Mycology newsletter

The ECMM/CEMM Mycology Newsletter is mailed to the members of the national societies affiliated to the European Confederation of Medical Mycology (about 3000 in 24 different countries)

1/2008

ECMM
European Confederation of Medical Mycology

CEMM
Confédération Européenne de Mycologie Médicale

Getting things done

A few weeks ago the ECMM has moved its domicile to Basel, Switzerland. Many societies and organizations in Europe and beyond have done this in the past. We are now officially a non profit organization stationed in Switzerland. Our Fellow societies in this country are among others the FIFA, UEFA and European Society for Clinical Microbiology and Infectious Diseases. However, it remains a pity that our Swiss medical mycology colleagues were not able yet to erect a Swiss Society for Medical Mycology. We would like to have them among the national societies affiliated to the ECMM. Some of our current members countries do not have a national mycological society but are rather working groups from dermatological or microbiological societies. ECMM should try to stimulate and help erecting multidisciplinary societies for medical mycology in every European country.

At the recent ECMM Council Meeting several decisions were made regarding the new Executive Board and the venue of the 2010 ECMM educational meeting and the 2011 TIMM meeting. After 6 years of commitment to the Confederation, Prof. Emmanuel Roilides and Prof. Martin Schaller resigned as General Secretary and Treasurer respectively. Six years is the maximum term for participating in the Executive Council and I would like to thank them both for their invaluable efforts and support to create the Confederation as it is now. The new executive committee was elected after a close race. Prof. George Petrikkos will serve as the new General Secretary and Prof. Cornelia Lass-Flörl as the new Treasurer. The Council gave me the green light to continue my term as President for another 3 years. The three of us will do our utmost best to fulfill the primary goal of the Confederation, organizing and promoting the science and all aspects of medical mycology in Europe and if necessary world wide. Regarding the last objective we have been promoting medical mycology in Africa by supporting already three Pan African Medical Mycology (PAMMS) meetings. The next, ECMM and ISHAM sponsored, PAMMS III will take place in February 2009 in Nigeria and I suggest you to attend this meeting in this exciting continent, if possible. You can read more elsewhere in this newsletter. Our 2008 ECMM educational meeting was held during the International Union of Microbiological Societies gathering in Istanbul early August of this year. Two ECMM working groups

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Jacques F. Meis

Join the new ECMM website!
http://www.ecmm.eu

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ECMM Affiliated Societies

(Information provided by the member Societies)

All-Russian National Academy of Mycology
President: Y.V. Sergeev
Vicepresident, *Head of Medical Section*: S.A. Burova
Secretary: A.Y. Sergeev *(ECMM delegate)*
Treasurer: V.M. Leschenko
Membership 2008: 246
Website: www.mycology.ru

Asociación Española de Micología (AEM) Sección de Micología Médica
President: JG. Quindós Andrés
Vicepresident: M.C. Rubio
Secretary: J. Pemán García
Treasurer: F.L. Hernando Echevarría
President Medical Mycology Section: F. Sánchez Reus *(ECMM delegate)*
Membership 2008: 223
National meeting: Every two years. A workshop meeting ("Forum Micológico") is scheduled the years between National Meetings
Journal: Revista Iberoamericana de Micología
Website: www.reviberoammicol.com/AEM

Associação Portuguesa de Micologia Médica (ASPOMM)
President: M. Rocha
Vicepresident: R.M. Velho
Secretary: M.L. Rosado *(ECMM delegate)*
Treasurer: M. Gardete
Membership 2008: 50

Austrian Society for Medical Mycology (ASMM)/Österreichische Gesellschaft für Medizinische Mykologie (ÖGMM)
President: B. Willinger
Vicepresident: C. Lass-Flörl (*ECMM delegate*), H.-J. Dornbusch
Secretary: W. Buzina
Vicesecretary: G. Ginter-Hanselmayer
Treasurer: C. Speth
Vicetreasurer: J. Rainer
Membership 2008: 112
National meeting: twice a year
Website: www.oegmm.at

British Society for Medical Mycology (BSMM)
President: E. M. Johnson *(ECMM delegate)*
General Secretary: S. Howell
Meetings Secretary: G. Moran
Treasurer: D.M. MacCallum
Membership 2008: 302
National meeting: 28 th - 31st March 2009, London
Newsletter: BSMM Newsletter
Website: www.bsmm.org

Bulgarian Mycological Society (BMS)
President: T. Kantardjiev *(ECMM delegate)*
Vicepresident: G. Mateev
Secretary: A. Kouzmanov
Treasurer: T. Velinov
Membership 2008: 80
National meeting: 26-29 May 2009
Website: www.bam-bg.net

Committee for Medical Mycology of

Czechoslovak Society for Microbiology (CSSM)
President: K. Mencl *(ECMM delegate)*
Secretary: P. Hamal
Treasurer: J. Gabriel
Membership 2008: 16
Newsletter: Bulletin of CSSM

Danish Society for Mycopathologia
President: J. Stenderup *(ECMM delegate)*
Vicepresident: B. Andersen
Secretary: B. Knudsgaard
Treasurer: J. Stenderup
Membership 2006: 25
National meeting: twice a year
Newsletter: Report from the Danish Society for Mycopathology

Deutschsprachige Mykologische Gesellschaft e.V. (DMykG)
President: O. Cornely
Vicepresident: M. Schaller *(ECMM delegate)*
Secretary: P.-M. Rath
Treasurer: C. Hipler
Membership 2008: 478
National meeting: 3-5 September 2009, Cologne
Journal: Mycoses
Newsletter: Mykologie Forum (4 issues/year)
Website: www.dmykg.de/start2.html

Federazione Italiana di Micopatologia Umana e Animale (FIMUA)
President: S. Oliveri
Vicepresident: A. Novelli
Secretary: M. Sanguinetti
Treasurer: L. Vallone
ECMM delegate: M.A. Viviani
Membership 2008: 160
National meeting: Milano 2010
Website: www.fimua.it

Finnish Society for Medical Mycology
President: R. Visakorpi
Vicepresident: T. Putus
Secretary: V. Ratia
Treasurer: O. Lindroos
ECMM delegate: P. Kuusela
Membership 2008: 88
National meeting: February 11, 2009
Newsletter: Sienet ia Terveys (Fungi and Health)

Hellenic Society of Medical Mycology
President: G. Samonis
Vicepresident: G.L. Petrikkos *(ECMM delegate)*
Secretary: A.M. Ziouva
Treasurer: H. Papadogeorgaki
Membership 2008: 81
Website: www.hsmm.gr

Hungarian Dermatological Society Mycology Section
President: G. Simon *(ECMM delegate)*
Secretary: N. Erős
Membership 2008: 61

Israel Society for Medical Mycology
President: E. Segal
Vicepresident: I. Polacek
Secretary: I. Berdicevsky *(ECMM delegate)*
Treasurer: D. Elad
Membership 2008: 50
National meeting: twice a year

Mycology Group of Bosnia Hercegovina
President: L. Ozegovic *(ECMM delegate)*
Secretary: M. Babic
Membership 2005: 19
National meeting: twice a year

Netherland Society for Medical Mycology (NVMy)
President: J.F.G.M. Meis *(ECMM delegate)*
Secretary: E.P.F. Yzerman
Treasurer: M.H. Dammer
Scientific Secretary: G. S. de Hoog
Membership 2008: 119
Newsletter: NVMy Newsletter
Website: www.nvmy.nl

Nordic Society for Medical Mycology (NSMM)
President: M.C. Arendrup *(ECMM delegate)*
Vicepresident: Lena Klingspor
Secretary: P. Gaustad
Treasurer: D.M.L. Saunte
Membership 2008: 214
Newsletter: at web site
Website: www.nsmm.nu

Polish Dermatologic Society-Mycology Section
President: E. Baran
Vicepresident: Z. Adamski, R. Maleszka
Secretary: R. Bialynicki-Birula *(ECMM delegate)*
Treasurer: A. Hryncewicz-Gwozdz
Membership 2008: 100
National meeting: Krakow 2012
Journal: Mikologia Lekarska (Medical Mycology)

Romanian Society of Medical Mycology and Mycotoxicology (RSMMM)
President: M. Mares *(ECMM delegate)*
Vicepresident: B. Doroftei
Secretary: A. Stefanache
Treasurer: L. Malic
Membership 2008: 80
National meeting: National Meeting every two years and Congress every four years
Journal: Fungi & Mycotoxins
Books: Syntheses of Medical Mycology (for Continuing Medical Education)
Website: www.fungi.ro / www.journal.fungi.ro

Société Belge de Mycologie Humaine et Animale/Belgische Vereniging Voor Menselijke en Dierlijke Mycologie
President: I. Surmont
Vicepresident: K. Lagrou *(ECMM delegate)*, F. Symoens
Secretary: M. Van Esbroeck
Treasurer: P. Huynen
Scientific Secretary: E. De Laere
Membership 2008: 181
Website: www.medmycol.be

Société Française de Mycologie Médicale
President: B. Dupont *(ECMM delegate)*
Vicepresident: N. Contet-Audonneau, R. Grillot, C. Guiguen
Secretary: P. Roux
Treasurer: A. Datry
Membership 2008: 350
National meeting: June 17-19, 2009, Poitiers
Journal: Journal de Mycologie Médicale
Website: http://pagesperso-orange.fr/sfmm

Swedish Society for Clinical Mycology
President: J. Faergemann
Vicepresident: T. Kaaman
Secretary: L. Klingspor *(ECMM delegate)*
Treasurer: M.L. von Rosen
Membership 2008: 80

Turkish Microbiological Society Mycology Section
President: Ö. Ang
Secretary: A. Ağaçfidan
Treasury: D. Yaylali
ECMM delegate: S. Arikan
Membership 2008: 150
Newsletter: Bulletin of the Turkish Microbiological Society

Getting things done

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convened a symposium on Candidosis in the Intensive Care (Chair: Lena Klingspor) and Zygomycosis (Chair: George Petrikkos). Recruitment of patients in the latter study was closed earlier this year after 3 years. Given the highly successful outcome of this ECMM working group on Zygomycosis, headed by Prof. Petrikkos, we have liaised with ISHAM to start a new joint ECMM/ISHAM working group on Zygomycosis covering Europe, North- and South America, the Indian subcontinent and if possible Africa. This project is truly in line with the ECMM objectives set forth in our Charter. Previous ECMM working groups were on candidemia, cryptococcosis, histoplasmosis, nocardiosis, scedosporiosis, black yeasts and tinea capitis. All studies were successfully concluded with one or more publications on behalf of the ECMM. To those members who walk around with good ideas, it is always possible to come up with initiatives for new working groups. The latest initiatives from ECMM members are future working groups on fusariosis and coccidioidomycosis in Europe.

The 2010 ECMM educational meeting will be together with our fellow Italian medical mycologists and TIMM-5 will take place in the autumn of 2011 in Valencia, Spain. For all of you it is now time to think ahead of TIMM-6 in 2013 and the educational ECMM meeting in 2012. This might be a change for your national society to host the largest medical mycology meeting in the world.

The digital focus of our society, www.ecmm.eu, has undergone a major reconstruction. The ECMM Website committee under the direction of Prof. Alexey Sergeev has prepared a new digital communication board for the ECMM members. I hope you will visit the website regularly to browse and gather information.

The year 2008 runs to an end. I wish that you were able to achieve all your personal goals set for this year and are prepared for the changes that will take place in the new year. ECMM will fly into a new exciting new year of change and things to look forward to in 2009.

Jacques F. Meis
ECMM President

Join the new ECMM website!

The new version of ECMM website is ready at http://www.ecmm.eu.

Under the recommendations of ECMM website committee (M.C. Arendrup, S. Arikan, J. Brandão, J. Meis, E. Roilides, A.Y. Sergeev, M.A. Viviani), it was decided to maintain the initial site design proposed by Prof F.C. Odds, but to extend its functionality. To do so, we have installed the famous European open-source framework, Drupal.

The four major parts of the website are dedicated to Confederation itself, ECMM events, projects and Newsletter. The "About ECMM" page tracks ECMM past and describes its current state, holding important documents and historical images. The "Events" page keeps records of all meetings and conferences held by Confederation since 1993. The "Working groups" page lists all completed surveys and follows active working groups. It will be further extended to allow members joining working groups online. The "Newsletter" page provides information on ECMM Mycology Newsletter and allows users to download all of its issues.

The new website will update you on all important events in Medical Mycology, new publications, research projects and meetings. In development stage is citation tracker that aggregates the latest publications from medical mycology journals. Events calendar is planned to provide the announcements of all mycological meetings and courses.

What extras does the new ECMM website offer? First of all, now it is really interactive. Everyone may register and use the site. Registered ECMM members can contact each other, keep in touch with researchers from other countries, subscribe for events and join projects. Now you can comment on each event or publication, vote in online polls, use forums. ECMM members and National Societies can start their own blog and share their news and other information with colleagues.

Your opinion is important. As the registered member online, you may comment on almost any page of the website. Propose your ideas of website development, ask for new features and improvements. We are waiting for your input.

Join the new ECMM online community! Register at http://www.ecmm.eu

Alexey Sergeev
Website Editor

ECMM Survey of Infections due to *Fusarium* species in Europe

Fusarium species cause a variety of infections in humans, including superficial, locally invasive, and disseminated infections. The clinical presentation largely depends on the immune status of the host and the fungal portal of entry.(1) Superficial infections, such as keratitis and onychomycosis, are usually observed in immunocompetent individuals, whereas invasive and disseminated infections occur in immunocompromised patients and are mainly associated with prolonged and profound neutropenia or severe T-cell immunodeficiency.(2)

Among the more than 50 *Fusarium* species identified, twelve have been described as causes of human infection. *F. solani* is the most frequently reported *Fusarium* species and is the cause of approximately 50% of the *Fusarium* infections; the next most prevalent species, in order, are *F. oxysporum* (20%), *F. verticilloides* (10%), and *F. moniliforme* (now classified as *F. verticilloides*, 10%).(1,3) In contrast with data from the literature, in Italy *F. verticilloides* resulted the most prevalent species (41%) followed by *F. solani* (25%). In particular, *F. verticilloides* was the most frequent species (57%) in deep-seated infections and *F. solani* is more common in superficial infections (46%).(4)

Fusarium species are relatively resistant to most antifungal agents. Careful analysis, however, shows that different species have different patterns of susceptibility.(1) The majority of *F. solani* isolates exhibited reduced susceptibility to azoles.(1,4-8)

The prognosis of fusariosis in immunocompromised host is poor and also the treatment of skin and nail infections is frustrating and failure of systemic and local treatment is common.

The main purpose of this study is to understand the epidemiology of fusariosis in Europe, collecting information on the patients infected by *Fusarium* (risk factors, localization/extent of infection, diagnosis, antifungal treatment and outcome) and on the infecting isolates (identification by molecular methods, *in vitro* susceptibility to antifungal agents).

Study design

Cases of fusariosis, deep seated as well as superficial infections, for which the infecting isolate is available, will be recorded on a questionnaire and the isolate collected and characterized. The form and the corresponding isolates will be collected in the national coordinator laboratory and strains studied.

The prospective study is planned to start on January 1st, 2009 and it will last for two years. Two years (2007 and 2008) retrospective data will be also collected.

Investigators interested in participating to this study as national coordinator for their country are welcome.

Anna Maria Tortorano

REFERENCES

1. Nucci M, Anaissie E. 2007. *Fusarium* infections in immunocompromised patients. Clin. Microbiol. Rev. 20:695-704.
2. Nucci M, Anaissie E. 2002. Cutaneous infection by *Fusarium* species in healthy and immunocompromised hosts: implications for diagnosis and management. Clin. Infect. Dis. 35:909-920.
3. Seifert KA, Aoki T, Baayen RP, et al. 2003. The name *Fusarium moniliforme* should no longer be used. Mycol. Res. 107: 643-644.
4. Tortorano AM, Prigitano A, Dho G, Esposto MC, Gianni C, Grancini A, Ossi C, Viviani MA. 2008. Species distribution and in vitro antifungal susceptibility patterns of 75 clinical isolates of *Fusarium* from Northern Italy. Antimicrob. Agents Chemother. 52:2683-2685
5. Arikan S, Lozano-Chiu M, Paetznick V, Nangia S, Rex JH. 1999. Microdilution susceptibility testing of amphotericin B, itraconazole, and voriconazole against clinical isolates of *Aspergillus* and *Fusarium* species. J. Clin. Microbiol. 37:3946-3951.
6. Azor M, Gené J, Cano J, Guardo J. 2007. Universal in vitro antifungal resistance of genetic clades of the *Fusarium solani* species complex. Antimicrob. Agents Chemother. 51:1500-1503.
7. Paphitou NI, Ostrosky-Zeichner L, Paetznick VL, Rodriguez JR, Chen E, Rex JH. 2002. In vitro activities of investigational triazoles against *Fusarium* species: effects of inoculum size and incubation time on broth microdilution susceptibility test results. Antimicrob. Agents Chemother. 46:3298-3300.
8. Alastruey-Izquierdo A, Cuenca-Estrella M, Monzon A, Mellado E, Rodriguez-Tudela JL. 2008. Antifungal susceptibility profile of clinical *Fusarium* spp. isolates identified by molecular methods. J. Antimicrob. Chemother. 61:805-809.

