The two Colombian environmental isolates were VGIII and the 13 clinical isolates were VGII. Of these, 12 isolates were mating type **a** and one α as determined by mating type PCR. *In vitro* mating reveled that only eight isolates were fertile when mated with the MATα tester strains. Seven of these 13 isolates presented a mucoid morphology and 11 displayed phenotypic switching. 19 of the 20 Vancouver Island outbreak isolates showed a smooth colony phenotype and switching. The cell size ($p = 0.0037$, mean 5.50 ± 0.833 nm) and capsular size ($p = 0.041$, mean 1.23 ± 0.046 nm) was greater in the Colombian isolates. There is evidence of a significant increase in proteolytic activity in the Canadian isolates ($p = 0.000$). The highest growth was observed at 25°C, however there is no significant difference between the two groups. The presence of VGII *C. gattii* clinical isolates in Colombia and the similarity observed in some virulence factors between the Colombian and Canadian outbreak isolates open up the possibility for further studies into the association between South America and the emergence of highly virulent outbreak strains.

PP-04-36

Comparison between microscopy, culture, and two different PCR-methods for the detection of dermatophytes directly from clinical specimens

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The current "gold standard" for the diagnosis of dermatophytes from clinical specimens is microscopy and culture. These methods are, however, very unspecific or time consuming (up to 4 weeks). Furthermore it is known that only 15-50% of microscopic positive specimens do not grow a culture. Thus molecular methods became very popular during the last years and many so called "in house" PCR assays have been developed for the identification of dermatophytes directly from clinical specimens. To evaluate two of the in house PCR-ELISAs, ca. 200 nail and skin specimens of 100 patients with suspected onychomycosis and/or tinea pedis were collected and analyzed by conventional and molecular methods. The molecular methods were both based on an ELISA method using species specific probes for the detection of *Trichophyton rubrum*. The genomic target was, however, distinct. The first PCR-ELISA was using a part of the topoisomerase gene (TI) while the second was targeting a microsatellite (MS) region. The specificity and sensitivity between the standard diagnostic methods and the molecular methods was compared as well as between both PCR based methods.
Two specimens per patient were examined microscopically with calcofluor for the presence of fungal material and a culture was grown. The QIAmp DNA Mini Kit (Qiagen) was used to extract the DNA from clinical specimens. The PCR assays were conducted in different laboratories.
The results show that microscopy as well as both PCR-ELISAs were more sensitive than culture. Microscopy and molecular methods had nearly identical sensitivities but PCR was species specific. The comparison of both molecular procedures revealed that the microsatellite ELISA was more sensitive when compared to the topoisomerase ELISA. These findings suggest that the sensitivity of the molecular methods is independent of the DNA extraction method used but depend on the target selected.

PP-04-37

Molecular identification and susceptibility of *Trichosporon* species isolated from clinical specimens: Isolation of *Trichosporon dohaense* sp. nov

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Trichosporon species have been reported as emerging pathogens and usually occur in severely immunocompromised patients. In the present work, 27 clinical isolates of *Trichosporon* species were recovered from 27 patients. The patients were not immunocompromised except one with acute myeloid leukemia. Sequence analysis revealed the isolation of *T. dohaense* Taj-Aldeen, Meis & Boekhout sp. nov. with CBS 10761T as holotype strain belong to ovoides clade. In the D1/D2 LSU rRNA gene analysis, *T. dohaense* is a sister species to *T. coremiiforme*, and in the ITS analysis the species is basal to the other species of this clade. Molecular identification of the strains yielded 17 *T. asahii*, three *T. inkin* and two of each, *T. japonicum*, *T. faecale*, and three isolates belong to *T. dohaense*. The former four species exhibited low MICs for five antifungal azoles but showed high MICs for amphotericin B. *T. dohaense* demonstrated the lowest amphotericin MIC (1mg/L). *T. asahii* was resistant for amphotericin B with a MIC 90 of >16 mg/L, and except for fluconazole (MIC90 8mg/L), had low MIC90 for itraconazole (0.5 mg/L), voriconazole (0.25 mg/L), posaconazole (0.25 mg/L) and isavuconazole (0.125 mg/L). The echinocandins, caspofungin and anidulafungin, demonstrated no activity against *Trichosporon* species.

PP-04-38

Identification of pathogenic yeasts species based on PCR-fragment size polymorphism (PCR-FSP) by using normal agarose gel electrophoresis

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The clinical importance of yeast infections has increased in recent decades. The current morphological and physiological methods for identification of the species are generally not easy to interpret and may be expensive or time-consuming. In the present study, we introduce and use a new approach for the identification and differentiation of medically important yeast species. In this method, size polymorphism of both internal transcribed spacer regions, ITS1 and ITS2, in the ribosomal DNA in various yeasts is used as the basis of species differentiation.

The genomic DNA of each 31 standard strains and 162 clinical isolates was extracted and ITS1 and ITS2 regions were PCR-amplified, separately. Both PCR products were mixed and analyzed after standard agarose gel electrophoresis. The species of the tested yeasts were identified by the electrophoretic patterns of the mixed PCR products of each sample, comparing the data obtained from the sequence analysis of ITS1 and ITS2 molecules.

By this method, with the exception of *C. albicans* and C. dubliniensis, we were able to clearly differentiate nearly all common pathogenic yeast species, including *C. albicans*, *C. glabrata*, *C. gulliermondii*, *C. parapsilosis*, *C. tropicalis*, *C. krusei*, *C. kefyr*, *C. lusitaniae*, *C. rugosa*, *C. famata*, *T. asahii*, *C. neoformans*, *S. cerevisiae* and some other rare pathogenic yeasts. All standard and clinical strains were identified correctly, without need to expensive methods such as sequencing or capillary electrophoresis.

It seems that the PCR-FSP method introduced in this study is the easiest molecular approach for the identification of a wide range of pathogenic yeast species and is applicable for diagnostic and epidemiological purposes in reference laboratories.

PP-04-39

A new species of the genus *Malassezia* based on the sequence analysis of 26S (D1/D2) and internal transcribed spacer 1 in ribosomal DNA

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Malassezia species are the members of the microbiological flora in human and warm-blooded animals' skin. They are considered to be etiological agents of pityriasis versicolor and *Malassezia* follicolitis in human. It is strongly suspected that *Malassezia* species have also a role in the pathogenesis of some other dermatological conditions such as seborrheic dermatitis, atopic dermatitis and pseoriasis. Recently on the basis of molecular data, six new species were added to the genus, totally 13 species were described and accepted so far. In the present study we describe a new species of *Malassezia* based on the nucleotide sequence of 26s rDNA and Internal Transcribed Spacer 1 (ITS1) regions, as the critical markers to differentiate between species.

The yeast was isolated from a Hamster, by culturing on the medium modified Leeming and Notman Agar (mLLA). Genomic DNA was extracted by glass-beads preparation. Two primer pairs, one for amplification of 26s(D1/D2) and another for ITS1 were used in PCR. The PCR products were sequenced and analyzed In Silico in comparison with other similar sequences already deposited in GenBank. 26SrDNA PCR product was also digested with the restriction enzyme CfoI.

Malassezia-specific primer pairs were successfully amplified the 26srDNA and ITS1 regions of the newly isolate, providing a single PCR product of about 580 and 280bp, respectively. After digestion the 26s(D1/D2) PCR product with the enzyme CfoI, unique and different RFLP pattern was observed. The analysis of 26s and ITS1 regions implicated new sequences comparing the same regions in all already described *Malassezia* species. Phylogenetic tree of both regions showed that the isolate is a different *Malassezia* species. Phenotypical characterization is under investigation.

Regarding the new RFLP pattern of D1/D2-26SrDNA and the unique nucleotide sequence of both 26S and ITS1 regions, we propose the isolates as a new species of *Malassezia*.

PP-04-40

Comparison of nested PCR and RFLP of *Malassezia* yeasts from healthy human skin

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Malassezia yeasts are normal flora of the skin that are discovered in 75-80% of healthy subjects. It is acknowledged that *Malassezia* yeasts are related to various skin diseases such as *Malassezia* folliculitis, seborrheic dermatitis and atopic dermatitis. So exact identification of the *Malassezia* species using accurate instrument is more important to investigate the pathogenesis of the *Malassezia* yeasts to various skin diseases. This research was conducted to investigate the molecular biology method for identifying and classifying the *Malassezia* yeasts, which is more accurate and cost effective. We compare the accuracy and efficacy of restriction fragment length polymorphism (RFLP) and nested polymerase chain reaction (PCR) method, known as relatively accurate method for identifying the *Malassezia* yeasts. In the result, although both methods show relatively fast and accurate identifying results, nested PCR method is faster than RFLP and time saving advantages, but lower consistent rate with genebank. Therefore, our results shows that RFLP is more useful and reliable in the detection of various *Malassezia* species than nested PCR, and the 26S rDNA, which was targeted in this study contains highly conserved base sequences and enough sequence variations for inter-specific identification of *Malassezia* yeasts.

PP-04-41

Poor performance of routine laboratory techniques for the identification of lipophilic yeast of the genus *Malassezia* Baillon

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The expansion of the genus *Malassezia* revealed the growing need for efficent, accurate, and rapid identification methods. Indeed it is largely recognised that molecular techniques are, particularly for these so peculiar yeasts, the best for an accurate identification, against the techniques based on morphology, physiology, and biochemistry. In this investigation, we studied 34 reference strains, representing 10 different species, for both microscopic morphology and colony diameter, pigment production and colony color on chromogenic *Malassezia* medium, catalase reaction, utilization of Tween compounds, assimilation of Cremophor EL, splitting of esculin, and germ-tube test.

We observed the non-utility of current routine laboratory testing procedures for laboratory diagnosis. This study has also demonstrated that 1. microscopic morphology and colony diameter as well as precipitate production were not reliable methods, 2. colony color or pigment production on OCCA *Malassezia* medium failed to distinguish such *Malassezia* species, 3. features thought to be characteristics for such species might not be expressed, e.g. catalase or beta-glucosidase, 4. utilization of Tween compounds might not yield reliable differentiation, 5. assimilation of Cremophor EL was not effective in laboratory diagnosis, and 6. none of the study strains produced germ-tubes even at 24 h.In conclusion, a critical evaluation of the efficacy of various conventional techniques for species identification of *Malassezia* was presented.

PP-04-42

Mucin 2 phenylethanol selective agar in mycology

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The increasing incidence of bacterial resistance has allowed for the development of increasingly resistance strains of Enterobactericeae on fungal-selective agars. Bacterial colonies grow much faster, thereby reducing the nutritive value of the medium, which in turn either slows or inhibits fungal growth. *Pseudomonas* and *E. coli* species (due to their antifungal activity) present a particular problem, followed by the slime producing species *Klebsiella* and *Enterobacter*. The objective of this study was to fund an adequate replacement for antibiotics used in agar in the isolation of funguses from samples contaminated with various species of Gram positive and negative bacteria. This study presents the replacement of antibiotics in Emmons agar with chemicals to which bacteria do not form resistance. A good replacement for antibiotics, which also proved to have selective activity, was the combination of mucic acid (Sigma M-4778), 2-phenylethanol (Merck 807006) and cupric sulphate ($CuSO_4$) or cupric chloride ($CuCl_2$). This selective combination was added to Emmons agar, heated to the boiling point, and is then ready for use (no need for sterilization in the autoclave). Emmons agar was used due to its widespread laboratory usage. In this study, clinical patient samples were used: sputum, bronchial secretions, wound swabs, skin swabs, faeces, urine sediment, etc. Samples were directly applied to the surface of the agar and incubated at 26 or 37°C for 24 or 48 hours, and isolated species determined using standard laboratory techniques. The most common isolates were *Candida* and *Aspergillus* species. This new diagnostic method represents an effective, economical and ecologically acceptable new possibility in mycological work.

PP-04-43

A newly pathogen of protothecosis as a novel achlorophyllic alga isolated from the inflamed skin caused by protothecosis

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A single strain, JCM 15793, of a newly pathogen of protothecosis as a novel pathogenic achlorophyllic alga species belonging to the genus *Prototheca* was isolated from the inflamed skin caused by protothecosis of the patient in a Japanese hospital. It was diagnosed that this strain was pathogen by histopathology and culture result. Analyses of the nuclear 18S rDNA and 26S rDNA D1/D2 domain sequences and chemotaxonomic studies indicated that this strain represents a new species with a close phylogenetic relationship to *Prototheca wickerhamii* and *Auxenochlorella protothecoides*. This strain grew well at 28-30 °C, did slowly and weakly at 37 °C, and did not grow at 40 °C. This strain grew at vitamin free medium and assimilated soluble starch and L-arabinose as a carbon source. Because JCM 15793 was different from the existing species in these characteristics, it was suggested that it was a new species of the genus *Prototheca*.

PP-04-44

Connoisseur's delight -what a fungal surprise!

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The last 2-3 decades has witnessed increase in the incidence of opportunistic fungal infections mainly due to modern medical advances, changes in man's environment and his immune defense.
The new millennium in Medical Mycology certainly belongs to opportunistic fungal infections in immunocompromised patients .
Patients and Methods: During the study period of January 2000-August 2008, 1600 samples from various clinical departments were received in the Mycology division.
All the specimens were subjected to KOH and culture and correlated with histopathology wherever relevant.
Results: Out of the 1600 specimens, there were 8 isolates from unusual clinical presentation *Cunninghamella bertiollatiae, Rhinosporidium seeberi, Engyodontium album, Trichophyton rubrum, Apophysomyces elegans, Absidia corymbifera, Histoplasma capsulatum, Rhizopus homothallicus*.
The rare isolates were confirmed by Dr. Arunaloke Chakrabarti in the center for advanced studies in Mycology reference laboratory PGI Chandigarh, India.
Conclusion: The diagnosis of fungal disease is a multidisciplinary approach requiring co-operation and collaboration of many people with diverse expertise. As most of the fungi causing fungal disease are saprophytic in nature, close communication with physicians is important to interpret the result of the laboratory.

PP-04-45

Fungi culture collections at the Centro Venezolano de Colecciones de Microorganismos (CVCM)

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CVCM is an open collection, formed by collections of public or private institutions, grouped in a node system with academic and administrative autonomy. It is responsible for the national inventory of microorganisms preservation, belongs to the World Federation for Culture Collections (CVCM-WFCC815), and is a founder member of the Federación Latinoamericana de Colecciones de Cultivos (FELACC). It maintains a database (CVCMdata), periodically publishes a catalog (www.cvcm.ucv.ve), and the most representative microorganisms are registered in the World Data Centre for Microorganisms (WFCC MIRCEN: www.wdcm.riken.go.jp). The central node has its main office at the Instituto de Biología Experimental (IBE) of the Universidad Central de Venezuela (UCV) and maintains a wide collection of bacteria, actinomycetes, plasmids, bacteriophages, transposons and clonation vectors. The node of the Laboratorio de Procesos Fermentativos (IBE-UCV) maintains a collection of entomopathogens, phytopathogens and antagonistic fungi of the genera *Aspergillus*, *Beauveria*, *Metarrhizium*, *Nomurea*, *Paecilomyces*, *Sclerotium*, *Trichoderma* and *Verticillium*. The node of INHRR is a collection approximately formed by 1500 fungi strains, mainly isolated from clinical and environmental samples. It had preserved more than 135 species and varieties of filamentous fungi representative of the genera *Absidia*, *Alternaria*, *Aspergillus*, *Chrysosporium*, *Cladophialophora*, *Coccidioides*, *Conidiobolus*, *Curvularia*, *Exophiala*, *Fonsecae*, *Fusarium*, *Histoplasma*, *Mucor*, *Paecilomyces*, *Paracoccidioides*, *Phialophora*, *Piedraia*, *Pseudoallescheria*, *Pyrenochaeta*, *Rhinocladiella*, *Rhizopus*, *Scedosporium*, *Sporothrix* and *Syncephalastrum*, among others, and a collection of yeasts mainly represented by the genera *Candida* and *Cryptococcus*. This node is the national reference center in mycological diagnostic, guarantees the preservation of autochthonous strains and offers support in investigation to the universities and postgraduate degrees in mycology. The CVCM system is a decentralized organizational model that locally assures the ex situ preservation of the microbial diversity in the region, and it has been taken as an organization model for the creation of the national system of culture collections.

PP-04-46

ClinicalSurveys.net - a web-based research portal for rare infectious diseases

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Studying rare medical events is a difficult and tedious task. Obtaining the necessary funds for randomized multi-center trials is challenging. The same is true for the observation of other uncommon events, e.g. rare drug toxicities or drug efficacy against certain disease subtypes, e.g. rare fungi. In this situation, clinical research often resorts to case registries, surveys or cohort studies. However, many investigators are lacking time, know-how and financial resources to setup a secure, convenient and efficient online survey on their own. Also, clinicians willing to contribute to clinical surveys may lack overview on what data are currently needed by whom. There is a need for a common platform bundling related topics to increase awareness for current research topics and create synergies between projects.

We have developed ClinicalSurveys.net to provide ID researchers and clinicians with a user-friendly online portal, allowing common access to a whole range of clinical surveys. Employing a customized version of Globalpark's (Huerth, Germany) internationally acclaimed 'EFS Survey' technology, ClinicalSurveys.net allows rapid and intuitive survey development with powerful features for design and data-control. The documenting user is provided with a simple, self-explaining documentation system accessible from virtually every browser on every available platform.

Fungiscope.net is the first survey conducted via the ClinicalSurveys.net platform, with 21 researchers from America, Asia and Europe registering 83 cases of rare invasive fungal infections. Surveys on spondylitis and meningitis in HIV patients and the customary treatment uses of antifungal drugs are already under development by different working groups.

ClinicalSurveys.net was created to answer pressing research needs. The service is provided to other academic researchers at cost price. None of the authors receive any financial gain from the site operation. We cordially invite ID research groups to conduct their case registries and surveys using the ClinicalSurveys.net platform.

PP-04-47

Rapid identification and diagnosis of *A. fumigatus* and Aspergillosis

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A. fumigatus is the most common pathogen which is only the second to *Candida albicans* in clinical fungal infection. Therefore, to find a rapid and specific identification method to *A. fumigatus* has considerable significance of diagnoses, therapy and epidemiologic studies for aspergillosis.

In this study, we established three kinds of methods to diagnose aspergillosis, like multiplex PCR (M-PCR), simple sequence repeat-PCR (SSR-PCR) and real-time PCR (RT-PCR). We designed genus specific primers of *Aspergillus, Fusarium* and *Mucor* which are very common in clinical infection, according to the TEF1 gene sequences published in Genbank. And using these primers performed M-PCR. Each genus was able to gain specific band of different length with *Aspergillus* 370bp, *Fusarium* 130bp and *Mucor* 560bp, while other pathogen could not amplified with these primers. We designed four kinds of microsatellite primers with repeat sequences about 15~20 nucleotides long, according to the complete gene sequence of fungi, then performed SSR-PCR. Using each primer, the strains of *A. fumigatus* all showed the clear, specific banding patterns, while other pathogen amplified had great different banding patterns from *A. fumigatus*. We also designed the Taqman probe and primers of mitochondrial translation optimization protein gene (MTO1) according to the published sequence of *A. fumigatus*, and the fragment amplified is about 450~500 nucleotides long. Then we performed two-step RT-PCR with 23 species of *Aspergillus* and 4 genera of other fungi. Only *A. fumigatus* was able to be amplified, while other species of *Aspergillus* had never amplified. And there had no cross-reaction with other pathogenic fungi. This study indicates these three methods can identify *A. fumigatus* rapidly, sensitive and specific. It will supply scientific basis to diagnose and therapy of aspergillosis and it has a better application prospect.

PP-04-48

Identification of *Aspergillus* section *Nigri* by Cyt *b* gene, rDNA and morphology

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Aspergillus section *Nigri* is included in very important species because they are used in fermentation industries and they also are encountered as human and plant pathogens and produced mycotoxins. The concept of black aspergilli has been classified as the *Aspergillus niger* group by Raper and Fennel and *Aspergillus* section *Nigri* by Games et al. In the past, the identification, classification and taxonomy of this group had mainly been based on morphological characteristics.

D1/D2 region of ribosomal DNA was broadly used for identification of fungi and other organisms. However, it did not sufficient for identification of species on fungi. The observation of conidiospore surface by scanning electromicroscope (SEM) is useful methods for identification of section *Nigri*. The typical morphology of conidiospore surface of strain is ease however, some strains show intermediated morphology.

Although some of the species can be readily distinguished morphologically, results obtained in several attempts at classifying this section are debatable and identification of some species is still difficult.

The partial mitochondrial cytochrome *b* gene (Cyt*b*) was analyzed for identification, classification and phylogeny of pathogenic fungi by L. Wang et al., S.K. Biswass et al. and K. Yokoyama et al.. DNA type of section *Nigri* were divided to 14 types and amino acid type were divided to 5 types. *A. japonicus* were divided into 4 DNA type, include in *A. aculeatus*. *A. niger* were divided into 4 DNA type,

We compared among D1/D2, Cyt *b* and SEM, The results of SEM observation show continuous variation of conidiospore surface. Phylogenetic tree of D1/D2 and Cyt*b* sequences were difference. These were different evolution of nucleus, cytoplasm and total genetic expression (morphology).

PP-05-1

Composting piles as an emission source of *Aspergillus* spores

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Composting facilities have emerged in recent years in which exposure to bioaerosols can be abundant. Handling of organic wastes which is used for the production of compost is a source of exposure to bioaerosols. Inhalation of bioaerosols can cause a variety of inflammatory, hypersensitivity and allergic responses in the lungs, especially in sensitized individuals. Among etiological factors of these diseases an important role is played by fungi especially *Aspergillus* spores. *Aspergillus* spores are well known sources of allergens that play a role in the development of hypersensitivity. A specific exposure with high risk of occupational disease is that *Aspergillus fumigatus*, a known opportunistic pathogen which under prolonged exposure conditions may cause invasive aspergillosis. The aim of this study was to evaluate fungi emissions from an urban waste composting facility in Isfahan, Iran. Anderson biosampler was used to collect samples for a six-month period at 8 point on the composting site and one off-site as the background sample. Air temperature, relative humidity and wind speed were also recorded at the time of sampling. Total fungi and *Aspergillus* spore concentrations were 763 and 408 cfu/m3 at composting piles as compared to 260 and 86 cfu/m3 background concentrations, respectively. *Aspergillus* spore concentrations at composting piles had a range of 71-2571 cfu/m3, of which *Aspergillus fumigatus* were the predominant taxon with a range of 0-1553 cfu/m3. Levels of *Aspergillus fumigatus* were significantly highest at composting piles and depleted by distance to background concentrations. There was a significant decrease of *Aspergillus fumigatus* concentrations with increasing of humidity and temperature in composting pile samples. These results indicated that composting process could increase levels of exposure to *Aspergillus* spores and poses a potential health risk for composting workers.

PP-05-2

Occurrence of aflatoxin M_1 in dairy products in Southern Italy: Results of a screening program

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Background. In accordance with the current European norms, the aflatoxin M_1 (AFM_1) concentration in milk must not exceed 0.05 microg/kg, while there is not a common European threshold limits for dairy products. The Italian Ministry of Health established a maximum permissible value of 0.45 microg/kg of mycotoxin in hard, long term ripened cheeses. Other states adopted lower limits: 0.25 microg/kg in Switzerland, Iran and Turkey, 0.20 microg/kg in Holland.
The present research is a screening program aimed to evaluate the presence of AFM_1 exceeding the threshold limits in cheeses produced in the Apulia Region (Southern Italy).
Materials and Methods. The presence of AFM_1 was tested, by using the ELISA test, on 265 samples of cheese made from cow, buffalo, goat, sheep, sheep-goat milk. Selected samples included unripened, medium and long-term ripened cheeses. The chi^2 test was used to assess the significance of comparisons of the positive percentages of cheeses at different stages of ripening and milk origin, setting the significance level at $p<0.05$. The software package SPSS version 16 Italian was employed.
Results. AFM_1 was found in 16.6% of the analyzed samples. No levels of AFM_1 exceeded the thresholds of 0.25 microg/kg. The highest positive incidence was for medium and long-term ripened cheeses, especially those made from sheep-goat milk, while buffalo cheeses tested consistently negative. No differences were shown considering the different kind of cheeses.
Conclusion. The level of contamination by AFM_1 in dairy products from Apulia Region are lower than in other Italian and European regions. Anyway, since AFM_1 is well known to be mutagenic and carcinogenic, a common international regulation ensuring a minimal presence of this AFM_1 in cheeses is needed. In this way will it be possible to better guarantee the distribution of safer, healthier foods, particularly in light of the norms on internal controls and HACCP.

PP-05-3

Aspergillus spp and building yards in hospital: A possible correlation?

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Background. The presence of building yards close to hospitals is considered a risk factor for epidemics caused by *Aspergillus* spp. In fact, air is considered the first vehicle of this fungus, whose spores persist in the air for a long period. Aim of the present study was to evaluate the hospital environmental conditions both before (T1) and during (T2) the presence of a building yards, by investigating the microbiological contamination of its rooms.
Methods. During the study period 4 building yards (2 outside and 2 inside the hospital) were observed. To evaluate the Air Total Microbial Count (TMC) and Total Fungi Count (TFC) the Surface Air System (S.A.S.) sampler - with respectively Wurtz Agar plates and Sabouraud Agar plates (SAPs) - was enrolled. For each hospital room 5 air samples were collected (4 corners and 1 centre) both at T1 and at T2. Moreover, the air suction drain gates contamination was evaluated by using a swab that was seeded onto a SAP. Information regarding the special control measures that the rooms underwent during the building yards were collected.
Results. When the investigated rooms were effectively isolated from the building yards, no differences were observed in the TMC and TFC between T1 and T2. On the contrary, the rooms not well isolated showed higher CMT and the presence of *Aspergillus nigere Aspergillus Fumigatus* at T2 than T1. The suction drain gates tested always negative for *Aspergillus* spp.
Conclusion. The results of the presents study underline that a effective isolation of the hospital environments from the building yards is able to ensure a better air quality and an healthier life for patients and hospital personnel. Moreover, when a building yards is close to an hospital it is important: a preliminary risk assessment, an environmental monitoring and an adequate cleaning program.

PP-05-4

Allergic rhinitis in office worker and airborne fungi in a building with Heating, Ventilation, and Air Conditioning system

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Visible mold in a large building with Heating, Ventilation, and Air Conditioning system (HVAC system) is of concern since prevalence of allergic rhinitis in office workers was dramatically increased. Several factors contributed to allergic rhinitis and air borne fungi were subjected to one of these contributions. The objective of this study was to examine the effect of air borne fungi on allergic rhinitis in office workers who worked in a large building with HVAC system in Bangkok, Thailand. The replication of bioaerosol was collected using an air collector model Sampl'Air Pro packed with sabouraud dextrose agar at the flow rate of 100 ml/min for 1 minute along with environmental-related factors monitoring. The plates were incubated at 25°C for 7 days. *Penicillium*, *Cladosporium*, *A.Niger*, *A.flavous*, *Uniden*, *Rhizopus*, *A.fumigatus*, *Stachybotrys*, *Fusarium*, *Clurvuria*, *Rhodotorula* were isolated. This amount of airborne fungi showed 198 CFU/m3 of mold. Amount of airborne fungi significantly associated with carbon dioxide and relative humidity ($p < 0.05$). History of asthma, visible mold, and a carpet decoration were shown as factors significantly related to allergic rhinitis in the workplace ($p < 0.05$).

PP-05-5

Development of analyzing system for microbial flora on board International Space Station and astronauts

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Humans in space are exposed space radiation, microgravity and stresses. Moreover, astronauts will face a variety of microorganisms growing on board space station, especially fungi. To survey the micro-biota especially fungal flora on board a Japanese International Space Station (ISS) module KIBO from brand-new to well-use condition, we need to prepare the analyzing system for microbial flora on board ISS and astronauts. We recognize the microbiological problems on ISS as followings. 1) The environment on board a space-station is controlled to be comfortable for astronauts and also for saprophytic microorganism. Therefore, it is possible that the crews and equipments exposed to a high concentration of microbes especially fungal spores in the closed system. This makes it essential to investigate the microbial flora present in spacecraft and space stations in order to be able to control microbial infection, allergy and disaster. The isolates will be potential pathogenic and they may cause different or atypical clinical features in the environment of space station. 2) It is well known that the incidence and changing of normal flora represent the host-immunology and host-parasite relationship. Especially in-flight, we need to intensively investigate not only the environmental micro-biota but also the normal micro-biota in flight Crew members.

PP-05-6

Toxicity of indoor moulds

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Sick building syndrome (SBS) is a term used for ill health symptoms commonly associated with staying in buildings with poor indoor air quality. Indoor humidity is a major factor related to (microbial) fungal contribution to the SBS. Respiratory toxicity of the most frequent indoor moulds - under Slovak dwellings' conditions (*Aspergillus versicolor, A. ustus, Penicillium expansum, P. chrysogenum* with extrolites' profile characterized by LC/MS/MS) and top risky *Stachybotrys chartarum* - from public health point of view - was studied in vitro by a bioassay on chick tracheal cultures and by analyses of histological and biochemical alterations of rat organs (lungs) or cell cultures (lung cells type II, Clara cells), and in vivo after the intratracheal instillation in wistar male rats (ca. 200 g) in 3-days experiments. Pure solvent (2 % dimethylsulphoxide - negative control) and standards of mycotoxins potentially produced by the fungi tested (sterigmatocystin, patulin, ochratoxin, diacetoxyscirpenol; 20 or 4 microg/ml) were used. Hematological parameters (leukocyte, erythrocyte and trombocyte cell counts, hemoglobin and hematocrit), cytotoxic (phagocytic activity and viability of alveolar macrophages - AM, the lactate dehydrogenase and acid phosphatase acitivites) and inflammatory response biomarkers (broncholaveolar lavage fluid -BALF cell counts, number of AM, granulocyte and AM differentials in BALF cell counts), incl. oxidative stress biomarkers and DNA-damage potential (comet assay) were measured in blood or the BALF. All fungal metabolites tested showed certain toxic effects that were concentration and cell origin of the toxicant (exo- or endometabolite) dependent. Generally, exometabolites (produced by fungi into their growth medium) were able to damage upper and lower airways and cause hematological disorders in rats in vitro, or in vivo respectively, much stronger. Lasting/repeated exposure to the indoor moulds and their metabolites may contribute to severe, even irreversible, health mutations in occupants of affected buildings, esp. young children.

PP-05-7

Comparative performance of two air samplers and mycological media for quantification of fungal aerocontamination in a poultry facility

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Fungal elements represent a significant part of the biological contaminants that could be detected in the air of animal facilities especially densely populated and enclosed poultry facilities. The aim of this study was to assess the performance of two air samplers and to compare different culture conditions for quantitative evaluation of fungal exposure in a poultry facility in France. The study was carried out in a 400 m2 building with 4300 broiler chickens. Air samples were collected every week throughout a 15-week period. Two devices were simultaneously used: CIP 10-M (Arelco, France), which allows the collection of airborne particles in a liquid, and AirPort MD8 (Sartorius, Germany), which allows the collection of airborne particles onto a gelatine membrane. Malt extract agar (ME) and dichloran glycerol-18 (DG18) were used. The effects of environmental parameters (temperature, relative humidity and animal density) on retrieval of airborne fungi were evaluated. Considering the whole sampling period, the mean of total indoor fungal concentration with CIP 10-M and AirPort MD8 were 161.5 and 95.7 cfu/m3 on DG18 (at 25C), 123.6 and 101 cfu/m3 on ME (at 25C), 95.1 and 85.80 cfu/m3 on ME (at 37C), respectively. Strong variations of cfu values occurred during the sampling period. CIP 10-M and AirPort MD8 were shown to display comparable performances but significant differences were observed between culture conditions for *Aspergillus* spp, *Scopulariopsis* spp. and the group of fungal organisms that could not be identified at the genus level. For *Aspergillus* spp. higher cfu values were obtained with DG18 and ME at 25C than with ME at 37C. For *Scopulariopsis* spp. higher cfu values were obtained with ME at 37C than with DG18.

PP-05-8

Year to year screening of airborne fungi in hotel environments

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High levels of indoor airborne fungi could have negative impact on human health since many them are known to induce respiratory diseases particularly allergies and mucous irritations. It is well known that low efficiency filters, stagnant water and dust of air-conditioning systems can increase counts of indoor airborne fungi. The objective of this study was screening of airborne fungi in hotels (Croatia) which have air-conditioning (cooling and heating) systems. Samples were collected between March and June in 2006 (N=22), 2007 (N=22) and 2008 (N=22) using Petri plate gravitational method on 2% Sabouraud-glucose agar supplemented with antibiotics. Average levels of airborne fungi were 4.5 CFU/plate/h (2006), 4.1 CFU/plate/h (2007) and 6.6 CFU/plate/h (2008) and were not statistically differ. Aeromycological study of outdoors in Croatia conducted in the same period showed that levels of airborne fungi were significantly higher (20-40 CFU/plate/h). Also previous studies in naturally ventilated indoors obtained similar levels as it was observed outdoors. Frequency and composition of aeromycota in hotels showed year to year similarity. Species of *Cladosporium* (59-63%) and *Penicillium* (41-63%) dominated over *Alternaria* (18-27%), *Aspergillus* (18-22%), *Acremonium* and *Epicoccum* (13-18%). Other detected fungi recovered from 4.5 to 9% of samples. In the second step 4 isolates of penicillia and 10 isolates of aspergilla were screened for ochratoxin A (OTA) and sterigmatocystin (ST) production by TLC semiquantitative method. Among isolates only one strain of *P. verrucosum* was able to produce OTA (6 mg/kg). Our earlier screening of outdoor *Aspergillus* isolates detected 6/18 *A. verisicolor* ST-producers (3.8-120 mg/kg). Taking into account detected levels of airborne fungi in hotels we could conclude that risk of fungal respiratory diseases and toxicoses is very low. The results also suggest that air-conditioned systems which are well designed and well-maintained could reduce the levels of potentially toxigenic airborne fungi in particular indoor environments.

PP-05-9

Professional exposure to airborne fungi in industrial environments-a pilot study

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Poultry production and wood industry are significant sources of microbial air contamination. There are numerous reports on health outcomes in occupants exposed to airborne fungi including mucous membrane irritations, imunotoxicity and allergic diseases. The objectives of this pilot study were screening of airborne fungi in poultry farm (PF) and wood-producing factory (WPF) in Croatia and evaluation of potential risk of respiratory diseases. Thirty samples were taken at each industrial facility and outdoors (approx. at 200 m of distance), in October 2008 using airsampler Mas 100 (Merck) and Malt agar plates supplemented with antibiotics. Significantly lower concentrations of airborne fungi were detected in WPF (mean $1.8x10^3$ CFU/m3) compared to PF ($3.2x10^3$ CFU/m3). 43% of samples from WPF were taken near working saws and had significantly higher levels of airborne fungi (mean $3.5x10^3$ CFU/m3) than samples collected at few meters of distance from saws (mean $5x10^2$ CFU/m3). Levels of airborne fungi in PF were in the same range. Outdoor concentration of airborne fungi was five times lower (mean 430 CFU/m3) than in PF and WPF suggesting the potential risk of developing respiratory diseases in occupants. Species of Rhizopus (63%), *Penicillium*, Cladosporium and *Scopulariopsis* (about 50%), *Aspergillus* and Trichoderma (16%), Alternaria and Paecilomyces (13%) prevailed in PF, while *Penicillium* (80%) and Cladosporium (63%) dominated over Paecilomyces and *Aspergillus* (37%), Chrysonilia and Trichoderma (about 30%) and Mucoraceae (10%) in WPF. Majority of identified fungi are potential allergens and some of them are potential producers of mycotoxins (species of *Penicillium* and *Aspergillus*), which possess imunotoxic activity. This pilot study contributes to the better understanding of the levels and composition of airborne fungi in particular industrial facilities and could be applied for creation of measures for reducing their levels in occupational environments as well as for standard drafting.

PP-05-10

Two cases of allergic bronchopulmonary mycosis caused by *Schizophyllum commune*

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Schizophyllum commune (*S. commune*) is a basidiomycetes fungus found in Japan, commonly on the surface of dry logs, dead wood and fallen trees. It recently has been identified as a causative agent of mucoid impaction of the bronchi (MIB) and allergic bronchopulmonary mycosis (ABPM). MIB is an uncommon condition indicating segmental and subsegmental bronchi characterized by the dilatation and filling of bronchi with characteristic thick mucoid material. MIB is thought to occur most commonly as a manifestation of a hypersensitivity state in association with ABPM.

We describe two patients with MIB/ABPM caused by *S. commune*. They showed eosinophilia and serum IgE levels increased. A mold was isolated from their mucous plug which was identified as *S. commune*.

ABPM is considered to be the result of an immunologic inflammatory reaction in the bronchi and the surrounding parenchyma in response to antigens (fungi) growing in mucous plugs in the airways. Steroid therapy generally is used for allergic aspect of ABPM, however, an infectious aspect has recently been recognized on the basis of the effectiveness of antifungal therapy for ABPA and histological findings of ABPM. A case 1 patient improved with administration of itraconazole (ITCZ) and corticosteroid (CS). A case 2 patient improved with CS administration.

There are some reports about lung infection due to *S. commune*, however, to our knowledge, only 18 cases (including the present cases) have been reported. We report two cases of ABPM caused by *S. commune* infection about the way to make a diagnosis and clinical course.

PP-05-11

Early identification of ochratoxin A-producing species using an electronic nose

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An electronic nose system using an array of metal oxide sensors (FOX3000, Alpha M.O.S.) was used to detect and discriminate between two ochratoxigenic species, *A. carbonarius* and *A. niger*, responsible for contamination of wine and other wine grape products, using volatile production patterns. The sensor format of this system is based on metal oxides. The oxide materials in these sensors contain chemically adsorbed oxygen species, which can interact with the volatile molecules, thus altering the conductivity of the oxide. This induces a change in the resistance of the sensor that is monitored as a raw signal. The data is normalised and then used for analyses. These ochratoxigenic species were grown on three culture media, Czapek Dox modified (CDm) agar, yeast extract sucrose (YES) agar and white grape juice (WGJ) agar, and the qualitative volatile fingerprints produced in the headspace were evaluated over periods of 48-120 h. The system was able to differentiate between the two species within 48h of growth on YES and WGJ agar using PCA which accounted for 99.9% and 97.2% of the data respectively, in principal components 1 and 2, based on the qualitative volatile production patterns. This differentiation was confirmed by cluster analysis of data. However, it was not possible to separate these species on CDm agar. Our results show that the two closely related ochratoxigenic species responsible of the contamination of wine and other wine grape products can be discriminated by the use of qualitative volatile fingerprints. This approach could have potential for rapid identification of *A. carbonarius* and *A. niger* on wine grape samples, reducing significantly the time of detection of these ochratoxin A potentially producing species.

PP-05-12

Ochratoxin A producing species of the genus *Penicillium* in retail wheat flours and feedstuffs from the Spanish market

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In Spain, low ochratoxin A (OTA) levels have been detected in wheat products and in several pig products but there is no information about the fungi involved in this OTA contamination. About twenty species belonging to the genera *Penicillium* and *Aspergillus* are known to form OTA but few of them are known to contaminate foods with this mycotoxin. *Penicillium verrucosum,* an important OTA producer typical of temperate and cold climates, is much more frequently found on cereals in countries where they occasionally have OTA problems as in North European countries compared with South Europe where levels of OTA generally seem to be lower or not detected. The aim of this study was to determine, identify and characterize the occurrence of potential OTA producing *Penicillium* spp. from retail wheat flours used for human consumption and feedstuffs purchased in the Spanish market. A total of 28 suspected *P. verrucosum* isolates were recovered from these substrates and were confirmed by RAPD analyses. From these isolates, 22 (79%) were OTA producers. Our results confirm the potential risk of OTA production in these foods and feedstuffs if stored improperly and corroborate the occurrence of *P. verrucosum* in South European countries.

PP-05-13

Isolation and identification of aflatoxin-production inhibitory bacteria from soil samples of Pistachio orchards, Damghan-Iran

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A total number of 100 soil samples (200 grams each) obtained from Pistachio orchards, Damghan-Iran during October 2008 were studied for *A. parasiticus* growth and aflatoxin (AF)-inhibitory bacteria. The soil sample suspensions prepared in sterile normal saline solution were cultured on glucose 2% - yeast extract 0.5% (GY) agar in presence of a non-aflatoxigenic nor mutant of *A. parasiticus* which accumulated the aflatoxin precursor norsolorinic acid (NOR) under conditions conducive to aflatoxin production. Inhibitory soil samples were selected based on the visual inspection on inhibition of orange-red pigment (NOR) production by the fungus. All bacteria were isolated from the inhibitory soils and they were examined for inhibitory activity toward *A. parasiticus* growth and/or NOR production by a visual agar plate assay. Among a total number of 287 bacteria isolated, 145 bacteria were found to inhibit *A. parasiticus* growth (45 isolates), NOR accumulation (28 isolates) or both (72 isolates). Morphological and chemical identification of the bacterial isolates showed that they were mainly belonged to the genera Bacillus, Arthrobacter, Pseudomonas, Entrobacter, and Ralstonia. Molecular identification of the isolated bacteria and purification of AF-inhibitory compounds from the most effective ones are being performed in our laboratory.

Poster Forum PF-01

PP-06-1

Usefulness of PCR-Elisa assay for detection of *Trichophyton rubrum*, *Trichophyton interdigitale, Epidermophyton floccosum* and *Microsporum canis* in skin scrapings and nails in routine laboratory diagnostics

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A uniplex PCR-Elisa assay has been used for direct assessment of dermatophytes in clinical samples. The specific target sequence of the primers (one of them labeled by digoxigenin) was the topoisomerase II gene. DNA isolation was carried out by Qiagen QIAamp DNA Mini Kit. The PCR products were hybridized by biotinylated probes. The biotinylated hybrids were immobilized on streptavidin-coated wells, and detected by using peroxidase-labeled anti-digoxigenin antibody in a colorimetric reaction. Nail specimens were investigated for *Trichophyton (T.) rubrum*, *T. interdigitale*, and *Epidermophyton floccosum*. Skin scrapings were tested for Microsporum canis, additionally. The sensitivity of the PCR was compared to those of the fluorescence preparation (Calcofluor®) and cultural isolation. In 1414 out of 3664 samples (duration of the study 10 months) dermatophytes could be detected using cultivation and/or PCR. 960 (68 %) samples were positive both in culture and PCR, in 201 (14 %) samples a dermatophyte grew, but PCR was negative. In 253 samples (18 %) the culture was negative, however PCR was positive. The diagnostic sensitivity of the Calcofluor® preparation was 80.1 %, when compared to culture. The specificity was found to be 80.6 %. The diagnostic sensitivity of the dermatophyte culture was 82.1 %, the specificity 100 %. The sensitivity of the PCR was 85.8 %, higher than that of cultivation. Among 1414 detected dermatophytes 68.8 % were *T. rubrum*, 20.1 % *T. interdigitale*, 0.8 % Epidermophyton floccosum, and 0.3 % *Microsporum canis*. In conclusion, the PCR-Elisa assay completes conventional laboratory diagnostics of dermatophyte infections. The assay appears to exhibit high sensitivity and specificity. This rapid method reduces duration of laboratory diagnostics and is a cost-effective procedure. The technique could prove suitable for use in the routine examination of clinical specimens in dermatology and is a promising option for rapid and direct identification of dermatophytosis.

