Now Approved in the EU

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Making a Difference in Infectious Diseases

Astellas' involvement in developing medical treatment of infectious diseases has been ongoing since the 1970s and continues today. Our commitment to the future development of new medications has been demonstrated by:

• Pioneering the conduct of empiric antifungal clinical trials
• Initiating some of the world's largest clinical trials in fungal infections
• Implementing pediatric and neonatal trials
• Collaborating with the medical community
• Sponsoring a clinical surveillance program to examine therapeutic management and patient outcomes for invasive fungal infections

At Astellas, we're committed to providing the best possible medications to patients and caregivers in their battle against infectious diseases.
Dear Advances Against Aspergillosis Colleague,

We are excited to have once again assembled many of the leading clinicians and scientists from around the world to drive forward the scientific and medical research agenda in *Aspergillus* and aspergillosis for the 2nd Advances Against Aspergillosis conference. There was an overwhelming feeling of success from the 1st Advances Against Aspergillosis meeting in 2004 in San Francisco where we had 364 attendees from 28 countries exchange ideas and forge new collaborations.

The *Aspergillus* field is in a state of rapid advancement, including the publications of the genomes of *Aspergillus fumigatus*, *Aspergillus nidulans* and *Aspergillus oryzae* providing a huge impetus for basic research. Additionally, the launch of several newer antifungals in the last few years and anticipated clinical trials of several more is a tremendous important therapeutic step forward. Despite the incidence of invasive aspergillosis increasing and the disease the leading fungal cause of patient mortality, prior to the 1st Advances Against Aspergillosis meeting there had been insufficient communication among experts in the area. This is another rare chance to gather the world’s aspergillosis experts in one venue. A fundamental tenet of this research colloquium continues to be to engender collaborative relationships amongst clinicians, scientists, and industry to further advance the field.

We thank the many corporate and foundation sponsors, listed in this program; without their support, this conference would not have been possible. We also thank the Scientific Committee for helping to assemble a truly international speaker list from the largest medical and scientific centers in the world, with a focus on contemporary topics in *Aspergillus* research. Indeed, by our design, much of the newest published literature and hypotheses in the field have originated from the organizers and speakers of this conference. In the program, we have introduced a majority of speakers who did not speak at the 1st Advances Against Aspergillosis, including many young scientists and clinicians, a pattern we would like to repeat in future years.

We also thank all the speakers and poster presenters, and every one of you, for contributing to the success of this effort. We hope you will enjoy the meeting, the conference hotel (which was used by the International Olympic Committee during the 2004 games) overlooking the Acropolis, as well as the beautiful city of Athens. Please also join us at the welcome reception, the satellite symposia, and the special conference social event highlighting the exciting culture of Greece. An essential part of this meeting is the new friendships we expect will result, and the support of young scientists entering the field.
The proceedings of this meeting will once again be published in a special supplement of the journal *Medical Mycology*, creating what we hope will be highlights of the newer insights from the many disciplines that encompass Aspergillus research and care.

Our plan is to continue this conference every other year, alternating between continents. You will notice that there is a special open planning session for the next conference at the end of this meeting. We invite you to come and offer any constructive criticism of this meeting and suggestions for new sessions or topics you would like to see in the future.

Best regards,

David W. Denning  
William J. Steinbach  
David A. Stevens

2nd Advances Against Aspergillosis Conference Co-Chairmen
# 2nd ADVANCES AGAINST ASPERGILLOSIS

*February 22-25, 2006 Athens, Greece*

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2nd ADVANCES AGAINST ASPERGILLOSIS
February 22-25, 2006 Athens, Greece

CONFERENCE CHAIRMEN AND SCIENTIFIC COMMITTEE

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David W. Denning, MD
University of Manchester, UK

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Stanford University, USA

William J. Steinbach, MD
Duke University, USA

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Hans-Knoell-Institute, Germany

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University of Liverpool, UK

Juergen Loeffler, PhD
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February 22-25, 2006 Athens, Greece

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Swiss Institute of Allergy and Asthma Research, Switzerland

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Hôpital de Hautepierre, France

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*University of Szeged, Hungary*

Aimee K. Zaas, MD  
*Duke University, USA*
## 2nd ADVANCES AGAINST ASPERGILLOSIS
### February 22-25, 2006 Athens, Greece

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**FINAL PROGRAM**

### WEDNESDAY, 22 FEBRUARY 2006

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<td>Welcome Reception (Terpsichore D)</td>
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<td>6:30 - 9:00 pm</td>
<td>Aspergilosis: Update and New Therapeutic Options (Terpsichore A)</td>
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<td><strong>Moderator: David W. Denning, MD</strong></td>
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<td></td>
<td>Aspergilosis in the USA, UK, and Japan in the 20th Century</td>
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<td>Aspergilosis in the ICU - the new 21st Century problem</td>
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<td>Managing drug interactions in patients with aspergilosis</td>
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<td>Combination therapy - is it indicated?</td>
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<td>William J. Steinbach, MD</td>
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<td>8:30 - 11:00 pm</td>
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<tr>
<td>7:15 - 8:30 am</td>
<td>Meet the Professor Sessions</td>
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<td>Manipulating innate immunity against aspergilosis (Santorini 1)</td>
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<tr>
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<td>Luigina Romani, MD PhD</td>
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<td>Challenges in diagnosis and treatment of invasive aspergilosis (Santorini 2)</td>
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<tr>
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<td>Challenges in pediatric and adult patients: a case-based analysis</td>
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<td>Thomas J. Walsh, MD</td>
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<td>Conference Introduction</td>
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<td>David W. Denning, MD</td>
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<td></td>
<td><strong>Plenary Session 1: Genetic and respiratory tract risk factors for aspergilosis</strong> (Terpsichore A)</td>
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<td><strong>Moderators: David A. Stevens, MD and George Petrikkos, MD</strong></td>
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<td>8:40 - 9:00 am</td>
<td>Invasive aspergilosis</td>
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<td>Aimee K. Zaas, MD</td>
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<td>9:00 - 9:20 am</td>
<td>ABPA and asthma with fungal sensitization</td>
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<td>Alan P. Knutsen, PhD</td>
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<tr>
<td>9:20 - 9:40 am</td>
<td>Chronic pulmonary aspergilosis/aspergilloma</td>
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<td>Helen Sambatakou, MD</td>
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9:40 - 10:00 am  Risk factors for allergic Aspergillus sinusitis  
*William K. Dolen, MD*

10:00 - 10:30 am  Discussion

10:30 - 11:00 am  **Break**

### Plenary Session 2: Separating out the components of the pathogenesis of invasive aspergillosis  
*Moderators: Jean-Paul Latgé, PhD and Karl V. Clemons, PhD*

- 11:00 - 11:20 am  Growth and virulence  
  *Judith C. Rhodes, PhD*

- 11:20 - 11:40 am  Global control of *Aspergillus* virulence  
  *Nancy P. Keller, PhD*

- 11:40 - 12:00 pm  Endothelial cells, platelets and vessel damage  
  *Scott G. Filler, MD*

- 12:00 - 12:20 pm  Alveolar macrophage interactions  
  *Oumaima Ibrahim-Granet, PhD*

- 12:20 - 12:40 pm  Th1/Th2 paradigm in aspergillosis  
  *David A. Stevens, MD*

- 12:40 - 1:00 pm  Discussion

1:00 - 2:30 pm  Lunch

### Plenary Session 3: Clinical trials - Methodology appraisal  
*Moderators: Raoul Herbrecht, MD and Thomas J. Walsh, MD*

- 2:30 - 2:45 pm  Challenges of patient recruitment for invasive aspergillosis trials  
  *Patricia Ribaud, MD*

- 2:45 - 3:00 pm  Defining clinical failure for salvage studies  
  *Georg Maschmeyer, MD*

- 3:00 - 3:15 pm  Antifungal dose finding in aspergillosis  
  *Claudio Viscoli, MD*

- 3:15 - 3:35 pm  Critique of trials in ABPA and fungal allergy  
  *Richard B. Moss, MD*

- 3:35 - 4:00 pm  Discussion/Break

- 4:00 - 4:45 pm  Critique of randomized trials in invasive aspergillosis - Debate  
  *Now we know what to do (most of the time)*  
  *John E. Bennett, MD*  
  *The trials tell us very little*  
  *Archie G. Prentice, MD*

- 4:45 - 5:00 pm  Discussion
5:00 - 7:00 pm  **Poster Session 1**  (Erato A-C)
(With tea and alcohol and snacks served at 6:00pm sponsored by Schering-Plough)
Sage Session Rounds at posters

7:00 - 9:30 pm  **Bridging the Gaps in Aspergillosis:**  (Terpsichore A)
_**Prophylaxis to Diagnosis**_
_Schering-Plough Satellite Symposia with Dinner_
**Moderator: Helen Giamarellou, MD PhD**
Antifungal prophylaxis in patients at high-risk for fungal infections
_ Oliver A. Cornely, MD_
Antifungal strategies: Prophylaxis, pre-emptive, or empiric therapy
_ Monica A. Slavin, MD_
Techniques used in the diagnosis of aspergillosis: Antigen and radiology
_ Dimitrios P. Kontoyiannis, MD_

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**FRIDAY, 24 FEBRUARY 2006**

7:15 - 8:30 am  **Experience with an Echinocandin in Aspergillosis -**  (Hesperides)
_MSD Satellite Symposia with Breakfast_
**Moderator: George L. Petrikkos, MD**
Clinical microbiology of invasive mould infections
_Cameron M. Douglas, PhD_
Clinical efficacy of caspofungin in the treatment of aspergillosis
_Michael Aoun, MD_

**Plenary Session 4: Laboratory contribution to the**  (Terpsichore A)
_diagnosis and management of aspergillosis_
**Moderators: Dimitrios P. Kontoyiannis, MD and Aristea Velegraki, PhD**

9:00 - 9:40 am  Galactomannan antigen testing - Debate
Defining a case of invasive aspergillosis with galactomannan
_Johan Maertens, MD_
Problems with galactomannan testing
_Paul E. Verweij, MD PhD_

9:40 - 9:50 am  Discussion

9:50 - 10:05 am  Glucan testing for aspergillosis
_Minoru Yoshida, MD (Sponsored by Seikagaku Corporation)_

10:05 - 10:25 am  PCR - platforms, strengths and weaknesses
_Lewis White, PhD_

10:25 - 10:45 am  Status of susceptibility testing in _Aspergillus_
_Cornelia Lass-Florl, PhD_

10:45 - 10:55 am  Discussion
10:55 - 11:20 am  Break

**Plenary Session 5: Aspergillus and the environment**  (Terpsichore A)

*Moderator: Maria-Anna Viviani, PhD*

11:20 - 11:40 am  What do molecular typing studies tell us about acquisition of Aspergillus?  
*Janos Varga, PhD*

11:40 - 12:00 pm  Cleaning up an Aspergillus contaminated air source - what works?  
*W. Elliott Horner, PhD*

12:00 - 12:20 pm  The impact of environmental fungi on housing and building design  
*Martin D. Chapman, PhD*

12:20 - 12:40 pm  Discussion

12:45 - 2:00 pm  **Clinical and Non-Clinical Antifungal Efficacy Trials:**  (Terpsichore A)

*Issues with Trial Design and Interpretation*

*Gilead Satellite Symposia with Lunch*

*Moderator: David A. Stevens, MD*

Animal models of infections: What can we learn from them?  
*Karl V. Clemons, PhD*

Consensus definitions for invasive fungal infections: Strengths, limitations, and revisions  
*Ben E. DePauw, MD PhD*

Clinical antifungal efficacy trials: Consensus standards for trial design and room for improvement  
*Raoul Herbrecht, MD*

Evidence-based approach to development of treatment guidelines and clinical management of invasive fungal infections  
*J. Peter Donnelly, PhD*

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**FRIDAY AFTERNOON - SPLIT SESSION**

**Parallel Plenary Session 6A: Developing areas of Aspergillus physiology**  (Terpsichore A)

*Moderators: Mark X. Caddick, PhD and Gregory S. May, PhD*

2:20 - 2:40 pm  Gene essentiality in Aspergillus  
*Geoffrey Turner, PhD*

2:40 - 3:00 pm  Amino acid and nitrogen acquisition  
*Gerhard H. Braus, PhD*

3:00 - 3:20 pm  Handling oxidative stress  
*Jesus Aguirre, PhD*

3:20 - 3:40 pm  New resistance mechanisms  
*Emilia Mellado, PhD*

3:40 - 4:00 pm  Programmed cell death in Aspergillus and other filamentous fungi  
*Geoffrey D. Robson, PhD*

4:00 - 4:30 pm  Discussion
Parallel Plenary Session 6B:

Allergic Aspergillus disease

Moderator: Richard B. Moss, MD

2:20 - 2:40 pm Asthma and mould allergy - does it matter?
Robert Niven, MD

2:40 - 3:00 pm Airborne fungal fragments and allergenicity
Brett J. Green, PhD

3:00 - 3:20 pm Discussion

3:20 - 3:40 pm Aspergillosis and long term corticosteroids
Carlos Agusti, MD PhD

3:40 - 4:00 pm Management of allergic fungal sinusitis
Bradley F. Marple, MD

4:00 - 4:30 pm Discussion

4:30 - 5:30 pm Oral Presentations from five submitted abstracts

5:30 - 7:30 pm Poster Session 2

(Poster Session 2)
(With tea and alcohol served at 6pm)
Sage Session Rounds at posters

7:30 pm Conference Social Event
Buses will leave as of 7:30 pm from the Athens Hilton.

SATURDAY, 25 FEBRUARY 2006

7:15 - 8:30 am Current issues in the management of invasive aspergillosis

Pfizer Hellas Satellite Symposia with Breakfast

Moderators: Emmanuel Roilides, MD and Helen Giamarellou, MD PhD

Treatment of invasive aspergillosis: Polyenes, azoles, or echinocandins?
Thomas F. Patterson, MD

Novel preventative strategies against invasive aspergillosis
George Samonis, MD

Management of cerebral aspergillosis and other rare cerebral mycoses
Markus Ruhnke, MD

8:35 - 8:40 Announcement of Awards and Introduction of Scholars
David A. Stevens, MD

Plenary Session 7: Genomics and Post-genomics

Moderators: Masayuki Machida, PhD and Gustavo H. Goldman, PhD

8:40 - 9:00 am Whole genome comparison of A. flavus and A. oryzae
Gary A. Payne, PhD
2nd ADVANCES AGAINST ASPERGILLOSIS  
February 22-25, 2006 Athens, Greece

9:00 - 9:20 am  What’s new in the Aspergillus niger genome?  
Scott Baker, PhD

9:40 - 10:00 am  Gene regulation  
Mark X. Caddick, PhD

10:00 - 10:20 am  Antigens in Aspergillus - predictions based on genomics  
Paul Bowyer, PhD

10:20 - 10:40 am  Discussion

10:40 - 11:10 am  Break

Plenary Session 8: The new Aspergillus taxonomy  
(Terpsichore A)  
Moderators: Josep Guarro, PhD and Jens Frisvad PhD

11:10 - 11:30 am  Old and new concepts of species differentiation in Aspergillus  
Robert A. Samson, PhD

11:30 - 11:50 am  Identifying clinically relevant Aspergilli  
Maren A. Klich, PhD

11:50 - 12:10 pm  Whole genome comparison of A. fumigatus group  
Jennifer Wortman, PhD

12:10 - 12:30 pm  New Aspergillus mycotoxins/secondary metabolites  
Thomas O. Larsen, PhD

12:30 - 12:50 pm  Discussion

12:50 - 2:00 pm  Lunch  
(Terpsichore D)

Plenary session 9: Prospects for improvements in management of invasive aspergillosis  
(Terpsichore A)  
Moderators: William J. Steinbach, MD and Thomas F. Patterson, MD

2:00 - 2:20 pm  Early diagnosis of invasive aspergillosis in small children  
Emmanuel Roilides, MD

2:20 - 2:40 pm  Antigens in A. fumigatus - experimental validation  
Reto Crameri, PhD

2:40 - 3:00 pm  Implications of Toll-like receptors discoveries for aspergillosis control  
Frank Ebel, PhD

3:00 - 3:20 pm  Granulocyte infusions or T cell therapy?  
Elias J. Anaissie, MD

3:20 - 3:40 pm  Discussion

3:40 - 3:45 pm  Conference Farewell  
William J. Steinbach, MD

Meeting close

4:15 - 5:00 pm  Open meeting to discuss AAA 2008 plans  
(Santorini I)
GENERAL CONFERENCE INFORMATION

Instruction for authors

Oral presentations
All meeting rooms are equipped with computers and data projectors. Please bring your presentation on memory stick or CD-ROM to the meeting room of your session in the break before the start of your session. It is not permitted to use your own laptop. Speakers may use room Ikaria to rehearse their presentation or to relax.

Poster presentations
Please mount your poster in the poster area (Erato A-C). You will find the assigned poster number on the poster boards. The numbers on the poster boards correspond with the abstract numbers in the abstract book.

Poster numbers from P001 - P075:
Poster mounting: Wednesday 22 February from 1:00 - 8:00 pm.
Presence of authors: Thursday 23 February from 5:00 - 7:00 pm
Poster removal on Thursday 23 February, from 7:00 pm (after the poster session).
Posters that have not been removed by the author at 8:00 pm are removed and disposed by the congress staff.

Poster numbers from P076 - P160:
Poster mounting: Friday 24 February from 8:00 - 2:00 pm
Presence of authors: Friday 24 February from 5:30 - 7:30 pm
Poster removal on Friday 24 February, from 7:30 pm
Posters that have not been removed by the author on Saturday 25 February at 2:00 pm are removed and disposed by the congress staff.

Congress language
The official Congress language will be English. No simultaneous translation will be available.

Messages
You may leave and collect messages at the registration desk during opening hours.

Exhibition
The exhibition is located on Terpischore D during the following hours:

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<td>Saturday</td>
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Insurance
In registering for the joint meeting of the 2nd Advances Against Aspergillosis conference, delegates agree that neither the organization nor the congress agency Congress Care is responsible for individual medical, travel or personal insurance. Delegates are requested to make their own travel and health insurance. The organizers can not assume liability for changes in the program due to external circumstances.

Disclaimer
The participant acknowledges that he/she has no right to lodge damage claims against the organizers should the holding of the congress be hindered or prevented by unexpected political or economic events or generally by force majeure, or should the non-appearance of speakers or other reasons necessitate program changes. With registration, the participant accepts this proviso.

Accreditation
• The 2nd Advances Against Aspergillosis is accredited by the European Accreditation Council for Continuing Medical Education (EACCME) to provide the following CME activity for medical specialists. The EACCME is an institution of the European Union of Medical Specialists (UEMS).
• The congress 2nd Advances Against Aspergillosis, 22 - 25 February 2006, is designated for up to 18 European CME credits (ECMEC). Each medical specialist should claim only those hours of credit that he/she spent in educational activity.
• EACCME credits are recognised by the American Medical Association towards the Physician’s Recognition Award (PRA). To convert EACCME credit to AMA PRA category 1 credit, contact the AMA.

Certificates of attendance including the European CME credits will be available upon return of the completed evaluation form at the registration desk Saturday February 25.

Registration desk
The registration desk will be open:

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<td>Wednesday</td>
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Conference Registration Fees

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<td>Early</td>
<td>On or before Nov 15, 2005</td>
<td>EUR 225</td>
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<td>Standard</td>
<td>Nov 16, 2005 - Jan 15, 2006</td>
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A letter of verification from a Department Head is required to register at Student/Trainee rates. Single day registration: 75% of the total for that category (Single day registration does not include the dinner social event).

The registration fee covers the following: admission to the meeting, welcome reception on Wednesday, programme / abstract book, certificate of attendance, 3 lunches and all coffee breaks.

**Name badge**
Access to all scientific events and to the exhibits is only possible with your personal name badge, which you will receive upon registration. All participants are requested to wear their name badges during the whole congress. EUR 30 will be charged for replacement of a lost badge.

**Social event only** EUR 75

**Accompanying person** EUR 100
*(NOT including the Social event)*
Covers access to Exhibit Area and Welcome Reception on Wednesday.

**Cancellation Policy**
If your registration must be cancelled, your fee, less EUR 50 for administrative costs, will be refunded upon receiving a signed, written request either by fax or mail. Email requests cannot be accepted. No refunds will be made after February 10, 2006. Please allow 6-8 weeks for refund.

**Social Event**
*Friday evening February 24, 7:30 - 11:00 pm*
Join your colleagues and friends for a festive Greek evening and a sumptuous dinner with excellent local wine at the Old Stables restaurant. The Old Stables are located approximately 30 minutes drive south east from Athens in the beautiful Attica landscape named Messoghia, surrounded by vineyards. The restaurant was established in 1929 and has an enchanting rustic character. It is housed in an old horticultural estate surrounded by a garden with its own water sources, water mills, fountains, pistachio trees and vineyards. On our arrival we will be welcomed with a glass of ouzo, the local spirit, by the hospitable, friendly staff of the restaurant dressed in traditional Greek costumes. We'll enjoy a delicious dinner with red or white local wine from the barrels while we attend a folkloric show with songs and dances from all parts of Greece. The professional Greek dancers dressed in colorful authentic costumes together with the bouzouki player will help us spend a memorable Greek evening.

**Buses will leave as of 7:30 pm** from the Athens Hilton. Please present your ticket at the bus. The charge for an accompanying guest is EUR 75; tickets are available at the registration desk.
Maps Athens Hilton
ABSTRACTS OF INVITED FACULTY
Although Aspergillus was first seen microscopically in 1729 (Micheli), it was not until 1848 that Bennett described an aspergilloma, in a patient with tuberculosis. Superficial, saprophytic and allergic manifestations of aspergillosis were then described in the late 1800’s. Exacerbation of asthma by Aspergillus and other moulds was noted in the 1920’s (Van Leeuwen). The remarkably high frequency of skin test positivity (15%) in asthmatics to Aspergillus and Penicillium was matched by challenge tests (Hansen). Asthma ‘caused’ by Aspergillus was described in the USA in 1930. Cerebral aspergillosis was described in 1931 (Just) and bony disease in 1936 (Shaw). The likely first case of subacute invasive aspergillosis complicating chronic granulomatous disease was seen in 1947 (Cawley). In 1953, Rankin described invasive aspergillosis in an immunocompromised patient (aplastic anaemia caused by chloramphenicol), presaging the current growth in cases. The same year ABPA was properly described as an entity, although similar cases had been clinically described previously (Hinson). In 1955, Zimmerman described aspergillosis in infancy. By this time, the introduction of cancer chemotherapy and corticosteroid treatment had increased the number of cases being seen, and Finegold attempted the first classification of aspergillosis in 1959. Amphotericin B was first used for treatment of invasive aspergillosis in 1959, fluocytosine in ~1970, itraconazole in ~1985, voriconazole in 1992 and caspofungin in ~1999.

This presentation also illustrates how the specific geographical, medico-technical and social conditions in UK, USA and Japan affected the evolution of medical mycology in each country in the 20th century. In the USA, a specific academic collaboration among researchers in botany, dermatology and laboratory sciences eventually resulted in the establishment of a medical mycology laboratory and training program as early as the 1920’s. Endemic fungal infections in the USA (eg coccidioidomycosis and histoplasmosis) encouraged authorities to embrace the study of mycoses in the public health arena in the 1940’s, from which emerged the effort to develop antifungal agents. In the UK, medical mycology developed in tandem with economic concerns over the Empire. Over the first half of the 20th century, tropical hygienists in the metropolis and in colonies pleaded for the collection and study of fungi pathogenic not only to man but also to commercially-viable plants and animals. Moreover, epidemics of human and animal ringworm in the immediate post-war period resulted in the unique and strong presence of veterinary medicine in British medical mycology. In Japan, the first case of invasive aspergillosis was described in a fisherman caught in the radioactive fall-out from the first atomic test in Bikini Atoll (1955) (Okudaira) the publicity from which posed an important turning point for the development of medical mycology in Japan.

The classification of invasive and chronic (non-allergic) manifestations of aspergillosis has recently been updated and detailed criteria for the diagnosis of common and rare manifestations of disease provided (Hope et al, 2005). Invasive aspergillosis can be divided
into acute (angioinvasive or non-angioinvasive) and subacute (or chronic necrotizing pulmonary). Chronic forms of pulmonary aspergillosis include simple aspergilloma, chronic cavitary pulmonary, chronic fibrosing pulmonary aspergillosis. Sinus aspergillosis follows a similar pattern - acute invasive, chronic invasive, chronic granulomatous and sinus aspergilloma. Allergic forms of disease include allergic bronchopulmonary aspergillosis, allergic aspergillus sinusitis and severe asthma with fungal sensitization (SAFS). Some forms of airway disease are more difficult to classify including Aspergillus bronchitis and obstructing bronchial aspergillosis (mucoid impaction).
MANAGING DRUG INTERACTIONS IN A PATIENT WITH ASPERGILLOSIS

Russell E. Lewis
University of Houston College of Pharmacy and The University of Texas M. D. Anderson Cancer Center, Houston Texas, USA
Wednesday, February 22, 2006, 7:30 - 8:00 pm

Drug interactions remain one of the most common, recurring challenges associated with the safe and effective treatment of aspergillosis in the immunocompromised patient. The introduction in the recent past of a new class of antifungals (echinocandins); safer or reformulated versions of older agents (lipid amphotericin B formulations, itraconazole); and broader spectrum azoles (voriconazole, posaconazole) has provided new opportunities for effective treatment of aspergillosis while minimizing toxicity and the potential for drug interactions. Nevertheless, newer antifungals can interact with a number of medications through a variety of pharmacokinetic and pharmacodynamic mechanisms. Therefore, knowledge of drug interaction profiles has become essential for tailoring antifungal therapy in the medically-complex patient.

Most antifungal drug interactions are pharmacokinetic in nature, arising from changes in the absorption or elimination of the interacting drug as well as the antifungal agent. In the GI tract, changes in pH, complex formation with ions, or interference with transport and enzymatic processes in the intestinal lumen could interfere with absorbance of itraconazole, and to a lesser degree, posaconazole. Induction of cytochrome P-450 (CYP P450) metabolism of antifungals in the liver by anti-epileptic agents, rifamycins or nevirapine can accelerate, to varying degrees, the clearance of all azoles and caspofungin resulting in potentially ineffective drug exposures. All azoles can inhibit, to various degrees, the CYP P450 - mediated metabolism of other drugs resulting in increased serum concentrations and potential toxicity. Amphotericin B-mediated decreases in glomerular filtration, active tubular secretion, or other mechanisms can lead to accumulation of potentially toxic drugs and enhance their nephrotoxic potential. Other possible mechanisms of drug interactions include interference of tissue-specific transport proteins (i.e. P-glycoprotein) or other yet undefined pathways for drug uptake into tissue.

While all drug interactions may not be clinically significant, those affecting the pharmacokinetics of antifungals used to treat Aspergillus infection, or interactions that decrease the metabolism/elimination of a drug with a narrow index for efficacy (anti-retroviral therapy) or safety (immunosuppressants, chemotherapy) have the potential for serious harm to the patient. Importantly, many of these interactions may go undetected unless clinicians adopt a proactive approach towards identifying these potentially serious drug interactions in patients on multiple medications. When a potentially serious interaction has been identified, steps for preventing or minimizing the effect of the interaction include: 1) avoiding one of more of the interacting drugs, 2) substitution of one of the drugs with a non-interacting drug, 3) staggering the time or modifying the dose strength or interval of drug administration, 4) changing the route of administration, and/or 5) more intensive monitoring.
for drug serum levels or toxicity. Studying the pharmacogenetic differences in metabolism and improving absorption in the presence of mucositis for the triazoles are important directions of future research.

References

COMBINATION THERAPY – IS IT INDICATED?

William J. Steinbach  
Duke University, USA  
Wednesday, February 22, 2006, 8:00 – 8:30 pm

There has been a recent surge in antifungals with activity against Aspergillus, now creating the opportunity to explore newer treatment strategies. In earlier years, when choices were limited to amphotericin B, itraconazole and several others such as 5-flucytosine and rifampin, combination therapy was in its infancy. We are now seeing an increasing number of clinical reports utilizing combination antifungal therapy for invasive aspergillosis, but we unfortunately continue to lack any definitive trials to substantiate its use. As with any amount of anecdotal or uncontrolled data, there is often a large heterogeneity of patients analyzed which therefore limits the ability to draw firm conclusions. There is also immense difficulty in accurately interpreting the experimental data, both in vitro and animal models, because the experimental conditions are often not consistent between studies. A few trends do appear to emerge from the available data, but the goal remains if we can follow other disciplines in medicine and demonstrate that multiple agents are more efficacious than a single antifungal.
PROTECTIVE TOLERANCE TO ASPERGILLUS: DENDRITIC CELLS, TREG AND BEYOND

Luigina Romani
Dept. of Experimental Medicine and Biochemical Sciences, University of Perugia, Perugia, Italy
Thursday, February 23, 2006, 7:15 - 7:45 am

The balance of pro-inflammatory and anti-inflammatory signaling is a prerequisite for successful host/fungal interactions. The occurrence of regulatory T (Treg) cells in Aspergillus infection and allergy and its interference by the fungus offers a valuable conceptual framework for accommodating events occurring at the host/fungal interface. The ability to control both class and magnitude of the immune response may confer an evolutionary advantage whereby the host would effectively fight infection but limit collateral immunopathology. Alternatively, fungus-induced immunosuppression could be viewed as a powerful immunoevasion strategy for the invading pathogen. Different DC subsets show specialization and complementarity in priming and tolerization to the fungus with plasmacytoid DC fulfilling the requirement for both Th1 priming and tolerization against the fungus. Upon adoptive transfer in vivo in experimental hematopoietic stem cells transplantation, plasmacytoid DC also induce tolerization toward alloantigens and diversion from alloantigen-specific to antigen-specific T cell responses in the presence of donor T lymphocytes. At the level of the cross-talk between DC and Treg the tryptophan metabolic pathway plays an essential role.
INVASIVE ASPERGILLOSIS: ELUCIDATION OF GENETIC RISK FACTORS FOR DISEASE

Aimee K. Zaas
Duke University Medical Center, Durham, NC, USA
Thursday, February 23, 2006, 8:40 - 9:00 am

Aspergillus fumigatus is a common and deadly pathogen in immunocompromised hosts. Invasive aspergillosis (IA), typically caused by A. fumigatus (AF), is the most common filamentous fungal infection following bone marrow transplantation, occurring in approximately 10% of allogeneic bone marrow transplant recipients. Other high risk groups include solid-organ transplant recipients as well as patients receiving chronic corticosteroid therapy. Research over the last several years has provided a better understanding of the epidemiologic risk factors for IA; however, the currently identified risk factors explain only a minor component of the susceptibility to IA among immunocompromised hosts. It has only recently been recognized that susceptibility to IA is greatly influenced by host genetic background. Polymorphisms in genes regulating innate and adaptive immune function are likely important determinants of host susceptibility to fungal infections and may become critically important during times of immunosuppression. Studies using both animal models of IA and human cohorts are attempting to elucidate the host genetic contribution to IA susceptibility. These studies are aimed at identifying host genetic polymorphisms that confer susceptibility or resistance to IA. Studies using animal models have focused on candidate genes, utilize positional cloning, database driven haplotype mapping as well as gene expression studies. In addition, human cohort studies are using genetic association studies to correlate clinical outcomes with genetic background. Understanding the role of host genetics in this complex phenotype can lead to numerous clinical benefits and hopefully improved outcomes. An understanding of genetic susceptibility profile will allow for the implementation of specific prophylaxis strategies for high risk patients, improved donor-recipient selection, and potentially novel therapies for IA.
GENETIC AND RESPIRATORY TRACT RISK FACTORS FOR ASPERGILLOSIS:
ABPA AND ASThma WITH FUNGAL SENSITISATION

Alan P. Knutsen
Saint Louis University, St. Louis, Missouri USA
Thursday, February 23, 2006, 9:00 - 9:20 am

Allergic bronchopulmonary aspergillosis (ABPA) is a Th2 allergic hypersensitivity lung
disease due to bronchial colonization of *Aspergillus fumigatus* that affects 1-2% of asthmatic
and 7-9% of cystic fibrosis (CF) patients. We hypothesize that genetic risk factors predispose
these patients to develop ABPA. We previously reported HLA-DR2 and DR5 restriction as
a risk factor for the development of ABPA. We further propose that HLA-DR restriction is
necessary but not sufficient for the development of ABPA. Recently, we reported that IL-
4R single nucleotide polymorphisms (SNP) and in particular the ile75val SNP in the IL-4
binding region is another risk factor and is associated with increased sensitivity to IL-4
stimulation. It has been reported that the combination of IL-4R and IL-13 SNP, ile75val/
arg110gln, is associated with more severe asthma. In preliminary studies, we have observed
increased frequency of this combination in ABPA asthmatic and CF patients. Another genetic
risk factor reported by Brouard et al is the -1082 GG genotype in the IL-10 promoter in
CF patients for the colonization of *A. fumigatus* and development of ABPA. This genotype
was associated with increased plasma IL-10 levels, and perhaps may be associated with
increased skewing of Th2 *Aspergillus* responses rather than down-regulation of inflammatory
responses. We hypothesize that increased sensitivity of IL-4 mediated activities secondary
to polymorphisms IL-4R in conjunction of other polymorphisms such as IL-13 and IL-10 in
conjunction with HLA-DR2/DR5 restriction to *Aspergillus* antigens in ABPA patients result
in increased B-cell activity, monocyte/dendritic cell phenotype that skews Th2 responses,
and skewing of *Aspergillus*-specific Th2 cells. This model system may be applicable to
other fungi such as *Alternaria* and *Cladosporium* which is associated with increased asthma
severity.
Aspergillus fumigatus causes a wide spectrum of illnesses in humans, including severe invasive infection in those with immunocompromised states, allergy in atopic patients and indolent progressive infection in those without immunodeficiency but prior pulmonary insults. This spectrum of disease offers a special opportunity to understand the relationship of the host and pathogen.