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ECMM survey: *Fusarium* Infections in Europe
Epidemiological and clinical form

Country: National Coordinator:
Centre: City:

PATIENT

Patient code: Sex: | M | F Birthdate (mm/yyyy) | | / | | | | |
Country of birth: Country of residence: Occupation:
Ward of hospitalization: | Hematology | ICU | Other, *specify*

FUSARIUM PATHOLOGY

Date of diagnosis (dd/mm/yy) | | / | | / | | | | Disseminated infection | Localized infection | Colonization
Involved site/s:
| blood | lung | pleura | peritoneum | cornea | skin | nails | other, *specify*
Detailed data (clinical manifestations, localization, imaging .....)

UNDERLYING DISEASE/FACTORS

Autoimmune disease, *specify* date of diagnosis		/		/		
Leukemia /	Lymphoma, *specify* date of diagnosis		/		/		
Solid cancer, *specify* date of diagnosis		/		/		
Hemopoietic stem cell transplant date		/		/		
autologous	allogeneic:	matched related;	matched unrelated;	mismatched;	aplotype;	umbelical cord
myeloablative:	related;	unrelate	non myeloablative:	related;	unrelated
Bone marrow transplantt date		/		/		
Graft versus host disease:	acute;	chronic date of diagnosis		/		/		
Severe neutropenia (<500/mm3), *specify time and duration of neutropenia*
Immunosuppressive drugs, *specify drugs, dosage, period of treatment*
Corticosteroids, *specify drugs, dosage, period of treatment*
Chronic obstructive pulmonary disease, *specify grade*
Surgery, *specify* date		/		/		
Solid organ tranplant, *specify* date		/		/		
Diabetes:	type I;	type II date of diagnosis		/		/		
AIDS CD4 number/mm^3: date of diagnosis		/		/		
Accidental trauma, *specify* date		/		/		
Dialysis:	hemo;	peritoneal
Stay in ICU APACHE II score: SAPS III score: period of stay:
Use of contact lens, *specify type* Contact lens solution used:
Other, *specify*

TREATMENT OF *FUSARIUM* INFECTION

Antifungal therapy Patient weight:

Drug 1: daily dose: from (dd/mm/yy) | | / | | / | | to (dd/mm/yy) | | / | | / | | |
Drug 2: daily dose: from (dd/mm/yy) | | / | | / | | to (dd/mm/yy) | | / | | / | | |
Drug 3: daily dose: from (dd/mm/yy) | | / | | / | | to (dd/mm/yy) | | / | | / | | |

Surgery, *specify* date | | / | | / | | |

OUTCOME OF FUSARIUM INFECTION

Cure, date	Death, date	Lost, date	Relapse, date

Last culture/s positive for *Fusarium* (*specify sample and date*)

MYCOLOGY

Direct microscopy and culture

	Direct microscopy				Culture				
Blood	not done	done	neg.	pos.*	not done	done, *specify system*	neg.	pos.	date / /
Bronchial secr.	not done	done	neg.	pos.*	not done	done	neg.	pos.	date / /
Oral secretions	not done	done	neg.	pos.*	not done	done	neg.	pos.	date / /
Nasal secretions	not done	done	neg.	pos.*	not done	done	neg.	pos.	date / /
Pleural fluid	not done	done	neg.	pos.*	not done	done	neg.	pos.	date / /
Peritoneal fluid, *specify if dialytic*	not done	done	neg.	pos.*	not done	done	neg.	pos.	date / /
Biopsy, *specify*	not done	done	neg.	pos.*	not done	done	neg.	pos.	date / /
Skin, *specify site*	not done	done	neg.	pos.*	not done	done	neg.	pos.	date / /
Corneal scraping, *specify*	not done	done	neg.	pos.*	not done	done	neg.	pos.	date / /
Nails, *specify*	not done	done	neg.	pos.*	not done	done	neg.	pos.	date / /
Other, *specify*	not done	done	neg.	pos.*	not done	done	neg.	pos.	date / /

* presence of hyphae

Histopathology

Biopsy, *specify sample/s and date*	not done	done	neg.	pos.*
Autopsy, *specify date*	not done	done	neg.	pos.*

* presence of hyphae

Note

FUSARIUM ISOLATES SENT TO THE NATIONAL/EUROPEAN COORDINATOR

Ref.number:	Identification:	Cultured from:	date / /
Ref.number:	Identification:	Cultured from:	date / /
Ref.number:	Identification:	Cultured from:	date / /

CORRESPONDING PHYSICIAN/MYCOLOGIST

Name of physician	Phone	Email
Name of mycologist	Phone	Email

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ECMM Survey of Coccidioidomycosis in Europe

Background

Coccidioidomycosis is a disease endemic to parts of South-West USA (Arizona, California, Utah, New Mexico), Central and South America, caused by the dimorphic fungi *Coccidioides immitis* and *C. posadasii*, desert soil-dwelling ascomycetes. These fungi grow as a filamentous saprobe in the soil and as endosporulating spherules within the host. Inhalation of arthroconidia results in a symptomatic respiratory tract disease, usually mild and self-limited, in up to 40% of infected patients. But the disease can last months and in 1% of cases disseminates beyond the lung. CNS, lymph nodes, bone tissue and skin are primarily involved. Disseminated infections can be fatal or require lifelong therapy.

Coccidioidomycosis in Europe

While not endemic in Europe, cases of coccidioidomycosis occur in individuals who have lived or travelled in endemic areas. Reactivation of infections from several years previous may result from a failing immune system, as for example occurs in some AIDS patients. Information of the general prevalence of this mycosis in Europe is not available.

Because the international tourism and the immigration from endemic countries are increasing, physicians in Europe need to become more familiar with the manifestations and with the approach to diagnosis. Travel history should always be sought in the evaluation of patients. Since few fungal elements can be present in biopsy, histology with special stains and the more sensitive culture examination should always be performed. In addition, to avoid risk of accidental exposure, laboratory workers should be informed of the clinical suspicion.

Objective of the survey

The objective of this survey is to discover the prevalence of coccidioidomycosis in Europe, where and how the infection was acquired, the group at risk, the fungal species responsible and the method by which the infection was diagnosed. The antifungal therapy and the outcome will also be analysed. This will lead to a better understanding of this imported mycosis and will enable a coordinated effort to target at risk populations and to standardize methods for the diagnosis and treatment of the disease.

Investigators interested in participating to the study as "national coordinator" for their country are requested to contact Prof. B. Dupont

Study period

Notification of new cases will start on the 1st January 2009. Retrospective cases since 1983 will also be collected

Collection of data and isolates

Notification of cases should be made using the epidemiological form for coccidioidomycosis reported at page 9. Data from the retrospective study should be compiled as soon as possible and send to the national coordinator. For the prospective study the information should be sent as the cases arise. The isolate, if available, should be stored in the laboratory where it was identified. Please contact your national coordinator to know if molecular identification of species is available before sending the isolates according to safety national rules. Postal regulations on the safe packaging of these dangerous pathogens need to be strictly followed.

Bertrand Dupont

Further information can be obtained from Prof. Bertrand Dupont (bertrand.dupont@nck.aphp.fr).

Risk for European Laboratory Workers

Biosafety Classification Directives recognizes these thermally dimorphic fungi as Hazard Group 3 pathogens. The risk of laboratory workers is a serious one, owing to the large number of spores many isolates produce in culture. Serious infections have occurred in laboratories without proper contaminant facilities. Clinicians should inform laboratories if clinical material from a patient with a suspected imported fungal infection is submitted for microbiological or histopathological investigation.

ECMM survey: Coccidioidomycosis in Europe
Epidemiological and clinical form

Center

Country: City: Hospital:
Physician's name:
Microbiologist/mycologist's name: email:

Patient information

Nationality: Country: City:
Birthdate (mm/yyyy) | | / | | | | Sex: | M | F Weight: Ethnicity:
Occupation:

Underlying disease / Risk factors

None | |
Immunosuppression | | HIV: | | CD4 cells *specify*: corticosteroids: | | anti-TNF: | |
Solid organ transplant: | | *specify:*
Risk factors | | BPCO: | | pregnancy: | | diabetes: | |
Other: | | *specify:*

Contamination

Date: (mm/yyyy) | | / | | | |
Country/state: area/county: town:
Mode of contamination *specify:*
Time (days, months or years) between contamination and first symptoms:
and treatment:

Disease

Date of diagnosis: | | / | | / | |
Primary infection: | | Disseminated infection: | | Relapse: | |
Asymptomatic infection: | | Symptomatic: | | *specify*:
Localisations: lung: | | infiltrate: | | nodule: | | cavity: | | lymphnode: | |
CSF: | | HTIC: | | vasculitis: | |
skin: | | ulceration: | | nodule: | |
joint/bone: | | *specify:*
other: | | *specify:*
Diagnosis procedures
biopsy: no: | | yes: | | site *specify*:
presence of spherules: no: | | yes: | | site *specify*:
positive culture: no: | | yes: | | site *specify*:
spp identification: *immitis*: | | *posadasii*: | | not available: | |
positive serology: no: | | yes: | | not done: | |
body fluid tested *specify* test(s) used *specify*
Other (antigen, skin test) *specify*

Treatment

Systemic antifungal: No: | | Yes: | |

Drugs	Route - Dosage/d	Date started	Date stopped	comment

intrathecal antifungal: No: | | Yes: | | CSF shunt: No: | | Yes: | |
Other treatment *specify*

Outcome

Cure: No: | | Yes: | | follow up: | | / | | / | | | | *specify* Improvement: No: | | Yes: | |
Failure: | | Death: | | cause of death *specify*
Progressive chronic infection *specify*

Was this case published: No: | | Yes: | | Please provide reference of publication:
or email a PDF copy

Other remarks:

Please email this form to your country coordinator

Special report on...

7th International Conference on *Cryptococcus* and Cryptococcosis

11-14th September, 2008, Nagasaki, Japan

by
Tihana Bicanic
Teun Boekhout
Maurizio Del Poeta
Angie Gelli
Stuart M. Levitz
Jenny K. Lodge
Kirsten Nielsen
Michal Olszewski
Peter G. Pappas
Tania C. Sorrell

From their humble beginnings of discovery as a human pathogen in the 1890s, *Cryptococcus neoformans* and *Cryptococcus gattii* have exploded onto clinical practice in this millenium. For instance a recent outbreak of *C. gattii* infections from Vancouver Island to Northwest USA has demonstrated the ability of this encapsulated yeast to change its pathobiology and ecology. Even more impressive is that the CDC now estimates the global burden of HIV-associated cryptococcosis at 1 million cases per year with estimated deaths of 680,000 per year. When placed in number of estimated deaths from infectious diseases in Sub Saharan Africa excluding directly AIDS only malaria and diarrheal illnesses rank higher.

John R. Perfect

Nagasaki, Japan
11-14 September 2008

Opening Address

Dr. Shigeru Kohno, the Conference Chair and Dean, Nagasaki University School of Medicine, warmly welcomed the conference participants to Nagasaki and noted that this was the first time the ICCC was being held in Far East Asia. He then introduced John Perfect, who gave the Opening Address. Dr. Perfect cited the latest Center for Disease Control (USA) estimates that there are over 1,000,000 cases of cryptococcosis per year and, of those cases, approximately two thirds will die. He then went on to focus on why *Cryptococcus* is a "brainy" yeast – in other words, why it is neurotropic. Dr. Perfect and his laboratory have examined what the requirements are for *C. neoformans* to survive in the cerebrospinal fluid (CSF) with the hypothesis that certain gene products promote dissemination and survival in the brain. Of 1700 mutants that were tested, 19 could not grow in CSF. The nature of these mutations was being studied.

Dr. Perfect then informed the audience that the IDSA Practice Guidelines for the treatment of cryptococcosis were in the final stages of revision and should be published soon. He emphasized that amphotericin B plus 5-flucytosine remains the optimal choice for induction therapy of cryptococcal meningitis. Fluconazole is not as good, but if practical considerations dictate its use, it should be used at doses >800 mg/day. In patients found to be co-infected with HIV and *C. neoformans*, it remains uncertain when is the best time to start antiretroviral therapy. One must balance the risk of further complications of AIDS with the risk of an immune response inflammatory syndrome to the fungus. Finally, Dr. Perfect addressed the issue of management of elevated intracranial pressure with the opinion that those with pressure about 25 cm should have measures taken to relieve the elevated pressure, including repeated lumbar punctures and, if necessary, shunting.

Stuart Levitz

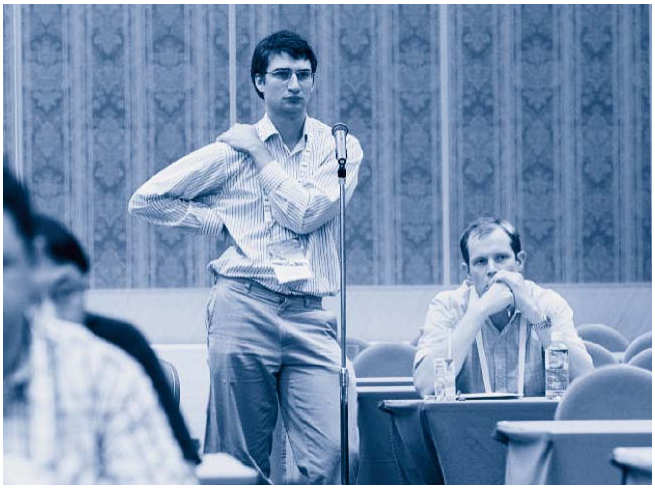
Shigeru Kohno

John Perfect

The Immune Response

The Immune Response I session began with a presentation by Thomas Kozel (University of Nevada School of Medicine, Reno, Usa) on the interaction of antibody and complement proteins with the cryptococcal capsule. Dr. Kozel emphasized that the capsule gets densest as it gets closer to the cell wall. There are differences *in vitro* versus inside the mouse as the capsule gets denser and has more O-acetylation when the fungus is *in vivo*. In addition, there are serotype-specific differences in the expression of O-acetylation groups in the fungus' buds. Next, Christopher Mody (University of Calgary, Canada) presented the results of his research on the mechanisms by which NK cells inhibit and kill *C. neoformans*. He emphasized

Stuart Levitz and Thomas Kozel



Simon Johnston

Kazuyoshi Kawakami

that perforin is required. While NK cells degranulation and lose perforin when exposed to *C. neoformans*, the NK cells subsequently "re-arm" with more perforin.

Anna Vecchiarelli (University of Perugia, Italy) then reviewed the immunosuppressive effects of the major capsular polysaccharide of *C. neoformans*, GXM. She then asked the question whether GXM could be used to treat autoimmune diseases. Beneficial effects were seen in an experimental model of collagen-induced arthritis.

The session concluded with two presentations selected from the submitted abstracts. First, Mark Krockenberger (University of Sydney, Australia) presented data on a new rat model of *C. gattii* pulmonary infection and contrasted it to what is known about rat models of *C. neoformans*. Finally, Simon Johnston (University of Birmingham, UK) presented intriguing data that *Cryptococcus* can escape from macrophages without killing the host cell by a process termed reverse phagocytosis or vomocytosis.