PP-06-2

Trichophyton rubrum detection in scales and nails by use of conventional methods and polymerase chain reaction (PCR)

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In Germany Trichophyton (T.) rubrum is the most common cause of chronic dermatophytosis. However, the conventional diagnostic methods are time-consuming and not of satisfactory sensitivity. Straight proof of fungal DNA in tissue samples by PCR-methods is hoped to improve diagnostic speed and sensitivity.

In this study we compared the detection of T. rubrum by conventional methods and by a PCR-based proof of fungal DNA within lesional tissue. Material collected from clinically suspected tinea and onychomycosis sent to our laboratory for diagnostic purpose was used for KOH-mounts, fungal cultures and direct PCR analysis. DNA was extracted by use of a commercially available kit and 30 cycles of a hot start PCR were performed with a primer set specific for T. rubrum-DNA.

408 samples of scales and 193 samples of nails were evaluated. Out of them, for scales and nails corresponding positive PCR and culture findings of T. rubrum were seen in 18.4 % and 20.7 %. A positive PCR but negative culture was found in 9.8 % and 24.4 % of scale and nail samples, and a positive culture but negative PCR was found in 3.2 % and 4.1 %. KOH-mounts showed some additional positive results that were not related to positive findings in T. rubrum-cultures or -PCR and could not be explained by growth of other fungi.

Our results show that the applied PCR-based detection of T. rubrum-DNA allows rapid identification of this fungus in cases of tinea and particularly of onychomycosis that are missed by cultures. However, it is not sufficiently sensitive to identify all infections with T. rubrum proven by positive cultures. Therefore it is currently a valuable diagnostic complementation but no substitute for conventional diagnostic methods.

PP-06-3

Enhanced gene replacements in Ku80 disruption mutants of the dermatophyte, Trichophyton mentagrophytes

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The frequency of targeted gene disruption via homologous recombination is low in the clinically important dermatophyte, Trichophyton mentagrophytes. The Ku genes, Ku70 and Ku80, encode key components of the non-homologous end joining (NHEJ) pathway involved in DNA double-strand break (DSB) repair. Their deletion increases the homologous recombination frequency, facilitating targeted gene disruption. To improve the homologous recombination frequency in T. mentagrophytes, the Ku80 ortholog was inactivated. The nucleotide sequence of the Ku80 locus containing a 2788-bp open reading frame encoding a predicted product of 728 amino acids was identified, and designated as TmKu80. The predicted TmKu80 product showed a high degree of amino acid sequence similarity to known fungal Ku80 proteins. Ku80 disruption mutant strains of T. mentagrophytes were constructed by Agrobacterium tumefaciens-mediated transformation. Average homologous recombination frequency was 75% for the areA/nit-2-like nitrogen regulatory gene (tnr) in Ku80- mutants, about 7-fold higher than that in wild-type controls. A high frequency (~67%) was also obtained for the Tri m4 gene encoding a putative serine protease. Ku80- mutant strains will be useful for large-scale reverse genetics studies of dermatophytes, including T. mentagrophytes, providing valuable information on the basic mechanisms of host invasion.

PP-06-4

A chromogenic substrate made of pulverized human nails to study the proteolytic activity of *Trichophyton rubrum*

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Trichophyton rubrum is a strictly antropophylic dermatophyte. To study part of the interactions with the human host, we developed a chromogenic substrate made of pulverized human nails conjugated with the dye remazol brilliant blue. In liquid media containing salts and 1% stained nail powder, *T. rubrum* progressively degraded the insoluble substrate and blue products were detected in the supernatant, whose absorbance was inversely correlated with the amount of keratin extracted from residual nail powder. Using this assay, twelve *T. rubrum* samples of six different genotypes were tested measuring the supernatant absorbance every 3 days, for 15 days. We observed differences in the ability to degrade the nail substrate among individual samples, but no correlation with the genotypes. Protein supernatants from samples with strong and poor degradation profiles were concentrated by ultrafiltration and analyzed by zymogram. Due to the particulate nature of our substrate, we first separated the proteins by polyacrylamide gel electrophoresis (without reducing agent), and then transferred them to another gel containing either gelatin or the chromogenic substrate. We identified four distinct proteolytic bands of 31-32kD, 36-37kD, 42-44kD and 46-48kD that could degrade gelatin. Only the two heavier polypetides (42-44kD and 46-48 kD) could degrade the nail powder. Gelatin degradation was abolished from the 31-32kD and 36-37kD bands when gels were incubated with 1mM PMSF, suggesting that they are serine proteases (subtlysins). The 42-44kD and 46-48kD bands corresponded to metalloproteases (fungalysins) since they were inhibited by 5mM EDTA. We concluded that this colorimetric assay has a good sensitivity and reproducibility to define different profiles of proteolytic activity among dermatophytes strains. The results indicate not only that *T. rubrum* degrades pulverized nail using metalloproteases, but also that the distinct activities observed may be due to differences in the rate of synthesis and secretion or in molecular structure of the enzymes.

PP-06-5

The microbiome of human skin infections

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The fungal infections of skin and nails, known as dermatomytosis, affect a high number of individual world-wide. Further, several studies suggest that about 30% of adults are non-symptomatic carriers of clinical relevant fungi. Within dermatophytosis, the athlete's foot disease and nail infections are prevailing. These typically nonfatal conditions are difficult to treat and require long and persisting treatments with antifungal agents, mostly ergosterol biosynthesis inhibitors as azols (e.g. ketoconazole, itraconazole and fluconazole), allylamines (e.g. terbinafine) and morpholines (e.g. amorolfine).

These dermatomytosis are caused by filamentous fungi such as Trychophyton, Microsporum or Epidermophyton species. These filamentous fungi have a high affinity for keratin, an important component of hair, skin and nails, which are the primary areas of infection by dermatophytes. However, it is believed that entire community of microorganisms (fungi, yeasts and bacteria) of infected areas is still far from being unveiled. In order to shed some light on the complete fungal communities in the dermatomycosis events, we have used next generation sequencing approaches to sequence all the ribosomal DNAs present in asymptomatic and symptomatic areas, which allowed an identification of all the microorganisms present in the samples. Further, we have also studied and compared the population of women and men. In global, we have been able to identify up to 100 different microorganisms, among fungi, yeasts and bacteria. Interestingly, we have identified several organisms which were not related with dermatomycosis, and we have also observed a significant difference between the male and female microbiome, which we are currently further exploring.

Poster Forum PF-01

PP-06-6

2006 epidemiological survey of dermatomycoses in Japan

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An epidemiological survey of dermatomycoses and the causative fungus flora of dermatomycoses in Japan for 2006 was made on a total number of 63029 outpatients who visited 16 dermatological clinics throughout Japan. The results were as follows.

1) Dermatophytosis was the most prevalent cutaneus fungal infection (7582 cases) seen in these clinics, followed by candidiasis (842 cases) and then *Malassezia* infections (283 cases).

2) Among dermatophytoses, tinea pedis was the most frequent (4779 cases: male 2358, female 2241), then in decreasing order, tinea unguium (2582 cases: male 1376, female 1206), tinea corporis (564 cases: male 341, female 223), tinea cruris (309 cases: male 254, female 57), tinea manuum (145 cases: male 92, female 53), and tinea capitis including kerion (17 cases: male12, female 5).

3) Tinea pedis and tinea unguium are seen to increase in the summer season, among the aged population. When compared to the last survey 2002 by clinical form, t. unguium patients increased 459 cases.

4) As the causative dermatophyte species, *Trichophyton rubrum* was the most frequently isolated among all dermatophyte infections except tinea capitis. Microsporum canis was slightly increased. M. gypseum and Epidermophyton floccosum are small number. T. tonsurans was increased up to 37 cases.

5) Cutaneous candidiasis was seen in 842 cases (305 male, 537 female). Intertrigo (298 cases) was the most frequent clinical form, followed by erosion interdigitalis (136 cases), oral candidiasis (135 cases), onychia et paronychia (108 cases), genital and diaper candidiasis in total (88 cases).

6) Tinea versicolor was seen in 175 cases. *Malassezia* folliculitis were collected 108 cases, 63 cases are reported from one clinic.

PP-06-7

Dermatophytoses for the past fourty years at one clinic in the northeast Japan. Tagajo, Miyagi, Japan

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This report shows the statistical changes of dermatophytoses and its causative fungi for the past 40 years from 1968 to 2007 at one clinic in the northeast Japan.The total number of dermatophytoses in this period was 15,730. The number of each clinical types as follows: tinea pedis; 12,767, tinea unguium; 4,321, tinea corporis; 2,084, tinea cruris; 1,424, tinea manuum; 737, tinea capitis; 83 and tinea barbae; 18. The annual incidence of tinea pedis and tinea unguim constantly increased for this period, and its age distribution sifted gradually to more older generation. Tinea cruris, on the contrary, decreased after 1980s especially in young generation. Tinea corporis also decreased in the same period gradually, but after year of 2000 it increased again among the young men. During the initial decade verious kinds of dermatophytes were isolated from all clinical types. In the recent years, however, its limited to a few species, and *Trichophyton(T.) rubrum* became the main species of all clinical types. Only in tinea pedis *T.rubrum* and *T.mentagrophytes* were equally isolated; *T.rubrum* was 4,120 and *T.mentagrophytes* was 3,750. *Microsporum canis* infection incresed in 1980s among the children and female adults, but after 1990 it gradually declined its number. *T.tonsurans* appeared first in the year of 2000, and after this year it spread rapidly in the whole country. In my clinic the most cases of this infection were consisted of the judo-athletes of several highschools or colleges and in addition one case of familiar infection. *T.verrucosum* was sporadically isolated among the family menbers of cattle breeders. From all cases of tinea barbae except one case *T.rubrum* was isolated.

PP-06-8

The most common dermatological disease in home medical care: Fungal infection

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Dermatological diseases in the elderly have been a major concern in recent years in Japan, especially in those receiving home care, because it is difficult for such subjects to be seen by dermatologists. According to the statistical survey published by the Japan Organization of Clinical Dermatologists, approximately 70% of patients in nursing homes or receiving home medical care have skin diseases. Among these diseases, fungal infection of the skin account for the largest percentage, with a high morbidity rate of 40% in patients receiving home medical care and 38.5 % in patients in nursing homes. In patients who are unable to care for themselves, caution must especially be exercised against the development of fungal infection in the external genital, gluteal, and buttocks regions as a result of inadequate care related to the toileting and bathing needs of these patients, as well as the daily use of diapers. Currently, one of the most important considerations is deemed to increase the number of home care dermatologists for these patients.

PP-06-9

Survey of keratinophilic fungi isolated from city park soils of Gorgan, Iran

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Soil can become a potential source of infection for humans. Keratinophilic fungi are a small but well defined and important group of fungi that degrade keratin. This survey is geophilic dermatophytes and related keratinophilic fungi isolated from city park soils of Gorgan using hair baiting technique. A total number of 244 samples were obtained from 22 park soils. keratinophilic fungi were isolated from 133 (54.5%) soil samples. The most commonly species in soil: Chrysosporium (23.8%), M. gypseum (19.3%), both (9.4%), paecilomyces (0.8%) *Aspergillus* flavus (0.4%).

Key words: Gorgan, dermatophytes, keratinophilic fungi, Iran

PP-06-10

Genotyping study of *Trichophyton schoenleinii* and *Microsporum canis* isolated from tinea capitis in Xinjiang province, west China

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Xinjiang is the largest and most westerly province in China. It is reported that tinea capitis has been epidemic in Uyghur (a minority of Chinese living in Xinjiang) school children in this province for recent 40 years, and caused by *Trichophyton violaceum*, *Microsporum ferrugineum*, *T. schoenleinii* and *T. verrucosum*, but rarely by *T. tonsurans* and *M. canis*. This time, we surveyed the tinea capitis of Uyghur school children in Hotan area (located at the extreme south west corner of the Taklamakan Desert). Their causative agents were *M. ferrugineum*, *T. violaceum*, *T. schoenleinii*, *T. verrucosum*, *M. canis*, and *T. tonsurans*. *M. canis* is one of common cause of tinea capitis in Central and Eastern China, but not previously reported from the Xinjiang. But recent two years cases of tinea capitis caused by *M. canis* are increasing. *M. canis* was isolated from 11 cases of tinea capitis in this survey. Genotyping study was performed for 26 strains of *T. schoenleinii*, and *M. canis* (including 5 Japanese strains used for reference) respectively by the inter-single-sequence-repeat (ISSR) PCR method reported by Jose Cno et al. (2005). We decided genotypes according to the electrophoresis by normal agarose, not by GeneScan™ polymer. Twenty-six *T. schoenleinii* strains showed 15 genotypes with primer ACA and 11 types with the primer CCA. Meanwhile, our previously study for genotyping of *T. schoenleinii* from Xinjiang showed this species had high genetic homogeneity. Twenty-six *M. canis* strains were divided into 17 genotypes with primer CCA and 5 types with ACA. Our results suggest that ISSR-PCR method have high reliability and reproducibility and it is available method for molecular epidemiological study of *T. schoenleini* and *M. canis*, to be able to determine the detail genotypes.

PP-06-11

Tinea capitis cases appearing in a Kumamoto clinic over a 5-year period

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We encountered 12 cases of tinea capitis at a clinic in Kumamoto prefecture from April 2004 to December 2008. Etiologic agents were Microsporum canis (6 cases) and Trichophyton tonsurans (6 cases). The affected patients were 11 boys, ranging in age from 2 to 18 years, and a 50-year-old woman. All 6 cases of tinea capitis caused by T. tonsurans occurred in judo athletes between 12 and 18 years of age. Patients with tinea capitis caused by M. canis included the middle-aged woman and 5 young boys, up to 8 years of age. The disease was non-inflammatory in 5 of the 6 M. canis cases and in 5 of the 6 T. tonsurans cases. The remaining 2 cases of tinea capitis were inflammatory. Patients infected with M. canis were treated with oral administration of itraconazole (ITZ) at doses of 2.4 - 4.0 mg/kg/day (3 patients) and or with oral administration of terbinafine hydrochloride (TBF) at doses of 2.6 - 4.6 mg/kg/day (3 patients). Treatment duration was 8-14 weeks with ITZ and 4-8 weeks with TBF. In the T. tonsurans cases, TBF was orally administered at doses of 1.4 - 2.4 mg/kg/day for 8-12 weeks. The patient with inflammatory T. tonsurans infection (Case 8) and one of the patients with non-inflammatory M. canis infection (Case 11) did not show improvement in symptoms after TBF and ITZ treatments, respectively, were initiated at the dosages recommended in Japan. Dosages were increased twofold according to European and North American guidelines and both patients recovered. It may be appropriate to re-examine treatment guidelines in Japan, as tinea capitis is frequently refractory to treatment.

Poster Forum PF-01

PP-06-12

Adult tinea capitis in Taiwan

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Tinea capitis is an infection of scalp and associated with hair which is common in children but rare in adults. The pathogen varied greatly to geographic regions and changes with time. During July, 2004 to December, 2008, 61 adult cases (57 female and 4 male) were diagnosed as tinea capitis in Mackay Memorial Hospital, Taiwan. Ages range from 35 to 88 with 9 aged 35-49 (15%), 8 aged 50-59(13%), 15 aged 60-69(25%), 22 aged 70-79(36%), and 7 aged 80-88(11%). 62 pathogens were isolated, including 32 *Trichophyton violaceum* (52%), 18 *Microsporum canis* (29%), 5 *Trichophyton tonsurans*, 4 *Trichophyton rubrum*, 1 *Trichophyton mentagrophytes*, 1 *Trichophyton verrucosum*, and 1 *Microsporum ferrugineum*. Combined *T. violaceum* and *M. canis* infection was noted in one case. The clinical presentations varied, including inflammatory papules, pustules, alopecic patches, black dots asymptomatic broken hairs. Patients responded to systemic antifungal agent treatment well. However, long carrier state of patient infected by zoophilic *M. canis* after treatment was noted.

PP-06-13

Success of posaconazole in the treatment of tinea capitis profunda caused by *Trichophyton verrucosum*: A case report

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Introduction

In the Czech republic the typical causative agent of tinea capitis is Microsporum canis. Cases caused by *Trichophyton verrucosum* occur very seldom. Following is a description of tinea capitis profunda caused by *Trichophyton verrucosum* infection. The deep wound was succesfully treated with the new azole derivate posaconazole.

Patient and treatment

An eight-year-old boy suffered an injury in the parietal region and stayed on his grandmother's farm. A number of complications arose during the healing process. Initially, an empiric therapy with cefazolin was advised. The cultivation of the wounds showed Staphylococcus aureus cefazolin senzitive. Fourteen days after the accident the patient was hospitalized because the wound was not healing. At this time the wound measured 10 cm square and the cranium was actually exposed. New abscesses developed in the vicinity of the wound. A surgical revision to remove necrotic material and draining of the wound was performed. A direct microscopic examination showed fungal elements. Amphotericin B was locally instilled, followed by a treatment with a new azole derivate posaconazole. A culture showed evidence of *Trichophyton verrucosum*. Based on the very positive clinical effect, the treatment with posaconazole was continued. After another four weeks eczema appeared. The patient was prescribed terbinafine in order to complete the healing process. At a follow-up exam after three months, a hairless scar was remaining. Plastic surgery was recommended.

Conclusion

Tinea capitis profunda caused by *Trichophyton verrucosum* is very uncommon in our geographic area. The surprising extent of the area affected was caused not only by the aggressiveness of *Trichophyton verrucosum* but also very likely by the delay in establishing the exact diagnosis. The new preparation of posaconazole was found to be very effective in the healing process.

PP-06-14

Chronically recurrent and disseminated tinea faciei/corporis; autoinoculation from asymptomatic tinea capitis carriage

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We report clinical findings in a 14-year-old girl with long-term recurrent and disseminated multiple eruptions of tinea faciei and tinea corporis, which persisted for ten years. The eruptions disappeared in response to topical antifungal treatments but recurred within a short time on different areas of her body. Mycological examination revealed the dermatophyte Trichophyton tonsurans in both scale samples from the body lesions and in brushing samples from her asymptomatic scalp. These observations suggested that she was an asymptomatic dermatophyte carrier on the scalp and autoinoculation of the dermatophyte was responsible for the recurrent and disseminated tinea faciei/corporis. This case indicates the importance of surveying the scalp as a source of pathogenic dermatophytes in patients with chronically recurrent tinea faciei/corporis.

PP-06-15

Molecular epidemiology of Trichophyton tonsurans isolated in Japan between 2006 to 2008

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Background:
Trichophyton tonsurans has been widely isolated from an epidemic of dermatophytoses among judoists, wrestlers and sumo wrestlers in Japan. A previous study using polymorphisms of non-transcribed spacer region (NTS) of ribosomal RNA gene(rDNA) revealed the epidemic among judoist and that among wrestlers had different sources of causative fungus(1). Since that study, many fungal strains have been isolated from these sports continuously.
Objective:
The present study was conducted to evaluate the fungal characteristics of the epidemic in Japan using the same molecular method used previously (1,2) with newly isolated strains.
Methods:
PCR-RFLP was performed on 158 strains of T. tonsurans isolated between July 2006 and December 2008. Of the strains, 120 were isolated from judoists, 16 from wrestlers and 17 from sumo wrestlers. PCR targeted on NTS of rDNA were performed and then the RFLP types were observed by digestion with Mva I and Ava I.
Results and Discussion:
Four molecular types, NTS I-IIIand VII(1,2) were detected. As the previous report (1), NTS I was the most predominant type, being in 146 strains. All the strains isolated from sumo wrestlers were NTS I. This supports the argument that the epidemic of sumo wrestlers was caused by T. tonsurans brought by judoist contaminated by NTS I strains. NTS II was found in 8 strains, 7 from wrestlers and one from a judoist. NTS III was found in 3 strains and NTS VII was found in one strain.
References:
(1)Mochizuki T et al. Jpn J Infect Dis 60:188, 2007.
(2)Mochizuki T et al. Jpn J Infect Dis 61:219, 2008.

PP-06-16

Screening examination and treatment of T. tonsurans infection in judo athletes affiliated with the University Judo Federation of Tokyo

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In Japan, the spread of T. tonsurans infection among combat sports participants continues to be a problem.
We used the hairbrush method and a questionnaire to survey athletes registered with the University Judo Federation of Tokyo who participated in National Championship judo matches in 2008. Treatment was offered to athletes who tested positive for T. tonsurans infection.
We surveyed 902 individuals (754 males and 148 females) affiliated with Judo clubs in 21 universities (males in 16 universities, females in 5 universities) and found 102 positive cases (11.3%). Males had a higher infection rate (95 cases; 12.6%) than females (7 cases; 4.7%).
Ninety individuals (88.2%) with positive hairbrush-culture results reported a history of tinea corporis, and 88 individuals (86.3%) with positive hairbrush-culture results considered themselves 'asymptomatic' at the time of the examination.
Ninety-six of the 102 individuals who tested positive for T. tonsurans infection accepted treatment with oral and topical antifungal agents. After 3 months of treatment, 85 (90%) were culture-negative.
Compared to earlier survey findings, the current survey indicates that the number of asymptomatic carriers of T. tonsurans has increased among college judo athletes, and infected athletes are less likely to be aware of their status. Screening examinations should be conducted periodically throughout the entire judo federation, followed by appropriate treatment of infected athletes. Development of treatment protocols for infected athletes, including asymptomatic carriers, is a high priority.

PP-06-17

The spread of *Trichophyton tonsurans* epidemic among the team members of body contact sports and their families

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Although *Trichophyton tonsurans* (*T. tonsurans*) is well-known as one of the causes of tinea in Europe and the USA, it was previously rare in Japan. However, the number of tinea patients caused by *T. tonsurans* has been increasing in judo, wrestling, and sumo team members since 2001 in Japan. We had five patients with tinea corporis and two patients with tinea capitis caused by *T. tonsurans* among judo and sumo wrestlers. Their skin eruptions were hair loss, erythema and papules on face, auricle, upper extremities, axilla and trunk, but they had no eruption on the lower half of the body including feet and toe nails. Skin and/or hair samples from all the patients were examined by microscopic examination with 10% KOH and cultured in Sabouraud's dextrose agar. From these examinations, *T. tonsurans* was identified in all 7 patients. Oral administration and topical treatments with antifugal agents were effective. However, one of the sumo wrestlers repeated the same symptoms several times, and his mother also had erythema with scale on the lower leg. A diagnosis of tinea corporis was made on her, because *T. tonsurans* was identified from the eruption. We consider that *T. tonsurans* is spread to sumo and judo wrestlers through body contact each other and also to their families. From these findings, it is necessary to explain the spread of the *T. tonsurans* epidemic among body contact sports to the team members and their families. In addition, we recommend that the team members of these sports should adhere to the following: 1) checking the skin before and after practice and matches, 2) showering after practice and matches, and 3) washing the training gear after practice and matches.

Poster Forum PF-01

PP-06-18

Tinea barbae caused by *Microsporum canis*

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We report a case of sycosiform tinea barbae caused by *Microsporum canis*. An 85-year-old male developed a severe inflammation of the bearded area of the face. He had used topical steroid on his face for 3 months. He had kept the cat for five months. Clinical diagnosis was verified by direct microscopic examination using potassium hydroxide (KOH) and Wood's light examination. Under examination of the dermoscopy with Wood's light (3Gen Dermlite UVA®, CA), the infected hair showed fluorescence. KOH examination revealed many positive spores and fine filamentous structures. Infected hair without fluorescence showed lower incidence of spores and filaments. The trichophytin skin reaction was positive. .Identification of *M. canis* was confirmed by both micro-morphology and genetic analysis using internal transcribed spacer region of ribosomal DNA. The patient was successfully treated with terbinafine 125mg/day for 2 months. The passive finding of Wood's light turned to negative during the therapy.
Tinea barbae is an uncommon superficial dermatophyte infection of the beard and moustache areas which in most cases is caused by *Trichophyton mentagrophytes* or *T. rubrum*, but isolation of *M. canis*is rare in Japan. As the clinical presentation of tinea barbae can mimic several other skin disorders including sycosis, contact dermatitis, perioral dermatitis, and actinomycosis, direct microscopic examination is essential. Wood's light examination is an important tool for diagnosis and evaluation of clinical course of not only tinea capitis but tinea barbae.

PP-06-19

A case of Tinea barbae due to *Trichophyton rubrm* with dermoscopic findings

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Tinea barbae is an uncommon dermatophytosis that affects the hair and hair follicules of the beard and mustache. We report a case of tinea barbae caused by *Thrichophyton rubrum* in a 74-year-old Japanese man who was misdiagnosed as acne vulgaris and was receiving oral antibiotics. On physical examination he was also suffering from tinea pedis et unguium caused by the same dermatophyte species. Diagnosis was based on direct microscopic examination of the scrapings taken from the involved site and culturing. Dermoscopy has been proposed as a diagnostic tool in the case of skin infections but no specific dermoscopic criteria have been described for Tinea barbae. We describe dermoscopic features that we believe to be characteristic to tinea barbae.

PP-06-20

First case of tinea corporis by Microsporum Gallinae in Japan

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Microsporum gallinae rarely cause of ringworm of the scalp and other parts of the human body and more often seen as cause of ringworm in chicken or other fowl.A Japanese male, 96 years old and in good health was bitten by a fighting cock. A few weeks later, two erythemas with minor itching lesions were noted on the right forearm. The eruptions gradually increased in size. On the flexor aspect of the right forearm, we observed scaly anular erythemas approximately 2.0 cm in diameter with a well-defined border associated with fine scales and with a center that showed clearing. After a month treatment with terubinafine cream, the eruptions healed.Microscopic examination of potassium hydroxide specimen prepared from the eruptions revealed many hyphae. Scales were cultured on Sabouroud's glucose agar (SDA) supplemented with antibiotics and actidione at 25 ^{0}C for 14 days. White mycelial colonies sprouted from the scales. The colony on SDA at 35^{0}C for 37 days was white floccose surface with strawberry-red pigment diffused into the medium. The isolate produced 4 to 8 celled fusiform macroconidia, and ovoid to pyriform unicellular microconidia. The isolate was identified as M. gallinae based on the morphology and ITS regions of rRNA gene sequences with more than 99% in identity to EF581136 derived from M. gallinae. In addition, an ecological study to isolate M. gallinae from fowls, feathers, and soils at elementary schools, chicken farms, and/or the fighting cocks at the patients' residence is under investigation. The present case was the first case of M. gallinae infection in our country. The disease limited to cutaneous, however an immmunocompromized host showed a severe dissemination all over the skin. We should pay attention to M. gallinae as a causative agent for zoonotic mycosis.

PP-06-21

Clinical correlation between human dermatophytosis and animal exposure

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Background: Dermatophytosis is a common disease affected both humans and animals. Zoophilic dermatophytes in humans are usually identified in patients who have closer contacted with their animals and are frequently found in exposed skin of patients.

Objectives: To find the clinical manifestations and to compare the relationship of the owners and the pets between patients with dermatophytosis caused by zoophilic and anthropophilic species.

Materials and methods: All data were taken from the medical records and fungal laboratory. Additional information is derived from telephone interview. The relationship of owner and pets are determined by connection feeling, frequency of touch, place of pets in the house and type of touch.

Results: Enrolled patients were 76 patients with zoophilic dermatophytosis and 154 patients with anthropophilic dermatophytosis. Zoophilic species produced lesions with statistically significant shorter duration before presentation (median = 17.5 days) compare to the anthropophilic species (median = 90 days) ($p<0.001$). Considered from site of the lesions, exposured areas were infected from zoophilic species (59.4%) which was statistically significant difference from anthropophilic species which mostly presented lesion on unexposed area (84.5%) ($p<0.001$). There were also statistical significant difference in all catagories of the relationship of owners and pets between zoophilic and anthropophilic groups ($p<0.05$).

Conclusions: Zoophilic dermatophytosis showed clinically more virulence than anthropophilic species. Close relationship between owners and pets are risk factor for transmission dermatophytes from animals to humans.

Key words: zoophilic dermatophytosis, relationship, animal

PP-06-22

An endemic cross-infection between humans and cats and a non-endemic human infection caused by *Arthroderma vanbreuseghemii* and molecular epidemiology

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We report an outbreak of an endemic infection of ringworms and a non-endemic human infection caused by *Arthroderma vanbreuseghemii* in Chiba Prefecture, located in the central region of the main island of the Japanese Archipelago. Moreover, we discuss the molecular epidemiology of this condition.

Endemic cases: An employee of Japan Agricultural Cooperatives working at a local station picked up 4 sibling kittens from an empty gutter that was situated close to the station and brought them to his office. Of the 4 kittens, 3 were kept at the houses of 2 colleagues and 1 was kept in the office. One month later, 4 persons, 2 of whom had raised the kittens at home and 2 family members, developed ringworm on their ears, arms and abdomen. Moreover, all the kittens had scaly skin and hair loss.

Non-endemic case: A 68-year-old man with malignant lymphoma from another village developed extensive ringworm on his right forearm.

Nine cultures obtained from the 4 humans, 4 cats and the man with the non-endemic infection showed yellowish-orange colonies with a granular surface on PDA. All the isolates produced abundant spherical microconidia and spiral bodies along with a few clavate macroconidia. Some isolates were determined the mating type as "-" based on successful crossings with *A. vanbreuseghemii* "+" (IFM56670). The internal transcribed spacer (ITS) rRNA sequences of the isolates were all identical to AB455530, which was derived from one of the cat isolates. The phylogenetic position of the genotype was in a cluster of 115 related sequences. The cluster consisted of *A. vanbreuseghemii* isolates from humans, rodents and cats in Asia and Europe. Furthermore, the sequences of the present isolates were identical to those of human and rodent isolates from China and South Korea. This suggests the existence of a genotype that is specific to East Asia.

PP-06-23

The problem of tinea pedis and tinea manum in adults in a rural area of Eskisehir City and therapy approaches

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Introduction: Superficial mycoses are important community health problems that affect millions of people in the world with a lifelong risk as 10-20%. The aim of this study is to determine the frequency of tinea pedis and manum in adults in rural area, the risk factors and usage of alternative therapy.

Material and methods: This study includes population older than 20 years old registered in Eskisehir Osmangazi University, Education and Research Region, living in towns in Middle Anatolian Region of Turkey. The study group was composed as randomly sampling from the records of the health unit and 2574 people were enrolled. The study had two stages: In the first stage, the study group was asked demographic data, hygienic habits and chronic diseases in a questionnaire. Their weight and height were measured. All participants were examined for tinea pedis and manum, and appropriate microbiological samples were collected from suspicious lesions, and KOH preparations and culture were performed in the microbiology laboratory. In the second stage of the study, therapy methods were asked from the participants diagnosed by microbiological methods. In the statistical analysis of the data, ki-square and logistic regression tests were used.

Results: The mean age of the participants was 46.59± 16.26. Most of the participants were females (51.4%). Microbiological samples were taken from 285 (11.1%) participants. In 109 (4.2%) of samples culture was positive. The most common localization of the lesions was on feet (88.1%), and most common agent was *Trichophyton rubrum*. The predisposing factors were age older than 40, male gender and obesity. Presence of chronic diseases, bad hygienic habits and physical activity were not found associated. Forty-nine (45%) of patients had taken a medical therapy, 56 (51.4%) had performed non-medical methods (cologne, *Lawsonia inermis*-Henna and softener creams). For the control of these infections, appropriate therapy methods should be used.

PP-06-24

Tinea pedis and tinea unguium in patients with depression

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Tinea pedis and tinea unguium are the most common fungal diseases of foot and nail. Epidemiological studies on tinea pedis and unguium have been performed across Europe and East Asia. The prevalence of these diseases was 20%, respectively. As to patients with depression, the prevalence of these diseases has not been scarcely reported so far.

We investigated prevalence of tinea pedis and tinea unguium in 187 patients with depression who were newly hospitalized in a mental hospital. They were 67 men and 120 women with a mean age of 52.8 years. Of these patients, 118 (63.1%, 45 men and 73 women) presented positive signs by direct microscopy. Clinically, interdigital and/or vesicular form of tinea pedis were observed in more than half patients. 55 patients(29.4%, 21 men and 34 women) had tinea unguium. The mean clinical score for tinea pedis was high as 5.4, while the mean SCIO score which indicates the severity of tinea unguium, was 17.7. As to the patient's age, in male patients tinea pedis and tinea unguium were most frequently observed among patients over 70 years of age. In female patients 50's was the peak of prevalence of tinea pedis and tinea unguium. There was no gender difference in clinical score of tinea pedis. Male patents of tinea unguium (especially over 50 years of age) tended to have higher SCIO score than female patients. Positive cultures were observed in 51 patients (43.2%). *Trichophyton rubrum* was isolated in 38 (74.5%), T. mentagrophytes in 12 (23.5%).

In this study, the prevalence of tinea pedis and unguium of patients with depression was high as 63.1% and 29.4%,compared with the prevalence in outpatients. Also clinical symptoms tended to be severer than those of outpatients. It was thought because that these patients didn't care their own feet by themselves.

PP-06-25

Histopathological study on the experimental onychmycosis in rabbit

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To know in details in pathophysiology of onychomycosis, the present paper describes that sequential histopathological and morphometric analysis on novel animal model using steroid-treated rabbit with infection of Trichophyton mentagrophytes (TIMM2789). Result of the study is summarized as follow:

1. The distances between from the edge in the nail matrix and to fungus were extended, sequentially.
2. The number of fungus indicated with occupancy rates in area in the nail plate were increased with the extension of the period after infection.
3. The distances from the edge in the nail or nail bed side to fungi were analyzed images of tissue sample by 2D. Therefore, position of the center of mean of PAS positive fungus moved from the edge of nail matrix to a distance and to deeper layer with time.

As a result, it became clear that fungi in the nail plate moved and change with time after the end of treatment with infection, and we suggested that this experimental onychomycosis model is useful to evaluate clinical condition and some medicine.

PP-06-26

Fungal identification in onychomycosis

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Dermatophytes are the main cause of onychomycoses, but various non-dermatophyte filamentous fungi are often isolated from abnormal nails. The correct identification of the infectious agent of nail infections is necessary to recommend appropriate treatment. Whether a NDF is really the infectious agent of onychomycosis, or whether it has to be considered as a casual and transient contaminant, often remains an open question. Direct identification of the infecting agent in nail was shown to be feasible using Polymerase Chain Reaction (PCR) and sequencing or Restriction Fragment Length Polymorphism (RFLP).

Identification of fungi in nails using PCR/sequencing/RFLP provides significant improvement in comparison to results obtained by cultures: (i) NDF can be identified with certainty as the infectious agents of onychomycosis, and discriminated from dermatophytes as well as from transient contaminants. (ii) It is possible to identify the infectious agent when direct nail mycological examination showed fungal elements, but negative results were obtained from fungal culture. This represents a substantial improvement of the sensibility of identification of fungi in nails since the frequency of culture negative results when direct nail mycological examination showed fungal elements is in the range of 40%. (iii) Identification of the infectious agent can be obtained in 24 h with PCR/sequencing/RFLP, whereas results from fungal culture can take as long as 1-3 weeks. The simplicity and the reliability of the assays, and high NDF detection frequency support that PCR/sequencing/RFLP can be routinely and advantageously used to identify infectious fungi in nails, provided that enough nail material is collected by the clinician.

PP-06-27

Identification, antifungal susceptibility and scanning electron microscopy of a keratinolytic strain of Rhodotorula mucilaginosa: A primary causative agent of onychomycosis

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Onychomycosis is a dermatological problem of high prevalence that affects mainly the hallux toenail. An onychomycosis caused by the yeast Rhodotorula mucilaginosa was identified by colony morphology, light microscopy, urease and carbohydrate metabolism (Vitek 2, bioMérieux) in a 57 years-old immunocompetent patient of Rio de Janeiro, Brazil. High resolution scanning electron microscopy of nail fragments, processed by a non-coating method, leaded to the observation with fine detail of the structures of both nail and fungus involved in the infection. Yeasts were mainly found inside grooves in the nail. Budding yeasts presented a spiral pattern of growth and nearly formed blastoconidia were found into the nail groove region. Keratinase assays and keratin enzymography revealed that such isolate was highly capable to degrade keratin. Antifungal susceptibility tests showed that the fungus was susceptible to low concentrations of amphotercin B and 5-flucytosine and resistant to high concentration of fluconazole, itraconazole, voriconazole and terbinafine. This findings showed first time data about the interaction of R. mucilaginosa in toenail infection and suggest that such emerging yeast should be consider also as an opportunistic primary causative agent of onychomycosis.

PP-06-28

Onychomycosis in Tehran

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BACKGROUND: Onychomycosis, a common nail disorder results from invasion of the nail plate by a dermatophytes, yeasts or mould species that these fungi give rise to some diverse clinical presentations.Objectives: The purpose of present study was to isolate and determine the causative fungi of onychomycosis in the population in Tehran, Iran. METHODS: Totally nail materials of 504 patients with prediagnosis of onychomycosis during 2005, were examined both with direct microscopy observation of fungal elements in KOH preparations and culture to identify the causative agent. All samples were inoculated on (1) Sabouraud dextrose agar (SDA, Merk) (2) SDA with 5% chloramphenicol and cycloheximide in dublicate for dermatophyte and (3) SDA with 5% chloramphenicol triplicate for mould isolation. RESULTS: Out of a total of 504 cases examined, 216 (42.8%), were mycologically proven cases of onychomycosis (144 finger nails, 72 toe nails). Among the positive results, dermatophytes were diagnosed in 46 (21.3%), yeasts in 129 (59.7%) and non dermatophytic mould in 41(19%). Trichophyton mentagrophytes was the most common causative agent (n=22), followed by *Trichophyton rubrum* (n=13), *Candida albicans* (n=42), C. spp. (n=56) and *Aspergillus* spp. (n=21) CONCLUSIONS: near the half of clinical suspected fungal nail infections is onychomycosis and yeast is responsible for most of the infections in Iran.

PP-06-29

Reduction rates of involved area in onychomycosis patients receiving anti-fungal agents

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When we treat onychomycosis, drug efficacy should be evaluated from the perspectives of both clinical and mycological effects, requiring clinical improvement, negativities of microscopic findings or mycological culture. As a clinical index, the degree of turbidity of an infected nail have been usually scored by measuring the distance from the distal edge to the proximal edge of the involved area, with the distance from the distal edge of the nail bed to the proximal nail fold being defined as 10 (highest). But the degree of turbidity was sometimes difficult to evaluate precisely, when the shape of affected area with mycosis had changed irregularly as time passed.
The author examined onychomycosis patients of toenail to evaluate clinical course correctly. The involved area was measured using software of a computer with clinical photographs. Reduction rates were compared among patients receiving anti-fungal agents.

PP-06-30

Cure rate, duration required for complete cure and recurrence rate in onychomycosis according to clinical factors

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Onychomycosis, a fungal infection of nail apparatus including nail bed, nail matrix, and nail plate, is the most common nail disease, representing up to half of all onychopathies. Despite great advances in the treatment of onychomycosis in recent years, many factors affect the remedial curative value. The purpose of this study was to evaluate the cure rate, duration for complete cure, and recurrence rate in onychomycosis according to its clinical features. The study was conducted with 207 patients with onychomycosis on the great toenail were treated with antifungal agents in our department. Overall, Complete cure rate (CCR) as a whole was 78.3%, duration for complete cure (DC) was 31.7 weeks, recurrence rate (RR) was 36.0%. As the degree of nail involvement increases, the CCR decreases, DC and RR increases. We did not find any statistically significant difference in CCR, DC, RR according to clinical type of onychomycosis and gender. According to age, CCR were higher in the younger group, and DC were longer in the elderly group. RR were not significantly different in the elderly group, but higher in the younger group. There were no significant differences of CCR between the itraconzaole-treated (ITR) group and terbinafine-treated (TER) group, but the only topically applied (TO) group showed significant low CCR. DC and RR were not different statistically between ITR, TER and TO group. The differences in DC and RR according to existence of Diabetes mellitus (DM) were statistically significant. In the DM group, DC were longer, and RR were higher. But CCR were not different statistically between diabetic and non-diabetic. In conclusion, there were statistically significant differences in CCR, DC, and RR associated with various clinical factors of each patient; therefore, these results will be used to establish therapeutic plan and predict prognosis of onychomycosis.

PP-06-31

Detection of dermatophytes and nondermatophytes from onychomycosis patients after systemic antifungal treatment: Evaluation of cure by nested PCR

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Systemic antifungals (itraconazole and terbinafine) are the first line of treatment for onychomycosis, including tinea unguium. However, the choice of antifungals and the evaluation of the cure rate are sometimes difficult due to lack of a clinical definition of a 'cure' for onychomycosis. We conducted experiments to detect fungal DNAs from 12 patients before and after antifungal treatment, and compared the results with the clinical manifestations. Fungal DNAs were directly extracted from the affected nail plates and amplified by nested PCR. Fungal 28S ribosomal RNA gene was used to develop primer pairs for fungus universal primers (FUP), dermatophytes, and non-dermatophytes. At the commencement of treatment, *Trichophyton rubrum* was predominantly detected in the affected nails. After treatment, 7 out of 12 cases were negative for fungal DNA, a fact which coincides with, or precedes the loss of, the yellow, opacificated nail plates. On the other hand, in another group of 5 cases in which the chosen treatments proved ineffective, we identified *Fusarium* spp., *Acremonium* spp., *Trichosporon* spp. and *Candida albicans* which had a high MIC for either of itraconazole or terbinafine. These data suggest that the detection of fungal DNA by nested PCR using the samples directly collected from the affected nail plates is a sensitive and specific means of identifying dermatophytes and nondermatophytes, and is useful for formulating an appropriate method of treating onychomycosis.

PP-06-32

Onychomycosis caused by *Phaeoacremonium parasiticum*

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A 55-year-old healthy male came to our dermatologic department due to a color change of his toe nail for months. Upon examination, a long, narrow blackish streak was noted on his right big toe nail, starting from the free end and expanding proximally to about 2/3 length of the nail. The nail plate overlying the blackish streak was removed and blackish nail debris from the invasion end of the streak was collected for examination. By microscopy, dense fungal masses composed of brownish septate, branching hyphae were noted. A diagnosis of onychomycosis possibly caused by nondermatophyte was accordingly made. The specimen was sent for culturing. The blackish nail debris was inoculated on plates containing Sabouraud's dextrose agar with or without antibiotics in a duplicate set. Many colonies of a single grayish mould with a moderate to slow growth rate were formed in agar plates without antibiotics; on the other hand, colonies of the mould were not formed in those plates with antibiotics. The mould is characterized by brown septate hyphae, prominent hyphal warts, and phialides bearing masses of ellipsoidal conidia at the apex. It was identified as *Phaeoacremonium parasiticum*. Although a few yeast and Acremonium colonies were also present in the plates along with *P. parasiticum*, they were regarded as contaminants due to low colony counts and disagreement with the microscopic observation. No dermatophytes had ever appeared in culturing. The β-tubulin gene sequence matches that of the CBS101007 isolate of *P. parasiticum* with a 100% identity.