Chronic pulmonary aspergillosis (CPA) is a rare manifestation of aspergillosis and usually affects middle-aged persons who are mildly or not immunosuppressed, with a predominance of males, and comprises chronic cavitary (CCPA) and chronic fibrosing pulmonary aspergillosis (CFPA) as well as simple aspergilloma (1). The clinical distinction between CPA and a simple aspergilloma is difficult.

Investigating genetic factors in patients with acute invasive aspergillosis is challenging, because there are multiple other confounding factors (such as temporary neutropenia and variable corticosteroid doses).

Genetic susceptibility factors have been suggested in the case of ABPA by the familiar occurrence of the disease and have been extensively studied. However, in the case of CCPA and CFPA the background cause has been little investigated.

It has been previously identified a subtle immune defect in innate immunity (mannose binding protein polymorphisms and more recently in surfactant) in these patients (2,3), suggesting that many of the differing manifestations of CPA might be genetically determined. The genes encoding MBL protein and surfactant are in proximity on chromosome 10, which could imply linkage of defects. The role of functional polymorphisms in certain candidate cytokine genes - derived from pathways of Th1 and Th2 immune response that are critical for effective antifungal activity - that could contribute to the risk of developing CCPA has only recently begun to be elucidated (4). Both innate and T helper (Th) immunity play a central role in fungal infection. A bi-directional function exists between the two compartments of the immune system, mainly through cytokine production.

Distinct underlying host genetic factors appear to predispose to different forms of aspergillosis. However, these defects can not be readily amenable to testing in a routine immunology workup.

These novel findings of noncellular, immunogenetic defects in patients with CCPA could explain the devastating consequences of the disease in otherwise healthy individuals without any obvious immune defect.

Future multicenter studies, including a larger cohort of CPA patients, are required and the genetic investigation of each group separately, according to the recent classification, in order to explain on a genetic ground the clinical, radiological, histological interindividual differences in the immune response among patients with CPA.
References
**ASPERGILLUS FUMIGATUS: GROWTH AND VIRULENCE**

Judith C. Rhodes  
University of Cincinnati, USA  
Thursday, February 23, 2006, 11:00 - 11:20 am

*Aspergillus fumigatus* is a ubiquitous fungus that plays an important role in carbon and nitrogen recycling in nature. Because *A. fumigatus* is thermotolerant, it is a predominant organism during the high-temperature phase of the compost cycle. The ability to grow at elevated temperatures and to utilize numerous varied sources of both carbon and nitrogen to support its growth have made *A. fumigatus* an important opportunistic pathogen of humans as well as a vital part of the nutrient-recycling ecosystem (5). Data correlating the growth rate and germination potential of *A. fumigatus* with its pathogenic potential suggest that these are related (3). Experiments with PABA auxotrophs have shown that alleviation of the growth defect restores virulence in vivo (2). In addition, studies have shown that thermotolerant growth also contributes to virulence in some experimental systems (1). Nutritional versatility has been cited as an important contributor to virulence, as well. Indeed, in studies in which pathways involved with nitrogen or carbon sensing have been perturbed, the resulting strains have shown reduced virulence in animal models, even if their in vitro growth rates have not been altered (4). Therefore, the remarkable ability of *A. fumigatus* to grow efficiently under a variety of environmental conditions and to utilize a wide variety of substrates to meet its nutritional needs have also contributed to its role as the predominant mould pathogen of immunocompromised patients.

**References:**


GLOBAL CONTROL OF ASPERGILLUS VIRULENCE

Nancy P. Keller
Department Plant Pathology, University of Wisconsin, Madison, WI, USA
Thursday, February 23, 2006, 11:20 - 11:40 am

We have characterized an A. fumigatus mutant in a developmentally expressed transcriptional regulator, laeA, that is reduced in virulence. Impairment is associated with loss of gliotoxin levels and associated reduced mortality and growth in a pulmonary murine model, increased susceptibility to macrophage phagocytosis and decreased ability to kill neutrophil cells (Bok 2005). LaeA function is conserved in the Aspergilli and likely other ascomycetes (Bok 2004). Microarray analysis of laeA mutants indicate global regulation of secondary metabolite gene clusters (Bok 2006), many of the clusters located in sub-telomeric regions of the genome, including the gliotoxin gene cluster. However loss of gliotoxin production only partly contributes to the laeA phenotype, implicating other factors, possibly other toxins, involved in A. fumigatus pathogenicity. Efforts to understand the mechanism of LaeA regulation of diverse secondary metabolite gene clusters, using the genetic model A. nidulans, indicate LaeA activity may impact heterochromatin status in the fungus. A model of LaeA activation of gene expression at the chromatin level will be presented.

References
ENDOTHELIAL CELLS AND VESSEL DAMAGE

Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, CA, Huntington Memorial Medical Institute, Pasadena, CA, and McGill University, Montreal, Canada
Thursday, February 23, 2006, 11:40 - 12:00 pm

A characteristic feature of invasive aspergillosis is angioinvasion. This process is important in the pathogenesis of the disease because it results in thrombosis of the affected blood vessels and infarction of the adjacent tissues. During invasive pulmonary aspergillosis, Aspergillus fumigatus hyphae invade the vasculature by passing from the abluminal to the luminal surface of endothelial cells. In contrast, during hematogenous dissemination, blood-borne hyphae escape from the vasculature by passing from the luminal to the abluminal surface of endothelial cells. To understand the mechanisms and potential outcomes of angioinvasion, we have investigating the interactions of A. fumigatus hyphae with vascular endothelial cells in vitro and in vivo.

We have found that when the hyphae are added to the luminal surface of endothelial cells in vitro, they are endocytosed by these cells within 1-2 hours. After approximately 4 hours, the organisms begin to cause detectable endothelial cell damage, which progressively increases over time. Damage to the luminal surface of endothelial cells is caused by a factor that is associated with the hyphal cell wall because killed hyphae cause as much endothelial cell damage as do live hyphae. Endothelial cells respond to luminal infection by expressing E-selectin, VCAM-1, IL-8 and TNF-α. Collectively, the expression of these pro-inflammatory molecules likely results in recruitment of leukocytes to the site of infection. The hyphae also stimulate endothelial cells to express tissue factor, which contributes to intravascular thrombosis.

When hyphae are added to the abluminal surface of endothelial cells, they cause significantly less damage than when added to the luminal surface. In fact, by scanning electron microscopy, the hyphae can be seen to penetrate completely through the endothelial cells without causing visible evidence of endothelial cell damage. Interestingly, although abluminal infection of endothelial cells causes less damage than luminal infection, it stimulates greater expression of E-selectin, IL-8, TNF-α, and tissue factor.

Using the mouse model of invasive pulmonary aspergillosis, we have verified some of our in vitro findings. We have observed vascular thrombosis at sites of A. fumigatus angioinvasion. Also, leukocytes were seen be adhering to endothelial cells lining the adjacent blood vessels, suggesting that there was expression of leukocyte adhesion molecules. Finally, by RT-PCR, we determined that A. fumigatus infection induced the expression of E-selectin, VCAM-1, and TNF-α mRNA. The expression of these pro-inflammatory genes coincided with recovery from neutropenia and the influx of leukocytes into the lung. Therefore, it is possible that in vivo, maximal stimulation of endothelial cells is induced by the combined effects of A. fumigatus and leukocytes.
INTERACTIONS OF A. FUMIGATUS WITH THE ALVEOLAR MACROPHAGE

Oumaïma Ibrahim-Granet
Unité des Aspergillus, Institut Pasteur, Paris France
Thursday, February 23, 2006, 12:00 - 12:20 pm

Aspergillus fumigatus is able to cause invasive aspergillosis in immunosuppressed patients. In the immunocompetent situation inhaled conidia are easily cleared by the alveolar macrophage, the key defender of the lung. Knowledge of the cellular pathways involved in the innate immunity against A. fumigatus is poorly represented. We investigated the immune response against A. fumigatus in alveolar macrophages in terms of MAP kinases, NFκB and cytokine signalling. In addition, the contribution of TLR2 and 4 as well as the adaptor protein MyD88 in this response was studied. Our investigations revealed that in murine alveolar macrophages, both MAP kinases, ERK and p38 are activated under in vitro conditions, following addition of A. fumigatus conidia. In vivo experiments, however, showed that ERK is playing a major role directly involved, because activation of p38 was negligible. Immunosuppression with corticosteroids inhibited phosphorylation of ERK and was directly accompanied with a strongly decreased level of TNF-alpha and additional cytokines. In addition, killing of A. fumigatus conidia is reduced when ERK is inhibited. Therefore, ERK appears to be an essential MAP kinase in the defence against A. fumigatus. Activation of the transcription factor NFκB appeared only at late times after infection suggesting an association with the intracellular swelling of conidia.

Due to controversial opinions about the involvement of TLR2, TLR4 and MyD88 in signalling and responses towards A. fumigatus we investigated the response towards conidia in different knock-out mice. Interestingly, the in vitro phosphorylation of ERK and p38 in response to conidia was not affected in TLR2, TLR4, MyD88 knock-out mice and none of these receptor mutants showed an in vivo altered cytokine expression. Finally, immunocompetent mice were not more susceptible to invasive aspergillosis under immunocompetent situations. Therefore we conclude, that these proteins are dispensible for the clearance of A. fumigatus under immunocompetent situations.

We have initiated studies to investigate the contribution of other pattern recognition receptors and Dectin 1 in signalling A. fumigatus in the alveolar macrophage.

References
3. S. M. Levitz. Interactions of Toll-like receptors with fungi. Microbes and Infection, 6: 1351
TH1/TH2 IN ASPERGILLOSIS

David A. Stevens
Sta. Clara Valley Medical Ctr. and Stanford University, San Jose and Stanford, CA
Thursday, February 23, 2006, 12:20 - 12:40 pm

The outcome of the Th1/Th2 balance is a critical determinant of the outcome in invasive aspergillosis. The innate immune system encounters the pathogen first. Dendritic cells (DC) appear to be the critical fulcrum at the intersection of the innate immune system, and which receptors are engaged, particularly which Toll-like receptors are triggered by the pathogen, likely determines which co-stimulatory molecules are expressed during the DC maturation process, and thus which cytokine pathway will eventually dominate. Some proinflammatory cytokines initially produced by naïve phagocytes may also direct dendritic cell direction. Thus DC are the main connections of the innate and adaptive immune systems. The cytokine pathway may be affected by the antigen load, whether and what type of immunosuppressive drug or immunosuppressive co-morbidity is present, the pathogen’s success in converting from conidia to hyphae, the pathogen’s production of toxins, and later whether appropriate antimicrobial chemotherapy is given. Chemokines triggered by cellular interaction with the pathogen call different host cell populations to the site of infection, collectins affect the phagocyte-fungus interaction, and the cytokines produced by the adaptive immune system then regulate the antifungal power of mononuclear phagocytes and polymorphonuclear neutrophils. In these ways the innate and adaptive immune systems work together in host defense. The key cytokines associated with a successful outcome of Aspergillus infection are upregulation of Th1 and proinflammatory cytokines IFNg, TNF, IL-12, GM-CSF, IL-1, IL-6 and IL-18, and down-regulation of IL-4 and IL-10. The converse is associated with progressive disease. The most data about favorable cytokine effects on phagocytes vs. Aspergillus concerns IFNg, GM-CSF and G-CSF, and for unfavorable effects, IL-10 and IL-4. GM-CSF and IFNg have also been shown to possess the ability of reversing the down-regulating effects of immunosuppressants on anti-Aspergillus phagocyte function. Neutralization of several Th1 cytokines in vivo has been shown directly to result in a bad outcome of infection, whereas administering Th1 or proinflammatory cytokines or neutralizing Th2 cytokines has been shown to produce a favorable outcome. Antifungal chemotherapy is associated with a switch to a Th1 profile, and antifungal chemotherapy combined with Th1 cytokine immunotherapy acts synergistically. Thus improved definition of the Th1/Th2 balance is essential for future prospects for immunotherapy, antimicrobial chemotherapy, and vaccination.
DEFINING CLINICAL FAILURE FOR SALVAGE STUDIES

Georg Maschmeyer
Dept. of Hematology and Oncology, Klinikum Ernst von Bergmann, Potsdam, Germany
Thursday, February 23, 2006, 2.45 - 3:00 pm

In patients with invasive aspergillosis (IA), there may be a clearly documented resistance to a particular antifungal agent. Clinical failure may be present already after a few days of therapy for IA. It has been shown that the switch to another licensed antifungal has not been able to equalize to overall clinical outcome. In contrast, there are numerous clinical settings where progression of findings or deterioration of the patient’s condition does not indicate failure, and, conversely, response to a “salvage” antifungal does not necessarily represent a superior efficacy of the drug. If stable disease is regarded as a failure and a patient improves after switch to another antifungal, this could inadequately be attributed to the “salvage” antifungal. Patients with pulmonary aspergillosis emerging during profound neutropenia may show enlargement of their lesions on CT scans, eventually accompanied by clinical deterioration during hematopoietic recovery. This may in fact represent the recruitment of neutrophils and monocytes to the pulmonary “battlefield”, resulting in a favorable clinical outcome also without changing antifungal treatment. If persistence of *Aspergillus* spp. in samples taken from a focus of IA, this could falsely give reason to assess antifungal treatment as failure. Infarcted tissue may contain vital filamentous fungi, because it is poorly penetrated by the antifungal, not indicating a lack of efficacy of this drug against the respective fungal pathogen. In patients treated with an echinocandin, serum galactomannan levels may increase despite successful treatment. Piperacillin-tazobactam or other semisynthetic beta lactam antibiotics may cause “false-positive” *Aspergillus* galactomannan levels. Patients primarily treated with a lipid formulations of AmB switched to a “salvage” antifungal will necessarily be treated with a combination therapy because of the persistence of high drug concentrations in tissue. Criteria to define “clinical refractoriness” or “resistance” or “non-response” or “failure” should be re-defined. One option to establish a valid definition would be to use a composite score including (a) clinical as well as (b) radiological and (c) microbiological or mycological criteria. The latter may include non-culture based methods such as serum galactomannan. Assessment should not be made earlier than after 7 days of full-dose systemic antifungal treatment. If more than one of the above-mentioned criteria shows progression or persistence or worsening, a patient’s course may be assessed as failure. However, in individual cases, e.g. a patient with hematopoietic recovery showing increasing volume of pulmonary aspergillosis and clinical deterioration, it may be recommended to refrain from switching the patient to another regimen and continue the current antifungal treatment for another 7 days before failure is stated. Clinical studies on second-line antifungal treatment for IA should be randomized and blinded, patients should separately be evaluated with respect to their reason for “failure” of primary antifungal treatment, and stratified according to their previous antifungal treatment. Ideally, the first-line regimen would be standardized. Host criteria such as neutropenia or GVHD should be clearly defined and documented with respect to their course over time, and patients should be stratified according to these criteria. A three-arm study (continuation of primary antifungal vs. combination of primary antifungal with a “salvage” drug vs. the “salvage” drug alone) would be ideal.
ABPA was recognized in 1952 in asthma and in 1965 in cystic fibrosis. Treatment protocols emerged from uncontrolled series of patients responding to regimes of oral glucocorticosteroids lasting several months. Oral steroids remain the mainstay of treatment, but dose regime and duration have never been standardized. Wang et al suggested an oral steroid regime based on experience with 25 patients with asthma-ABPA, and similar results were reported in 33 patients by Capewell et al.1,2 Unfortunately, oral steroids in ABPA are problematic due to frequency of relapse after taper or discontinuation; lack of steroid effect on airway fungal burden; and toxicities, several of which are exaggerated in patients with CF due to their underlying disease, as well as a possible increased risk of non-tuberculous mycobacterial infection.

Other therapies were tested in several small case series or uncontrolled trials in ABPA patients with underlying asthma or cystic fibrosis, and in prospective randomized double-blind placebo-controlled trials of inhaled corticosteroids (n=1) or oral itraconazole (n=2) in patients with asthma-ABPA. While inhaled steroids have been used with apparent success in several case reports and small series, the only controlled study was unsuccessful.3 However this study employed beclomethasone in modest dose without spacers, and more recent positive reports employed higher doses, spacers and newer agents such as budesonide. Use of inhaled budesonide with itraconazole can lead to adrenal suppression due to itraconazole inhibition of hepatic cytochrome P4503A4.

Case reports and uncontrolled series with improved treatment success upon addition of itraconazole to steroids were validated by a randomized controlled trial in 55 asthma-ABPA patients, in which more responders were found in the itraconazole group.4 A study in stable asthma-ABPA patients showed clinical benefit and an anti-inflammatory effect of itraconazole in that sputum eosinophils and eosinophil cationic protein as well as serum total IgE and *Aspergillus* IgG antibodies declined.5 In ABPA patients with CF, itraconazole has also been reported to be clinically beneficial in several uncontrolled studies.

Despite combined use of oral steroids and itraconazole, ABPA relapses, and steroid dependence or toxicity, have led to examination of alternative agents in several small uncontrolled studies. Reports of nebulized amphotericin B in several patients with ABPA and CF suggest a potential benefit. Voriconazole has also been used with some success but also some toxicity. Both itraconazole and voriconazole have been used in some CF-ABPA patients as monotherapy with mixed results. A recent report described treatment of refractory CF-ABPA with monthly high-dose intravenous methylprednisolone, also with mixed results. No controlled trials of voriconazole, inhaled amphotericin, or intravenous pulse steroids have been published.
Conclusions
1. Systemic corticosteroids remain the mainstay of treatment, but have never been evaluated by randomized controlled trials, and toxicity is high.
2. Itraconazole is an effective steroid-sparing agent with anti-inflammatory aspects.
3. A possible role for inhaled corticosteroids, voriconazole, nebulized amphotericin, and pulse iv corticosteroids is suggested in case reports but there have been no controlled trials.
4. Future studies should focus on controlled trials of antifungal and immunomodulatory agents since conventional steroid therapy remains problematic.

References
RANDOMIZED TRIALS IN INVASIVE ASPERGILLOSIS:
WE HAVE LEARNED A LOT

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Thursday, February 24, 2006, 4:00 - 4:45 p.m.

Randomized trials in invasive aspergillosis have evolved over the past decade. Definitions of disease now include specifics of the underlying disease and how this effects interpretation of certain tests, including high resolution CT and smears or cultures of sputum and bronchoalveolar lavage. The intent of improving the case definitions is to limit the number of patients in the study who do not have aspergillosis. Outcome of these patients is less likely to be influenced by the choice of antifungal drug. These patients dilute out the effect of the antifungal and tend to can make the two arms of the study have the same outcome. Study hypotheses have changed from underpowered superiority trials to adequately powered noninferiority trials. Consensus building between Europe and North America has allowed trials to be conducted with the same protocol in both regions, thereby increasing study enrollment. In aggregate, the following outcomes can be drawn from randomized trials. Liposomal amphotericin B is possibly superior to C-amB at 14 days and less toxic (1). Whether the dose of liposomal amphotericin is 1 or 4 mg/kg daily is not as important as other factors in determining outcome of possible aspergillosis (2). Amphotericin B colloidal dispersion (ABCD) is less nephrotoxic but has more acute infusion-related reactions than conventional amphotericin B. In an underpowered study, ABCD may have provided an inferior response (3). Starting treatment with voriconazole is superior to starting with conventional amphotericin B (4).

There are limitations in extrapolating data from study populations to the usual patient. Those limitations are less severe than interpreting data from case reports and historically controlled trials. Better tests for diagnosis and outcome analysis have the potential of improving the ease and validity of conducting randomized trials. In an era of increasing cost containment, it will be the randomized trials that provide the clinician with the information needed to avoid inappropriate use of expensive drugs and drug combinations.

References
Aspergillosis is the leading infectious cause of mortality in patients undergoing stem cell transplant or treatment for acute leukemia. Although in the past antifungal prophylaxis strategies focused on the prevention of invasive candidiasis, invasive aspergillosis and increasingly other mould species are currently the more pressing problems. This change may be driven in part by the use of prophylaxis for yeast and viral infections and changes in transplantation practices and supportive care. The diagnosis of invasive fungal infection (IFI) is often not made until infection is well established and mortality remains high. Recently new antifungal agents including the echinocandins and broader spectrum triazole drugs such as voriconazole and posaconazole have become available. With these less toxic and in certain settings more effective agents, comes the question as to how best to use these new drugs. Whilst attractive, prophylaxis has been associated with problems such as drug toxicity, interactions and breakthrough infection with resistant moulds such as Zygomycetes which may limit the benefits of this approach (1). Prophylaxis may be better targeted to only those at highest risk of fungal infection and during the period of highest risk. Patients at high risk for IFI have recently been better defined (2). Although empiric antifungal therapy remains the standard of care for high risk patients who have prolonged or recurrent fever and neutropenia, this approach has recently been re-examined, especially in those already receiving systemic antifungal prophylaxis. As can be seen from recent empiric antifungal therapy trials in patients receiving antifungal prophylaxis, few episodes of fever and neutropenia were diagnosed as due to invasive fungal infection with less than 5% due to invasive aspergillosis (3). Disadvantages of empiric antifungal therapy are unnecessary antifungal exposure with related cost and toxicity, tendency not to use full treatment doses and complacency about aggressively pursuing a definitive diagnosis (4). Further, an increasing proportion of invasive fungal infection occurs in the absence of neutropenia, such as in patients with graft versus host disease, those receiving treatment for Cytomegalovirus infection or potent monoclonal therapies eg Alemtuzumab for lymphoproliferative diseases. Surveillance of high risk patients with newer diagnostic tests such as fungal PCR and antigen testing to trigger pre-emptive antifungal therapy may offer an alternative to prophylaxis and empiric antifungal therapy. However, studies evaluating the impact of this approach on patient outcomes have not yet been completed. Practical issues such as the utility of this approach in patients receiving systemic antifungal prophylaxis or empiric antifungal therapy as well as the choice of the most appropriate test, combination of tests and frequency of testing to use for surveillance need to be resolved.

There have been recent changes in both populations at risk for IFI as well as the epidemiology of infections responsible for IFI and a better understanding of high risk groups. Despite major new developments in antifungal drugs and diagnostic testing, the challenge of devising effective antifungal therapy strategies in order to reduce morbidity and mortality remains.
References
RECENT ADVANCES AND FUTURE CHALLENGES IN THE DIAGNOSIS OF INVASIVE ASPERGILLOSIS (IA)

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Thursday, February 23, 2006, 8:00 - 8:30 pm

IA is a leading cause of mortality in severely immunocompromised patients. Late diagnosis remains a major impediment to the successful treatment of IA. Despite significant advantages associated with the development of standardized diagnostic criteria for IA and promising non-culture-based diagnostic methods, over one third of IA infections are still diagnosed postmortem.

Conventional diagnostic methods based on histology and culture remain the cornerstone in diagnosis of IA, however they have a low yield and their performance is frequently precluded in heavily immunocompromised patients with cytopenias and/or underlying co morbidities. High-resolution chest CT imaging has significantly improved the diagnosis and outcome of IA in persistently neutropenic patients. However, CT imaging does not discriminate between IA and other opportunistic mold infections of the lung.

In recent years, efforts have been directed toward identifying non-culture-based markers for rapid, reliable diagnosis of IA by detection of antigens such as galactomannan (GM) and 1-3 β-D-glucan (BG), and measurement of *Aspergillus* DNA by using PCR. Studies in profoundly neutropenic patients and/or BMT recipients have shown high sensitivity (67% to 100%) and specificity (86% to 99%) rates for GM assay. However, the performance of GM assay in other settings, including pediatric patients, those receiving antifungal prophylaxis, and solid organ transplant recipients is suboptimal. Other factors, such as the pretest probability of infection, the patient’s immune status, antifungal therapy, antibacterials, and diet may also affect both the performance and interpretation of the GM assay. A colorimetric assay for the detection of BG, an integral cell-wall component of most pathogenic fungi, has shown promising The sensitivity (ranging from 67% to 100%) and specificity (ranging from 84 to 100%) in limited studies thus far. Nevertheless, the BG assay appears to be less sensitive and reproducible and becomes positive later in the course of IA than GM antigen assay.

PCR detection of *Aspergillus* DNA is a promising method for early detection of IA, and also allows for diagnosis of other opportunistic fungal infections. The sensitivity of PCR is excellent, but its specificity for invasive infections can be problematic. Multiple unresolved issues accompany the use of PCR for diagnosis of IA, including the sample type, amplification strategy, protocol, primer selection, and account for the lack of a standardized, commercially available assay. There have been no extensive studies examining how PCR performs in comparison with galactomannan detection for the early diagnosis of IA. Comparative prospective evaluation of the existing non-culture based diagnostic methods would facilitate their incorporation into preemptive strategies for the optimal management of IA. There is a need for innovation in this area. Preclinical studies suggest that the detection of *Aspergillus* secondary metabolites or peptidomimetic approaches for imaging are potentially
new avenues for the development of novel diagnostic tests. In view of the evolving and complex epidemiology of opportunistic mold infections, developing clinical, laboratory and radiologic parameters that favor the diagnosis of pulmonary aspergillosis from other lung mycoses is also important an research direction for the future.

References
The efficacy of echinocandins against Aspergillus species has been established through in vitro assays, in animal models of infection, and in clinical practice. Caspofungin and other inhibitors of 1,3-β-D glucan synthesis (GS) produce dramatic morphological changes, but incomplete clearing, in cultures of growing hyphae. Other filamentous fungi, including many rare moulds, exhibit a similar in vitro response to GS inhibitors. Despite the apparent fungistatic in vitro activity against filamentous fungi, several compounds in this class have strong efficacy in vivo. For example, caspofungin prolongs survival in chronically immunosuppressed mice with induced disseminated aspergillosis, even when neutropenia is maintained for weeks after a short dosing regimen. Kidneys of these mice showed no evidence of recrudescent A. fumigatus burden after the infection had been cleared. One possible explanation for echinocandin-mediated clearance of A. fumigatus in vivo stems from the newly-discovered role of β-glucan in the inflammatory response. Binding of cell wall β-glucan to the dectin-1 receptor of macrophages leads to production of proinflammatory cytokines, which augment the innate immune response to swollen conidia and germlings. Dramatic changes in A. fumigatus cell wall structure, such as those produced by exposure to echinocandins, may increase the opportunity for interactions between β-glucans and dectin-1, and lead to a heightened response to “wounded” hyphae. Further defining the structural changes that occur in the cell wall when a filamentous fungus is challenged with an echinocandin may provide additional insight into the significant efficacy of these compounds in vivo.
CLINICAL EFFICACY OF CASPOFUNGIN IN THE TREATMENT OF ASPERGILLOSIS

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Friday, February 24, 2006, 7:45 - 8:15 am

The eucaryotic nature of fungi has been for long, a major hindrance for the development of active antifungal agents with low toxicity. However, during the recent years, a better understanding of the fungal cell wall structure, allowed to target cell wall components not present in mammalian cells. Caspofungin, the first representative of the echinocandin family, acts by inhibiting the synthesis of 1-3 β D glucan which is essential for the integrity of the cell wall structure. The clinical experience with caspofungin is being built up step by step, covering gradually the whole spectrum of the standard care of invasive aspergillosis, from the empiric treatment of patients at risk during neutropenia, to the first-line, the salvage and the combined therapy, in probable or proven cases. One of the first issues to be explored was the salvage treatment with caspofungin in case of refractoriness to or intolerance of previous antifungal treatment in invasive aspergillosis. In this trial, among the 83 patients evaluable for efficacy, the response rate was 45 % (1). What distinguishes this study from other similar trials evaluating lipid formulations of amphotericin B or triazoles, is the high proportion of refractory cases (83 %) and that of proven invasive cases (41 %). Empiric therapy with caspofungin in patients with persistent fever despite 4 days of antibiotics and neutropenia was compared with a liposomal formulation of amphotericin B in a large, randomised double-blind trial enrolling 1123 patients (2). The overall success rate was similar for the two drugs, around 34 %. However, among the subgroup of patients with baseline invasive aspergillosis, the response rate was higher in the caspofungin arm (41.7 % vs 8.3 %). Also, the survival to 7-days follow-up was greater in the caspofungin group (92.6 % vs 89.2 %, p=0.05). Breakthrough infections with resistant moulds such as zygomycetes, Fusarium or Trichosporon species did occur on caspofungin, but in a very limited proportion. Combination antifungal therapy is a new issue that is being explored nowadays. Polyenes and azoles act through the same target which is ergosterol. Caspofungin, because it has a different target, is well suited for combination with either class. In an open, non randomised study, caspofungin was combined with amphotericin B formulation or a triazole in the treatment of invasive aspergillosis in patients refractory to or intolerant of previous antifungal therapy. Among the 53 patients enrolled, the success rate at end of therapy was 55 % and for those who received more than 7 days of combined therapy, it was 66 % (3). Another retrospective review of patients with invasive aspergillosis refractory to initial therapy with amphotericin B formulation and who received as salvage therapy, either voriconazole alone (31 patients) or a combination of voriconazole and caspofungin (16 patients), showed a significant reduction in mortality (4). The safety data derived from all the clinical trials done so far, showed that caspofungin has one of the best safety profiles of all available antifungals active against Aspergillus. Histamine-release reactions have been rarely reported and increased caspofungin exposure by concomitant cyclosporin administration with elevated liver enzymes observed in healthy volunteers. However, this interaction with cyclosporin does not seem to have a
significant clinical impact (5). In summary, what the clinical data tell us today is that caspofungin has added an important weapon to the therapeutic armamentarium we dispose against invasive aspergillosis. It constitutes the best option for empiric antifungal therapy in persistently febrile neutropenics and for salvage therapy. The first-line and the combination therapy are still being furtherly evaluated.