Kazuyoshi Kawakami (Tohoku University Graduate School of Medicine, Japan) opened the Immune Response II session by examining selected aspects of the innate immune response to *C. neoformans*. He presented data that the beta-glucan receptor, dectin-1, was dispensable for immunity to *C. neoformans.* Then, he reviewed studies that established a critical role for the adapter protein,

Giuseppe Teti

MyD88, in host defenses against cryptococcosis. As TLR9 utilizes MyD88 to signal, Dr. Kawakami examined whether TLR9 senses DNA from *C. neoformans*. He found cytokine production following stimulation of dendritic cells with *C. neoformans* DNA. Next, Stuart Levitz (University of Massachusetts Medical School, Worcester, USA) discussed immune responses to mannoproteins. These immunodominant proteins are heavily mannosylated via both N-linkages and O-linkages. Dr. Levitz presented data showing that mannoproteins are poor stimulators of cytokine production by dendritic cells. However, combining mannoproteins with TLR ligands synergistically boosted cytokine production. Dr. Levitz speculated that this combination could make a good vaccine.

Type I interferons are known to play a critical role in viral infections, but their contribution to host defenses against the mycoses has received scant attention. Therefore, Giuseppe Teti (University of Messina, Italy) looked at the role of type I interferons in cryptococcosis. Mice deficient in either IFNbeta or the IFNalfa/beta receptor had reduced survival in a pulmonary challenge model. Small, but significant, amounts of IFNalfa were produced by macrophages after cryptococcal stimulation.

Two speakers selected from the submitted abstracts finished the session. Kirsten Nielsen (University of Minnesota, Minneapolis, USA) examined the role of pheromone signaling during *in vivo* infection. In a co-infection model, she studied dissemination of **a** and alfa strains that had disrupted pheromone signaling. The **a** cells were increased in size and had decreased central nervous system penetration. Finally, Hansong Ma (University of Birmingham, UK) presented data positively correlating virulence of *C.neoformans* and *C. gattii* with the capacity to proliferate intracellularly in macrophages. Interestingly, strains isolated from the Vancouver Island outbreak were amongst the most rapid intracellular replicators.

Stuart Levitz

Nagasaki, Japan
11-14 September 2008

Cell Wall and Capsule

The session opened with Tamara Doering (Washington University Medical School, St. Louis, USA) who presented a two part talk. First, she asked the fundamental question, "Where is capsule made?". Using a conditional mutant that doesn't secrete, accumulation of vesicles containing the major capsular polysaccharide, GXM, was observed, suggesting that at least the building blocks are manufactured inside the cell. Next, Dr. Doering tackled the question of how xylose gets incorporated into the capsule. A mutant deficient in cryptococcal xylosyltransferase 1 (Cxt1) had modestly reduced xylose in GXM but markedly reduced xylose in GalXM. Guilhem Janbon (Institut Pasteur, Paris, France) then discussed galactose metabolism in *C. neoformans*. He found that UDP-glucose epimerase 1 (UGE1), but not UGE2, was necessary for virulence. Moreover, UGE1 is temperature regulated, raising the question of whether it is part of the stress response.

The next speaker, Jennifer Lodge (Saint Louis University School of Medicine, USA), discussed the fungal cell wall, of what is "underneath the capsule". She emphasized that *C. neoformans* is known to have alfa-1,3-glucans, beta-1,3-glucans, beta-1,6,-glucans, >50 predicted GPI-anchored mannoproteins, chitin and chitosan. Unknown still is whether *C. neoformans* makes beta-1,4-glucans. A genome-wide search for putative enzymes involved in cell wall synthesis reveals 8 chitin synthases, 3 chitin synthase regulators and 4 chitin deacetylases. The function of each and the reason for this apparent redundancy are being explored by making deletion strains.

Two speakers chosen from the submitted abstracts concluded the session. Michael Botts (University of Wisconsin-Madison, USA) demonstrated a new density gradient technique which yielded basidiospores that were 90% viable. As basidiospores are postulated by some to be the infectious propagule, Dr. Botts characterized their surface composition. He found evidence for a thin layer of GXM as well as partially exposed beta-glucans and mannans. The session concluded with a presentation from Karen Wozniak (University of Massachusetts Medical School, Worcester, USA) examining intracellular events following phagocytosis of *C. neoformans* by dendritic cells. *C. neoformans* traffics to compartments containing endosomes and lysosomes. Moreover, crude lysosomal extracts from dendritic cells potently killed the fungus in a dose-dependent manner.

Stuart Levitz

West Meets East

A report of the epidemiology and clinical manifestations of cryptococcosis in the Far East, Europe, the United States and Australia

Presentations in this session allowed a comparison of the similarities and differences in cryptococcosis in China (Jianghan Chen and Zhu Yanjie), Korea (Jun Hee Woo), Japan (Yoshitsugu Miyazaki), Thailand (Khuanchai Supparatpinyo), South Africa (Tom Harrison), the USA (Peter Pappas), France (Françoise Dromer) and Australia (Tania Sorrell), and stimulated discussion around clinical questions that are still unanswered. Selected topics of interest are summarized below.

Non-AIDS associated cryptococcosis is currently much more common than that associated with AIDS in China, Japan and Australia. HIV-associated cryptococcosis has more than halved in the US and France since the advent of highly active antiretroviral therapy. In contrast, the overwhelming majority of cases in Thailand and South Africa remain HIV-associated. Though the incidence in Thailand has declined since the introduction of HAART, cryptococcosis is still the third commonest opportunistic infection in HIV-infected patients. In South Africa, despite the roll-out of antiretroviral therapy programs, many patients with HIV present late with low CD4 counts, hence the incidence of cryptococcosis appears little changed. In fact *Cryptococcus neoformans* is the commonest cause of adult meningitis in areas of high HIV seroprevalence, especially in Southern and East Africa. Among other classical causes of immunocompromise, solid organ transplan-

Tania Sorrell

Khuanchai Supparatpinyo

tation is now of particular importance (but not haematopoietic stem cell transplantation). In the US, cryptococcosis is the third commonest invasive fungal infection in recipients of solid organ transplants, often presenting more than 12 months post- solid organ transplantation.

C. neoformans serotype A is the predominant pathogen in all countries. In Australia, where infection due to *C. gattii* is endemic and affects apparently healthy individuals, approximately half of the cases in this group are still caused by *C. neoformans*, whereas in other countries reporting *C. gattii* infection, the proportion of cases in HIV-negative individuals is lower. Notably, data from British Columbia, Canada, which is the site of the most recent outbreak of cryptococcosis due to *C. gattii*, were not presented in this session. In France, serotype D causes a significant minority of cases compared with serotype A but is infrequent outside of Europe.

A number of presenters noted that cirrhosis/chronic liver disease, diabetes mellitus and/or end-stage renal disease were present in a significant number of non-AIDS associated cases, though in the absence of case control or prospective studies it was not always clear that these constituted independent risk factors for cryptococcosis. Nevertheless several speakers spoke to their clinical impression that outcomes are significantly worse in patients with cirrhosis, due largely to poor tolerance of the best therapeutic regimens in this group. More work to elucidate optimal therapy is required.

There was a consistent finding that abnormal mental status at presentation is associated with a worse outcome regardless of therapy. It was of interest to hear that 22% of patients in Shanghai present with optical disease, significantly higher than in Western countries represented. A similarly high rate of papilloedema/optic neuritis was reported from Papua New Guinea prior to the AIDS epidemic and was attributed to a typically late presentation of illness. Other statistically significant poor prognostic markers in CNS diseases included underlying cirrhosis and high CSF protein (Korea) and CSF cryptococcal antigen >512, infection with serotype A rather than D and failure to receive 5-flucytosine as part of the induction antifungal therapy (France).

Several delegates raised the possibility that differences in epidemiology, clinical features and response may result from individual genetic and/or ethnic genotypic differences. There was general agreement that further investigation of host determinants is warranted.

Approaches to induction therapy for cerebral cryptococcosis varied depending on the availability of drugs in different countries. In those where amphotericin B and 5FC are available this combination was generally considered to be the treatment of choice. In organ transplant recipients in the US, liposomal AMB is preferred to conventional AMB because of the high risk of renal failure in this group. In Thailand, where 5FC is not available and most cases are associated with AIDS, an initial 2-week course of AMB is followed by 400mg/d of fluconazole for 8 weeks and then maintenance fluconazole therapy is used. The high prevalence of cryptococcosis and HIV co-infection in Thailand is the rationale for the unique recommendation that primary prophylaxis be given. Itraconazole, which is also effective against another problematic opportunistic fungus in Northern Thailand, *Penicillium marneffei*, is recommended for those with a CD4 lymphocyte count <100.

Two speakers, Zhu Yuanjie and Tania Sorrell, emphasized the prolonged time that more than one of CSF glucose, leukocyte count, India Ink stain and cryptococcal antigen titre remain positive, even with successful therapy. It can be concluded that a better means of monitoring the therapeutic response to allow individual optimization of therapeutic regimens is needed.

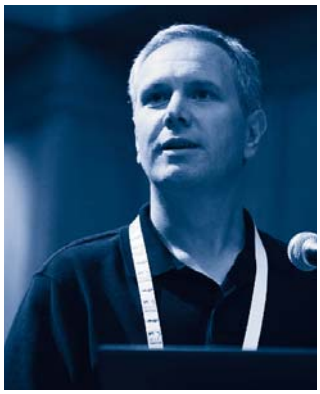
Tania C. Sorrell

Nagasaki, Japan
11-14 September 2008

Gene Regulation and Signaling

Two sessions on gene regulation and signaling highlighted the complexities of the signaling pathways in *Cryptococcus neoformans*. For example, a host-specific signal like elevated temperature leads to a myriad of responses including altered shape, size and volume. Andrew Alspaugh (Duke University School of Medicine, USA) presented nice data demonstrating that the differential localization of Ras1, either to the cell surface or to endomembranes, may represent a mechanism by which *C. neoformans* Ras1 can specifically activate distinct signaling pathways in response to different upstream signals. Ping Wang (Louisiana State University Health Sciences Center, New Orleans, USA) showed that Crg2, a regulator of G protein signaling in *C. neoformans* functions as a multiregulatory protein that controls mating distinctly from Crg1 and also inhibits Gpa1-cAMP-dependent signaling. Among the repertoire of responses to different stresses is a calcium-mediated signaling pathway that promotes survival of *C. neoformans* to ergosterol biosynthesis inhibitors. Angie Gelli (University of California, USA) presented evidence demonstrating that the calcium channel Cch1-Mid1, a central mediator of this pathway is activated by the depletion of ER/secretory calcium stores and demonstrated the possibility of identifying inhibitors of Cch1-Mid1 that could function to promote fungicidal activity of ergosterol inhibitors such as azoles. Interestingly and perhaps not surprisingly some signaling pathways may operate differently in *C. gattii*. Sudha Chaturvedi (Wadsworth Center, USA) presented evidence suggesting that several genes in *C. gattii* involved in oxidative stress, mating and secretion appear to be functionally and structurally different when compared to the same genes in its sibling species, *C. neoformans*. These unique differences between similar sets of genes could constitute diverse virulence mechanisms in *C. gattii*.

Angie Gelli



Jim Kronstad

June Kwon-Chung

Andrew Alspaugh (right) and Guilhem Janbon

Studies in cell signaling would benefit enormously from more genetic and biochemical tools such as the ones being developed by Hiten Madhani's group (University of California-San Francisco, USA). Among the newest developments are DNA tiling microarrays for *C. neoformans*. These arrays go beyond the measurement of mRNA expression levels because they can uncover characteristics of large-scale chromosome function and dynamics as well as promote discoveries of regulatory pathways. The usefulness of genome-wide approaches that identify and characterize global patterns of gene expression was evident in the nice work presented by Jim Kronstad (University of British Columbia, Canada) where he discussed the use of combined transcriptional profiling by serial analysis of gene expression (SAGE) and DNA microarray analysis in understanding the role of iron as a regulator of virulence factor expression and as a central nutrient during infection. DNA microarrays were also used to study the mechanism of heteroresistance in *C. neoformans*. In work presented by June Kwon-Chung (NIAID, NIH, Bethesda, USA), transcriptome comparisons between H99 and clones resistant to fluconazole revealed several hundred genes that were upregulated in the resistant subpopulations. Many of the differentially regulated genes were located on chromosome A and L and both chromosomes were duplicated in resistant clones. Heteroresistance appears to be independent of Hog1 signaling, unlike the cellular response to other types of stresses.

Angie Gelli

7th ICCC

Nagasaki, Japan
11-14 September 2008

Epidemiology, Molecular Typing, Population Genetics

Teun Boekhout

A number of presentations illustrated the recent advances made in the fields of taxonomy, epidemiology, molecular typing and population genetics. In a summarizing presentation Teun Boekhout (CBS Fungal Biodiversity Centre, Utrecht, The Netherlands) described the presence of six monophyletic lineages present within the *Cryptococcus neoformans* species complex that may represent separate species. Based on nuclear genes these lineages were found to be genetically isolated, but apparently recombination has been observed to be present in *C. gattii* in some mitochondrial genes. Moreover, interspecific *C. neoformans* x *C. gattii* hybrids were discussed. Dee Carter (University of Sidney, Australia) addressed the issue of sexual recombination in *C. neoformans.* Most *Cryptococcus* populations show a highly unbalanced mating type ratio, yet the infectious agent is thought to be a sexually generated basidiospore. Intererestingly, recombining populations were observed to occur by analyzing small clusters of isolates occurring on phylograms based on AFLP analysis. *C. neoformans* isolates belonging to the VNI and VNII genetic types isolated from small animals in the Sydney region, showed recombination. Apparently, recombination occurs in the environment in both *C. neoformans* var. *grubii* and *C. gattii*. Reiko Ikeda (Meiji Pharmaceutical University, Japan) presented her novel work on the interaction between cryptocccal cells and those of *Staphylococcus aureus*. Coculturing with *S. aureus* resulted in killing of cryptococcal cells. This mechanism seems dependent on the biochemical composition of the cryptococcal capsule and the presence of triosephosphate isomerase (TPI) present in the cell wall of *S. aureus*. Following adherence of *S. aureus*, apoptosis-like cell death is induced in *C. neoformans* cells. Massimo Cogliati (University of Milan, Italy) presented date on a novel serotype C population of *C. gattii* that belonged to genotype VGIV in India. Using Multi Locus Sequence Typing (MLST) analysis the Indian isolates could be linked to a population in Botswana.

Anastasia Litvintseva

Reiko Ikeda

In a presentation on behalf of several South American colleagues, Elisabeth Castañeda (Universidad El Bosque, Bogota, Colombia) presented evidence on the presence of the VGII genotype of *C. gattii* (6.2% of the isolates) in South America, especially in the Amazon area in Brazil and in Colombia. Both *C. neoformans* var. *grubii* and

Massimo Cogliati (left) and Wieland Meyer

C. gattii were detected on various species of trees. Jianghan Chen (Second Military Medical University, Shanghai, China) reported on the high incidence of *C. neoformans* var. *grubii* among apparently immunocompetent patients in China. Anastasia Litvintseva (Duke University Medical Center, Durham, USA) presented a highly interesting hypothesis suggesting that genotype VNB and VNI-B isolates of *C. neoformans* var. *grubii* originated in Southern Africa, where they occupy

Elisabeth Castañeda and David Ellis

a specific ecological niche, especially related to the mopane (*Colophospermum mopane*) tree. VNI isolates, in contrast, seems to be imported in Africa by migration of humans and/or birds (especially pigeons). Different subpopulations of VNB are geographically isolated, they show recombination within the environment, and hybridization occurs between strains of VNI and VNB. Finally, Wieland Meyer (University of Sidney, Australia) showed his results of an ongoing effort to study the global molecular epidemiology of the *C. neoformans* species complex. Using PCR-fingerprinting and MLST eight major molecular types were observed, including two subtypes in *C. grubii* and the serotype AD hybrids. In addition, evidence was presented that the high and low virulent genotypes VGII-A and VGII-B also occur in South America, and that these may be ancestral to the Australasian populations as well as the population causing the Vancouver Island outbreak.

Teun Boekhout

Nagasaki, Japan
11-14 September 2008

7th ICCC

Virulence Factors

During the last two decades we have learned much about the pathogenesis of cryptococcosis and yet the understanding of the molecular mechanisms of pathogenicity of *Cryptococcus neoformans* in the contest of the status of the host immune response is only very recently taken under consideration. There is now a consensus that the virulence factors of *C. neoformans* are not static components of its pathogenic fitness but rather fungal features that change dynamically during the infection perhaps according to the host environment in which the fungus is located.

The session on "Virulence factors", have shed some light on possible mechanisms by which this fungus adapts to host environments.