The diseased portion of the nail was trimmed off and a sulconazole solution was prescribed for topical use. The patient came back for follow-up 5 months later. New healthy nail grew and very little diseased nail was noted at the distal end of the nail. Specimens were collected for culturing again. The result was negative.

PP-06-33

Onychomycosis due to *Candida* parapsilosis: Four cases in patients with autoimmune disorder

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Primary nail invasions by *Candida* species with minimal paronychia; *Candida* onychomycosis are a rare disease that have been provided to be exclusively seen in patients with immunological disorders, however, secondary *Candida* infection to the nail plate following *Candida* paronychia are rather common (Watanabe S et.al, J Dermatol. 1983, Hay RJ et.al, Br J Dermatol. 1988). We report here four cases of *Candida* onychomycosis due to *Candida* parapsilosis. Although *Candida* parapsilosis has been implicated in nail infection, a few cases have been reported from Japan. Repetitive and duplicate cultures with or without cycloheximide cultures, and direct microscopy established diagnosis in each cases.

Two patients who presented with distal and lateral subungal onychomycosis were positive for rheumatoid factor. Two patients have been suffered from associated autoimmune diseases. One case of total dystrophic onychomycosis was associated with type 1 diabetes mellitus. The fourth case of *Candida* paronychia in the fingernail and superficial white onychomycosis in the footnails have been affected with type 1 diabetes mellitus and Hashimoto's thyroiditis.

Clinical features of the infected nails were sometimes indistinguishable from onychomycosis due to dermatophytes species except the limited response to oral antifungal therapy with terbinafin or itraconazole. The previous reports and our findings suggest that *Candida* onychomycosis limited to nailplate, can also be regarded as a subtype of chronic mucocutaneous candidiasis.

PP-06-34

Prevalence of candidia species in onychomycosis is patients refer to dermatological clinic in Northern Iran (Tonekabon)

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Onychomycosis is common in our community. The most complaint of this patients is worry from visual form and it's frequent relapses. Because of high prevalence in wet climate such as studied region and lack of researches in this subject, we decided to study this disease and determine the presentation factors. Recent study done on patients refer to Tonekabon hospital clinic of dermatology in 2008. Diagnosis of disease is based on clinical manifestation and in next level (if suspected in diagnosis based on culture). From 210 patients suspected to onychomycosis due to candidia 75 patients (35/7%) were positive culture. Most of them were men (64%) and most of the patient were in 61-70 years old (41/1%). The disease had high prevalence in farmers (52%) and house wives (24%) and who had very contact to water (80%) and history of nail trauma (68%). The most common clinical presentation that reported was DLSO (Distal lateral subongual onychomycosis) (100%). There wasn't any defined relations Between sex and clinical view of onychomycosis. Because of onychomycosis is due to candidia is a relapsing disease and control of causing factors have a high role in presentation of it's relapses with more studies on causing factors we can prevent this disease significantly.

PP-06-35

A case of nail Candidiasis due to *Candida* parapsilosis, successfully treated with 3 cycles of itraconazole pulse therapy

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66-years-old woman consulted about the nail deformities of her left thumb and index finger. She was under treatment with 5mg/day of prednisolone for rheumatoid arthritis. She first found her nail change on her thumb nail, 2 years before her first visit. Her deformed nails were accompanied with onycholysis and subungual hyperkeratosis, partly with green colored stain, which she first found 8 months before her first visit. There was no change observed on here periungual site of her deformed nails. Under microscope, fungal elements were detected in the deformed nails. In each time of the five consecutive series of her visits, colonies of yeasts were cultured and fungal elements of pseudohyphae were found under microscope. The cultured yeast was determined as *Candida* parapsilosis with VITEK 2 technology system. Thus the nail disorder was substantially diagnosed as nail candidiasis. MICs (micro gram/ml) for the causative yeast, *Candida* parapsilosis, was as follows; AMPH 0.125, 5-FC <=0.125, FLCZ 0.5, ITCZ 0.125, MCZ 1, MCFG 1, VRCZ 0.06. Three cycles of itraconazole pulse therapy, 400mg/day for a week in each cycle, were applied. Tropically luliconazole 1% solution was complementally applied. The nail deformities were totally disappeared 8 months after her first visit, when she started the treatment. It then was estimated that green nail was obtained secondly, because fungual therapy without any treatment for Pseudomonas induced total cure of the nail change. Nail candidiasis is usually caused by *Candida albicans*, and it is quite rare that causative fungus is C. parapsilosis. Even on this rare case, itraconazole pulse therapy was found to be substantially effective.

Poster Forum PF-01

PP-06-36

Aspergillus ochraceopetaliformis as a cause of onychomycosis

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Aspergillus (A.) ochraceopetaliformis is a mould that was only recently characterized as a fungal species that is genetically clearly different from A. ochraceus but conspecific with A. flocculosus. It has not yet been identified as a proven human pathogen. Here we report a case of a typical distal subungual toenail infection in an otherwise healthy woman that was caused by *A. ochraceopetaliformis*. A filamentous fungus was demonstrated microscopically in lesional nail material. Identical pathogens were isolated from the nail lesion at 3 visits separated by considerable intervals of time and no other relevant fungi were grown in cultures with and without cycloheximide. Species identification was performed by scrutinizing the phenotypic and genetic characteristics. Cultures were grown on several mycological agars and morphological and physiological criteria were assessed. Molecular identification was done based on amplification and sequencing of the ITS regions of rDNA and parts of the calmodulin and β-tubulin genes. These methods led to an accordant identification of *A. ochraceopetaliformis* by conventional and genetic methods. Treatment with terbinafine plus ciclopiroxolamine was then initiated and resulted in gradual healing. To the best of our knowledge this case is the first verified *A. ochraceopetaliformis* infection to be published. Two of the isolated strains are preserved in the public CBS Collection of Fungi (accession nos.CBS 123363 and CBS 123362).

PP-06-37

Emericella stella-maris, a new opportunist involved in onychomycosis?

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Onychomycosis can be caused by yeasts, dermatophytes and non-dermatophytic molds.
There have been increasing reports of nail mycoses due to non-dermatophytes in the recent years. The incidence of onychomycosis due to non-dermatophytes varies according to geographic region. In Greece, yeasts represent the major part of isolates from fingernail onychomycosis (72-82.9%) followed by dermatophytes (10%), the non-dermatophytes representing 1.9-5.6 %. Among the non-dermatophytes, molds more commonly involved in onychomycosis worldwide are *Scopulariopsis* brevicaulis and species of *Aspergillus*. *Aspergillus* species such as A. nidulans and A. sclerotiorum are rare whereas only one case of onychomycosis due to Emericella quadrilineata (anamorph *Aspergillus* tetrazonus) has been reported.
We present a case of fingernail onychomycosis with repeated positive culture of a slow growing mold. The patient, a 38 year old male, had typical signs of distal lateral onychomycosis on most of his fingernails since several months. He had no further complaints and no apparent predisposing factors.
Microscopically the fungus was characterized by filaments and abundance of Hülle cells. After subculturing, septate conidiophores with biseriate conidial heads were grown. In older cultures a plethora of deep red-purple cleistothecia containing asci with star-shaped ascospores were observed.
Based on sequences of ITS, beta-tubulin and calmodulin genes, this fungus was 100% identical to Emericella stella-maris, a new taxon of Emericella, published by Zalar et al. in 2008. This is the first clinical case associated with this new species of Emericella.

Zalar, P., Frisvad, J. C., Gunde-Cimerman, N., Varga, J. & Samson, R. A. (2008): Four new species of Emericella from the Mediterranean region of Europe. Mycologia 100(5): 779-795.

Poster Forum PF-01

PP-06-38

Fusarium paronychia sine paronychia

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We describe a case of *Fusarium* paronychia of the big left toe, in a 40-years-old native Italian female.
The patient referred attacks of intense pain affecting the entire left limb with simultaneous herpes simplex of the upper lip. The phenomenon had been going on for approximately one year.
She was in good health and neurological and orthopaedic history and examinations were unremarkable.
2 years before she had been diagnosed with distal subungual onychomycosis due to *Fusarium* solani. Although the therapy was prescribed, it was not carried out, because a spontaneous apparent healing was achieved, and the nail unit looked normal again. The husband, who is an experienced physician, disagreed with the treatment. Scrapings taken from deep bottom of the nail fold and lateral nail groove yielded mix mould growth with a small colony of *Fusarium* solani, in 2 consecutive samples taken during the pain remission period.
Treatment with terbinafine 250 mg/day for 3 months with spirit whitfield locally produced a complete recovery, i.e. disappearance of the pain and no more relapses of herpes with negative mycological cultures.

Conclusions:
1.This is the first case reported, to our knowledge, of *Fusarium* paronychia sine paronychia (FPSP)
2.We advise to consider FPSP in differential diagnosis of monolateral pain of the limb.
3.FPSP should be added to the clinical *Fusarium* paronychia spectrum:
a.Paronychia with onychomycosis
b.Isolated paronychia with its variants
c.Paronychia superimposed on subungual melanoma

Poster Forum PF-03

PP-06-39

Onychocola canadensis onychomycosis: Report of 23 new cases from France

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Onychocola canadensis is a keratinophilic cycloheximide resistant mould responsible for onychomycosis and cutaneous adjacent lesions in temperate climates. Since the first description in 1990 only 45 cases have been reported.
Herein we report 23 cases diagnosed in two French University Hospitals between 1996 and 2009. Eleven patients were women, age ranged from 50 to 82. Fourteen patients lived in rural areas and 9 were involved in gardening or farming. Ten presented with peripheral vascular disease complicated with leg ulcers in 3. Other associated factors were overweight (4 cases), generalized dermatitis (4 cases), diabetes (5 cases), alcoholism (4 patients). Five patients had a cardiopathy, 3 a renal insufficiency associated with lower limbs edema in 2 and one had a severe bullous dermatosis with fatal outcome. In 4 cases the diagnosis was established during a hospitalisation for erysipela. Only toenails were affected. Distolateral subungual type (8 cases) and total onychodystrophy (9 cases) were the most frequent clinical aspects. *O. canadensis* was isolated as well from toewebs in 2 cases. *O. canadensis* was isolated in pure culture in all but 2 patients where *Candida* ciferrii was associated.
Classical associated factors such as older age, peripheral vascular disease and contact with earth were found. However our study disclosed no female predominance unlike previous studies did. The potential risk of erysipela should be emphazised as onychomycosis can represent a portal of entry as in dermatophytosis. Scarcity of reports may be due to the very slow growth of this mould, starting with tiny colonies in the agar at 3 to 4 weeks when cultures are usually discarded in the routine lab. Since *O. canadensis* is resistant to systemic antidermatophytic antifungals, care should be given to sample all suspected nails before treatment is considered.

PP-06-40

Candida albicans abrogates the expression of interferon-γ-inducible protein-10 in human keratinocytes

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Candida albicans is the predominant causative agent of human cutaneous candidiasis. Epidermal keratinocytes play an important role in the cutaneous immune response through the production of cytokines and chemokines, including interferon (IFN)-γ-inducible protein 10 (IP-10). Here, we investigated the influence of *C. albicans* infection on IP-10 production by normal human epidermal keratinocytes (NHEK) in vitro. Our results showed that IFN-γ-stimulated NHEK showed enhanced IP-10 mRNA and protein expression; this expression was down-regulated by *C. albicans* infection. *C. tropicalis* also impaired IFN-γ-induced IP-10 expression, but *C. glabrata* did not. Heat-killed *C. albicans* did not impair IFN-γ-induced IP-10 expression. We found that co-incubation of NHEK with live *C. albicans* without cell-to-fungi contact impaired IFN-γ-induced ·IP-10 mRNA and protein expression in NHEK, suggesting the role of soluble factors derived from live *C. albicans* in this impairment. Enzyme-linked immunosorbent assay (ELISA) analysis revealed that *C. albicans* and *C. tropicalis* could produce marked levels of prostaglandin (PG)E_2, while *C. glabrata* produced low levels of this PG. Treatment with E-series prostaglandin receptor antagonists–AH6809 and AH23848, not SC19220–restored IFN-γ-induced·IP-10 expression in *C. albicans*-infected NHEK. Thus, *Candida*-derived PGE_2 may impair IFN-γ-induced IP-10 expression in human keratinocytes, and play a role in the pathogenesis of cutaneous candidiasis.

PP-06-41

Utility of oligonucleotide microarrays investigating the interaction of host and *Candida albicans* in vulvovaginal candidiasis

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Vulvovaginal candidiasis caused by the opportunistic yeast *Candida albicans* are a significant problem in women of child-bearing age. Although numerous researches have been conducted over the past two decades to investigate the pathogenesis of VVC, the results of these studies often conflict with each other. Which protective host defense mechanism actually protects women from vaginitis and what virulence factor from *C. albicans* occur or are associated with acute or recurrent symptomatic infection are still not understood. In the present investigation, we made a microarray to include lots of the genetic factors reported recently to be correlated to the pathogenesis of vulvovaginal candidiasis and hybridized it with total RNA of clinical specimens from patients and asymptomatic carriers to directly investigate the pathogenesis of vulvovaginal candidiasis by observing the transcript profiling changes from host and *C. albicans*. We selected those genes with expression ratios (patient sample vs carrier sample) greater than or equal to 2, or less than or equal to 0.5 to observe the transcript profiling changes from host and *C. albicans* and make an analysis. Of the genes that were analyzed, there were 43 and 16 factors could increase and decrease 2 folds respectively in the samples of patient group when comparing the carrier sample; and among them, LIP4, SAP5, HWP1, EFG1, CPH1, MCP-1, MIP-1a, MIP-2, IL-1, NF-kB, TGF-b1, TLR4 changed most prominently. Expression of these genes was associated with extracellular hydrolysis, hyphal formation, phenotypic switching and native immunity. The data presented here indicate that the deficiency of adaptive immunity and the increased virulence of pathogen strains are all related to the pathogenesis of vulvovaginal candidiasis, and innate immunity factor TLR4 possible plays an important role in the local immunity of the host with vulvovaginal candidiasis.

Poster Forum PF-03

PP-06-42

Molecular study of *Candida* species isolated from candidial stomatitis and thier related predisposing factors in patients using complete dental implants

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The *Candida* species are one of the main causes of stomatitis. Considering the scanty of information about the prevalence of *Candida* species in denture wearers as well as the variation of these yeasts in sensitivity to antifungal drugs,we undertook a study of prevalence and predisposing factors of candidiasis in patients using complete denture.

114 elderly individuals resident in retirement homes or referring to retirement society were included in this study. After examination of oral cavity and prostheses by a prosthodontist, the specimens were taken with swapping the mucosa or the internal parts of prostheses.All the samples were inoculated into plates containing sabouraud dextrose agar with chloramphenicol.The isolated yeasts were identified based on their physiological (Germ tube test, ability to produce chlamydoconidia and color formation in chrome agar *Candida*) and molecular properties (PCR-RFLP).

From the 114 elderly individuals with complete dentures, 69.3% of the cases were female.The most commonly isolated species were *Candida albicans*(41.5%) followed in frequency by *Candida glabrata* (18.4%) and *Candida tropicalis* (12.9%). In addition, *C.dubliensis* with the frequency of 10.9% was reported for the first time from clinical specimens in Iran. Moreover, rare species such as *T.capitatum*, *T.beigeli*, *S.cerevisiae, C.lipolytica* and *Prototheca wickerhamii* was also isolated.

In the present study, no statistically significant difference was observed between clinical symptoms of stomatitis and factors such as smoking habit, sex, type of denture cleanser, removing denture at night, cleanness of denture, vertical dimension and having suction in denture. However, statistically significant differences were found between having xerostomatia and poor denture hygiene with angular cheilitis and leukoplakia. Among studied factors,significant differences were seen only between duration of using the denture and candidiasis (>50 Colony Forming Unit).

PP-06-43

Recent incidence of *Candida* spp. oral disease, colonization and fluconazole resistance in HIV/AIDS patients using microbiological and molecular detection methods

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Background: The current prevalence of symptomatic oropharyngeal candidiasis (OPC), colonization, and rate of fluconazole (FLU) resistance due to *Candida* in HIV/AIDS has not been well described. This study was designed to detect and identify the occurrence of oral *Candida* colonization/disease and FLU susceptibility in patients using standard microbiological and molecular techniques. Methods: HIV/AIDS patients were eligible for enrollment with CD4+ count <200 and/or symptomatic OPC. Oral rinse samples were obtained from 171 patients over 311 visits and colonization was assessed microbiologically and by direct amplification of yeast DNA from oral samples by standard PCR using *Candida* pan-fungal primers. Species ID was confirmed on CHROMagar *Candida*, germ tube assessment, API 20C, and molecular sequencing. FLU susceptibility (MIC <=8 ug/ml) was assessed by CHROMagar dilution. Results: Of these 171 pts, the median CD4 cell count at enrollment was 87 (range, 2-348). Of 311 patient visits, 243 (78%) were from patients on Highly Active Antiretroviral Therapy (HAART). 260 (84%) patient visits showed *Candida* carriage and 76 (24%) showed symptomatic OPC. While carriage was detected in 260 visits, microbiology was positive in 250 and PCR in 217; 10 were positive by PCR exclusively. 341 isolates were obtained, in which *C. albicans* was detected in 54%. *C. dubliniensis* (16%), *C. glabrata* (17%), *C. tropicalis* 5%, *C. krusei* 4%, *C. parapsilosis* 3% and *C. guilliermondii* and *C. lusitaniae* < 1% each. *C. albicans* occurred in 66/107 (62%) OPC visits. Decreased FLU susceptibility occurred in 110/341 (32%) isolates. 75/110 (68%) isolates with reduced susceptibility were non-*albicans* spp. 41/110 (37%) of these isolates with decreased susceptibility to FLU were obtained from OPC patients. Conclusions: Even with active antiretroviral therapy, oral yeast colonization and symptomatic OPC, including yeasts with reduced FLU susceptibility remain common in patients with advanced HIV/AIDS.

PP-06-44

Microevolution of *Candida albicans* in chronic oral mucocutaneous candidosis of APECED patients

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Introduction: Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) is an autosomal recessive disease caused by loss-function mutations of the autoimmune regulator (AIRE) gene which has been mapped on chromosome 21 q22.3. Often the first visible sign of the disease in childhood is chronic oral mucocutaneous candidosis (CMC) mainly caused by *Candida albicans*. Most APECED patients receive repeated treatment and prophylactic courses of antifungals throughout their lives which has lead to a decrease in the susceptibility of the colonizing strains to azole antifungal agents. The aim of our study was to determine whether the patients have been colonized by the same *C. albicans* strains throughout the years and whether strain microevolution had occurred.

Materials and methods: A total of 31 *C. albicans* isolates of varying fluconazole susceptibility recovered during the years 1995-2007 (2-5 isolates/patient) from 11 APECED patients were analysed. E-tests were performed to determine the fluconazole MICs and the strains were multi locus sequence typed (MLST). Briefly, sequences of bases in PCR fragments of seven housekeeping genes (AAT1a, ACC1, ADP1, MPI1b, SYA1, VPS13, and ZWF1b) were determined. The sequences were all given a sequence type (ST) and compared to those included in the MLST database (www.mlst.net).

Results: The samples of five of the patients showed changes in one of the seven alleles giving them different STs. Two patients had mixed populations of two different strains and the samples of three patients had no change in the colonizing *C. albicans* strains. Changes in fluconazole susceptibility could be observed in all patients regardless of ST.

Conclusions: Results of our typing showed strain maintenance with microevolution in APECED patients suffering from CMC. Changes in the MICs in strains with identical STs isolated years apart suggest molecular changes in genes linked with fluconazole resistance.

PP-06-45

First isolation and identification of Trichosporon inkin colonized in vagina in China

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Objects A case of vaginal colonization due to Trichosporon inkin was presented, while the clinical and experimental studies related to the strain were held.

Methods A 34-year-old female presented with the increasing of vaginal discharge and local abnormal odor for two months. Clinical laboratory examination was carried out, and the experimental research included purification of the strain, slide microculture, temperature test, urea enzyme test, biochemistry identification, antifungal susceptibility test, and molecular sequencing gene identification were held, too. Results Nugent scores of vaginal discharge were 5 to 6 and stramineous, reductus, and yeast-like colony were isolated twice. The strain had the ability of growth in 42 Celsius degrees. By the slide microculture of corn agar, appressorium on the top of hypha and typical sarcinae were observed. Yeast malt agar was the optimal growth medium of the strain. The urea enzyme test of the strain was positive, while the API 20C AUX biochemistry test and gene identification based on molecular sequencing were consistent with Trichosporon inkin. The isolate were sensitive to Amphotericin B and azoles such as Clotrimazole and fluconazole, while resistant to flucytosine and caspofungin.

Conclusions It was the first case of vaginal colonization due to T. inkin in China. The accurate identification of the species relied on the aggregate analysis based on phenotype characters, biochemistry test, and molecular sequencing.

PP-06-46

Thymic stromal lymphopoietin secretion from human keratinocytes during exposure to *Malassezia* species

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[Introduction] The lipophilic yeast *Malassezia* is part of the cutaneous microflora, and is thought as an exacerbating factor in atopic dermatitis (AD). Among *Malassezia* species, *Malassezia* globosa and M. restricta are particularly dominant on the skin of AD patients. However their precise roles in AD remain unknown. Thymic stromal lymphopoietin (TSLP) is an IL-7-like cytokine expressed by epithelial cells including epidermal keratinocytes and play an important role in allergic inflammation. TSLP instructs myeloid dendritic cells (mDCs) to induce inflammatory T helper 2 (Th2) cells. Recent studies have shown that TSLP is highly expressed by keratinocytes in skin lesions from patients with AD but not in normal skin and nonlesional skin of patients with AD. However factors inducing TSLP production from human keratinocytes of AD are uncertain. In this study, we investigated the secretion of TSLP from human keratinocytes during the exposure to M. globosa or M. restricta.
[Materials and Methods] Normal human epidermal keratinocytes (NHEK) were exposed to M. globosa or M. restricta at a multiplicity of infection (MOI) of 20 in vitro. TSLP secretion from NHEK cells was measured by ELISA. The expression of TSLP mRNA was determined by reverse transcription-polymerase chain reaction (RT-PCR).
[Results and Discussion] The exposure of NHEK cells to M. globosa resulted in a marked secretion of TSLP. Similarly, M. restricta-exposed NHEK cells showed an increased TSLP secretion. RT-PCR analysis confirmed that up-regulated TSLP mRNA expression paralleled the enhanced TSLP secretion by M. globosa- or M. restricta-exposed NHEK cells. These results suggest that M. globosa and M. restricta may play a role in initiating or exacerbating AD through the induction of TSLP secretion from epidermal keratinocytes.

PP-06-47

Evaluation of a simple method for isolation of genomic DNA from *Malassezia* species

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Malassezia can be found as a part of the normal skin flora of humans and are associated with several skin diseases and even systemic infections under some circumstances. For identification, several physiological and molecular techniques were described. For molecular studies of fungi, different DNA extraction methods have been described previously. A reliable DNA extraction method which is suitable for PCR assay is the most important step for molecular studies.
In this study we aimed to perform PCR directly from *Malassezia* colonies instead of using a special DNA extraction step. Such a method has been described previously by Luo et al (JCM, 2002;40:2860-65) for *Candida* spp, Cryptococcus neoformans and some molds. However, applicability of this simple method for *Malassezia* species was tested for the first time in this study. Shortly, a single colony was taken from a subculture with a sterile micropipette tip and suspended in 20 microliter of sterile distilled water in a microcentrifuge tube. One microliter of this suspension was used in PCR mix as a DNA source without further purification. Additionally, we tested the success of this method directly from maintenance culture slants. Amplification reaction was performed by using the universal primers ITS1 and ITS4.
Colonies taken from both subcultures and also maintenance slants of all tested *Malassezia* strains (7 M.furfur, 7 M.sympodialis, 2 M.globosa) could be successfully amplified and produced amplicons of 630 bp and 830 bp with primer couple of ITS1 and ITS4.
As a conclusion, *Malassezia* colonies on growth media can be used directly for PCR studies without necessiating an extra DNA extraction and purification step, as a simple, rapid, cheap and reliable alternative method.

Poster Forum PF-03

PP-06-48

Problems in diagnosing *Malassezia* folliculitis

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Malassezia folliculitis (MF) is a discrete, often pruritic, papulopustular eruption, localized mainly on the upper portion of the trunk and shoulders. It is often co existing with acne vulgaris. We report 2 (two) cases of severe cases MF with acne vulgaris. At the beginning it was diagnosed clinically and mycologically as MF and treated with ketoconazol 200 mg/day to no avail. Further direct mycological examination revealed that not all lesions yielded spores. Isotretinoin 200 mg/day was then started for several weeks giving moderate results. In order to establish the diagnosis of MF, it needs more than just clinical and mycological results but also ruling out acne vulgaris.

PP-06-49

Filamentous hyphal form of *Malassezia* spp. found in some erythematous lesions of seborrheic areas of skin

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Although *Malassezia* spp. is one of the normal floras of seborrheic areas of skin in healthy individuals, it is generally accepted that the presence of the yeast form of *Malassezia* spp. plays an important role in seborrheic dermatitis. It is known that spores of *Malassezia* are almost always found in Parker-KOH direct preparations of specimens scraped from lesions of seborrheic dermatitis, but hyphae of the organisms are rarely found. On the other hand, it is well established that the mycelial form of *Malassezia* spp. causes tinea versicolor, and the application of an antifungal agent is a logical and effective treatment for the condition.

In this study, we made direct microscopic examination of scales scraped from the scalp, the area around nostrils and eyebrows, and the chin. The subjects studied included patients with seborrheic dermatitis, atopic dermatitis, dermatomyositis and some healthy individuals. The finding of morphological parasitic forms, i.e. hyphae and/or spores was recorded.

Our results showed that not only spores, but also hyphae of *Malassezia*, were found in scale scraped from slightly erythematous lesions in several cases. We present these cases, together with discussion of some contributing risk factors for forming the hyphal phase of *Malassezia* spp.

PP-06-50

Identification of *Malassezia* species isolated from patients with seborrhoeic dermatitis, atopic dermatitis, Tehran, Iran

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The *Malassezia* yeasts are members of the normal human skin mycoflora. These yeasts cause various human skin diseases in certain conditions. Although the yeasts have been described in late 19th century, their classification was not clear until 1996. Following several reclassifications, twelve species of the genus *Malassezia* have been identified so far. Whereas, a few studies have been carried out on isolation and identification of *Malassezia* and its association with Seborrhoeic dermatitis (SD) and Atopic dermatitis (AD), the aim of this study was to determin the *Malassezia* species of the normal skin flora as well as the species in patients with SD and AD, according to the method devised by Guillot et al.
Methodes: in this study, 81 patients (34 with AD and 47 with SD) plus 40 normal subjects were examined for *Malassezia* cotamination. A direct microscopic examination and culturing were carried out on the skin samples. The isolated yeasts from Dixon media were identified by their morphological features as well as physiological characteristics by use of tween model.
Results: 56 patients (69.1%) were female and the rest (30.9%) were male. The highest prevalence of skin lesions was seen in patients with 21-30 years of age (41.3%). Cultures yielded positive results in 85.1% of patients with SD and 47.1% of patients with AD as well as 77.5% of the normal subjects. The culture results showed a statistically significant difference between the patients and normal subjects (2χ, $p = 0.001$). The positive results of cultures in patients with SD were more than patients with AD.
Conclusion: M. globosa was the most frequent species isolated from AD and normal subjects and M. furfur was the most prevalent species isolated from SD.

PP-06-51

Identification of *Malassezia* species isolated from Iranian seborrhoeic dermatitis patients

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Background and aims: In recent years, the genus *Malassezia* has come to be considered important in the etiology of seborrhoeic dermatitis (SD). The aim of present study was identification of *Malassezia* species on the lesions of Iranian SD patients.
Methods: 100 patients with SD were enrolled in study. Diagnosis of *Malassezia* was made after budding yeast cells with or without hyphae were microscopically observed on skin scales stained with methylene blue staining. All samples were cultivated on Leeming and Notman and Sabouraud's dextrose agar culture media. The agar plates were incubated at 32 C for 2 weeks and evaluated for the existence of growth every day for a total of 7 days. Identification of isolated yeast was based on morphologic and physiologic tests, namely Tween assimilation profiles and catalase reaction.
Results: From 100 patients with SD, 60% of the cases were female. The age range was 12to65 years with median 27.3 years. The highest prevalence of SD was seen in 20to29 years age group. 59% and 41% of patients had local and generalized lesions, respectively. 58% of patients showed lesion on head. Microscopic examination of skin scales was positive in 100% of SD lesions. 96% of patients showed more than 1to3 yeasts in each microscopic field whereas only 4% patients showed 1to3 yeasts in whole slide. Totally, 77% of the specimens yielded *Malassezia* in culture. The most commonly isolated *Malassezia* species was M. globosa (55.8%). M. globosa was most common isolated species from head and face. M. furfur had most frequency on trunk lesions.
Conclusion: The results of our study showed high recovery rate of *Malassezia* species on lesions of patients with SD. So we think as some other investigators that the colonization of *Malassezia* on the lesions of SD could play an important role in SD pathogenesis.

PP-06-52

Malassezia microbiota and specific IgE antibody production in patients with atopic dermatitis

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Lipophilic yeasts, *Malassezia* are part of the cutaneous microbiota of healthy subjects, which have been thought to be an exacerbating factor in atopic dermatitis (AD). Of the 13 currently accepted *Malassezia* species, we found that both M. globosa and M. restricta were predominant species in the skin of AD patients. In this present study, the cutaneous *Malassezia* microbiotain AD patients were analyzed using a PCR-based culture-independent method with species-specific primers, and anti-*Malassezia* species-specific IgE antibody was measured using ELISA with antigen for each species. In lesional skin, M. globosa and M. restricta were detected in all of the AD patients, whereas the other species were present in fewer than 60%. In addition, M. restricta predominated over M. globosa. Anti-*Malassezia* IgE antibody was detected from 72% of the cases. Species-specific IgE antibody against M. globosa and M. restricta was detected in 66 and 70% of the cases, respectively. Moreover, the species with low detection rate in skin also had low detection rates for specific IgE antibody in serum.

PP-06-53

Identification of the major allergen of *Malassezia globosa* relevant for atopic dermatitis

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[Introduction] *Malassezia* is a part of the normal flora of human skin, but it may induce IgE production in atopic dermatitis (AD) patients. *M. globosa* is particularly dominant on the skin of AD patients. However, little information is available regarding its allergens. In this study, we attempted to identify major allergens from *M. globosa* using a proteomics analysis.

[Materials and Methods] The supernatant of the crude lysate from *Malassezia globosa* CBS7966 was analyzed by IgE-immunoblotting with sera from AD patients. The IgE-reactive components were analyzed by a proteomics approach, combining two-dimensional Western blotting and matrix-assisted laser desorption ionization time-of-flight MS (MALDI-TOF MS) postsource decay (PSD) mass spectrometry.

[Results and Discussion] Immunoblotting showed that IgE-reactive components with molecular masses of 40-45kDa proteins were detected by 100% (28 of 28) of sera from AD patients. Highly IgE-reactive protein spot with a molecular mass of 42 kDa and pI of 4.8, designated MGp42, was identified by two-dimensional immunoblotting, and sequenced partially by MALDI-TOF MS and PSD analysis of the peptide digest. The *M. globosa* allergen was then cloned and the amino acid sequence deduced from the cDNA sequence. 5'/3'RLM-RACE PCR analysis revealed that the full-length cDNA contained 1,908 bp of open reading frame (ORF) encoding 635 amino acid residues with a calculated molecular mass of 69.7 kDa and a pI of 6.02. The N-terminal sequence of MGp42 protein started from the 250th residue, Asp-250, of the deduced amino acid sequence. MGp42 protein consisted of 386 amino acid residues with a calculated molecular mass of 42 kDa and a pI of 4.8, in agreement with the result of 2D-immunoblotting. MGp42 showed similarity to the heat shock protein 70 family, suggesting that MGp42 may be a cleavage product of intact HSP70. The novel allergen from *M. globosa* could be useful in diagnosis of AD.

Poster Forum PF-03

PP-06-54

Evaluation of specific IgG and IgA levels against *Malassezia* species in sera of patients with atopic dermatitis

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[Introduction] The lipophilic yeast *Malassezia* is considered to be an exacerbating factor in atopic dermatitis (AD). Among *Malassezia* species, *Malassezia globosa* and *M. restricta* are particularly dominant on the skin of AD patients. Elevated serum IgE levels to *M. globosa* and *M. restricta* are found among AD patients in high frequencies. However, little information is available about serum levels of other subclass antibodies in AD patients. In this study, we investigated the IgG and IgA levels to *M. globosa* and *M. restricta* in sera of AD patients.
[Materials and Methods] Sera from 37 AD patients, including 20 infants (mean age, 9.5 ± 3.6 years) and 17 adults (mean age, 25.2 ± 6.3 years), and 11 healthy donors (mean age, 32.1 ± 4.8 years) were used in this study. The IgG and IgA antibody levels against *Malassezia* species were determined using the AlaSTAT Microplate system with slight modifications.
[Results and Discussion] Adult AD patients had higher IgA levels (1.01 ± 1.03 IU/mL) to *M. globosa* compared with infant AD patients (0.58 ± 0.72 IU/mL) and healthy controls (0.48 ± 0.88 IU/mL). Similarly, the *M. restricta*-specific IgA level in adult AD patients (1.16 ± 1.64 IU/mL) was higher than that in infant AD patients (0.68 ± 0.90 IU/mL) and healthy controls (0.65 ± 1.13 IU/mL). No difference in serum IgG levels against *Malassezia* species was found between AD patients (adults and infants) and healthy donors. These results suggest that the mucosal immunity to *Malassezia* may be elicited in AD patients. The participation of mucosal immunity to *Malassezia* in the pathogenesis of AD should be further investigated.

PP-06-55

Characterization of melanogenesis in fungal skin infections

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Fungal skin pathogens cause some of the most common infections, dermatophytosis and pityriasis (tinea) versicolor, known to man and are globally responsible for a significant burden of diseases. Investigation into the pathogenesis of disease can often lead to a greater understanding of the causes, progression and outcomes of human infections.
Melanins are synthesized by organisms of all biological kingdoms; they make up a heterogeneous class of natural pigments that are implicated in the pathogenesis of several important human fungal pathogens. This study investigated whether the fungal skin pathogens, *Malassezia furfur* and dermatophytes, produce melanin or melanin-like compounds in vitro. A melanin-binding monoclonal antibody (mAb) labelled yeast cells of *M. furfur* and conidia of dermatophytes as determined by immunofluorescence microscopy. Treatment of *Malassezia* yeast cells with proteolytic enzymes, denaturant and concentrated hot acid yielded dark particles that were similar in size and shape to their propagules. Electron spin resonance spectroscopy revealed that black particles derived from yeast cells of *M. furfur* contained a stable free radical compound, consistent with their identification as melanins. These findings indicate that *M. furfur* and dermatophytes can produce melanin or melanin-like compounds in vitro. Based on what is known about the function of melanin in the virulence of other fungi, this pigment may affect the pathogenesis of fungal skin infections.

PP-06-56

Two phase itraconazole treatment of atopic dermatitis

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Fungi such as *Malassezia* furfur and *Candida albicans* are known to be involved in the development of atopic dermatitis (AD), due to increased sensitivity to these allergens or increased serum IgE in AD patients. I previously reported the successful treatment of AD with oral administration of the antimycotic agent, itraconazole. This study reports on the post treatment follow-up data to take an extended look at the regimen, which was determined to be the best regimen as evaluated by over-all efficacy of therapy and to be the effective minimum dosage in terms of period, tolerance, and safety. This regimen was constructed as a dual phase treatment with itraconazole.
1st phase (introduction phase): 100mg/day for 1 week
2nd phase (maintenance phase): after 1st phase, 200mg/week, repeating
Treatment period ranged from 3 to 8 months (Mean: 6.5 months),
with use of anti-allergic (epinastine: 20mg/day) every day during the treatment.
All clinical symptoms in patients showed great improvement (P<.001), the majority of patients`condition improving significantly within the first week (introduction phase). Follow-up of patients was continued up to 4 years. Patients' condition continued to remain good across the follow-up period with little or no recurrence.

PP-06-57

Morphological and physiological characterization of *Sporothrix schenckii*

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The dimorphic fungus *Sporothrix schenckii* is the etiologic agent of sporotrichosis. Although it is a ubiquitous species, its morphological and physiological characteristics have been underinvestigated. In the present work, 35 clinical isolates of S. schenckii were analyzed with regard to their halophilic or osmophilic growth properties, their growth in different culture media, the morphology of their colonies, and their enzymatic activity. The isolates were inoculated into media containing 1-14% of sodium chloride for assessing halophily, 10-40 % of glycerol for analyzing osmophily, Sabouraud dextrose agar and potato dextrose agar for assessing growth and morphology, Christensen's medium for urease, polysorbate 80 for lipase, albumin for protease, and egg medium for phospholipase. All isolates were incubated at 25 oC (filamentous mycelial form) and at 37 oC (yeast-like form) in the analyzed media. Halophily, osmophily and urease were assessed qualitatively, while morphology and the remaining enzymatic activities were evaluated quantitatively at 7, 14, 21, and 28 days. After 7 days of incubation, the yeast-like form showed a higher growth rate in both media. However, no growth differences were identified between both forms for the other periods of time. The mycelial form was more resistant to osmophily, while the yeast-like form was more resistant to halophily. All isolates showed a positive reaction to urease. Seventy-two percent of the strains analyzed were good lipase producers, while 61% were good phospholipase producers. Protease activity was higher after 14 days, but 50% of the strains showed low activity. Thus, urease and lipase activities may be used for biochemical characterization, whereas phospholipase and protease activities vary amongst the strains analyzed. Therefore, the phenotypic characterization of S. schenckii allows improving the diagnosis and treatment of sporotrichosis. We acknowledge the financial support granted by CAPES, CNPq, and FAPERGS.

Poster Forum PF-02

PP-06-58

Sporothrix globosa: Is the only newly described Sporothrix species causing human infections in India?

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Sporothrix schenckii, the etiological agent of sporotrichosis has a world-wide distribution. Multiple outbreaks have been reported indicating that the disease can cause significant morbidity. In India, the disease is endemic Assam, West Bengal and Himachal Pradesh and the cases have been reported from the other states of the country including Uttar Pradesh, Delhi, Punjab, Karnataka, Tamilnadu and Manipur. The taxonomy of this *Sporothrix schenckii* is complex and several molecular studies has shown the genetic diversity within this species complex. Recently, Marimon et al have proposed the existence of three new species viz. Sporothrix braziliensis, Sporothrix mexicana and Sporothrix globosa within the *Sporothrix schenckii* complex which were closely associated with their geographical origins. With the use of limited Indian strains of Sporothrix, the study has revealed that all those strains of Indian origin belonged to Sporothrix globosa. The present study was planned to identify the species of *Sporothrix schenckii* complex prevalent in the geographically diverse regions within the Indian subcontinent. A total of 37 clinical strains of *Sporothrix schenckii* isolated form geographically diverse places of India were identified based on macroscopic and microscopic morphology, physiological studies, and DNA sequence of the calmodulin gene. The macroscopic and microscopic features were similar to that described for Sporothrix globosa. Analysis of the Calmodulin gene sequence revealed that all the isolates belonged to Sporothrix globosa with minimum variations among the different strains. All the isolates produced two types of conidia and the sessile conidia which were brown to dark brown, thick walled, globose to subglobose, measuring about 3-5 micrometer were characteristic of this species. None of the isolates grew at 37^{0}C. All the isolates assimilated sucrose and were negative for ribitol and raffinose. The present study confirms the findings of the earlier study that all the Indian isolates tested belong to one species, Sporothrix globosa.

PP-06-59

Sabouraud dextrose broth for in vitro evaluation of the sensitivity of *Sporothrix schenckii* to antifungal agents

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Sporothrix schenckii is a dimorphic fungus that causes sporotrichosis, a ubiquitously distributed mycosis. It is the subcutaneous mycosis with highest incidence in the state of Rio Grande do Sul, Brazil. The Clinical and Laboratory Standards Institute (CLSI) standardized the M38-A method for establishing the in vitro sensitivity of filamentous fungi, including S. schenckii, to antifungal agents. M38-A is the current broth dilution reference method for determining the antifungal activity in these microorganisms. In the present study, we used the RPMI-1640 culture medium buffered with 3-(N-morpholino)-propanesulfonic acid; however, this medium has some disadvantages: its high cost and the cumbersome procedure involved. Sabouraud dextrose broth (SDB) is the major culture medium used in mycology, and it is easy to prepare and inexpensive. The purpose of the present study was to evaluate the feasibility of using SDB in lieu of the RPMI-1640 medium for evaluating the in vitro sensitivity of S. schenckii. Thirty-five clinical isolates of S. schenckii and three antifungal agents were used: itraconazole, ketoconazole, and terbinafine. The M38-A protocol standardized by the CLSI was used. *Candida* krusei ATCC 6258 and *Candida* parapsilosis ATCC 22019 were used for quality control. The statistical analysis was performed with Student's t test. Both media showed to be statistically equivalent for itraconazole (p=0.70), ketoconazole (p=0.96), and terbinafine (p=0.87). Thus, SDB may be used in lieu of RPMI-1640 to evaluate the antifungal activity of S. schenckii.

PP-06-60

A case of lymphangitic sporotrichosis developing under the Nail

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A 75-year-old woman working on a farm consulted a nearby clinic because of a 4-week history of a nodule under the nail of the right thumb. She was given a diagnosis of whitlow and treated by nail avulsion. Later, multiple dark-red nodules, some as large as a thumb tip and crusted in the center, appeared sporadically on her right arm, in the area from the dorsum of her hand to the upper arm. The patient was referred to our department.

Histopathologic analysis of a skin biopsy specimen revealed granulomatous and pyogenic changes. An isolate cultured from the biopsy specimen was identified as *Sporothrix schenckii* based on colony morphology and microscopic morphology. A sporotrichin test was positive, and the patient was given a diagnosis of sporotrichosis. The patient was successfully treated with local thermotherapy and oral potassium iodide.