References
USEFULNESS OF β-D-GLUCAN ASSAY IN THE DIAGNOSIS OF DEEP MYCOSIS
- EXPERIENCES IN JAPAN

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Friday, February 24, 2006, 9:50 - 10:05 am

The G-test, which measures β-D-glucan (BDG), was developed in 1992 by Obayashi et al. for the serological diagnosis of deep mycosis1. Clinical trials are being conducted in the areas of hematological and respiratory diseases, and exceptional results of 90% sensitivity and 100% specificity in diagnosing deep mycosis have been achieved2. Moreover, with candidemia positive findings have been obtained earlier than with blood culture in some cases3. BDG has also been reported to allow detection of relatively rare mycoses such as those due to Trichosporon, Fusarium, Acremonium, and Saccharomyces in addition to Candida and Aspergillus4. In the area of hematological disorders, the detection of causal fungi is usually difficult, and guidelines such as those of the Infectious Diseases Society of America (IDSA) recommend empiric therapy with an antifungal agent in instances of febrile neutropenia that does not respond to broad-spectrum antimicrobial agents in 4-5 days. In Japan, though, BDG measurement is widely used in this instance. While the turbidimetric BDG assay is also used for this purpose in Japan, the chromogenic kinetic BDG assay is recommended based on its sensitivity. Medical sources of BDG can lead to false positives in the absence of deep mycosis, including dialysis membranes and filters made from cellulose, gauze, blood products (β-globulin and albumin), and drugs containing BDG (sizofiran and lentinan).

Most pathogenic fungi besides Zygomyces originally contain BDG, so identification of species is not possible. However, detection of BDG in both candidiasis and aspergillosis, two major deep mycoses is actually not a major drawback. In the area of hematology, distinguishing between the two is relatively easy based on the underlying illness and radiological findings. Most mycoses encountered in the areas of surgery and emergency medicine are candidiasis, so along with Candida colonization, as has been used in the past, BDG has become as an important indicator in additional diagnosis for early treatment. Like Aspergillus galactomannan, BDG is used in Japan as a microbiological factor in diagnostic guidelines for deep mycosis. A new BDG assay (Fungitell) using Limulus polyphemus was recently developed5. While there are some differences in cutoff levels and reactivity between Fungitell and the G-test, its sensitivity is satisfactory, and it is considered a useful test. In addition, BDG can also be used to monitor clinical efficacy, and examples of its effective use are presented.

References
2. Lancet 345: 17-20, 1995
As highlighted in the 1st Advances Against Aspergillosis Conference the development and use of molecular methods to aid in diagnosis of invasive aspergillosis is extensive, but consensus over an optimal method has not been achieved (1). With high sensitivities and specificities the benefits of Aspergillus PCR assays are clear and the use of real-time PCR platforms for quantifying fungal burden, reducing the sample turn around time and minimising the opportunity for contamination have further enhanced their clinical relevance. Apart from contamination (2) the other weaknesses of Aspergillus PCR methods are often less evident, albeit possibly widespread:

1) Choice of specimen (BAL, CSF, Serum or Whole Blood).
The wrong choice may limit target availability and/or introduce false positive and false negative results due to colonisation or contamination and PCR inhibition, respectively.

2) Extraction Procedure (Manual or Automated).
The performance of any PCR assay is dependent on the quality of the DNA extracted and the use of a robust and reproducible extraction procedure is paramount as the variation in the quality and quantity of DNA released by different methods can be great.

3) PCR assay design.
In designing a PCR assay time must be taken to minimise oligonucleotide cross hybridisation. Non-specific oligonucleotides may generate false positive results. However, the use of non-specific primers in a PCR assay utilising a specific probe may lead to the generation of false negative results.

4) PCR Platform
The choice of PCR platform is critical. The use of real-time platforms over block-based thermocyclers is widely preferred, although the choice of real-time platform can also be significant (3).

5) PCR results
Result interpretation in a clinical context is also important. It is generally accepted that two consecutive, reproducible positive PCR results are required in determining a true positive result. In a clinical setting this may be difficult with patients receiving antifungal therapy possibly reducing, already low, fungal burden below reproducible PCR thresholds.

In conclusion PCR is a useful tool to aid in the diagnosis of invasive aspergillosis, although it is essential that an optimal method be agreed to allow inclusion in future consensus diagnosis criteria. It should be used in conjunction with other methods (e.g. GM ELISA and HRCT) to enhance the opportunity for detection of this devastating infection.
References
The increased incidence of invasive infections due to *Aspergillus* and the growing number of new antifungal agents have multiplied the demand and interest for in vitro antifungal susceptibility testing. At time, the M38-A reference method for filamentous fungi, published by the Clinical Laboratory Standard Institute (CLSI) is available for the determination of MICs (minimum inhibitory concentration) of *Aspergillus* spp. against antifungals. However, the M38-A methodology exhibits some limitations and it is well known that the size of inoculum, the use of growth medium, the time of incubation and the inoculum preparation method can influence MIC values. Recently, it was shown that an inoculum size of 105 CFU/ml distinctly differentiated amphotericin B or itraconazole-resistant *Aspergillus* strains in vivo from the susceptible ones. The MICs of amphotericin B and itraconazole were > 2 and > 8 µg/ml, respectively. Several studies have demonstrated that conidia counting in haemocytometer for inoculum preparation is an accurate, reproducible and universal procedure, independent of the colour and size of conidia.

Based on these findings the European Committee on Antimicrobial Susceptibility Testing (EUCAST) has left the Subcommittee on Antifungal Susceptibility Testing (AFST-EUCAST) in charge of the preparation of guidelines for in vitro susceptibility testing of antifungals against *Aspergillus* spp. This committee adopted the M38-A reference method and developed a proposed EUCAST broth dilution method for susceptibility testing against *Aspergillus* (EUCAST-AST-ASPERGILLUS) with the following main modifications: (i) RPMI 1640 supplemented with 2% glucose (RPMI 2%G) as assay medium, (ii) inoculum preparation by conidium counting in a haemocytometer and (iii) an inoculum size of 2 x 105 - 5 x 105 CFU/ml. The incubation time is about 48 hours at 35°C and MIC is read visually. The concentration of drug in the first well in which there is no growth is the MIC value. Amphotericin B, itraconazole, voriconazole and posaconazole at concentrations of 0.015 to 8µg/ml are recommended as antifungal drugs.

At time, members of the AFST-EUCAST perform extensively interlaboratory exercise studies to validate this method and to define quality control strains. The standard method described herein is intended to provide a valid, easy, rapid and economic method for testing the susceptibility to antifungal agents of *Aspergillus* spp. and to facilitate an acceptable degree of conformity, e.g. agreement within specified ranges, between laboratories in measuring the susceptibility.
References


Aspergillus species have been the subject of numerous epidemiological studies. Several phenotypic and genotypic techniques have been used for examining the genetic variability of A. fumigatus and its relatives. The most useful typing techniques are DNA based methods including the random amplified polymorphic DNA (RAPD) technique, microsatellite and minisatellite length polymorphisms, DNA-DNA hybridization to moderately repeated inactive retrotransposon-like sequences, and multilocus sequence typing (MLST). The outcomes of molecular typing studies are numerous. The results of typing clinical isolates indicate that most of the invasive aspergillosis patients were infected by a single strain, although mixed infections with multiple strains of A. fumigatus can also occur. In contrast, 5 to 10 different genotypes per patient were isolated from cystic fibrosis patients. Genetic analysis could not discriminate between clinical and environmental isolates of A. fumigatus, indicating that every strain present in the environment is a potential pathogen if it encounters the appropriate host. The ability of any A. fumigatus isolate to become pathogenic also provides evidence for the absence of host specificity (1). The source of infection (community acquired vs nosocomial) can also be monitored by typing. The isolation of identical strains from a patient and from the hospital environment of that patient indicates that the infection was nosocomially acquired (about 40% of IA cases appear to have a nosocomial origin). The potential sources of A. fumigatus conidia are potted plants, construction work, showers, personal and medical materials (eg. pillows), etc. Typing studies led to the discovery of a new pathogenic species, A. lentulus (2), and to the identification of species not known previously to be pathogenic, including Neosartorya hiratsukae, N. udagawae and several unnamed aspergilli related to A. fumigatus, A. viridinutans and N. spinosa. Multilocus microsatellite sequence typing revealed the existence of two genetically isolated groups (the ‘fumigatus’ and the ‘occultum’ clade) within a global A. fumigatus population (3). A. fumigatus was suggested by the authors to be the first example of a true cosmopolitan fungus with no evidence of correlation between a genotype and its geographic location. Additionally, the results obtained based on statistical tests support the premise that recombination played an important role in A. fumigatus populations (3). The observation of repeat induced point mutations in the Aftul transposon sequences, the presence of numerous transposons in the A. fumigatus genome, and the lack of correlation between trees constructed based on independent data sets also support the presence of recombination in A. fumigatus populations. The observed recombining population structure could be caused either by past or present meiotic exchanges or parasexuality. The discovery of functional mating type genes in A. fumigatus indicates that recent sexual processes could be the cause of such a population structure (4). The detection of nearly identical ratios of the two mating type genes in a global survey is also consistent with the presence of sexual reproduction in the global A. fumigatus population (4). Regarding the evolution of MAT loci among aspergilli, the presence of a partial MAT1-2 sequence within the idiomorph-flanking
region is consistent with the proposal that asexual species such as *A. fumigatus* arose from a homothallic ancestor in which *MAT1-1* and *MAT1-2* HMG genes originally were adjacent, but the genes then became separated as a result of a translocating break and aberrant segregation (5). This hypothesis is also supported by the extensive conserved synteny observed at the MAT loci of *A. nidulans*, *A. fumigatus* and *A. oryzae* (5). Experiments are in progress to cross *A. fumigatus* (and *A. oryzae*) isolates in order to induce a sexual cycle in these species.

References
Total avoidance of Aspergillus is difficult since these fungi are widespread. Risks increase greatly when dusts accumulate or water damage occurs in buildings, which occur many ways; all must be avoided to minimize exposure to high-risk patients.

Design issues to consider for hospitals include pressurization between rooms and across building envelopes. As shown in Wiseman (2003), a 2.5 Pa pressure differential induces up to 200 L/s of air infiltration. In a humid climate, this can cause enough moisture accumulation to permit mold growth in the walls. Use and placement of insulation, air barriers and vapor retarders in the envelope must work with the ventilation system to avoid mold growth in walls, especially in cold climates and in hot, humid climates (Straube 2005). Such growth typically includes Aspergillus spp. and spores from such growth can infiltrate interior spaces through unplanned (and hence unfiltered) airways (Morey 2003). Construction materials that do not support mold growth should be considered for use where moisture may occur. Outdoor air intakes should be above grade, and materials used in HVAC interiors should be moisture resistant and/or able to withstand cleaning. In cooling climates, HVAC equipment should be able to adequately temper both humidity and temperature.

Construction and renovation activities disturb dust with Aspergillus spores, so strict dust control dust is required to prevent escape from work areas into patient areas. HVAC equipment should be easy to maintain, have at least MERV 8 filters, up to MERV 17 in critical areas and not favor dust accumulation. UVGI helps control contagious pathogens, as discussed by First (1999), but less is understood for building-related pathogens. In humid climate cooling seasons, chronically damp walls of ventilation systems collect dust and organic material in which mold can grow. The efficacy of UVGI illumination to reduce mold growth on these walls was described by Levetin (2001).

The discovery of mold growth in a hospital should prompt diligent efforts to remediate it. Any affected area must be cleaned with adequate dust control measures, considering the extent and location of the colonization, the type of water, and the time materials have remained wet. The success of mold remediation may be assessed by dust loading on surfaces, and in health care facilities the efficacy should also be evaluated by air sampling for culturable, thermotolerant molds.

Multiple factors in hospitals must be managed to minimize risks of Aspergillus exposure for high-risk patients. Building envelope and ventilation systems must be adequately designed, installed and maintained. Construction and renovation activities should be carefully planned, closely monitored and coordinated with infection control officers to prevent fugitive dust entry into occupied areas. UVGI can be useful in certain applications as part of an infection control scheme, but the limitations of UVGI for molds should be recognized. The value of routine air monitoring remains unresolved, but building operation should promptly address moisture problems and mold growth with proper caution. Additionally in hospitals, the diligent control of dusts and culturable thermotolerant molds are critical for minimizing risk to patients.
References
Animal models, particularly those studied in rodents, are an integral part of antifungal drug development. The model must be well defined and the characteristics of a good model have been detailed previously (1, 2). The capacity to control different variables are beneficial, allowing a well defined model system to be used to address various issues of efficacy with monotherapy, combinations, immunotherapy, and look for novel indications, and potentially preventative vaccines, prior to a clinical trial or where a clinical trial is impractical. Animal models also are useful for studying diagnostic assays, as well as pharmacology and toxicity. Thus, we are able to address issues in vivo that cannot be answered by in vitro tests. In our laboratory we have standardized three murine models of aspergillosis: systemic infection in noncompromised mice, cerebral infection in pancytopenic mice and pulmonary infection in steroid-suppressed mice. Other laboratories have used inhalational murine models, and pulmonary or systemic models in rabbits and guinea pigs, as well as birds and insects (1).

There are two primary parameters of efficacy followed. Survival studies results in clear data sets, but ask much of a potential therapeutic. In addition, ethical questions arise concerning the humane care and use of animals requiring euthanasia of the animals prior to death, which can skew the data because of subjective judgments made by the investigators. Infectious burden can be a more sensitive parameter of efficacy, but the best method of determination for Aspergillus is controversial, with some investigators choosing a PCR methodology and others using CFU determinations. Each method has benefits and drawbacks, and our own studies demonstrated that drug efficacy of caspofungin and amphotericin B against CNS aspergillosis could be demonstrated at a single time point using either method. Surrogate markers of infection are much sought after and have included radiographic imaging techniques applicable to pulmonary models in rabbits, as well as body weight and temperature. In addition, PCR methods and assays for antigenemia (galactomannan and glucan) have been used to study disease progression and for diagnostic purposes.

Are animal models predictive of clinical efficacy? We have found the systemic model has been predictive of future clinical efficacy for azoles, echinocandins and amphotericin B preparations. However, a single model cannot answer all efficacy questions and different models can give different results. As an example we found micafungin to be highly efficacious in murine systemic and cerebral models of aspergillosis, whereas it was ineffective against pulmonary disease in steroid-suppressed mice. A benefit of using models is the ability to examine various antifungal combinations for potential enhancement of efficacy or drug antagonisms. We, and others, have found conventional amphotericin B and itraconazole to show some antagonism in vivo. Against CNS infection, where it would be impractical to do a clinical trial, we have used our model to show significant enhancement of efficacy by both survival and reduction of infectious burden by combinations of a
lipid-carried amphotericin B and voriconazole, and that lipid-carried amphotericin B in combination with an echinocandin showed nonsignificant improvements in efficacy, and were not antagonistic. However, these enhanced efficacies again did not carry-over to the pulmonary model of invasive aspergillosis in steroid-suppressed mice. Lastly, we use animal models for the purposes of studying pharmacological responses such as PK tissue penetration and drug distribution, dose-related damage to different target organs in relation to the capacity to effect cure of infection and determination of whether the trade-off of damage to an organ is acceptable. Thus, the use of animal models for various studies provides us with useful information that has historically been shown to reflect what happens clinically.

References
It is clear that the presentation of the definitions of invasive fungal infections in immunocompromised patients fulfilled a need. In virtually every scientific paper and clinical trial protocol produced since 2002 these definitions are used, which has helped greatly in interpreting the results and in comparing reported data (1). These guidelines represented an attempt to establish specific, reproducible guidelines to allow entry of patients into clinical trials on the basis of standardized criteria. These criteria were explicitly not recommended to manage patients in daily clinical practice.

Soon after their introduction it became apparent that the definitions were used for research purposes in patients other than those with cancer and, more troublesome, also for clinical decision making, which carried the risk of severe under-treatment when therapy would be postponed until an invasive fungal infection met the criteria for a proven or probable infection. Subira and colleagues called an alarm that 64% of their patients with proven invasive aspergillosis at autopsy did not meet any decisive criterion for diagnosis prior to death (2). Whereas inappropriate use as such should not be regarded as a shortcoming of the definitions, the perceived need to modify the criteria to ensure quantitatively sufficient entry of proven and probable cases into clinical studies should (3). Indeed, it is beyond question that significant numbers of patients with eventually proven fungal infection may be excluded from entry into a trial on the efficacy of an antifungal regimen if the criteria for the proven and probable categories are applied strictly. A typical representative of this non-eligible group is a neutropenic patient with a characteristic pulmonary infiltrate such as a halo or air-crescent sign. The categories of proven and probable infections were recognized as reliable and reproducible, albeit that new diagnostic tools could serve to make these categories larger. Conversely, the criteria required to classify as a possible invasive fungal infection appeared so loose-fitting that the vast majority of patients meeting them have, in fact, no fungal infection at all. Antibiotic-refractory fever in a persistently neutropenic host proved a dominant factor; an additional nonspecific symptom sufficed to classify as a possible invasive fungal infection. Inclusion of many such patients in clinical trials dilute a study population and inevitably will frustrate assessment of the antifungal potency of a given compound or strategy. Hence, reduction of the number of trivial cases became a major trigger to consider a refinement of the fairly recent set of definitions (4). A second reason was the increasing evidence on the value of several indirect diagnostic tests, for example, the high resolution CAT-scan of chest and abdomen, detection of fungal DNA by PCR in body fluid, of β-D-glucan in plasma, and of galactomannan in blood as well as in bronchoalveolar lavage fluid. The aim of valid surrogate markers is that they are not only precise but also accurate and fit to convey useful information (5).

Upfront two strategic restrictions were unanimously accepted: the definition set should remain easily reproducible and had to offer the opportunity for a reasonable comparison.
of the future data sets with those collected during clinical trials conducted in patients with proven and probable invasive fungal infections according to the original definitions. Otherwise historical would become impossible.

References
This presentation will focus on the aspergillosis trials. The voriconazole comparative trial is a reference for trials in invasive aspergillosis. The major reason is obviously the successful outcome in demonstrating a superiority of voriconazole over amphotericin B including a survival advantage. However the innovative design of his study also helped to make it a new standard in clinical trials in IA: inclusion criteria anticipated most of the EORTC/MSG consensus definition criteria published several years later, acceptance of a radiological sign – the halo sign – as a major sign for the disease, evaluation of a strategy of treatment with modification in case of failure or intolerance, response assessment using strictly predefined criteria and confirmation of eligibility and of response assessment by a data review committee (DRC) of 12 members including radiologists.

The strength of this design had also some limitations. The halo sign on CT-scan was not precisely defined leading to discrepancies between investigator and DRC in the interpretation. These discrepancies were increased by the variable quality of the CT scan, and of the copy of the films. Sixteen percent of the cases were excluded from the modified intent to treat (MITT) population by the DRC for non confirmation of the halo sign. However intent to treat (ITT) analysis showed that these non-eligible patients had the same response advantage to voriconazole than the eligible patients suggesting that they really had aspergillosis although they did not fit the very strict inclusion criteria.

The halo sign is now better known by both investigators and DRC members. Improvement in CT scan technology, including transmission of electronic images to the DRC, helps to identify appropriately this sign. In the recently completed Ambiload study (comparison of two doses of liposomal amphotericin B for primary therapy in invasive aspergillosis) using similar entry criteria, the DRC rejected less than 10% of the cases for non-confirmation of the halo sign demonstrating an improvement of assessment by investigators.

Recruitment of invasive aspergillosis in clinical trials is a concern: number of cases are limited and is likely to decrease with more extensive use of prophylaxis; diagnosis is still difficult despite the use of antigen detection tests; competition now exist with the development of several potentially active drugs; and finally the use of combination therapy is more frequent although there is no demonstration of benefit in first line therapy. An elegant way to facilitate inclusion is to enroll patients with a possible invasive fungal infection till the results of the baseline tests are available. Patients remain in the study if probable or proven infection is confirmed within a few days. This flexibility made the success of the Ambiload study with a recruitment of 201 eligible patients among a total of 339 enrolled patients over a 18-month period.
Antigen testing is now routinely performed in most centers and is an effective criterion for identifying the cases. However various cutoffs are used in local centers and also in clinical trials. A uniform cutoff, lower than the cutoff recommended in Europe by the manufacturer, is mandatory for the next clinical trials.

Stratification according to the major prognostic factors is essential. Currently little is known about factors of poor prognosis in invasive aspergillosis with the exception of GVHD and steroid therapy in allogeneic HSCT recipients. The recent Ambiload study identified two baseline prognostic factors: allogeneic HSCT, a factor usually taken into account for the stratification, and progressive malignancy, a factor obviously associated with poor survival but not yet used for stratification in clinical trial. Other prognostic factors exist and should be considered at least to compare the severity of the disease at baseline if not used for stratification.
Treatment guidelines are based on evidence and as such form an integral part of the management of fungal infections. Until recently almost all such guidelines attained the level of expert consensus and little more. The successful completion of the randomised controlled trial of voriconazole versus other licensed antifungal therapies changed this (1) providing us with the first such study to meet the rigorous standards required of a randomised controlled trial which on its own allowed efficacy of be determined providing the highest level of evidence. As voriconazole was more effective in treating invasive aspergillosis (IA) and in reducing attributable mortality it has become the drug of choice for first line therapy of this invasive mould disease. However there are patients who tolerate the drug poorly and others who cannot be given theazole as it might interfere to their detriment with another more clinically important drug e.g. ciclosporin necessary to prevent GVHD. An alternative drug is therefore necessary. Preclinical studies indicated a dose-response to increasing doses of liposomal amphotericin B and in patients dosages as high as 15 mg/kg/day appeared well tolerated (2) To test this a formal RCT was conducted that was also sufficiently powered to address the question. However there was no difference found in efficacy between the 10 mg/kg/d arm and the 3 mg/kg/d arm proving once again that mice are not men (3). The potential of an echinocandin as first-line therapy is also undergoing a proof of concept phase with the expectation that a further formal comparative trial will be conducted EORTC (4). There is here an analogy between what was found for monotherapy of bacterial infections in the immunocompromised host.

Irrespective of the drug used for initial therapy many patients will respond to treatment with a single agent but a substantial proportion will need to switch treatment because of an inadequate response or intolerance (so called “Salvage therapy”) or have therapy complemented with another drug. Drugs that have been shown of value for salvage therapy include caspofungin and posaconazole. Though the evidence for this indication is meagre most clinicians are comfortable in using either drug. Others driven more by emotion than evidence feel compelled to adapt initial therapy to combination therapy by adding another drug like caspofungin to first-line therapy and maybe even 2 drugs for instance adding the echinocandin with a lipid formulation of amphotericin B to voriconazole Although irrational this approach is at least understandable if not commendable given the obvious feeling of desperation in the face of failing treatment. Formal trials are clearly needed but these will necessarily have to be more of a proof of concept design rather than a classical RCT because of limited numbers and lack of resources. Moreover the regimen should be based on what is known about the pharmacokinetics and pharmacodynamic of the drugs in question as well as the nature of any interactions. The laboratory has a clear role to play in supplying in-vitro daya and data from animal studies. The purpose of the clinical trial would be to see whether
these results are in any way reflected in patients. One way of tackling the problem has been to employ therapy empirically on the flimsiest of evidence namely persistent fever refractory to antibiotic therapy. This is increasingly being abandoned with the availability of improved diagnosis in favour of a more pre-emptive approach. That is to initiate therapy before all the evidence is in for probable and proven IA but when there is some evidence of invasive fungal diseases likely due to Aspergillus. This strategy attempts to strike the best balance between science and clinical practice to optimise survival if not resolution of IA. Clearly a formal trial still needs to be conducted but a recent single-centre study did indicate a significant reduction in antifungal use without any loss of effectiveness.

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GENE ESSENTIALITY IN *ASPERGILLUS*

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Friday, February 24, 2006, 2:20 - 2:40 pm

Essential gene products are seen as potential targets for developing antifungal drugs, and genome wide screening methods to identify such genes have been devised in a number of laboratories. These have made use of random insertion approaches in diploids (Firon and D’Enfert 2002), where failure to recover haploid segregants after haploidisation is taken as evidence for essentiality. Genes identified by such a procedure might be expected to include those whose deletion leads to impaired growth, rather than no growth, since the latter could also result in failure to recover haploid mutant segregants from heterozygous diploids. In many cases, bioinformatics can provide further information about the probable function of such essential genes. However, confirmation of gene function requires experimental analysis, including mutation, and this is particularly important for orphan genes, where bioinformatics gives either limited information, or none at all. Since mutants are not recovered from haploidisation, other approaches are needed for functional analysis. These include recovery of haploid spores carrying lethal deletions from balanced heterokaryons (Oakley et al. 1990), and the use of conditional promoters in haploids. For example, the native promoter can be replaced by the glucose-repressible *alcA* promoter of *A. nidulans* (Romero et al. 2003).

Though is possible that full repression may not be obtained at some genome loci, it offers an option for investigating the mutant phenotype of a down-regulated gene of interest. Bioinformatics has been used to find genes whose putative orthologues are essential in other species, particularly *Saccharomyces cerevisiae*, and such genes have been tabulated in Nierman et al. (2005). However, there is also the risk that apparent orthologous genes may have not share exact functionality and essentiality in different fungi, so these still need validating. For example, two genes involved in polar growth, *BEM1* and *CBK1*, are not essential in *S. cerevisiae*, but are essential in *A. nidulans*.

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AMINO ACID ACQUISITION, CROSS-PATHWAY CONTROL AND VIRULENCE IN A. FUMIGATUS

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Friday, February 24, 2006, 2:40 - 3:00 p.m.

The regulation of metabolism and the control of developmental programs are interwoven processes in fungi. Amino acids are essential precursors of translation and are either (i) taken up from the environment by transport processes, or (ii) synthesised from precursors which are derivatives of the carbon and nitrogen primary metabolism, or (iii) result of the degradation of proteins which are no more required under specific conditions. Fungal amino acid biosynthesis is therefore carefully regulated. This includes the transcriptional and the post-transcriptional control of genes encoding biosynthetic enzymes or the regulation of enzymatic activities. In A. fumigatus as well as in other fungi, an imbalanced amino acid diet results in amino acid starvation and activates a complex genetic network including a signal transduction pathway and the transcriptional activator CpcAp. This genetic network, which has been named ‘cross-pathway control’, coordinately regulates hundreds of genes in numerous biosynthetic pathways. The A. fumigatus transcription factor CpcAp is required for full virulence in the mouse model (Krappmann et al., 2004). The molecular mechanism for this observation is unknown, because only the basal activity of CpcAp and not the induction of the cross-pathway control seems to be required for virulence. In yeast, activation of this network results in cell-cell and cell-surface adhesion (Braus et al., 2003), in A. nidulans, activation of the cross-pathway control impairs developmental programs as the formation of fruitbodies (Hoffmann et al., 2001). The amount of CpcAp in the fungal cell is regulated by the translational control of the corresponding mRNA in the cytoplasm. For Gcn4p, which is the yeast counterpart of CpcAp, an additional control of protein stability in the nucleus (Pries et al., 2002), which depends on the availability of amino acids, has been demonstrated. The genes which are necessary for CpcAp control are present in the genome of A. fumigatus suggesting a conserved fungal control of this regulator. The present state of our knowledge of the cross-pathway control of A. fumigatus will be presented.

References
AIRBORNE FUNGAL FRAGMENTS AND ALLERGENICITY

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Friday, February 24, 2006, 2:40 - 3:00 pm

Exposure to fungi, particularly in water damaged indoor environments has been thought to exacerbate a number of adverse health effects including subjective symptoms such as fatigue, cognitive difficulties and problems with memory to more definable diseases such as allergy, asthma and hypersensitivity pneumonitis. Understanding the role of fungal exposure in these environments has been limited by methodological difficulties in enumerating and identifying fungi in environmental air samples. Consequently data on personal exposure and sensitization to fungal allergens has been restricted to the spores of a few select and easily identifiable species. The contribution of airborne spores, hyphae and fungal fragments of other genera to exposure and allergic sensitization are poorly characterized. There is increased interest in the role of aerosolized fungal fragments following reports that the combination of hyphal fragments and spore counts improved the association with asthma severity [1]. Such fragments are categorized as either sub-micronic particles or larger fungal fragments. In vitro studies have shown that sub-micronic particles of several fungal species are aerosolized in much higher concentrations (300-500 times) compared to spores [2], and that respiratory deposition models suggest that these particles of Stachybotrys chartarum may be deposited 230-250 fold higher than spores [3]. The practical implications of these models are yet to be determined for actual human exposures.

We have developed novel immunodetection techniques to determine the extent to which larger fungal fragments, including hyphae and fractured conidia function as aeroallergen sources. These were based on the Halogen Immunoassay (HIA), an immunostaining technique that detects membrane-bound antigens derived from collected airborne particles >2 µm with human serum IgE [4]. Our studies demonstrate that the numbers of total airborne hyphae were often significantly higher in concentration than conidia of individual allergenic genera [5]. Approximately 25% of all hyphal fragments expressed detectable allergen and the resultant localization of IgE immunostaining was heterogeneous among the hyphae. Furthermore, conidia of ten genera that were previously uncharacterized could be identified as sources of allergens. These findings highlight the contribution of larger fungal fragments as aeroallergen sources and present a new paradigm of fungal exposure [5].

Direct evidence of the associations between fungal fragments and building related disease is lacking and in order to gain a better understanding, it will be necessary to develop diagnostic reagents and detection methods, particularly for sub-micronic particles. Monoclonal antibody-based assays enable the measurement of individual antigens but interpretation can be confounded by cross-reactivity between fungal species. The recent development of
species-specific monoclonal antibodies, used in combination with a fluorescent-confocal HIA technique should, for the first time, enable the speciation of morphologically indiscernible fungal fragments. The application of this method will help to characterize the contribution of fungal fragments to adverse health effects due to fungi and provide patient-specific exposure and sensitization profiles. This will ultimately contribute to better patient management.