Peter Williamson (University of Illinois at Chicago, USA) talked about the autophagic process of *C. neoformans* and suggested that, during starvation conditions in the host, autophagy may allow the fungus to survive within host macrophages (1). The intracellular compartment is a nutrient deprived environment and *C. neoformans* responds by inducing mechanisms, such as the autophagic process, that would allow it to grow.

Maurizio Del Poeta

Interestingly, this process may also favor the survival of the fungus within macrophages during the latent infection.

Bettina Fries (Albert-Einstein College of Medicine, USA) talked about the phenotypic switching of *C. neoformans* and its contribution to virulence (2). During infection, this fungus can undergo reversible switching from a smooth parent to a mucoid variant. Interestingly, the mucoid is more virulent than the smooth variant. Dr. Fries identified two genes (ALL1 and ALL2) that are downregulated in the mucoid variant and, thus, deletion of these genes significantly enhanced viru-

Tamara Doering and Joe Heitman

Julianne Djordjevic

References

1. Hu, G., M. Hacham, S. R. Waterman, J. Panepinto, S. Shin, X. Liu, J. Gibbons, T. Valyi-Nagy, K. Obara, H. A. Jaffe, Y. Ohsumi, and P. R. Williamson. 2008. PI3K signaling of autophagy is required for starvation tolerance and virulence of *Cryptococcus neoformans*. *The Journal of clinical investigation* 118:1186-1197.
2. Jain, N., L. Li, Y. P. Hsueh, A. Guerrero, J. Heitman, D. L. Goldman, and B. C. Fries. 2008. Loss of Allergen1 (ALL1) confers a hypervirulent phenotype that resembles mucoid switch variants of *Cryptococcus neoformans. Infection and immunity*.
3. Siafakas, A. R., T. C. Sorrell, L. C. Wright, C. Wilson, M. Larsen, R. Boadle, P. R. Williamson, and J. T. Djordjevic. 2007. Cell wall-linked cryptococcal phospholipase B1 is a source of secreted enzyme and a determinant of cell wall integrity. *The Journal of biological chemistry* 282:37508-37514.
4. Garcia, J., J. Shea, F. Alvarez-Vasquez, A. Qureshi, C. Luberto, E. O. Voit, and M. Del Poeta. 2008. Mathematical modeling of pathogenicity of *Cryptococcus neoformans. Molecular System Biology* 4:183-195.
5. Savageau, M. A. 1969. Biochemical systems analysis. II. The steady-state solutions for an n-pool system using a power-law approximation. *J Theor Biol* 25:370-379.
6. Savageau, M. A. 1969. Biochemical systems analysis. I. Some mathematical properties of the rate law for the component enzymatic reactions. *J Theor Biol* 25:365-369.
7. Torres, N. V., and E. O. Voit. 2002. *Pathway analysis and optimization in metabolic engineering*. Cambridge University Press, Cambridge, UK.

lence. Studies addressing how these genes are regulated during the infection are underway.

Julianne Djordjevic (University of Sidney, Australia) talked about cryptococcal phospholipases and the fungal secretory pathway (3). This pathway is essential for making an intact cell wall, capsule and exporting key virulence factors, such as laccase and phospholipase B1. Dr. Djordjevic has identified fungal proteins such as phosphatidylinositol-specific phospholipase C (PI-PLC) and potentially beta glucanases, as key regulators of the secretory mechanism and the establishment of cryptococcal cell wall integrity.

Finally, my talk was about the mathematical modeling of cryptococcal pathogenicity (4). We recently created a mathematical model of the Cn sphingolipid pathway within the framework of the Biochemical System Theory (BST), which uses power-law representations for enzymatic and transport processes (5-7).

Bettina Fries

On the basis of predictions using the mathematical model empirical results, we were able to study and validate the changes in the sphingolipid pathway when *C. neoformans* cells switches form the extra- to the intracellular environment and to propose new mechanisms of intracellular adaptation.

Maurizio Del Poeta

Nagasaki, Japan
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7th ICCC

Tissue Tropism

The studies presented at the Tissue Tropism session explored different mechanisms by which cryptococci are enabled to persist in the lung, and to gain access to the brain and survive within the CNS. The opening talk by Ambrose Jong (Keck School of Medicine, Los Angeles, USA) revealed an exciting mechanism that could contribute to high cryptococcal CNS tropism. His studies explored the concept of blood brain barrier invasion in the context of a direct interaction of brain microvascular endothelial cells with hyaluronic (HA) present in *Cryptococcus neoformans* capsule. These studies reveled a relationship between HA synthesis by *C. neoformans,* HA retention in the cryptococcal capsule and the engagement of HA with host's CD44 molecules to achieve firm adhesion between the organism and the host's

Michal Olszewski

Françoise Dromer

Yun Chang

endothelial cell. Furthermore, the interaction of HA with CD44 triggered signaling pathway that lead to phosphorylation of protein kinase alpha, down-stream recruitment of beta-actin to the endothelial membrane rafts and their rearrangement. Dr Yong proposed that *C. neoformans* exploits this pathway, to transverse across the endothelium in a "zipper like" fashion following firm adhesion to the endothelial cell surface.

Françoise Dromer (Institut Pasteur, France) presented evidence for another mechanism of *C. neoformans* crossing from blood into the brain. In a series of elegant studies she demonstrated that *C. neoformans* can exploit monocytes to cross blood brain barrier as a "passenger" carried by these cells. The proof for this "Trojan Horse" hypothesis was demonstrated by comparing intravenous inoculations of the yeasts in a free form with injections of yeasts that were ingested by cultured monocytes. Inoculation of the "Trojan Horses" enhanced fungal burden in the brain, while the sustained phagocyte depletion from the blood decreased the rate of cryptococcal blood to brain crossing in this model.

The role of cryptococcal virulence factor urease on pulmonary growth of *C. neoformans* and during CNS invasion was demonstrated by Michal Olszewski (University of Michigan, Ann Arbor, USA). Studies of his group demonstrated that urease expression enhances cryptococcal persistence in the lungs, by potentiating the non-protective Th2-arm of the T cell mediated immune response. Deletion of urease gene from *C. neoformans* resulted in a 100-fold decrease in lung burden, reduced Th2 cytokine expression/IgE production, and an absence of the Th2-driven pathology in the lungs of C57BL6 mice. Furthermore, this study suggested that dentritic cells are the upstream cellular target of urease-mediated virulence, as urease expression increased the frequency of immature dentritic cells in regional pulmonary lymph nodes. Dr. Olszewski also presented evidence that urease enhances blood-to-brain invasion by enhancing *C. neoformans* sequestration within small capillaries. These studies provided evidence that urease acts as virulence factor in both pulmonary and microvascular compartments, and thus promotes cryptococcal tissue tropism in both the lung and brain.

Lung and brain tissue represent extreme conditions in the host environment with respect to oxygen concentration. Metabolic shift from the growth in high (the lungs) to low oxygen conditions (brain) is required for cryptococcal brain invasion. Yun Chang (NIH, Bethesda, USA) explored genetic basis of *C. neoformans* adaptation to low oxygen environment using genetic screening of T-DNA insertional mutants. Multiple pathways that enabled *C. neoformans* growth in low oxygen levels have been identified, including genes regulating ergosterol biosynthesis, iron homeostasis, as well as mitochondrial functions and sensitivity to reactive oxygen species.

In summary, studies presented at the Tissue Tropism session of 7th ICCC, highlighted the progress in our understanding of mechanisms that contribute to the pathogenesis of cryptococcal infection. Some of these mechanisms, in particular the "Trojan Horse" hypothesis and the hypothesis for a direct interaction of *C. neoformans* with brain endothelial cells during CNS invasion were initially proposed as alternatives. However, the data presented at this conference were not conflicting. Both mechanisms were convincingly shown to be used by *C. neoformans* in different clinical circumstances. Future studies are likely to determine whether cryptococcal urease and the genes important in hypoxic conditions are important for *C. neoformans* survival in the "Trojan Horse" monocytes. These genes could also contribute to CD44 dependent and CD44-independent interactions of *C. neoformans* with the cerebral endothelium. In the future, we expect to learn about new cryptococcal genes and new mechanisms that aid *C. neoformans* persistence in the lungs and its CNS invasion. These studies will deepen our understanding of cryptococcal pathogenesis and provide a new opportunity for the development of novel therapeutic strategies.

Michal Olszewski

7th ICCC

Nagasaki, Japan
11-14 September 2008

Sex, Mating, and Evolution

Kirsten Nielsen

This session contained talks about micro evolutionary events, sexual reproduction in *Cryptococcus* and related species, and sexual development. James Fraser (University of Queensland, Brisbane, Australia) gave an intriguing talk on the role of subtelomeric regions in evolution and adaptation to new environments. *Cryptococcus* has karyotypic variation from strain to strain and relapse infections often display gross chromosomal rearrangements. Translocations, duplications, inversions, and deletions could provide a selective advantage, but would likely lead to sterility. Changes in subtelomeric regions would allow subtle changes that are tolerated during sexual development. Examination of sub-telomeric regions revealed genes associated with niche adaptation. The Fraser lab has characterized one of these regions on the right arm of chromosome 3 that contains many hexose transporters. They observed amplification of the arsenic transporter gene ARR3 with 3-18 copies of the gene. Arsenic is a toxic metalloid that is pumped out of cells by ARR3. Analysis of arsenic resistance revealed that strains with increased ARR3 gene copy number had higher resistance.

Joe Heitman (Duke University Medical Center, Durham, USA) presented a fascinating talk on the structure and evolution of the mating type locus in *Cryptococcus* and closely related species. *Cryptococcus* is a member of the basidiomycete phylum. Most basidiomycetes have a tetrapolar mating system with two unlinked loci. Yet *Cryptococcus* has a bipolar mating system with only a single locus. Analysis of this locus suggests it was generated by fusion of two loci. To better understand the mechanism of the evolution of the MAT locus, the Heitman lab has cloned and analyzed the mating loci from other closely related species. By characterizing the mating loci in *C. amylolentus* they have been able to identify strains of opposite mating type and characterized mating for this species. In addition, Dr. Heitman also presented additional data supporting the prevalence of same-sex mating in the environment and showed that it may be linked to a specific Sxi1alpha phenotype.

Emilia Kruzel from Christina Hull's lab (University of Wisconsin-Madison, USA) presented an elegant study to characterize gene expression during sexual development using microarray analysis. They identified early, intermediate, and late genes and also found spatial differences in expression of many genes known to be involved in mating. For example, pheromone gene expression was up-regulated early in the mating process and then repressed later in sexual development. They also identified a class of unknown genes which may be involved in dikaryotic growth.

There were also a few talks in other sessions which relate to sex, mating, and evolution. Dee Carter (University of Sydney, Australia) described population genetic studies which show evidence of recombination and same-sex mating in limited geographic or temporal ranges suggesting that sex and spore production are probably common in the pathogenic *Cryptococcus* species but may be masked by clonal expansion. Kirsten Nielsen (University of Minnesota, Minneapolis, USA) showed coinfection with both mating types blocks central nervous system penetration by one of the sexes in a pheromone dependent manner. The pheromone signaling results in giant cell production which may alter host cell interactions to affect virulence. Finally, Michael Botts, from the Hull lab, showed that spores are more resistant to environmental stresses. Electron microscopy revealed that the spore coat contains capsule which plays a role in proper sexual development and spore dispersion. These studies underscore the importance of sex and evolution in many aspects of *Cryptococcus* biology and virulence.

Kirsten Nielsen

James Fraser

Joe Heitman

Nagasaki, Japan
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Clinical Sessions

First Session

Robert Larsen, from the University of Southern California, discussed a new method of susceptibility testing for *Cryptococcus neoformans* and attempts to correlate results with clinical outcomes. The fundamental approach to his method, which has not been validated in clinical trials, is based on the concept that quantitative cultures are a useful tool in assessing mycologic response, and that susceptibility testing should be linked to quantitative cultures providing more of a "continuum" rather than a simple 'susceptible or 'resistant' result. Dr. Larsen has not been able to correlate these data with clinical outcome, but his hypothesis is that the current method of susceptibility testing is somewhat flawed, and that relationship between susceptibility data and clinical outcome should be further explored.

Li-Ping Zhu, from Fudan University in Shanghai, reported a retrospective 11-year survey of non-HIV infected patients who have been diagnosed with cryptococcal meningitis. During this 11-year period spanning the years 1997 through 2007, 154 cases of non-HIV-associated cryptococcal meningitis were diagnosed at this institution. Surprisingly, only 27% of patients had significant underlying conditions. Out of 72 cases who underwent CD4 lymphocyte testing, only 25% were found to have counts >200 cells/mm^3. Most patients received initial therapy with amphotericin B with or without 5-flucytosine, and there was approximately 20% mortality at the end of antifungal therapy (10 weeks). About 60% of these deaths were attributable to cryptococcal meningitis. The authors note that clinical manifestations in immunocompromised patients were less severe than their "normal" counterparts, and that response to therapy was significantly higher in immunocompromised patients than otherwise normal patients (P = 0.046). The mortality was similar for immunocompromised and normal patients. Dr. Zhu's interesting findings suggest that the incidence of non-HIV-associated cryptococcal meningitis is increasing in China and that treatment outcomes tend to be better among immunocompromised compared to otherwise normal individuals.

Peter Pappas, from the University of Alabama at Birmingham, discussed cryptococcosis among transplant recipients. Most of Dr. Pappas' discussion focused on the results of TRANSNET, a prospective surveillance program among 25 US transplant centers, which was conducted between 2001 and 2006. During that time, 98 cases of transplant-associated cryptococcosis were identified. Dr. Pappas underscored the importance of this infection among solid organ transplant recipients and indicated that three-month mortality for this disorder was approximately 25%, among the lowest of the invasive fungal infections in this vulnerable population. He emphasized that cryptococcosis remains an important complication in the late post-transplant period in solid organ transplant recipients.

Tania Sorrell, from the University of Sydney, discussed cryptococcal phospholipase B as a potential antifungal drug target. Cryptococcal phospholipase B1 facilitates invasion of the lung and is essential for hematogenous dissemination of infection. Using structure-activity relationship experiments, the author attempted to correlate antifungal activity of several compounds through inhibition of phospholipase B1. Interestingly, miltefosine, an anti-protozoan agent with broad-spectrum fungicidal activity, inhibited phospholipase B1 activity, but only at concentrations greater than six times the MIC, suggesting that phospholipase B1 inhibition is not its primary mode of action. Other compounds demonstrated significant antifungal activity, but this was restricted to yeasts. The most promising of these compounds was miltefosine.

Peter G. Pappas

Peter Pappas

Li-Ping Zhu

Second Session

John Bennett (NIAID, Bethesda, USA) opened the session by providing a historical overview of clinical trials in cryptococcal meningitis (CM), and outlining key gaps in our knowledge of its management. The evidence of the need for a second drug (5FC) in combination with amphotericin B was reviewed, as well as the timing of switch to, and best azole to use as maintenance therapy. More information is required on the optimal treatment of raised CSF opening pressure and the ideal time to start antiretroviral therapy post diagnosis of CM, as well as better definitions and guidelines for management of cryptococcal immune reconsti-

tution inflammatory syndrome.

Previous studies have shown a relationship between baseline CSF opening pressure and outcome in cryptococcal meningitis. Tihana Bicanic (St George's University of London, UK) presented findings from three studies of HIV-associated CM from Thailand and South Africa (n=163), showing that aggressive management of raised CSF opening pressure using repeat lumbar punctures over the first 2 weeks of treatment resulted in no significant differences in mortality at 2 and 10 weeks between patient groups categorized according to baseline opening pressure (<20, 20-30, >30cm H_2O). Opening pressure correlated with fungal burden, both at baseline and day 14 of treatment.

Tom Harrison (St George's University of London, UK) concentrated on important issues in the treatment of CM in developing countries. In phase II studies, rate of clearance or early fungicidal activity has been shown to be a suitable marker of treatment response. Pooled data from studies in Thailand, Uganda and South Africa (n=262) demonstrate that a poor rate of clearance is independently associated with mortality at 2 and 10 weeks. In places without facilities to administer amphotericin B, studies of the best oral treatment regimen have shown best rate of clearance and good tolerability of oral fluconazole at 1200mg/day. A study in Malawi is comparing this dose alone with a

Tihana Bicanic

combination with oral flucytosine. Given the high frequency of impaired conscious level at presentation, earlier diagnosis is paramount and a collaboration is planned to develop a urinary cryptococcal antigen test for outpatient screening. Campaigns to improve access to drugs (amphotericin B, flucytosine) and CSF manometers are ongoing.