To date, about 3500 cases of sporotrichosis have been reported in Japan. This disease is more likely to occur in winter than in summer, and usually affects children or older adults, particularly farmers and outdoor workers. Sporotrichosis typically affects the face in children. In adults, the arms are most frequently affected, followed by the face. The present case is the first reported case of this disease developing under the nail and is noteworthy in considering the differential diagnosis of this disease.

PP-06-61

Case report. Sporotrichosis successfully treated with oral itraconazole

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A 65-year-old male was referred to our department for evaluation of eruptions on his arm. The eruption appeared on the inner side of his right upper arm 9 months ago, and had gradually enlarged without subjective symptoms. Another eruption appeared at the proximal site of the first eruption 5 months ago. There was no history of trauma or taking immunosuppressive agents.

Physical examination revealed that the distal eruption was a red, oval-shaped, flat-elevated, plaque, 28 x 17mm in size, and that the proximal one was a pinkish, dome-shaped, nodule, 10 x 10mm in size. Laboratory findings showed no remarkable abnormality. The distal plaque was completely excised for histologic and mycological examination. Hematoxyline-eosin (H&E) stain showed epidermal pseudocarcinomatous hyperplasia and dense, band-like, inflammatory infiltration in the upper-middle dermis which consisted of lymphocytes, plasma cells and histiocytes forming epithelioid granuloma focally. A skin test using sporotrichin antigen was positive. The pathogen was identified as *Sprothrix schenkii* based on findings of the culture on Sabouraud's dextrose agar at 25℃ and the slide culture of the fungal isolate. DNA extracted from the sample amplified with the oligonucleotide primers specific for *Sporothrix schenkii*, and direct sequencing of the obtained DNA fragment revealed a high degree (99%) of sequence similarity to *Sporothrix schenkii*. After 12 weeks administration of oral itraconazole (200mg/day), the eruption disappeared leaving only mild erythema, and there has been no recurrence during the 5 months of follow-up until the present.

Poster Forum PF-02

PP-06-62

Sporotrichosis in pregnancy: Report of four cases of a zoonotic outbreak in Rio de Janeiro, Brazil

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Sporotrichosis is the most common of the subcutaneous mycoses. The disease has diverse clinical manifestations being the most common the lymphocutaneous form. In most cases the transmission occurs through trauma with contaminated organic material. However, in the last 11 years in Rio de Janeiro, Brazil, this epidemiological profile had changed and the transmission by sick cats was commonly observed, resulting in a zoonotic outbreak of sporotrichosis. Pregnant patients with sporotrichosis constitute a special group due the immunologic status and the drug choice for treatment is limited; most of the antifungals available are teratogenic toxic or cause thyroid damage to the fetus. Four pregnant patients with confirmed sporotrichosis from the Hospital Universitário Pedro Ernesto/UERJ were assessed. All four patients lived in Rio de Janeiro and repported direct contact with cats. The mean age was 31.3 years and the mean period of infection was 3.8 weeks. The lymphocutaneous form predominated (75%) and none had signs or symptoms of systemic manifestation. Amphotericin B and local heat terapy were selected as treatment. There were no pregnancy intercorrences and all babies were born in time and normal, except one that died hours after delivery. All patients were clinically cured of sporotrichosis. The diagnosis was confirmed by isolation of *Sporothrix schenckii* of the skin lesions. Serodiagnosis and serological follow-up was performed in all patients using an ELISA test with the SsCBF antigen, we had previously described. Good correlation between clinical improvement of patients and the decrease of IgG antibodies level were observed. Supported by: Brazilian Healthy Ministry, Faperj and CNPq.

PP-06-63

Survey of 157 sporotrichosis cases examined in Nagasaki prefecture between 1951 and 2008

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This paper surveys a total of 157 sporotrichosis cases examined in Nagasaki prefecture, Japan. No significant differences regarding either gender or the affected sites of the body were found between 134 cases diagnosed from 1951 to 2000 and 23 cases diagnosed from 2001 to 2008. Both males and females were equally affected during these periods. The lesions were frequently seen on the face (26.3% in males, 26.1% in females) and upper limbs (64.7% and 60.9%, respectively). Regarding the clinical forms, the fixed type (61.0% in males, 60.9% in females) was much more frequent than the lymphocutaneous type (39.0% and 39.1%). The ratio of patients over 50 years of age increased from 72.0% to 87.0%. No patients under 10 and two patients over 90 were examined after 2001. There has been a remarkable increase in the number of cases in the Shimabara area (from 26.8% to 47.8%). With respect to the therapy, before 1994, potassium iodide (KI) was used in almost all cases (99.1%). Since 1995, however, itraconazole has been used in more than 50% of the cases. Cases treated with terbinafine have also increased. KI has been used in about 20% of the cases diagnosed after 1995. The use of KI temporarily decreased between 1995 and 2000 (8.3%), but has recently increased again (27.2%). The treatment period until complete cure was 17.0 weeks for itraconazole and 10.9 weeks for KI prescriptions.

PP-06-64

Purifying selection in Exophiala dermatitidis leads to adaptation to the human environment

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Exophiala dermatitidis is an asexual fungus able to produce melanized budding cells. The fungus is able to pass through stomach and intestinal tract of laboratory animals without decrease of vitality, probably aided by its ability to convert to meristematic growth at low pH. The fungus occurs consistently, though at a low incidence, in the digestive tract of otherwise mostly asymptomatic humans. Using rDNA ITS sequencing, two main genotypes **A** and **B** are known. The group of genotype **A** strains contains more clinical isolates, whereas genotype **B** strains are preponderantly found in the (natural and man-made) environment. With multilocus analysis, ITS and TUB1 showed limited recombination, but no evidence was found in EFα-1, probably because of polymorphism being nearly restricted to a multi-T region. Genotypes **A** and **B** show diversification by phenetic differences, such as in thermotolerance, ultimately leading to ecological speciation processes which mark its transition from its natural habitat as an asymptomatic associate of frugivorous animals in the tropical rain forest to human-dominated environments. We hypothesize that it is transported to this new environment by humans after ingestion of contaminated tropical fruits. Further dissemination, e.g. to public bathing facilities, must be brought about by human-carriage. The transition was calculated to be accompanied by purifying selection. A set of 178 strains from natural and human-dominated environments in Thailand with a worldwide selection of strains from CBS collection were analyzed by AFLP fingerprinting method to establish the haplotype distribution. It is concluded that virulence is likely to be linked to individual clones, some having remained local and others having spread on a world wide scale by human vectors. The main genotypes **A** and **B** may be viewed as clusters of haplotypes that on average show different ecological trends.

PP-06-65

Isolation of black yeast in endemic areas of lethargic crab disease (LCD)

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Chaetothyrialean fungi represent a large and heterogeneous group of species which are characterized by having a dark-pigmented cell wall (melanin) in vegetative and reproductive cells. These fungi are found in nature mainly in soil and decomposing organic matter. The main fungal diseases caused by these agents include eumycothic phaeohyphomycosis and chomoblasthomycosis, which occur by the traumatic implantation of the fungus in the host tissue. Recently, an emerging infirmity known as Lethargic Crab Disease (LCD), caused by a chaetothyrialean fungus of the genus Exophiala, is causing massive mortalities in populations of the mangrove crab (Ucides cordatus) in Brazil. Crabs affected by LCD show lethargy and poor motor control. The present study aimed to isolate chaetothyrialean fungi from the Bay of Guaratuba, Paraná (a region that has never been exposed to mortalities caused by LCD), to characterize the obtained isolates through morphology and molecular markers, to identify fungi related to the etiological agent of LCD, and to compare the obtained results with previous isolates collected in regions where the disease is endemic (states of Sergipe and Bahia). Only 0.17% of chaetothyrialean fungi from Guaratuba showed similarity in their ITS sequence with the reference strains of Neofussicoccum parvum, Cladosporium tenuissimum, C. cladosporoides, and C. chlorocephalum. This is much lower than the observed frequency in Sergipe, in which 31% were identified morphologically and using molecular data as Exophiala bergeri, E. salmonis, E. spinifera, C. oxysporum, Phialophora americana,and Cladophialophora chaetospira. In Bahia, the frequency of dematiaceous fungi was lower (18,69%) but still substantially higher than in Paraná. A total of 85 chaetothyrialean isolates were obtained (9.38%), of which 29 had morphologies similar to that of the genus Exophiala. These results underscore the importance of understanding the ecological distribution of chaetothyrialean fungi to uncover the epidemiology of LCD.

PP-06-66

Antifungal activity of different pterocaulon alopecuroides extracts on fonsecaea pedrosoi

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Chromoblastomycosis belongs to a group of chronic mycoses caused by several species of dematiaceous fungi, but Fonsecaea pedrosoi is its most frequent etiologic agent. Many treatments have been used against chromoblastomycosis, but efficacy is low, and therefore it is not possible to have a procedure or drug of choice. An ethnoveterinary study found that plants of the Pterocaulon (Asteraceae) genus are popularly used for treating epithelial lesions of fungal or bacterial etiology. The raw methanolic extract of Pterocaulon alopecuroides, a species native to southern Brazil, showed relevant antifungal activity on a large number of chromoblastomycosis agents. The purpose of the present study is to evaluate the antifungal activity of extracts from the aerial parts of Pterocaulon alopecuroides, obtained with solvents with increasing polarity, in 15 clinical isolates of Fonsecaea pedrosoi. The minimum inhibitory concentration (MIC) was determined by the microdilution method recommended by the M38-A document of the Clinical and Laboratory Standards Institute, and which was modified by us. Results showed that the fractions had different activities on the tested chromoblastomycosis agents. The MIC of the hexane fraction was 2,500 to 625 μg/mL, and for 67% of the isolates, the MIC amounted to 1,250 μg/mL. The MIC of the methanolic fraction ranged between 1,250 and ≤ 156.25 μg/mL, and 40% of the isolates presented an MIC of 625 μg/mL. The highest antifungal activity was observed in the dichloromethane fraction, with an MIC of ≤ 156.25 μg/mL in 100% of the assessed strains. Coumarins and flavonoids, to which, according to the literature, biological properties of several plants are attributed, are present in this fraction. These results demonstrate that the identification of the bioactive compounds of Pterocaulon alopecuroides may be an important strategy for the treatment of chromoblastomycosis. We acknowledge the financial support granted by FAPERGS, CNPq, CAPES, and PROPESQ.

PP-06-67

Susceptibility of fonsecaea pedrosoi to propolis and analysis of correlation with the phytochemical profile

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Fonsecaea pedrosoi is the major etiologic agent of chromoblastomycosis. To date, no drug or procedure has been completely efficient against this disorder. Propolis is a resinous mixture collected by bees from several plants. Its chemical composition is very complex and variable, and is related to the surrounding vegetation, season of the year and area where it is collected. Phenolic compounds such as flavonoids have been related to its biological properties. The antifungal activity of propolis against some fungi has been documented in the literature, but little is known about its activity on Fonsecaea pedrosoi. The purpose of this study was to evaluate the in vitro susceptibility of 12 clinical isolates of Fonsecaea pedrosoi to eight propolis samples collected in Rio Grande do Sul, Brazil, and to correlate their activity to their phytochemical composition. The minimum inhibitory concentrations (MICs) of propolis were established according to the microdilution method proposed by the M28-A document of the Clinical and Laboratory Standards Institute. The phytochemical characterization was performed by thin layer chromatography and quantification of the phenolic compounds. The MICs obtained ranged from 156.56 to 2,500 μg/mL, and the most active samples were those collected in Santo Antonio da Patrulha and Candelaria. The samples showed compounds with a chromatographic profile of terpenoids, compounds which showed higher concentrations in the more active samples, as well as compounds with a chromatographic profile of flavonoids. With this technique, a smaller variety of compounds was observed in the more active samples when compared with the other propolis samples analyzed. The quantification of phenolic compounds showed that there is no correlation between concentration and antifungal activity. The propolis samples tested showed a high antifungal potential against F. pedrosoi; however, no phytochemical pattern was found that might be related to this activity. Financial support: FAPERGS, CNPq, CAPES, and PROPESQ.

PP-06-68

A case of chronic chromoblastomycosis treated with a combination of voriconazole and local heat therapy

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Introduction: Chromoblastomycosis (CBM), a chronic cutaneous or subcutaneous disease caused by black fungi, is notoriously resistant to treatment especially in severe clinical forms, and its management is challenging. Usually itraconazole (ITZ) or terbinafin is chosen as a first line chemotherapy. Voriconazole (VCZ) is a second generation antifungal triazole that has demonstrated *in vitro* activity against many pathogens of CBM, but its clinical usefulness in treatment has not yet been evaluated. Here we present a chronic severe case of CBM treated successfully with VCZ and local heat therapy.

Case report: A 55-year-old Filipino, with a 20-year history of multiple skin lesions on his left lower limb, presented to seek treatment for progressive lesions on his leg in spite of undergoing treatment. In the Philippines, since 1999 he has had several prolonged courses of therapy for his fungal condition including surgery and CO_2 laser treatment in addition to systemic antifungal drug administration.

At our first examination in October 2008, he was treated with ITZ at doses of 100mg per day. The lesions on the thigh and upper part of the leg had healed by scarring, but prominent cauliflower-like lesions were observed on cicatricial skin of the lower part of the leg. The diagnosis of CBM was established by the direct observation of sclerotic cells in skin scrapings and biopsy specimens, and it was confirmed by the isolation of dematiaceous mold. Response to oral VCZ in a dose of 400mg per day, with topical heat therapy using disposable pocket warmers for several hours per day, was evident by month 1. At 2 month tumoral masses were reduced with negative fungal culture and the elevations of transaminases detected at the beginning had become normalized. The patient remains on combination therapy and clinical improvement was continued at month 4.

PP-06-69

A chronic chromoblastomycosis model by *Fonsecaea monophora* in Wistar rat

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Chromoblastomycosis is a chronic, cutaneous and subcutaneous infection characterized by verrucose lesions, mainly caused by *Fonsecaea monophora* in southern China, and poorly responds to available therapies. For investigating the pathogenicity of *Fonsecaea monophora*, we established a chronic chromoblastomycosis model with *Fonsecaea monophora* in Wistar rats. The suspensions of 2×10^6cfu conidia and fragment hyphae were injected by intracutaneous (ic) and subcutaneous (sc) routes at either side back of Wistar rats. Small nodules were formed at the inoculation sites in the first week after inoculation. In the second week, the nodules enlarged and became soft, and pus could be aspirated from the nodules. In the forth week, the nodules in ic group ulcerated and sclerotic bodies were observed in pus smear by both inoculation routes. In the 3rd month, the nodules by ic route became flat with thin black crust on the surface. For ic group, sequential biopsy revealed the extensive necrosis with neutrophil infiltration and sclerotic bodies and some debris of fungi around in the 1st month; sclerotic bodies inside multinucleated giant cells in the 2nd month and widespread granulomatous inflammations in the 3rd month.

This study presents a promising animal model that can be used to investigate the pathogenicity of the different etiologic agents, the immune response of the host involved in the pathogenic process and to explore the effective antifungal agents for chromoblastomycosis therapy in vivo.

PP-06-70

Two cases of subcutaneous phaeohyphomycosis due to unidentified fungi in immunocompromised hosts

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[Case 1] A 64-year-old man presented with a 3-year history of a nodule on his left knee. He had taken prednisolone, 10mg dairy, for autoimmune haemolytic anaemia for 10 years. His hobby was gardening. On examination, a brownish and elastic hard nodule, 20mm in diameter, was present on the left knee. A biopsy specimen showed numerous fungal elements by PAS staining. We performed a total resection, and the lesion did not recur. In a culture study, a dark gray colony slowly grew. Its surface was wetly villous and partly velvety. We could not identify it, because this fungus had no conidial formation despite culturing it in various kinds of culture media / conditions.
[Case 2] A 59-year-old woman, who had been treated with oral corticosteroids and tacrolimus for her systemic lupus erythematosus and autoimmune hepatitis, had a 6-month history of an ulcerative nodule on her left hand. Histologic examination of the excised nodule showed an abscess surrounded by multinuclear giant cells that included numerous conidia and hyphae. Culture materials from biopsied nodules and crusts yielded a dark grayish green villous colony on potato dextrose agar and Sabouraud's dextrose agar, and a white velvety colony on brain heart infusion agar. We could not identify this fungus, because the formation of conidiospore was poor.
She developed metastasized lesions with elevated serum β-D-glucan. Currently, she was treated with an oral antifungal agent.

PP-06-71

The cases of subcutaneous phaeohyphomycosis

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Phaeohyphomycosis is a rare fungal infection that is more commonly associated with immunocompromised patients. The term "Phaeohyphomycosis" is intended for opportunistic infections caused by dermatiaceous fungi regardless of their taxonomic classification. The E.jeanselmei taxon is a popular fungi which is isolated from phaeohyphomycotic cyst. However, the E.jeanselmei taxon is reportedly rather heterogeneous with various opportunistic Exophiala species that have been isolated from humans and that have been previously identified as E.jeanselmei, differing in terms of predilection, clinical behavior and ecology. In recent years, diagnositic approaches have been supplemented by molecular tools. By using this methods, many strains identified as E.jeamselmei morphologically, have been re-identified as another Exophiala species, e.g. E, xenobiotica. Here we report two cases of Phaeohyphomycosis. One case is caused by E.xenobiotica, the other case is caused by E.jeanselmei. We compared the two cases and discussed the difference of their clinical behavior and ecology.

Poster Forum PF-02

PP-06-72

Chromoblastomycosis and subcutaneous phaeohyphomycosis caused by *Exophiala bergeri* and *E. xenobiotica* in immuno-compromised patients

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Case 1: An 83-year-old woman with rheumatoid arthritis was injured with a splinter and 1 month later developed a nodule on her left forearm. Culture of the excised nodule revealed black yeast-like colonies. The nodule exhibited hyperkeratosis, acanthosis, and dermal granulomatous infiltrates with multinucleated histiocytic giant cells. Sclerotic bodies and their chains were present in the inflammatory tissue, especially within the giant cells. Terbinafine was administered postoperatively.

Case 2: A 61-year-old man with systemic lupus erythematosus presented with progressive subcutaneous abscesses on his left hand and forearm since1 month. Culture of a biopsy specimen revealed black yeast-like colonies. The specimen showed suppuration, granulomatous reactions, giant cells, and massive pale brown filamentous and toruloid hyphae. Surgical excision followed by flucytosine administration for 2 months was successful.

Mycological findings: On potato dextrose agar (PDA), felty, grayish-to-black or partly wet and black colonies (Case 1) and felty, grayish-to-black colonies (Case 2) were observed. Unicellular conidia formed from the small projections on the walls of filamentous and torulose hyphae and from ampuliform conidiogenous cells. Yeast-like reproduction was dominant in the Case 1 isolate. The internal transcribed spacer (ITS) sequences on the rRNA gene of the Case 1 and 2 isolates coincided with those of *Exophiala bergeri* and *E. xenobiotica*, respectively.

E. bergeri was originally described as a causative agent of chromoblastomycosis; *Torula bergeri*, in 1949, the fungus name had been regarded as a synonym below several species related with *Exophiala*. In 1999, *T. bergeri* was considered an independent species and reclassified under the genus by Haase et al. Case 1 is the second reported case of "*bergeri*" infection, and ITS sequences on >10 clinical isolates of this fungus have been recorded, worldwide, including Japan. *E. xenobiotica* infection is rare in Japan. These infections will be detected more frequently by genotyping clinical isolates.

PP-06-73

Mycetoma due to a novel species of *Pleurostomophora* in an indigenous woman from the Kimberley region of Western Australia

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Mycetoma is a local, chronic, slowly progressive, often painless infection of subcutaneous tissues caused by fungi. We report a 73-year-old indigenous woman from the remote rural Kimberley region of Western Australia, who was admitted for management of left ankle mycetoma (neglected for at least 10 years). She had a large, non-tender indurated 10cm x 15cm mass with multiple draining sinus tracks near her left lateral malleolus. MRI showed changes consistent with extensive mycetoma.

The tumour was surgically removed; latissimus dorsi muscle flaps and skin grafts were required to cover the defect. Histopathology of the biopsy demonstrated multiple micro-abscesses with fungal grains surrounded by hyaline-like material (Splendori-Hoeppli reaction) consistent with fungal mycetoma. Operative tissue cultures yielded two fungi with distinct phenotypic features: a melanized isolate identified as a Pleurostomophora sp. (nov. species) by The CBS (Utrecht, The Netherlands) and another isolate identified as Phialamonium curvatum. The *Pleurostomophora* sp. isolate could only be identified by complete sequencing of the 18S rRNA gene. Using CLSI M38A methodology, the isolates had matching antifungal susceptibility test (AFST) results (Women's and Children's Hospital, North Adelaide, Australia), testing voriconazole 0.5 mg/L (S) and itraconazole 1.0 mg/L (R). She was initially treated with voriconazole, but had intolerable nausea and vomiting. Itraconazole was therefore tried empirically, despite the in vitro AFST results, with complete wound healing. Treatment in the Kimberley was given for approximately 18-months with reasonable adherence, and was associated with good clinical efficacy. Currently, at 5-years follow-up, she remains well with a good functional outcome and without any signs of relapse.

We report a novel species of *Pleurostomophora* sp. associated with mycetoma. This case also illustrates the challenges in identification of the phylogenetically diverse dematiaceous agents of mycetoma, and the imperfect correlation between AFST results and the clinical efficacy of antifungals used to treat mycetoma.

Poster Forum PF-02

PP-06-74

A case of Actinomycetic mycetoma

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The case was a 25-year-old Japanese male patient was consulted at dermatological clinic of Tokai university hospital at our department on 29 November 2007 complaining of the skin nodes of the right knee. He was injured with the right knee by falling down in 1999. The part of injury cicatrized and spread slowly. The patient had no particular medical or family history. On the physical examination at the initial visit, the lesion containing small verruciform masses with sores and crusts was an 16 X 18 cm in size and had satellite masses. It extended from skin to the fascia in the MRI views. Lesions in the other internal organs can't be found out by image inspection. Histopathological examination revealed abscess formation consisting of neutrophiles, lymphocytes and epithelioid cells. In the center of abscess, lumps dyed by PAS and Grocott's stains were recognized in the histological sample. The culture views on Sabouraud's dextrose agar and Potato Dextrose Agar from the skin organization at the first examination showed colonies of the woolliness of the light gray-black. Because of the result of the culture, the dosage of itraconazole 200mg/day internal use therapy carried out. The skin nodes increased again from treatment the fifth month though it were showed a tendency to healing temporarily. The operative treatment was carried out. The culture views from the skin organization at the operation showed a fold, and the center was orange, and the border was white. In addition, it smelled of the soil. By the further mycology and a pathological close inspection, the colony at the first examination was considered a contamination. We diagnosed this case as Actinomycetic mycetoma / plimary cutaneous nocardiosis. We administered minocycline and trimethoprim-sulfamethoxazole combination to the patient by addition. We are carrying out to define identity of the genus.

PP-06-75

Study of 62 cases of mycetoma in Iran

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We present a retrospective study of 62 cases of mycetoma in Iran that were diagnosed clinically and microbiologically from 1972 to 2005. In 1972 mycetoma has been first report in Iran by medical mycology department of Tehran University. We retrospectively compared the overall prevalence of mycetoma and the prevalence of infective agent in Iran during 33 years. In our study age, sex, job, infective agent and anatomical site of infection have been considered. Retrospective analysis of the records revealed that the ratio of actinomycetoma and eumycetoma was 42:20 that differed significantly ($p<0.01$). Actinomadura madurae (n=11) in actiomycetoma, and Psudallescheria boydii (n=9) in eumycetoma were predominant agents. The male to female ratio was 41:21and the peak age for infection was between 40-50 years old but there is no a significant differences between age groups (P>0/05) and the earliest age of onset was 18 and the latest 65.The single most common site of infection is foot but generally hand and other limbs can infected and 54.4% of infected area was in palm and disease can infected other area with less frequency. A total of 49 cases were pedal mycetoma and 13 cases were in extra pedal areas .The results show that farmers with 45.2% are at greater occupation risk of mycetoma ($p<0.01$).

PP-06-76

Protothecosis in Japan. Literature review of Japanese cases

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Protothecosis is rare infectious disease caused by *Prototheca sp.* which is achlorophyllic algae related to the green algae *Chlorella* and reported only more than 100 cases in English literature around the world. Some immunocompromised status, trauma, contact with contaminated water, tropical area ware recognized as predisposing factor. We experienced a case of protothecosis in Okinawa, Japan in March, 2007 and report it in a Japanese congress. Since then, we have interested in protothecosis, gathered reported cases in Japan, although almost of those are in Japanese and including cases only reported in congress, via internet tool named ″*ICHUSHI Web* ″ like a ″PubMed″ in Japan and did some evaluation. Total incidences of protothecosis in Japan are 30. 18 are men, 12 are women. One is a 15-year-old boy and both 40 and 42-year-old women reported but remains of all were over at age of 50. 26 people are cutaneous protothecosis, 3 are systemic and there is no olecranon bursitis as clinical manifestation. Exceptionally, *Prototheca* recovered from larynx in one case. Causative agents of 17 identified as *Prototheca wickerhamii* and remained 13 are not yet speciation. More than half of patient has immunocompromised status due to underlying disease and/or immunosuppressive drugs. Interestingly, geographical distribution of protothecosis clearly has some tendency in Japan. Northern part of Japan has no case. From middle to southern part of Japan have some incidences. It seems like that more southern have more incidences from analysis of incidences per population. We presume that southern part of the Temperate Zone tend to more incidences of Protothecosis than northern part although the tropical zone mentioned as predisposing factor ever and actually southeast of the U.S reported as place where Protothecosis tend to more occur in literature. We also plan to additionally evaluate cases in the U.S mainly about its geographical distribution.

PP-06-77

Cutaneous protothecosis and review of Chinese reports

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Protothecosis is a relatively uncommon infection of humans and animals, which is caused by either of two species belonging to the genus Prototheca. Protothecosis has been classified in three clinical forms: cutaneous protothecosis, olercranon bursitis and disseminated infections. About half of protothecosis cases are cutaneous infection. We present here a case of cutaneous protothecosis due to Prototheca zopfii. A 23 years old woman presented slowly enlarging macule appeared in the right face for more than 2 years without itching or ache. Spherical sporangia containing multiple endospores were detected by direct microscopy in tissue specimen, which could also be found in tissue sections with Gomori methenamine silver stain and PAS stain. The culture colony is dull white and yeast-like. The API20 system provides the assimilation pattern as trehalose negative and propanol positive. Large-subunit region of ribosomal RNA gene was amplified with universal fungal primers D1 and D2. The consensus sequence of the isolate aligned with 99% sequence similarity to multiple sequences of Prototheca zopfii var. portoricensis available in the GeneBank database. The cutaneous protothecosis reports in Chinese were reviewed.

Poster Forum PF-02

PP-06-78

A case of cutaneous *Pseudallescheria boydii* infection

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Pseudallescheria boydii is a ubiquitous filamentous fungus. We report a case of cutaneous *P. boydii* infection in the bilateral dorsal hands in a 69-year-old female Japanese patient who was receiving oral predonisolone (15 mg/day) and azathioprine (75 mg/day) for the treatment of rheumatoid arthritis. She presented with a lesion on the bilateral dorsal hands that persisted for 1 month. A biopsy specimen from the skin lesion revealed granulomatous inflammation with hyphae. DNA sequencing and macroscopic and microscopic characteristics of the culture were used to identify the causative agent as *P. boydii*. The patient was treated with a 3-month course of oral itraconazole pulse therapy at a dosage regimen of 400 mg/day for 1 week per month. No change was detected at month 4, and the patient was then treated with 125 mg/day of oral terbinafine for a month. No change was detected at month 6. Further, the lesion gradually increased in size and also disseminated to the face. Preliminary studies have reported that the mean minimum inhibitory concentration of voriconazole against *P. boydii* is lower than that of itraconazole, which has been viewed as the treatment of choice for *P. boydii* infections. We will now attempt to treat the infection with voriconazole. An opportunistic infection in immunocompromised patients can be life-threatening; therefore, prompt treatment based on accurate diagnosis is important.

PP-06-79

Paecilomyces lilacinus cutaneous infection in a case of Rheumatoid arthritis treated with oral voriconazole and topical Mycostatin solution

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This 66 y/o female, who has been relatively well before this admission, complained of redness over anterior portion of left leg for one month, and painful ulceration with swelling over the same area for one week. Past history: Rheumatoid arthritis. She has visited our dermatology department one day before this admission, and multiple hemorrhagic vesicles, pustules, bullae with erosion and exudates over left leg were noted. She denied trauma history recently, using herb or any medication for the wound, but cleansing the wound with beta-iodine once a day. She has habits of bathing herself (including the open wound) in hot water 20-30 minutes a day. She has productive cough (green sputum) for 2 days, but she denied fever, rhinorrhea, sore throat, muscular soreness, headache, dizziness. She has nocturia (2-3/night), but no dysuria, frequency, or urgency. She did not have pets, not exposure to countryside, animals, insect-bitten, open wound, and no recent traveling history. She presented to our ER as multiple ulceration and swelling. CBC: no leukocytosis. CRP: 0.63. Under the primary impression of cellulitis, she was admitted for further evaluation and management. 11/26 Skin biopsy: Paecilomyces species. We treated her with oral voriconazole 1# BID and local treated with Mycostatin solution. The response was good and the wound heal well.

PP-06-80

Soft tissue infection caused by the coelomycetous fungus microsphaeropsis arundinis

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Coelomycetes are emerging agents of locally invasive subcutaneous opportunistic infections in immunocompromised hosts. Herein we report the third well documented human infection caused by Microsphaeropsis arundinis. A 62-year-old man with long-standing myelodysplastic syndrome, recently transformed into acute myeloid leukaemia, presented with a lesion on his left foot. The mass had been slowly increasing in size following his first cycle of induction chemotherapy. Examination showed a 2cm x 3.5cm painless, deep granulomatous, purplish-black, non-exudative plaque on the dorsum of his left foot.

Cytology of a fine needle aspirate demonstrated non-specific mixed inflammatory cells consisting predominantly of histiocytes and lymphocytes, with scanty neutrophils. Direct microscopy and methenamine silver and periodic acid-Schiff stains were negative, but fungal cultures yielded a pure culture of a dematiaceous hyphomycete after 7-days incubation, which was identified as Microsphaeropsis arundinis by phenotypic features and molecular studies. Phenotypic features included typical ostiolate pycnidial conidiomata, ampulliform conidiogenous cells, and small, smooth-walled, brown, cylindrical conidida. Colonies on tap water agar produced typical pycnidia after 7-weeks with a 12hr/12 hr alternate dark-light regimen. Molecular identification of Microsphaeropsis arundinis was performed by PCR amplification and DNA sequencing of the internal transcribed spacer regions (ITS1 and ITS2) of the ribosomal RNA gene complex. Antifungal susceptibilities tests (AFST) were performed using Yeast-One Sensititre colorimetric microbroth dilution method (96hrs, 28°C). MIC's by AFST were: itraconazole 0.03mg/L, voriconazole 0.06mg/L, posaconazole 0.016mg/L and amphotericin B <0.125mg/L. The isolate had susceptible-dose-dependent MIC's to fluconazole 32mg/L and 5-flucytosine 16mg/L. Terbinafine tested susceptible by a disc diffusion methodology.

The patient was treated with oral terbinafine, and the mass completely resolved after 9-weeks of therapy, with terbinafine being stopped shortly thereafter. Formal excision of the lesion was not required. Henceforth, there has been no recurrence despite on-going aggressive chemotherapy, after almost 12-months follow-up.

PP-06-81

Primary cutaneous zygomycosis due to *Absidia corymbifera* in a patient with cutaneous T cell lymphoma

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Zygomycosis is the most rapidly fatal fungal disease in human by organisms of the class Zygomycetes. Cutaneous zygomycosis is the third most common clinical presentation in this patient population. Cutaneous zygomycosis caused by *Absidia corymbifera* is very uncommon. We present here a case of primary cutaneous zygomycosis caused by *A. corymbifera* in a patient with primary cutaneous CD4 positive small/medium-sized pleomorphic T-cell lymphoma. A slowly enlarging arm ulcer appeared in a 61-year-old man with cutaneous T cell lymphoma. Skin biopsy revealed proliferation of aseptate hyphae and nodular small/medium-sized pleomorphic CD4+ T cell infiltration. Immunopathology study and molecular genetic study confirm the diagnosis of cutaneous T cell lymphoma. Colonies on Sabouraud dextrose agar at 37 degreeafter 2 days were woolly and whitish-grey with raised mycelium, and the reverse of the culture was colorless. Maximum growth temperature was 46 degree. Upon examination of a wet mount preparation stained with lactophenol cotton blue, branching sporangiophores arising from the stolons were evident and arranged in whorls. The sporangia were pear shaped and had prominent conical columellae. A flask-shaped apophysis was evident beneath the sporangium. Ribosomal DNA ITS domains was amplified, and the product was sequenced, which was aligned with 99% sequence similarity to multiple sequences of *Absidia corymbifera* available in the GenBank database. Since no organ involvement was detected by thorough examination, the patient was diagnosed as having primary cutaneous zygomycosis. The patient received local radiation therapy for treatment of cutaneous T cell lymphoma, and received intravenous amphoteracin B for only 3 weeks due to its side effect. This is the first case report of cutaneous zygomycosis caused by *A. corymbifera* with primary cutaneous CD4+ small/medium-sized pleomorphic T-cell lymphoma. The primary cutaneous zygomycosis due to *A. corymbifera* were reviewed.

PP-06-82

Primary cutaneous zygomycosis caused by rhizomucor variabilis: A new endemic zygomycosis? Cases report and overview of 7 cases reported in China

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We present two cases of primary cutaneous and subcutaneous zygomycosis caused by Rhizomucor variabilis, and review 7 cases well reported in the past twenty years in China. Two female patients without any underlying diseases both presented with gradually enlarged plaque and nodules on the face. R. variabilis was isolated from the lesions of the two patients. Both cases showed resistance to itraconazole, but being treated successfully with 3-5 months of amphotericin B therapy. By overviewing the all 7 cases, we observe that most of these cases share the following characters:long clinical course, occurrence on patients with farming experience and surgery or injuries histories, locations in faces or extremities, slowly expanding nodules and patches in early phase, no cellular immunity dysfunction or underlying diseases, and histopathology showing mixed granulomatous inflammatory infiltration without obvious vascular involvement. R. variabilis or R. variabilis var. regularior was isolated from the lesions. The disease showed low mortality but high mutilation, with sensitivity to amphotericin B treatment while resistance to azoles. The features mentioned above are obviously different from cutaneous zygomycosis cases caused by other Zygomycetes species. It is noteworthy that all the 7 cases are observed in China, and further more, in four geographically adjacent provinces of East China. This coincidence suggests further research to explore the relationships between endemic circumstance and potential morbility of the disease in the area.

PP-06-83

Rhinofacial entomophthoromycosis due to *Conidiobolus coronatus* - a case report from north-India and an overview

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Rhinofacial entomophthoromycosis due to *Conidiobolus coronatus* is a rare, chronic granulomatous disease occurring mainly in tropical Africa, South and Central America and south-east Asia including India. We report an autochthonous case diagnosed by culture and histopathology in a 30-year-old male farmer, resident of Gorakhpur city in Uttar Pradesh, a northern state. The patient presented with a slowly progressive swollen nose with obstruction since one year. He was non-diabetic, non alcoholic, without history of trauma and negative for HIV antibodies. His general physical and systemic examination was unremarkable. The nose showed a diffuse, mildly tender erythematous, non pitting, bilateral swelling, mild mucosal crusting and hypertrophy of inferior turbinates but no regional lymphadenopathy. A CECT scan showed bilateral frontal, maxillary and ethmoid sinusitis with nasoethmoid polyposis. Culture of nasal biopsy on Sabouraud dextrose agar medium with antibiotics yielded multiple, white, glabrous, slightly granular and radially furrowed colonies of a mold with satellite colonies at periphery. Notably, the culture plate lid had identical mold growth resulting from forcibly discharged conidia. PDA slide culture of the isolate revealed numerous globose conidia with a basal papilla, borne singly on short slender conidiphores. Besides, replicate conidia and a few villose conidia typical of *Conidiobolus coronatus* were present. Identity of the isolate was further confirmed by direct DNA sequencing of internal transcribed spacer (ITS) region of rDNA. Haemotoxylin and eosin stained tissue sections of biopsy revealed irregular epidermal acanthosis, marked inflammatory and granulomatous reaction with sparse hyphae. The patient was treated successfully with a standard regimen of saturated potassium iodide solution, administered orally in conjunction with oral itraconazole, 100 mg twice a day for 8 weeks. An overview on epidemiologic and laboratory diagnostic aspects, including molecular diagnosis and therapy of the disease, will be presented.

PP-06-84

Chaetothyriales associated with leafcutter ants: Opportunistic species

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Although species of chaetothyrialean fungi are recurrently observed on humans, their environmental niches are still not well clarified. Their presence in natural substrates has been considered uncommon. Recent studies show that when selective methods are applied, black yeasts belonging to the Chaetothyriales may be easily encountered in nature. Investigations on the ecology of these agents can help the understanding of their epidemiology. Leafcutter ants are social insects with a complex fungus mutualism. This symbiosis was recently reviewed when molecular phylogenetic analyses revealed the occurrence of black yeasts forming a derived monophyletic lineage, in the Apterostigma ant-microbe association. Aiming the study of black yeasts in other ant's microniches, the oil flotation isolation technique was used. Thus, three opportunistic species were found in the ant waste deposit and in the fungus garden of lab nests of the leafcutter ants Atta sexdens rubropilosa: Coniosporium epidermidis; Exophiala xenobiotica and Cladophialophora minourae. C. epidermidis is considered a lineage basal of this group of fungi, among relatives from rocks. It is found in humans either asymptomatic or symptomatic. E. xenobiotica is a relatively common agent of cutaneous infections, although environmental strains are frequently reported in habitats rich in hydrocarbons. Cl. minourae is reported as saprophytic, usually found in plant litter, but also present in polluted soil; phylogenetic analyses show this group is sister to the pathogenic species Cl. arxii. It is known that virulence and pathogenicity in Chaetothyriales show three distinct behaviors: saprobes; low-virulence and highly specific pathogenicity. Our results arise questions to be elucidated, e.g.: the role of black yeasts and relatives in Attini ant colonies and the relationship between the fungus niche and its virulence. To achieve these goals, more samplings were done and the strains isolated are being identified.

PP-06-85

Repeated isolations of *Scedosporium apiospermum* from skin of manatees (*Trichechus manatus*)

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Since the spring of 2006, a male Caribbean manatee *(Trichechus manatus)*, named "Ryu" introduced in the Okinawa Churaumi Aquarium in 1997, has been showing coin sized white spots and anthemas on the dorsal distal tail tip of the fin and side of body. The blood profiles, chemistries, general behaviors and appetite were normal. The animal was scrubbed with iodine compound, however there was no improvement. Thereafter, the legions became expanding all over the body. In August 2006, abundant fungal elements were detected on the scale samples under microscopy. White mycelial colonies sprouted from the scales cultured on potato dextrose agar plates supplemented with chloramphenicol (CPDA) at 25°C. The isolates were identified as *Acremonium* sp. morphologically.In February 2007, we noticed that the scales had abundant fungal elements and *Diplogastrid*sp. The species name of the nematoda is under investigation. The repeated culture of scales at 37°C showed abundant colonies of *Acremonium* sp. and *Scedosporium apiospeermum*. Then we cultured the scales of other manatees on CPDA at 35°C. *S. apiospermum* were isolated from the male manatee and the male cohabiter showing no cutaneous lesions. *S. apiospermum* isolates were also positives in April 2007 and March 2008 both males. Nineteen isolates of *S. apiospermum* were collected from 3 times sampling including one isolate from the nursing pool water. There was 3 different genotype of the isolates based o the internal transcribed spacer (ITS) 1-5.8S-ITS 2 regions of ribosomal RNA gene sequences; 2 genotypes were limited to "Ryu" and the rest one was common in male manatees and the pool. Simultaneous isolations of *S. apiospermum* and nematoda on the skins of manatees were not transient. The manatees are under observation without treatments of antifungal drug in January 2009 because of no serious changes on the lesions.

Poster Forum PF-03

PP-06-86

Rapid detection of fungal keratitis using DNA stabilizing FTA^R filter paper

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Purpose: Polymerase chain reaction (PCR) is becoming increasingly important for the rapid detection of fungal keratitis. However, the technique of specimen collection and DNA extraction before PCR may interfere with test sensitivity. The aim of this study was to investigate the use of DNA stabilizing FTA^R filter paper for specimen collection without DNA extraction for a single step, non-nested PCR for fungal keratitis.

Methods: Specimens were collected from ocular surfaces using FTA^R filter discs which automatically lysed collected cells and stabilized nucleic acids. Filter discs were directly used in single step PCR reactions to detect fungal DNA. Test sensitivity was evaluated using serial dilutions of *Candida albicans*, *Fusarium oxysporum* and *Aspergillus fumigatus* cultures. Specificity of the test was analysed by comparing 196 and 155 healthy subjects from Switzerland and Egypt, respectively, with 14 patients diagnosed with microbial keratitis.

Results: PCR using filter discs was able to detect 3 organisms of *C. albicans*, 25 *F. oxysporum* organisms and 125 *A. fumigatus* organisms. In healthy volunteers, fungal PCR was positive in 1.0% and 8.4% eyes from Switzerland and Egypt, respectively. Fungal PCR remained negative in 10 cases of culture proven bacterial keratitis and became positive in 3 cases of fungal keratitis but missed 1 case of culture proven *A. fumigatus* keratitis.

Conclusions: FTA^R filter paper for specimen collection together with direct PCR is a very promising method for detecting fungal keratitis; i) the analytical sensitivity is high, without the need for a semi-nested or nested second PCR; ii) the clinical specificity is 91.7-99.0%; iii) the method is rapid and cheap.

PP-06-87

Fungal etiology in eye specimens submitted to clinical mycology laboratory from January 1998 through September 2008 in a turkish tertiary-Care Military Hospital

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Fungal eye infections are increasingly recognized as an important cause of morbidity and blindness. In this retrospective study, it is aimed to determine the frequency of fungal agents in eye specimens submitted to the mycology laboratory in a ten years period, from January 1998 through September 2008. Gulhane Military Medical Academy is the largest tertiary-care military hospital in Turkey with a 2000 bed capacity. The clinical mycology laboratory established in Department of Medical Microbiology processes approximately more than 900 clinical specimens in a year.
During the study period, a total of 9432 mycological specimens was submitted, and of them, 287 (3%) was from the Department of Ophthalmology. When the specimen types were analyzed, most of the specimens were corneal scrapings (n:98, 34%), followed by corneal swabbing (n:74, 26%) and vitreal fluid specimens (n:69, 24%). The remaining specimen types were as follows; irrigation fluid, evisserated eye tissue, corneal RIM, anterior chamber fluid, eye-biopsy material, lens, and aqueous humor samples. Of the cultured mycological eye specimens, only 7% (n:20) yielded fungal growth. Of the 20 isolated fungi, while majority was *Aspergillus* spp (n:12, 60%), 8 A. ustus, 3 A. fumigatus, and one A. flavus, it was followed by *Candida* spp. (n:6, 30%), one Fusaium oxysporum, and one Scedosporium apiospermum.
As a conclusion, *Aspergillus* spp. was found to be accounted for the majority of fungal isolations. The identification of any causative agent should be performed at species level due to susceptibility pattern variability observed among etiological agents.