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Fungal Responses to Reactive Oxygen Species

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Friday, February 24, 2006, 3:00 - 3:20 pm

Reactive oxygen species (ROS) such as hydrogen peroxide, produced externally or
during normal metabolism, can damage different cell components and usually trigger
a counteracting antioxidant response. Most of what we know about how fungal cells
deal with ROS comes from studies in which the yeasts Saccharomyces cerevisiae and
Schizosaccharomyces pombe have been challenged by external ROS. In contrast, little is
known about the consequences of increased endogenous ROS formation. Current evidence
suggests that S. pombe represents a good paradigm to understand the antioxidant response in
filamentous fungi. In this organism, a multistep phosphorelay, a stress-response MAP kinase
pathway and an AP-1 type transcription factor regulate a global oxidative stress response
that includes the activation of the only catalase gene present in this yeast (1). The fact that
animals and humans utilize ROS and related nitrogen reactive species (RNS) to prevent
fungal infection has generated great interest in defining the components of the antioxidant
response and studying their role as virulence determinants in Aspergillus and other fungi.

As an approach to understand the antioxidant response and its relation to development, our
group described four different catalases in Aspergillus nidulans, characterized genes catA-C
and showed that the stress-MAP kinase SakA transmits oxidative and osmotic stress signals
in this fungus, and regulates the induction of catA and catB genes. We also established that
SakA regulates conidiospore viability, resistance to oxidative and heat shock stress and
expression of the noxA gene, which encodes a conserved NADPH oxidase (NOX) involved in
ROS production and sexual development (2). SakA activation by both osmotic and oxidative
stress has been found to be mediated by the response regulator SskA (3). In Neurospora
crassa Nox-1 also regulates sexual development, whereas Nox-2 is required for sexual spore
viability/germination (Cano et al., unpublished). Homologous catalases and MAP kinase
genes seem to play similar roles in the opportunistic human pathogen A. fumigatus. A mutant
lacking Cat1 and Cat2 catalases shows decreased resistance to hydrogen peroxide reduced
and a decreased virulence in a rat model (4). Although the role of sakA in virulence has
not been evaluated yet, this gene is induced by osmotic and hydrogen peroxide stress and is
required for full conidiospore germination in a suboptimal nitrogen source (5). Compared
to S. cerevisiae and S. pombe, filamentous fungi appear to have additional mechanisms to
handle ROS, such as the presence of a larger number of antioxidant enzymes and secondary
metabolites with antioxidant function. In addition, filamentous fungi have enzymes like the
NADPH oxidases, which regulate development through ROS production. Therefore, fungi
like A. nidulans and A. fumigatus offer the opportunity to study the interplay between
ROS production, perception and detoxification, as well as the role of these processes in cell
differentiation and pathogenesis.
References
NEW RESISTANCE MECHANISMS TO AZOLE DRUGS IN ASPERGILLUS FUMIGATUS AND THE EMERGENCE OF THE NEW ANTIFUNGAL DRUGS RESISTANT A. FUMIGATUS ATYPICAL STRAINS

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Friday, February 24, 2006, 3:20 - 3:40 pm

In the last years, the incidence of the invasive infection by moulds has increased. Also, the characteristics of invasive mycosis are changing due to the description of new pathogenic species and also because strains and species resistant to antifungal drugs are appearing. The development of mould antifungal drug resistance is the inevitable and logical consequence of their clinical use, although the frequency and clinical relevance of this fact is unknown. Current Aspergillus fumigatus antifungal drugs resistance mechanisms are reviewed. Occurrence of azole drug resistance in A. fumigatus is a known fact. Also, the methodology to detect resistance in the laboratory is available and it has a good correlation with in vivo outcome. Analysis of resistance mechanisms at the molecular level have identified the bases for A. fumigatus azole resistance. In A. fumigatus, there are two distinct but related 14α-sterol demethylase (Cyp51) proteins encoded by cyp51A and cyp51B genes. Recently, the role of Cyp51A in cyp51A knock out strains have been assessed and it has been established that this enzyme is responsible for A. fumigatus azole susceptibility. To date, the most prevalent mechanism of azole resistance in A. fumigatus appears to be the modification of Cyp51, specifically mutations in cyp51A. In strains from clinical origin these mutations, have been associated with three different susceptibility profiles: (i) cross-resistance to itraconazole (ITC) and posaconazole (POS) that has been associated with amino acid substitutions at glycine 54 (G54), (ii) elevated MICs to all azole drugs has been associated with amino acid substitutions at methionine 220 (M220), and (iii) cross-resistance to all azole drugs related to the presence of Cyp51A substitutions at leucine 98 for histidine (L98H) linked to a duplication in tandem of a 34 bp repeat in the cyp51A promoter region with an increase of cyp51A gene expression between 8 to 10 times.

Increasing reports of isolation of genetic variants of A. fumigatus, originally identified as poorly sporulating strains of A. fumigatus, as a causative agents of invasive infection, are worrisome. These isolates have been found to have reduced in vitro susceptibility to multiple antifungal drugs, but mainly to voriconazole (VCZ MICs > 4.0 mg/L), and also to amphotericin B (AmB MICs 1-2 mg/L) and itraconazole (ITC MICs 0.5-1 mg/L). We have collected 14 isolates of these A. fumigatus genetic variants and found different antifungal drugs MICs patterns between them. Therefore, they have been grouped according to their antifungal drugs susceptibility profiles and also to their cyp51A and cyp51B gene sequences. The study of molecular mechanisms of antifungal drug resistance is the most valuable strategy to resistance development control and also in helping to develop safer and more active molecules able to avoid them. In the meanwhile is important the correct use of the available tools: epidemiological surveillance of resistance emergence and to use all the efforts towards prompt diagnosis in order to accomplish an adequate and effective treatment.
Bibliography:


Invasive pulmonary aspergillosis (IPA) usually occurs in severely immunocompromised patients. The expanded use of glucocorticoids (GC) in clinical practice accounts for the increasing number of fungal infections reported in mildly or non-immunocompromised hosts. We report a series of 8 patients with fungal pneumonia in whom long term high dose GC treatment was the only risk factor for opportunistic infections. All patients except one had chronic underlying disorders (asthma, idiopathic fibrosis, chronic obstructive pulmonary disease -COPD-). Seven patients were diagnosed of pulmonary aspergillosis and one presented a Candida tropicalis pneumonia. Etiological suspicion of fungal infection was obtained during lifetime in seven cases and in one case was confirmed only in the post-mortem examination. Although in most cases, bronchoscopic techniques allowed to identify the microorganism, delay in establishing the diagnosis (mean 19 days) precluded a prompt initiation of a specific treatment. The course of the fungal infection was ominous. All but one patients experienced progressive respiratory failure requiring ICU admission and mechanical ventilation support. Despite this, all of them died. The only survivor was a patient receiving early empirical antifungal treatment due to a high clinical suspicion of fungal infection. Based on the present and previous findings, antifungal treatment should be considered in chronic respiratory patients requiring high or repetitive doses of GC when there is clinical evidence of pneumonia and isolation of Aspergillus spp from respiratory secretions.
PROGRAMMED CELL DEATH IN ASPERGILLUS AND OTHER FILAMENTOUS FUNGI

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Friday, February 24, 2006, 3:40 - 4:00 pm

Aspergillus fumigatus is causes over 90% of all cases of invasive aspergillosis in immunocompromised patients as well as causing asthma, allergies, and mycotoxicosis. Despite the often high levels of morbidity and mortality caused as a result of aspergillosis, only a small number of antifungal agents are available for treatment and not all are particularly effective. It is therefore important to identify novel pathways and target which could be used to develop new antifungal agents. Programmed cell death, in which cells actively participate in their own death through the activation of defined pathways, has long been established as an important developmental pathway in metazoan systems (Kaufmann & Hengartner, 2001) but it is only recently that evidence for a primitive form of PCD in the fungi has come to light. While much of the evidence for this comes from studies in the yeast Saccharomyces cerevisiae, (Madeo et al, 2004) recent evidence strongly suggest the presence of a metacaspase (primitive orthologues of the mammalian caspases) independent and dependent pathways in the Aspergilli which can be activated by deleterious environmental stimuli such as starvation, oxidative stress and antifungal agents (Mousavi & Robson, 2003; 2004; Fedorova et al, 2005) as well as developmentally during sporulation (Thrane et al, 2004). The experimental and bioinformatic evidence for these pathways and the implications for future antifungal development strategies will be discussed.

References
ALLERGIC FUNGAL RHINOSINUSITIS: A REVIEW OF CLINICAL MANIFESTATIONS AND CURRENT TREATMENT STRATEGIES

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Friday, February 24, 2006, 3:40 - 4:00 pm

Allergic fungal sinusitis is a relatively newly characterized disease entity that commands a great deal of interest. Large amounts of information are being generated addressing the underlying etiology of the disease, its clinical presentation, and forms of treatment. Although controversy still exists, recent evidence supports the theory that AFRS represents an immunologic, rather than infectious, disease process. While early reports focused on the role of Aspergillus sp. as the causative fungus involved in the etiology and pathogenesis of the disease, later literature reveal that and association with a number of dematieacious fungi. An improved understanding of this underlying disease process has lead to an evolution in the treatment of AFRS. Medical therapy has shifted from an emphasis upon systemic antifungal therapy to various forms of topical treatment and immunomodulation. Likewise, surgical treatment of AFRS, still a crucial component of the overall treatment plan of the patient, has shifted from radical to a more conservative yet complete approach. Although important, surgery alone does not lead to a long-term disease free state. A comprehensive management plan incorporating both medical and surgical care remains the most likely way to provide long term disease control for AFRS.

This review of the current literature is intended to serve as an overview of the current state of information available for AFRS focusing upon the impact of eosinophilic inflammation. Further, it is intended to discuss current treatment strategies as they relate to this underlying pathogenesis.

References
NOVEL PREVENTATIVE STRATEGIES AGAINST INVASIVE ASPERGILLOSIS

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Saturday, February 25, 2006, 7:40 - 8:05 am

Invasive fungal infections, invasive aspergillosis (IA) in particular, represents a major threat to immunocompromised patients, especially patients with hematologic malignancies or who receive hematopoietic stem cell transplantation. Hence, prevention of IA is a critical strategy that requires a clear understanding of the mold’s environmental sources and how it is transmitted to immunocompromised patients. Knowledge of the exposure, mechanisms of transmission, and host susceptibility to IA are vital in selecting appropriate preventive strategies to those settings where infection is more likely to occur. Among the strategies to reduce the incidence of IA is the maintenance of high quality air, i.e., air with low spore counts in hospital areas visited by patients at risk. Housing patients in laminar airflow facilities with high-energy particulate air-filtered rooms helps prevent IA, but it is only realistic and cost-effective for the highest-risk groups and for limited time periods. Air filtration is a costly preventive strategy of questionable value when done with portable filtration units. Moreover, air control measures outside the hospital are extremely difficult to implement and this is important since the majority of cases of IA after allogeneic stem cell transplantation occurs during the post-engraftment period. For these reasons, targeted antifungal prophylaxis remains the most promising of the potential prevention strategies against IA. Many older and newer antifungal agents have been used for this purpose. Amphotericin B, being the oldest and most widely used antifungal, has been used prophylactically in various doses and schedules, but has largely been replaced by its lipid and liposomal formulations that have improved safety profile. Although prophylactic fluconazole prevents candidiasis, this drug has no activity against molds, including Aspergillus spp. On the other hand itraconazole appears to prevent IA in those patients who can tolerate the drug, since its poor tolerability limits its use. The newer extended spectrum triazoles voriconazole and posaconazole have also been used in prophylactic trials with encouraging results in highly selected populations of patients at risk. Trials using echinocandins as prophylaxis have not been published, yet. Early, repeated, high-resolution computerized tomography scans of the chest and sequential monitoring of Aspergillus galactomannan and DNA in the serum and other body fluids can lead to earlier diagnosis and pre-emptive therapy. Despite the above, more studies are clearly needed to better define patient populations who will clearly benefit from such a strategy. For example, patients undergoing transplantation for hematologic malignancies from mismatched or unrelated donors are clearly at higher risk compared to patients undergoing autologous transplantation, since among other risk factors they frequently receive moderate doses of corticosteroids for extended periods for GVHD, a well-known risk factor for IA. Hence, results of studies in specific populations should be analyzed with caution and prophylaxis should be applied to similar patients, because antifungals are not devoid of side effects and overuse selects for resistant fungi. In conclusion, the topic remains open in order not only to find the most effective regimens against IA, but also the ideal patients in real need for antifungal prophylaxis.
References
Invasive aspergillosis is an increasing cause of morbidity and mortality in immunocompromised patients. Extension of invasive aspergillosis to the central nervous system (CNS) is associated with an exceeding high mortality which approaches 100%. One major factor contributing to this devastating outcome is a poor penetration into the CNS of frequently used antifungal drugs, such as amphotericin B or itraconazole. Data from case-reports and a recent retrospective study suggest that neurosurgical interventions, such as abscess resections, stereotactic drainages, or the use of intraventricular catheters, might improve the outcome in CNS aspergillosis. Voriconazole, a triazole antifungal agent with broad activity against various fungi, including *Aspergillus* species, shows superior activity in invasive aspergillosis compared to treatment with conventional amphotericin B. Voriconazole readily penetrates the blood-brain barrier yielding fungicidal drug concentrations within the CNS. In a recent retrospective study, the outcome and survival of 81 patients who were treated with voriconazole for definite (n = 48) or probable (n = 33) CNS aspergillosis were evaluated retrospectively. Complete and partial responses were recorded in 35% of patients and varied by the underlying disease group. Thirty-one percent of patients survived CNS aspergillosis for a median observation time of 390 days. There were 31 patients who underwent neurosurgical procedures, including craniotomy/abscess resection (n = 14), abscess drainage (n = 12), ventricular shunt (n = 4), and Ommaya-reservoir (n = 1). Multifactorial analysis revealed that neurosurgery was associated with improved survival (P = .02). It may be concluded from this study that the combined approach of systemic antifungal therapy together with neurosurgical treatment is currently the best approach to treat patients with CNS aspergillosis.

In patients with haematological malignancies, opportunistic infections with *Candida* or *Aspergillus* remain the most common infections affecting the CNS. However, opportunistic infections with less common fungi (e.g. zygomycetes) are becoming more common and must be considered in the differential diagnosis. Infections with zygomycetes (which include the order *Mucorales*) have been described more common in immunocompromised patients during the past years. Mucormycosis can manifest as rhinocerebral, pulmonary, disseminated, cutaneous, or gastrointestinal disease. Rhinocerebral involvement begins with the nasal mucosa, from which the organism extends to the palate, paranasal sinuses, orbit, face and brain. Treatment of CNS mucormycosis should also include appropriate debridement of devitalised tissue, although surgery may be difficult in some situations. Treatment of mucormycosis consists of either „high-dose“ amphotericin B (1-2-1.5 mg/kg/d) or „high-dose“ amphotericin B lipid formulations (doses in excess of 10 mg/kg/d). In a retrospective review of 59 patients with haematological malignancies, the use of liposomal amphotericin B was the only factor associated with recovery from mucormycosis. As for the triazoles, posaconazole has shown to have good activity *in vitro* against *Mucor* spp. and several other zygomycetes and may serve as an alternative as well. However, published data on the treatment of CNS...
mucormycosis with posaconazole are still very limited. Other novel treatment approaches, such as combination therapy, are also being explored. Early investigations have produced encouraging results; however, large, prospective studies involving many patients are necessary to validate the widespread use of these approaches.

References
WHOLE GENOME COMPARISON OF *A. FLAVUS* AND *A. ORYZAE*.

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Saturday, February 25, 2006, 8:40 - 9:00 am

*Aspergillus flavus* is a plant and animal pathogen that also produces the potent carcinogen aflatoxin. It grows as a saprophyte of a variety of substrates, but can be pathogenic to corn, peanuts, cotton and tree nuts. *Aspergillus oryzae* is a closely related species that has been used for centuries in the food fermentation industry and is generally regarded as safe (GRAS). Whole genome sequences for these two fungi are now complete providing us with the opportunity to examine any genomic differences that may explain the different ecological niches of these two fungi, and perhaps to identify pathogenicity factors in *A. flavus*. Our initial analysis of these two genomes shows that while these two fungi are very similar they do differ in interesting features. The *A. flavus* genome has been assembled into 79 scaffolds ranging in size from 4.5 Mb to 1.0 Kb. Over 75% of the genome is in the 10 largest scaffolds and 99.6% of the predicted genes are in the largest 16 scaffolds. The estimated genome size (36.3 Mb) and predicted number of genes (13,071) for *A. flavus* is similar to that of *A. oryzae* (36.8 Mb and 14,007, respectively). These two fungi have significantly larger genomes that *A. nidulans* and *A. fumigatus*. The *Aspergillus flavus* and *A. oryzae* genomes are enriched in genes for secondary metabolism, but do not differ greatly from one another in predicted number of polyketide synthases (A.f.=34, A.o.=31), nonribosomal peptide synthases (A.f.=21, A.o.=24), or cytochrome P450 enzymes (A.f.=122, A.o.=151). Each species does have a set of unique genes, most of which have no known function. These may be targets for identifying differences between these two species. *A. oryzae* scaffolds have been assigned to chromosomes by optical mapping, alignment of *A. flavus* scaffolds to the *A. oryzae* genome shows high correspondence to the *A. oryzae* chromosomes. The 16 largest scaffolds of the *A. flavus* genome essentially correspond to the 16 arms of the predicted 8 chromosomes.

In comparison with *A. oryzae*, there has been a translocation event in *A. flavus* between chromosomes II and VI. The break site is associated with a family of uncharacterized repeat elements. Three types of transposable elements have been predicted. In each case their frequency of occurrence is greater in *A. oryzae* than *A. flavus*. The initial genomic analysis indicates that these two fungi are very similar. A closer examination of their difference may reveal genes involved in the two different ecologies of these fungi.
A fundamental aspect of any organism’s success is the ability to monitor and respond appropriately to the environment, a process which is largely achieved through the appropriate regulation of gene expression. There are few better examples than fungi, which inhabit diverse and often hostile environments, ranging from leaf litter to the human body. The ability of the Aspergilli to survive in and exploit so many different environments is coupled to their ability to overcome a wide range of stresses as well as their metabolic versatility. Fundamental to both are the complex signalling and regulatory systems which monitor the intracellular and extracellular environment. Gene regulation has been well studied in Aspergillus, most notably A. nidulans. This has led to a broad range of mechanisms being uncovered and characterised through genetics and molecular biology. The regulatory strategies employed by the Aspergilli include chromatin remodelling, a wide array of DNA-binding transcription factors and co-activators acting through the promoters, differential splicing and transcript stability, as well as translational and post-translational events. Through the availability of the sequenced genomes new insights into these mechanisms and their importance in the organism’s biology have emerged. Additionally this revolution has provided a powerful range of new tools to investigate these processes. In this paper we will review recent data demonstrating the various levels at which gene expression is regulated and assess the importance and consequences of the different processes. We will briefly look at the genome sequences as a source of new information about gene expression as well as the evolution of the mechanisms involved and ask what predictions can be made. The future contribution and potential of proteomics and transcriptomics will also be reviewed.
Although Aspergillus is commonly associated with invasive and chronic disease, it is probable that it is a far more common agent of allergy and severe asthma. Allergy is a common ailment seen with increasing frequency in the developing world. Symptoms may range from mild irritation to life threatening asthma. Although allergies are known to be caused by an enhanced type 2 immune response little is known about the causative agents of allergy beyond identification of the molecules involved. Aspergillus fumigatus is associated with several forms of allergy ranging from mild allergy to severe debilitating asthma type conditions such as allergic bronchopulmonary aspergillosis (ABPA). In particular we do not understand why some organisms cause allergy and other closely related organisms do not. Over 20 allergens have been characterised from A. fumigatus with the probable existence of a further 50 or more less well characterised allergenic proteins. It is unclear which allergens are involved with the various conditions caused by A. fumigatus. Cross reactivity of allergens when challenged with serum is a well known phenomena and it has been suggested that this cross reactivity arises from similarities in linear or 3D epitopes of related proteins from different organisms. Similarly pan allergens have been described where proteins with similar functions from different kingdoms have been shown to be allergenic. Although the prevalence and relatedness of the cross reacting proteins has been well described for a number of allergens the extent to which this may occur and the potential number of cross reacting allergens present within an organism has yet to be defined. Furthermore cross reaction begs the question of whether certain organisms are actually primary allergens or merely contain cross reactive epitopes that react with IgE originally targeted at another organism or protein. Additionally it is also uncertain whether allergenic proteins described in the literature are primary allergens or merely cross reacting against a primary IgE triggering allergen. Many organisms are known to produce families of closely related proteins and it may also be the case that allergens within an organism’s repertoire might be mis-identified because of the presence of cross reactive epitopes. Advances in genome sequencing have revolutionised approaches to the study of living organisms. Recently the genome sequences of 3 species of Aspergillus have been determined. Here we use the recently completed genome sequence of the allergenic A. fumigatus (Af) to compare allergen homologues in the supposedly non-allergenic A. nidulans and A. oryzae and to investigate whether Af might possess cryptic homologues of previously published allergen proteins from other fungal species. This analysis suggests that most Af allergens are present in the other Aspergilli and that homologues of allergens from more distantly related fungi are present in Aspergillus. Additionally the analysis suggests individual fungal species may contain multiple proteins that cross react with sera from individuals sensitised to the primary allergen. Af allergens with undefined function have been tentatively assigned functions on the basis of homology to genes of known function. Analysis of allergens across all fungi
suggests that a core set of pan-fungal allergens exists alongside a number of species specific allergens which might be an extremely useful diagnostic or predictive tool.

References
OLD AND NEW CONCEPTS OF SPECIES DIFFERENTIATION IN *ASPERGILLUS*

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Saturday, February 25, 2006, 11:10 - 11:30 am

The classification of species in the genus *Aspergillus* has been studied by many taxonomists. The most important monograph on which most taxonomies are based, however, goes back to the excellent publication by Raper & Fennell (1965). Later revisions of certain *Aspergillus* sections have been predominantly nomenclatural changes and are largely based on morphology. Many new taxa were added particularly in the genera *Emericella* and *Neosartorya*. Identification of the most common and often important species remains problematic due to the variability in the phenotypic characters. This has caused errors in the literature, especially concerning the links to mycotoxin formation.

The new taxonomies are based on a polyphasic approach using phenotypical characters together with multigene DNA sequences. In particular extrolites profiles have proven to be specific for the taxa and this has contributed to a stable species concept. Examples of new classifications for species in section *Circumdati*, *Flavi*, *Fumigati* and *Nigri* are presented (Samson et al., 2004; Frisvad et al., 2004a and b; Hong, 2005, 2006) Although the polyphasic approach might reveal clear cut species, problems may arise for some species if they are to be separated based only on their microscopic features and few physiological features. Suggestions for new methods in order to carry out more fast and precise identifications will be discussed. Full genome sequencing and DNA arrays offers exciting new bases for identifying the Aspergilli, but recent methods based on image analysis of accurately fingerprinted phenotypes are also very promising. However both methods require a stable and well resolved taxonomy and nomenclature. Validated careful phenotypic classification (taxonomy) together with phylogenetic treatment of DNA sequence data is a prerequisite for reliable rapid identification methods and database formation. DNA bar coding will be possible in the future based on molecular methods or chemical (chromatographic and spectrometric), physiological and morphological methods or both.

References
IDENTIFYING CLINICALLY RELEVANT ASPERGILLI

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Saturday, February 25, 2006, 11:30 - 11:50 am

Historically, classification and identification of aspergilli was accomplished using morphological characteristics. A number of molecular, immunological and biochemical methods are now available. Some of these methods are designed to identify only a few species, whereas others address most of the important species in the genus. For the most part, the results of the various approaches concur, yielding similar results in identifying aspergilli. The identification method used should be a function of the needs of the clinician or researcher. In most clinical settings, immediate identification of the species causing a fungal disease is less important than rapid determination of the treatment regime that will be most efficacious for the patient. This may not require identification of the isolate to the species level. Speciation and ‘fingerprinting’ of strains become important in determining etiology and epidemiology of the disease. In these situations, accuracy of the identification is more important than the speed at which the identification is performed. In such cases, researchers frequently use more than one method of identification. The number of species reported to cause aspergillosis is growing, therefore, any accurate system of identification of clinical isolates must include species not yet reported in clinical cases.

Each identification method has advantages and disadvantages. The morphological approach is the least expensive and quite accurate, but it requires use of a standardized plating and incubation regime, some skill with a microscope and an understanding of the characteristics of the genus. Like the morphological approach, the biochemical approach requires culturing the fungi, but is a bit limited because not all isolates of a species consistently produce the same metabolites. Although inexpensive, use of thin layer chromatography plates can yield good information for identification, but metabolites can only be accurately identified with more sophisticated equipment for HPLC and mass spectroscopy. Immunological methods may be used on tissue samples, but are currently limited because they have been developed for only a few taxa. Molecular methods have the advantage of not necessarily requiring isolation or culturing of the fungi and require only a skilled technician for acquiring data, however, the equipment necessary for molecular work is very expensive, accepted molecular methodologies change frequently, and the process needs to be overseen by a well-trained molecular biologist to avoid potential errors in identification.
WHOLE GENOME COMPARISON OF THE A. FUMIGATUS GROUP

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Saturday, February 25, 2006, 11:50 - 12:10 pm

The publication of the genomes of A. fumigatus, a human pathogen, A. oryzae, used in food production, and A. nidulans, a genetic model organism, represents an important milestone for the Aspergillus research community, expanding knowledge of their physiology and mechanisms of gene regulation. Although members of the same genus, phylogenetic analysis of these species revealed that each pair is approximately as evolutionarily distant as mammals and fish, with A. fumigatus and A. oryzae more related to one another than A. nidulans.

Three additional genome projects are being funded by the NIAID with the goals of better elucidating the A. fumigatus genome and improving the genome annotation: N. fischeri (A. fischerianus), A. clavatus and A. terreus. A. terreus and N. fischeri are capable of causing disease in human patients, while A. clavatus cannot survive at body temperature, and is therefore not a viable pathogen. Preliminary analysis of genome structure supports the previously determined phylogeny of these species, with N. fischeri the most related to A. fumigatus, with the longest uninterrupted syntenic blocks; A. clavatus intermediate, with numerous local rearrangements and inversions; and A. terreus the most distant, with inter-scaffold breaks common.

This presentation will cover our preliminary examination of the genome sequences and predicted gene complements of N. fischeri and A. clavatus in comparison to two strains of A. fumigatus, Af293 and CEA10. Even within the single species, A. fumigatus isolates vary significantly in their morphology and pathogenicity. Af293 and CEA10 have a number of unique, strain-specific genes (2%), located predominantly in non-syntenic subtelomeric blocks. A few strain-specific regions contain putative gene clusters involved in secondary metabolism, osmotolerance, or arsenic resistance, highlighting the potential role of the subtelomeric regions in maintaining species variability.

Comparative genomic analysis at the level of these more closely related Aspergilli should provide important additional insight into the evolutionary forces at play and their effect on gene content, regulation and expression. Characterization of strain and species specific genes, polymorphisms and differential regulation will foster our understanding of mechanisms of pathogenicity, environmental adaptation and resistance to anti-fungal treatments.
Filamentous fungi are known to produce various types of secondary metabolites including polyketides, terpenoids and alkaloids. For fungi that can cause aspergillosis this includes biological active compounds such as gliotoxin, territrem B, fumagillin, fumitremorgens and several other metabolites.

At Center for Microbial Biotechnology (CMB) we have shown that profiles of metabolites are produced very consistently at the species level. This means that important human pathogenic aspergilli and related species can be distinguished from each other based solely on their “chemical fingerprint”. This paper will illustrate how important species such as *A. fumigatus*, *A. novofumigatus* and *A. lentulus* can be recognized both by simple methods such as TLC and advanced methods such as direct injection mass spectrometry, allowing identification of species characteristic fingerprints within few minutes.

Target analysis for the detection of individual compounds such as gliotoxins from crude extracts of pure fungal cultures or purified extracts from other biological matrices is done by the use of HPLC coupled to diode array detection and mass spectrometry. Our studies have shown that all species produce a number of unknown metabolites often as major metabolites. Significant unknown compounds are isolated by standard preparative methods followed by structural elucidation based on the use of NMR and X-ray.

References
Among pediatric patients invasive aspergillosis (IA) can be seen primarily in neonates, patients with primary immunodeficiencies (e.g., chronic granulomatous disease, Job syndrome), hematological malignancies or HIV infection. Microscopic examination and culturing of appropriate specimens remain the gold standard of *Aspergillus* mycological diagnosis of IA in any patients. High resolution CT serially performed constitutes a sensitive mode of diagnosis in hematological patients. Central cavitation of small nodules without air crescent formation is found in younger patients. Diagnosis of IA with new antigen tests, such as galactomannan (GM) assay is difficult in children because specificity and sensitivity are inferior compared to adults. The clinical specificity of GM in neonates and children appears to be lower than that in adults in whom it is generally estimated to be >90%, which is possibly due to the ingestion of extraneous galactomannan (in food and water) and translocation across an immature or damaged gut wall. Antibiotics (e.g., piperacillin-tazobactam) represent an additional source of extraneous galactomannan that may compromise clinical specificity. Although several GM studies have included at least some children in their cohorts, there have been a paucity of pediatric-predominant studies. In one prospective study, the false positive rate in adult patients was 2.5% vs. 10.1% in children. While the sensitivity and specificity of the test (using values >1.5 in at least 2 sequential samples) were 88.6 and 97.5%, respectively, the sensitivity increased to 100% in adult patients, and the specificity dropped to 89.9% in children. In another study, the false positive rate in the fever of unknown origin group was 0.9% in adults and 44% in children. There are numerous theories attempting to explain the increased false positivity in children, ranging from *Bifidobacterium bifidum* in the intestinal microflora that mimics the epitope recognized by the EB-A2 in the enzyme-linked immunoabsorbent assay kit to GM-positive infant formula used in neonates, but the complete answer remains elusive. Galactomannan testing in children also is plagued by false negative results in some particular pediatric patients such as those with CGD. One study evaluated patients with CGD or Job’s syndrome and IA and found that GM antigenemia was detected in 4 of 15 cases of CGD and Job’s syndrome versus 24 of 30 cases of all other immunocompromised conditions (P=0.0004). Another interesting diagnostic assay, (1,3)-beta-D glucan assay, has been studied in adult patients with fungal infections but not specifically with IA. There are no data that address the clinical sensitivity of the (1,3)-beta-D glucan assay specifically for *Aspergillus* spp. On the other hand, detection of nucleic acids of *Aspergillus* spp. by polymerase chain reaction (PCR) is a powerful tool for IA diagnosis. No studies, however, address the issue in neonates, whereas in older pediatric patients PCR is probably as good as in adult patients. High degree of suspicion in an immunodeficient pediatric host, suggestive clinical and radiologic findings and mycological data by the application of multiple diagnostic methods are the most important means of increasing our capacity to diagnose IA in young patients.
References
Breathing leads to the uptake of a wide variety of particles from the environment, among them various microorganisms including fungal spores. While most inhaled microorganisms are harmless, others, like Aspergillus fumigatus conidia, are potentially dangerous and have to be rapidly recognized and eliminated. The innate immune system of the lung is adapted to a permanent exposure to A. fumigatus conidia and alveolar macrophages are primarily responsible for their elimination, as they are able to track down and kill these spores. Additionally, they may recruit neutrophils to the site of infection and communicate with the adaptive immune system, thereby determining whether an inflammatory response is triggered or not. The ability of macrophages to recognize relevant microorganisms largely depends on their expression of various pattern recognition receptors (PRRs), which recognize conserved pathogen-associated molecular patterns (PAMPs). The family of Toll-like receptors (TLRs) represents an important subset of these PRRs and has been the subject of intense research in recent years. While the list of PAMPs is growing, it became obvious that certain TLRs are able to recognize several, biochemically distinct PAMPs.