Peter Pappas (University of Alabama at Birmingham, USA) presented the findings from an open-label phase II randomized study of CNS cryptococcosis conducted in Thailand and USA (n=140), comparing amphotericin B at 0.7 mg/kg alone versus in combination with fluconazole 400 or 800mg/d for 14 days, followed by maintenance fluconazole. All three arms were well tolerated. At day 14, successful outcome (composite clinical/ mycological endpoint) was seen in 41%, 27% and 54% of patients in the 3 arms respectively. There was a trend towards better outcomes in the combination arms at 6 and 10 weeks. The results should be validated in a phase III trial.

The ensuing discussion focused on the need for collaborative international efforts and mobilization of political will and funding to address the above questions, probably in the form of a Phase III trial including both developed and developing countries, coupled with primary prevention in the form of antigen screening and targeted primary fluconazole prophylaxis.

Tihana Bicanic

Tom Harrison

John Bennett

Nagasaki, Japan
11-14 September 2008

7th ICCC

Genomics Workshop

Eddie Byrnes representing the Heitman laboratory from Duke University opened the session with a discussion on the variation within the *Cryptococcus neoformans* var. *grubii* type strain H99. Gaining insights into *C. neoformans* var. *grubii* virulence mechanisms and genomic architecture through a detailed analysis of passaged H99 isolates. Since the "birth" of isolate H99 on February 14th, 1978 many things have happened. The isolate lost virulence through lab passage (possibly multiple independent times), was passaged through a rabbit to increase virulence, and distributed globally to many labs. Some version of this isolate was used to sequence the genome, construct a congenic strain pair (KN99a/alfa), construct large-scale mutant libraries (Madhani/Lodge), and most recently used to construct a tiling array. This has been the major type strain for serotype A, and has been used in countless publications over the last 2 decades. Acknowledging and understanding the differences between these passaged isolates, and increasing awareness of isolate choice for experiments is important for many future studies. To examine differences in phenotype and genotype, we are conducting classical genetic experiments with mating and artificial diploid construction, comparative genomic hybridizations with NimbleGen (Heitman and Kronstad), cDNA microarray studies in YPD, CSF, and L-DOPA medias (Perfect and Heitman), murine virulence experiments (Lodge), and whole genome sequencing of several variants with single and paired end Solexa sequencing (Dietrich). It is our goal that this focus will allow a greater understanding of genetic, and possibly epigenetic determinants of virulence, and mating.

Jim Kronstad from the University of British Columbia followed with a description of analysis of the *C. gattii* genomes. He utilized comparative genome hybridization (CGH) to compare gene copy number among *gattii* isolates with different virulence. Although changes in copy number were seen, there was not a clear correlation between virulence and a specific locus. CGH analysis of serotype A strains also revealed a large number of changes so that it is difficult to pick out specific changes to test. Disomy of chromosome 13 may be relatively common, and may result in lack of melanization.

Cheryl Chun from the Madhani laboratory at University of California, San Francisco, then described the high throughput gene deletion project. The lab generated over 1200 deletion strains, and these were tested *in vitro* and *in vivo* using a mixed model of infection. They found 164 strains with defects in growth in the lung. Many of these strains have defects in growth at 37°C, melanin or capsule, but approximately one fourth have no known defects, and may represent novel mechanisms. She also described a putative transcription factor, GAT201, that may regulate many of genes involved in virulence. The Madhani lab gene deletion set is available from the ATCC and the Fungal Genetics Stock Center.

Kim Gerik from the Lodge laboratory at Saint Louis University described a bioinformatics approach to identifying signaling components for the cell integrity pathway. She and colleagues have identified 25 potential signal transduction proteins through domain searches. Gene deletions have identified ten potential candidates that could be responsible for signal transduction of stresses including cell wall stress as well as oxidative and nitrosative stress. The Lodge lab gene deletion set is available from the Fungal Genetics Stock Center.

Jennifer Lodge

Eddie Byrnes

Tamara Doering from Washington University described progress by the microarray consortium. Arrays with *C. neoformans* var. *neoformans* genes have been available through the Washington University Genome Center since 2005. Many of the probes also hybridize to var. *grubii* genes. Now that the annotations for the var. *grubii* strain H99 have been updated, the arrays are currently being augmented with probes specific for H99, as well as mating type loci genes, and additional var. *neoformans* probes. The probes are ready for printing, and a new .gal file generated by Steve Giles in Christina Hull's laboratory is available. The Broad will be releasing a new annotation of H99 soon.

Cheryl Chun

Fred Dietrich

Teun Boekhout from Utrecht University, provided AFLP and MLST evidence for six distinct molecular types (VNI/II/B, VNIV, VGI, VGII, VGIII, and VGIV) that likely represent cryptic species within the *C. neoformans/C. gattii* species complex. This evidence is being marshaled to support a proposal that *C. neoformans* var. *neoformans* and *C. neoformans* var. *grubii* be recognized as distinct species rather than simply as varieties, and similar proposals should be forthcoming assigning four species within *C. gattii* (VGI, VGII, VGIII and VGIV). Genomic sequences are currently available for VNI (H99), VNIV (JEC21 and B3501A), VGI (WM276) and VGII (R265) and representatives of each other lineage (expect the VGIII AD hybrid lineage) should be advanced for sequencing. In addition, in select lineages it will likely be advantageous to sequence other isolates, such as for the VGII Vancouver Island Outbreak lineages VGIIa major (R265, already available) and VGIIb minor (R272), for example. In addition, closely related nonpathogenic species such as *Cryptococcus amylolentus*, recently discovered to have a novel teleomorphic form (*Filobasidiella amylolenta*) should also be sequenced for comparisons. This robust genomic database would considerably advance the field.

Fred Dietrich from Duke University discussed Solexa, the next generation of DNA sequencing. Sequences obtained from Solexa sequencing can be problematic to assemble since the sequence reads are so short. He described an approach that he is applying to the fungal pathogen of cotton, *Ashbya gossypii*, which utilizes paired end reads and improves assembly.

Sarah Brown from the Lodge laboratory at Saint Louis University described a proteomic approach to examining the response to oxidative stress in *Cryptococcus*. There were very few changes in the *Cryptococcus* proteome induced by exposure to oxidative stress compared to the response to nitrosative stress.

Overall there was excellent progress reported on gene deletions, analysis of strains with differences in phenotypes, and availability of microarrays (http://genome.wustl.edu/activity/ma/cneoformans/). *C. neoformans* deletion strains sets (UCSF or SLU) are available through the Fungal Genetics Stock Center (http://www.fgsc.net/) at low cost. There was discussion regarding the pressing need for a central database to house genomic, annotation, microarray, gene deletion, proteomic and other related data.

Jennifer Lodge

Joe Heitman, Jim Kronstad and Jennifer Lodge

Report on...

3rd Advances Against Aspergillosis

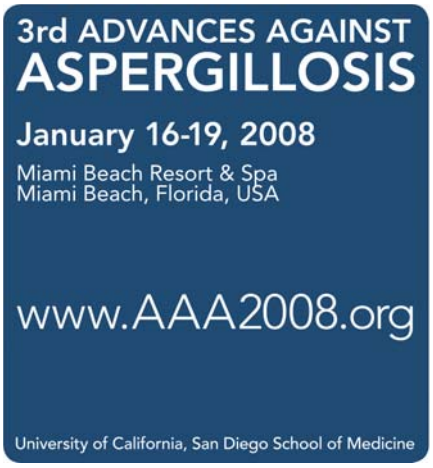
The 3rd Advances Against Aspergillosis international meeting was in Miami, Florida, January 16-19, 2008. The scientific program proved busy and comprehensive with 13 plenary sessions and 3 satellite symposia. In addition, two poster sessions were held to accommodate presentation of the 95 posters accepted for presentation.

The program covered molecular diagnostics, fungus-host-interactions, *Apergillus*-sinusitis, controlling *Aspergillus* in building up to *Aspergillus* and asthma. The topics provided a huge impetus for basic and translational research. Herein we will give a short overview on selected topics.

In the session 'Pathogenesis and clinical manifestations in different hosts' Brahm Segal (Roswell Park Cancer Institute, USA) focused on the role of *Aspergillus* in chronic granulomatous disease (CGD). He explained that CGD is an inherited disorder of the NADPH oxidase complex in which phagocytes are defective in generating superoxide anion. As a result of the defect in this key host defense pathway, CGD patients suffer from recurrent life-threatening bacterial and fungal infections. Invasive aspergillosis is the most important cause of mortality in CGD. He underlined also, that there exists important differences between invasive aspergillosis in CGD compared to other immunocompromised conditions. In neutropenic patients, invasive pulmonary aspergillosis is characterized by hyphal angioinvasion, coagulative necrosis and paucity of inflammatory cells. In contrast, angioinvasion is not a feature of invasive aspergillosis in CGD patients. CGD is also characterized by excessive inflammatory responses that are independent of the host defense deficit. These findings demonstrate a key role of NADPH oxidase in downregulating inflammation induced by specific ligands of pathogen recognition receptors. Recent studies point to defective tryptophan catabolism underlying impaired host defense and pathogenic inflammation in CGD. Moreover, Dr. Segal pointed out that prophylaxis with a mould-active agent should be offered to CGD patients. Itraconazole was safe and effective as prophylaxis in a randomized study. Bone marrow transplantation is curative in CGD, but is associated with expected frequencies of transplant-related morbidity and mortality.

Dimitrios P. Kontoyiannis (University of Texas, Usa) gave an overview on invasive aspergillosis and steroid-treatment. He reported that glucocorticosetroids (GCs) have pleiotropic effects on the immune system that account for the propensity of patients to potentially life-threatening invasive aspergillosis (IA). In addition, GC might enhance the "fitness" of the fungus to cause disease. Although the exact prevalence and attributed mortality of IA in GC-treated patients is difficult to assess, *Aspergillus* species are significant pathogens in patients with multiple myeloma, collagen vascular diseases, solid organ and especially allogeneic stem cell transplant recipients. In the latter setting, he told, high cumulative doses of GCs administered for graft-versus-host disease (GVHD) prophylaxis and/or treatment have been shown to be associated both with the risk of acquisition and the poor outcome of IA. There are distinct differences in the histopathologic features of invasive pulmonary aspergillosis in GC-induced immunosuppression compared to IA caused by neutropenia. The lesions in GC-associated IA consist mainly of neutrophilic and monocytic infiltrates, inflammatory necrosis, scant intra-alveolar hemorrhage and a paucity of hyphae and angioinvasion; in contrast, coagulative necrosis, intraalveolar hemorrhage, scant mononuclear inflammatory infiltrate and higher "burden" of invading hyphal elements is observed in granulocytopenic animals. Not suprisingly, the performance of non-culture based antigen detection diagnostic methods is suboptimal in GC-associated IA, because the fungal burden is low. He concluded with the following message: the severity of IA appears to be associated with the intensity of GC treatment therefore, every effort should be made toward the use of the lowest GC dose for the shortest possible time.

In the session '*Aspergillus* sinusitis' Arunaloke Chakrabat (Postgraduate Institute of Medical Education



& Research, Chandigarh, India) discussed in detail the controversies surrounding the categorization of fungal sinusitis. He pointed out that sinusitis, more accurately rhinosinusitis, is a common disorder affecting approximately 20% world population at some time of their lives. He stated that acute rhinosinusitis (ARS) is well categorized, yet controversies encompass chronic rhinosinusitis (CRS), especially the fungal rhinosinusitis (FRS). Based on histopathological findings, FRS can be divided into two categories: the invasive and non-invasive form, depending on invasion of mucosal layer. Three types of FRS are invasive: acute fulminant, chronic invasive and granulomatous invasive. The two non-invasive FRS disorders are fungal ball, and fungus related eosinophilic rhinosinusitis (of which allergic fungal rhinosinusitis (AFRS) appears to be a distinct disorder). Still, categorization of FRS remains controversial and open to discussion. Especially as it was suggested, that fungi might play an important role in CRS. Diversity of opinion exists on whether FRS should be characterized as an infection or an inflammatory condition. He pointed out, that currently there are more questions than answers concerning the categorization of FRS. Recognizing the problem several societies try to reach a consensus on these definitions. ISHAM has also formed a working group on 'Fungal sinusitis' to exchange ideas in the direction of resolving the problems.

In the session '*Aspergillus* species and strain differences' Corne Klaassen (Canisius Wilhelmina Hospital, Nijmegen, The Netherlands) gave on overview on how to best run molecular typing in *Aspergillus*. He stated, that only two molecular methods available, which are highly reproducible and yield unambiguous, user independent, typing data: the Multi Locus Sequence Typing (MLST) and the microsatellite analysis. Each of the two methods offers several advantages over the other. The main advantage of MLST is the DNA sequence format that is accessible to a growing number of clinical laboratories. DNA sequence data are easily compared and exchangeable between labs. Yet we have to know that the costs are high, the turn-around time long and the discriminatory power relatively low. The latter is a direct consequence of the relatively low mutation rate of a given DNA sequence due to the presence of mismatch repair mechanisms. Microsatellites are unique in their extremely high discriminatory power which is a direct result of the inherent instability, during DNA replication, of tandemly repeated DNA sequences. Fortunately, microsatellites are still sufficiently stable to allow longitudinal studies within an appropriate time window. However, interpretation of genotypically different isolates should be done with care and should take this instability into account. He underlined, that microsatellites are amendable to rapid and high-throughput analyses in a modular fashion allowing large numbers of isolates to be analyzed. The cons of microsatellite markers are that they are species specific. Basically, the choice for either of the two methods could be appropriate and should primarily be based on the exact reason for performing strain typing. He concluded that MLST seems to be most informative at the genus and/or population level whereas microsatellites are best used when high-resolution strain typing is required.

In the session 'T-cell immunity' Teresa Zelante from University of Perugia, Italy, gave on overview on host response signatures to invasive aspergillosis. She spoke on dendritic cells (DCs), which comprise several different forms or subsets, each having distinct receptors for antigen uptake and signalling, different pathways for antigen processing and different functional outcomes. *In vitro* studies suggested pulmonary DCs to be able to internalize *Aspergillus fumigatus* conidia and hyphae.

She concluded her presentation with the perspective that the IDO+DCs/Tregs axis has a protective role in fungal allergy and suggested that induction of IDO could be an important mechanism underlying the anti-inflammatory action of corticosteroids.

In the session '*Aspergillus* growth towards pathogenicity' Alessandro Pasquallotto (Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil) gave on summary on the clinical syndromes due to *A. fumigatus* and *A. flavus*. He stated that most of the information available about *Aspergillus* infections has been originated from *A. fumigatus*, the most frequent species in the genus. *A. flavus* is however particularly prevalent in regions of the world with dry and hot climate such as the Middle East and Sudan. Interestingly, *A. flavus* seems more virulent and more resistant to antifungal drugs than most of the other *Aspergillus* species, which has been demonstrated both *in vitro* and in animal models. He suggested that aflatoxin does not seem to be a major factor in the pathogenesis of *A. flavus* infections (*A. flavus* isolates produce aflatoxin B1, the most toxic and potent hepatocarcinogenic natural compound ever characterized). *A. flavus* is a common etiology of fungal sinusitis and cutaneous infections, but not fungal pneumonia. Only chronic cavitary pulmonary aspergillosis has rarely been associated with *A. flavus*. Although *A. fumigatus* is responsible for the vast majority of cases of allergic bronchopulmonary aspergillosis (ABPA), *A. flavus* has also been implicated in some series, mostly in India. The bigger size of *A. flavus* spores, in comparison to *A. fumigatus* spores, may favor their deposit in the upper respiratory tract. *A. flavus* also accounts for circa 80% of *Aspergillus* keratitis cases. Other clinical syndromes often linked with *A. flavus* include postoperative wound infections, *Aspergillus* osteomyelitis following trauma or inoculation, and chronic granulomatous sinusitis. Outbreaks of aspergillosis involving the skin, oral mucosa, or subcutaneous tissues are usually associated with *A. flavus*. He stated that most of these outbreaks have been associated with a single or a few different strains, which contrasts with what has been documented for infections caused by *A. fumigatus.*

Cornelia Lass-Flörl

Report on...