PP-06-88

Fungal keratitis associated with contact lens wear

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Fungal keratitis is a serious and painful corneal disease that sometimes leads to loss of the eye and frequently caused by Fusarium species and comprises up to 35 percent of microbial keratitis cases.

Early diagnosis of fungal keritiitis in patients is crucial for prompt antifungal therapy. The infection has been associated with various risk factors such as trauma (especially by vegetable material), chronic ocular surface disease, immune suppression, and contact lens wear. In this study we report 6 cases of fungal keritiitis due to contact lens wear in Iran.

The corneal scraping was soaked in 10% KOH and analyzed by direct microscopic and culture on Sabouraud Dextrose agar (SDA). Incubation was performed at 250C and 37 0C for 2 weeks. At the same time, a piece of scraping was smeared onto clean glass slides and the material was Gram stained. All of the patients were female, student, between 18-20 years old and used from contact lens. In all of the cases in direct microscopy we found septet mycelium. Culture results of corneal scrapings basis of their macroscopic and microscopic features were positive for Fusarium species in 5 of patients and *Aspergillus* species in one patient.

This study reports increase of keratomycosis attributable to Fusarium species among contact lens wearers in Iran.

PP-06-89

Fungal endophthalmitis caused by *Emericella nidulans* in a patient following cataract surgery

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We report a case of fungal endophthalmitis caused by *Emericella nidulans* in a 50-year-old female patient after an uncomplicated cataract surgery. On patient's 7-day-postoperative regular check-up, mild increase of anterior chamber reaction was determined. Despite trea tment with frequent application of topical fortified antibiotics (vancomycin and cefazolin) and steroid eye drops, the patient's vision decreased and anterior chamber reaction increased together with the development of hypopion at the end of second week. Treatment at that time included anterior chamber and vitreous tap with intravitreal antibiotic injections (vancomicin and amikacin). The patient's vision continued to deteriorate with no improvement in biomicroscopic findings, and anterior chamber paracentesis, vitreous tap and finally complete vitrectomy with removal of the capsular bag including the intraocular lens were performed. Some surgical specimens were sent to clinical microbiology laboratory for culture and direct microscopic evaluation. Meanwhile, intravitreal amphoterecin B injection was applied. Finally, several sets of culture yielded growth of an *Aspergillus* sp. and these isolates were identified later as *Emericella nidulans* by sequence-based identification in Radboud University Nijmegen Medical Center, Nijmegen, the Netherlands. To our knowledge, this is the first reported case of fungal endophthalmitis due to *Emericella nidulans*. The antifungal susceptibility pattern of the fungus could not be determined in this period. But, after replacing the antifungal therapy with vigorous systemic and local voriconazole administration, significant improvement was observed in vision and ocular inflammatory reaction, and she has remained infection free for a 3-month follow-up period with a good final visual acuity. In conclusion, a rarely observed case of *Emericella nidulans* endophthalmitis was successfully treated with topical and oral voriconazole use.

PP-06-90

Expanded evaluations of contact lens cleansing solutions reveals impaired fungicidal activities against *Fusarium solani* and *Fusarium oxysporum*

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A multinational outbreak of *Fusarium* keratitis resulted in withdrawal of MoistureLoc Contact Lens Cleansing Solution, but the exact causes of this outbreak remain elusive. We hypothesized that product inadequacies and inappropriate usage might have contributed to this still to be resolved outbreak. In the present study, we developed a more rapid and accurate method for an expanded evaluation of current and discontinued cleansing solutions for their fungicidal efficacy. Four multipurpose cleansing solutions (MoistureLoc, Equate, MultiPlus and OptiFree) were tested against planktonic and biofilm- derived cells of *F. oxysporum* and *F. solani*. The methods included a traditional colony forming assay (CFU) and a novel flow cytometry (FC) assay based on dual fluorochrome probes (Sytox Red and CellTracker Green). The tests were designed to simulate manufacturer recommended cleaning regimen (240 min) and inappropriate use (15 - 60 min). The challenge inocula simulated both low and high fungal contaminations of lens cleansing solutions (10^3 -10^5 CFU). Both FC and CFU assays provided comparable results (r^2=0.97), but FC assay results were available in 5 hrs compared to 24-48 hrs for CFU. Furthermore, FC assays allowed ready identification of viable but dormant fungal cell populations, which are missed in CFU assay. In general, a time and inoculum dependent survival pattern was evident in both *F. oxysporum* and *F. solani*, and biofilm-derived cells were more resistant than planktonic cells. MultiPlus and Equate brands were highly efficacious in 100% sterilization. Biofilm derived cells of *F. oxysporum* and *F. solani* survived up to 4 h in MoistureLoc and OptiFree solutions. In conclusion, FC method provided a rapid and reproducible evaluation of lens cleansing solutions. *F. oxysporum* and *F. solani* survived for significant periods of time in discontinued MoistureLoc and still available OptiFree solutions.

PP-07-1

Epidemiology and in vitro susceptibility of yeasts isolated from cardiovascular patients in Iasi, Romania during a 7-year period

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INTRODUCTION: Fungal infections have increased dramatically in recent years and candidemia is a major risk factor for morbidity and mortality in intensive care units.
METHODS: We evaluated the in vitro susceptibility (using ATB FUNGUS, Bio Meriéux, France) of yeasts isolated from various clinical specimens of infected patients treated in the wards (cardiology and cardiovascular surgery) of the Institute of Cardiovascular diseases, Iasi, Romania. Yeasts were identified using the API (ID 32C) system (Bio Meriéux, France) and filamentous fungi were identified by conventional methods (culture and microscopic aspects).
RESULTS: A total of 4863 patients were treated at the Institute of Cardiovascular diseases between May 2001 and December 2008. Fungal infections represented 3.24% of the total of infections. The most commonly isolated yeast was *Candida* non-albicans (51.35%), followed by *Candida albicans* (32.43%) and filamentous fungi (16.22%: Pneumocisits jirovecii, Acremonium spp, Blastomyces dermatitidis, Trichosporon spp.). Tracheal aspirates were the most prevalent site of infection/colonization (40%), followed by bloodstream infections (25.71%), urinary tract (22.85%), biopsie pieces, catheter colonization and wounds (11.48%). The percentage of yeast isolated with decreased susceptibility or resistance to fluconazole was 14.28%, and for voriconazole was 2.85%.
CONCLUSIONS: 1.Our study found comparable frequency of fungal infections and azoles susceptibility as literature reports. 2. We isolated rare fungus reported in cardiovascular surgery infections (Pneumocisits jirovecii, Blastomyces dermatitidis - in pneumonia, and Acremonium spp, Trichosporon spp - in endocarditis).

PP-07-2

Fungal peritonitis in chronic ambulatory peritoneal dialysis patients-A 7 year study in a tertiary care center in South India

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Introduction: Chronic ambulatory peritoneal dialysis (CAPD) is an increasingly popular replacement therapy in end stage renal disease.

However peritonitis continues to be a frequent complication of CAPD. Fungal peritonitis remains a serious complication associated with high rates of morbidity and mortality.

Aim of the study: We did a study to determine the risk factors and outcome of fungal peritonitis in CAPD patients.

Patient and Methods: This retrospective study period was from the year 2000-2007.Patients were evaluated for previous episode of peritonitis, clinical manifestations such as abdominal pain, vomiting, fever, abdominal tenderness. cloudy dialysis effluent, exit site infection and ultrafiltration failure.

The diagnosis was based on elevated CAPD effluent count of 100 or more WBCs per microlitre and isolation of fungi.

Results: The total number of CAPD patients were 185 during the period of study. The incidence of fungal peritonitis was 16.2% with 30 patients among 185 developed fungal peritonitis. Age varied between 8-75 years with mean age of 57 years. The mean duration of CAPD before development of fungal peritonitis was 17.29 months (range3-48). Seventeen patients (56.6%) had previous episodes of bacterial peritonitis that was treated with multiple antibiotics.

The fungi isolated were *Candida albicans* in 15 patients (50%). Others included *Candida* non albicans3 (10%), *Aspergillus* spp. was 6 (20%)-*Aspergillus* flavus 4 and one each of *Aspergillus* nidulans, *Aspergillus* terreus, other fungi constitued of 20%comprising of 2 isolates of Penicilium sp, and one each of Cunninghamella bertholletiae, Acremonium sp. Cladosporium sp and Fusarium solani.

CAPD catheter removal and initiation of antifungal therapy was done for all patients. Re-insertion was done for 3 patients. Mortality rate was 16.6%.

Conclusion: Patients with previous bacterial peritonitis and antibiotic usage are at greater risk of developing fungal peritonitis.

PP-07-3

Prevalence of fungal rhinosinusitis in Delhi / New Delhi metropolitan area- A mycoserologic, histopathologic and clinical study

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Fungal rhinosinusitis (FRS) has emerged world-wide as an increasingly important clinical entity. The paucity of information, however, on its prevalence in Delhi and many other parts of India prompted the present study. The study material comprised 69 patients clinically diagnosed as chronic rhinosinusitis (CRS) in the ENT Department, Lady Hardinge Medical College, New Delhi, during 2005 to 2008. The patients underwent computed tomography (CT) of paranasal sinuses, absolute eosinophil count, total and differential leukocyte count. Endoscopically removed polyps / sinus mucosa and intrasinus debris were investigated for fungal etiology by direct microscopy and culture. Besides, about one half of each specimen was fixed in formal saline for histopathologic examination. Of the 69 cases of CRS investigated 27 (39%) showed fungal etiology. Based primarily on histopathologic criteria, 15 (22%) of the cases showing presence of mucin infiltrated with hyphae were classified as allergic fungal rhinosinusitis (AFRS); the remaining 12 (17%) showed massive growth of fungal hyphae without mucin suggestive of fungal ball (FB). Forty-two cases had non-mycotic etiology including 16 cases of Eosinophilic mucin rhinosinusitis (mucin without fungal elements). Detailed mycological investigations established *Aspergillus flavus* as the predominant etiologic agent i.e. in 13 of the 16 (81 %) culture positive FRS cases. The *A. flavus* positive cases included 8 of AFRS and 5 of FB. *Aspergillus fumigatus* and *A. terreus* proved to be the etiologic agents in one case each of AFRS. Species identification of *Aspergillus* remained undetermined in one case of FB. Serum precipitins against the etiologic aspergillii occurred in 75% and 50% of the FB and AFRS cases, respectively. We conclude that FRS is not an uncommon disease in Union Territory of Delhi and *Aspergillus flavus* its predominant etiologic agent. It is probably under-diagnosed in many parts of India due to inadequate awareness and / or diagnostic facilities.

PP-07-4

(1→3)-β-D-Glucan assay for the diagnosis of invasive fungal infections: Review of the literature

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Background: The measurement of plasma (1→3)-β-D-glucan (BDG) was developed for the diagnosis of invasive fungal infections (IFI) in 1992. Since then, BDG assay has been widely used in Japan especially in patients with hematological diseases. To evaluate its usefulness, we have analyzed the sensitivity of BDG in patients with IFI by review of the literature.
Methods: Related English and Japanese papers were searched for with terms of BDG and any of IFI due to *Candida*, *Aspergillus*, *Cryptococcus*, *Pneumocystis*, and other fungi from PubMed (English) and JMEDICINE (Japanese).
Results: Sixty-five pieces of literature were identified and BDG was measured by 5 different methods. The earliest method estimated plasma BDG as Fungal Index from the difference between measurements by a conventional limulus test, which reacts with both endotoxin and BDG, and an endotoxin-specific test. In 1995, test kits for direct measurement of plasma BDG was developed (Fungitec G-test and G-test MK). In the next year, a turbidimetric assay (β-glucan Wako) was introduced and in 2001, another colorimetric assay (β-glucan Maruha). Recently, yet another colorimetry, Fungitell, became available in USA/EU. IFIs were identified in 1136 patients, and 888 (78.2%) were positive. Fungemias were found in 375, and 329 (87.7%) were positive: 305 of 350 (87.1%) in candidemia and 24 of 25 (96.0%) in other fungemias including cryptococcemia. As for pulmonary infections, 15 of 22 (68.2%) were positive in *Candida* pneumonia, 182 of 240 (75.8%) in invasive pulmonary aspergillosis and 143 of 149 (96.0%) in *Pneumocystis* pneumonia. In contrast, the positive rate of cryptococcal pneumonia was 10.9% (5 of 46).
Conclusions: The high incidence of positive plasma (1→3)-β-D-glucan in IFI was reconfirmed.

PP-07-5

Recent trends in fungal isolation from clinical specimens of blood culture and central venous catheter

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We analysed the isolation frequency of fungi from clinical specimens of blood culture and central venous (CV) catheter in Juntendo University Hospital from 1994 to 2007.

The fungi isolated was between 5 to 14% from the blood culture, before year 2004. Since 2005 the frequency of fungi has declined to 2 to 5% from the blood culture. However the isolated fungi from the CV catheter shows no remarkable changes (between 6 to 16%).

In fungi isolated from blood culture, *Candida albicans* (38.2%) was the most dominant, followed by *Candida parapsilosis* (24.5%), *Candida glabrata* (13.7%), *Candida tropicalis* (10.8%), *Candida guilliermondii* (2.5%), *Candida krusei* (2.0%), *Cryptococcus neoformans* (2.0%), *Candida lusitaniae* (1.0%), *Trichosporon* sp. (1.5%) and others.

In fungi isolated from CV catheter, *C. albicans* (34.5%) was the most dominant, following *C. parapsilosis* (32.0%), *C. glabrata* (11.4%), *C. tropicalis* (7.5%), *Trichosporon* sp. (3.9%), *C. guilliermondii* (2.5%), *C. krusei* (1.8%), *C. lusitaniae* (1.1%) and others.

The correlation of isolating fungi, suggest that the most isolated fungi from blood cultures are caused by catheter associated blood stream infection (CA-BSI). We estimated the clinical significance of fungi from specimens of blood culture and CV catheter between 2007 to 2008, from clinical course and laboratory data (such as beta-D gulcan). Obviously, contaminant cases are rare, and almost all isolations are caused by CA-BSI.

Since increased immunocompromised host in hospitalised patients, careful management of CV catheter management should be recommended.

PP-07-6

Development and application of *in situ* hybridization with peptide nucleic acid probes on tissue sections for histological diagnosis of invasive fungal infections

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Histopathological diagnosis for invasive fungal infection has depended on recognizing characteristic in shape of fungi in tissue by detailed observation *via* microscope. It, however, is not rare to be difficult to identify fungi only by histological examination, because shape of fungi varies from case to case to which virulence of fungi and impaired defense mechanism affect, significantly. In order to achieve accurate and rapid diagnostic procedure using pathological and cytological preparations, the present paper discusses an application of *in situ* hybridization (ISH) to identify fungi in tissue section with peptide nucleic acid (PNA) probes targeting the 28S rRNA of panfungal and three organisms; *Candida albicans, Fusarium* spp., and *Histoplasma capsulatum*. The performance of ISH with PNA probes was evaluated by using formalin-fixed and paraffin-embedded sections of lungs from mice experimentally infected with *Aspergillus fumigatus, A. terreus, A. flavus, C. albicans, Rhizopus oryzae* and *F. solani* and biopsy and autopsy specimens confirmed by culture. As the result, specific and strong signal intensity was obtained for each probe. In addition, panfungal PNA probe was useful to know whether the fungus observed in paraffin-embedded tissue section can maintain reliable RNAs for FISH or ISH.

PP-07-7

Primary exploration of two-round PCR on the rapid molecular diagnosis of clinical fungal infection specimens

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[Objective] To establish a diagnosis method for clinical fungal infection using two-round PCR, and to evaluate its sensitivity by comparison with traditional culture and routine PCR methods.

[Methods] Totally 29 clinical sputum and alveolar wash solution specimens were collected from patients with suspicious fungal infection. Direct microscopy with 10%KOH, fungal culture, routine PCR and two-round PCR with fungal universal primer for ITS regions of rDNA were performed, and fungal positive rates and detection rates of multiple species were compared and analyzed.

[Results] Positive rates of fungal detection by direct microscopy, fungal culture, routine PCR and second-round PCR were 20.69% (6/29), 37.9% (11/29), 17.2% (5/29) and 48.3% (14/29), respectively; and the rates of more than 2 species identified by fungal culture, routine PCR and second-round PCR were 6.9% (2/29), 3.45% (1/29) and 24.1% (7/29), respectively. According to the data, two-round PCR method were better than routine PCR in fungal positive detection rate; as to multiple species fungal detection, two-round PCR showed significantly higher positive rate than routine PCR and traditional fungal culture methods.

[Conclusions] Using two-round PCR, the sensitivity of molecular diagnosis in clinical specimens could be obviously improved.

Poster Forum PF-04

PP-07-8

How different is Neosartorya udagawae from *Aspergillus fumigatus*?

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Neosartorya udagawae, a heterothallic fungus, has an anamorph that is morphologically indistinguishable from *Aspergillus fumigatus*. Because of the similarity in their conidial morphology, clinical isolates of N.udagawae are frequently identified as A. fumigatus on phenotypic characteristics alone. We recently documented four cases of invasive aspergillosis caused by N. udagawae in patients with CGD (n=3) and MDS (n=1). In contrast to typical disease caused by A. fumigatus, disease caused by N. udagawae appeared more chronic, with infection spreading across anatomical planes in a contiguous manner. Since N. udagawae is poorly recognized, we characterized the species in comparison with A. fumigatus. A total of 11 N. udagawae strains, including two Type strains and 9 clinical isolates, were compared with A. fumigatus B-5223. The rodA and benA sequence analysis showed the 11 N. udagawae strains to be distinct from B-5233. Using PCR, we identified alpha box in CBS114217 and HMG domain in CBS114218, the two type strains of opposite mating type, indicating that CBS114217 has MAT1-1 locus and CBS114218 has MAT1-2. Five of the 9 clinical isolates have MAT1-1 and 4 have MAT1-2 indicating an equal representation of the two mating types as clinical isolates (similar to A. fumigatus). Growth of the N. udagawae strains was considerably slower than A. fumigatus at temperatures between 30°-37°C. While A. fumigatus grows at 55°C but fails to grow at 10°C, N. udagawae failed to grow at the temperatures >42°C but formed colony at 10°C. N. udagawae was more susceptible than A. fumigatus to neutrophils as well as hydrogen peroxide and significantly less virulent in CGD mice. Both N. udagawae and A. fumigatus produced gliotoxin. It is plausible that the differences in growth characteristics and susceptibility to host responses is associated with subtle distinction in disease progression in humans.

PP-07-9

Fatal central nervous system *Aspergillus granulosus* in a lung transplant recipient

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Summary: Disseminated disease by *Aspergillus granulosus* has been reported only once previously. We report CNS *Aspergillus granulosus* in a patient receiving both a bone marrow and a lung transplant.

Case Report: An 18-year-old male presented for a bilateral lung transplant in October 2006. Ten years previously, he had been diagnosed with pre-B cell acute lymphocytic leukemia for which he underwent bone marrow transplantation one year prior to lung transplantation. His post-BMT transplant course included graft-versus-host disease, *Aspergillus terreus* pneumonia, CMV, *Mycobacterium avium*-complex, and influenza A infections. He had no known infections at the time of lung transplant and was discharged 10 days post-transplant. His post-operative period was complicated by seizures, and MRI showed multi-focal ring-enhancing lesions at the gray-white junction in both cerebral hemispheres along with early ventriculitis. The size and location of the lesions precluded resection. A fungus was suspected, and the patient was treated empirically with amphotericin B (AMB), voriconazole (VCZ), and posaconazole (PCZ). The patient became more neurologically impaired throughout December & January, and serial MRIs showed progressive vascular occlusion. He was continued on AMB and PCZ until made DNR and expired 2 days later.

Mycology: Culture from the autopsy brain stem yielded *A. granulosus*. Colonies were yellowish-tan and granular at maturity due to large clumps of irregularly-shaped Hulle cells. Other microscopic features include sparse, biseriate fruiting structures with small, oval-shaped vesicles borne on long, pale brown, thick-walled conidiophores. Growth occurred at 40°C supporting its neurotropic potential. The isolate was confirmed as *A. granulosus* based on sequence identities with the type cultures of *A. granulosus* (NRRL 1932) and *A. ustus* (NRRL 275) using ITS, D1/D2 regions and the β-tubulin gene.

Conclusion: *Aspergillus granulosus* is an uncommon but potentially lethal species in transplant recipients. It shares features with other species in the *Aspergillus* Section *Usti*, including *A. calidoustus*.

PP-07-10

Underlying disease frequency in patients with chronic pulmonary aspergillosis

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Background: Chronic pulmonary aspergillosis (CPA) is defined as the presence of at least one pulmonary cavity on chest imaging, with or without a fungal ball (aspergilloma) together with symptoms (usually weight loss, fatigue, cough, haemoptysis and breathlessness) for at least 3 months, and serology or cultures implicating *Aspergillus* spp. Numerous underlying diseases have been associated with CPA, as have defects in innate immunity.
Methods: Details of the underlying diseases of 94 CPA patients attending our referral clinic were collected, and the distribution of these underlying diseases analysed. Mannose binding lectin genotype was also determined (INNO-LiPA MBL2, Innogenetics).
Results: Many patients presented with multiple underlying diseases, such that a total of 183 underlying diseases were identified for the 94 CPA patients. For each patient, one underlying disease could be identified as the primary cause of the CPA. As reported elsewhere, previous tuberculosis (TB) (classical or atypical) was the most common underlying disease (31%). Amongst other conditions, pneumothorax/bullae, emphysema/COPD, sarcoidosis and previous lung resection were also identified as primary underlying diseases, in 13%, 12%, 7% and 6% of referred patients respectively. Multiple other less well documented underlying diseases included ABPA (10%), prior treated lung cancer (9%), pneumonia (7%), rheumatoid arthritis (3%) and SAFS/asthma (2%). The last three diseases were found more often as one of multiple underlying diseases, in 22%, 5% and 20% respectively. Mannose binding lectin genetic defects were also common (39% of the 36 patients tested).
Conclusions: TB and atypical TB remain the predominant risk factors for development of CPA, however multiple other diseases, some not previously reported, were also identified as the primary underlying disease leading to the development of CPA.

PP-07-11

Invasive oro-facial fungal infections in patients with hematological malignancies: Report of 27 cases due to *Aspergillus* and non-*Aspergillus* species

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Purpose: Little is known about the characteristics and backgrounds that occur during the progression of invasive oro-facial fungal infections in immunocompromised patients. The aim of this study was to examine the clinicopathological features, antifungal susceptibilities, epidemiology and prognosis of these patients helping the early diagnosis and treatment of the disease.

Patients and Methods: The patients with hematological malignancies who developed invasive oro-facial infections between 1990 and 2002 were studied. Fungal infections were identified with histological, microbiological, and molecular methods. The in vitro susceptibility studies were performed by the broth microdilution method M-38P. Clinical records of the patients were reviewed and the following information was collected: underlying illness, neutropenia, and antifungal medication.

Results: Twenty seven patients had positive fungal infections in histological examinations and 15 patients showed a positive culture for one of the following organisms: *Aspergillus* species in 13 patients (A. flavus in 10, A. terreus in 2, and A. fumigatus in 1), as well as Exophiala dermatitis, Trichoderma longibrachiatum, and Fusarium moniliforme in 1 patient each. Eleven patients were diagnosed as aspergillosis by in situ hybridization technique. The infection dramatically developed stage by stage into hemifacial necrosis with serious pain during neutropenia. All patients received surgery and were basically treated with amphotericin B in combination with itraconazole, or micafungin. In vitro susceptibility studies showed that non-*Aspergillus* species had elevated MICs against itraconazole and micafungin. Twenty two of 27 patients survived with recovery of neutrophils.

Conclusion: The high survival rate associated with invasive oro-facial fungal infections could be achieved with early diagnosis based on clinical findings and aggressive therapy in addition to the improvement of the patient's hematologic status.

Myoken Y et al. Clinical Infectious Disease 33: 1975-80, 2001
Myoken Y et al. Journal of Oral and Maxillofacal Surgery 66:1905-12, 2008

PP-07-12

Pathophysiological study of chronic necrotizing pulmonary aspergillosis associated with sequelae of tuberculosis

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Objectives: The pathophysiology and definition of chronic necrotizing pulmonary aspergillosis is controversial because variety of underlying diseases, spread of causative fungi included degenerated exudates along the terminal airway still remain obscuring. The aim of this study is to clarify the clinical and histopathological features of chronic necrotizing pulmonary aspergillosis (CNPA).
Patients and Methods: We conducted a histological study of 8 patients clinically diagnosed as CNPA following sequelae of tuberculosis who had hemoptysis for 5 years.
Results: The mean age of the patients was 69.5 year-old. The underlying disorders were HCV-related or alcoholic liver cirrhosis in 3 and diabetes mellitus in 1. All patients had fever, general fatigue, and hemoptysis. Chest computed tomographic images of them revealed fungus balls, cavity wall thickening, and consolidation surrounding cavity. Serologically, 2 patients were positive for *Aspergillus* galactomannan antigen, all patients were positive for anti-*Aspergillus* antibody, and 5 patients showed an elevation of (1, 3)-β-D-glucan. Micafungin alone was administered in 2 patients, voriconazole in 2, amphotericin B in 1, and itraconazole in 1, respectively. Furthermore, 4 patients received bronchial arterial embolization therapy, and 5 patients underwent lung resection. As a result, 4 patients who could not receive surgery died from respiratory failure due to massive hemoptysis or deterioration of CNPA. Histopathological examination revealed that the cavity wall consisted of three layers comprised of necrotic, granulation, and fibrous tissue layers. Aspergilli were found in both the fungus ball and necrotic tissue comprising inner layer of the cavity wall. In addition, most of the vessels was occluded incompletely with thrombosis and involved by necrosis and local invasion of Aspergilli.
Conclusion: The destruction of blood vessels at the cavity wall involved by necrosis and local invasion of Aspergilli may cause massive hemoptysis. Therefore, surgical intervention should be considered as a prior procedure for CNPA patients.

PP-07-13

Utility of mass spectrometry for studies of invasive pulmonary aspergillosis in the rat

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State-of-the-art: The high mortality rate of invasive pulmonary aspergillosis (IPA) results partly from the lack of a reliable, sensitive and specific method for establishing an early diagnosis. Mass spectrometry (MS) analysis of the proteins present in bronchoalveolar lavage (BAL) fluid may provide a solution to this problem, through the isolation and identification of new biomarkers.
Methods: Immunocompromised rats (treated with cyclophosphamide and fed a low-protein diet) were infected with a strain of *Aspergillus fumigatus* through a Microsprayer IA-1B in situ nebulizer. Animals were killed when their clinical condition became critical, and BAL was carried out. BAL fluid was then analyzed by MS, using MALDI-TOF technology. The protein profiles obtained were compared with those of control rats and with the results of classical complementary analyses: determination of *Aspergillus* galactomannan antigen levels in the blood and BAL fluid, mycological culture and pathological examinations.
Results: Protein profiles very different in qualitative and quantitative composition were obtained for the two groups of animals (rats with confirmed IPA close to death and control rats). All the rats dying after a similar duration of disease progression had very similar protein profiles. By contrast, profiles differed as a function of the duration of disease progression.
Conclusion: It is not particularly surprising that different protein profiles were obtained for healthy and diseased rats. However, the modifications observed, the changes in these modifications during the course of the disease and the reproducibility of the method are highly encouraging. We hope to be able to isolate and to characterize markers specific for invasive pulmonary aspergillosis in this model.

PP-07-14

The utility of *Aspergillus* galactomannan assay (GM EIA) for monitoring pediatric allogeneic bone marrow transplant (BMT) patients

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GM EIA is approved for early diagnosis of invasive aspergillosis in high risk patients. We assessed the utility of serial GM EIA for diagnosis and guidance for antifungal therapy in a cohort of pediatric BMT patients.

METHODS: From July 2006 through June 2008, 65 pediatric patientss received allogeneic BMT at MSKCC. Standard antifungal prophylaxis consisted of fluconazole. Pts with history of pre-transplant IFI, on corticosteroids, GVHD or prolonged neutropenia received mold- active prophylaxis (MAP) with voriconazole or micafungin. Pts were monitored prospectively by serial GM. Work-up for invasive fungal infections (IFI) and choice of antifungal therapy was at the discretion of the physician. EORTC/MSG criteria were used for IFI diagnosis. The impact of GM result on clinical management was assessed by an independent reviewer.

RESULTS: 63 patients had 1,887 GM (median 27, range 8-54). Mean age 8.1 years; 69% hematologic malignancy; 58% peripheral stem cells; 69% unrelated donor; 63 % T-cell depleted. 17% pts had presumed aspergillosis pre-transplant. Nine (14%) pts developed acute GVHD (grade 2-4).
Thirty-seven (57%) and 26(40%) pts were on MAP at 1 and 6 months post transplant. Six (9.2%) pts were treated for IFI; (Possible 3, probable based on GM 2, definite 1). All patients treated for IF had been on MAP. Overall GM monitoring lead to change in management in 1 patient. The number of GM needed to diagnose 1 case of IA was >600 at a cost of 160,000$.

CONCLUSIONS: 1) In our pediatric cohort GM did not have an impact on earlier diagnosis or management of IFI.
2) The financial burden of monitoring fungal burden was 510,000 US dollars; More than 600 GM were performed to diagnose a single case of IA by GM.
2) The utility of GM needs to be reconsidered in clinical settings with high use of mold active therapy.

PP-07-15

Evaluation of an in situ imagery technique for the follow up of an experimental aspergillosis in chickens (Gallus gallus)

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Infection by *Aspergillus fumigatus* is a common respiratory disease in birds and significant economic losses are associated with this mycosis in breeding turkeys and chickens. The aim of the present study was to evaluate the possibility of following the localisation and development of fluorescent *Aspergillus fumigatus* conidia in the respiratory tract of chickens. Air sack inoculation was performed on 18 animals with 108 conidia which expressed the DsRed protein and 30 controls were inoculated with a suspension of 108 red fluorescent microspheres (Merck). The kinetics of inoculation were followed. Groups of 2 to 3 birds were slaughtered 87, 63, 39, 15, 3 hours and less than 5 min after inoculation. The chicks were examined by a fluorescent camera (IVIS Spectrum system) then autopsied. The type and the spread of the lesions were measured and the left lung was removed and treated by imagery. The amount of each fluorescent signal was quantified for the whole animal and the isolated lung. The results showed a progressive development of the signal in the respiratory tract which appears to be correlated with the production of fluorescent hyphae. The surface which produced the hyphae and the macroscopic pulmonary lesions were very closely superimposed. The choice of fluorochrome appears to be very important in order to optimise the in vivo follow-up of an experimental aspergillosis infection.

Poster Forum PF-04

PP-07-16

High resolution typing of *Aspergillus fumigatus* by multi locus VNTR analysis (MLVA)

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The genetic relationship between clinical and environmental isolates of *Aspergillus fumigatus* can be studied using genetic fingerprinting with DNA-based methods. In the present study, we describe a new molecular typing method based on the detection of small genomic sequences (greater than 9 bp) repeated at least 3 times. A research of such sequences, called VNTR (Variable Number Tandem Repeat), was performed on whole available genome of A. fumigatus strain Af293 and a total of 77 VNTR were detected. These VNTR markers were tested on 30 clinical isolates and ten of them, spread on 4 different chromosomes, were finally selected for their applicability and discriminatory power. A total number of 223 independent isolates (including 146 isolates from birds with or without aspergillosis, 76 isolates from patients with or without invasive aspergillosis and the reference strain CBS 144.89) were tested with the panel of 10 VNTR markers. After DNA extraction, amplification was obtained with the ten different couples of primers with the same PCR program for all markers. Amplicons were separated on agarose gel and the results were analysed using a specific software. This analysis generated 210 different patterns. The Simpson's Diversity Index for the individual markers ranged from 0.4471 to 0.8344. The combination of all 10 markers yielded an index of 0.9995. Specific advantages and disadvantages of the MLVA method were evaluated in terms of applicability, ease of use, exchangeability and reproducibility within a laboratory. In the present study, the amplicons were easily analysed on agarose gel, but the reproducibilty of the MLVA method can be increased by the use of fluorescently-labeled primers and access to high-resolution electrophoresis platforms.

Poster Forum PF-04

PP-07-17

Iron chelator Deferasirox (Exjade®) alone or in combination with lipid formulations of amphotericin B (AmB) is effective in treatment of murine invasive pulmonary aspergillosis

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Background: Increased bone marrow iron level in patients with hematologic malignancies is an independent risk factor for developing Invasive pulmonary aspergillosis (IPA) suggesting an important role for iron uptake in the pathogenesis of IPA. Therefore, chelation of host iron with an appropriate iron chelator might prove to be effective in treating IPA. We investigated the role of deferasirox (Def) mono or combination therapy with AmB in treating murine IPA.

Methods: BALB/c mice with cyclophosphamide/cortisone immunosuppression were infected via inhalation with A. fumigatus. Treatment with Def (20 mg/kg/d qod, p.o.) was started at day -2 (continuous therapy) or day +1 (delayed therapy) relative to infection and continued for a total of 5 doses. Liposomal AmB (LAmB) or AmB lipid complex (ABLC) were administered i.v. at 3 mg/kg/d for 5 days starting at 24 h post infection. Placebo control mice received vehicle control. The primary and secondary endpoints were time to survival and CFU, respectively.

Results: Def-treated mice (n=24) improved median survival time compared to placebo-treated mice (6 d for placebo- vs. 9 d to Def-treated mice, $P<0.007$). In delayed therapy, combining Def with LAmB or ABLC improved survival of mice (n=8) compared to placebo or Def monotherpay ($P<0.04$). Further, Def+LAmB demonstrated significant improvement of mice survival compared to LAmB ($P<0.02$). Combination therapy with LAmB or ABLC reduced lung CFU compared to placebo-treated mice ($P<0.04$). In the continuous therapy, combination therapy with LAmB prolonged survival of mice (n=16) compared to placebo- or Def-treated mice ($P<0.005$) and strongly trended to improve survival vs. LAmB-treated mice ($P=0.077$). Additionally, all treatment regimens reduced lung CFU compared to placebo-treated mice ($P<0.05$).

Conclusion: Iron chelation therapy with Def alone or in combination with AmB is effective in treating experimental IPA. Further development of the FDA-approved Def is warranted as adjunctive therapy for IPA infections.

PP-07-18

Presumptive therapy for persistent febrile neutropenia in onco-hematological patients

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Purpose: The effectiveness and safety of a presumptive therapy (PT) by using serum tests for fungi with galactomannan antigen and β-D-glucan tests and/or computed tomography (CT) in the high-risk patients for invasive aspergillosis (IA) was compared with those of an empirical treatment (ET).

Method: We retrospectively reviewed the records of 66 adult patients with cancer or hematological disorders who experienced persistent febrile neutropenia (FN) for 5days or longer and were treated with anti-*Aspergillus* agents between July 2003 and June 2008. The treatment was classified as presumptive if anti-*Aspergillus* agents were started after the serum tests became positive and/or infiltrates or nodules were found on either chest radiographs or CT scans. Probable or proven IA was defined according to the new EORTC/MSG criteria.

Result: The median age was 62 (range;16-76) for the ET and 63 years (range;20-80) for the PT group. The underlying diseases included acute leukemia in 21 patients for ET and 25 for PT, malignant lymphoma in 6 and 3, and others in 4 and 7, respectively. The median duration of neutropenia was 25 days in ET and 21 days in PT, respectively, and the median time from the rise of body temperature to the start of treatment was 8 days in both groups. No difference was observed in the survival rate in 12 weeks from the start of the anti-*Aspergillus* agents, i.e. 67.7% for ET vs 68.6% for PT, and only one patient who died of systemic fungal infection (candidemia) was experienced in the ET group among 21 expired patients.

Conclusion: This retrospective study suggests that PT is as effective and safe as ET in treating patients with persistent FN. The PT strategy can be applied to a high-risk group for IA at no expense of increased morbidity or mortality.

PP-07-19

Exogenous *Aspergillus fumigatus* endophtalmitits

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This 50 year-old female was quite healthy before. She suffered from blurred vision and eye pain after her eye injured by the rebounding wood plate. She went to emergency room where corneal laceration with traumatic cataract (os) was noted. Under the impression of eyeball rupture, she was admitted for emergent operation. After admission, primary suture and intravenous antibiotics were performed smoothly. However, corneal wound infiltration with anterior chamber reaction increased 4 days later. Lensectomy, anterior chamber fluid culture, and repeated intravitreous amphotericin-B was performed under the impression of fungal endophthalmitis (os). Topical amphotericin-B 1.5 mg/cc q2h and oral voriconazole 200 mg q12h were used. However, hypopyon was noted and natamycin ophthalmic solution q1h was used instead. Fungus culture showed *Aspergillus fumigatus*. B-scan still showed vitreous opacity (V.O.) (++). Left eye pain with hyphema, corneal infiltrates and fibrin exudates were found. Anterior chamber irrigation (os) was performed on 7/14. However, sudden onset of fever with chills was noted on 7/17. White blood cell (WBC) was 2500, suspect neutropenic fever. Therefore, systemic amphotericin B and intravitreous amphotericin B q3d was done. Because suspected central nervous system (CNS) infection, systemic antibiotics with maxipime and metronidazole were used. On 7/24, Penetrating keratoplasty (PKP) was performed smoothly under general anesthesia. Graft condition was clear and suture wound intact. No more V.O. was noted and no more fungal material was found on the retina. Serial intravitreous amphotericin B was done. Her general condition was stable and she was discharged.

Poster Forum PF-04

PP-07-20

Gene expression in *Candida albicans* fatty acid desaturase null mutant

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Polyunsaturated fatty acids (PUFAs), including linoleic acid (C18 : 2) and a-linolenic acid (C18 : 3), are major components of membranes. PUFAs are produced from monounsaturated fatty acids by several fatty acid desaturases (FADs) in many fungi, but *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* and humans do not have these enzymes. Although the fungal pathogen *Candida albicans* produces C18 : 2 and C18 : 3, the enzymes that synthesize them have not yet been investigated. The level of PUFAs tended to be higher in the mycelial form than in the yeast form.

We identified two genes in *C. albicans* that encode delta12 and Ω3 FADs, and named them *CaFAD2* (orf19-118) and *CaFAD3* (orf 19-4933), respectively. To elucidate the functions of these enzymes, we constructed *CAfad2*delta and *Cafad3*delta null mutants (Microbiology. 152:1551, 2006). However, phenotypic characteristics such as germ tube formation, hyphal morphogenesis, and chlamydospore formation did not differ among the wild-type strain, *Cafad2*delta, and *Cafad3*delta. Moreover these PUFAs did not affect the virulence to mice or morphogenesis in the culture media used to induce morphological change of *C. albicans*. We also observed both mutants by transmission electron microscope and the mutant strains were subjected to transcriptional profiling by microarray analysis validated by real-time RT-PCR. Significantly modulated genes were by determined by comparing with the revertant strain. Among 6,165 genes 15 genes were significantly modulated (more than 4-fold difference) using Wilcoxon T-test and another 34 genes were detected using a 1.5-cutoff and Bonferroni correction. Differential gene expression results from microarray analysis were validated using real-time reverse transcription (RT)-PCR. Among them 13 genes were down regulated. However, functions were unknown for a large part of the modulated genes in the microarray which will be analyzed further in order to define their relationship with FADs.

PP-07-21

First report of *Candida nivariensis* pneumonia in a HIV infected patient in India

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Candida nivariensis is a recently described pathogenic yeast closely related to *Candida glabrata* and *C. bracarensis*. Since its first isolation in 2005 from three patients in a Spanish hospital, it has been reported from Indonesia, Japan and United Kingdom. We report its first isolation in India from the respiratory tract of a 45-year-old male, resident of Delhi, diagnosed 2 years ago as HIV positive and suffering from pneumonia not responding to antibacterial antibiotics. Direct DNA sequencing of D1/D2 region of 28S rRNA gene and internal transcribed spacer (ITS) region of rDNA confirmed that the isolate was *C. nivariensis*. Also, the patient's serum was positive for (1,3) β-D-glucan (108 pg/ml) and *C. nivariensis* DNA detected by PCR amplification of ITS region with species-specific primers. On CHROM *Candida* medium, the isolate grew as white colonies in contrast to the pink colonies of *C. glabrata*. Carbohydrate assimilation profile with the ID 32 C yeast identification system (bioMeriux) revealed only glucose assimilation and low species discrimination. The assimilation results were confirmed by auxanographic method. Also, the isolate fermented glucose and trehalose. *In vitro* antifungal profile of the isolate determined by broth microdilution and Etest methods revealed its susceptibility to the antifungals tested. The MICs obtained by microbroth dilution method were as follows: fluconazole (2μg/ml), itraconazole (0.25μg/ml), voriconazole (0.25μg/ml) and amphotericin B (1μg/ml). The Etest MICs were: fluconazole (2μg/ml), voriconazole (0.25μg/ml), posaconazole (0.38 μg/ml), amphotericin B (0.5 gμ/ml), 5-flucytosine (0.094 μg/ml), and caspofungin (0.25μg/ml). Repeated isolations from 3 consecutive sputum cultures and demonstration of *C. nivariensis* DNA as well as (1, 3) β-D-glucan in the patient's serum documented its etiologic significance. This report highlights the importance of molecular methods in definitive identification of *C. nivariensis* and its etiologic role.

PP-07-22

Karyotype differences of the Czech and Japanese *Candida glabrata* bloodstream isolates

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Background: Invasive candidiasis belongs to the most serious nosocomial infections, threatening particularly immunocompromised patients. *Candida glabrata* has been referred as the most common non-albicans bloodstream pathogen in the U.S. hospitals and its frequency increases steadily worldwide. Karyotyping using pulsed-field gel electrophoresis (PFGE) is one of the most reliable genetic techniques for detecting inter-strain differences in yeasts.

Goal: To determine occurrence and rate of geographical differences in karyotypes between Czech and Japanese *C. glabrata strains* isolated from the bloodstream.

Material and Methods: Thirty-nine isolates were compared: 13 from 12 Czech patients and 26 from 26 Japanese patients hospitalized in seven Czech and nine Japanese hospitals, respectively. Isolated DNA was run in 1% Pulsed-Field Certified Agarose using the CHEF-Mapper system. The running buffer was 0.5 × TBE chilled to 14°C and three consecutive blocks of parameters for PFGE were chosen: (i) 140 V, 130 s for 17 h (ii) 150 V, 300 s for 12 h and (iii) 110V, 300 s for 18 h. Karyotypes were compared using the GelCompar software.