Attempts to pinpoint the relative importance of the various TLRs for recognition of A. fumigatus have been undertaken by several groups. In vitro experiments using transfected cells and isolated macrophages from TLR-deficient mice provided evidence for an involvement of either TLR2 and/or TLR4. Sometimes controversial results were obtained, due to different experimental set-ups and the types of macrophages used (genetic background, peritoneal versus alveolar, human versus murine). However, the analysis became even more complicated by the fact that germination is an integral part of the infectious process. This morphological transformation, leading from inhaled resting conidia to swollen conidia, germlings and finally hyphae, results in exposure of different surface structures, being mainly proteinaceous in resting conidia and carbohydrate-rich in all other cell types. Moreover, recent findings demonstrated a stage-specific surface exposure of certain cell wall carbohydrates, like β1-3 glucan and exposure of additional PAMPs by fungal lysis may provide another level of complexity. While the relevant A. fumigatus TLR ligands are still unknown, β1-3 glucan recently turned out to be an important PAMP. Its recognition by the C type-lectin-like receptor dectin-1 has been implicated in the inflammatory response to A. fumigatus, but also in conidial phagocytosis. Since TLR2 has also been implicated in both processes it will be interesting to analyse a potential interaction or cross talk of both receptors.

Although the relative importance of distinct PRRs still has to be determined, infection experiments in TLR-deficient mice provided convincing evidence for an involvement of TLR2 and TLR4 in the host response to A. fumigatus. Interstingly, liposomal amphotericin B has recently been shown to shift TLR signalling from TLR2 to TLR4, thereby enhancing fungizidal activity while reducing deleterious inflammatory cytotoxicity. Activation of
certain TLRs and modulation of their signalling has been discussed in other forms of disease and might be a promising strategy to strengthen the immune response in patients at risk to develop systemic A. fumigatus infections.

References
ORAL ABSTRACT PRESENTATIONS
IDENTIFICATION OF NOVEL ASPERGILLUS FUMIGATUS CELL WALL PROTEINS INVOLVED IN THE HOST-PATHOGEN INTERACTION

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Aspergillus fumigatus is the most common mold pathogen of human beings, causing both invasive diseases in immunocompromised patients and allergic disease in patients with atopic immune systems. Those at risk include leukemic patients undergoing aggressive chemotherapy, transplant recipients, and patients with AIDS, multiple myeloma, severe combined immunodeficiency and chronic granulomatous disease.

The A. fumigatus cell-surface consists of cell wall glycophosphatidylinositol/GPI-modified proteins (GPI-CWPs). These proteins contain a C-terminal hydrophobic domain which is cleaved off and replaced with a glycophosphatidylinositol /GPI-anchor and an N-terminal hydrophobic signal peptide sequence that targets them to the endoplasmic reticulum (ER). The GPI-anchor directs the attachment of these proteins to either the fungal plasma membrane or its cell wall.

Fungal GPI-anchored proteins are involved in cell-wall biosynthesis, remodeling and adhesion.

Upon conidia inhalation, CWPs are the first component which the immune system cells encounter; determining macrophage recognition, action and the appropriate immune response.

The overall aim of this study is to identify novel A. fumigatus cell-surface proteins involved in adhesion, pathogenesis and host pathogen interactions.

We have performed computational analyses of the A. fumigatus genome suggesting the existence of 80 putative genes containing a leader sequence and a glycophosphatidylinositol /GPI-anchor-containing motif.

From this list we identified 12 putative cell wall genes containing 2-3 of the motifs (leader sequences, serine-threonine rich areas, GPI-anchors, CFEM motifs) found in cell wall and adhesion molecules discovered in other fungi. Three of these genes were shown to be expressed during growth of the fungus and in the lungs of infected mice using RT-PCR on A. fumigatus mRNA and further disrupted by a rapid transposon-based deletion method (Afu6g14010, Afu1g09590 and Afu4g06820- AfuEcm33). Interestingly, AfuEcm33p is up-regulated throughout growth (up to 24h), the AfuEcm33 disrupted mutant strain exhibited precocious conidial germination, conidial clumping, and resistance to Congo red, a growth inhibiting reagent and to the antifungal drug Caspofungin. Upon administration to mice, the AfuEcm33-disrupted strain showed a higher level of virulence, compared to the wild type strain. These results suggest that the glucan content of the cell wall of this mutant may be altered. GPI-anchored genes play a role in pathogen/host interactions.

Consequently, there is a high probability that a thorough analysis of this mutant will provide important insights into the interaction of molds with the immune system. Additionally, the results of these experiments may provide important insights into the mechanism by which the mutant strain becomes hypervirulent.
DELETION OF THE ASPERGILLUS FUMIGATUS CALCINEURIN GENE DECREASES VIRULENCE IN A LOW DOSE MURINE INFECTION


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Ca+2 is one of the important messengers that mediate responses to hormones, developmental cues and external stimuli. It is implicated in regulating such diverse and fundamental cellular processes as cell division and polarity, morphogenesis and stress responses. The conformation of Ca2+-binding protein changes on binding Ca2+, resulting in modulation of its activity or its ability to interact with other proteins or nucleic acids and modulate their function or activity. The genes that encode EF-hand-containing Ca2+-binding proteins are one of the classes of Ca2+ sensors. This motif is a helix-loop-helix structure that binds a single Ca2+ ion. A. fumigatus is the most common species of Aspergillus that cause life-threatening pulmonary disease in severely immunocompromised patients. Aiming to identify EF-hand-containing Ca2+-binding proteins, we performed a BLAST search of the recent completed A. fumigatus genome. We were able to identify at least 28 genes that encode proteins with EF-hand motifs. Sixteen, five, three, and four of these proteins have 1, 2, 3, and 4 EF-hand motifs, respectively and also display several other motifs, such as solute carrier, major facilitator superfamily transporter, and amino acid rich regions. As a preliminary step to characterize the A. fumigatus genes that encode EF-hand motifs and investigate their role in pathogenesis and virulence in this species, we decide to inactivate the calcineurin A (calA; Afu6g04540) gene. Calcineurin is a serine-threonine specific phosphatase heterodimer consisting of a catalytic subunit A and a Ca2+-binding regulatory B subunit. The association of the two subunits is essential for activity. The deletion strain displayed a pronounced phenotypic defect, i.e., small sporulating colonies that showed comparable growth at different temperatures. The morphology of the delta calA germlings showed increased branching when compared to the corresponding wild-type strain. As expected, the delta calA strain is not sensitive to immunosuppressor drugs, such as cyclosporin, tacrolimus, and sirolimus. Surprisingly, delta calA strain shows reduced susceptibility to oxidative stressing agents, such as paraquat and menadione. In a low dose murine infection model, the conidia of the delta calA strain was 60 % less virulent than the corresponding wild type strain. Taken together, these findings suggest that the calA gene is important for A. fumigatus pathogenicity.

Financial support: FAPESP and CNPq, Brazil
INTRAPULMONARY TREM-1 EXPRESSION CONFERS SIGNIFICANT PROTECTION AGAINST CHRONIC FUNGAL ASTHMA IN MICE BY PROMOTING CLEARANCE OF A. FUMIGATUS

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Rationale
Recent observations suggest that Triggering Receptor Expressed on Myeloid cells-1 (TREM-1) and its associated signalling adapter protein KARAP/DAP12, play a critical regulatory role in immune responses during acute infection and inflammation. TREM-1 is an orphan pattern recognition receptor (PRR), which is highly expressed on neutrophils and monocytes, and is a crucial mediator of the acute inflammatory response such as in sepsis. TREM-1 appears to be involved in inflammatory responses in multiple tissues, including in the lung during ventilator-induced pneumonia. TREM-1 expression has also been reported in Aspergillus-containing hepatic granulomas, suggesting that this PRR may be involved in antifungal responses of the host. Recently it was demonstrated that TREM-1 and DAP-12 make important contributions to the innate immune response during invasive pulmonary aspergillosis. Thus the present study was designed to investigate the role of TREM-1, and its signaling molecule, DAP12, in the course of A. fumigatus-induced fungal asthma in mice.

Methodology
We used an established protocol for induction of chronic fungal asthma in mice. This model features chronic changes in airway physiology and function and is used for investigation of ABPA and allergic asthma. CBA/J mice were sensitized to soluble A. fumigatus antigens by intra-peritoneal and intra-nasal routes over 4 weeks. To modulate TREM-1/DAP-12 signaling 3 Adenoviruses (Ad) were constructed containing expression vectors for either FLAG/DAP12 (AdDAP12), the extracellular portion of TREM-1 and the Fc portion of human IgG (AdTREM-1Ig), or a type-5 replication deficient vector (Ad70); to enhance or antagonize TREM-1/DAP12 signaling pathways or control for viral-related effects, respectively. At day 0, 3 groups (n=5) of sensitized mice were challenged with an intra-tracheal (i.t.) injection of 5x10⁶ live A. fumigatus conidia combined with 2x10⁸ pfu adenovirus, a fourth group (n=5) received conidia and saline alone.

Results and conclusions
Seven days following conidia challenge, the AdTREM-1Ig-treated group exhibited significantly greater airways hyper-reactivity (AHR) upon methacholine challenge compared with the DAP12 and control Ad70 groups. Conversely, the AdDAP12 group had significantly lower AHR compared with the control Ad70-treated group. This difference in lung function was evident as early as day 3 following adenovirus instillation but in correlation with the transient expression of the viral products, was not maintained at Day 28. Histological sections
showed extensive interstitial inflammation in the lungs of the AdTREM-1Ig group, which contained markedly higher levels of fungal material compared to the Ad70 control group at Day 7. In contrast, fungal material was absent from the AdDAP12 group at this time and interstitial inflammation was diminished. Further biochemical analysis of whole lung and BAL samples indicated that blockade of TREM-1/DAP12 signaling exacerbated fungal asthma by a mechanism involving increased CCR4 chemokine ligands (CCL17 & CCL22) and reduced levels of IL-12 and CCL1 production. Adenoviral treatment in an acute model of allergic airways disease, induced by soluble A. fumigatus antigens, had no significant effect upon the course of this model, suggesting that the mechanism of recognition of intact conidia is critical to the innate antifungal immune response mediated by TREM-1 and DAP12 during chronic fungal asthma.
The major defense mechanism against invasive aspergillosis is phagocytosis by lung resident or immigrating leukocytes. Within the lung, phagocytosis can occur in the lumen of alveoli, mainly by alveolar macrophages (AM) and invading neutrophil granulocytes (Nph). Additionally, dendritic cells (DC) have been shown to efficiently take up A. fumigatus antigens before transporting them to lung-draining lymph nodes for antigen presentation to T cells. However, while a number of studies have addressed the process using fixed samples and focusing on either one of the mentioned cell types, the dynamic aspects of phagocytosis, especially the cell biological events and timing in the transition from initial leukocyte-aspergillus contact to finally completed uptake of the pathogen are largely unknown. In addition, the lung provides different environments, namely the 2 D like mucus-covered inner surface of alveoli as well as the 3-D interstitial tissue in between. It is not known, whether phagocytic processes can occur in both areas to the same extent and whether all types of phagocytes are equally functional.

In order to clarify these questions, we freshly isolated AM, Nph and DC from mice and incubated them together with conidia of A. fumigatus in an interstitial tissue-like system based on 3-D collagen as well as in a 2-D system consisting of cells in a tissue plate covered with medium. Conidia were brightly red by transgenic expression of DsRed, thus allowing the distinction between cells and external as well as phagocyted fungal elements. Phagocytosis was imaged on the single cell level by time lapse video- as well as 2-Photon microscopy over periods of up to 5 hours.

While DC were very efficient in rapidly taking up or dragging conidia in both 2-D and 3-D systems, AM and Nph were almost unable to take up or even drag conidia within a 3-D collagen based system. Transfer into 2-D systems induced a 2-9 fold increase of phagocytic capabilities in these cells. This was not dependent on inhibition of migration or reduced contact rates to conidia in 3-D. However, observable contacts between A. fumigatus conidia and AM or Nph in 3-D rarely induced any detectable interaction, while almost 100% of these events led to either phagocytosis or dragging of conidia in 2-D. Thereby, the onset of dragging occurred almost immediately after contact formation, while hagocytosis not infrequently required several contacts over a period of minutes up to hours. Thus, since DC have to take up and transport fungal elements both in 3-D interstitial tissue as well as within alveoli, while AM and Nph mostly act within the lumen of alveoli, we assume, that the cells have optimized their phagocytic capabilities for the environment, where encounter with the pathogen is most likely.
Aspergillosis is a high risk complication in lung transplantation, specially in cystic fibrosis (CF) patients. Different strategies may be used to conduct antifungal treatment. Among them, azoles are currently used besides conventional or liposomal amphotericin B and more recently, caspofungin as the first echinocandin. Voriconazole (VRZ) is a new imidazole drug introduced in 2003 without TDM recommendation, arguing its good bioavailability (96%) and a classical half-life (6h) as compared to itraconazole (ITZ) (30h). Nevertheless, we introduced the UV-HPLC determination of VRZ plasmatic concentrations in order: 1- to manage the drug-drug interaction between calcineurin inhibitors and the imidazole antifungals, in relation with their high metabolism (CYP2C19/VRZ, 3A4/ITZ and VRZ) and their CYP3A4 potential inhibition, 2- to assess the achievement of therapeutic VRZ levels in CF transplanted patients. The incidence of aspergillosis morbidity in our CF lung transplanted patients was 20%. VRZ was then administered to 40 patients between 2003 and 2005. Treatment duration ranged from a few days up to 6 months. VRZ TDM data (> 800 determinations) analyzed in this group concerned trough (C0) and peak levels collected + 2h after dosing (C2). We referred the therapeutic concentration range to the pivotal trials pharmacokinetics data (C0 : 1.5 +/- 0.5 mg/L - C2 : 4.0 +/- 1.0 mg/L). Indeed, the recommended standard dosage (200 mg/day BID following a loading dose) of this non linear pharmacokinetics drug was able to achieve significant levels in only 20% of these patients [detectable level was considered as > 0.2 mg/L and Apergillus ssp MIC as > 0.5 mg/L]. Adaptations conducted up to 800 mg/day appeared to be necessary to reach detectable levels. Despite adaptation, trough VRZ concentrations remained < 0.5 mg/L in 20 to 25% patients after one week of treatment. The dose regimen necessary to achieve therapeutic VRZ levels was as an average higher and more variable as compared to non CF transplanted patients or non transplanted patients. The contribution of higher clearance in these youngest patients (25 yrs) was expected, as well as the possible lack of absorption in CF patients, but the variability justified an individualized basis approach. This was also observed even using IV route, underlying the VRZ pharmacokinetics variability in this population, maybe enhanced by CYP2C19 polymorphism. The high risk of inefficacy during underdosed periods was supplied by the use of antifungal associations, specially with caspofungin, supported by an individualized concentration-controlled adaptation. High trough levels (> 5 mg/L), usually at risk of hepatic abnormalities or intolerance, were less common than in other groups but occurred as well (3%). VRZ concentrations correlated significantly (r2 = 0.34 ; p<0.01) with aspartate aminotransferase but not bilirubin. Alanine aminotransferase and alkanlin phosphatase were related only in case of previous hepatic alteration. This represents an intensive antifungal strategy, based on antifungal associations waiting for documented therapeutic levels when azoles are addressed. Further investigations, including the ongoing 2C19 polymorphism determination, are needed to validate this costly approach, despite the absence of invasive aspergillosis mortality in our CF lung transplanted population since 2000.
POSTER ABSTRACTS
RNAi - A TOOL FOR TARGET FINDING IN NEW DRUG DEVELOPMENT

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Abstract - RNAi (RNA interference) refers to the introduction of homologous double stranded RNA (dsRNA) to specifically target a gene’s product, resulting in null or hypomorphic phenotypes. Long double-stranded RNAs (dsRNAs; typically >200 nt) can be used to silence the expression of target genes in a variety of organisms and cell types (e.g., worms, fruit flies, and plants). The long dsRNAs enter a cellular pathway that is commonly referred to as the RNA interference (RNAi) pathway. RNAi is being considered as an important tool not only for functional genomics, but also for gene-specific therapeutic activities that target the mRNAs of disease-related genes. RNAi plays a very important role in endogenous cellular processes, such as heterochromatin formation, developmental control and serves as an antiviral defense mechanism. RNAi has shown great potential for use as a tool for target finding in new drug development, molecular biological discovery, analysis and therapeutics. The involvement of the RNAi pathway in post-transcription silencing, transcriptional silencing and epigenetic silencing as well as its use as a tool for forward genetics and therapeutics.

Keywords- RNAi, Gene silencing, RNA silencing, Antisense RNA, neurological disorders
P002

CYTOKINES IN BRONCHOALVEOLAR LAVAGE FLUID (BALF) FROM EXPERIMENTAL PULMONARY ASPERGILLOSIS

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Recently, the incidence of invasive pulmonary aspergillosis has dramatically increased but host response to the fungus is not thoroughly investigated. The objective of this study was to examine the effect of fungal metabolites including gliotoxin on production of cytokines in the lung infected by Aspergillus fumigatus. In this study, two different clinical strains were employed: a gliotoxin-producing strain and a non-producing strain. The fungal conidia of each strain were inoculated intratracheally into mice of two groups, and after 24 or 48 h bronchoalveolar lavage was done with 3 ml saline. We quantified levels of cytokines in BALF employing purchased ELISA kit (Endogen or R&D Systems). Survival rate of mice infected by each strain were not statistically different. Pro-inflammatory cytokines such as IL-1 beta, TNF-alpha and IL-6 increased in the both groups. Chemokines such as MCP-1 and MIP-2 increased, particularly in mice infected by the gliotoxin-producing strain. IL-12 and IFN-gamma but not IL-4 increased. Curiously, IL-2 did not increased in both groups, which suggests that T-cell proliferation might not have occurred. In conclusion, these results lead us to conjecture that the fungal infection promotes T helper 1 differentiation without regard to the production of gliotoxin by the fungi.
P003

CLONING AND HETEROLOGOUS EXPRESSION OF A LACCASE ORTHOLOGUE FROM ASPERGILLUS FUMIGATUS AND FUNCTIONAL ANALYSIS OF THE ASSOCIATED GENE CLUSTER

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Laccase (E.C. 1.10.3.2, p-benzenediol:oxygen oxidoreductase), which is an enzyme belonging to the multi-copper oxidase family, catalyzes the oxidation of a broad variety of polyphenols with a preference for p-isomers, which are converted to p-quinones. Previously cloned laccase genes of Aspergillus nidulans, Aspergillus fumigatus, and laccase signature sequence were used to analyze A. fumigatus genome for laccases. This sequence analysis resulted in 4 probable laccase genes, one of which was the previously cloned abr2 gene. In this study, one of these genes (Aflac1) was further characterized. After sequence alignment and characterization studies, Aflac1 was predicted to have 2128 bp having six introns, which makes the protein 606 amino acid long, and the predicted protein sequence showed 63% homology with the dihydrogeodin oxidase of Aspergillus terreus and 38% homology to the laccase 2 of Botryotinia fuckeliana. Aflac1 gene is found within an uncharacterized gene cluster containing 13 genes which are predicted to be involved in the biosynthesis of A. fumigatus metabolites, trypacidin and monomethylsulochrin. By a comparative genomics approach, a putative cluster of 13 genes including dihydrogeodin oxidase was identified in A. terreus responsible for geodin biosynthesis. Aflac1 gene, amplified by the primers designed using the information obtained from sequence analysis, was cloned onto pAN52-1 and pAN52-4 vectors for heterologous expression in Aspergillus sojae. Furthermore, gene silencing studies will be performed to prove the function of Aflac1 and the associated gene cluster.
Inhalation of resting conidia is usually the first step of a systemic A. fumigatus infection. In the lung the inhaled spores encounter an environment which permits germination. However, the relative importance of certain environmental conditions for conidial activation and subsequent hyphae formation have so far not been analysed in detail. In this study we studied the role of oxygen during germination. For this purpose we first established an assay to quantify germination. We found that inhibitors of the respiratory chain were nearly as efficient in blocking germination as cycloheximide, an inhibitor of protein synthesis, which is well known to prevent germination. Electron microscopic studies revealed that all inhibitors blocked the formation of germlings, but were unable to prevent conidial swelling. This indicates that both protein biosynthesis and respiration are not required for sensing of the environment and the very early step of germination, which is characterized by shedding of the hydrophobic surface layer and osmotic swelling. In line with these data we found that A. fumigatus was not able to grow or germinate under anaerobic conditions. For microscopic studies we additionally used mitotracker, a fluorescent dye that specifically stains mitochondria with an activated respiratory chain. We found that mitochondria are indeed activated at the stage of swollen conidia. In summary the present study provides evidence that respiration is required for germination of A. fumigatus conidia.
SYSTEMATIC ANALYSIS OF ALKALINE ADAPTION IN PATHOGENIC FUNGI

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Regulation of alkaline adaptation has been identified as a critical virulence determinant in models of invasive aspergillosis and invasive candidiasis. This study further defines the mechanisms of alkaline adaptation in Saccharomyces cerevisiae, Candida albicans, Candida glabrata and Aspergillus fumigatus. The cellular effects of alkaline adaptation on S. cerevisiae were assessed by global phenotype testing at pH 4 and pH 8. The response to alkaline adaptation in different fungi was assayed by microarray analysis of S. cerevisiae, C. albicans, C. glabrata and A. fumigatus cultures at pH 4 and 8. A global S. cerevisiae interaction network was created, representing all published protein-protein and protein-DNA interactions. S. cerevisiae microarray and phenotypic data sets were mapped to the network to create an integrated alkaline adaptation map. Hubs of significant expression or phenotypic differences were identified. Reciprocal blast analysis identified gene orthologues between fungal species, and the resulting matrix was used to identify those genes with conserved expression profiles between species. The S. cerevisiae network identified several genes required for iron assimilation activated at pH 8, regulated by the transcription factor Rcs1. Phenotypic data identified a group of genes involved in osmotic stress responses and cell wall stabilisation required for growth at pH 8. Many vacuolar protein sorting genes were also required for growth at alkaline pH. Comparative analysis of transcript profiles between species identified conserved expression of amino acid biosynthetic genes in S.cerevisiae and A. fumigatus under alkaline conditions. Alkaline adaptation has been identified as a critical component of fungal virulence. The construction of an integrated alkaline adaptation network in S.cerevisiae, and comparative microarray profiling of alkaline adaptation in four fungal species, have enabled detailed characterisation of the fungal pH response across species. Current studies aim to investigate the role of critical components of alkaline adaptation in murine models of fungal infection.
THE METACASPASE GENES, CASA AND CASB, ARE NOT REQUIRED FOR
DEATH INDUCED BY OXIDATIVE STRESS IN A. FUMIGATUS

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Background
Recent studies have shown that fungi possess intrinsic suicide programs that are analogous
to mammalian programmed cell death (PCD) pathways. In the yeast S. cerevisiae, oxidative
stress induces a PCD response that requires the metacaspase protein Yca1p (1). In Aspergillus
fumigatus it has been shown that growth to stationary phase induces the expression of
caspase-like proteolytic cleavage, but the contribution of the A. fumigatus metacaspases to
this activity has not been reported (2).

Methods
To study the role of metacaspases in A. fumigatus we have disrupted each of the two
metacaspases in the A. fumigatus genome, casA and casB, using the split marker
recombination approach. The sensitivity of the (delta)casAB mutant to oxidative stress was
compared to that of wild type using conidial viability and radial growth assays. To determine
if CasA and/or CasB mediate caspase-like activity, we compared the caspase-like activity of
protein lysates from the (delta)casAB mutant to the wild type organism using fluorescence-
based caspase substrates that have been shown to identify caspase-like activity in Aspergillus
spp.

Results
Conidia and germlings from the (delta)casAB mutant showed wild type sensitivity to
oxidative stress mediated by hydrogen peroxide, t-butyl hydrogen peroxide, menadione, and
paraquat. Caspase-like activity was induced in wild type protein lysates at stationary phase,
and in response to carbon starvation, but no difference in activity was observed between the
wild type and the (delta)casAB mutant.

Conclusion
We conclude that casA and casB are dispensable for cell death induced by oxidative stress.
We also conclude that caspase-like substrate cleavage observed during stationary phase can
be accomplished in a metacaspase-independent manner.

References
2. Mousavi, S.A. and G.D. Robson, Entry into the stationary phase is associated with a rapid
loss of viability and an apoptotic-like phenotype in the opportunistic pathogen Aspergillus
IN VIVO ANALYSIS OF ASPERGILLUS FUMIGATUS DEVELOPMENTAL GENE EXPRESSION DETERMINED BY REAL-TIME RT-PCR

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Background
As A. fumigatus hyphae mature, they become developmentally competent in that they gain the capacity to form conidia. We have shown that the transition from pre-competent to competent hyphae in vitro is associated with change in the expression of numerous genes. Among genes expressed by competent hyphae in vitro are a number of putative virulence genes, many of which are under the control of the transcription factor, StuAp. However, it was unknown whether hyphae are competent during invasive aspergillosis, and whether the expression of competent-associated virulence genes is governed by StuAp in vivo. We hypothesized that during experimental infection, A. fumigatus hyphae are developmentally competent and express competence-associated genes that may mediate virulence.

Methods
Mice were immunosuppressed with cortisone acetate and cyclophosphamide and then infected intranasally with 5 X10⁵ conidia of either wild-type AF or a stuA null mutant. Mice were sacrificed on days 1 and 3 after infection, after which their lungs were harvested for RNA extraction. To evaluate longer duration of infection, mice were infected with strain AF293 in an aerosol chamber (1-3X10³ conidia per mouse) and organs harvested after 5-8 days of infection. For in vitro comparisons, A. fumigatus strains were grown in liquid YPD medium at 37 degrees C. At various time points, samples were collected for RNA extraction. Expression of candidate A. fumigatus genes was analyzed by real-time RT-PCR, and the expression of these genes was normalized to the expression of TEF1.

Results
Expression of the competence associated genes stuA and sspA (which encodes a putative cell wall protein) was detected from all samples extracted from animals infected with wild-type A. fumigatus. Further, the mRNA levels for these genes were comparable with those found in competent hyphae in vitro. In contrast, expression of the pre-competence genes hsp70 and ura7 was not detected at any significant level in vivo, or in competent hyphae grown in vitro, but was detected at high level in pre-competent hyphae grown in vitro. Interestingly, while sspA expression in vitro was entirely dependent on stuAp, sspA transcription was detected in mice infected with the stuA null mutant strain in vivo, albeit at reduced levels. The expression of StuA-dependent toxin biosynthetic genes (including AspF1, dmaW and metAP, among others) was detected in mice infected with strain AF293, but not in mice infected with the stuA null mutant.
Conclusion
Our results suggest that developmentally competent hyphae predominate during invasive pulmonary aspergillosis. Further, StuAp governs the expression of multiple toxin biosynthetic genes in vivo, although some genes that are regulated by StuAp in vitro are governed by other pathways in vivo.
INTERLABORATORY REPRODUCIBILITY OF AN EXPERIMENTAL MURINE MODEL OF INVASIVE PULMONARY ASPERGILLOSIS

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Background
The development of a simple reproducible animal model of invasive aspergillosis (IA) provides an important tool for the study of virulence mechanisms and the development of novel therapeutics and diagnostics. Reproducibility both within and across laboratories is a particularly important criteria in the evaluation of such models. We have recently developed a murine inhalational model of IA that displays good intralaboratory reproducibility, and we therefore evaluated the inter-laboratory reproducibility of this model at two geographically distant sites.

Methods
Experiments were performed in parallel at two independent laboratories (LABiomed and UTHSCSA) using identical protocols. Male Balb/C mice (20-22g) were immunosuppressed with cortisone acetate (250mg/kg on D-2 and D+3 of infection) and cyclophosphamide (250mg/kg on D-2 and 200mg/kg on D+3). Immunosuppressed mice were then challenged with A. fumigatus in an aerosol chamber as previously described (Sheppard, AAC, 2004). We compared overall survival after infection, and in a second set of experiments determined the time course of fungal burden by quantitative culture. Finally, the effects of liposomal amphotericin B (Ambisome) (10mg/kg/d ip from D1 to D8 of infection) on survival and fungal burden were compared.

Results
The overall survival of untreated infected mice was not statistically different between the 2 sites, ranging from 60% to 70% mortality. Total lung fungal burden was also highly reproducible between both laboratories over all time points. Treatment of infected mice with 10mg/kg/d of liposomal amphotericin was protective, reducing the mortality of treated mice to 30% at both sites. In addition, treatment significantly reduced pulmonary fungal burden at both institutions in a similar manner.

Conclusion
Our results show that this aerosol inhalational model of invasive aspergillosis is reproducible not only within a single institution, but also between institutions. This model therefore can serve as a useful tool to facilitate the comparison of in vivo experimental results performed at different laboratories.
A PHENYLALANINE TO LEUCINE SUBSTITUTION AT POSITION 389 IN SQUALENE EPOXIGENASE CONFERS TERBINAFINE RESISTANCE IN ASPERGILLUS FUMIGATUS

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The allylamine antimycotic terbinafine is highly effective for the treatment of cutaneous human mycoses and is active on a wide range of pathogenic fungi including dermatophytes, moulds, dimorphic fungi, and some yeasts. Terbinafine acts by blocking the biosynthesis of ergosterol, the major sterol in fungal membranes, by inhibiting squalene epoxidase. The widespread use of oral and topical terbinafine suggests that resistant strains may emerge, although little is known about the mechanism of resistance to this drug. To better understand such mechanisms, we isolated terbinafine-resistant mutants of Aspergillus nidulans following UV mutagenesis. A T4216C point mutation was found within AnErg1, which encodes squalene epoxidase, resulting in a F1406L amino acid substitution. The equivalent mutation was introduced into A. fumigatus (F389L) and was also found to confer resistance with a MIC>160.0 µg/ml compared to wild type MIC= 0.3 µg/ml. The presence of the mutation was dominant when expressed from an ectopic chromosomal location or from an autonomously replicating plasmid (pRG3-AMA1) in the presence of the wild type gene. Loss of the plasmid containing the AfErg1-T1664C mutation restored sensitivity to the strain. Similarly expression of plasmid-encoded wild type AfErg1 failed to confer resistance. A. fumigatus mutants resistant to terbinafine did not show cross-resistance to itraconazole, ketoconazole, fluconazole and clotrimazole, as expected. These results suggest that a F389L amino acid substitution in Erg1p in A. fumigatus confers prominent resistance to terbinafine. This finding may have clinical significance and could be of value as a new selectable marker.
Aspergilli are common fungi in the environment and cause different aspergillosis especially in immunocompromised patient AIDS, and transplant recipients. Despite development of treatment, the numbers of case of various form of aspergillosis have increased markedly over the past decade. The ability of aspergillus fumigatus isolates to produce protease such as serine protease and their ability to cause invasive aspergillosis, suggested that this protease is an important virulence factor.