ECMM has convened three joint symposia with the International Union of Microbiological Societies during the XII Mycology Division Congress held in Istanbul from 5 to 9 August 2008. The symposia focused on Fungal biofilm, on Invasive fungal infections in the intensive care, and on Zygomycosis. Following the synopsis of the three symposia are reported

Three ECMM Symposia at the IUMS 2008

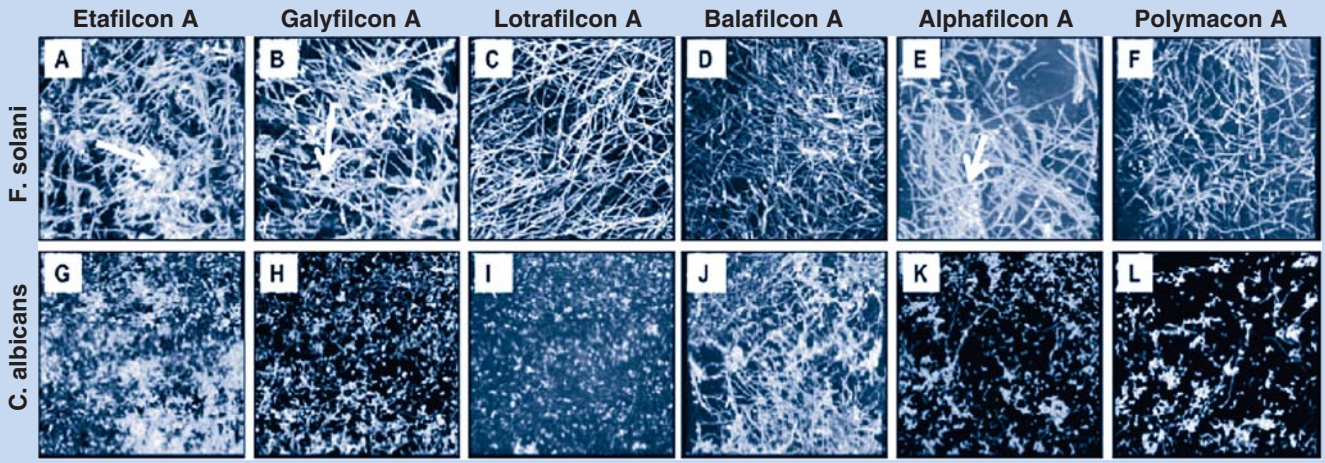
Fungal Biofilm: the New Frontier

Chairs: M. Ghannoum, J.P. Latgé

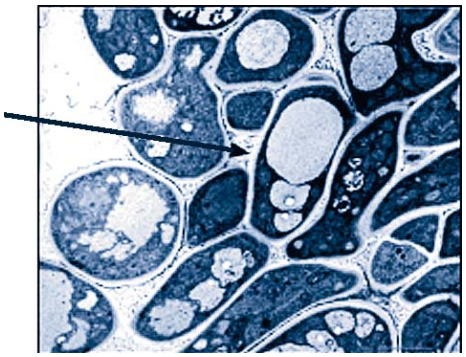
Biofilms are defined as a community of micro-organisms that are attached to a surface and embedded in an extracellular polysaccharidic matrix (ECM). Biofilms are particularly important in human pathology since growing as a multicellular community helps to colonize the substratum and resist external aggressions. Biofilms formed by the pathogenic yeasts *Candida* and *Cryptococcus neoformans* on medical devices show an increase in the MIC50 to almost all antifungals. Filamentous fungi are also forming biofilms. Recently many keratitis with contact lens were attributed to biofilm formation of *Fusarium* species. Extracellular material surrounding the *Aspergillus fumigatus* mycelium has been seen during growth in infected tissues or *in vitro* under static aerial conditions. This matrix is composed of galactomannan, melanin and other components specific to *in vivo* or *in vitro* conditions. *In vitro* alfa1,3 glucans, antigens and hydrophobins are present in ECM whereas *in vivo*, these components are only cell wall constituants, suggesting that ECM *in vivo* is constituted by other fungal components, still unknown, or by host-made molecules.

C. neoformans biofilm formation is dependent on the presence of the capsular polysaccharide glucuronoxylomannan (GXM) and correlated with the ability of GXM to bind the devices. The protective monoclonal antibodies IgG1MAb 18B7 directed against GXM prevents the biofilm formation by complexing the released GXM and inhibiting further cell binding to the surface. Complexing IgG1MAb 18B7 to Bismuth^{237} damages the biofilm by penetration of the antibody through the channels. However, combination therapy with amphotericin B has an antagonistic effect since the antibody links the capsule of the cells and prevents the penetration of the antifungal to the cells, suggesting that the host immune response may contribute

Mahmoud Ghannoum and Jean Paul Latgé



Confocal scanning laser microscopy analyses of *Fusarium* biofilms formed on different soft contact lenses, showing their varying architecture.
Fusarium or *Candida* isolates were allowed to form mature biofilms on different types of contact lenses: (A,G) etafilcon A, (B,H) galyfilcon A, (C,I) lotrafilcon A, (D,J) balafilcon A, (E,K) alphafilcon A, and (F,L) polymacon lenses. Bioflms were stained with ConA and FUN1 dyes, indicating the presence of polysaccharides and metabolic activity, respectively. (Imamura et al. (2008). *Antimicrob. Agents Chemother.* **52**(1): 171-182).



Aspergillus fumigatus biofilm in vitro. The arrow indicates extracellular matrix (ECM) in the biofilm

to drug resistance of biofilms formed *in vivo*.

For *C. albicans* biofilm formation, surface components such as O- and N- mannosylated glycoproteins (adhesins) are essential. They promote the attachment of the fungus to a surface. Two MAP kinases Mkc1p and Cek1pand their effectors Pmt1p and Pmt4p (O-mannosyltransferases) as well as Efg1p regulator (transcription factor regulating the adhesin synthesis) mediate a significant portion of responses to conditions required for biofilm formation, since deletion of *PMT1*, *PMT4* or *EFG1* genes prevented it. Transcriptomic analysis shows that Efg1p is also required for the induction of the genes implicated in hypoxic conditions such as the ones existing inside the biofilm and in deep host tissues.

C. albicans as well as *Fusarium* form biofilms on all types of lenses but the biofilm architecture varied with the lens type and with the strain as demonstrated with different *Fusarium* strains. The eradication of the contaminants by contact lens care solutions is highly dependent on the type of solution used. *Fusarium* and *C. albicans* cells in biofilms are resistant to several sterilizing solutions conversely to the planktonic state, suggesting a possible role for biofilms in the recent outbreaks of fungal keratitis.

Seeing the fungus as a separate entity is a complete remote idea. Pathogenic fungi have to be studied as populations since their behaviour *in vivo* will be totally different and their physiology will be totally depending on the extracellular matrix forming the biofilm in which these populations leave.

Mahmoud Ghannoum
Anne Beauvais
Jean-Paul Latgé

Invasive Fungal Infections in the Intensive Care

Chairs: J. Meis, L. Klingspor

The heterogenous population of severely ill patients admitted to an intensive care unit (ICU) shares a high susceptibility to nosocomial fungal infections. A symposium entitled "Invasive fungal infections in the intensive care" was organised by ECMM at the IUMS 2008 held in Istanbul last August. This symposium, chaired by Jacques Meis and Lena Klingspor, broached different topics: infections caused by *Candida* and by filamentous fungi, risk factors and prophylaxis, and antifungal therapy. The *ad interim* results of the ECMM survey on deep-seated *Candida* infections in ICU surgical patients were reported by Lena Klingspor, convenor of the Working Group.

An overview on yeast and mould infections in ICU patients was presented by Anna Maria Tortorano and Sevtap Arikan, respectively.

The gastrointestinal insults that may arise as a consequence of ICU management procedures are responsible for the vulnerability of these patients to haematogenous dissemination of *Candida* species, such as *C. albicans, C. glabrata, C. tropicalis*, that form part of their commensal flora of the gastrointestinal tract. The alteration of the skin barrier, as in the presence of IV lines, favours the acquisition of yeasts, such as *C. parapsilosis*, colonizing the patient's skin or the hands of the healthcare workers. In addition, the vascular catheters, as well as other implantable devices, may be hematogenous seeded by *Candida*, such as *C. albicans, C. glabrata* etc, coming from distant local infection. Formation of biofilm on implanted biomaterials increases resistance to antifungal agents, protects *Candida* from host defences, and causes failure of devices.

Infections caused by filamentous fungi, such as aspergillosis, fusariosis, zygomycosis, are now known to occur with increasing frequency in patients other than those with classical risk factors such as profound and prolonged granulocytopenia. Air and water are the most important sources of these infections. Inhalation of spores is the common route of infection by *Aspergillus*. Ventilation systems, contaminated air during renovation activity, water, food and ornamental plants remain the major reservoir of *Aspergillus* in hospital setting, although a significant number of patients acquires infection before hospitalization. Inhalation of spores, ingestion, trauma and non sterile wound dressing or other contaminated devices may lead to infection caused by other filamentous fungi such as *Fusarium* and zygomycetes.

Murat Akova analysed the published randomised clinical trials of antifungal prophylaxis with fluconazole in different adult patient populations in the ICU. *Candida* infection, as well as *Candida* colonization, occurred less frequently in the fluconazole group, but no differences in mortality could be demonstrated between the treatment and placebo group. A shift towards fluconazole-resistant isolates was not shown in any of the studies. Even according to the most recently published trial, an early empirical treatment was not useful. On the contrary, efficacy and safety of fluconazole prophylaxis was demonstrated in preterm infants at high risk of neurodevelopmental impairment associated to *Candida* bloodstream infection. To identify adult patients who will benefit by an early treatment, a prediction rule, based only on clinical data or on both clinical and microbiological data, was set up by different groups of American and European intensivists.

Anna Maria Tortorano

The armamentarium of antifungals available to manage the fungal infections in the ICU setting was reviewed by George Samonis. While lipid and liposomal amphotericin B compounds have been the mainstays of treatment for more than 10 years, azoles have recently shown considerable efficacy. Fluconazole is effective against most of *Candida* infections, voriconazole was proven very potent against aspergillosis and significantly improved the outcome of this disease. Posaconazole as well as echinocandins, cover both *Candida* and *Aspergillus*. Unfortunately, while treatment options are increasing, new fungal threats, such as *Fusarium* and zygomycetes, have emerged. Amphotericin B compounds and possibly posaconazole are indicated against these "new" threats. Combinations of antifungal agents are under investigation, but conclusions have not yet been drawn. Prof Samonis concluded that the patient in ICU is often too sick for anything to work and the outcome of the fungal infection highly depends on an early diagnosis and on the recovery of the immune function of the patient.

Lena Klingspor, closed the symposium reporting the results of the first 18 months of the ECMM survey. A total of 420 episodes of deep *Candida* infection (76% bloodstream infections) were reported from the participating countries, that is 169 from Italy, 88 from Austria, 69 from Greece, 39 from Sweden and 10-29 from UK, Finland and Czeck Republic. Most of the patients (46%) underwent an abdominal surgery and 17% a thoracic intervention. A solid organ transplant was performed in 2.7% of the cases. A total of 78 (19%) patients had repeated surgical interventions. A solid tumour was the underlying disease in 34% of the patients and diabetes in 18.3%. *C. albicans* caused 59% of the episodes, followed by *C. glabrata* (15%), *C. parapsilosis* (13%), *C. tropicalis* (6%). *C. krusei* was reported as cause of infection in 11 cases (3%) and *C. lusitaniae* and *C. dubliniensis* in 8 episodes each. Overall crude mortality at day 30 was 31%, highest in *C. glabrata* (40.5%), *C. krusei* (46%) and *C. lusitaniae* (50%) infections. A total of 15% of the patients was under systemic antifungal prophylaxis when *Candida* infection was diagnosed. The management of the infection consists of fluconazole in 52% of the episodes, caspofungin and liposomal amphotericin B in 21% and 16%, respectively. Prof. Klingspor concluded outlining the characteristics of the typical surgical patient in ICU affected with deep-seated candidosis: male, >60 year old, undergone to abdominal surgery, presenting several risk factors, not receiving antifungal prophylaxis.

Anna Maria Tortorano

Zygomycosis

Chairs: G. Petrikkos, E. Tümbay

George Petrikkos (Athens University, Greece) presented the current epidemiology of zygomycosis in Europe, showing the data from the ECMM Working Group on Zygomycosis. Fifteen countries submitted 230 cases (Italy 60, Greece 36, Germany 35, Switzerland 22, France 21, Belgium 16, Austria 12, Spain 9, Russia 6, Norway 5, Finland 2, Czech Republic 2, Turkey 2, Netherlands 1 and UK 1). Israel also submitted cases but they will be included in 2008.

The mean age of the patients was 50 years and 60% were male. The main underlying diseases were hematologic malignancies (45%), bone marrow transplantation (10%), trauma (17%), diabetes (9%), other malignancies (5%) and solid organ transplantation (4%). The main sites of infection were the lung (29%), rhinocerebral (14%), the sinuses (13%), soft tissues (25%) and disseminated (15%). Statistical analysis showed correlation between hematological malignancy and pulmonary disease, as well as between diabetes and rhinocerebral disease. Zygomycosis was proven in 114 cases and probable in 116. Various methods of diagnosis were used including histology, culture, direct microscopy and molecular methods. The isolated fungi were mainly *Rhizopus* sp (24%), *Mucor* sp (22%) and *Absidia* sp (14%).

Mortality was 44.7%. On multivariate analysis, the factors found to be related to the outcome were age, previous administration of caspofungin, trauma as an underlying factor, treatment with amphotericin B and surgical treatment.

The pathogenesis and host defenses against *Zygomycetes* were analyzed by Emmanuel Roilides (University of Thessaloniki, Greece). He pointed out that although the pathogenesis of zygomycosis has not been fully understood yet, many interesting aspects of it have been studied, including the role of monocytes and neutrophils, various pro- and anti-inflammatory cytokines etc. He concluded that the genetic mapping of important *Zygomycetes* will help unrevealing pathogenesis of zygomycete infections and help creating more and better diagnostic and therapeutic targets.

Eric Dannaoui (Institut Pasteur, Paris, France) talked about conventional and molecular diagnostic methods. He pointed out that morphological-based identification of fungi can be erroneous in >20% of cases. He presented data showing that sequencing of ITS region is a reliable method for accurate identification of *Zygomycetes* and he also talked about the use of PCR testing on histology specimen.

Grit Walther (CBS Fungal Biodiversity Center, Utrecht, The Netherlands) presented the ongoing study of his group, the aim of which is to achieve a reliable diagnosis of mucormycosis by ITS barcoding of the *Mucorales*. In order to further cover the diversity of the *Mucorales* species, the group is in the process of generating ITS barcodes for all species of the *Mucorales* present in the CBS collection. These sequences will be used to set up a database for an accurate and rapid routine identification of *Mucorales* species that will be made publicly available through the CBS website. This set of ITS DNA barcode database will not only improve the reliability of the species recognition, it will also facilitate the detection of unknown pathogenic species and the search for a potential correlation between species and underlying diseases.

The pharmacology of antifungal agents against zygomycosis was presented by Andreas Groll (Children's University Hospital, Muenster, Germany).

Livio Pagano (Università Cattolica del Sacro Cuore, Rome, Italy) talked about antifungal prophylaxis and therapy of zygomycosis. He talked about the traditional approach of treating the infection with amphotericin B, as well as the emerging role of posaconazole. He also presented *in vitro* studies, where the majority of *Zygomycetes* demonstrated resistance to fluconazole, itraconazole and echinocandins, whereas investigational triazoles, such as posaconazole were found to be active against *Mucorales*.

Summing up, this was a very interesting symposium on zygomycosis, covering many aspects of these rare infections, which have been rising in recent years.

George L. Petrikkos

Report on...

Cape Sounion, south of Athens, Greece, 30th May – 1st June 2008

1st International Forum on Zygomycosis

Zygomycosis (mucormycosis): an emerging or re-emerging disease?

The 1st International Forum on Zygomycosis was organized by the Hellenic Society for Medical Mycology under the auspices of the ECMM, and supported by a generous unrestricted educational grant from Gilead Sciences International. One of the most spectacular Greek temples, The Temple of Poseidon, is positioned on Cape Sounion so adding to the atmosphere of the conference. Some 98 faculty and participants gathered to update each other and exchange new information of zygomycosis. The meeting was the first of its kind. The Forum-style symposium was designed to answer the central question: is zygomycosis an emerging or re-emerging infectious disease, and what do we really know about zygomycosis?