Results and Discussion: No clear geographical difference was found. Except five isolates with the overall lowest similarity rate, all isolates were divided into two groups with 52% similarity. One included 10 Czech and four Japanese isolates, the other two Czech and 18 Japanese isolates. This is consistent with the published data from similar studies of *C. albicans* where only ¨enrichment¨ of the regional yeast population by certain specific strains was observed, accompanied by a mixture of common global strains.

Conclusion: Presence of different *C. glabrata* strains in the Czech Republic and Japan is not absolute, however, certain geographical differences were found.

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Poster Forum PF-04

PP-07-23

Molecular relatedness based on the analysis of the ribosomal R NA of *Candida albicans* isolated from patients hospitalized in eight medical centers in Brazil. A practical method to evaluate molecular epidemiology

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Molecular epidemiological surveys search for fast-economic and reliable methods. We analyzed *Candida albicans* isolates using the analysis of PCR products of the transposable intron in the large sub unit (25S) of the rRNA gene region (rDNA). 157 isolates from patients hospitalized in eight hospitals located in different regions in Brazil were analyzed. 126 isolates were obtained from blood cultures of 46 candidemic patients assisted in seven hospitals and 31 isolates from different anatomic sites, from patients of a single hospital. Genotyping was performed using the pair of primers: CA-INT-L (5' ATA AGG GAA GTC GGC AAA ATA GAT CCG TAA-3') and CA-INT-R (5' CCT TGG CTG TGG TTT CGC TAG ATA GTA GAT-3'). 28 episodes (84 isolates) were genotype A; 12 episodes (36 strains) genotype B; 4 episodes (6 strains) genotype C and two episodes, genotypes A+B. Genotypes was distributed similarly in all hospitals where genotype A was predominant, except one where genotype B was predominant suggesting a hospital cross dissemination in the neonate ICU. The collection of isolates analyzing different anatomic sites included: genotype A: 17 isolates (urine:9; blood:4; secretions:4); genotype B (urine:3; secretions: 8) and genotype C (urine:1; secretion:1). Comparing genotypes of blood isolates with non-blood isolates, the proportion of genotype A was not significantly higher than B (p=0.20). However, excluding the endemic strain genotype B isolated from the neonates, genotype A was significantly higher in invasive candididasis (p=0.003). In conclusion, in Brazil the genotypes had a similar distribution of worldwide studies and reflected the trend predominance of genotype A in invasive candidiasis.

PP-07-24

Micro-CT analysis of experimental *Candida* osteoarthritis

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Candida arthritis is uncommon in comparison with superficial or deep-seated candidiasis. However, recent advances in sophisticated therapeutic technologies, in particular, insertion of artificial joint may increase the incidence of joint infection with *Candida* species. The increasing elderly population with osteoporosis may also increase the incidence of prosthetic joint procedures.
Candia albicans osteoarthritis in the rat model was investigated using micro-computed tomography (micro-CT). Total 40 rats were intravenously injected with *C. albicans* cells at a nonlethal dose. Joint swelling was observed in 24 rats. Two or more joints were invaded in 10 of the 24 rats. The tarsal region of the hind paw was the main target of this osteoarthritis followed by the foreleg elbows. Erosions of the affected tarsal joint bones were observed as an initial sign of the osteoarthritis several days after the onset of swelling. Following this initial sign, severe surface roughness and disintegration of the joint bones progressed.

Moreover, three-dimensional (3D) microstructures of trabecular bone and changes in 3D bone parameters were characterized with calcaneal bones from invaded hind paws. Typical changes of the 3D images in arthritic bones were coarsening of the trabecular bone distribution and weakening of the trabecular bone connectivity. These morphological transitions were quantitatively confirmed by changes in 3D bone parameters measured from consecutively scanned bone slices.

In conclusion, Micro-CT has been shown to be useful for quantifying morphological changes occurring in *Candida* arthritic bones and observing the focus of affected joint bones in living rats.We hope to use Micro-CT to investigate potential therapeutic strategies using the experimental *Candida* arthritis rat model.

PP-07-25

Epidemiologic analysis and antifungal susceptibility of candidemia at four hospitals in Belo Horizonte, Brazil

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The availability and use of aggressive chemotherapeutic and immunosuppressive agents as well as broad-spectrum antibacterial agents has created a large population of patients who are at increased risk of acquiring bloodstream infections with fungal organisms, especially *Candida* species. In order to evaluate the epidemiology of candidemia in Belo Horizonte, State of Minas Gerais, Brazil, we performed a prospective surveillance study conducted in four tertiary care hospitals from February 2003 to November 2007. A total of 4824 episodes of bloodstream infection were identified, and *Candida* species accounted for 168 cases (3.5%). All the 168 *Candida* isolates were identified by routine microbiological techniques and had the following distribution: *Candida albicans* (45.2%), C parapsilosis (21.4%), C. tropicalis (16.7%), *C. glabrata* (9.5%), C. guilliermondii (4.8%) and C. krusei (2.4%). The crude mortality rate was 50%, and Acute Physiology and Chronic Health Evaluation score II (APACHE II score), hematology malignancy, central venous catheter, neutropenia, and acute renal failure were factors associated with a higher probability of death in candidemic patients. *C. albicans* was significantly associated with mortality when compared with all other non- albicans species. *Candida* spp. isolates were assayed for in vitro susceptibility to amphotericin B, fluconazole, 5-flucytosine, itraconazole, terbinafine and voriconazole. Resistance to fluconazole was detected in 22 isolates (8.9%). Resistance to amphotericin B, flucitosine and voriconazole were low. We confirmed that fluconazole-resistant *Candida* strains are a rare finding in Brazilian candidemic patients. The knowledge of the local epidemiological trends in *Candida* species associated with candidemia is important to guide prevention strategies and therapeutical choices.

PP-07-26

Prevalence of *Candida* dubliniensis among cancer patients in Kuwait

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Introduction: Although *Candida* dubliniensis forms only a minor component of the normal oral flora, it appears to have a world-wide distribution. Despite its close genetic and phenotypic relationship with *Candida albicans*, its role in human disease is mostly restricted to oral colonization, particularly among HIV-infected patients. The prevalence of C. dubliniensis in association with other disease conditions has been infrequently reported. In this study, we present data on the prevalence of C. dubliniensis among yeast species isolated from cancer patients over a five-year period. Methods: A total of 1445 yeast isolates were recovered from respiratory specimens, blood, urine and oral swabs. *Candida* dubliniensis isolates were provisionally identified by germ tube test, formation of rough colonies with chlamydospores on sunflower seed agar, and Vitek 2 assimilation profile. Their identity was confirmed by species-specific PCR and sequencing of internally transcribed spacer (ITS) region of rDNA. Antifungal susceptibility for fluconazole was determined by Etest. Results: Of 1445 yeast isolates identified, the prevalence of C. dubliniensis was 5% (n = 71), *C. albicans* 60% (n = 862) and other yeast species 35% (n= 512). All the C. dubliniensis isolates originated from respiratory specimens (5.9%) and oral swab cultures (3.2%), but none from blood or urine specimens. Using Etest, the MIC90 and MIC range values for fluconazole were 4 µg/ml and 0.047 -8 µg/ml, respectively. Thus, all the C. dubliniensis isolates were susceptible (<8 µg/ml) to fluconazole. Conclusion: The overall prevalence of C. dubliniensis among yeast isolates recovered from cancer patients in Kuwait was 5%. The exclusive isolation of C. dubliniensis from respiratory or oral specimens and not from blood or urine specimens suggests that this species has preference to colonize these sites of human body. In vitro resistance to fluconazole was not encountered. The study was supported by KFAS grant No. 2005-130-205.

PP-07-27

Pattern of *Candida* colonisation and invasive Candidiasis in the ICU

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Fungal colonising flora and invasive candidiasis (IC) were assessed during an Australian study of risk prediction for IC. 569 patients were in Westmead Hospital general ICU >72h from June 2007-December 2008. 4,152 samples, taken 72h post admission then twice weekly until discharge, were obtained from throat, perineum and urine and cultured onto *Candida* Chromogenic agar (CHROMagarTM, BioMerieux). *C. albicans, C. tropicalis* and *C. krusei* were speciated by colour; other isolates were designated '*Candida* species'. Fungal load was estimated (+ to +++) after 48h. 528 throat and 531 perineal swabs from 205 patients were analysed for eight *Candida* species by multiplex-tandem PCR (MT-PCR).

On initial culture, 90%, 55% and 17% patients were colonised in throat, perineum and urine, respectively. Over a month, throat colonisation dropped to 36% but perineum and urine colonisation increased to 85% and 40%, respectively, peaking at three weeks. 49% patients were colonised in >1 site with the majority (30%) in the throat and perineum; 98 (17%) were never colonised. *C. albicans* predominated at all sites; it was isolated from 67% patients. MT-PCR yielded similar results; however, the primary screen showed less *Candida* in the throat (71%) and perineum (44%) due to greater specificity of the MT-PCR (*S. cerevisiae* accounted for 70 false positives). MT-PCR and cultures were concordant for five patients who developed IC. Each was colonised with the infecting *Candida* in throat and/or perineum on all screens preceding infection (up to 4). Based on colonisation data, the sensitivity, specificity, PPV and NPV for IC from patients with throat and/or perineal colonisation in all of the first 3 screens was 100%, 61%, 4% and 100% for culture (n=74) and 100%, 33%, 4% and 100% for MT-PCR (n=66), respectively. In conclusion, MT-PCR is a practical, rapid (2h) and cost-effective tool with a high NPV for prediction of IC.

PP-07-28

Presence of *Candida* spp. at the peg-and-socket articulation (gomphosis) in patients with periodontal disease: The diabetes as a risk factor

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Background. In the oral cavity, some mycetes may be simply commensals or can develop into opportunistic pathogens. They mainly colonize the oral mucosa but can also affect the tongue, teeth and dental support apparatus, even causing degeneration of the peg-and-socket articulation. Bearing in mind that diabetic patients have a greater predisposition to mycotic complications, aim of the present work was to assess - in patients with periodontal disease - the association between diabetes and presence of mycetes at the level of the teeth-periodontal joints.
Materials and Methods. A case control study was conducted in a group of 130 selected patients with periodontal disease, 65 diabetics and 65 non diabetics. The presence of mycetes was tested by inserting cones of sterile bibulous paper between the teeth and periodontal apparatus, in contact with the peg-and-socket articulation and kept in situ for 2 minutes. Subsequently, the material was seeded onto Sabouraud Dextrose Agar with CAF plates. To assess associations between variables, the chi square test was used, setting significance at a value of $p<0.05$. The statistical software package SPSS version 16 Italian was employed.
Results. Twenty-seven patients were positive for *Candida* spp.: 22 subjects were diabetic and 5 non diabetic. *Candida albicans* was the most frequent species (74.1%), followed by *Candida krusei* (14.8%) and *Candida parapsilosis* (11.1%). The resulting Odds Ratio was 6.14 ($P<0.001$).
Conclusion. The results of present study show that colonization by *Candida* spp. in patients with periodontal disease is favoured by diabetes, and can extend and involve the dental joints. In these patients, early diagnosis of mycotic infection is essential to avoid other infectious complications. It would, therefore, be good practice always to take into account the possibility of a mycotic infection when making a differential diagnosis of periodontal disease.

PP-07-29

Nosocomial *Candida* in intensive care unit (ICU): Epidemiology, transmission and prevention

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The incidence of nosocomail fungal infections has increased in recent years, and antibitotic resistance is an issue in some hospital. The incidence of candidemia is higher in ICU than in other parts of the hospital. Candidemia caused by *Candida albicans* in ICU suggest that patients proximity and the possible transport of the these microorganism by the hands of members of the clinical staff may be risk factors these patients. predisposing factors including, the admission and a prolonged permanence of a patient in an ICU, the presence of intravascular catherers,antifungal prophylaxis at time of surgery,the use of wide- spectrum antibiotics and disequilibrium indigenous microbiota that can the increasing use of antifungal agents has led to the development severity of candidemia and antifungal resistance for patients in the ICU. Prospective studies will be needed to defined more clearly the patients and hospital reservoirs to infection, appropriate treatment and the role of new drugs in the treatment and prophylaxis. Role of susceptibility testing as a guide to selecting appropriate therapy for nosocomail candidemia. The efficacy of antibiotic prophyaxis for ICU patients .To exist the effect of an infection control program on the incidence of hospital-acquired infection (HAI) and associated mortality. The clinical mycology laboratory is an essential component of an effective infection control program. Laboratory personnel have a broad range of technologies, from traditional methods of detecting and identifying organisms to modern molecular typing methods, that they can use to support and enhance the efforts of the infection control staff .If the infection control team applies these technologies appropriately, it can prevent problems and solve nosocomial mysteries efficiently, both programs will be successful and the patients and the hospital will benefit because the risk of nosocomail fungal infections and the frequency of resistant organisms will be reduced.

PP-07-30

A retrospective molecular screening for *Candida* orthopsilosis and C. metapsilosis among Danish C. parapsilosis blood isolates

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C. parapsilosis groups II and III are proposed to be replaced by two new species C. orthopsilosis and C. metapsilosis, respectively. C. parapsilosis is retained for group I isolates. We studied the prevalence and susceptibility profiles of these recently described species among 79 blood isolates of C. parapsilosis which collected from Danish patients with candidemia in the years 2002 to 2008.

The isolates were screened by PCR amplification of SADH (secondary alcohol dehydrogenase) gene followed by restriction digestion with the enzyme BanI as previously recommended (Tavanti et al. 2005). C. metapsilosis ATCC 96144 and C. orthopsilosis ATCC 96139 were included as controls. Isolates with RFLP pattern distinct from the C. parapsilosis strain were characterized by sequence analysis of ITS1-5.8SrDNA-ITS2, 26SrDNA(D1/D2) and SADH regions.

By PCR-RFLP of SADH gene alone, four isolates (5.1%) had restriction pattern identical to C. orthopsilosis ATCC 96139. However, sequence analysis of SADH and 26SrDNA genes and ITS regions identified 2 of these 4 isolate as C. metapsilosis. Phylogenetic analyses using the software DNASIS and ClustalW showed that the sequence grouping of different DNA targets are compatible with each other.

We conclude that PCR-RFLP profile of SADH gene which has been used for differentiating of new species of C. parapsilosis group is not valid enough and more reliable DNA-based method is needed for diagnostic and epidemiological purposes.

PP-07-31

Mixed fungal colonization in non-surgical intensive-care patients

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Background: Knowledge of risk factors is important for properly applying and evaluating diagnostic tests to derive therapeutic conclusions. Colonization with fungi has been identified as an independent risk factor for invasive mycosis. In addition to colonization with a single species, the combination of different fungal species may be of interest.

Methods: In a prospective study we analysed samples taken from 411 patients after admission to our ICU. Swabs from nostril, throat and anus and specimens of tracheal secretions and urine were taken and cultured on CHROM-Agar. Results: Positive results were found in 798 (42.7%) of all 1868 samples. Of these, 618 were positive for a single species, 158 for two species, and 22 for three species. Concerning distribution of species, we found *Candida albicans* in 69.3%, *Candida* glabrata in 34.8% and *Candida* tropicalis in 8.1% of all positive specimens. In 90 cases, cultures grew *Candida albicans* together with *Candida* glabrata, in 23 cases, *Candida albicans* together with *Candida* tropicalis, in 12 cases, *Candida albicans* together with *Candida* glabrata and *Candida* tropicalis. Most frequently, a mixed colonization was detected from throat swabs (74 mixed, out of 281 positive cultures, 26.3%), followed by tracheal secretions (35 mixed, out of 153 positive cultures, 22.9%) and anal swabs (48 mixed, out of 235 positive cultures, 20.4%). In contrast, a mixed colonization was significantly less frequent in nasal swabs (18 mixed, out of 136 positive cultures, 13.2%) and in urine (5 mixed, out of 56 positive cultures, 8.9%).

Conclusions: A large proportion of samples showed growth of yeasts. Out of culturally positive, in 22.6% were found more than one species. Colonization with more than one species was found to be significantly more frequent in throat, trachea and anus compared to nose and urine.

PP-07-32

Recent experience with fungemia: Change in species distribution and azoles resistance and its correlation with outcome

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Owing to a rise in frequency and change in pattern of cases with fungemia due to yeast at our tertiary care center, we conducted a prospective study for four months to understand the epidemiology and outcome of this infection. Detailed case histories of those cases including management protocol and outcomes were noted. Records of 140 cases with fungemia (27.1% adult and 72.9% pediatric patients) were analyzed. Though C. tropicalis was the overall commonest (42.1%) yeast isolated, significantly higher isolation of C. guilliermondii (30.4%), and Pichia anomala (17.6%) was noted in pediatric patients; and *C. albicans* (26.3%), *C. glabrata* (10.5%) in adult patients. Rare species isolated include C. ustus (0.7%) and Trichosporon asahii (2.1%). In a case-control study in pediatric surgery ward, central line, prematurity, abdominal surgery, and ICU stay were significantly associated with fungemia (P<0.05). However, while comparing risk factors in adult and pediatric patients, significant association (P<0.05) with severe/recurrent pneumonia, ICU stay was observed in pediatric patients; diabetes, renal failure, central line, and extended period of broad-spectrum antibiotic and hospital stay were significant in adults. Mortality was high (56.9% & 47.4%) in both groups of patients and attributable to fungemia in 35.9%; 45 (32.1%) patients died before initiation of therapy, and 20 (14.3%) were cured after removal of central venous line only. Resistance to azoles (fluconazole, itraconazole, voriconazole) emerged in *C. albicans* (12.5 - 18.8%), C. tropicalis (10.2 -13.6%). Antifungal susceptibility testing report modified the therapy from fluconazole to conventional or liposomal amphotericin B in eight patients, five patients responded to modified therapy. In conclusion, the study highlighted the rise of non-albicans *Candida* species in our hospital with differential distribution in pediatric and adult wards and emergence of azole resistance in common species of *Candida*. Antifungal susceptibility testing could guide in proper management of some patients.

PP-07-33

Candidaemia with uncommon *Candida* species in Australia: Predisposing factors, outcome, antifungal susceptibility and implications for management

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The risk factors for and clinical features of bloodstream infection with uncommon *Candida* spp. are incompletely defined. To identify clinical variables associated with these species that might guide management, we analysed 57 cases of Candidaemia resulting from uncommon *Candida* spp. in comparison with 517 episodes of *Candida albicans* Candidaemia encountered in Australia from 2002-2004. Infection with uncommon *Candida* spp. (5.3% of Candidaemia) was significantly more likely to be outpatient-, than inpatient-acquired (p =0.01). Prior exposure to fluconazole was uncommon (n=1). *Candida* dubliniensis was the commonest species (39%) followed by *Candida* guilliermondii (19%) and *Candida* lusitaniae (12%). C. dubliniensis fungaemia were independently associated with recent intravenous drug use (p =0.01) and chronic liver disease (p =0.03) whilst infection with species other than C. dubliniensis was associated with age <65 years (p =0.02), male sex (p =0.03) and HIV infection (p =0.05). Sepsis at diagnosis and crude 30-day mortality rates were similar for C. dubliniesis-, non-C. dubliniensis species- and *C. albicans*-related Candidaemia. Haematological malignancy was the commonest predisposing factor (28% cases) for C. guilliermondii and C. lusitaniae Candidaemia. The 30-day mortality of C. lusitaniae fungaemia was higher than the overall death rate for all uncommon *Candida* spp. (42.9% vs. 25%, p =NS). Resistance to azole and amphotericin B antifungal agents was rare but five strains (9%) had fluconazole MICs of 16-32 mg/L. All strains were susceptible to voriconazole, posaconazole and caspofungin. Candidaemia caused by uncommon *Candida* spp. is emerging among hospital outpatients; certain clinical variables may assist in recognition of this entity.

PP-07-34

Retrospective analysis of diagnosis, management, and outcome of candidemia in non-neutropenic patients

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Background. Candidemia is the most prevalent form of invasive mycosis worldwide. Diagnosis and management underwent major changes during the past few years.

Aim of the study. (1) To document and correlate diagnosis, management, and outcome of recent cases of candidemia in non-neutropenic patients. (2) To recommend strategies for management.

Patients and methods. Thirtyone non-neutropenic patients were diagnosed with candidemia by at least one blood culture growing *Candida* sp. at a single secondary care hospital in central Germany during the years 2005 to 2008. The clinical records were analyzed retrospectively.

Results. *Candida* spp. recovered from the blood cultures included: *Candida albicans* 21, *Candida parapsilosis* 3, *Candida glabrata* 3, *Candida tropicalis* 2, and *Candida lusitaniae* 2. Additional primarily sterile sites with *Candida* sp. included the tips of central venous catheters (n=7), port sites (n=3), and peritoneal fluids (n=2). Clinical symptoms included fever of unknown origin, leukocytosis, elevated C-reactive protein, and elevated procalcitonin. In all cases, the clinical diagnosis of candidemia was made after a preliminary oral report from the laboratory, that a yeast grew in a blood culture. Management strategies included removal of central venous catheters (n=8), removal of infected ports (n=3), and abdominal surgery (n=2). Antifungal chemotherapy was installed 2 to 5 days after the onset of fever. Fluconazole was given to 21 patients, mostly 200 mg per day for 2 to 17 days. Caspofungin was given to 7 patients, 50 mg per day for 10 days. One patient received only nystatine orally. Two patients died without receiving antifungal chemotherapy. Outcome: 14 patients died, 17 survived (mortality 45%).

Conclusions. The diagnoses of candidemia depended on the report of positive blood cultures. Managements included removal of catheters and low to regular dose short term fluconazole or caspofungin. Mortality was related to late onset of management.

PP-07-35

The distribution of species and susceptibility of amphotericin B and fluconazole of yeast pathogens causing invasive infections in Taiwan

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To study the species distribution and antifungal susceptibility of yeasts causing invasive infections in Taiwan, we have analyzed 155 and 210 isolates obtained from sterile sites from19 hospitals in 2002 and again from the same ones in 2006. Blood was the most common source, accounting for 73.7% of the total isolates, followed by ascites (21.6%), cerebrospinal fluid (CSF, 3%), and synovia (1.7%). *Candida albicans* was the most frequently isolated species (50.4%), followed by *Candida tropicalis* (20.6%), *andida glabrata* (11.5%), *Candida parapsilosis* (8.5%), *Cryptococcus neoformans* (3.9%), *Candida krusei* (0.8%), and other nine species (4.3%). There were one (0.3%) and seven (1.9%) isolates with minimum inhibitory concentrations (MICs) of amphotericin B greater than or equal to 2 mg/l after 24 hours (h) and 48 h incubation, respectively, whereas there were 15 (4.3%) and 31 (8.5%) isolates with MICs of fluconazole greater than or equal to 64 mg/l under the same condition. The MIC50 and MIC90 of amphotericin B were 0.5 mg/l and 1 mg/l, respectively after either 24 h or 48 h incubation. The MIC50 and MIC90 of fluconazole were 0.25 mg/l and 4 mg/l after 24 h incubation and 0.5 mg/l and 32 mg/l after 48 h incubation. Interestingly, MICs of fluconazole greater than or equal to 64 mg/l was significantly higher for isolates in 2006 than those in 2002.

Poster Forum PF-04

PP-07-36

Epidemiology and sensitivity profile of *Candida* strains from invasive infections in a tertiary hospital in Greece

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The changing epidemiology of *Candida* isolates in nosocomial settings with increasing numbers of more resistant non-albicans species, constitutes a major challenge in the management of invasive candidiasis (IC). The aim of this study was to examine shifts in the distribution of *Candida* strains from cases with IC in our hospital and test their antifungal sensitivity.

During the period from January 2006 to October 2008, 41 *Candida* strains from 40 patients with IC were isolated from blood cultures. They were identified to species level and tested in vitro for susceptibility to antifungals.

Of the 41 strains isolated, 11 were *C. albicans* (27%), 16 C. parapsilosis (39%), 8 *C. glabrata* (19%), 2 C. sake, 1 C. intermedia, 1 C. tropicalis, 1 C. lusitaniae, 1 Pichia ohmeri and 1 was non-albicans no further identified. The incidence of C. parapsilosis and *C. glabrata* was increased considerably compared to the years 2001-2005 (19% and 14% respectively, *C. albicans* representing 63% of cases).

All species were sensitive to 5-Flucytocine and more than 90 % of all species were sensitive to Amphotericin B, Caspofungin and Voriconazole. Only 1 *C. albicans* was resistant to Fluconazole. In contrast, 7 of the 8 *C. glabrata* strains had dose dependent sensitivity or were resistant to Fluconazole, Itraconazole and Ketoconazole.

In conclusion, a shift towards non-albicans and more resistant strains was noted in our hospital during the last 33 months compared to previous years. Our data show that although a small proportion of all *Candida* strains are resistant to antifungals usually administered as standard therapy, shifts in species distribution may result in therapy failures. Ongoing monitoring of the local epidemiology and susceptibility testing when appropriate may help the selection of initial therapy considerably.

PP-07-37

Antifungal susceptibility testing and genomic DNA profiles of *Candida* isolates from oral cavity in AIDS patients under prolonged antiretroviral therapy

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In HIV patients, oral colonization by *Candida* spp. has predicted the subsequent development of oral pharyngeal candidiasis(OPC).A cross sectional study was conducted April 2003 to April 2004. Patients HIV positive, undergoing antiretroviral treatment for at least 1 year, without clinical and symptomatic evidence of OPC; aged > 18 years; and had not undergone antifungal therapy in the last 3 months. Samples from the oral cavity were obtained by swabbing the oral mucosa. Antifungal susceptibility tests were performed according to the document M27-A2. *Candida* strains were genotyped by electrophoretic karyotype (EK). 331 patients were enrolled. 147(44%) patients harbored *Candida* spp in the oral cavity. Oral colonization was similar in patients diagnosed in period A: 1996-1995 (90 patients; 43 colonized) and in period B: 1996 - 2003 (241 patients; 104 colonized) (p=0.53). 161 *Candida* strains were recovered of 147 patients: *C. albicans*: 137 (85%); (117 *C. albicans* serotype A and 20 strains serotype B); 24 non-albicans species included: 7 *Candida* glabrata, 4 *Candida* tropicalis, 2 C. dubliniensis ; 11 others. No difference was observed among patients in the two periods concerning *C. albicans* serotypes. Period A: 83 - serotype A and 14 serotype B. Period B: 31-serotype A and 7 serotype B (p=0.20). No resistance to azoles was detected, including *C. albicans* and non-albicans. SDD occurred in *C. glabrata* to azoles and one isolate of C. tropicalis was SDD to itraconazol. 74 colonized patients had previously used antifungal agents and higher MIC levels were detected only in non-albicans isolates, independently of use of antifungal agents. EK identified 15 DNA profiles among 117 *C. albicans* serotype A whereas serotype B had three different EK profiles.. In conclusion, the use of antifungal drugs was not related to high MICs and colonization occurred independently of the time of use of ARV.

PP-07-38

Histopathological study of central nervous system candidiasis

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In recent years, incidence of invasive fungal infection has been increasing, mostly due to advance of medicine that may produce immunocompromised individuals. Candidial infection in central nevous system (CNS) is one of the most serious form of blood stream infection of *Candida* sp. of which mortality has been known as more than 50%

In this research, we employed 27 autopsies with invasive CNS yeast infection which were confirmed. In addition to details morphological analysis on shape of yeast, in situ hybridization with originally designed *Candida*-specific PNA probe was carried to identify *Candida* infection of each patient. This was followed by histopathological investigation; invasiveness, shape, and distribution yeast or yeasts with pseudohyphal growth, and a study regarding to correlation between histological characteristics and number of leukocyte in the peripheral blood just before death.

As a result, supratentorial region was the commonest area of disseminated candidial infection in CNS, and that density was highest in the cerebral white matter followed by the gray matter, basal ganglia. On the other hand, regarding to the lesions developed in the cortical area, the average distance from the brain surface was 4.026 mm. This area corresponding to deeper cortex has a characteristic arterial structure that refers hair-pig curving reverse. The structure may attribute to high incidence of development of candidial foci in the deeper cortex, because of that increasing of shear stress.

PP-07-39

Disseminated trichosporonosis caused by *Trichosporon* species in patients with hematological malignancies: A retrospective multicenter study from Japan

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Background: Disseminated Trichosporonosis (DT), infection by *Trichosporon* species, is an uncommon but frequently fatal mycosis. Though it is observed around the world, little is known about this disease. We organized a nationwide study group to clarify the clinical characteristics and useful treatments for DT in Japan.

Methods: A questionnaire about experimental DT was sent to about 700 hospitals throughout Japan. In this study, DT was defined as the cases with both infectious symptom and blood culture yielding *Trichosporon* species. Case reports with clinical information on past cases from 2000 to 2007 were returned. The risk factors, presence/absence of breakthrough infection, outcome, and efficacy of antifungal regimens for DT were investigated.

Results: 77 cases of DT were reported from 40 institutions. 81.8% of patients had sever neutropenia. Anti-fungal agents were administered to 68 patients before the onset of DT; the most common antifungal agent was micafungin (MCFG) (46 of 68 cases; 67.6%). Though various treatment regimens were used for DT, the mortality rate within 30 days was 70.1%. Univariate analysis revealed that overall survival after DT was significantly longer in patients treated with regimens including voriconazole (VRC) than those of patients without VRC (median survival time, 88 vs 8 days, p=.034). In contrast, no difference in overall survival was apparent according to the treatment with regimens including amphotericin B (9 vs 17 days, p=.423).

Conclusions: In Japan, many cases of DT developed as a breakthrough infection in patients with use of MCFG. VRC was an effective agent for DT. This is the first report of a large-scale nationwide study of trichosporonosis.

PP-07-40

Clinical, epidemiological and evolutive features of 77 patients with cryptococcocal meningitis in Uberaba, Minas Gerais, Brazil

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Cryptococcosis is an opportunistic mycosis caused by two species of the Cryptococcus complex, C. neoformans and C. gattii that affect immunodeficient and immunocompetent hosts respectively. The aim of this study is to present, epidemiological and clinical profile of patients with cryptococcal meningitis diagnosed at the teaching hospital from 1998 to 2007. From 77 patients, 68 (88.3%) presented AIDS, the remaining, only three had other immunosuppression conditions. The median age was 35.6 and 57 (74%) were male. Cryptococcal meningitis was the first AIDS defining condition in 38 (55.9%) cases being that 25 (65.8%) presented simultaneously both diagnosis. Thirty six (48.7%) referred evolution of symptoms related to Cryptococcus infection of one week. Headache, fever, weight loss and altered mental status were present in 87.7%, 72.6%, 70.5% and 52.1%, respectively. The cerebrospinal fluid (CSF) analysis showed features of lymphocytic meningitis with positive cryptococcal antigen and India ink in 83.6% and 87.7% of cases. CSF and blood cultures were positive in 77 (100%) and 18 (57.4%) cases respectively. From 77 isolates, 72 (93.5%) were C. neoformans and 5 (6.5%) C. gattii. This specie were recovered from two AIDS patients. Forty seven (82.4%) out 57 AIDS patients presented CD4+ < 50 cells/mm3. Therapy with Amphotericin B was started in 74 patients, but 44 (59.5%) died during the first days or weeks on treatment. Eight (32%) of 32 evaluated patients, presented the Immune Reconstitution Inflammatory Syndrome (IRIS) after several weeks on HAART. The high mortality rate observed was similar to that reported in developing countries, but it is unacceptable in Brazil where the HAART is disposal in all public health services since 1996. Futhermore, inadequate adhesion and advanced AIDS diagnosis often contribute to explain the poor outcome observed in cryptococal meningitis patients.

Key words: Cryptococcal meningitis, AIDS, IRIS, HAART

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PP-07-41

Ecological niche of Cryptococcus neoformans species complex in the soils of Betul, a city of central India

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The aim of present study was to search for the presence of C. neoformans species complex in the environment of frequented areas of Betul city. Out of 245 soil samples investigated 38 (15.5%) samples were found positive for the pathogen. Out of 38 positive human urine soaked sites, 18 (47%), were positive for C. neoformans var, neoformans and 20 (52%) for C. neoformans var. gattii. Out of 15 samples investigated from ceilings and walls of patient's room in the hospitals and nursing-homes of Betul, 6 (40%) were positive for C. neoformans var. neoformans, and 9 (60%) for C. neoformans var. gattii. The present investigation suggest that human urine soaked soil can act as a reservoir for the C. neoformans species complex in India.

PP-07-42

Molecular epidemiology of Cryptococcus neoformans in Taiwan

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Cryptococcosis is caused by 2 species in the genus Cryptococcus, Cryptococcus neoformans and Cryptococcus gattii. Global data as well as our previous data suggest that the geographic distribution, environment reservoir, clinical presentation and antifungal susceptibility of these two species are different. Cryptococcus was classically subdivided into the three varieties: C. neoformans var. grubii (serotype A), C. neoformans var. neoformans (serotype D), C. neoformans var. gattii (serotypes B and C), and the hybrid serotype AD. Clinical isolates were collected retrospectively and prospectively from at least three hospitals in Taiwan. Clinical data (immune status, pigeon exposure), use of antifungal agents, antifungal response will be reviewed. The variety of these isolates will be determined by biochemical methods and genotypes will be evaluated and compared by using M13 polymerase chain reaction-fingerprinting and orotidine monophosphate pyrophosphorylase (URA5) gene restriction fragment length polymorphism analysis with HhaI and Sau96I in a double digest. Clinical isolates will be grouped into one of the eight molecular types, 5 serotypes and three varieties.

PP-07-43

Cryptococcosis in Saint Petersburg, Russia, 1990-2008

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Cryptococcosis is an emerging life-threatening disease in Saint Petersburg. We report clinical cases of cryptococcosis confirmed by culture in Reference mycological laboratory (Kashkin Research Institute of Medical Mycology). During years 1990-2008 49 cases of cryptococcosis have been diagnosed and 15 (30,6%)of these cases were revealed in the last year. Clinical manifestations were: meningitis - 35 (71,4%) patients, acute disseminated cryptococcosis - 12 (24,5%), cryptococcemia - 1 (2%), vertebral osteomyelitis - 1 (2%). Risk factors were: HIV-infection - 36 (73,5%)patients, haematological disease - 7 (14,3%), renal transplantation - 3 (6,1%), idiopathic lymphocytopenia - 1 (2%), unknown - 2 (4,1%). Aetiologic agents in all cases except one were Cryptococcus neoformans. The case of vertebral osteomyelitis was caused by C. albidus. On murine model it was shown that C. neoformans isolates varied from low to highly virulent. The strains also differed in NO-production and resistance to phagocytosis in vitro.
Conclusions. Prevalence of cryptococcosis in Saint Petersburg has increased significantly. C. neoformans clinical isolates varied in virulence and other biological properties.

Poster Forum PF-06

PP-07-44

The occurrence of the primary pathogenic yeast *Cryptococcus gattii* in Europe

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Cryptococcosis is caused by the basidiomycetous yeasts *Cryptococcus gattii* (serotype B and C) and *C.neoformans* (serotype A, D and AD). Infections with *C.gattii* occur almost only in immunocompetent individuals, while *C. neoformans* var. *grubii* and var. *neoformans* have a predilection for immunodeficient humans. Another epidemiological difference between both species is the apparent restriction of *C.gattii* to (sub-)tropical regions, whereas *C. neoformans* can be found worldwide. The distribution pattern of *C.gattii* has changed by an outbreak in the temperate climate of Vancouver Island (Canada). Several case-reports were published which suggests that *C.gattii* is becoming more prevalent in the temperate climate of Europe as well. This prompted us to set up an epidemiological study, in collaboration with the ECMM *Cryptococcus* and cryptococcosis workgroup, to investigate the occurrence of *C.gattii* in Europe.

We have investigated 60 *C.gattii* isolates, from which 44 were of clinical origin, ten came from the environment and six had a veterinary origin. Thirty-five isolates were identified as genotype AFLP4/VGI, while the remaining isolates belongs to AFLP5/VGIII (n=2), AFLP6/VGII (n=12), AFLP7/VGIV (n=2), AFLP8 (n=6), and AFLP9 (n=1). Two isolates belonged to a novel genotype, AFLP10. The majority of isolates was mating-type α (n=36), eighteen isolates were mating-type **a**, and five hybrid isolates harboured both mating-types. Phylogenetic analyses, based on ten sequenced loci, confirmed the presence of five different haploid AFLP genotypes of *C.gattii* in Europe.

Most of the clinical cases were caused by isolates belonging to genotype AFLP4/VGI and AFLP6/VGII. It is possible that most of the patients acquired the infection with *C.gattii* during their stay in (subtropical or tropical) regions outside Europe. However, infections were also reported in patients who never travelled outside Europe. This indicates that *C.gattii* isolates of both genotypes AFLP4/VGI and AFLP6/VGII occur in the Mediterranean area.

PP-07-45

Antifungal susceptibility profile and molecular typing of *Cryptococcus neoformans* and *Cryptococcus gattii* isolates from India

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The pathogenic yeasts *C. neoformans* and *C. gattii* are widespread in decayed wood inside trunk hollows of taxonomically divergent tree species and frequently occur in their surrounding soil in India. In this study, we report the antifungal susceptibility, mating type and molecular types of environmental isolates of *C. neoformans* and *C. gattii*. Our PCR results showed that all of the 121 environmental isolates (*C. neoformans* = 61, *C. gattii* = 60) were mating type α (MAT α).Our PCR fingerprinting and / or molecular sequence analyses identified that all environmental strains of *C. neoformans* belonged to *C. neoformans* var *grubii*, molecular type VN I, whereas all *C. gattii* isolates belonged to molecular type VG I. Notably, 10 of the 61 (16 %) isolates phenotypically identified as *C. neoformans* were, in fact, C. gattii by molecular analysis underlining the pitfall of potential phenotypic mis-identifications. Antifungal susceptibility testing was performed for the 121 environmental isolates using CLSI microdilution method (M 27A2). All of the isolates were susceptible to the five tested antifungal agents (amphotericin B, 5 flucytosine, fluconazole, itraconazole, and voriconazole). In contrast 3 of the 110 clinical isolates (C. neoformans = 108, C. gattii = 2) originating from north-western India were resistant to 5 flurocytosine and all three belonged to C. neoformans var grubii. A comparison of the geometric means of MICs revealed that *C. gattii* isolates were more susceptible to amphotericin B (0.255 versus 0.296, P< 05) and 5 flucytosine (2.00 versus 8.00, P< .05) and less susceptible to fluconazole (7.37 versus 3.91 P< .05), itraconazole (0.255 versus 0.141, P< .05) and voriconazole (0.143 versus 0.066, P< .05) than *C. neoformans* isolates. We discuss the implications of these results in the epidemiology of *C. neoformans* and *C. gattii* infections in India.

PP-07-46

A case of cryptococcal meningitis of which morphological examination on yeasts in cytological specimen was useful for accurate assessment for antifungal chemotherapy

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Introduction: The present paper describes a case of cryptococcal meningitis focusing on sequential change in shape and number of yeasts in cytological specimen; cerebrospinal fluid, of which morphological examination on yeasts in cytological specimen was useful for accurate assessment for antifungal chemotherapy.

Case: 65 years old man had been admitted with headache. He was diagnosed as cryptococcal meningitis with confirmation of presence of encapsulated yeasts in and positive culture of *Cryptococcus neoformans* from cerebrospinal fluid, and increasing of antigen in both peripheral blood and cerebrospinal fluid. The treatment with intravenous administration of liposomal amphotericin B and flucytosine, and subdural injection of classical amphotericin B were carried out. Culture became negative, promptly, but yeasts are continuously found in the cerebrospinal fluid by cytological examination. He was finally diagnosed as adult T cell leukemia by bone marrow aspiration biopsy due to his hyperpotassemia on 180 days after admission.

Cytological findings: Within 60 hospitalized days, there are numerous yeasts which were spherical, smooth-surfaced, and clearly encapsulated. The yeasts measured 4-17 µm in diameter and their capsule was Alcian-bleu positive. During 196 hospitalized day, we had 40 times of cytological diagnostic occasions with the cerebrospinal fluid, which showed decreasing in number of yeasts and change of their shape, gradually. Deformed yeasts; grooved, wrinkled, and hemilunar-shaped, became to be found, occasionally, and the reactivity became to be irregular for methenamine silver.

Conclusion: Detailed and sequential observation focused in change of shape and number of yeasts in cytological specimen must be useful to surmise the efficacy of ongoing antifungal chemotherapy.

PP-07-47

Cryptococcus gattii meningoencephalitis in an immunocompetent person 13 months after exposure

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A 53-year old immunocompetent Swiss female is described who developed severe meningoencephalitis due to infection with *Cryptococcus gattii* 13 months after exposure on Vancouver Island, Canada. Diagnosis was achieved from cerebrospinal fluid (CSF) by positive India-ink microscopy, positive latex particle agglutination, and positive culture. Species identification was performed by sequencing the intergenic and internal transcribed spacer regions (IGS, ITS) of the rRNA genes and by growth on L-canavanine-glycine-bromthymol blue medium. After initial therapy with fluconazole by which the patient did not improve, therapy was changed to amphotericin B and flucytosine, and later to high dose fluconazole and amphotericin B. Despite long-term treatment and external drainage of CSF, the patient's condition improved only slowly. The patient was discharged after 132 days of hospitalization.

PP-07-48

A fatal case of blastomycotic meningoencephalitis with neutrophilic pleocytosis in an immunocompetent patient

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INTRODUCTION: Blastomycosis is endemic to the Ohio and Mississippi River valleys. Blastomycotic central nervous system (CNS) infection is generally characterized by lymphocytic pleocytosis. We report a fatal case of blastomycotic meningoencephalitis with neutrophilic pleocytosis from a non-endemic area.

CASE: A 59-year-old immunocompetent male from North Dakota developed headache, confusion, hearing loss and dizziness during June 2005. He was prescribed 10 days of amoxicillin but demonstrated no clinical improvement and was admitted to the hospital during July 2005.
Lumbar puncture revealed a white blood cell count of 525 cells/mm^3 (70% neutrophils and 29% lymphocytes), glucose 24 mg/dL, and protein 180 mg/dL in the cerebrospinal fluid (CSF). CSF was sent for Gram stain, culture, cryptococcal antigen and herpes simplex DNA. All tests were negative. Blood was sent for culture and was tested for Lyme antigen, West Nile virus, and *Blastomyces dermatitidis* serology, which were all negative. Chest radiograph and head CT findings were unremarkable.
Partially treated bacterial meningoencephalitis was suspected because of prior exposure to amoxicillin. Intravenous vancomycin, ceftriaxone, and ampicillin were started empirically. Despite treatment with three antibiotics, the patient's neurologic symptoms gradually deteriorated. Repeated head CT remained unremarkable and repeated CSF analysis demonstrated no improvement. Therapy was changed to intravenous gatifloxacin, cotrimoxazole, and fluconazole, however, the patient expired during August 2005 despite this change. Autopsy revealed *B. dermatitidis* in the lungs, brain, and meninges. Fungal cultures of the blood and CSF were negative.