In this study we examined 27 clinical isolated of aspergillus fumigatus for elastase activity and their pathogenesis and inhibition of disease in animal model. 26 clinical isolate (from human and animal) and one standard strain of A. fumigatus (as control) was chosen from Mycology Research Center, Faculty of Veterinary Medicine, University of Tehran. Conidia were plated on agar medium containing elastin for hallo around the colony, which indicated elastin degradation, and estimate of elastase production by isolates. The spores of these isolates were inoculated in to liquid medium containing elstin for elastase activity in filtrated cell wall and cytoplasmic extract (for some of isolates). Two isolates conidia (with high and low elastase ac-
tivity) and the standard strain were injected to 8 groups of mice, and mortality rate evaluated for 5 weeks. Blood were collected in 4 times for elastase ctivity and IgG concentration. The mice after the end of this time, sacrificed and different organs such as brain, heart… were re-

The sections of tissue were stained with PAS and Verhoff, s techniques for observation of fungal elements and elastin degradation, respectively. In addition to tissue cultures were taken for growing A. fumigatus. The result of fungal cultures on plate with elastin showed that colonies diameter and hallo diameter around the colonies were 30.59±11.92 and 34.5± 10.984 mm, respectively. Mean of elastase activity index (hallo diameter /colony diameter) was 0.918 ±0.265. The elastase activity on broth medium was 0.195±0.037 IU; bovine mastitis has most elastase ac-
tivity. This activity in cell wall and cytoplasmic extract were lower significant differences be-
tween control group (saline injection) and other group, whereas no observed these differences in sera IgG concentration. Mortality rate in group which injected with positive elastase was higher than group which used chymostation as an elastase inhibitor (P<0.05). Microscopic observation of stained section showed that aspergillus hyphaen exist in some of the tissue and defense cells such as PMN and lymphocyte cells were observed in others. Elastic staining for lung and artery showed that no different in the elastin couantity between control groups and cases groups. Several plates from tissues cultures were positive for A. fumigatus, CFU from tissues indicated that kidney was high infected from others. Regarding to these finding it seems that elastase plays an important role in virulence in aspergillus and pathogenesis.
TRANSCRIPTOME ANALYSIS OF ASPERGILLUS FUMIGATUS EXPOSED TO VORICONAZOLE

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For a comprehensive evaluation of genes that have their expression modulated during exposition of the mycelia to voriconazole, we performed a large-scale analysis of gene expression in Aspergillus fumigatus using a microarray hybridization approach. By comparing the expression of genes between the reference time and after addition of voriconazole (30, 60, 120, and 240 min), we identified 2,271 genes differentially expressed in the wild-type strain. To validate the expression of some of these genes during exposition to voriconazole, we analyzed thirteen genes showing higher expression in the presence of voriconazole by real-time RT-PCR. Although the magnitudes of induction differed between the two experimental systems, in all cases they were in good agreement with the microarray data. To our knowledge that is the first study of microarray hybridization analysis for a filamentous fungus exposed to an antifungal agent. In our study, we have observed: (i) a downregulation of various ergosterol biosynthesis genes; (ii) upregulation of genes involved in a variety of cell functions, such as transporters, transcription factors, proteins involved in cell metabolism, and hypothetical proteins; and (iii) the involvement of the cAMP-PKA signaling pathway in the overexpression of several of these genes.

Financial support: FAPESP and CNPq, Brazil and NIAID, USA
Sequencing of the Aspergillus fumigatus genome has permitted the development of functional genomic studies, many of them focussed on the discovery of fungal molecules with relevance in virulence. Deletion of a candidate gene by replacement with a genetic marker and demonstration that the knockout strain is partial or completely avirulent is the methodology usually employed.

Among the possible transformation markers to be utilised in this methodology, the fungal auxotrophic markers allowing dual (positive and negative) selection, such as the pyrG gene, encoding orotidine 5’-monophosphate decarboxylase, are really valuable. Firstly, it permits clear identification of transformants. Secondly, such a marker has been used extensively by molecular mycologists and thus it is already present in many genetic constructs. Thirdly, corresponding A. fumigatus auxotrophic mutants exist (D’Enfert et al. 1996). However, at present, only the bacterial genes conferring resistance to hygromycin, and rarely phleomycin, are utilised for virulence studies of A. fumigatus knockout strains, despite the inherent difficulties in the transformation process, especially in the selection of real transformants. The reason for not using the pyrG based transformation system in A. fumigatus virulence studies, so far, is the concern of using a genetic marker with an intrinsic pathogenic role (D’Enfert et al. 1996), a fact that could question the interpretation of such studies. This concern has also been stressed after recent reports in Candida albicans describing the influence on virulence of the locus of integration of the URA3 marker cassette, routinely used for obtaining knockout strains for many years (Brand et al. 2004). In this work, we analysed the utility of the pyrG/pyr-4 transformation system to generate knockout strains without affecting the intrinsic virulence of A. fumigatus. Three A. fumigatus knockout strains containing deletions in three different metabolic genes, the pyrG, oleA and oleB genes, were constructed by replacing their ORFs with the Neurospora crassa pyr-4 gene. The uridine auxotroph A. fumigatus CEA22 (pyrG) strain was employed as the recipient strain and prototroph transformants were selected and further analysed by Southern blotting to identify those containing the appropriate gene replacement event.

The virulence of the three knockout strains was tested in a murine model of invasive pulmonary aspergillosis (Smith et al. 1994) and compared with that of three reference isolates: A. fumigatus 293, 237 and ATCC46645. As a control, the non-pathogenic recipient CEA22 (pyrG) strain was also included in this study. Enzyme assays, survival curve data and histopathological studies showed that the replacement of the pyrG gene by pyr-4 does not alter the intrinsic virulence of A. fumigatus. Surprisingly, by using this methodology
we found that deletion of two putative pathogenic genes, oleA and oleB, have no effect on virulence. This result demonstrates that expression of the pyr-4 marker, regardless its genomic location, fully restores virulence of an A. fumigatus pyrG mutant strain. Taken together, all these results validate the use of the pyrG/pyr-4 transformation system for future identification of virulence determinants in A. fumigatus.
EFFICIENT PHAGOCYTOSIS OF ASPERGILLUS FUMIGATUS CONIDIA BY MURINE MACROPHAGES REQUIRES RECOGNITION OF SSI-3 GLUCAN BY DECTIN-1 AND THE PRESENCE OF TLR2

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Aspergillus fumigatus is an opportunistic fungal pathogen responsible for severe, life-threatening infections in immunocompromised patients. Aspergillus conidia are ubiquitously found in the environment and it has been estimated that humans inhale several hundred of them per day. In the lung of immunocompetent hosts, resident macrophages are supposed to rapidly ingest and kill invading spores, while in immunocompromised patients some conidia may escape the attack of the innate immune system and establish a systemic infection. Although conidial phagocytosis is crucial to protect the host, we still know little about the mechanisms and molecules involved in this process. Since humans have adapted to the daily confrontation with fungal spores, it seems likely that so-called 'pathogen associated molecular patterns' (PAMPs), conserved structures present on the fungal surface, are not only important for the activation of macrophages, but also for the phagocytosis of inhaled conidia. In this report we provide evidence that laminarin, a soluble β1-3 glucan, efficiently blocks phagocytosis of A. fumigatus conidia by macrophages. We also found that preincubation of conidia with a commercially available Limulus polyphemus preparation, containing the β1-3 glucan-specific lectin factor G, blocks phagocytosis by macrophages. These finding were corroborated by the efficient inhibition of phagocytosis by a dectin-1 specific monoclonal antibody. We also found that swollen conidia, which contain higher concentrations of β1-3 glucan on their surface, are phagocytosed much more efficiently than resting conidia. Finally, we provide evidence that TLR2, but not TLR4, is essential for efficient phagocytosis of A. fumigatus conidia, suggesting that the interaction between dectin-1 and TLR2 is of crucial importance not only for the immunological recognition of fungal pathogens, but also for their phagocytosis.
Recent evidence has shown that the immune response in individuals that succumb or stave-off infection by Aspergillus fumigatus differs. Healthy volunteers, who cleared the infection, showed a TH1-type response whereas patients who developed invasive aspergillosis (IA) showed raised levels of IL-10 indicating a TH2 skewing of the immune responses (Hebart et al, 2002).

No similar data has been collected from patients with Chronic Granulomatous Disorder (CGD), who are particularly at risk of developing IA. As a prelude to an in depth analysis of the immune response of the host to A. fumigatus infection in CGD we characterized the transcriptional response of immunocompetent and immunosuppressed CD1 mice, 8 and 24 h post-infection. This was done using a murine immunoarray, (MRC Rosalind Franklin Centre for Genomics Research), which contains oligonucleotides designed to 1104 genes that are annotated as either being involved in the immune response or that encode transcription factors.

Multiple ANOVA, SAM analysis and t-tests were performed in TIGR MeV. These statistical tests allowed us to distinguish genes that were upregulated in direct response to A. fumigatus as opposed to saline inoculation under both immunocompetent (18 genes upregulated) and immunocompromised conditions (42 genes upregulated). It also enabled us to distinguish the difference in response to A. fumigatus in immunocompetent and immunocompromised conditions (54 genes upregulated). Several genes have been shown to be upregulated that are important in immune response coordination and TH1 vs. TH2 phenotype. In an immunocompetent murine model there appears to be a tightly controlled TH1-type immune response and down regulation of regulatory T cells resulting in clearance of infection. In a neutropenic model both TH1 and TH2 specific genes are upregulated. The loss of neutrophils causes mis-regulation and an uncoordinated immune response resulting in death due to infection. This shows the important role of neutrophils in controlling immunity to A. fumigatus infection.

This work has revealed the nature of the murine immune response, at the transcriptional level, to A. fumigatus infection and serve as a platform for the analysis of the immune response in CGD p47phox-/- mice.
Aspergillus fumigatus (A. fumigatus) is an opportunistic pathogen and is the most common cause of invasive pulmonary aspergillosis (IPA), a life-threatening disease of immunocompromised patients that is acquired by inhalation of airborne spores. Especially, penetration of the lung tissues of these fungi is a key step in the infectious process. Therefore, various putative virulence factors of A. fumigatus have been studied over the past decades. But, the cytotoxic and biological effects of these virulence factors between clinical and environmental isolates of A. fumigatus were not fully understood. Although some environmental A. fumigatus strains seem to be less virulent than clinical isolates, the attenuation is not absolute, and these strains are probably still capable of causing invasive infection by various virulence factors.

In this study, we have tried to compare clinical isolates (10 strains) with environmental isolates (10 strains) of A. fumigatus on the expression of cytokine genes in human T cell (HuT 78), monocyte (THP-1), and lung cells such as lung epithelial cell (A-549), normal lung fibroblast (NHLF), and normal lung micro-vascular endothelial cell (NHLMVEC), using multiplex PCR (MPCR) including cytokine genes (IL-2, IL-3, IL-4, IL-5, IL-13, and ICAM-1), and inflammation cytokine genes (IL-1beta, IL-6, IL-8, TNF-alpha, TGF-beta, and GM-CSF). And, we have also investigated the matrix metalloproteinase by zymography and there genes (MMP-1, 2, 3, 7, and 9) by MPCR in lung cells, because these activity may be represented in both local and systemic inflammatory events and tissue invasion of fungi during the fungal infection, and in how these could affect to host.

Our data in this study showed that all of the cytokine genes expressed in human cells were not differ from between clinical and environmental isolates, although, the amounts of expressed cytokine genes have difference slightly in accordance with strains.

These results suggest that the genetic variability between strains exits, but that there is no difference on the expression of cytokine genes in the human cells. Therefore, we proposed that environmental isolates of A. fumigatus have also capable of causing infection. Certainly, more tests and in-depth studies of this process in the level of protein are needed. Nevertheless, this study represents the first step of our studies in showing the expression of cytokines by A. fumigatus in various human cells.
HUMAN GRANULOCYTES ARREST GROWTH OF ASPERGILLUS FUMIGATUS CONIDIA BUT NOT HYPHAE THROUGH AN OXYGEN-INDEPENDENT ANTIMICROBIAL MECHANISM

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Invasive aspergillosis is a frequent complication in systemic immuno-suppression and is a common cause of morbidity and mortality in patients with chronic granulomatous disease (CGD). Polymorphonuclear granulocytes (PMN) from CGD patients are unable to generate reactive oxygen species and as a consequence are unable to kill otherwise opportunistic microbes such as Aspergillus fumigatus. It has been thought that after inhalation, A. fumigatus conidia are killed primarily by alveolar macrophages whereas hyphae are killed by PMN in an oxygen-dependent manner. Using XTT to assay A. fumigatus viability, we re-evaluated the role of neutrophils in host defense against this important fungal pathogen. As expected, PMN from CGD patients were unable to inhibit hyphal growth when compared with normal PMN. However, PMN from both the normal as well as the CGD patients caused equivalent growth inhibition of conidia in a PMN dose-dependent manner. Time-lapse microscopy demonstrated equally rapid (<10 minutes) ingestion of conidia by both normal and CGD PMN. Conidial germination, as observed for 16-24 hrs, was significantly impaired in the presence of PMN. Vital staining of conidia with neutral red demonstrated that while > 97% of conidia untreated with PMN took up and compartmentalized the dye within vesicles (indicating viability) in 2 hrs, only 40% of conidia treated with PMN from both normal and CGD patients were able to do so. Furthermore, electron microscopy revealed substantial ultrastructural damage to conidia ingested either by normal or CGD PMN. Work is currently underway to identify the factor(s) responsible for the previously unrecognized contribution of oxygen-independent antimicrobial systems to the innate host defenses against this opportunistic human pathogen.
DISRUPTION OF THE NON-RIBOSOMAL PEPTIDE SYNTHASE GENE, GLIP, IN THE ASPERGILLUS FUMIGATUS GLIOTOXIN BIOSYNTHETIC PATHWAY

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Aspergillus fumigatus produces a variety of secondary metabolites during hyphal growth including gliotoxin, helvolic acid, fumagilin and others. Of these secondary metabolites, gliotoxin has received significant attention because of its putative role in the pathogenesis of A. fumigatus. Molecular studies on gliotoxin production by A. fumigatus has thus far been carried out by disrupting the laeA gene, a global regulator of secondary metabolites both in A. fumigatus and A. nidulans. We have isolated glip and laeA deleted strains via Agrobacterium tumefaciens mediated transformation of the strain B-5223. The glip gene, a homolog of sirp of Leptosphaeria maculans, encodes a non-ribosomal peptide synthase which is in the putative 12- gene cluster involved in the biosynthesis of gliotoxin in A. fumigatus. The glip deletant strain showed no obvious modification in morphological characteristics. Preliminary data obtained by HPLC analysis, however, showed that the culture supernatant of glip deletant strain lacked gliotoxin while LaeA deletant strain produced reduced level of gliotoxin compared to B-5223. A chemiluminescence assay of the PMA-induced-oxidative burst of human polymorphonuclear granulocytes in the presence of culture supernatants of B-5233,glip deletant, and laeA deletant and laeA deletant complimented with wild type laeA gene showed that glip deletant is the only strain that lacks the ability to inhibit oxidative bursts. Animal studies using glip deletant, laeA deletant and the wild type strain B-5223 are underway to assess the role of gliotoxin in the pathobiology of A. fumigatus.
ACUTE INVASIVE ASPERGILLOSIS: THE MOUSE MAKES THE DIFFERENCE

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Background
Many models of invasive aspergillosis use intravenous infection which heavily targets the spleen and kidneys and almost completely spares the lungs. In contrast, clinical aspergillosis almost invariably follows inoculation of the upper or lower respiratory tract and the lungs are the most heavily targeted organs. Our group has developed a model for murine invasive aspergillosis which reproduces clinical pathogenesis (Sheppard, AAC 2004). However, the optimal animal model, including mouse strain, and the methods of immune suppression to facilitate the invasive fungal infection are still undergoing definition. These parameters are explored in the present studies.

Methods
Outbred ICR, and inbred DBA, C57 and Balb/c mice were infected with Aspergillus fumigatus #AF293 using two types of aerosol chambers. The first is an acrylic chamber that allows simultaneous challenge of 50 mice. The second is a Madison chamber which allows simultaneous challenge of >100 mice. In the acrylic chamber we nebulized conidia of Aspergillus fumigatus at 10⁹ CFU/ml of fluid for 1 h for infection and in the Madison chamber we nebulized conidia at 10¹⁰ CFU/ml for 1 h. Mice were immunosuppressed with cortisone acetate and cyclophosphamide on day -2 and day +3 of infection and treated with ceftazidime daily beginning on day -2 before infection. The course of infection was analyzed by length of survival (Log rank test, n=10-20) and semi-quantitative tissue cultures (Mann Whitney test, n=5-20) done on day 1, 3, 5, 7 and 11 after infection. Results: 1) Similar results were obtained in mice infected with either chamber. 2) DBA mice were unsatisfactory: immunocompetent DBA mice did not succumb following infection nor did Aspergillus persist in their lungs; immunosuppressed DBA mice succumbed by day 9, whether or not they were infected with Aspergillus. 3) C57 mice were also unsatisfactory: they did not succumb to aspergillosis and they rapidly reduced their lung tissue fungal burden counts. 4) Both Balb/c and ICR mice were more satisfactory: following infection with Aspergillus in the acrylic chamber mortality was 60% and 10-40% (respectively) in the Madison chamber with both mouse strains. Persistence of Aspergillus in their lungs occurred to day 11, with approximately 10⁴ total lung fungal burden/mouse with the acrylic chamber and with persistent but lower counts using the Madison chamber.
Conclusions
For different reasons neither DBA nor C57 mice were suitable models of invasive aspergillosis in this system. In contrast, both outbred ICR and inbred Balb/c mice were acceptable models of invasive aspergillosis particularly in the acrylic chamber. This should reassure investigators who want to use an inexpensive, readily available outbred mouse for studies of acute invasive aspergillosis.
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PROFILE OF HOST GENE EXPRESSION DURING INVASIVE PULMONARY ASPERGILLOSIS

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Background
During invasive pulmonary aspergillosis, hyphae of Aspergillus fumigatus invade the blood vessels of the lung. The endothelial cell response to angioinvasion has the potential to influence the type and number of leukocytes that are recruited to foci of infection. We have previously found that in vitro infection with A. fumigatus hyphae induces endothelial cells to express the leukocyte adhesion molecules, E-selectin and VCAM-1, but not ICAM-1. We therefore used real-time RT-PCR to investigate whether A. fumigatus induces a similar pattern of leukocyte adhesion molecule expression in a murine model of invasive pulmonary aspergillosis.

Methods
BALB/c mice were immunosuppressed with cyclophosphamide and cortisone acetate, and then infected with A. fumigatus spores in an inhalational chamber. Control mice were immunosuppressed similarly, but not infected. On days 5-8 after infection, groups of 3-8 mice were sacrificed, and total RNA was extracted from their lungs using a freeze-crush hot phenol extraction. The expression of genes encoding murine E-selectin, VCAM-1, ICAM-1, and TNF-α was measured using real time RT-PCR, and the results were normalized to the expression of murine GAPDH.

Results
Interestingly, ICAM-1 and VCAM-1 expression tended to be lower in the mice infected with A. fumigatus compared to the uninfected controls after 5-6 days of infection, suggesting that A. fumigatus might actually suppress the host inflammatory response. After day 7-8 of infection, the infected mice had significantly increased expression of E-selectin, VCAM-1, and TNF-α compared to uninfected, immunosuppressed control mice at the same time points. This increase in gene expression coincided with recovery from neutropenia and the dramatic influx of neutrophils into the lungs. Therefore, it is likely that the leukocytes themselves stimulated the expression of E-selectin and VCAM-1. Finally, immunosuppression by itself influenced gene expression, as the expression of all 4 genes in the uninfected mice was higher at the earlier time points compared to the later time points.
Conclusions
In murine invasive pulmonary aspergillosis, there are two phases of inflammatory response seen in vivo. During the early phase of A. fumigatus infection, there is down-regulation of leukocyte adhesion molecule expression. In the late phase of in vivo infection, as seen in vitro, A. fumigatus stimulates the expression of E-selectin and VCAM-1. In vitro, A. fumigatus directly stimulates expression of these leukocyte adhesion molecules, whereas in vivo the presence of leukocytes is required for these leukocyte adhesion molecules to be expressed.
Aspergillus fumigatus is a pathogen of man, in particular of immunocompromised patients. Considerable research is being directed to the identification of pathogenicity determinants which may explain the virulence of A. fumigatus over other common thermotolerant fungal species. Extracellular phospholipases have been shown to be important virulence determinants in the pathogenesis of several bacterial infections including those caused by Clostridium perfringens, Pseudomonas aeruginosa and Listeria monocytogenes where they cause significant tissue damage and necrosis. Phospholipase B production by Candida albicans and Cryptococcus neoformans has been correlated with virulence and phospholipase B knockout mutants in C. albicans and C. neoformans are shown to have reduced pathogenicity in animal models. It was recently demonstrated that clinical isolates of A. fumigatus produce significantly higher levels of extracellular phospholipase C activity compared with environmental isolates [Birch et al., 2004]. This may be important in disease development, particularly as infection usually occurs through the inhalation of airborne conidia and the surface of the alveoli is coated in surfactant which is composed of >80% phospholipid. In this study, we identified from the TIGR genome sequence data a number of putatively secreted phospholipase and lipase genes and attempted to examine their regulation in the presence and absence of lecithin, a phosphotidylcholine phospholipid using the TIGR (version 2) oligomer slide-based microarrays. Analysis shows the significant differential regulation of a number of secreted lipases as well as known A. fumigatus allergens and virulence-related genes.

BINDING OF BASEMENT MEMBRANE LAMININ TO ASPERGILLUS TERREUS CONIDIA

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Basement membrane laminin has been suggested to play a role in adherence of Aspergillus fumigatus conidia to bronchopulmonary epithelium. Interactions with this protein were therefore investigated in two other filamentous fungi colonizing the airways of patients with cystic fibrosis, Scedosporium apiospermum and Aspergillus terreus. Whereas S. apiospermum did not bind laminin as revealed by immunofluorescence and flow cytometry, an intense labelling was seen for A. terreus. Electron microscopy showed the uniform distribution of laminin binding sites at the surface of both conidia and germ tubes. In addition, flow cytometry experiments demonstrated the specificity of the binding and a cross-reactivity between laminin and fibronectin. Laminin binding was saturable and reversible, and seemed to be mediated by a sialic acid-specific lectin. Moreover, microtiter plate adherence assays suggested a role for this interaction in adherence of the conidia and confirmed the involvement of a sialic acid-specific lectin. Finally, the influence of culture conditions was also investigated. The expression of laminin receptors at the surface of conidia increased with their maturation and was affected by the presence of Zn++ cations in the culture medium. Beside, analysis of mycelial extracts by 2-D electrophoresis revealed the presence of at least 2 protein spots underrepresented in cultures grown in the presence of Zn++. The recent sequencing of the whole genome of A. terreus would allow us to identify these Zn-regulated proteins, which could be involved in the recognition of laminin.
Aspergillus flavus is well known as one of the principal producers of aspergilloses and aflatoxins, a class 1 carcinogen. Because of the importance of this mould on animal and human health, it is essential to determine their life histories and distribution in nature. Argentina is a predominantly agricultural country and the grain exportation has great acceptance in the world markets. As an attempt to contribute to the quality of those grains and improve the conditions for the agricultural workers, the objective of our study was to characterize the genetic diversity of A. flavus populations from different agroecological zones giving important information to determine which control measures are most effective in reducing A. flavus contamination. Geographical changes in the population structure of Aspergillus flavus from stored peanut seeds were analysed by using toxigenic potential, aflatoxin types B and G and cyclopiazonic acid (CPA) production, culture characteristics and vegetative compatibility groups (VCG) via complementation tests between nitrate-nonutilizing mutants. The A. flavus isolates were identified as members of either the L strain or S strain. In addition an effort was made to compare S strain from different countries using VCGs. The results indicated that more than 90% of the isolates were aflatoxin producers and almost 100% of them produce cyclopiazonic acid. The majority of the S strain produces aflatoxins type B and G. The percentage of the L strain is higher than S strain but this difference varies between agroecological zones. The results obtained indicate that genetic diversity of A. flavus in Argentina is elevated, with 0.65. VCG diversity index. This diversity also was indicated by the presence of many single-isolate VCGs as well as by the lower number of VCGs that have members of different agroecological zones. Among 100 heterokaryotic isolates of A. flavus, 66 VCGs were found and none of the S strain from Argentina was compatible with those of other countries. Scarce VCGs contained isolates from widely separated peanut growing regions examined.

S and L strains acts different, the aflatoxin producing potential of Aspergillus communities is higher when S strain is present but the conidia production in less than in L strains. The heterogeneity in the populations derived from peanut seeds at diverse locations in Argentina suggests that A. flavus populations have undergone changed spatially and temporally. Pairising of testers from Argentinean S strain with testers from Bénin, USA and Australia produced not surprising results because of the big VCG diversity in A. flavus. VC has been widely used to provide insights into the genetic structure of fungal populations and is a strong predictor of cladistics groupings In order to accomplish the study of A. flavus in Argentina, it is necessary the identification of A. flavus isolates belonging to the same genotype or clone. The ability to characterize and monitor genetically identical strains from A. flavus populations should allow one to determine how the disease is spread and which of the subpopulations are associated with aflatoxin and/or aspergilloses in our country.
Aspergillus fumigatus metabolises propionate and propionyl-CoA generating carbon sources like odd chain fatty acids and some amino acids via the methylcitrate cycle. This pathway leads to an alpha-oxidation of propionate (propionyl-CoA) to pyruvate and is initiated by the key enzyme methylcitrate synthase. Methylcitrate synthase possesses a Km for the substrates oxaloacetate and propionyl-CoA in the micromolar range preventing an accumulation of propionyl-CoA. A deletion of the gene coding for methylcitrate synthase leads to an accumulation of toxic propionyl-CoA at respective growth conditions.

Propionyl-CoA inhibits enzymes from primary metabolism like the pyruvate dehydrogenase complex or the succinyl-CoA synthetase. In addition, the synthesis of polyketides like the spore colour polyketide DHN-melanin becomes impaired. To elucidate the impact of the methylcitrate cycle on virulence the methylcitrate synthase mutant was compared with the wild type in a murine infection model. The ability of the mutant to kill mice was reduced by a factor of ten. In addition, in vivo kill assays showed that conidia of the mutant strain were more efficiently killed by primary alveolar macrophages than that of the wild type. Thin sections of lungs infected with either the wild type or the mutant displayed a reduced growth rate of the mutant within that tissue. The wild type was able to grow invasive through the lung, whereas the mutant strain is embanked by polymorphonuclear immune cells. Therefore, it is concluded that propionyl-CoA generating amino acids are used as carbon sources during infection and efficient removal of propionyl-CoA is essential to retain full virulence. Results will be confirmed by investigation of the behaviour of a complemented strain in the murine infection model.
INVESTIGATION OF THE MECHANISMS OF THE INHIBITION OF APOPTOSIS BY CONIDIA ASPERGILLUS FUMIGATUS.

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A main innate-immune response to inhaled conidia of Aspergillus fumigatus (Af) is the synthesis of pro-inflammatory cytokines, which include TNF-alpha a known inducer of apoptosis. Modulation of host cell apoptosis is one of the mechanisms whereby pathogens can overcome host cell defences. Af propagates through airborne conidia, which are inhaled into the small airways, where they may germinate and initiate an infection. Inhibition of apoptosis by Af conidia was shown in a transformed type II pneumocytes cell line A549, in human tracheal epithelial 16HBE, as well as in primary human respiratory cells . Inhibition of apoptosis by Af conidia was also observed when apoptosis was induced by co-cultivating A549 cells with activated human alveolar macrophages cells in absence of the addition of apoptotic inducers: this points to the biological significance of our findings. For TNF-alpha-induced apoptosis, the observed anti-apoptotic effect of Af conidia was associated with a significant reduction of caspase-3. The fact, that conidia decreased apoptosis in TNF-treated cells despite the presence of a translational inhibitor cycloheximide, argue for an upstream effect such as an interaction with caspase-8, blocking DISC formation. The results of Western blotting have shown significant decrease in the intensity of the signal of bands corresponding to activated caspase-8 in the presence of conidia.

Involvement of caspase during TNF induced apoptosis was confirmed by the use of the caspase inhibitor Z-VAD. FMK. Z-VAD. FMK inhibited TNF-induced apoptosis in a dose-dependent manner, while control cultures without the reagent showed no change in the number of apoptotic cells. Furthermore, it was shown that the inhibitory effect of Z-VAD. FMK was additive to the effect induced by conidia, suggesting that the inhibitions by Z-VAD. FMK and conidia might both target caspase.

The nature and mode of action of the conidial factors that block apoptosis are unknown but these factors could be conidial molecules either present in the airborne conidia and released upon contact with the cell or synthesised de novo in presence of the epithelial cells.
Host defense against invasive aspergillosis (IA) is multifactorial and depends both on innate and acquired immunity. Macrophages and neutrophils play a major role in the defense against fungi by different mechanisms including iron deprivation. The importance of iron acquisition for in vivo growth of Aspergillus fumigatus (Af) fungi was shown by the group of Haas using Af siderophore mutants that were avirulent in a mouse model of IA. The purpose of our study was to estimate whether the natural resistance-associated macrophage protein (Nramp1), a bivalent-metal/iron transporter that is expressed within late endosomes/lysosomes of macrophages, plays a role in the susceptibility to IA. Earlier it was shown that cells with inactivated Nramp1, that exhibit impaired bivalent cation transport, enable excessive growth of intracellular pathogens. RAW264.7 transfectant cell lines R21 (Nramp1 deficient) and R37 (Nramp1 expressing) alongside with newly established bone marrow derived macrophage cell lines from DBA2 (Nramp1-r/Nramp1-r), BALB/c (Nramp1-s/Nramp1-s) and BALB/c congenic Nramp1-r counterparts C.CB mice were used in this study. No significant difference in the internalization of Af conidia was found between Nramp1 deficient, resistant or sensitive macrophages. All types of macrophages were able to internalize in vitro from 78 to 92% of bound conidia after 24 hours, as was estimated by flow cytometry using FITC labeled Af conidia. To estimate intracellular killing of Af conidia, macrophages were incubated for 24, 48 and 72 hrs at different Af:Macrophage ratio in the presence of Amphotericin B (to kill extracellular conidia) and then lysed using 0.1% Tween-20 in Sabouraud medium. No significant difference in the killing of Af conidia was seen between genetically different cells. These data show that in vitro i) intracellular killing of Af conidia requires more than 24 hrs and depends on the number of internalized Af conidia per cell; and ii) there is no dependency of Af conidia intracellular survival on the presence of Nramp1. To estimate the resistance to Af infection in vivo DBA2, BALB/c and C.CB mice were immunosuppressed and challenged with 6x10E6 of Af conidia intranasally. DBA2 strain was the most sensitive to Af, followed by BALB/c. C.CB was the most resistant as estimated by both mortality rate, lung fungal load and dissemination into kidney, spleen and brains. Thus, other factors than genetic difference in Nramp1 gene can mediate susceptibility or resistance to invasive aspergillosis in immunocompromised mice.
Oxylipins are oxygenated lipids which play a signaling role in various biological functions in plants, fungi, and animals. We have identified three genes - ppoA, ppoB, and ppoC - encoding fatty acids oxygenases in the opportunistic human pathogenic fungus, Aspergillus fumigatus. Ppo proteins showed high amino acid sequence similarity to prostaglandins (PGs) which are one of the essential oxylipins in mammalian systems. Furthermore, Ppos were shown to mediate PG production in A. fumigatus as determined by ELISA. We have previously generated a silenced ppoABC mutant which had higher resistance to reactive oxidative substances (ROS) and increased virulence comparing to wild type (WT) in a mouse pulmonary model. To identify which ppo(s) contributed to this silenced phenotype, we have created knockout mutants of each ppo gene. ppoA and ppoB null mutants produced more conidia, but ppoC mutant less conidia than wild type. In addition, conidia of the ppoC mutant germinated faster and were 30% larger than wild type. This mutant was more resistant to ROS challenge than other strains, both mutant and wild type. We propose that PpoC might be a strong candidate affecting virulence in A. fumigatus.
GLIZ, A TRANSCRIPTIONAL REGULATOR OF GLIOTOXIN IN ASPERGILLUS FUMIGATUS

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Gliotoxin is a non-ribosomal peptide produced by Aspergillus fumigatus and other fungi. This compound has been proposed as an A. fumigatus virulence factor due to its cytotoxic, genotoxic and apoptosis stimulating properties. Recent identification of the gliotoxin gene cluster identified several genes (e.g. gli genes) likely involved in gliotoxin production including gliZ encoding a putative C2H2 zinc binuclear transcription factor. Both silencing of gliZ expression using RNAi technology and replacement of gliZ with a marker gene decreased gliotoxin production and gene expression of other gli cluster genes. However, production of other metabolites were also affected by gliZ disruption. Placement of multiple copies of gliZ in the genome increased gliotoxin production. The murine pulmonary model indicated a decrease in virulence in gliZ mutant and altered ability to kill PMN cells.
Aspergillus fumigatus is the most common human and animal mold pathogen among the 182 recognized species of the Aspergillus genus, causing both invasive diseases in immunocompromised patients and allergic disease in patients with atopic immune systems. The cell wall of A. fumigatus is composed of a polysaccharide skeleton interlaced and coated with cell-wall proteins (CWPs). The major class of fungal CWPs is the glycosylated phosphatidylinositol/GPI-modified proteins. These proteins contain a C-terminal hydrophobic domain which is cleaved off and replaced with a glycosylphosphatidylinositol/GPI-anchor and an N-terminal hydrophobic signal peptide sequence that targets them to the endoplasmic reticulum (ER). The GPI-anchor directs the attachment of these proteins to either the fungal plasma membrane or its cell wall. Fungal GPI-anchored proteins are involved in cell-wall biosynthesis, remodeling and adhesion. Many GPI-anchored CWPs involved in fungal adhesion also have multiple tandem repeat units containing a high percentage of heavily O-glycosylated serine and threonine residues.