The content of the symposium went a long way in achieving the goals of the meeting. Background topics covered what is known (not a lot!) about the environmental sources of *Zygomycetes* (Malcolm Richardson) and a useful update on the taxonomy of the agents of zygomycosis (Elizabeth Johnson). The classification of the *Zygomycota* is in a state of flux so this presentation was particularly timely. The remainder of the symposium was divided into a number of sessions.

Session 1 was devoted to recent trends in the epidemiology of zygomycosis with talks on changing epidemiology (Jacques Meis), incidence of zygomycosis in transplant recipients (M. Cuenca-Estrella), and hospital acquired zygomycosis illustrated by a recent outbrek in Athens (A. Antoniadou).

Session 2 explored risk factors and pathogenesis. Lectures included zygomycosis related to trauma (Anna Skiada), deferrioxamine vs. Deferasirox: what is the role of iron (A. Symeonidis); zygomycosis and neutropenia (Livio Pagano), zygomycosis and diabetes (Olivier Lortholary), and considering the apparent increase in cases of zygomycosis in the setting of voriconazole prophylaxis, a very timely lecture entitled: Is voriconazole a risk factor? (J. Parada).

The clinical presentation and diagnosis of zygomycosis was covered in session 3 with lectures on the clincal presentation in adults (George Samonis), zygomycosis in neonates and children (Emmanuel Roilides), conventional methods of diagnosis (Cornelia Lass-Flörl), molecular methods of diagnosis (Eric Dannaoui), and susceptibility testing: *in vitro – in vivo* correlations (Juan Rodríguez Tudela).

The second days programme concluded with a session on treatment. The challenges in the management of zygomycosis was presented by George Daikos and L. Vrana. The use of liposomal amphotericin B (AmBisome) was reviewed by Georgios Petrikkos. Oliver Cornely posed the question: posaconazole: an alternative or an add-on-choice? Andreas Groll presented the current thinking on the use of hyperbaric oxygen and other adjunctive methods of management and the session was concluded with a video presentation on the surgical approach to treatment: perspectives from a maxillo-facial surgeon (A. Rapidis). The final session was an interactive audience and panel discussion on definitions and proposals for formulating diagnostic and clinical guidelines. The meeting concluded with a meeting of the ECMM Working Group on Zygomycosis. In summary, the Ist International Forum on Zygomycosis was an excellent, exhaustive meeting on an underrepresented disease area. The presentations of the symposium will be published as a collection of reviews in a forthcoming supplement of *Clinical Microbiology and Infection*, facilitated by additional welcomed support from Gilead Sciences. The Hellenic Society for Medical Mycology and the organising committee (G. Petrikkos, J. Meis, A. Mitroussia-Ziouva, E. Roilides, G. Samonis and A. Skiada) are to be congratulated on organising such a fine meeting. We look forward to similar symposia in the future.

Malcolm Richardson

Report on...

34th Annual Meeting of the EBMT

Florence, Italy, 30 March - 2 April 2008

Haematopoietic stem cell transplantation (HSCT) is widely used in the treatment of blood and lymphoid cancers, and a range of other immune diseases, with more than 30,000 autologous and 15,000 allogeneic procedures performed annually worldwide. But, as some 5000 delegates at the recent 34th Annual Meeting of the European Group for Blood and Marrow Transplantation (EBMT) congress heard, success is hindered not only by a shortage of fully matched grafts, but by complications such as invasive fungal infection (IFI) associated with the prolonged immunosuppression that accompanies HSCT.

Catherine Cordonnier

High-risk groups for *Aspergillus* infection

IFI is six times more common in patients undergoing HSCT than those who have autologous grafts, and the risk is also raised in patients who have umbilical cord blood transplants, according to data presented at the congress.

Livio Pagano, from the Policlinico Gemelli, Rome, Italy, reported an IFI rate of 3.8% in a retrospective cohort study of transplant patients treated at 11 Italian centres - 7.8% in those undergoing allogeneic transplants, compared to 1.2% in those who had an autologous graft. Aspergillosis mortality was also higher in allogeneic than autologous transplant patients - 77% and 14% respectively - with candidaemia associated with fewer deaths and less variation between the two types of transplant (57% and 44% respectively).

Data from a retrospective analysis of 306 patients undergoing HSCT from unrelated donors (60%), family mismatched (23%), mismatched unrelated (11%) or cord blood (6%), presented by Anna Maria Raiola from San Martino Hospital, Genoa, Italy, confirmed the excess risk of invasive aspergillosis (IA) in allogeneic transplants. In the study, 37 patients had probable and 8 had proven IA, with a prevalence of 15%. The median time to onset was 53 days after HSCT (range 4-449 days), with infections roughly divided between early and late onset. Mortality was 76%, with 67% related to IA, and IA the primary cause in 40%. Late take of neutrophils and steroid therapies were related to increased risk of IA, and ATG use in the conditioning regimen, steroid therapy, relapse, IgA and cholinesterase at diagnosis of IA were all identified as predictors of survival.

Dr Raiola concluded that IA is associated with high mortality, especially in patients whose immune system does not recover after HSCT.

Empirical versus pre-emptive antifungal therapy?

Pre-emptive antifungal therapy is a cost-effective alternative to empirical therapy in patients who are neutropenic for relatively short periods (under 15 days), but further refinement of diagnostic techniques is needed before it can be recommended more widely for patients who are likely to have a low neutrophil count for more prolonged periods.

This was the conclusion of Catherine Cordonnier, from the Hôpital Henri Mondor, Paris, France, at the end of a presentation during which 38% of the audience said that they used pre-emptive treatment in allogeneic HSCT patients and 34% said they used the empirical approach.

Professor Cordonnier's advice was based on the results of the PREVERT study, which compared empirical and pre-emptive treatment in 293 patients with haematological malignancies and an expected period of neutropenia of 10+ days during their treatment. All were screened twice weekly for galactomannan antigen.

Seventeen patients in the study had an IFI, 4 (2%) in the empirical group and 13 (9%) in those receiving pre-emptive therapy ($p<0.02$), though the overall survival rate was comparable ($p=0.12$). Further investigation revealed that there was no difference in infection rate between the two treatment approaches when neutropenia was short. But the longer the period of neutropenia, the greater was the risk of infection with pre-emptive therapy.

Professor Cordonnier therefore recommended that future pre-emptive strategies should include more refined techniques - imaging tools or biological markers - to increase diagnostic accuracy. She added that pre-emptive treatment should be evaluated against prophylactic approaches.

Guidelines updates

At an EBMT Infectious Diseases Working Party session held at the congress, Professor Cordonnier introduced the recently updated European Conference on Infection in Leukaemia (ECIL-2) guidelines, which elaborated on the original guidance on the prophylaxis and treatment of infection complications in leukaemia patients produced in 2005.

At a consensus meeting of 52 ex-

perts from 24 European countries and Australia, held in 2007, level A1 evidence-based recommendations for antifungal prophylaxis in allogeneic HSCT or induction chemotherapy of acute leukemia were made for posaconazole 200 mg tid oral or fluconazole 400 mg qd iv/oral.

Equivalent (A1) recommendations for empirical treatment of fungal infection were made for liposomal amphotericin B 3mg/kg or caspofungin 50mg. For first line treatment of invasive pulmonary aspergillosis, ECIL-2 made an A1 recommendation for voriconazole 2 x 6 mg/kg D1 then 2 x 4 mg/kg. No specific treatment received an A1 recommendation for salvage therapy, but posaconazole, caspofungin and voriconazole all received BII recommendations.

The ECIL-2 recommendations are broadly similar to those of the Infectious Diseases Society of America (IDSA), published earlier this year, and discussed at the EBMT congress.

Cost effectiveness of antifungal prophylaxis

Putting key European and US recommendations for antifungal prophylaxis into practice falls well within internationally accepted cost-effectiveness thresholds, according to new data, presented by Helmut Ostermann from the University of Munich Hospital, Germany.

He calculated that, in Germany, it costs €21,073 to treat an invasive fungal infection, in terms of hospital stay, diagnostic tests, blood products, antifungal and other therapies. Against this background, he analysed the cost per quality adjusted life year (QALY) of using posaconazole for antifungal prophylaxis, in line with the ECIL2 and IDSA guidelines.

Taking account of all treatment costs and the impact of induction chemotherapy and HSCT on quality of life, Professor Ostermann showed that the cost per QALY of using posaconazole instead of the previous standard treatment, itraconazole, was €8,342. This compares with the €30,000-€50,000 per QALY threshold generally accepted by government health services for new therapies.

Professor Ostermann added that similar analyses have been carried out in a number of other countries, with cost savings calculated for using posaconazole instead of itraconazole in the USA, Canada, Spain, Scotland, the Netherlands, Switzerland and France, and a cost per QALY of €1,173 in Belgium.

The Swiss experience

Using clinical trial data for a cost-effectiveness analysis of posaconazole versus standard azole therapy for the prevention of IFI in high-risk patients in Switzerland, health economist Roger-Axel Greiner and colleagues reported a mean cost saving of CHF 1,118 in neutropenic patients (CHF 9,089 vs 10,207) switched to posaconazole. In haematological patients with GVHD, switching to posaconazole was associated with a CHF 7041 increase in costs (CHF 17,720 vs 10,679). But, at CHF 48,324, the cost per life year saved fell below the CHF 60,000 threshold accepted as cost effective.

The Spanish experience

In another demonstration of the impact of guidelines implementation, Rafael Duarte, from the Hospital Duran i Reynals, Barcelona, Spain, reported that switching from itraconazole to posaconazole prophylaxis in allogeneic HSCT patients reduced prophylaxis failure and improved fungal infection survival, with a trend towards an improvement in overall survival. None of 13 consecutive patients given posaconazole prophylaxis since guidelines implementation in June 2007 required additional antifungal treatment for infection within 100 days of their transplant, compared with 31% of 13 consecutive patients who received itraconazole prophylaxis before guidelines implementation (p=0.04). Fungal infection-free survival was 85% at 100 days in the posaconazole group, compared to 46% with itraconazole (p=0.03). Overall survival was 85% and 69% respectively (p=0.08).

Dr Duarte concluded that posaconazole was well tolerated with no significant toxicity, though he drew attention to the need to reduce the dose of cyclosporin A in patients using this anti-rejection drug, because of its interaction with posaconazole.

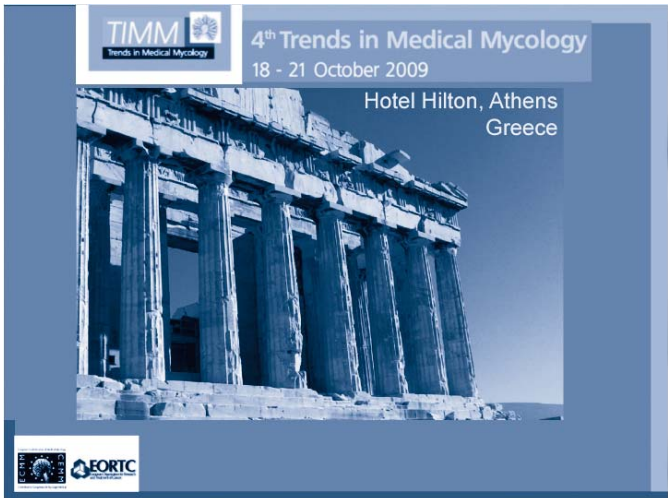
Future directions

A key study aimed at determining which part of the DNA extraction process needs to be improved in order to make *Aspergillus* PCR testing more practicable is expected to get underway in the next few months. Outlining the study, Peter Donnelly, from Radbourd University Nijmegen Medical Centre in The Netherlands, updated delegates on the progress of the *Aspergillus* PCR Working Group of the International Society for Human and Animal Mycology (ISHAM). He explained that an initial review had concluded that DNA extraction rather than the performance of the various PCR techniques is the main obstacle to wider use. The extraction process will therefore be explored at 24 centres, and the basic requirements for clinical validation have also been agreed. Professor Donnelly reported that it is hoped to propose a new standard for PCR testing by early 2009.

Serial galactomannan results could prove a useful predictor of survival in patients with IA, and tests should be included in future treatment studies, concluded Johan Maertens, from the University Hospital Gasthuisberg, Leuven, Belgium. He presented data from two recent studies showing that galactomannan index (GMI) correlates well with survival. In the first study, in 56 adults with haematologic cancer receiving antineoplastic therapy, there was a strong correlation between survival outcome and GMI ($p< 0.0001$), and the results were comparable for neutropenic and non-neutropenic patients. A similar finding has been reported in a second study of 43 patients. But data from larger numbers of patients are now needed, said Dr Maertens.

Jenny Bryan

Trends in Medical Mycology-4, Athens, October 2009



The 4th Congress on Trends in Medical Mycology (TIMM-4) will be held in Athens, Greece from the 18th till the 21st of October 2009. TIMM mycological international meetings are jointly organized by the European Confederation of Medical Mycology (ECMM) and by the Infectious Diseases Group of the European Organization for Research and Treatment of Cancer (EORTC-IDG). TIMM have taken one of the most important places among the meetings in the field of fungal infections globally, and has become a forum in which investigators and clinicians from all over the world exchange research results and opinions on medical practice. Well-known speakers discuss the most important advances in basic science and clinical research in mycology. The executive committee in collaboration with the national and international scientific committees works hardly in order to prepare an excellent scientific program and make the participation to the congress a long-lasting memory. The meeting is designed for infectious disease specialists, haematologists, oncologists, transplant physicians, microbiologists, immunologists, dermatologists, intensivists and other health workers with interest in medical mycology.

The preparation for the meeting is going very well. The program of the Congress has come close to finalization and experts on the field are invited to give lectures on a variety of interesting laboratory and clinical topics within human mycology. Within its 3-day duration the Congress will contain five high-quality plenary sessions discussing and updating hot issues in mycology. There will be sixteen workshops covering a broad spectrum of different mycological topics, four oral presentation sessions where the most interesting abstracts will be presented and sessions of poster rounds and viewing. The poster sessions will encourage one-to-one discussions between faculty, presenters and delegates. The Congress will also contain fifteen meet-the-expert sessions within which a selection of educational topics brought by the most expert mycologists will be presented.

There will be two innovations in this meeting compared to the previous TIMM's. First, in addition to the well-established Drouhet Lecture that is given during each of the last several congresses, a new distinguished lecture has been recently established and will be delivered during TIMM-4 for the first time. This is the Ben de Pauw Lecture that has been created through an unrestricted grant by Gilead to the TIMM. Prof. Ben de Pauw has been instrumental in the creation and success of the EORTC-Infectious Disease Group and it is a great honor to have the first distinguished Ben de Pauw Lecture delivered in Athens. A second innovation in the Athens meeting is that more grants will be given for the best abstracts and posters. This is expected to attract more high-quality presentations to the congress.

The venue for the TIMM-4 is in Athens, Greece. Athens is the most important classical city in Greece, the birthplace of democracy, science and philosophy. Athens is full of historical and cultural treasures throughout the downtown area and the surrounding region. Acropolis with Parthenon, many other classical monuments and a number of beautiful Byzantine churches as well as excellent museums make the visitors' experience unforgettable. Greece will undoubtedly give an irresistible background for this exciting scientific forum, providing not only a beautiful setting for a high powered meeting, but also a flavour of the Greek taste of life to all congress participants.

The meeting venue, Athens Hilton (www.athens.hilton.com), is located at the heart of the Greek capital, a few kilometers away from Acropolis and Athens historic town. Detailed information on the program will be published in January 2009 in the 2nd announcement of the congress and on the congress website www.timm2009.org. Abstract deadline is 1 June 2009 and for more information contact the congress secretariat Congress Care info@congresscare.com Phone: 31-73-690-1415 or www.congresscare.com.

The TIMM-4 in Athens will once again offer excellent science and medicine in a superb venue. We look forward to greeting you in Greece and discuss new developments in medical mycology!