DISCUSSION: This unusual case presentation of *B. dermatitidis* infection highlights the importance of including this organism in differential diagnosis in the case of CNS infection with neutrophilic pleocytosis, in the absence of travel to or residence in an endemic area. Fungal culture and *B. dermatitidis* serology were insufficiently sensitive to diagnose CNS or pulmonary blastomycosis. Furthermore, CT and chest radiographic findings may be misleading.

PP-07-49

Cross-reaction of *Blastomyces dermatitidis* accuprobe test with *Chrysosporium carmichaelii*

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Background: Isolates of *Blastomyces dermatitidis* have been traditionally identified by microscopic evaluation of the hyphal phase, followed by conversion to the yeast phase. Most laboratories now identify isolates suspected of being *B.dermatitidis* by microscopic evaluation of the hyphal phase followed by the DNA probe test ("Accuprobe") specific for *Blastomyces dermatitidis* [Gen-Probe, San Diego, CA]. Although a few mould species (e.g., *Emmonsia parva, Paracoccidioides brasiliensis* and *Gymnascella hyalinospora*), reportedly have produced false-positive results with Accuprobe, we describe the first case of a false-positive result with *Chrysosporium carmichaelii*.

Case report: A 48-year-old man presented to the surgical service with a nodular lung lesion suspicious for malignancy. A lung biopsy was performed and tissue was submitted for histology, direct microscopy and fungal culture.

Mycology: No fungal elements were seen on histologic or calcofluor white/KOH stains. Culture yielded mould growth after 2 weeks incubation. Initial microscopy appeared suggestive of *B. dermatitidis* and the isolate's identification was confirmed by the Gen-Probe assay at the Illinois Department of Public Health. Microscopic re-evaluation of the cultured isolate suggested *Chrysosporium* species, possibly *C. carmichaelii*, based on the formation of pyriform conidia on short, slightly curved stalks. DNA target sequencing of the internal transcribed spacer (ITS) and the D1 D2 regions was performed and the isolate shared 98.2 to 99.8% identity with *C. carmichaelii*.

Conclusions: Since isolation of *Blastomyces dermatitidis* from a clinical specimen prompts consideration of antifungal treatment, accurate identification of the fungus is of paramount importance. This case underscores the importance of having well-trained mycologists and laboratory professionals who do not rely on molecular results alone.

PP-07-50

Pulmonary cavity co-existence of hyphae and spherules in coccidiomycosis patients

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Pulmonary coccidioidomycosis is acquired by a susceptible host arthroconidia inhalation. Main endemic zones are in the USA and Mexico border in arid and semi-arid zones. Endospore-containing spherules are typical in Coccidioides immitis and C. posadasii infected tissue. Although non-classic mycelia structures parasitic forms have been observed in human specimens, they have been frequently overlooked.

In the present study the co-existence of hyphae and spherules, parasitic forms, in pulmonary cavity in patients with chronic coccidiodomycosis was evaluated. The study inclusion criterion was isolation of Coccidioides spp. and colonized cavity lesion. The study population comprised patients (n= 40) with coccidiodomycosis admitted to the Instituto Nacional de Enfermedades Respiratorias, Mexico, since September 1991 to November 2008. Biological specimens were sputum and bronchial wash or brushing, and, in a small proportion, lung tissue, lymph node, skin and secretions from fistulae. Diagnosis was made by Direct examination with 15% potassium hydroxide and pathology assessment included cytology and histopathology using periodic acid Schiff (PAS), Gomori methenamine silver (GMS), hematoxylin and eosin (H-E) smear stains and fungus isolation.

Co-morbidity in these patients was: type 2 diabetes mellitus, malnutrition and/or anemia, AIDS, cardiopathy and lymphopenia. Chest radiology and computer axial tomography revealed colonized cavity lesion, nodules, micronodules, lung opacity and, in some patients, pleural effusion, empyema and hydropneumothorax.

Parasitic forms of Coccidioides spp. observed were: Spherules with or without endospores, germination and filamentation of spherule, and plentiful of septate hyphae.

In conclusion: Microscopic analysis proved co-existence of hyphae and spherules 28/40 (70%) associated to cavity pulmonary lesion and chronic coccidiodomycosis. The microenvironment present in cavity lesion from these patients must have specific environment (for instance, ratio of O2/CO2, and CO2 partial pressure near 0 mmHg) to allow co-existence of hyphae and spherules parasitic forms of Coccidioides spp.

PP-07-51

Increase in Non-*Aspergillus* mold infections in recipients of allogeneic bone marrow transplantation (BMT) at Memorial Sloan-Kettering Cancer Center (MSKCC)

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Invasive Aspergillosis (IA) is the most common Invasive Mold Infection (IMI) in BMT. An increase in Non-*Aspergillus* Mold (NAM) infections has been reported in cancer patients. NAM infections had been extremely rare in BMT at MSKCC. We describe the epidemiology of NAM infections at MSKCC during 2006-2008.

METHODS: All patients had BMT for hematologic malignancies at MSKCC. The incidence of IMI was determined by prospective surveillance for two 3 year study periods: January 2000- December 2002 (Cohort A) and January 2006- December 2008 (Cohort B). EORTC/MSG criteria were used for diagnosis. Serum Galactomannan for diagnosis and voriconazole prophylaxis were available for Cohort B only.

RESULTS: Cohorts A and B were comprised of 240 and 397 patients respectively. The incidence of IMI was 7.5% and 5.5% respectively. NAM accounted for 6% of all IMIs in Cohort A and 28% in Cohort B.

In cohort A 1 patient (0.4%) had mucormycosis. In cohort B 6 patients (1.5%) had NAM: Rhizopus 4, Absidia 1, Scedosporium 1. Median time to diagnosis was 218.5 days (range 21-443). 3 of 6 patients (50%) with NAM infections also had Invasive Aspergillosis. All patients had prolonged courses of voriconazole prior to diagnosis of IMI. Diagnosis was antemortem in 4 of the 6 patients (66.6%). Mortality was 83%.

CONCLUSIONS: 1) During the time interval between January 2006 and December 2008 there was an overall decrease of 26% in IMI but a relative increase in Non *Aspergillus* Mold infections of 78%. 2) 50% of NAM cases also had IA. 3) Voriconazole and intense immunosuppression may partially account for the rise in NAM infections in BMT. 4) Local surveillance and clinical suspicion are important for timely diagnosis and treatment.

PP-07-52

An experience of zygomycosis in a tertiary care centre in North India

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Aim of the study:The aim was to study the spectrum of clinical presentation, epidemiology, diagnosis and management of zygomycosis in a tertiary care centre.

Methods: All cases of zygomycosis diagnosed at our hospital during 2005-2008 were reviewed. Diagnosis was made either by direct microscopy or culture. The sites of involvement, underlying disease, species of fungi isolated and outcome of therapy were analyzed.

Results: Nineteen patients were diagnosed at ante-mortem. Rhino-orbito-cerebral type (42.1 %) was the commonest presentation followed by pulmonary (26.3 %) and renal (15.7 %). Parotid and liver abscess were diagnosed in one patient each. Pulmonary zygomycosis was seen in renal transplant recipients. Uncontrolled diabetes mellitus and immunosuppression due to renal transplantation were significant risk factors. Three patients with renal zygomycosis were apparently healthy. Culture was positive in 15 patients; various species isolated were *Rhizopus arrhizus* (5), *Absidia corymbifera* (2), *Apophysomyces elegans* (2),*Rhizopus homothallicus* (2),*Cunninghamella bertholletiae* (2), *Rhizopus rhizopodiformis*(1) and *Mucor spp* (1). We report for the first time two cases of cavitary pulmonary zygomycosis caused by *R. homothallicus*. Adequate therapy was provided in 14 patients, two patients expired before antifungal therapy could be initiated and three patients received only antibiotic & fluconazole. Mortality was common in patients with rhino-cerebral zygomycosis (8/9).Surgical debridement and amphotericin B was found to be the best treatment.

Conclusion: Zygomycosis is not an uncommon infection in our Institute. Microbiological culture identified the implicated pathogens. Combination of amphotericin B therapy, surgical debridement, treatment of underlying disease or reversal of the immunosuppression are standard therapy. Isolation of *R. homothallicus* in two patients with pulmonary abscess in this study highlights the importance of this zygomycete as an emerging pathogen of this disease in India and requires further study. Increased awareness and high index of suspicion helps in early diagnosis of zygomycosis resulting in a favourable outcome.

PP-07-53

Species-dependent differences in virulence in experimental pulmonary mucormycosis are related to sporangiospore germination, hyphal metabolism, and circulating molecular biomarker levels

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Background. Mucorales are emerging causes of life-threatening pneumonia in patients with prolonged neutropenia and corticosteroid therapy. Little is known about the relation between different species of Mucorales and their pathogenesis in pulmonary mucormycosis.
Objectives. To study the relative virulence of *Rhizopus oryzae* (RO), *Rhizopus microsporus* (RM), *Mucor circinelloides* (MC), *Mucor indicus* and *Cunninghamella bertholletiae* (CB) in experimental pulmonary mucormycosis and the possible correlation with germination rate, metabolic activity, and circulating Mucorales-specific-DNA.
Methods. Interspecies virulence was studied in experimental pulmonary mucormycosis in persistently neutropenic rabbits by a panel of validated outcome variables. Sporangiospore germination kinetics were measured over 4 h. Hyphal metabolic activity was determined by XTT assays. Plasma levels of Mucorales-specific-DNA, as a surrogate biomarker for angioinvasion, were measured by qPCR of two regions within the 28S rRNA gene.
Results. CB caused the highest lung weights, most extensive pulmonary infarcts, and lowest survival of 0% (0/18), in comparison to 16% (3/18, $p<0.01$) of RM-, 81% (21/26) of RO- and 83% (15/18) of M-infections ($p<0.001$). There were significant inoculum-dependent differences in residual pulmonary fungal burden (CFU/g) among CB-, RM-, and RO-infected rabbits (10^2-10^4 CFU/g, $p<0.05$), and significant differences in organism-mediated pulmonary injury in RM-, and RO-infected rabbits ($p<0.05$). Differences in virulence correlated with different germination kinetics at 4 h: CB (67-85%)> RM (14-56%)>RO (4-30%)>MC and MI (0%). These data correlated with greater in vitro metabolic activity by XTT assay of CB at 6 h (OD450=1.22) in comparison to that of other species (0.37-0.84). Mean peak plasma Mucorales-specific-DNA concentration (log GE/ml) followed a similar pattern: CB> RM> RO> MC.
Conclusions. Cunninghamella bertholletiae and *Rhizopus microsporus* were significantly more virulent than *Rhizopus oryzae* and *Mucor* species in experimental pulmonary mucormycosis. Virulence parameters of mucormycosis in vivo correlate with species-dependent differences in germination kinetics, hyphal metabolic activity, and circulating levels of Mucorales-specific-DNA.

PP-07-54

Cutaneous Cunninghamella sp. infection and suspect lung infection in AML

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This 69 year-old male is a case of myelodysplasia syndrome. He has history of gastric ulcer with subtotal gastrectomy, appendectomy for more than 20 year had ever admitted to our hospital on 2006-6-12 to 6-14 for bone marrow examination. Bone marrow was done which showed AML (M1). He began to suffer from intermittent fever about 10 days ago before this admssion. Cough with scanty sputum production developed about 3 days ago. Associated symptoms of general malaise, dyspnea on exertion and poor appetite were noted. There was no chest pain, abdominal pain, diarrhea, dysuria, recent travel nor animal contact history. He was brought to our emergency department where neutropenia and elevated CRP (C-reactive protein): 32.05 was noted. Initial chest X ray showed pneumonia patch over left upper and lower lobe. Chest CT was arranged which revealed pneumonia over left upper, lower lobe and right lower lobe. Under impression of neutropenic fever with left pneumonia he was admitted to our ward for further management. 10/10 Chest CT: 1. Consolidations in left upper lobe, left lower lobe, and right lower lobe suggesting pneumonia. 2. Subcentimeter lymph nodes. 3. Small amount of left pleural effusion. 10/22-24 Skin biopsy culture: Cunninghamella sp. He was passaway in spite of amphotericin B treatment.

PP-07-55

Absidia corymbifera infections in leukemia patients: Two cases report in China

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Zygomycosis is an infection with fungi of the class zygomycetes. The organisms most commonly implicated belong to the genus Rhizopus. The infections due to Absidia corymbifera were rare. There were at least seventy cases of zygomycosis published in the Chinese literature. However, the pathogenic organisms were identified in only sixteen cases among them. The infections caused by A. corymbifera have never been reported in China. We described two cases of A.corymbifera infections in leukemia patients in the paper. The first case was a 15-year-old female diagnosed as acute lymphoblastic leukemia. During the period of neutropenic phase after chemotherapy, the patient presented soft tissue necrosis in her maxilla. The computerized tomography (CT) scan showed high-density shadow in both his antrum maxillas. The pathological examination of necrotic tissue expressed broad aseptate hyphea and A.corymbifera was recovered from necrotic tissue. The second patient was a 46-year-old male with acute myeloid leukemia. The patient appeared fever and cough three days after chemotherapy. The CT scan showed large areas of high-density shadow in his lung. The presentation with broad hyphae from bronchoalveolar lavage and the positive culture for A. corymbifera suggested the patient suffered pulmonary zygomycosis. The patient died two weeks after the termination of chemotherapy in spite of administration with amphotericin B.

PP-07-56

Cunninghamella bertholetiae (Cb) angioinvasive pulmonary infection in a patient with acute lymphoblastic leukemia (ALL)

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We report a case of 31-yo female with febrile neutropenia after aggressive chemotherapy and allogeneic HSCT for ALL. Chest CT revealed bilateral pulmonary nodules and voriconazole was started. Serum galactomannan and bronchoalveolar lavage were negative. Several weeks after engraftment she developed dyspnea, and new chest CT showed huge pulmonary infiltrates, large pericardial effusion and a mass in the right atrium. Analysis of the pericardial fluid, including cultures and panfungal PCR, was negative. A direct microscopy of a transbronchial lung biopsy showed broad nonseptate hyphae, but cultures were negative. Antifungal therapy was changed to liposomal amphotericin B and posaconazole with significant clinical improvement and a remarkable decrease in lung infiltrates. Hematological workup showed complete remission. In the following weeks the patient gradually developed clinical signs of superior vena cava (SVC) obstruction. CT scan showed a mass penetrating the SVC and nearly occluding it. An extensive inflammatory process involving the right lung, the heart and the SVC was observed at surgery. Cultures were negative, PAS and silver stains of multiple specimens identified fungal elements consistent with mucormycosis. PCR followed by DNA sequencing confirmed the diagnosis of Cb of the Zygomycetes class. Pericardial and pleural biopsies showed relapse of the ALL. The patient died from bacterial sepsis 3 weeks later. This is the first reported case of Cb pulmonary infection in Israel. Review of the literature revealed only 17 similar cases. Since July 2007 there has been an ongoing outbreak of pulmonary aspergillosis in the hemato-oncology unit in our hospital with extensive use of voriconazole. Physicians should be aware that increased use of voriconazole may lead to higher incidence of Zygomycetes.

PP-07-57

A significantly emergent clinical entity as primary cutaneous A A significantly emergent clinical entity as primary cutaneous zygomycosis in tertiaty care health services

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Background: Zygomycosis or mucormycosis is extensively invasive fungal infection with high mortality rate. Various infections caused by this group of fungi are becoming increasingly common and survival of patients remains grim. In most of patients, diabetes mellitus may be underlying factor but in primary cutaneous zygomycosis presentation may be without it. Objectives: To increase awareness of this group of emerging infections among medical staff and to emphasize importance of their early diagnosis and treatment. Patients: The patients diagnosed with primary cutaneous zygomycosis at the tertiary care hospital between 2001 and 2008 are being reviewed. They presented with diagnosis of necrotizing fasciitis. Methods: Detailed history of each patient was taken, clinical presentation, site of involvement, underlying illness and risk factor, if any, were noted. The diagnosis was established by direct microscopic evidence of broad, aseptate or sparsely septate ribbon-like hyphae with right angle branching in KOH wet mount and histopathological examination of stained sections with H&E, PAS and GMS stainings. Fungal cultures were put up for isolation and species identification. Outcome of medical and/or surgical therapy was analysed. Results: Out of nine patients reviewed, underlying illness i.e. diabetes mellitus was present only in one. Commonest risk factor was found to be injection abscess. Apophysomyces elegans was isolated in four cases, Saksenaea vasiformis in one and Absidia corymbifera in one. The fungal culture turned out to be sterile in three cases despite direct findings being positive. Mortality rate was very high as only four patients responded well to medical and/or surgical treatment. Discussion: There is an urgent need for high index of clinical suspicion thereby taking early biopsy of affected site so that benefits of prompt diagnosis and therapy may be achieved. The key to survival among these patients is an early diagnosis, prompt antifungal therapy combined with extensive surgical debridement.

PP-07-58

Cerebral phaeohyphomycosis due to *Rhinocladiella mackenziei* (formerly *Ramichloridium mackenziei*)

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Cerebral phaeohyphomycosis caused by *Rhinocladiella mackenziei* (C.K. Campb. & Al-Hedaithy) Arzanlou & Crous, comb. Nov. (formerly *Ramichloridium mackenziei*) is extremely rare, and geographically limited to the Middle East. The fungus targets the brain exclusively with a grave prognosis. Eighteen cases have been reported in the literature from the period 1983 to 2004 with almost 100% mortality. Our patient, case nineteen, 2008, presented with a brain abscess while receiving chemotherapy for carcinoma of the breast. Diagnosis was by craniotomy and aspiration of the brain abscess. Direct microscopy showed dematiaceous fungal hyphae. Cultures grew *Rh. mackenziei* and this was confirmed by molecular analyses. Histopathological sections of brain biopsy manifested moniliform hyphae characteristic for phaeohyphomycosis. The patient failed to respond to antifungal therapy with amphotericin B and voriconazole or amphotericin B and posaconazole and finally expired in 64 days after diagnosis. In vitro antifungal susceptibility testing showed this strain to be resistant to amphotericin B while susceptible to itraconazole, voriconazole, and posaconazole. Previously published antifungal susceptibility data indicate that although strains show variable susceptibility to amphotericin B the organism is generally refractory to treatment with this agent. Similar outcomes are seen with the azole agents used alone or in combination with other drugs. Although no specific risk factors have been identified, the majority of cases have occurred in immune compromised individuals. *Rh. mackenziei* is highly virulent agent of serious cerebral phaeohyphomycosis, and should be considered in the differential diagnosis of central nervous system disease in the Middle East.

PP-07-59

Dematiaceous moulds - Emerging pathogens in the pediatric oncology population

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Background: Infections caused by dematiaceous moulds have been reported with increasing frequency in immunocompromised patients. We report the occurrence of phaeohyphomycosis in a pediatric oncology hospital over the past 46 years.
Methods: Chart review was conducted of all cases diagnosed with invasive fungal infections from 1962 to 2008 at St. Jude Children's Research Hospital, Memphis, TN. Cases with proven or probable invasive fungal infections and positive cultures for dematiaceous moulds were included in the series. Clinical data, microbiology and pathology results, antifungal prophylaxis and treatment regimens were recorded. Outcome was assessed at 12 weeks after onset.
Results: Fifteen cases of phaeohyphomycosis were identified; 11 were diagnosed between 2000 and 2008, and 4 between 1986 and 1996. Thirteen patients had underlying hematological malignancies; 2 had solid tumors; 4 had undergone hematopoietic stem cell transplantation. The predominant site of infection was skin and soft tissue (8), followed by lower respiratory tract and sinonasal infections (3 each) and bloodstream infection (1). Organisms identified included Exserohilum rostratum, Pseudallescheria boydii, Cladosporium spp., Curvularia spp., Bipolaris spp., Alternaria spp., Chaetomium spp., Wangiella dermatitidis, and Macrophomina phaseolina. There were two mixed infections, each with two species of moulds isolated. Seven patients developed infections while receiving voriconazole prophylaxis. Antifungal therapy typically consisted of either polyene or triazole monotherapy. Two patients also had surgical debridement. At week 12, twelve had complete resolution of infection. Three patients died, two with active pulmonary fungal infection
Conclusions: This series shows a marked increase in the rate of phaeohyphomycosis in a pediatric oncology population during the past decade. While the spectrum of fungal species was similar to that reported in adults, there was a greater proportion of soft tissue infections among children. Although half of the cases arose in patients receiving voriconazole prophylaxis, most cases responded well to posaconazole or amphotericin B treatment.

PP-07-60

Validation and clinical application of a molecular method for the identification of histoplasma capsulatum in human specimens

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Histoplasmosis is usually diagnosed by identification of the causative agent, Histoplasma capsulatum var. capsulatum, in clinical samples using special stains, isolation in culture or indirectly by serological assessment. Nonetheless, these conventional tests present several difficulties delaying the diagnosis, and there is a need for molecular assays to be implemented. To improve diagnosis of histoplasmosis, we evaluated 146 clinical samples from 135 patients with suspicion of histoplasmosis using a previously reported nested PCR assay (Hc100). In order to determine the specificity of this molecular test, we used samples from healthy individuals (n=20), from patients with suspicion of respiratory disease with negative culture (n=29), and from patients with other proven infections (n=60). Additionally, DNA samples obtained from cultures of related respiratory pathogens were studied. A panfungal PCR assay amplifying internal transcribed spacers ITS-1, ITS-3 and ITS-4 was also used to identify all fungal DNA. All PCR amplified products were sequenced. From the 146 samples, 67 (45.9%) were positive by culture and PCR, while 9 samples negative by culture were positive by PCR. The sequences of amplified products presented ·97% identity with H. capsulatum. The Hc100 PCR exhibited a sensitivity of 100% and specificity of 92.4% and 95.2% when compared to the negative controls and with respect to samples from other proven clinical entities, respectively; PPV was 83% and NPV was 100%. Additionally, the positive and negative likelihood rates were 25 and 0, respectively. These results suggest that the Hc100 based nested PCR assay for detection of H. capsulatum DNA is a useful test in areas where this mycosis is endemic.

PP-07-61

Development and evaluation of an assay to detect Histoplasma capsulatum antigenuria: A diagnostic test needed in resource-limited settings

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Histoplasma capsulatum infection causes significant morbidity and mortality in HIV-infected individuals, particularly those in countries without access to diagnostic tests or antiretroviral therapies. The fungus grows unchecked in persons with AIDS, resulting in progressive, disseminated histoplasmosis (PDH), which can be fatal within weeks if left untreated. The availability of a simple, rapid method to detect H. capsulatum infection would dramatically decrease time to diagnosis and treatment of PDH in resource-limited countries. We have developed an antigen-capture ELISA to detect antigenuria in infected patients. The assay uses polyclonal antibodies against H. capsulatum as both capture and detection reagents and a standard reference curve is included to quantify antigenuria and ensure reproducibility. Urine specimens were collected prospectively from patients at a Guatemalan HIV clinic (n = 101), and from healthy residents of the USA (n = 33) and Guatemala (n = 50). Additionally, we evaluated urines from patients in prior studies who had been confirmed by culture to have non-histoplasmosis fungal infections (n = 61), for a total of 245 patients tested. The H. capsulatum antigen-capture ELISA showed a sensitivity of 81% (39/48) in detecting antigenuria in patients with culture-proven PDH and an overall specificity of 95% (187/197) against the negative control urine cohorts. Longitudinal analysis of serial urine specimens from 14 PDH patients with good follow-up showed that there was a marked decrease in detectable H. capsulatum antigenuria during antifungal therapy. Use of this simple, rapid ELISA in endemic resource-limited countries may lead to reduced PDH-related morbidity and mortality. The test may also prove useful to clinicians wishing to monitor PDH patient recovery.

PP-07-62

Histoplasmosis in two French university hospitals

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During a eight-year period (2001-2008), eleven cases of histoplasmosis were diagnosed in the mycology-parasitology department of Tenon and Saint Antoine hospitals, Paris, France. Ten were Histoplasma capsulatum var capsulatum infections. Seven were disseminated forms observed in HIV patients with CD4 < 100, native from Sub-Saharan Africa (4), South America (2), and the Caribbean (1). The most frequent presentation was febrile pancytopenia. One patient died 4 months after the diagnosis, one was lost to follow-up, the others had clinical cure with a follow-up of 16- 66 months. A disseminated form was also observed in a liver transplant recipient who never left France. Two patients were French travelers, one contaminated in Central America and the other in South East Asia, with acute and subacute pulmonary forms. Infection was due to Histoplasma capsulatum var duboisii in only one case. Diagnosis was made fortuitously during abdominal intervention for acute intestinal obstruction, the patient was HIV negative, native from Mali, West Africa. Histoplasmosis is the most frequent systemic imported mycosis in Europe and our series is demonstrative of its different epidemiological aspects in France.

PP-07-63

IgG to *Histoplasma capsulatum* high MM antigens (hMMAgs) and IgG-hMMAgs immunecomplex in experimental histoplasmosis in mice

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Histoplasma capsulatum var. capsulatum, a thermally dimorphic fungus that causes histoplasmosis, releases soluble components. The present study partially characterized the high MM antigens (hMMAgs) from *H. capsulatum* cell-free antigens (CFAgs) and investigated levels of IgG to hMMAgs and IgG-hMMAgs immunecomplex (IC) in experimental histoplasmosis. The CFAgs was fractionated by chromatography (Sephadex G-75) and the fractions obtained were analyzed by dot blotting, western blotting and carbohydrates evaluation. Groups of mice were infected with $2.2x10^4$ *H. capsulatum* yeast cells and IgG to hMMAgs and IC analyzed by immunoenzymatic assays (ELISA) at 28 days post-infection. The hMM fraction (hMMAgs) presented high percentage of the carbohydrates with MM larger than 150 kDa and constituted by at least two immunogenic components. Increased levels of IgG anti- hMMAgs and IgG-hMMAgs IC were observed in infected group. In conclusion, hMMAgs present MM larger than 150 kDa is rich in carbohydrates and it is constituted by at least two immunogenic components that induce high levels of specific IgG and form circulating IgG-hMMAg IC in experimental histoplasmosis.

Poster Forum PF-06

PP-07-64

The utility of recombinant proteins of H and M antigens of *Histoplasma capsulatum* in the detection of specific antibodies in patients' sera

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Histoplasma capsulatum is a dimorphic fungus which causes human histoplasmosis. Although the fungus is distributed worldwide (particularly endemic in the Ohio-Mississippi river valleys), it has never been isolated from soil in Japan. For this reason, histoplasmosis is classified as an "imported mycosis". Kamei et al. has reported that the number of histoplasmosis patients in Japan has significantly increased in the last decade. H and M antigens are major antigens that are known to be useful for the diagnosis of histoplasmosis. We examined expression and purification of these antigens and applied their recombinant proteins to ELISA.

The genes of H antigen and M antigen were cloned into the *E. coli* vector, pQE-80L, for the expression of recombinant proteins tagged with 6xHis. Although these antigens were efficiently expressed in *E. coli*, most of the proteins formed inclusion bodies, which were dissolved by the solution containing urea. Then 6xHis-tagged proteins were purified by using the Ni-Sepharose. CBB staining showed high purity of the recombinant proteins.

Then, we applied the purified proteins to ELISA. The proteins coated each well, and sera from 22 healthy volunteers and 10 patients of histoplasmosis in Japan were examined. On the plates coated with recombinant M antigen, the absorbance at 450nm (antigen coating well - non-coating well) in the histoplasmosis patients group was significantly higher than that of healthy volunteers ($p<0.01$, Mann-Whitney U test). Furthermore, in the H antigen coated plates, the absorbance of patients' group was significantly higher than that of healthy volunteers ($p<0.01$, Mann-Whitney U test). These data suggest that these purified proteins may be useful for the detection of antibodies reacting with each antigen in the sera of patients with histoplasmosis.

PP-07-65

Molecular characterization of two isolates of Histoplasma capsulatum from an outbreak in treasure hunters

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In Mexico, primary pulmonary histoplasmosis is considered the most relevant clinical form of this disease due to its severity and high mortality rate. H. capsulatum EH-53, reference strain, was isolated from fatal disseminated histoplasmosis from immunocompetent patient (Hidalgo, Mexico), in 1977. Recently, this strain was characterized by phylogenetic relationships between representatives of the three varieties of H. capsulatum from six continents. EH-53 was integrated to the LAm A clade for Latin America.

In this study we report a recent outbreak of histoplasmosis in treasure hunters and the molecular characterization of two isolates from these patients. Six immunocompetent patients presenting severe respiratory symptoms suggestive of histoplasmosis were admitted to Instituto Nacional de Enfermedades Respiratorias, Mexico City, in August, 2007. They were looked for a treasure on Tamarindos Ciudad Cardel Veracruz (VZ), Mexico, endemic zone of histoplasmosis. In all cases, a chest CAT scan revealed disseminated micronodular images throughout the lung parenchyma, as well as bilateral retrocaval, prevascular, subcarinal and hilar adenopathies. Four of the six patients developed disseminated histoplasmosis. Their abdominal CAT scans showed hepatomegaly and splenomegaly. Two patients only had a lung infection. The diagnosis was confirmed using Grocott and PAS staining techniques, immunological testing and isolation of the fungus in two patients. Isolates were identified by PCR using a probe designed from antigen M.

Two isolates from VZ and reference strains: EH-397, EH-398, EH-406, EH-408, EH-437, EH-449 and EH-53 were analyzed by RAPD-PCR using the 1253 oligonucleotide by itself and oligonucleotides 1281 and 1283 mixed. The same DNA-pattern was detected in both isolates and they proved 100 % relatedness with the EH-53 strain. Therefore, EH-53 is the main prevalent strain in the zone since 1977. Based on molecular profile, these isolates could be considered into to the LAm A clade for Latin America too.

PP-07-66

Characterization of potential virulence factors of *Penicillium marneffei* under oxidative stress condition inside murine macrophage

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Penicillium marneffei is an intracellular dimorphic fungus that can cause a fatal disseminated disease in HIV-infected patients. As an intracellular pathogen, survival within phagocytic cells as a yeast cell is the primary key to a successful invasion by *P. marneffei*. However, the mechanism of survival, given the oxidative stresses within the macrophage, remains unknown. The putative virulence genes involved in host infection have been isolated and characterized in *P. marneffei*. Genes of interest include those involved in stress response, such as Cu, Zn superoxide dismutase (*sodA*), catalase-peroxidase (*cpeA*), and heat shock protein 70 (*hsp70*). In addition, the genes responsible in cell adhesion and adaptation, such as glutaraldehyde-3-phosphate dehydrogenase (*gpdA*), and isocitrate lyase (*acuD*), have also been points of focus. In this study, semi-quantitative RT-PCR and Northern blot analysis were used to monitor the expression of selected *P. marneffei* genes in different growth phases and during macrophage infection. For *sodA*, differential expression was demonstrated in vitro wherein the transcript was more abundant in yeast cells than in either conidia or the mold phase. Additionally, during macrophage infection, *sodA* expression was upregulated. Transcript levels of *cpeA* also accumulated in yeast and conidial cells, but not in the mycelial phase. Consistent with this observation, the transcriptional *cpeA* response of conidia upon internalization by murine macrophages was upregulated after 2, 4 and 8 h of incubation. However, the differences in expression of *acuD*, *hsp70* and *gpdA* were not statistically significant by RT-PCR analysis. Nonetheless, the Northern blot analysis revealed that *acuD* and *hsp70* were induced in conidia after prolonged co-incubation with macrophages. In contrast, the expression of *P. marneffei gpdA* was repressed during macrophage infection, presumably due to nutritional deprivation and the glucose-poor intracellular environment. Collectively, these results provide insights into how pertinent genes may act as the virulence factors of *P. marneffei* infection.

PP-07-67

An endemic foci of Penicilliosis marneffei in India

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In India, penicilliosis marneffei was first noticed among the HIV infected patients of Manipur in late 1998. Manipur is a small state in the north eastern corner of India bordering Myanmar. Geographically, it is in close proximity to Thailand and southern China, an endemic area of penicilliosis marneffei.These cases had never visited the endemic area.

In the last 10 years, more than 500 cases of penicilliosis marneffei have been detected among HIV infected persons of Manipur along with other fungal infections such as candidiasis and cryptococcosis. The causative fungus have been isolated from skin lesions, lymph nodes,blood, bone marrow,urine and sputum samples. At the time of diagnosis CD4 count is almost always below 100 cells /cu mm. Being a common opportunistic infection, the patients are diagnosed on direct smear examination of the samples and not always processed for special stain and fungal culture.But the difficulty of differentiation of the yeast form of *Penicillium* marneffei from that of Histoplasma is well known.With culture confirmed cases of histoplasma being reported from the same state of Manipur, confirmation of presumptively diagnosed penicilliosis marneffei by fungal culture is an important approach. An interesting feature is the isolation of the fungus with different culture morphology from different sites of the same patient.Few cases reported from other parts of India are inhabitants of Manipur. The disease seems to be regionally confined to north east India where bamboo groves sheltering bamboo rats (?reservoir) are in plenty. The fungus have been recovered from the Cannomys badius locally known as Chabbi. So far, it is not yet isolated from environmental samplings such as soiland air. It is presumed that the infection is confined to NE India because of the geographical and climatic conditon prevailed there.

PP-07-68

Interaction of *Penicillium marneffei* with soil amoeba as a model of fungal pathogenesis

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*Penicillium marneffei*is an important dimorphic pathogenic fungus which causes human infection in Southeast Asia particularly in the northern of Thailand. Since, an interaction of *P. marneffei* with a mammalian host is not a requisite for fungal survival, the origin and maintenance of virulence is still enigmatic. Recently, *Cryptococcus neoformans* was shown to interaction with macrophages, slime molds, and soil amoeba in a similar manner, suggesting that fungal pathogens strategies may arise from environmental interactions with phagocytic microorganisms. In this study, we examined the interaction of *P. marneffei* with the soil amoeba *Acanthamoeba castellanii*. In both conidia and yeast cells of *P. marneffei* were ingested by amoeba and macrophages which resulted in amoeba death and fungal growth. This result is consistent with the view that soil amoeba may contribute to the selection and maintenance of certain traits in *P. marneffei* that confers on this fungus the capacity for virulence in mammals.

PP-07-69

Pulmonary involvement by Pseudallescheria/Scedosporium: An overview

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Some species of the Pseudallescheria / Scedosporium complex may cause serious and difficult to treat, localized or disseminated infections, particularly in lungs and/or brain, or otherwise may cause health problems, such as allergic bronchopulmonary reactions or transitient or chronic airway colonization in patients with mucosal dysfunction or bronchial disorders. We reviewed all the available reports on Pseudallescheria / Scedosporium pulmonary involvement, focusing on different aspects including epidemiology, underlying diseases and conditions, type of infections, classification of clinical manifestations, diagnosis, treatment and outcome. Conidia or hyphae of these fungi may often enter respiratory tract via inhalation or aspiration of polluted water, after a near-drowning experience. Clinical *features* were variable and related to patient immune status. In otherwise healthy patients the infection was usually characterized by non invasive types of involvement while invasive pulmonary and/or disseminated infections were seen in immunocompromised patients. Both non-invsive and invasive type infections may be radiologically indistinguishable from pulmonary aspergillosis. The mortality rate was closely related to the infection type and was rather high because of the lack of an effective therapy.

PP-07-70

Scedosporium aurantiacum virulence studies using a murine model

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Scedosporium species are clinically important emerging pathogens with Scedosporium prolificans noted to be the most virulent species. The recently described species Scedosporium aurantiacum comprises a substantial proportion of Australian clinical isolates and causes a wide range of serious human infections. Further, environmental surveys revealed a high prevalence of S. aurantiacum in urban areas around Sydney. PCR-fingerprinting using the microsatellite specific primer M13, and MLST analysis using 4 genes (EF1alpha, SOD2, CAL, BT2) have identified different genotypes among the isolates. Based on these findings, we conducted virulence studies using a murine model on a range S. aurantiacum strains and compared the results using S. prolificans. Eight S. aurantiacum and two S. prolificans strains with an inoculum size of 1x10^6 conidia/ml were inoculated intravenously into 7-week old immunocompetent BALB/c mice. S. aurantiacum was noted to be as virulent as S. prolificans, causing death in 60%-100% of mice. There were significant differences in virulence between the different genotypes of S. aurantiacum.

PP-07-71

Scedosporium aurantiacum: An emerging pathogen in Australia and New Zealand?

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Scedosporium aurantiacum is a new Scedosporium species. Reports of S. aurantiacum infection are as yet, uncommon. To understand the potential of this species to cause disease and to identify predisposing factors for its isolation, we analysed 52 episodes of S. aurantiacum isolations in 42 patients in Australian and New Zealand hospitals from 2001-08. Speciation of S. aurantiacum was performed by DNA sequencing.

The mean age of patients was 44.4 y (range 5-93); 55% patients were female. The major predisposing factors for isolation were chronic suppurative/obstructive lung disease (58% episodes), diabetes (17%), corticosteroids (12%) and trauma (6%). Eleven (21%) episodes occurred in non-transplanted cystic fibrosis patients and 7 (14%) in lung transplant recipients. The colonization (25 patients): invasive disease (17 patients) ratio was 1.5: 1. All cystic fibrosis patients were colonized. Fifty-four percent of isolates were recovered from sputum/BAL. Among 17 patients with invasive disease, the main sites of infection were lung (n=4), eye (n=4). Three patients each had sinus, inner ear and skin/subcutaneous abscess involvement, 2 had osteomyelitis and one had S. aurantiacum recovered from resected cardiac tissue. MIC90 results were: amphotericn, 16 mg/L, itraconazole, 1 mg/L, voriconazole, 0.25 mg/L, posaconazole, 0.5 mg/L. Thirteen patients (invasive disease) received antifungal drugs (all with voriconazole), 9 underwent surgery - 3 had surgery alone. All patients were alive at 90 days following isolation of S. aurantiacum. S. aurantiacum caused a range of serious infections although the outcome was apparently benign. Clinical variables associated with its isolation include chronic lung disease and cystic fibrosis.

PP-07-72

Non-pigmented conidia of *Scedosporium prolificans* in a histology section of disseminated infection

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Scedosporium prolificans is an emerging pathogen of opportunistic infection. Since it is still a rare infection and its morphology in tissue simulates that of *Aspergillus*, it is challenging for pathologists to diagnose its infection in histology sections. We report here an autopsied case of disseminated infection of *Scedosporium prolificans* in a patient of acute myeloid leukemia.

The diagnosis of acute myeloid leukemia was made in a 58 year old female after sore throat and swelling of gingiva of one month duration. Peripheral white blood cell count was 39,700 /micro liter with 96% leukemic cells. Anti-leukemic chemotherapy of one week duration was followed by pneumonia. She died on hospital day 19 due to multiorgan failure. Blood cultures for fungi that were taken within the week prior to death yielded *Scedosporium prolificans*.

Specimens for fungal culture were inoculated onto Sabouraud's dextrose agar at room temperature. Within one week of incubation, fungal growth was observed as cottony white colonies with abundant aerial mycelium. Microscopically, the hyphae were hyaline and septate. Ovoid conidia were borne on short flask-shaped conidiogenous cells. Conidia were olive to brown, one celled, and smooth with a slightly narrowed truncated base. On the basis of these morphological characteristics, the isolate was identified as *Scedosporium prolificans* and was confirmed by DNA sequence analysis.

At autopsy, almost all of the internal organs demonstrated hemorrhagic necrosis. Thin septate hyaline hyphae were seen in the area of necrosis and its vascular invasion was marked. Lemon-shaped hyaline conidia were found terminally or laterally on hyphae. These fungal elements were confirmed to be that of *Scedosporium* species by *in situ* hybridization.

When lemon-shaped conidia are histologically found along hyaline hyphae, *Scedosporium* with annelloconidia are suspected. Although these conidia are usually brown in HE sections, they were non-pigmented in the present case.

PP-07-73

Molecular typing of recurrent *Scedosporium apiospermum* isolates from a patient with cystic fibrosis

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Cystic fibrosis is an autosomal recessive disorder characterized by the dysfunction of the exocrine glands, leading to a production of abnormally viscous bronchial secretions. The abundant mucus may trap bacteria and fungi, allowing transient or chronic lung colonization. We report here a case of persistent colonization with *Scedosporium apiospermum* in a patient with cystic fibrosis (CF). In order to establish the persistence of a specific genotype or a possible reinfection with a new one, we performed a molecular typing of six consecutive isolates. Moreover, we studied in vitro susceptibility of isolated strains by means of both E-test and CLSI method. Fungal isolates from patient were typed by random amplification of polymorphic DNA (RAPD) using primers UBC701, UBC703, and GC70. A unique genotype was isolated over a period of 12 months, despite three months of antifungal treatment with voriconazole (VRC). As already observed by Defontaine et al, once a genotype of S. apiospermum establish a colonization it seems not to be replaced by other. Antifungal susceptibility testing with both commercial and CLSI methods showed low MIC values only for triazoles, confirming the resistance of *S. apiospermum* to antifungal drugs (particularly amphotericin B, the classical treatment for fungal infections), making it very difficult to cure.

PP-07-74

Invasive fusariosis among immunosuppressed patients: A matched case-control study in a tertiary care university hospital

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Fusariosis is an emerging hyalohyphomycosis, associated to high mortality, involving severely immunosuppressed patients. To define risk factors and associated-mortality of invasive fusariosis, a matched case-control study of proven cases of invasive fusariosis was carried out accounting patients cared for during 24 months at the Unicamp Hospital. Data collected included: demographics, underlying disease, previous use of antifungal, antibacterial, clinical presentation, specimens, therapy and outcome. Matching criteria were age, hospital ward and period of hospitalization.
Sixteen patients (18 episodes) and 31 controls were enrolled. Twelve patients (75%) presented disseminated disease (13 episodes); 7 had positive blood cultures and in 5 patients Fusarium was isolated from skin lesions. Sinusitis was present in 3 patients (18.8%) and vitritis in one. Underlying diseases: acute leukemia (9); chronic leukemia (2); lymphoma (4) and solid tumor (1). Hematological stem cell transplant (HSCT) was performed in 10 patients (7 allograft; 3 autologous). All patients received antifungal prophylaxis (15 fluconazole; 1 voriconazole). First-line therapy in all patients was amphotericin-B and in 12 (75%) patients this therapy was switched to voriconazole after a median of 8 days. Fusariosis-associated mortality was 81.3%, significantly higher than the overall mortality in the control group (25.8%; $p<0.001$). In patients with fusariosis, death was associated with disseminated disease, but not with any underlying condition, HSCT or therapy. Univariate analysis showed the following risk factors for invasive fusariosis: ciprofloxacin ($p=0.001$) and fluconazol ($p=0.002$) prophylaxis and presence of central lines ($p<0.001$). Neutrophils counts <1000, <500 and <100, HSCT, corticosteroids and underlying diseases were not risk for fusariosis. Stepwise logistic regression showed that prophylaxis with ciprofloxacin was risk factor for invasive fusariosis ($p=0.029$). Fusariosis-associated mortality rate reinforces the need of feasible interventions to decrease the incidence among high-risk patients. Long-term ciprofloxacin prophylaxis in neutropenic patients should be considered as a risk for invasive fusarios.