Genes containing multiple tandem repeats undergo frequent recombination-dependent expansion or contraction in size, creating alterations in adhesion and immunogenicity. Examples of such GPI-anchored CWPs important in pathogenesis include the Candida albicans Als and Hwp1 adhesins and the Candida glabrata EPA1 adhesin. Interestingly, no homologs for these adhesins can be found in A. fumigatus. Computational analyses of the A. fumigatus genome suggest the existence of 80 putative GPI-containing proteins, of which almost half are of unknown function.

In S. cerevisiae, most genes containing tandem repeats encode CWPs and typically show size variability between different yeast isolates. This variation provides the functional diversity in cell-surface antigens that allows rapid adaptation to the environment.

We have analyzed A. fumigatus genomic sequences to identify Tandem repeats using the ETANDEM software program, a numerical score for tandem repeats in a nucleotide sequence (http://emboss.sourceforge.net/apps/etandem.html). We have performed a PCR analysis of 7 tandem-repeat-rich GPI-anchored CWP-encoding genes. Genomic DNA from 12 independent patient isolates of A. fumigatus was analyzed using primers designed to amplify the tandem repeat domains. Four of seven genes (Afu4g09600, Afu2g05150, Afu3g08990 and Afu6g14090; ETANDEM* scores= 292, 301, 432 and 134, respectively) showed significant size variation between isolates, whereas three did not (Afu4g03240, Afu7g00970, Afu2g07800; ETANDEM scores = 72, 49 and 34, respectively). This is the first time that A. fumigatus CWP-encoding genes containing tandem repeats have been shown to vary in length from strain to strain, a finding that may have important biological consequences.
P031

SINGLE-SPORE CULTURES OF THE SEQUENCED STRAIN OF ASPERGILLUS FUMIGATUS (AF293) HAVE SIGNIFICANTLY DIFFERENT MORTALITY RATES IN A MURINE MODEL OF IPA

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Background
Aspergillus fumigatus AF293 was the clinical isolate selected for genomic sequencing from the culture collection assembled by Denning in Manchester. As a consequence, it has been distributed to many laboratories worldwide and has been used in numerous in vitro and in vivo experiments. Cultures that have been distributed from Manchester or the Fungal Genetics Stock Center (FGSC) (USA) are designated AF293 1 for single-spore culture 1 (Fung. Genet. Biol. 41:443 2004). As cultures were from a single spore, it has been assumed that they must be phenotypically and genetically identical. During experimental procedures the strain is likely to have been sub-cultured and refrozen numerous times on the assumption that these procedures will not affect its phenotype.

Methods
Four single-spore cultures were obtained from the oldest available frozen stock of the clinical isolate. Microsatellite-length-polymorphism typing has demonstrated that these sub-cultures are identical to the original clinical isolate. One stock of single-spore culture 1 (ssc1) has been routinely used many times in our animal studies. The stocks of ssc2-4 have been left untouched at minus 80°C. The original minus 80°C stock of ssc1 has been removed from the freezer multiple times for sub-culturing and distribution to other laboratories and to the FGSC, but has not itself been sub-cultured. Mice were persistently immunocompromised with multiple doses of cyclophosphamide and cortisone then infected with the original stocks of ssc1-4 and the routine stock of ssc1 using 1 x 10⁹ nebulized spores in an inhalation chamber. After exposure mice develop severe invasive pulmonary aspergillosis. Mice were observed for up to 15 days and any mice with severe disease were killed. Lungs were removed from all mice when killed due to severe disease or after 15 days, and quantitatively cultured. Aspergillus recovered from lung of mice infected with the routine stock of ssc1 (Rssc1) were repeatedly passed (6 rounds) through mice and the mortality and tissue burden recorded.

Results
The mortality of the Aspergillus cultures (combined data from 3 experiments) was ssc1 60%, ssc2 27%, ssc3 20%, ssc4 47%, Rssc1 27% (ssc1 v ssc2/ssc3 p<0.05). Lung burdens were 3.67, 3.80, 3.70, 3.75, 3.24 and 3.44 Log10CFU/g for ssc1, ssc2, ssc3, ssc4, Rssc1 and the passaged strain, respectively (p>0.05). Mortality of the Rssc1 laboratory aliquot of Clone 1 was consistently lower than the original stock (p=0.09). It was not possible to increase the mortality of the routine stock of ssc1 by serial passage in mice (despite re-isolating the strain from mice succumbing to infection).
Discussion
Stocks of ssc1-4 had significantly different mortality rates in a murine invasive pulmonary aspergillosis. More worryingly Rsscl that was used in our animal studies exhibited reduced mortality compared to the original stock. Serial passage of the Rsscl through mice did not increase the mortality associated with infection. These data highlight that caution must be exercised when storing and sub-culturing Aspergillus during ongoing studies as routine laboratory procedures can affect the virulence of isolates.
DISRUPTION OF A NON-RIBOSOMAL PEPTIDE SYNTHETASE, GLIP, ELIMINATES GLIOTOXIN BIOSYNTHESIS IN ASPERGILLUS FUMIGATUS

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Gliotoxin is a secondary metabolite produced by Aspergillus fumigatus (Af) known to have immunosuppressant activities. However, the role of gliotoxin in fungal virulence has not yet been elucidated. The purpose of this study is to examine the hypothesis that gliotoxin plays a critical role in the pathophysiology of invasive aspergillosis. In order to address this hypothesis, a non-ribosomal peptide synthetase, GliP, predicted to be involved in gliotoxin biosynthesis, was disrupted to create a strain of Af deficient in gliotoxin production. Lack of gliotoxin production by the null mutant was confirmed with high-pressure liquid chromatography (HPLC) analysis, confirming GliP’s role in gliotoxin biosynthesis. Real-time PCR confirmed a lack of GliP expression in the null mutant, and demonstrated that 11 genes in the predicted gliotoxin biosynthesis cluster were also not expressed in the null mutant. Null mutants displayed normal growth and conidiation rates in vitro, and scanning electron microscopy revealed no differences in conidia morphology. Preliminary experiments to address the definitive role of gliotoxin in fungal virulence are underway and will be presented. These results are the first experimental evidence to confirm the non-ribosomal biosynthesis of gliotoxin in Af.
IN SILICO, PHYLOGENOMIC, AND EXPRESSION ANALYSIS OF NON-RIBOSOMAL PEPTIDE SYNTHETASES IN ASPERGILLUS FUMIGATUS STRAIN AF293

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Non-ribosomal peptide synthetases (NRPS) are large multi-modular proteins responsible for the production of certain fungal and bacterial secondary metabolites. Currently, the role of secondary metabolites in invasive aspergillosis (IA), caused by the opportunistic pathogen Aspergillus fumigatus, is largely unexplored. The purpose of this study was to identify all NRPSs in the Af genome sequence to identify candidate NRPS genes that may produce secondary metabolites important in the pathophysiology of IA. Bioinformatic analyses of the AF293 genome sequence identified 13 complete NRPS genes, and 1 hybrid NRPS/polyketide synthetase. Phylogenomic analyses with NRPSs identified from other sequenced Aspergillus species revealed that Af contains several unique, less ancestral NRPSs that may produce novel secondary metabolites involved in fungal virulence, as well as conserved more ancestral NRPSs. Phylogenomic analysis of the NRPS adenylation domain amino acid sequences suggested multiple potential evolutionary processes that have led to the diversity of NRPS genes in the genus Aspergillus. Real-time PCR revealed differential gene expression of the Af NRPS genes in different in vitro culture conditions. These preliminary results demonstrate the potential diversity of secondary metabolites produced by Af, and present the first step towards identifying the corresponding non-ribosomally synthesized secondary metabolites.
P034

PSEUDOMONAS AERUGINOSA (PA) KILLS ASPERGILLUS FUMIGATUS (AF) HYphae IN VITRO

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Background
Recently, a pathogenic relationship has been described between PA and the fungus Candida albicans (Science 2002;296:2229). We examined whether PA possessed a similar capacity to kill AF hyphae or suppress hyphal development through quorum molecule (QM) signaling.

Methods
AF293 conidia were incubated at 37°C for 24 hours alone, or in combination with viable PA (AF 293 conidia: PSA ranging from 100:1 to 1:100), PA culture filtrate alone, or PA QM [3OC12HSL, farnesol, dodecanol; 0.1 to 10 mM] in RPMI growth medium. At serial timepoints, replicate tubes were analyzed by microscopy and the XTT assay to measure hyphal viability following exposure to PA or QM. Recovery of AF hyphal growth following co-incubation with PA or QM was determined by washing and resuspending fungal conidia in fresh PA or QM-free growth medium.

Results
Co-incubation of PA with AF resulted in inoculum-dependent inhibition of AF hyphal viability and conidia germination. Growth of AF 293 was completely inhibited at 24 hours at AF:PA ratio > 1:10 (P< 0.001 vs. control). Similarly, PA culture filtrate and QMs inhibited hyphal growth and germination in a dose-dependent fashion, with persistent effects noted at 10 mM (dodecanol) even after 24 hours growth in QM-free media. Microscopy at 24 hours revealed extensive attachment of PA to AF hyphae, and in the case of QM, swollen conidia with rare germlings even after 48 hours incubation in strong hyphal inducing conditions.

Conclusions
The viability and germination of AF is severely affected by PA and QM released by PA. The ability of QM to lock AF in the conidial form in the setting of hyphal inducing conditions may represent a strategy for AF survival in the presence of antagonistic organisms such as PA.
ASPERGILLUS FUMIGATUS INDUCES DIFFERENT ENDOTHELIAL CELL RESPONSES DURING ABLUMINAL VERSUS LUMINAL INVASION OF ENDOTHELIAL CELLS

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Background
During invasive pulmonary aspergillosis, A. fumigatus (AF) hyphae invade the vasculature by passing from the abluminal to the luminal surface of endothelial cells (ECs). In contrast, during hematogenous dissemination, blood-borne hyphae escape from the vasculature by passing from the luminal to the abluminal surface of ECs. Although ECs are known to be polarized, it is not known if ECs respond differently to AF when the organism is in contact with the abluminal versus the luminal surface. Therefore, we infected ECs with AF on either their abluminal or luminal surface and investigated their response to the fungus.

Materials and Methods
Human umbilical vein ECs were grown on either the top (for luminal infection) or underside (for abluminal infection) of cell culture inserts with 3 micron or 0.1 micron pores. ECs were infected by adding AF germlings to the top of the inserts. EC invasion by AF was observed using transmission and scanning electron microscopy (TEM and SEM). EC damage by AF was measured by a chromium release assay. EC stimulation was assessed by expression of genes encoding E-selectin, IL-8, tissue factor, and TNF-alpha, which was measured by real-time RT-PCR. Results. By TEM and SEM, we observed that AF hyphae grew through the pores of the filter inserts and penetrated the abluminal surface of the ECs after 8 hours of infection. Some of these hyphae passed completely through the ECs and protruded through their luminal surface. Hyphae added to the luminal surface of the ECs were internalized within 1-2 hours. We found that abluminal infection induced significantly less EC damage than did luminal infection at 8, 16 and 24 hours. When the ECs were grown on filter inserts with a pore size of 0.1 micron, direct contact between the abluminal surface of the ECs and AF was prevented. However significant EC damage was still detectable at 24 hours, indicating that a soluble factor released by AF is partially responsible for EC damage during abluminal infection. In contrast to the damage results, abluminal infection with AF stimulated significantly greater expression of E-selectin, IL-8, tissue factor, and TNF-alpha mRNA than did luminal infection.

Conclusions
These results indicate that ECs respond differently to AF depending on the surface that is infected. Specifically, AF hyphae cause less EC damage, but greater EC stimulation when they infect the abluminal surface compared to the luminal surface.
DETECTION OF MEDICALLY IMPORTANT ASPERGILLUS SPECIES INVOLVED IN INVASIVE ASPERGILLOSIS THROUGH DNA MICROARRAY WITH TYRAMIDE SIGNAL AMPLIFICATION

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Invasive aspergillosis (IA) has been increasingly posing major problems in hospitals especially in patients that are undergoing hematopoetic stem cell transplantation, solid organ transplantation and treatments for hematological malignancies. With the aim of keeping the mortality and morbidity rate caused by IA at minimal, we have designed and applied a microarray method that assists in the early detection of IA with increased sensitivity and specificity. 40 clinical isolates of Aspergillus spp. were obtained and 5 ATCC strains (A. fumigatus 36607, A. nidulans 10074, A. niger 16888, A. oryzae 10124 and A. terreus 1012) were purchased from American Type Culture Collection (ATCC). All the isolates and ATCC strains were grown on Sabauroud Dextrose Agar (SDA) at appropriate temperature at different duration of time depending on the growth. For DNA extraction, they were grown in Sabouraud Dextrose broth (SDB) and DNA were extracted using conventional method. A few primers sets and internal probe sequences were designed. All oligonucleotide probes were synthesized without any modifications. Slides were purchased and derivatised with epoxysilane (3-glycidoxypropyltrimethoxysilane). Probes were spotted on the slides by using manual array spotter. The spotted slides were UV-crosslinked and kept until use. PCR were carried out with the primer sets designed following the optimal PCR conditions and the PCR products that were obtained were ethanol precipitated and labelled with biotin for binding with streptavidin during the hybridisation step. Biotinylated PCR products were heat denatured and later hybridised to slides for overnight at 55 degree Celsius. In order to get higher sensitivity (higher fluorescence intensities), we incorporated a system called Tyramide System Amplification (TSA) biotin system into our detection method. The biotinylated PCR products that bound to the spots on the glass slides will give manifold signals through that system and finally the fluorescence signals are obtained through the incubation with streptavidin conjugated Alexa Fluor dye. The fluorescence images that were formed were scanned using microarray scanner and analysed using a microarray software. For assay sensitivity, 40 species and subspecies of clinical isolates of Aspergillus spp. (function as positive test strains) and another group of 40 strains of taxonomically or ecologically related fungi (functions as negative test strains) were included and tested. For sensitivity test, different amounts of genomic DNA were used as initial template for the PCR. As of today, we have managed to optimize the condition for PCR and also microarray hybridisation. Our initial results showed that slides that were hybridised with the PCR products generated from A. fumigatus ATCC have shown that all the spots that were expected to fluoresce gave signals. This implies that the species specific probe for A. fumigatus is specific. Nevertheless, more tests need to be carried out to test the specificity of the probe for every species. The initial results that were obtained seem to be very encouraging and prove that the DNA microarray with the incorporation of Tyramide Signal Amplification works.
P037

CLONING AND SEQUENCING GENES IN THE AGING CELLS OF ASPERGILLUS FUMIGATUS

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Subtractive hybridisation (SH) can be used to aid the isolation of genes showing altered expression in target cells following exposure to a stimulus. Most importantly, using such an approach, no prior knowledge of the specific genes is required. This procedure increases the effective concentration of induced sequences expressed in an experimental mRNA population (target) but not in a control mRNA population (driver) by hybridisation of target with an excess of driver to remove sequences common to both.

In this study, subtractive hybridization was used to isolate genes strongly or uniquely expressed during the early stages of the stationary phase. A total of 33 cDNA clones were recovered. Some of these encoded homologues of known proteins, such as, heat shock protein, whilst the others either had high homology to hypothetical proteins of unknown function or were not represented in the Blast database.
Aspergillus fumigatus (Afu), a medically important opportunistic fungus, is the major etiologic agent for invasive aspergillosis. Invasive form of aspergillosis is often encountered in patients with leukemia, HIV, transplant cases and is a leading cause of death in immunocompromised patients despite empirical treatments with currently available antifungal drugs (Amphotericin B, itraconazole etc). These drugs have serious limitations due to their cyto-, hepato-, and nephrotoxicity. In view of limitations of these drugs, there is a need to explore novel drug targets based on genes sequences. In this context, a total 250 phage clones were randomly selected from a non-normalized cDNA library of Afu and 175 expressed sequence tags (ESTs) were generated. Out of 175 ESTs, 107 novel ESTs were submitted in GenBank and putative functions were assigned to 83 ESTs. Based on comparative genomic analysis, we observed that the sequences homologous to isocitrate lyase (ICL), malate synthase, transcriptional activator for allantoin, guanylate kinase, thiamine regulated gene, thiamine biosynthesis protein, cobalamine independent methionine synthase, ATP synthase subunit-4, 29 hypothetical proteins and 2 predicted proteins are present in single copy in the Afu genome and these are absent in human genome. Presence of similar sequences in other fungi such as A. nidulans, C. albicans, S. cerevisiae suggests that it may be useful to explore them for their potential as broad spectrum antifungal drug targets. Twenty-four unique ESTs represent Afu specific genes which may be investigated for their role in pathogenesis and specific detection of Afu in clinical samples. A microarray was developed using these ESTs and analysis of differentially expressed Afu genes during conidia-macrophage interaction indicated up-regulation of transcripts of isocitrate lyase, malate synthase, malate dehydrogenase etc. The complete gene sequence of ICL of Afu (Af285) has revealed an open reading frame of 1614 bases (Accession no- AY224491). Its deduced amino acid sequence showed significant homology with the ICL gene of A. nidulans (Identities-84%), N. crassa (74%), S. cerevisiae (57%), C. immitis (81%), M. grisea (76%) and C. albicans (65%) etc. In vitro studies illustrated the ICL transcripts in Afu conidia with 0.2% glucose in the medium and a further increase in the ICL transcript in these conidia after its interaction with murine macrophages. However, an ICL transcript was not observed in Afu conidia (With 2% glucose in the medium) with and without interactions with macrophages under similar conditions. In an invasive pulmonary aspergillosis (IPA) murine model experiment, an ICL transcript was detected on the 2nd and 4th day in Afu obtained from infected lung tissue whereas there was no evidence of ICL transcript on 1st day of infection. These observations suggest that there is depletion of glucose in the hostile host environment and ICL expression is regulated by glucose concentrations. Deletion mutant of ICL has prolonged the survival rate of mice in
candidiasis in a murine model but did not affect the survival rate of mice in cryptococcosis. Up-regulation of ICL gene during host-Afu interaction and absence of its human orthologs, suggest that the inhibitors against ICL may be explored for the treatment concurrently with antifungal drugs.
INVASIVE ASPERGILLOSIS ANIMAL MODELS (IAAM): STANDARDIZED MURINE AND GUINEA PIG MODELS OF INVASIVE PULMONARY ASPERGILLOSIS

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Background
Invasive pulmonary aspergillosis (IPA) is clinically relevant with limited treatment AND DIAGNOSTIC options. Evaluations of diagnostic methodologies and therapeutic options against IPA have been performed in a wide variety of animal models. Recently, extensive investigations into the Aspergillus genome have received great attention; however, animal models have only been used sparingly for genomic evaluation of this disease organism. The variations of animal models and their design make comparison of in vivo studies difficult. Thus, we have addressed this critical need to develop uniform standard model systems to evaluate new diagnostic targets and methodologies.

Methods
A recently awarded contract from the National Institutes of Health/National Institute of Allergy and Infectious Diseases (N01-AI-30041), supports the development of standardized animal models of invasive aspergillosis to evaluate new diagnostic targets and tools to answer key questions in this disease. The IAAM study group is designed to be a resource to provide training and experimental studies for the Aspergillus research community at large. Results: We have developed both mouse and guinea pig models of IPA using two different types of inhalation chambers. Both models present the key features of reproducible pulmonary infection using either a low-cost acrylic chamber (Sheppard, AAC 2004) or a large-scale Madison aerosol chamber, which allows simultaneous challenge of up to 126 mice or 18 guinea pigs, predictable mortality, ease of duplication, and they recapitulate human disease. Simple serial blood sampling is a feature of the guinea pig model that enhances monitoring of surrogate markers of infection. These models serve to encourage the development of diagnostic methodologies and tools against IPA. To date, 15 investigators from academia and industry have initiated collaborative efforts under this contract to study the effects of gene expression or gene deletions, diagnostic methods and vaccines, as they relate to development of new diagnostic targets. Finally, the contract provides for the creation and maintenance of an Aspergillus database, whereby qualified investigators may share details of their strains and experiments. Conclusions: This contract, through these key features, provides the Aspergillus research community with the mechanism for evaluation of genomic targets using standardized animal models of IPA in order to improve the diagnosis and therapy of invasive aspergillosis. These studies are ultimately aimed at improving patient care and reducing mortality in this often fatal disease.
CHARACTERISATION OF PUTATIVE G PROTEIN-COUPLED RECEPTORS IN ASPERGILLUS FUMIGATUS

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The opportunistic human-pathogenic fungus Aspergillus fumigatus was subject to recent studies on cAMP signal transduction with regard to morphogenesis and virulence. To date, one of the most important questions is still unanswered: what are the external signals and the corresponding receptors sensing those ligands or stimuli which activate downstream signal transduction pathways?
In an attempt to close this lack of knowledge we identified 7 trans-membrane proteins (7TMR), a basic feature of G protein-coupled receptors, using a genome-wide approach. We could identify more than 30 putative G protein-coupled receptors with homologies to known pheromone, nutrient, cAMP and PHT11-related receptors.
In a first approach, Saccharomyces cerevisiae glucose receptor-like (S.cerevisiae Gpr1p) genes designated gprC and gprD were deleted in A. fumigatus. We made use of strain delta-akuB, which is deficient in non-homologous end joining and therefore preferentially shows homologous recombination events. Physiological characterisation of the mutants revealed severe defects in filamentation. Under various growth conditions, which included different carbon and nitrogen sources, temperatures and additives like vitamins or pharmacological substances, wild-type growth could not be fully restored. To identify components involved in downstream signal transduction stimulated by the putative receptors, we employed a proteomic approach. Several putative proteins were identified.
EFFICIENT DOWN REGULATION OF ALBI GENE USING AN AMA1- BASED EPISOMAL EXPRESSION OF RNAI CONSTRUCT IN A. FUMIGATUS

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RNAi has been used as a powerful tool in gene silencing at the post-transcriptional level in a wide range of eukaryotic cells. The presence of RNA silencing machinery in filamentous fungi has led to the successful application of RNAi methodology in these organisms. A recent study by Mouyna et al., has shown an efficient down regulation of both essential and nonessential genes in some integrative transformants of A. fumigatus. However, a range of interference has been reported which could be a result of random integration of integrative construct in different positions or rearrangement of construct and loss of transcriptional unit of RNAi during the integration.

In Aspergillus species, AMA1 (Autonomous maintenance in Aspergillus)-based plasmids have been successfully used in episomal expression of different constructs. High frequency of transformation, relatively high copy number of plasmid in the nuclei and easy recovery of plasmids are some advantages of AMA1 containing plasmids.

Here we have examined the efficiency of an AMA1-based episomal RNAi construct in down regulation of Albl gene involved in spore pigmentation in Aspergillus fumigatus. A sense-loop-antisense construct containing a 500 bp fragment of Albl gene in opposite directions with a 100 bp GFP sequence in the middle was prepared and cloned into a plasmid containing an inducible promoter of A. fumigatus and a fungal selection marker. Episomal RNAi vector was prepared by PCR amplification of whole promoter, loop structure and terminator and then cloning into NotI site of AMA1-NotI-pyrG3 plasmid. Transformation of A. fumigatus AF293 pyrG strain with the resulting plasmid led to a number of colonies which showed inactivation of the gene (albino phenotype) in induction condition. The phenotype was stable even after five subcultures in induction medium. Comparison of mRNA level in integrative, episomal and also co-transformants as well as phenotypic analysis will be presented.
DIFFERENCES IN EXTRACELLULAR ENZYMATIC ACTIVITY BETWEEN ASPERGILLUS SPECIES ISOLATES FROM PATIENTS WITH OTOMYCOSIS

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The otomycosis is a mycotic infection that attacks the ear canal and the tympanic membrane. The Aspergillus species are the most frequent causes of otomycosis, especially in the tropical and subtropical regions of the world.

The purpose of our study was to determine enzymatic biotypes by testing enzymes production of the Aspergillus strains isolated from patients with otomycosis and to assess whether this findings can be used for rapid identification of important clinical Aspergillus isolates. We evaluated enzymatic activity of Aspergillus strains (n=94), isolated from external auditory canals from patients with otitis during 2000 to 2004. Strains were stored at the Aspergillus Culture Collection of the Mycology Laboratory, School of Medicine, Belgrade, Serbia and Montenegro. Mycological identification was done by conventional methods, reference to the International Commission on Penicillium and Aspergillus. The enzymatic activities of 19 enzymes were assessed by API ZYM (bioMerieux) method.

Results showed that the all Aspergillus strains (n=94) produced enzymes no2 (phosphatase alcaline), no3 (esterase C4), no6 (leucine arylamidase), no11 (phosphatase acid), no17 (beta-glucosidase) and no18 (N-acetyl-beta-glucosaminidase).

The difference between A. flavus, A. fumigatus and A. niger were observed in production of enzymes no4 (esterase lipase C8), no7 (valin-aryilamidase), no12 (naphtol-AS-BI-phosphohydrolase) and no14 (beta-galactosidase).

The A. flavus strains (n=31) were positive for enzymes production no12 (naphtol-AS-BI-phosphohydrolase), no14 (beta-galactosidase) and negative for enzymes no4 (esterase lipase C8), no7 (valin-aryilamidase). The A. fumigatus strains (n=25) were positive for enzymes production no4 (esterase lipase C8), no7 (valin-aryilamidase), no12 (naphtol-AS-BI-phosphohydrolase) and negative for enzyme no14 (beta-galactosidase).

The A. niger strains (n=38) were positive for enzyme production no4 (esterase lipase C8) and negative for enzymes no7 (valin-aryilamidase), no12 (naphtol-AS-BI-phosphohydrolase), no14 (beta-galactosidase). The results obtained suggest that the differences in enzymatic activity between important clinical Aspergillus isolates could be useful for the identification of species of the genus Aspergillus, for epidemiological purposes and possible for virulence study.
INFLUENCE OF CULTURE FILTRATES OF ASPERGILLUS FUMIGATUS ON DIFFERENT HUMAN CELL LINES PROLIFERATION

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Culture filtrate (CF) of Aspergillus fumigatus obtained under aerobic conditions contains numerous mycotoxines with a variety of effects on mammalian cells and a potent cytotoxic effect on different cell lines. Gliotoxin has been well studied as a candidate for that activity and it was shown that production of gliotoxin depends on well aerated and oxygenated conditions. In contrast, influence of A. fumigatus CF obtained under anaerobic conditions on mammalian cells and human cancer cell lines are not described despite of the fact that inside of aspergilloma, fungus cells grow in the non-aerated conditions.

The aim of the study was to determine the influence of A. fumigatus CF obtained under anaerobic conditions on different human cancer cell lines. A. fumigatus enviromental isolate was grown for 5 days on Sabouraud dextrose agar at 30°C and the conidia were colected and resuspended in MAM culture medium at concentration of 1 X 10⁶/ml. A. fumigatus CF were prepared under oxygen and non-oxygen conditions at 37°C and supernatants were collected after 24 h, 3 and 6 days and tested for prostaglandin E2 concentration by ELISA. WISH, CaCo and DU-145 cell lines were treated with different CF and colorimetric and colonogenic assays was done in order to detect inhibition and stimulation of cell lines proliferation. Data demonstrated inhibition of WISH cell line and inhibition of proliferation of human cancer cell lines CaCo and DU-145 (100%) when treated with CF obtained in aerobic conditions. In contrast, A. fumigatus CF obtained under anaerobic conditions stimulated human cancer cell lines proliferation (50%), without any effect on WISH cell line.