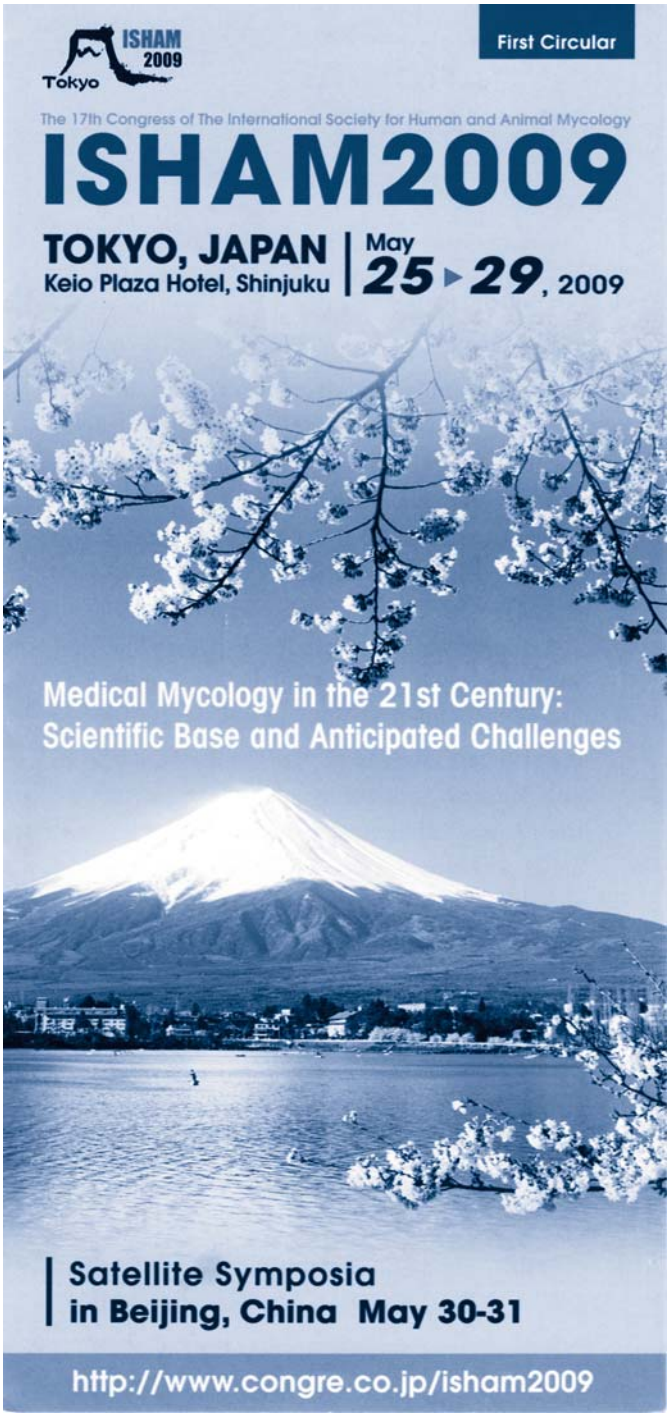
Emmanuel Roilides and *George Petrikkos*
on behalf of TIMM-4 Executive Committee

ISHAM 2009: The 17th Congress in Tokio

ISHAM
INTERNATIONAL SOCIETY FOR HUMAN AND ANIMAL MYCOLOGY

ISHAM has become one of the most active international organizations in medical mycology, and its congress is an event that you may not miss. The congress, held in Tokyo, 25-29 May, 2009 is very reasonably priced: this is a great chance to visit Japan! Registration for ISHAM2009 is now open. For accommodation, see online hotel online booking. There is a wide range of options between deluxe and budget.

The organizers have put together an excellent, densely informative program covering all aspects of modern medical mycology. More than 50 symposia and sessions are planned with distinguished speakers on themes in medical, veterinary and indoor mycology with a focus on human and animal health. More than 80 chairpersons have been confirmed for the majority of the Scientific Sessions and speaker selection is progressing well; a list of chairpersons and speakers will be available soon. The extremely wide range of experts will stimulate and expand the scope of your research.



A significant amount of attention will be devoted to posters, so that all participants will have ample opportunity to present their work. Every day there will be viewing as well as oral poster sessions in a poster forum, held in addition to the regular poster exhibitions. Poster presenters in the PF will have 5 minutes of oral presentation, may show 5 slides, and may receive 1 question. Case reports are particularly welcome. In addition, twelve poster prizes will be given to posters of highest quality.

The ISHAM congress will host a meeting for ISHAM-affiliated organizations. The meeting is open to anyone volunteering to stimulate medical mycology in all its aspects. Major theme is the promote networking and providing facilities for joint research. The agenda is posted on the ISHAM website, www.ISHAM.org.

Each day several luncheon and evening seminars are scheduled, and every evening a pleasant and interesting activity will be organized. Keynote lectures can be found on the ISHAM website. In addition, several ISHAM Working Groups will hold their meetings.

Mycologists under 35 and having a great career in mind become ISHAM member and get their money back when participating ISHAM2009. Young members present in Tokyo will receive an extra gift worth $ 100,-

Important dates:

Call for Papers: On-line Abstract Submission for Poster Presentations is open now.

Abstract Submission Deadline: December 26, 2008.

Early registration deadline at low fee: February 19, 2009.

ISHAM 2009 is one of the top international congresses, an optimum arena to present your latest research. Please join us in Tokyo and take advantage of this marvellous opportunity to enjoy intensive science, international contacts, and warm Japanese hospitality!

Hideoki Ogawa, Congress President
Sybren de Hoog, ISHAM President

The program for the ISHAM congress in Tokyo 2009, http://www.congre.co.jp/isham2009/

ISHAM Working Group on Filamentous fungi and chronic respiratory infections in cystic fibrosis Angers, June 7-8, 2009

The first meeting of the ISHAM Working Group on Filamentous fungi and chronic respiratory infections in cystic fibrosis will be organized in Angers University, Angers, France, on 7 and 8 June, 2009. Aim is to focus attention on the much overlooked respiratory infections caused by filamentous fungi in patients with cystic fibrosis.

Beside bacteria which remain the major causative agents of respiratory infections in the context of cystic fibrosis (CF), several filamentous fungi may also colonize the respiratory tract of these patients. This fungal colonization of the airways, facilitated by the frequent and prolonged cures of antibiotics and by the use of corticosteroids, may also lead to true respiratory infections whose frequency regularly increases along with the development of lung transplantation and the increase in life expectancy. Apart from *Aspergillus fumigatus*, numerous other species are reported increasingly, such as *Scedosporium apiospermum*, *A. terreus, Exophiala dermatitidis* and *S. prolificans*, some of them being poorly susceptible to current antifungals and therefore difficult to treat. However, the prevalence of these fungi in the context of CF is certainly underestimated and their clinical significance still remains to be defined. Large scale-multicenter studies should be designed in order to define the real prevalence of these species and the clinical significance of their recovery from respiratory secretions, but also to highlight possible geographic variations in their prevalence and to improve the biological diagnosis of airway colonization/infection. Additionally, numerous questions raise from the colonization of the airways by these filamentous fungi, and basic research on the ecology of these fungi, their biochemistry, and their pathogenic mechanisms should be promoted to define prophylactic measures or to develop more effective antifungal drugs.

The Workshop will be open to anyone who wishes to contribute to the study of chronic respiratory infections caused by filamentous fungi in patients with CF. It will be asked to each attendant to give a short presentation of his or her lab and of the work(s) that has been done in the past few years in our research field. Presentation of scientific projects in this area with search of partners is also encouraged. But a large part of this meeting will also be dedicated to discussions in order to plan future developments and collaborative studies. The number of participants is limited to 50 and there will be no fee.

Jean-Philippe Bouchara

3rd Pan African Medical Mycology Society (PAMMS) Conference Abuja, Nigeria, February 25-27, 2009

Previous meetings

Successful 1st meeting ("Medical Mycology: The African Perspective") was held at the Hartenbos Beach Resort near Mossel Bay in the Western Cape, South Africa on 25 January 2005. The Pan African Medical Mycology Society (PAMMS) was inaugurated during this meeting and a steering committee consisting of Hester Vismer (Cape Town, South Africa), Ifeoma Enweani (Ekpoma, Nigeria) and El Sheikh Mahgoub (Khartoum, Sudan) was elected to look after PAMMS during its first few years.

The 2nd meeting was also held in CTICC, Cape Town, South Africa between May 6-8, 2007. During this meeting PAMMS Council members were elected viz Hester Vismer (Cape Town) President, Ifeoma Enweani (Nnewi, Nigeria) Vice President, John Rheeder (South Africa) Secretary, Alf Botha (South Africa), Abdalla Ahmed (Saudi Arabia), and Ahmad Moharram (Egypt) as members. Membership of the PAMMS is free, as the Africa Fund for Fungal Biodiversity and Mycotic Infections, initiated by Sybren de Hoog and Jacques Meis of the Netherlands, will cover the initial costs of the Society.

Conference Announcement

It is a pleasure to invite you to attend the 3rd meeting of the PAMMS in Abuja to be held at the National Center for Women Development (NCWD) located in the Central Business District. The conference will provide medical mycologists from Africa with a unique opportunity to present their latest research findings, to foster collaboration and to establish long-term relations between scientists from Africa and abroad. A PAMMS General Meeting will be held to discuss various updates on Medical Mycology Studies. Invited speakers from the African continent and speakers from outside Africa, working on topics concerning African fungi will participate in the meeting.

Poster presentations will also form an important part of the programme.

In addition to the stimulating scientific programme planned for PAMMS 2009, Abuja is the current capital of Nigeria and is situated at the heart of Nigeria. It has a diverse culture with tourist attractions and hospitable people.

Organising Committee

Ifeoma Enweani (Chairperson), Grace Ayanbimpe (Treasurer), Members: Lyidia Abia-Bassey, Emeka Nweze, Harish Gugnani, Afe Ekundayo, Dennis Agbonlahor, Francisca Okungbowa, Onyechere Allison.

Scientific Committee

Hester Vismer (South Africa), David Katerere (Cape Town), Jacques Meis (Netherlands), Sybren de Hoog (Netherlands), El Sheikh Mahgoub (Sudan), Abdalla Ahmed (Saudi Arabia), Ifeoma Enweani (Nigeria), John Rheeder (South Africa).

Important Contacts and Addresses

All information regarding the PAMMS 2009 conference is also available at the following website:
http://www.cbs.knaw.nl/meetings

Enquiries

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ABBREVIATED PRESCRIBING INFORMATION

Presentation: A sterile lyophilised product for intravenous infusion. Each vial contains 50 mg of amphotericin B, encapsulated in liposomes. **Indications:** The treatment of severe systemic and/or deep mycoses where toxicity (particularly nephrotoxicity) precludes the use of conventional systemic amphotericin B in effective dosages. The empirical treatment of presumed fungal infections in febrile neutropenic patients, where the fever has failed to respond to broad-spectrum antibiotics and appropriate investigations have failed to define a bacterial or viral cause. **Dosage & Administration:** Preparation – Follow the reconstitution instructions exactly as given in the SmPC. Administration – AmBisome should be administered by intravenous infusion over a 30 – 60 minute period. The recommended concentration for intravenous infusion is 0.2 mg/ml to 2.0 mg/ml. Therapy for systemic and/or deep mycoses is usually instituted at a daily dose of 1.0 mg/kg of body weight, and increased stepwise to 3.0 mg/kg, as required. Data are presently insufficient to define total dosage requirements and duration of treatment necessary for resolution of mycoses. However, a cumulative dose of 1.0 – 3.0 g of amphotericin B as AmBisome over 3 – 4 weeks has been typical. Dosage of amphotericin B as AmBisome must be adjusted to the specific requirements of each patient. The recommended dose for empirical treatment in febrile neutropenia is 3 mg/kg body weight per day. Treatment should be continued until the recorded temperature is normalised for 3 consecutive days. In any event, treatment should be discontinued after a maximum of 42 days. Children have been successfully treated with AmBisome without reports of unusual adverse events and have received comparable doses to adults on a per kilogram body weight basis. There are no specific dosage recommendations or precautions for elderly patients. **Contra-Indications:** Hypersensitivity to any of the constituents of AmBisome, unless the condition requiring treatment is life threatening and amenable only to AmBisome therapy. **Warnings:** Although anaphylactic or severe allergic reactions are rare, administration of a test dose is still advisable. If a small amount of AmBisome (e.g. 1 mg) can be administered for about 10 minutes without severe allergic reactions within 30 minutes, the dose can be continued. Laboratory evaluation of renal, hepatic and haematopoietic function should be performed at least weekly and particular attention should be given to patients receiving concomitant therapy with nephrotoxic drugs. Levels of serum potassium and magnesium should be monitored regularly. **Use in diabetic patients:** Each vial of AmBisome contains approximately 900 mg of sucrose. **Use in dialysis patients:** Haemodialysis or peritoneal dialysis does not appear to affect the elimination of AmBisome and data suggest no dose adjustment is required, however administration should be avoided during the haemodialysis procedure. **Use in pregnancy:** As the safety of AmBisome in pregnancy has not been established, the risk/benefit ratio must be considered. **Interactions:** No specific pharmacokinetic interactions of AmBisome with other drugs have been reported in clinical trials to date; patients requiring concomitant drug therapy should be monitored closely. Concurrent administration of other nephrotoxic agents may increase the risk of nephrotoxicity in some patients. **Side effects:** In two double-blind, comparative studies, AmBisome treated patients experienced a significantly lower incidence of infusion-related reactions, as compared to patients with either conventional amphotericin B or amphotericin B lipid-complex. Less frequent infusion-related reactions may include back pain and/or chest tightness or pain, dyspnoea, bronchospasm, flushing, tachycardia and hypotension, and these resolved rapidly with cessation of the infusion. In a double-blind study involving 687 patients, nephrotoxicity with AmBisome was approximately half that for conventional amphotericin B. In another double-blind study involving 244 patients, the incidence of nephrotoxicity with AmBisome was approximately half that for amphotericin B lipid complex. Additional adverse events observed in clinical trials include nausea, vomiting, hypokalaemia, creatinine and BUN increase, hypomagnesaemia, hypocalcaemia, hyperglycaemia, hyponatraemia, liver function test abnormalities, hyperbilirubinemia, vasodilation, increased alkaline phosphatase, diarrhoea, abdominal pain, rigors, headache, rash and pyrexia. Convulsion, thrombocytopenia, anaphylactoid reactions, hypersensitivity, angioneurotic oedema and renal insufficiency/failure have been reported rarely. **Overdosage:** In clinical trials, repeated daily doses up to 15 mg/kg in adults and 10 mg/kg in children have been given without reported dose-dependent toxicity. If overdosage occurs, stop administration immediately and carefully monitor hepatic, renal and haematopoietic function. Pharmaceutical Precautions: Do not store above 25°C. Do NOT freeze. As AmBisome does not contain any bacteriostatic agent, from a microbiological point of view, the reconstituted and diluted product should be used immediately. In-use storage would not normally be longer than 24 hours at 2 - 8°C, unless reconstitution and dilution has taken place in controlled and validated aseptic conditions. Chemical and physical stability has been demonstrated for 24 hours at 25°C ± 2 and 7 days at 2 - 8°C for reconstituted product. Following dilution with 5% dextrose, chemical and physical stability have been shown for 24 - 48 hours at 25°C ± 2 and 4 - 7 days at 2 - 8°C (dependent upon final concentration). DO NOT STORE partially used vials. DO NOT RECONSTITUTE AMBISOME WITH SALINE, OR MIX WITH OTHER DRUGS. **Legal category:** POM. **Package Quantities:** Cardboard carton of 10 vials each. NHS price – Carton of 10 vials, £966.90. **Product Licence Number:** PL 16807/0001. Full prescribing information is available from the marketing authorisation holder: Gilead Sciences International Ltd, Granta Park, Abington, Cambridge CB21 6GT.

CONSULT THE SUMMARY OF PRODUCT CHARACTERISTICS BEFORE PRESCRIBING

Date of preparation: August 2006. AmBisome is a trademark.

References:

1. AmBisome, Summary of Product Characteristics, 5 July 2007.
2. Cornely O *et al.* Clin Infect Dis 2007; **44**: 1289-97.
3. Ringdén O *et al.* J Antimicrob Chemother 1991; **28(Suppl B)**: 73-82.
4. Data on file. Gilead Sciences International – AMB0700007.
5. Boswell GW *et al.* J Clin Pharmacol 1998; **38**: 583-592.

Date of preparation: January 2008.
INT/AMB/0108/CM/15

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