PP-07-75

First case report primary actinomycosis of the breast from Tamilnadu: Diagnosis by fine-needle aspiration cytology

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Actinomycosis is a chronic, suppurative, granulomatous disease characterized by the presence of multiple draining sinusis discharging sulfur granules • The causative agents are anaerobic or microaerophilic Actinomyces species. Actinomyces israelii is the predominant pathogen in human infection. Primary actinomycosis of the breast is a rare disease and only few cases have been reported till date. We report an uncommon case of a 49-year-old woman with primary actinomycosis of the breast. She presented with a hard mass in her left breast, clinically diagnosed as fibroadenoma of six months duration. Examination revealed a hard nodule 5 x 5 cm, fairly movable, with smooth skin. Diagnosis was established by fine- needle aspiration cytology (FNAC). Fifteen ml of serosanguinous fluid was aspirated and cytological examination revealed the presence of the characteristic Actinomyces colonies with Splendore • Hoeppli phenomenon. Direct examination in 10 % KOH, gram and Kinyoun acid-fast stains revealed short, branching, gram positive, nonacid- fast filaments of bacterial width and coccobacillary forms. Cultural method of study was undertaken and white, spidery colonies were grown on brain heart infusion agar anaerobically in 4 days and smear examination revealed gram positive, nonacid -fast, short, branching filaments of bacterial width and coccobacillary forms. Since facilities were not available for speciation, the isolate was identified as Actinomyces species. The case is being presented to increase the awareness of the clinicians and pathologists that primary actinomycosis of the breast could present clinically simulating malignancy and should be included in the differential diagnosis of mass in the breast.

PP-07-76

Pneumocystis jirovecii diagnosis by polymerase chain reaction technique

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Pneumocystis jirovecii pneumonia (PCP) is a severe respiratory infection, considered as one of the most common complications in immunocompromised patients. There are few researches on PCP in Venezuela, all of them carried out using direct immunofluorescence technique (DIF). Currently, it is necessary to have another detection method that increases the sensibility and specificity of PCP diagnosis, additionally to the use of diagnostic conventional methods, in order to provide an early diagnosis of this disease. The aim of this work was to implement the polymerase chain reaction technique (PCR) for the diagnosis of Pneumocystis jirovecii. Sixty two (62) clinical samples (spontaneous and induced sputa, bronchioalveolar lavage, and tracheal aspirates) collected from patients with AIDS, cancer and non-AIDS-non cancer low respiratory tract infections, were processed by DIF and nested PCR, using external (pAZ102-E and pAZ102-H) and internal (pAZ102-X and pAZ102-H) primers, targeting to the mitochondrial Large Subunit RNA region (mtLSUrRNA) of P. jirovecii genome, proposed by Wakefield et al. The PCR results were compared with DIF results (as a reference technique), using X2 test. Values of sensibility (S), specificity (E), positive and negative predictive values (PPV and NPV), positive and negative verisimilitude reasons (PVR and NVR), errors and agreement for the PCR technique were also calculated. P. jirovecii was detected by DIF in 14 patients and by PCR in 24 patients. PCR had values of S=100%, E=79.2%, PPV=58.3%, NPV=100%, PVR=4.8, NVR=0.3, and an agreement of 84%. PCR is a high diagnostic value technique that successfully predicts the absence of PCP with a negative result. A positive result does not discriminate among infection and colonization; therefore, it should be interpreted with caution taking into account the signs and symptoms of the patient.

PP-07-77

Pneumocystosis in Venezuelan patients: Epidemiology and diagnosis (2001-2008)

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The objective of this work was to investigate the epidemiology of pneumocystosis in Venezuelan patients using a retrospective study during an eight year period. Three hundred and seven (307) clinical samples collected from patients with AIDS (40.1%), cancer (20.2%), and non-AIDS-non-cancer (39.7%) low respiratory tract infections patients were processed by direct immunofluorescence technique. Pneumocystosis was diagnosed in 81 patients with a general frequency of 26.4%, which varied according to the patient's group: 35.8% in AIDS patients, 30.6% in cancer patients, and 14.8% in non-AIDS-non-cancer low respiratory tract infection patients. This study demonstrated the existence of differences in pneumocystosis frequency related to the patient's underlying disease, and that the illness is an important health problem in immunocompromised patients in Venezuela. Pneumocystosis must be suspected in non-immunocompromised patients with signs and symptoms of low respiratory tract infection, and the study of this illness must include patients with cancer and chronic obstructive pulmonary disease. Direct immunofluorescence is a usefull technique for pneumocystosis diagnosis; however, it requires an optimal sample and skilled personnel in the laboratory.

PP-07-78

Yeasts isolated from HIV-infected patients in Jakarta, Indonesia: A first report

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Indonesia is one of the countries with a fast growing incidence of HIV-infection. Opportunistic fungal infections are good indicators of AIDS as the final stage of HIV-infection, and these are usually caused by *Candida* and *Cryptococcus* species. In this study we report on various *Candida* and *Cryptococcus* species isolated from HIV-infected patients suffering from oropharyngeal candidiasis (OPC) and cryptococcal meningitis. The yeast isolates were analysed by conventional and molecular methods, namely amplified fragment length polymorphism (AFLP) and sequence analysis of the D1/D2 domains of the 26S rDNA and the ITS 1+2 regions. Out of 60 patients, *Candida* was isolated from 44 (73,3%) patients and 38 (86,4%) were suffering from OPC. The species identified were 37 *C. albicans*, three *C. dubliniensis*, eight *C. tropicalis*, nine *C. glabrata*, one *C. ethanolica*, one *C. nivariensis*, and one strain of *Isatschenkia tropicalis*. From patients with meningitis we obtained 54 (20,38%) isolates of *Cryptococcus neoformans* serotype A from 265 spinal fluids. It turns out that in HIV infected patients, various *Candida* species were isolated. It includes new species *C. nivariensis* and *C. ethanolica* which for the first time isolated from human. While patients with crytpococal meningitis were proved to be infected by *Cr. neoformans* serotype A.

PP-08-1

Urogenital and digestive infection due to *Candida albicans* in a cat with diabetes mellitus

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Candidosis is sporadically described in cats despite *Candida albicans* is a common commensal yeast of the digestive tract of mammals and birds. The present case concerns a 6-year-old-castrated shorthair cat with polyuria/polydipsia, anorexia, weight loss, weakness and dehydration. The clinical signs associated with elevated blood glucose and fructosamine levels allowed a diagnosis of diabetes mellitus (type 2). Insulin therapy associated with a low-carbohydrate diet were undertaken. Clinical improvement with recovery of normal water intake and previous weight was obtained. One year later, hospitalization was required because of pronounced dysuria, prostration, anorexia and fever. Clinical examination revealed dehydration, a marked vesical globe at palpation and the presence of a creamy material in the prepuce. Ultrasonography showed a thick-walled urine bladder containing abundant hyperechogenic sediment. Urine was cloudy with abundant sediment revealing numerous budding yeasts, pseudohyphae and hyaline septate hyphae at direct microscopic examination in lactophenol blue and after Giemsa staining. Microscopic examination of swabbed samples from prepuce and buccal cavity also showed presence of budding yeasts in both and also pseudohyphae in the mouth. Numerous fungal yeast colonies were obtained in pure culture from urine, prepuce, buccal swab and faeces. *Candida albicans* was identified after chlamydosporulation on potato-carrot-bile agar, germination test in serum and assimilation of carbohydrate compounds (API 32C). After emptying of urine bladder, rehydration and cefalexine therapy, the cat was treated during 3 months with itraconazole (5 mg.kg. day orally) which was then stopped for economical reasons. Improvement of health condition was patent after 12 days of antifungal therapy but *Candida* yeasts were still observed in urine at each of the numerous subsequent controls during the following months. The cat died three months later possibly from diabetic complications and/or hepatic disease. Necropsy could not be performed.

Poster Forum PF-10

PP-08-2

First autochthonous cryptococcosis by Cryptococcus gattii in a Spanish ferret (Mustela putorius furo)

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Human and animal cryptococcosis is mainly caused by Cryptococcus neoformans and C. gattii. The first one is prevalent in immunosuppresed individuals. Infections by C. gattii occur in immunocompetent patients in tropical and sub-tropical regions whereas C. neoformans can be found worldwide. However, the distribution pattern of C. gattii changed after an outbreak in Vancouver Island (Canada). In the last years, several publications showed that C. gattii is becoming more prevalent in Europe.
In Spain, C. gattii infection was first reported in 1998 when it was isolated in apparently immunocompetent goats, in Extremadura. In 2003 the first autochthonous human infection by C. gattii was detected in Alicante. Later, in 2004 and 2008, two new human cases were reported. One of them could be considered autochthonous as well. The other was a young African man travelling through Spain.
Case report:
A 17 month-old male neutered ferret presented with acute bilateral blindness and maxilar lymphadenopathy. Cytologic evaluation of the lymph node revealed pyogranulomatous lymphadenitis with Cryptococcus yeasts.
Two weeks later, prescapular lymph nodes were enlarged. Latex test showed a serum cryptococcal antigen titre of 1/32,768 and CSF qualitative analysis was also positive. A biopsy taken from prescapular lymph node confirmed the cytologic findings. Treatment with fluconazole (10 mg/kg/24 h/po) was initiated. C. gattii serotype B was isolated from the lymph node and the isolate was sensitive to fluconazole (8 mg/L).
In spite of a satifactory outcome and regression of the lymph nodes, blindness persists.
This case constitutes the first documented case of cryptococcosis in a ferret in Spain and the first isolate of C. gattii in Catalonia, although the disease has been previously reported in this species in other parts of the world.

PP-08-3

Moulds in the upper respiratory tract in dogs suffering from chronic rhinosinusitis - A pilot study

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Allergies and chronic rhinosinusitis (CRS) are one of the most prevalent chronic diseases affecting humans in the Western World. This is also an increasing problem among dogs, which basically live in the same environment as their owners. Several studies have been performed investigating the biodiversity of fungi in the nasal mucus of humans. However, practically no such studies have been performed in dogs. The aim of the present study was to increase the knowledge of the occurrence of fungi in nasal mucosa of dogs, and to compare the findings with similar studies in humans.

A total of 37 dogs with clinical features suggestive of chronic rhinosinusitis (chronic unilateral or bilateral seropurulent nasal discharge) were evaluated. Quantitative and qualitative mycological analyses of nasal lavage, collected during rhinoscopic examinations, and of swabs from nasal mucosa, were performed.

The study group consisted of dogs representing 19 different breeds with the dachshund as the most prevalent breed by means of seven affected individuals. Except for one brachyocephalic breed (lhasa apso) all were representing mesocephalic or dolichocephalic breeds. The average age was eight years and there were 19 males, 16 females and two of unknown gender.

29 (78 %) of the dogs were fungus positive. 24 different species of 15 different genera were identified, with a maximum of seven different species per dog. The most prevalent isolates belonged to the genera *Aspergillus*, with 20 (54 %) positive, hereby 13 *Aspergillus fumigatus*, 5 *Aspergillus niger* and 2 *Aspergillus flavus*. *Penicillium* was the second most prevalent genus with 12 (32 %) positive. Other genera found; *Alternaria, Cladosporium, Aureobasidium, Fusarium, Acremonium, Epicoccum, Ulocladium, Trichocladium, Geotrichum, Phoma, Botryotinia, Pilidium, Fomes*.

This pilot study indicates that the distribution of fungal genera in nasal mucus of dogs highly correspond to the distribution of fungal genera in nasal mucus of humans.

Poster Forum PF-10

PP-08-4

Radiographic evaluation of aspergillosis in 10 African gray parrot cases

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Aspergillosis is the most frequently occurring fungal infection in birds. A total of 10 clinical cases of aspergillosis in African grey parrot with symptoms of respiratory disease, which were referred to the small animal hospital of University of Tehran, are presented. The clinical signs and Ventro-dorsal and latero-lateral radiographs were taken from all birds; Hyperinflation of air sacs and visible nodular densities in the lungs and air sacs were detected in all birds. Loss of definition of the air sacs lining was occurred in 4 cases. Asymmetry of the air sacs as a result of air sac collapse, hyperinflation, or filling with necrotic material was seen in 7 cases. Mycological examinations via native microscopy and cultivation on Sabouraud dextrose agar were performed. *Aspergillus fumigatus* strain was isolated from the lungs and the air sacs of the birds.

PP-08-5

Aspergillus fumigatus metabolic drift mutants. Isolation, morphologic characterization and significance to vaccine development

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As a result of nearly unknown mutations occurring within specific metabolic compartments of fungi a decelerated rate of growth develops. Depending on the distinct clone involved, this leads to gradually diminishing colony sizes with correspondingly prolonged generation times and concomitantly correlative levels of attenuation. These so-called metabolic drift (MD) mutants arise quite strikingly in all microbe populations as a ubiquitous manifestation of an evolutionary principle whereby virulent pathogens adapt to highly susceptible hosts.

In addition to this and owing to the general validity of biological phenomena for bacteria and fungi alike, an indirect enrichment affecting such mutants takes place in germ suspensions which are in a gradual process of dying off (Data from Linde et al., manuscript in preparation, 2009). These increased environmental tolerance (Iet)-mutants with diminished metabolism in general express no resistance pattern. From a single *Aspergillus fumigatus* wild strain, by stepwise isolation we were able to produce one-, two- and three-marker-mutants, or, as it might be, the corresponding prototype vaccine strains. With the help of a careful selection of marker-specific colony sizes it is possible to establish the attenuation level appropriate to the susceptibility of the particular host-species involved.

These MD mutants showed marked changes of morphology, e. g. colony size decreased, color and staining differed from that of wild type. Microscopically, single MD mutants failed to produce sporulation. Number of conidia decreased when compared to the wild strain. At present, it is not possible to make conclusive statements regarding the stability of these clones. Most three-marker-clones of *Aspergillus fumigatus* showed stability over approximately 15 passages. Thus, very small three-marker-clones with a size below 10 % of the wild type are forming revertants during their passages. These revertants show good stability of their size. The MD mutants were further characterized using MALDI-TOF mass spectrometry.

PP-08-6

A canine case of histoplasmosis duboisii in Japan

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Histoplasmosis is a highly pathogenic mycosis caused by a thermally dependent dimorphic fungus; *Histoplasma capsulatum* (HC). Molecular epidemiological study based on the internal transcribed spacer regions of ribosomal RNA gene sequences (ITS) suggested that Japanese autochthonous histoplasmosis might be caused by HC. var. *farciminosum* (*Equidae* specific, pseudofarcy, HC-F), as a heteroecism of pseudofarcy in humans and dogs. However, some reports suggested that Japan might be one of endemic areas of HC var. *duboisii* (HC-D) based on the size of parasitic yeast form cells detected in the first autochthonous human case.

The present study describes a possibility that Japan is one of endemic area of histoplasmosis duboisii based on a canine autochthonous case.

The case was found in the 13-year-old spayed female Labrador retriever living in Chiba City, Japan. The dog was died of lymphoma confirmed by the presence of atypical lymphocytes or carcinomatous epithelial cells in the smear of nasal discharge and mammary exudates, and the postmortem examination. The chest X-ray image showed ground-glass opacity in all lobes three days before death. The dog was started on 0.5 mg/kg of amphotericin B intravenously on the fourth day of hospitalization, but died on the fifth day. The histopathological specimens stained by periodic acid Schiff's reaction and Gomori's methenamine silver techniques detected intracellular yeast cells from 1 to 6 μm in diameter in the lung. Histoplasmosis might infect as one of opportunistic infections confirmed by a partial ITS sequence located at a cluster consisted of HC-D predominantly derived from a paraffin-embedded lung tissue.

The present case suggested that most of the autochthonous cases of human and canine histoplasmosis in Japan might be caused by HC-F and a few of them might be caused by HC-D.

PP-08-7

A case of systemic infection of *Colletotrichum gloeosporioides* in a cat with feline immunodeficiency virus infection

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Colletotrichum gloeosporioides is a causative agent of anthracnose. Several human cases of corneal and deep skin mycoses have been reported, but feline cases have not get been known. Our case involved a 10-year-old spayed Japanese domestic cat, in which the following features were observed: lethargy, anorexia since 1 month, an ulcerative granuloma at the left foreleg end and swelling of the nose bridge. A 20 × 30 mm mass was detected by palpation, X-ray and ultrasound examination. The leukocyte count increased, while the platelet count decreased; further more, blood urea nitrogen, inorganic phosphorus, aspartate aminotransferase and alanine aminotransferase were elevated. The cat was found positive for feline immunodeficiency virus (FIV), but negative for feline leukaemia virus. The smear sample obtained from the abdominal mass by the fine-needle aspiration method consisted many lymphocytes and mycelia. Similar mycelia were also detected in the ulcer and nose bridge smears. Uniform fungal colonies sprouted from the biopsy and smear samples. The colonies had a white cottony surface with a dark green reverse side with appressoria and cylindrical conidia. The sequences of the genes encoding the D1/D2 domain of the large subunit ribosomal RNA were identical to AJ301909, and the isolates were identified as *C. gloeosporioides*. A partial fungal sequence in the nose bridge smear was also identical to AJ301909. The isolates were sensitive to AMPH-B (0.5 μg/ml), MCZ (0.5 μg/ml) and ITCZ (0.5 μg/ml) and resistant to 5-FC, FLCZ and MCFG. ITCZ was orally administered for 9 days at a dosage of 20 mg/day, and the symptoms improved. However, euthanasia was applied on the ninth day based on the owner's requwst because of the deterioration of the general condition. The fungal infection occurred as an opportunistic infection following immunodeficiency due to FIV. The present study suggests that patients with immunosuppresed diseases should be cautious of environmental pathogens.

PP-08-8

Occurrence of *Trichophyton erinacei* in pet hedgehogs in Spain: One year study

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Hedgehogs have become increasingly popular as exotic household pets. The two most familiar hedgehog species are the African pygmy hedgehog, *Atelerix albiventris* and the European hedgehog, *Erinaceus europaeus*.
Dermatophytosis is the most common mycosis in these exotic pets, being *Trichophyton erinacei* the dermatophyte usually isolated from the quills and underbelly of hedgehogs. In many cases, hedgehogs can be asymptomatic carriers of this fungus, and herein lays their potential for zoonotic transmission.
This study was conducted in order to know the occurrence of *T. erinacei* in pet hedgehogs in Spain. In 2008, 27 pet hedgehogs (23 African pygmy hedgehogs, 2 European hedgehogs and 2 Egyptian (long-eared) hedgehogs) were studied. Samples of quills and skin scrapings were submitted by practitioners from different Spanish Veterinary Clinics of Spain to our laboratory. They were inoculated on Mycosel agar plates and incubated at 25 C. White colonies were isolated from the quills of 6 African pygmy hedgehogs. Five of them showed scaly skin and spine loss, but one positive animal was asymptomatic. The six fungal isolates gave a positive reaction in the urease test before seven days of incubation at 25 C and were morphologically identified as *T. erinacei*. Molecular study of the isolates is in progress.

PP-08-9

An overview of ringworm infections in pets and domestic animals in Croatia within a six-year period

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Dermatophytes comprise primary zoophilic, geophilic, and sometimes anthrophilic species of keratinophilic fungi that can cause ringworm infections in animals. In this retrospective study, data records for a six-year time period were reviewed (from January 2002 to December 2007). During that time, 1662 pets (dogs, cats, and rabbits), and 116 domestic animals (ruminants, horses and swine) suffering from different cutaneous lesions suspect to ringworm infection, were admitted and examined at the Department of Microbiology and Infectious Diseases with Clinic, at the Faculty of Veterinary Medicine, Zagreb, Croatia. Hair, scale and crust samples were collected using different techniques: tooth brushes, scotch tapes, or scrapings. In order to detect the presence of fungal arthroconidia, hair samples were directly examined under the microscope in lactophenol or 10% potassium hydroxide. In addition, samples were processed for culturing on Sabouraud dextrose actidione agar (BioRad) and incubated at 27 °C within 3 weeks. Isolated dermatophytes were identified microscopically in lactophenol blue or red, and when necessary subcultured on potato dextrose agar or polished rice grains to stimulate the growth of conidia. From 1778 cultured samples, dermatophytes were recovered from 458 samples (25.76%), and arthroconidia were found in 102 positive samples (22.27%). Overall *Microsporum canis* was most common identified dermatophyte (76.20%), while *Trichophyton mentagrophytes*, *T. verrucosum* and *M. gypseum* were isolated less frequently (12.45, 7.42 and 2.62% respectively). *M. persicolor* was found only in one case (0.22%). In five cases (1.09%), animals were infected with two species (*M. canis* and *T. mentagrophytes*). As expected, *T. verrucosum* was the main cause of dermatophytoses in all positive cattle. *M. canis* prevailed in dogs and cats (78.40 and 94.02%), while manifest dermatophytoses in rabbits were caused by *T. mentagrophytes* (57.89%) and *M. canis* (42.11%).

PP-08-10

Biodiversity of dermatophytes isolated from animals in Italy

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Compared with anthropophilic dermatophytes, zoophilic species have been studied less extensively. Species identification in many of these studies was based on morphology. Molecular approaches covering the biodiversity of dermatophytes colonizing or infecting animals have the potential to reveal novel species and enable to evaluate the intraspecific variation of known species. In view of this, the internal transcribed spacer region (ITS) of dermatophytes strains isolated from samples submitted to the Mycology Laboratory of the Veterinary Faculty of Turin (Italy) was sequenced. Species identification was done morphologically and by entering the obtained ITS barcodes into a Dermatophytes ITS DNA barcode database (http://www.cbs.knaw.nl/dermatophytes/defaultpage.aspx).
Among 26 strains analyzed, 13 belonged to Microsporum canis (4 dogs, 7 cats, 1 dwarf rabbit, 1 chamois), 9 to Trichophyton interdigitale (3 dogs, 2 cats, 1 dwarf rabbit, 2 rabbits, 1 chamois), 1 to Microsporum gypseum (dog) and 1 to the Trichophyton anamorph of Arthroderma benhamiae (guinea pig). This last strain represents the first report of A. benhamiae in guinea pigs in Italy. The 2 remaining strains showed unique ITS sequences and might represent new species. One of these strains causing dermatological lesions in a dog was morphologically identified as Trichophyton erinacei but its ITS sequence differed from the type strain of this species by 8 basepairs while the intraspecific variation of all strains included in the database thus far represented only 2 basepairs. The other strain, isolated from wild chamois with no evidence of dermatological lesions, showed 89% similarity with its nearest neighbor, Microsporum cookei. This large molecular distance to its next akin suggested the detection of a hitherto undescribed species. Detailed morphological studies are needed to support these results. In conclusion, isolates from animals are still a rich source for detecting new species and ITS DNA barcoding provides an effective tool for this search.

PP-08-11

A case report of onychomycosis in a Japanese monkey

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Onychomycosis is a mycotic infection of the keratinized tissue of nail plate. Although, it is commonly considered to be caused by one of the dermatophytes, a variety of other fungi have been implicated as etiologic agents in the disease. Trichosporon species are yeasts-like organisms that are belonging to Basidiomycota. Several members of this genus are known to be pathogens in human and animals. A non pathogen species of this genus, *Trichosporon montevideense*, is thought to be responsible for an onychomycosis case in 4 years old Japanese monkey, *Macaca fuscata*. A severe onychomycosis was clinically diagnosed in its four limbs. Direct colony examination, cultural and molecular methods were used for a proper identification of the putative causative agents of this case. Comparison of results suggested *T. montevideense* as the pathogen of the onychomycosis case.

PP-08-12

Onychomycosis caused by chrysosporium keratinophilum in bennett's wallabies (Macropus rufogriseus)

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Seven Bennett's wallabies (Macropus rufogriseus) from a group of fifteen were presented with onychodystrophy, onychomadesis and severe digital tumefaction. The animals lived a zoological park in Lyon, France. Differential diagnosis included a traumatic origin with or without secondary bacterial infection, a primary bacterial disease, a fungal infection and, less likely, a neoplasia. Diagnostic tests consisted of cytology, histology, bacterial and fungal cultures. Histopathological findings included a pseudo-carcinomatous hyperplasia of the claw matrix. Using periodic acid Schiff stain, septate hyphae were detected inside a cavity with keratin in the matrix claw. The species Chrysosporium keratinophilum was identified on fungal cultures. The wallabies were orally treated with ketoconazole (15 mg/kg sid) for 20 weeks. Material and enclosures were cleaned and sprayed with enilconazole once a month for four months. No improvement of advanced cases was observed but no new case appeared during six months. Chrysosporium keratinophilum is a keratinophilic fungus commonly isolated from soil, plant, material, coat of mammals and birds. It lives on remains of hairs and feathers in soil. Chrysosporium keratinophilum may cause infections in humans, and is sometimes responsible for onychomycosis. To the knowledge of the authors, there is no existing case report of infection due to this fungus in animals.

PP-08-13

Malassezia dermatitis in dogs in Brazil: Clinical signs, diagnosis and molecular identification of causative agents

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Elevated populations of *Malassezia* pachydermatis can be detected on the skin of dogs with pruritic dermatitis. However, there is no standardized technique for the quantification of *Malassezia* yeasts and the relationship between the number of yeasts and the degree of damage of the skin has never been demonstrated. The present study evaluated two techniques of detection and quantification of *Malassezia* yeasts from the skin of 117 dogs with a suspicion of *Malassezia* dermatitis (pruritus, erythema, lichenification/seborrhea, excoriations and alopecia). The animals were presented at the Veterinary Hospital UFRGS, Porto Alegre, Brazil. The clinical signs were evaluated using the index CADESI (Canine Atopic Dermatitis Extend and Severity Index). The isolates were characterized by RFLP analysis. The efficacy of cytological examination and fungal culture was also evaluated. For cytological quantification, five anatomical sites were sampled using the tape strip technique. For mycological culture, a piece of sterilized carpet was applied on the skin lesions and further transferred onto Dixon modified medium. Yeast population sizes were expressed as mean CFU (colony forming units) by cm2. Out of 117 dogs with clinical signs compatible with *Malassezia* dermatitis, 62 had positive cultures for *Malassezia* spp. Dogs with positive cultures presented a higher degree of damage than negative ones, mainly erythema. Most of the isolates (61 out of 62) were identified as M. pachydermatis. Only one isolate showed the RFLP pattern of M. furfur. The specific identification was further confirmed by ITS, CHS-2 and rRNA sequencing.

PP-08-14

First report of a hyalohyphomycosis due to Acremonium strictum in a red-eared slider semi-aquatic turtle: Successful treatment by ketoconazole and clotrimazole

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The red-eared slider (Trachemys scripta elegans, syn. Pseudemys scripta elegans) is a semi-aquatic turtle belonging to the family Emydidae (order Testudines). It is native to the southern United States (Mississippi Valley drainage) but has become common in various areas of the world, e. g. in Germany - due to the pet trade. This 34-years-old female turtle was hold as a pet, during summer time in small water basin outside the house. It suffered from skin lesions for about two months. The normally typical attractive green carapace which is finely patterned with yellow-green to dark green markings showed hyperkeratotic and squamous lesions. Erosions and flat ulcerations appeared both at the upper and lower side of the carapace. The general conditions of the turtle deteriorated. First, empirical topical treatment using the antibiotic agent chloramphenicol was started without success. Skin scrapings from carapace were sent to microbiological lab for mycological investigation. A mould grew on Sabourauds dextrose agar. It was differentiated as Acremonium spp. In addition, a yeast fungus grew which was identified as *Candida* curvata using ID 32 C. According to molecular investigation using ITS rRNA region sequencing and MALDI-TOF mass spectrometry the fungus was identified as Acremonium strictum.

An antifungal treatment was started using ketoconazole systemically (tablets in fish food), plus topical clotrimazole. The skin and carapace lesions improved within two weeks as well as the general conditions. After two months of treatment the red-eared slider was cured.

Acremonium strictum is being isolated from dead plant material and soil. The mould is a plant, animal, and human pathogen. The species causes disease in humans and animals, e. g. mycetoma, onychomycosis, and hyalohyphomycosis. Prognosis of such an infection in animals is often bad. The reported red-eared slider has been cured due to intense antifungal treatment and excellent care by the pet holders.

PP-08-15

Eumycetoma caused by *Aspergillus fumigatus* in an alpaca (Lama pacos)

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A great variety of fungal species are able to form granules in vivo but only a few species are regularly reported as causative agents of mycetomas in humans or animals. The main fungal agents responsible for white grain mycetomas are belonging to the genera Acremonium, Scedosporium, Fusarium and less frequently *Aspergillus*. We report here a case of eumycetoma due to *Aspergillus fumigatus* in a 15-year-old female alpaca (Lama pacos) living in a zoological park in France. The animal presented a swelling on the left thigh, which was noticed 7 months after intramuscular injections of an antibiotic (oxytetracycline) and an anti-inflammatory drug (flunixine). Needle aspiration of the swelling yielded pus-like material. White grains (30-200 microns) were detected at direct examination. Histological examination revealed the presence of multiple pyogranulomas gathered around eosinophilic clusters containing hyaline fungal hyphae and vesiculous organs. The fungal species was identified as *Aspergillus fumigatus* on culture. The identification of the causative agent was confirmed by immunohistochemistry as well as by amplification and sequence analysis of fungal ITS 1 and 2 and 5.8S ribosomal DNA regions from tissue samples. The animal appeared in good health initially and no lameness was ever observed. During the evolution of the mycetoma, the alpaca got pregnant and its newborn was healthy and had a normal growth. Surgery or antifungal treatments were not possible. The lesion kept growing in size and the animal became weak and anorexic. The alpaca was eventually euthanized, 2 years after the diagnosis of mycetoma was made. Necropsic examination revealed a large abscess and several smaller abscesses in the muscles of the left posterior leg but no dissemination to other tissue. This is, to the best of our knowledge, the first case in which A. fumigatus was identified as the causative agent of a muscular mycetoma.

PP-08-16

Emerging fungal pathogens for pet reptiles

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Cutaneous mycoses in reptiles have been documented worldwide but are likely underdiagnosed because lesions often are indistinguishable from those of bacterial disease. Although different species of fungi can be incriminated, over the last few years some cases of dermatomycosis in several reptile species (chameleons, snakes, salt-water crocodriles, bearded dragons) has implicated the *Chrysosporium* anamorph of *Nannizziopsis vriesii* and *N. vriesii* as the causative agents. The source of infection in the reported cases and the ecology of this fungi remains to be defined. Furthermore, it is rarely found on the skin of healthy captive squamate reptiles.

In our laboratory we have recently reported the first case of cutaneous hyalohyphomycosis in two pet iguanas (*Iguana iguana*) and the first case in Europe of dermatomycoses in an inland bearded dragon (*Pogona vitticeps*) caused by *Chrysosporium* species related to *N. vriesii*. Since April 2008 three new cases of dermatomycoses in pet green iguanas have been diagnosed. In all cases, numerous white fungal colonies were recovered in pure culture from skin samples inoculated on Mycosel agar. When possible, histological examination confirmed granulomatous fungal dermatitis. The isolates were morphologically identified as members of the anamorphic genus *Chrysosporium* and all grew well at 37 C. The ITS-5.8S rRNA gene of the isolates were sequenced. Phylogenetic analysis of the sequences revealed that strains isolated from iguanas and from bearded dragon are related with *N. vriessii* AJ131687 but only showed an 81% of identity. Molecular and phenotypic studies of the isolates are in progress.

PP-08-17

In vitro susceptibility of *Prototheca zopfii* genotype 1 and 2

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Prototheca zopfii is as a pathogen of bovine mastitis associated with reduced milk production characterized by thin watery secretion with white flakes. *P. zopfii* was re-classified as genotypes 1 and 2 by based on 18S rDNA sequences. Moreover, all mastitis isolates investigated could be assigned to *P. zopfii* genotype 2, suggesting that this genotype is the etiologic agent of bovine mastitis. However, *in vitro* susceptibility tests of 2 genotypes of the isolates have not been well investigated. This study is the first to assess the susceptibility tests of genotype 2 isolates from bovine mastitis and that genotype 1 isolates from cow-barn surroundings.

Ten isolates of genotype 1, 10 isolates of genotype 2, a type strain of genotype 1 of *P. zopfii* (SAG2063T), a type strain of genotype 2 of *P. zopfii* (SAG2021T) and a type strain (SAG2064T) and 1 isolate of *P.blaschkeae* were tested for susceptibility against gentamicin (GM), kanamycin (KM) and itraconazole (ITZ) by E-test.

All strains of genotype 1 were susceptible to GM at 2.2 μg/ml (1-4 μg/ml), KM at 14 μg/ml (2-32 μg/ml) and ITZ at >14.4 μg/ml (1- >32 μg/ml). All strains of genotype 2 were susceptible to GM at 10 μg/ml (4-16 μg/ml), KM at 127.6 μg/ml (24-256 μg/ml) and ITZ at >32 μg/ml. Two strains of *P.blaschkeae* were susceptible to GM at 3 μg/ml (2-4 μg/ml), KM at 22.7 μg/ml (8-32 μg/ml) and ITZ at >17.3 μg/ml (1->32 μg/ml).

These results suggested that the susceptibility of all *P. zopfii* genotype 2 isolates was more sensitive against GM and KM than that of *P. zopfii* genotype 1. Moreover, genotype 2 and a type strain and 6 isolates of genotype 1 were not susceptible against ITZ (>10 μg/ml). The drug susceptibility of *P. zopfii* is confirmed to be different from each genotype.

Poster Forum PF-10

PP-08-18

The white nose fungus

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White nose syndrome (WNS) is a newly described condition affecting little brown, small-footed, pipistrelle and northern long-eared bat species that hibernate in mines and caves in New York and neighboring states. As the temperatures decrease in the fall, there is a reduction in the bats major food source, insects. To compensate, the bats enter torpor by lowering their body temperatures to ambient conditions (approximately 3-5°C), thereby reducing their metabolism rates. Inspection of caves and mines in 2006 and 2007, revealed areas of high mortality of over-wintering bats and the presence of white mould, primarily on the muzzles of a high percentage of these animals. However, no cause/effect relationship has yet been established between the fungus and die-off.

An isolate (R-4246) recovered from a little brown bat carcass found in a cave in upstate New York was the focus of the present investigations. Histopathology of stained muzzle tissue sections revealed a fungus colonizing the surface and on rare occasions, penetrating through the basement membrane of the skin. Best, albeit slow, growth was noted at temperatures from 5°C to 15°C on several standard mycology media, but limited or no growth on Sabouraud dextrose agar containing 5, 7, 10% salt and on Mycobiotic agar. Conidiogenous areas were dendritic, occurring in whorls, with green-colored conidia measuring 1-2 X 7-10 μm. The latter were truncate at the base, taping at the apices, and predominantly sickle shaped. Colonies, initially white, became green with conidial production. *In vitro* antifungal susceptibility tests of R-4246 suggested susceptibility to amphotericin B and the azoles, but resistance to echinocandins. Gene sequence investigations showed the organism to have 98% similarity with *Geomyces* spp. Based on all these characteristics, we propose an epithet for this new *Geomyces* species.

PP-08-19

Anti-fungal cell wall β-glucan antibody in animal sera

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We previously reported the presence of an antibody which reacts to fungal cell wall β-glucan, anti-β-glucan (BG) antibody, in human sera. Fungal infections can cause clinical problems in animals. In this study, we examined the existence and reactivity of anti-BG antibody in sera of livestock such as cows, horses and pigs and domestic pets such as dogs and cats.

The anti-BG antibody was detected and differed in titer and reactivity among species. In most animals, sera showed a high titer to a β-1,3-glucan containing a slightly branched long β-1,6-glucan segment, *Candida* solubilized β-glucan (CSBG), and a low titer to GRN, a 6-branched β-1,3-glucan from *Grifola frondosa* and yeast mannan (Y-Man). A significant titer of anti-BG antibody to AgHWE mainly composed of β-1,6-glucan from *Agaricus brasiliensis* or ASBG mainly composed of β-1,3-glucan from *Aspergillus niger* was also detected. The rate of reactivity to ASBG and AgHWE was different between each species.

This study showed that antibody to β-glucan, a major fungal cell wall component, was present in the serum of livestock and domestic pets. This antibody was highly reactive to the cell wall β-glucan of pathogenic fungi. It was thought that the anti-BG antibody participated in the immune-response to fungi and defense in the early stages of fungal infection.

PP-08-20

Antifungal activity of itraconazole and voriconazole against clinical isolates obtained from animals with mycoses

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Animal mycosis, particularly deep mycosis, is one of the most challenging conditions encountered by veterinarians. Pathogens causing mycotic infections in animals include fungi such as *Cryptococcus neoformans*, *Candida* spp., and *Aspergillus* spp. The antifungal drugs used for the treatment of deep mycoses in animals as well as humans are polyenes and azoles. However, the sensitivity of clinical isolates obtained from animals toward these drugs has rarely been assayed. In this study, the antifungal activities of itraconazole and voriconazole against clinical isolates of *C. neoformans*, *Candida* spp., and *A. fumigatus* isolated from animals with mycoses were examined using the broth microdilution method performed according to the guidelines provided by the Clinical and Laboratory Standards Institute. The minimum inhibitory concentrations (MICs) of itraconazole toward the *C. neoformans*, *Candida* spp., and *A. fumigatus* isolates were 0.125-1, 0.125-2, and 0.25-2 mcg/ml, respectively, and those of voriconazole toward the *C. neoformans*, *Candida* spp., and *A. fumigatus* isolates were 0.0625-0.5, <0.0313-0.0625, and 0.0625-1 mcg/ml, respectively. The results of the MIC analyses implied that the fungal isolates obtained from infected animals exhibit an equivalent degree of susceptibility to itraconazole and voriconazole, as is observed in the case of isolates obtained from humans. The appropriate antifungal therapeutic strategy for the treatment of mycoses in animals must be selected taking into consideration the host immune status and organ function as well as the *in vitro* sensitivity of the pathogens to antifungal drugs.

PP-08-21

Poisoning of dogs with tremorgenic Penicillium toxins

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Fungi in genus *Penicillium* are commonly found in mouldy food, feed and food waste. This genus includes known producers of a wide range of mycotoxins. Among *Penicillium* extrolites are tremorgenic substances like penitrems, nephrotoxins, such as ochratoxins and citrinin, and a range of other compounds including suspected tremorgens such as thomitrems and roquefortine C. *Penicillium* crustosum is among the known producers of the extensively studied tremorgenic mycotoxins penitrem A and E as well as less studied toxins such as roquefortine C and thomitrems.

Four cases of accidental intoxications of in total 6 dogs will be discussed. Poisonings with tremorgenic mycotoxins due to intake of mouldy feed or food waste were suspected. The clinical signs included vomiting, convulsions, tremors, ataxia, and tachycardia - all classical signs of various intoxications affecting the nervous system. Available samples of feed, stomach content and/or tissues from the intoxications were analysed by mycological and chemical analysis. Penitrem A was found in all reported poisonings and roquefortine C in some cases, where it was included in the analysis. The producer of these toxins, *Penicillium* crustosum, was detected in all mycological examinations, and the mycotoxins were assumed to have caused the intoxications. One dog was euthanized in the acute phase. Three dogs recovered completely within a few days. In two dogs, neurological symptoms were still observed several months after the poisoning. One of these dogs recovered completely within about six months, while the other still suffers from ataxia three years later.

Poster Forum PF-10

PP-08-22

Epidemiologic significance of the latent fungal carriage in animals

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Domestic animals represent the main source of dermatophytic infection for humans. However, clinically affected animals play a minor role in dermatophytic transmission because of therapeutic and preventive measures. On the contrary, latent fungal carriers represent more significant risk for humans, because animal owners have no any idea about dermatophytic carriage in their pets. Thus, asymptomatic pet can play a role as a latent source of the infection and environmental contamination for a long time with no any restriction.

Latent fungal carriage can be revealed in a wide range of domestic animals, including dogs, cats, rodents, ferrets, horses, cattle and camels. Children are more susceptible to dermatophytic infection their intensive contacts with animals. On the other hand the prevalence of zoophilic dermatophytes instead of antropohyilic species in aetiology of human dermatophytosis was revealed in resent studies (I. Takahashi, 2003, M. Lange et al., 2004, M. Ivanova, 2006). Evidently, these tendencies are in correlation.

The control of latent fungal carriage in animals represents the complex problem of social significance. It is well-known that the best method for prophylaxis of animal dermatophytosis is vaccination. Particularly, Russian vaccines such as LTF, Microderm, Equivac have established a reputation of the effective remedies both for prophylaxis and treatment of dermatophytosis. However, in case of fungal carriage the usage of the vaccines is not effective. The elimination of dermatophytes from the animal's hair-coat can be reached by means of antifungal drugs applied as shampoos and sprays. Nowadays the veterinary strongly lacks such kind of medications.

The procedure for a wide mycological screening of domestic animals is highly required. Such a procedure was designed and implemented in veterinary practice in Russia. It is based on cooperation between major veterinary centers and mycological laboratories. Improving of public knowledge in the field of mycological risk factors is also beneficial.

PP-08-23

A case of alternariosis successfully treated with local hyperthermia

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A 68-year-old painter visited our clinic 5 years after sustaining an injury of his right forearm in a fall. The injury healed but reddish papules appeared on the site 3 months later. He was advised to treat the eruption with topical steroids, but the clinical condition gradually worsened.

At the initial presentation, we observed irregular, granular lesions on the right forearm that appeared as dark-red papules of a rice-grain size fused to each other. We performed a skin biopsy. Histopathological examination showed epidermal atrophy and dense inflammatory cell infiltration of the dermis, accompanied by granulomas composed of polynucleated giant cells, plasma cells, neutrophils and histiocytes. Fungal elements were found within the giant cells and histiocytes. PAS and Grocott staining revealed mycelia possessing spores, spore chains and septae. Grayish-black, downy colonies grew rapidly from a skin specimen incubated in potato dextrose medium. The reverse side of the culture medium appeared black. Microscopic analysis showed beaked conidia in chains. DNA sequencing of 26S rRNA D1/D2 regions of the isolated fungus identified Alternaria alternata, and the patient was given a diagnosis of phaeohyphomycosis due to A. alternata.

We recommended surgery, but the patient refused. Treatment consisted of local hyperthermia using a portable chemical body warmer. At 10 months, the lesion was healed, with only mild depigmentation. Two years later, the patient shows no sign of relapse.

To date, 30 cases of cutaneous aterinariosis have been reported in Japan, but ours is the first report to describe successful treatment with local hyperthermia. We recommend local hyperthermia as a promising option for treating cutaneous alternariosis in patients who may prefer a non-surgical treatment.

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