This is the first study that demonstrates that A. fumigatus CF obtained under anaerobic conditions contains secondary metabolites which stimulate proliferation of human cancer cell lines, so the nature of A. fumigatus products obtained under anaerobic conditions needs to be determined.
A. FUMIGATUS-MATURED HUMAN DC EXPRESS CCR7 AND A SPECIFIC PATTERN OF IL-12, IL-23, AND IL-27 CYTOKINES ACQUIRING THE CAPACITY TO INDUCE A TH1 RESPONSE

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Aspergillus fumigatus (A. fumigatus) is the most prevalent airborne fungal pathogen that causes fatal invasive aspergillosis in immunocompromised patients. Given the essential role of dendritic cells (DC) in initiating and regulating immune responses, we investigated the impact of A. fumigatus conidia infection on human DC. The conidia were rapidly internalized by human DC and induced a significant release of TNF-alpha within the first 8 h. Besides its key contribution to anti-Aspergillus innate response, TNF-alpha represents an essential cytokine involved in DC maturation. Indeed, the TNF-alpha present in the supernatants from A. fumigatus infected-DC was able to induce partial maturation of DC characterized by the acquisition of CD83 and the absence of CCR7. Only DC having internalized the conidia undergo full maturation expressing the CCR7, which could give them the capacity to migrate to the lymph nodes to prime naïve CD4 T cells. Additionally, the analysis of regulatory cytokines showed that infected DC produced simultaneously IL-12p70 and significant amounts of IL-10. Interestingly, neither IL-10 neutralization nor CD40 ligand stimulation were able to further increase IL-12p70 production from infected DC. Whereas the central role of IL-12 in the generation of Th1 cells has long been appreciated, recently two other members of the IL-12 family, IL-23 and IL-27, were described to play important roles in the regulation of interferon-gamma production from naïve and memory T cells. A slight increase of IL-27p28 expression was found after A. fumigatus infection. Conversely, A. fumigatus-infected DC were able to induce a robust expression of IL-23p19 mRNA and the release of IL-23 protein. Based on these results, we studied the capacity of A. fumigatus-infected DC to prime a Th1 response. Interestingly, A. fumigatus-matured DC primed naïve T cells for an allogeneic response inducing a clear lymphoproliferation and a Th1 polarization. Since a very low expression of IL-27p28 was detected in A. fumigatus infected-DC, the observed Th1 response could be mainly dependent on the secretion of IL-12p70, which acts at an early stage of naïve Th cell differentiation. Moreover, the significant production of IL-23 could play an important role in the establishment and maintenance of a pool of Th1 memory cells specific for A. fumigatus in healthy individuals. This study on the expression of the new IL-12 family members controlling the Th1 response sheds light on a novel aspect of DC contribution to anti-Aspergillus immunity and might be instrumental to reinforce the new DC-based therapeutic approaches.
CALCINEURIN A IS LINKED TO FILAMENTATION, ANTIFUNGAL SUSCEPTIBILITY, AND VIRULENCE IN *ASPERGILLUS FUMIGATUS*

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The calcineurin pathway has been shown to be related to filamentation, antifungal susceptibility, and virulence in several other fungal pathogens. Calcineurin A (*cnaA*) is the catalytic subunit in the calcineurin pathway. We successfully replaced the *cnaA* gene in *A. fumigatus* 293 (wild-type strain), creating a ∆*cnaA* mutant strain which has a profound decrease in lateral filamentation compared to the wild-type strain. This decrease in filamentation leads to a drastic reduction in growth rate and amount of conidia produced at all temperatures tested. Conidiation appears to occur at a similar time point through aerial filamentation.

Scanning electron microscopy yielded profound differences in both the conidia as well as the hyphae of the ∆*cnaA* mutant versus the wild-type strain. While wild-type *A. fumigatus* conidia are coated in rodlets, the ∆*cnaA* mutant lacks rodlet structures and instead the conidia clump together in liquid culture. The hyphal structures of the ∆*cnaA* mutant are drastically blunted, suggesting a role for the calcineurin pathway in cell wall morphological development that we initially proposed with previously published echinocandin antifungal and calcineurin inhibitor data.

Light microscopy reveals that *in vitro* treatment of germinated wild-type strains with the pharmacologic calcineurin inhibitors (FK506, cyclosporine A) leads to a dose-dependent blunting of hyphal branching, with similar effects seen through caspofungin treatment. A combination of caspofungin + calcineurin inhibitors leads to a synergistic blunting of growing hyphae, with FK506 possessing greater antifungal activity than cyclosporine A as we have already previously described. Treatment of ungerminated wild-type conidia with calcineurin inhibitors results in a profound delay in filamentation (>60 hours vs. 9 hours). Real-time PCR reveals an approximate 2-fold increase in *cnaA* expression during wild-type strain exposure to antifungals, beginning only at the time points after filamentation. These additional data suggest that the calcineurin pathway is critical in filamentation and hyphal growth.

Further antifungal susceptibility testing reveals predicted fungistatic killing with caspofungin against the wild-type strain. However, when caspofungin is used against the ∆*cnaA* strain, the normally fungistatic caspofungin is improved into a fungicidal agent. FK506 has no effect on the ∆*cnaA* strain, likely due to the fact that the target has been genetically removed, however cyclosporine A retains some diminished antifungal activity. The use of calcineurin inhibitors in combination with caspofungin against the wild-type strain gives similar but diminished results to the effects of caspofungin on the ∆*cnaA* mutant, suggesting that genetic removal of the *cnaA* is more powerful than pharmacologic inhibition of the pathway. These data suggest that the calcineurin pathway could be harnessed to help improve killing of *A. fumigatus*. Murine inhalational, murine intranasal, murine intravenous, and wax moth models all
document decreased virulence of the ΔcnaA mutant compared to the wild-type strain. In our inhalational model of persistently immunosuppressed invasive pulmonary aspergillosis there was an 80% difference in mortality (90% wild-type vs. 10% ΔcnaA mutant, p < 0.0001). The reconstituted mutant yielded similar results to the wild-type strain. The intranasal, intravenous, and wax moth models all showed similar trends, confirming in four completely different animal models the importance of cnaA in invasive aspergillosis disease establishment.
Identification of Small Non-Protein-Coding RNAs and Small Peptide RNAs in Aspergillus Fumigatus

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In the past few years, a growing number of small non-protein-coding RNAs (ncRNAs) has been identified in various model organisms from E. coli to mouse (Mus musculus). NcRNAs do not encode proteins, but have important and essential cellular functions either on their own or in complex with proteins that are bound to the RNA. Those functions range from RNA processing, modification, transcriptional regulation, mRNA stability and translation up to protein secretion. Furthermore, these small ncRNAs can serve as targets for therapeutic agents. However, most genome projects neglect the identification of ncRNAs, although the complete range of mRNAs as well as ncRNAs is essential for understanding the function of cells and consequently whole organisms. In addition, identification of mRNAs, encoding small proteins, is difficult to achieve by bioinformatical approaches, only. Hence, the aim of this study is to experimentally identify the content and function of ncRNAs and small mRNAs in the genome of a pathogenic member from the genus of Aspergillus. For this study, we chose the most common pathogenic Aspergillus sp., Aspergillus fumigatus to generate a cDNA library encoding small ncRNAs as well as small peptide RNAs. To obtain RNAs expressed under different growth conditions or developmental stages, mycel from seven different culture conditions was harvested and total RNA was isolated. After size separation (two fractions: 15-70 nt and 71-600 nt, respectively) of total RNA the 3’-ends of RNAs were C-tailed, the cap-structures of small mRNAs removed and a DNA-linker ligated to the 5’-ends. After RT-PCR, cDNAs were ligated into a pGEM-T vector. We are currently carrying out sequence analysis of about 6000 cDNA clones followed by bioinformatical and expression analysis. The function of selected ncRNA/small peptide RNA-candidates will then be elucidated by the construction of corresponding deletion strains.
Signal transduction and stress-response genes of fungal pathogens play important roles for exerting pathogenesis and, in some cases, biosynthesis of mycotoxins. As such, they should serve as potentially viable targets for antifungal compounds. Results of our research, as presented in this poster, show that targeting genes in the mitochondrial respiratory chain pathways, MAPK or vacuolar H(+) -ATPase (V-ATPase) using safe, natural compounds can significantly elevate the sensitivity of fungi to commercial fungicides or antifungal drugs. The use of such compounds can result in lowering effective dosages, costs of treatment and potential for development of resistance.

Our rationale is based on the fact that cellular targets of several conventional antifungal compounds are already known. Examples of these targets include macromolecular synthesis (e.g., nucleic acids, amino acids, cell wall, etc.), cell division, signal transduction and respiration. We theorize that disruption of cellular redox homeostasis using phenolics may inhibit fungal development and invasiveness. Targeting these systems with drugs and additional safe, natural compounds leads to oxidative stress, with a resultant decrease in cell viability. We illustrate the use of this target-based strategy to significantly improve control of Aspergillus fumigatus.

The molecular target for strobilurin-related fungicides, such as azoxystrobin or kresoxim-methyl, is the mitochondrial respiratory bc1 complex. Inhibition of this complex eventually leads to cellular oxidative stress caused by abnormal release of electrons from the respiratory chain. Using deletion mutants, we found at least five phenolic compounds that disrupt the normal function of mitochondrial respiration. Combined treatments of these phenolic agents and commercially available fungicides that are inhibitors of the mitochondrial respiratory chain have a 100 to 1000-fold synergistic fungicidal effect due to disruption of respiration and inhibiting the oxidative stress-response of the fungus. We also found that the sakAdelta (MAPK mutant) strain of Aspergillus fumigatus was much more sensitive to phenolics, indicating sakA likely is involved in regulation of the antioxidative stress response system in A. fumigatus, perhaps involving mitochondrial function/respiration. In addition, we found that the alkaloid berberine targets the activity of oxidative stress genes, and combined treatment of this alkaloid and certain phenolics resulted in > 10,000 times greater fungicidal activity than either compound alone. In addition to directly targeting mitochondrial respiration we also found effective synergistic control targeting the vacuolar H(+)-ATPase (V-ATPase) system by using phenolics as synergists for the V-ATPase inhibitor concanamycin A. We conclude that natural compounds (i.e. phenolics or alkaloids) can be developed as useful antifungal agents when the molecular target is identified. The use of this approach leading to effective control of a broad spectrum of fungal pathogens is discussed.
CHARACTERIZATION OF TWO NONRIBOSOMAL PEPTIDE SYNTHETASES INVOLVED IN SIDEROPHORE BIOSYNTHESIS IN ASPERGILLUS FUMIGATUS

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The ability to acquire iron in vivo is essential for most microbial pathogens. Recently, we have shown that A. fumigatus produces two major siderophores, extracellular Desferri-Triacetylfusarinine C (TAFC) and the intracellular Desferri-Ferricrocin (FC) and that the lack of both siderophores results in absolute loss of virulence in a murine model of invasive aspergillosis.

We found that expression of two A. fumigatus genes encoding nonribosomal peptide synthetases, termed sidC and sidD, is repressed by iron, which indicates a possible involvement in siderophore biosynthesis. To analyze the function of SidC and SidD we constructed respective gene deletion mutants in A. fumigatus ATCC46645, termed sidC and sidD. SidC displays the same modular structure as the ferricrocin peptide synthetase An-SidC and consistently HPLC analysis of culture supernatant and cell extracts revealed that sidC produces TAFC but not FC. In contrast, sidD was able to synthesize FC but not TAFC. Iron starvation reduced the radial growth rate of sidC slightly and that of sidD greatly. Growth of sidD but not sidC was completely blocked by the extracellular iron chelator bathophenantroline disulfonat, which indicates that TAFC plays the major role in siderophore-mediated iron acquisition in A. fumigatus.

sidC displayed decreased production of spores during iron replete and iron-depleted conditions, which could be completely cured by supplementation with FC, which suggests a critical role of the intracellular siderophore in conidiation. Both sidC and sidD showed increased sensitivity to H2O2 during iron depleted conditions demonstrating that siderophore-mediated iron uptake and storage are important for resistance to oxidative stress. Upon infection of a host, A. fumigatus is attacked by reactive oxygen species produced by phagocytic cells. Therefore, siderophores might play not only a role in iron supply and storage but also in resistance against phagocytosis. The characterization of the peptide synthetases involved in TAFC and FC biosynthesis now enables to investigate the contribution of each siderophore to virulence of A. fumigatus.

Austrian Science Foundation Grant FWF-P15959-B07 supported this work.
P051

FUNGAL INFECTION IN SAMPLES OF BRONCHIAL WASHING IN BRONCHOSCOPY DEPARTMENT

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Introduction
Bronchoscopy can help us to take specimen from distal part of lung in order to diagnose deep-seated fungal infection. Diagnosis of deep-seated fungal infection in lung parenchyma is relatively difficult. Therefore the prevalence and prognosis of pulmonary fungal infection has been difficult to evaluate. The objective of this study is to determine the utility of bronchoscopy with bronchoalveolar lavage for diagnosis of fungal infections.

Materials and Methods
This study was a prospective study of patients submitted for routine bronchoscopy (in and outpatients) in Emam Reza University hospital. A total of 110 patients were included. Bronchoscopies by bronchoalveolar lavage (BAL) specimens were performed by standard methods. Specimens were sent to Mycology Laboratory in order to prepare direct smear and culture for isolation of fungus. In the case of lavage, an aliquot also was studied for cellular morphology.

Results
The most frequently isolated fungus was Candida spp. (33.7%) followed by Aspergillus species (18.4%). Malassezia species was isolated only from one patient. In 14.7% of samples direct smear was positive for fungus while in 43 cultured samples (45.3%), fungal colonies were observed. Microscopic study of the stained smear revealed that 68% of patients have significant inflammatory cells in their BAL.

Conclusion
Bronchoscopy with BAL is useful in diagnosing fungal infections. However, Candida albicans is one of the most common fungi that could be isolated from BAL, but Aspergillus spp. is the most suspected fungus causing fungal pulmonary infection. Key words: Fungal infection, pulmonary infection, Bronchoalveolar lavage (BAL), Bronchoscopy, Aspergillus, Candida.
Aspergillus fumigatus is the most common aetiological agent of aspergillosis infection in humans. Invasive aspergillosis is a major cause of death in leukaemia and organ transplant patients. One of the classes of antifungal drugs used in treatment are azoles, such as itraconazole (ITC). They inhibit the enzyme, 14 alpha-sterol demethylase, which acts at the third step of the ergosterol biosynthesis pathway.

Several mechanisms have been described for resistance to azoles. One mechanism is modification of the target enzyme. Another is drug efflux by membrane transporters that results in reduced intracellular drug concentration. Several mutations in the A. fumigatus cyp51A gene have been shown to cause azole resistance. Mutations that confer resistance to drugs may confer a biological fitness cost that is expressed as decreased growth rate, survival or virulence.

Three pairs of susceptible and resistant isogenic strains, from cases where azole resistance was acquired during treatment, were studied in addition to eight isolates obtained serially from a patient with an aspergilloma (chronic cavitary pulmonary aspergillosis)(patient D). MICs of ITC, voriconazole (VRC), ravuconazole (RVC), and posaconazole (POS) were determined. Two measures of growth rate (colony radial growth rate and specific growth rate) have been determined on various media. Finally, sequencing of the cyp51A gene was carried out. Three resistance profiles were observed in these clinical isolates. Resistance to ITC only was observed in the resistant isolate of two of the pairs and cross resistance to ITC and POS was observed in the third resistant paired isolate. A third resistance profile characterized by very high MICs of ITC (> 8.0 mg/l), VRC (8.0 mg/l), RVC (8.0 mg/l) and POS (4.0 mg/l) was observed in the isolates from patient D. Isolates 4 and 8 were more susceptible to POS (MIC of 1-2.0 mg/l).

A previously described mutation was present in the majority of the isolates from patient D and is therefore associated with cross resistance to the four azoles. However, two novel amino acid substitutions in the Cyp51A protein were identified in two of the isolates from patient D. Isolate 4 had a Tyr to Cys amino acid change at codon position 431 and isolate 8 had a Gly to Cys change at codon 434. These two isolates also exhibited reduced fitness, as indicated by reduced growth rate in one and decreased conidiation in the other. Interestingly, microsatellite-length-polymorphism typing indicates that all eight resistant isolates from patient D are identical. To show formally that the codon 431 and 434 changes cause cross resistance in 14 alpha-sterol demethylase, the mutant alleles will be expressed in a heterologous yeast system developed by Meneau et al., 2004 (Antimicrobial Agents Chemother 48 (7): 2610-6). This work is planned.
Conventional diagnosis of life-threatening and invasive aspergillosis (IA) is sometimes unsuccessful due to the high proportion of negative blood cultures and to the atypical features of some isolates. Molecular biology-based detection and identification is therefore a working alternative for rapid detection and concurrent pathogen identification. A LightCycler duplex PCR system was devised for detection and identification of the two most common IA aetiologic agents (sensitivity 93.9% and positive predictive value 96%). Oligonucleotide primers were designed from the ITS1 and flanking regions (DDBJ/EMBL/Gen-Bank database, accession nos.: X78537, A. flavus; M55626, A. fumigatus. The human beta-globin gene was used as internal control. This system detected and identified Aspergillus species in whole blood specimens of a 15-month old female child with ALL, who 30 days after induction chemotherapy was admitted in the ICU with respiratory distress requiring mechanical ventilation and support with inotropic agents. Sputum smears taken on admission were positive for septate hyphae and cultures were positive for Aspergillus flavus. Subsequent bronchoalveolar lavage (BAL) specimens were PCR negative for Pneumocystis jiroveci and culture-positive for A. flavus and A. fumigatus. Chest X-ray revealed consolidation in the right upper lobe (RUL) of the lung. The CT scan showed RUL aspergilloma. Successive serum and BAL galactomannan antigen tests were positive. Blood cultures were negative, but three serial blood PCR assays were initially positive for both A. flavus and A. fumigatus, while later on only A. fumigatus positive. Sequencing of the PCR products showed 100% homology with the published A. flavus and A. fumigatus sequences. Combination antifungal chemotherapy (caspofungin 10mg/d; voriconazole 40mg b.i.d) was administered. Microdilution susceptibility testing (NCCLS M38A) and Etest (AB Biodisk, Solna, Sweden) showed A. flavus and A. fumigatus MICs for caspofungin (0.03 and 0.06 mg/L), voriconazole (0.06 and 0.12 mg/L) posaconazole 0.01 and 0.03 mg/L), itraconazole 0.125 and 0.25 mg/L). Amphotericin B A. flavus MIC was 0.5 mg/L and A. fumigatus 2 mg/L. In two subsequent BAL cultures A. fumigatus was the single isolate. On day 10 the child showed signs of clinical improvement, inotropic agents were discontinued and mechanical ventilation detachment procedures were initiated. Despite antifungal chemotherapy and the apparent clinical improvement, after extensive pulmonary hemorrhage, the child succumbed 12 days after initiation of treatment. Antifungal susceptibility phenotypes for both A. flavus isolates were identical and so were the susceptibility phenotypes of the three A. fumigatus isolates. However, strain similarity was confirmed by amplifying ribosomal intergenic spacer (IGS) region repeat elements and with minisatellite PCR fingerprinting. Band pattern analysis
(Bionumerics, Applied Maths, Belgium) clustered (UPGMA) the A. flavus isolates in a single distinct group and assembled all A. fumigatus isolates in one separate group. The devised LightCycler PCR system successfully detected a mixed population of Aspergillus spp. Incorporation of probes for direct confirmation of the A. fumigatus and A. flavus identities can further improve the proposed system. Multiplex PCR schemes are of particular clinical relevance, may instigate appropriate treatment strategies and can account for the unexpected as every specimen, even from the same patient, may be different.
Utility of multi-locus sequence typing for speciation of isolates in the Aspergillus fumigatus group

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Background

Our recent phylogenetic analyses utilizing sequences from the ß tubulin and rodlet A regions revealed a new species A. lentulus, and two previously established species, A. udagawae and N. pseudofischeri among isolates identified phenotypically as A. fumigatus. These findings bring into focus the limitations of phenotypic methods of speciating Aspergilli. Herein we have investigated the potential of a multi locus sequence typing (MLST) approach using a panel of 7 genes to: (1) discriminate species among A. fumigatus isolates collected from diverse sources and (2) determine the population structure of A. fumigatus Methods. Fifty Aspergilli, all members of the A. fumigatus group were assembled from diverse sources - 36 A. fumigatus clinical isolates from various culture collections (Fungal Testing Laboratory, San Antonio, Texas; Centers for Disease Control, Atlanta; Institut Pasteur, France; Fred Hutchinson Cancer Research Center, Seattle), 4 A. lentulus, 3 N. udagawae, 2 N. pseudofischeri, the type strains of A. fumigatus Fresenius Af293 and N. fischeri and 3 A. fumigatus isolated from soil near Seattle. A panel of seven genes was selected and primers designed to amplify 450 bp-520 bp gene regions that included conserved coding regions flanking 1-2 non-coding regions and are: the ß tubulin [benA; 450bp], rodlet A [rodA; 490bp], calmodulin [cal; 510bp], class III chitin synthase G [chsg; 481bp], glyceraldehyde 3 phosphate dehydrogenase [gdp; 513 bp], catalase [cat; 486 bp] and carboxypeptidase 5 [cxp; 470 bp]. Genomic DNA was harvested from the fungi, subjected to PCR amplification and sequenced. The resultant sequences were aligned using Clustal W and maximum likelihood (ML) trees were generated using PAUP* software with bootstrap values generated from 1000 pseudoreplicates (1). Results. A total of 168, 500 nucleotides of sequences were generated with 3370 nucleotides generated per isolate. A total of 242 polymorphic nucleotides were detected among the seven loci that delineated the fungi into 11 sequence types with the cat-cal-cxp gene regions being the most hypervariable. The ML trees of the single locus and combined data set revealed that the fungi grouped into 4 distinct clades - A. lentulus, N. pseudofischeri, A. fumigatus clade and N. udagawae with high bootstrap values. However, 5 isolates that fell in the A. fumigatus clade in the benA and rodA regions grouped with the A. lentulus clade in all the other five-gene regions. Very few (zero to 5 nucleotides) polymorphic sites were detected between isolates that fell within individuals within the clades Conclusions. 1. A MLST scheme using the cat, cal, cxp and gdp gene regions is proposed for speciation in the A. fumigatus group. The proposed panel of gene regions grouped the isolates into well-resolved clades and was highly amenable to PCR amplification and sequencing. 2. The MLST scheme as proposed was not useful in strain determination among individuals within the A. fumigatus clade.
Reference
P055

IDENTIFICATION OF ASPERGILLUS SPECIES USING PECTIC ZYMOGRAM TECHNIQUE

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One hundred and three isolates showing Aspergillus specifications were obtained from different sources. Based on morphological characters 12 species: A. alliaceus, A. candidus, A. carneus, A. flavus, A. fumigatus, A. niger var. niger, A. niger var. awamori, A. niveus, A. ochraceous, A. sydowii, A. terrus, A. ustus, and A. versicolor were identified. The isolates were subjected to pectic zymogram electrophoresis. Based on the similarity of isozyme electrophoretic phenotypes, 45 zymogram patterns were identified. In the analyses of the electrophoretic pattern, 26 isozyme loci corresponding to the polygalacturonase and pectin esterase were observed. Although there was a considerable intraspecific variation for Aspergillus species, the species were distinguished using this technique and there was no common zymogram pattern among the species.
P056

ANALYSIS OF AMPHOTERICIN B RESISTANCE IN ASPERGILLUS TERREUS

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The majority of documented cases of invasive aspergillosis (IA) are caused by Aspergillus fumigatus (A. fumigatus). However, at our institution (The Innsbruck Medical University, Innsbruck, Austria) Aspergillus terreus (A. terreus) infection is a frequent cause of IA (up to 40%). This kind of infection is highly associated with a fatal outcome under Amphotericin B (AmB) therapy. The reasons for the AmB resistance are yet unknown. Therefore we examined the role of ergosterol content, cell wall modifications, lovastatin and catalase production of A. terreus on AmB resistance.

Ergosterol of A. terreus (n = 33) was isolated from whole cells by saponification with a 25% alcoholic potassium hydroxide solution, followed by extraction of nonsaponifiable lipids with heptane. Ergosterol was identified by its unique spectrophotometric absorbance profile between 230 and 300 nm. A. fumigatus (n = 12) and Scedosporium spp. (n = 2) served as controls. The influence of the cell wall from A. terreus (n = 12) and A. fumigatus (n = 5) on AmB resistance was investigated by cell wall lysis with the enzyme Glucanex (Sigma-Aldrich) and followed by MIC determination with the received protoplasts. The role of lovastatin was investigated by agar diffusion tests (Sabouraud agar, pH 7.0). The catalase activity was measured by hydrogen peroxide degradation. The decrease in absorbance (hydrogen peroxide degradation) was followed at a wavelength of 240 nm for 30 s. The catalase activity was calculated by interpolating the rate of hydrogen peroxide degradation of each test sample on the calibration curve obtained by plotting the hydrogen peroxide degradation against the catalase activity of the calibration samples.

The mean ergosterol content of the A. terreus isolates was 0,50% of the wet weight of the cells (range: 0,10% - 1,2%). A. fumigatus evinced a mean ergosterol content of 0,42% (Range: 0,10% - 1,1%). In comparison to the conidia the protoplasts showed no significant change in the MIC values. Lovastatin showed no depressing effect on AmB susceptibility in vitro. The catalase production in A. terreus was 25% higher than in A. fumigatus.

In conclusion neither the ergosterol content, nor the cell wall and lovastatin had influence on the AmB susceptibility. Catalase production in A. terreus could be one major factor distributing to AmB resistance, further study is necessary.
P057

DIAGNOSTIC VALUE OF PCR-BASED IDENTIFICATION OF ASPERGILLUS IN BLOOD SAMPLES OF PATIENTS AT RISK FOR INVASIVE ASPERGILLOSIS

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Background
Invasive aspergillosis (IA), an important cause of mortality among immunocompromised patients still represent a major diagnostic challenge especially in early stages due to the low sensitivity and technical difficulties associated with the various methods routinely used to identify this condition. Objectives: The aim of the present study was to assess the predictive performance of a PCR-based assay on blood samples as a screening/diagnostic tool in a group of patients at risk for IA, namely allogeneic and autologous hematopoietic stem cell transplant (HSCT) recipients and acute leukemia (AL) patients undergoing intensive chemotherapy.

Methods
Blood samples were obtained weekly from patients with AL and autologous HSCT recipients during periods of neutropenia, and from allogeneic HSCT recipients during 3 months post transplantation. DNA was extracted from a total of 720 whole blood samples collected from 129 patients during a 10 month-period. A nested PCR targeting 18S rRNA Aspergillus-specific sequences was performed on all stored samples in a blinded fashion.

Results
Of 129 patients, 106 were subsequently found not to have IA while the remaining 23 patients were considered to have definite (4), probable (12), or possible (7) IA according to the revised European Organization for Research and Treatment of Cancer/Mycoses Study Group classification (CID, 2002, 34:7). Positive PCR results were found in 22 of 106 (21%) patients with no IA, in 5 of 7 (71%) patients with possible IA, in 11 of 12 (92%) patients with probable IA and in 3 of 4 (75%) patients with definite IA. In 15 of 23 (65%) patients the PCR-positive blood sample preceded clinical diagnosis. The overall sensitivity, specificity, positive predictive value and negative predictive value of the PCR assay for the diagnosis of IA in all levels of certainty were 83%, 79%, 46%, 95% respectively.

Conclusion
This study demonstrates that PCR-based screening for aspergillus in blood of patients at high risk for IA is a useful adjunct to clinical and standard microbiological diagnostic approaches. Positive results do not necessarily indicate infection (positive predictive value is 46%) but warrant further investigation, while serial negative results essentially rule out IA (negative predictive value of 95%). PCR-based diagnosis decrease the time to diagnosis and increase the rate of detection.
P058

HIGH-RESOLUTION MOLECULAR FINGERPRINTING OF ASPERGILLUS FUMIGATUS FROM A SUSPECTED OUTBREAK IN THE INTENSIVE CARE UNIT

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Introduction
In October 2005, a possible outbreak of Aspergillus fumigatus among intensive care unit (ICU) patients was investigated using a high resolution molecular fingerprinting assay.

Methods
A total of 28 A. fumigatus isolates (mainly from respiratory samples) from 4 different patients and 4 environmental isolates were studied. All isolates were analyzed using the STRAf (Short Tandem Repeats of Aspergillus fumigatus) assay. This is an exact typing method for A. fumigatus, which yield a genotype of up to 9 different markers that can easily be compared to each other.

Results
All but one of the intrapatient isolates were of the same genotype, all interpatient isolates belonged to different genotypes. The isolates collected from the environment were all unique; two samples proved to be a mixture of two or more strains. Two out of 4 patients had probable aspergillosis and were treated with systemic antifungal (itraconazole and caspofungin) at autopsy invasive aspergillosis was diagnosed.

Conclusion
Since all environmental isolates were of a different genotype, they most likely do not fully represent the actual variation of strains present in the ICU which may be much larger. To have an accurate reflection of the environmental strains, multiple isolates will have to be of the same genotype. Therefore, it was of no surprise that we didn’t uncover any relation (if present) between the patient isolates and the few environmental isolates collected in the same period. Molecular epidemiology was of little refuse in this outbreak. We conclude that we were dealing with nosocomial aspergillosis during construction work, but to be able to prove such relationships, continuous monitoring of the endogenous population will be mandatory.
DO RECOMBINANT ANTIGENS HAVE A FUTURE AS DIAGNOSTIC MARKERS FOR INVASIVE ASPERGILLOSIS?

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Recombinant proteins of Aspergillus fumigatus were tested by ELISA to quantify the anti-Aspergillus antibodies in sera of patients with aspergilloma and invasive aspergillosis (IA). In spite of the variability observed in the immune responses of individual patients, quantification of the antibody titers against the 18-kDa ribonuclease, the 360-kDa catalase and the 88 kDa dipeptidylpeptidaseV were useful for the diagnosis of aspergilloma. In the group of immunocompromised patients with IA, no antibody response was mounted in response to the Aspergillus infection in any of the patients. Interestingly, about half of the patients with proven IA came to the hospital with high titers of anti-Aspergillus antibodies, suggesting that they were infected upon entry to the hospital. These results suggest that recombinant ribonuclease, catalase and dipeptidylpeptidaseV have a great potential in the serodiagnosis of aspergillosis in the immunocompromised and immunocompetent patient.
Guttural pouch mycosis in horses is an upper respiratory tract infection. This unfamiliar fungal disease was first described by Rivolta in 1868 and appears to have been contracted by stabled horses, usually during the warmer season of the year. Until now, though there have been so many reports on the clinical findings and therapy of the guttural pouch mycosis, the paucity of reports on biological and epidemiological studies of the causative agents prompted us to investigate it. In this presentation, we report on the mycological and ecological studies of the Emericella nidulans (E. nidulans) seized with guttural pouch mycosis in horses. It was carried out on the data concerning the distribution of the causative agents, and the biological characteristics such as enzyme protease activities of casein and bovine haemoglobin substrates under pH 4 to 9, the requirement of urea as the nitrogen source, the property of temperature and relative humidity dependence to grow up, the survival activities of fungal spores under several environmental conditions, and the fungicidal effects by disinfectants and UV irradiation.

The causative agent of guttural pouch mycosis was predominantly identified as E. nidulans (teleomorph of Aspergillus nidulans). The followings were, however, also detected as the rare clinical cases: Aspergillus fumigatus, Absidia corymbifera and Myceliophthora thermophila. E. nidulans was generally distributed in straw bedding and hay feeds by the mycological investigation.

It had a character of the high activity of protease both substrates, apparent urea property, beta-haemolysis positive, thermophile to grew up to 53 centigrade, long-term survival in stable environments, susceptibility against disinfectants such as alcohol and halogen compounds, and UV resistance to become non-viable cells under conditions of 100-150 milliwatt second per square centimeter.

As the results, the guttural pouch mycosis caused by E. nidulans in Japan was usually propagated in the horses. As the mycological and ecological studies of pathogenic E. nidulans were short of, it has been definitely shown the relation of the fungal infection in point of the incidence, biological characteristics, distribution, survival and susceptibility of disinfectants.
In this study,a total of 50 patients sera and 55 samples from healthy individuals were obtained. These patients were hospitalizined in Maseeh_e Daneshvari Hospita, Tehran, Iran. From the 50 patients,18(36%)were female and 32(64%)were males. According to the duration of hospitalization and resistance to anti tuberculosis drugs, patients were divided into 2 groups(Recurrent tuberculosis& new Tuberculosis).

In our study,by using Eliza technique,the levels of antiaspergillus IgG and IgM & anti-candida IgG in sera of the patients were investigated. Direct microscopic examinations and sputum cultures were done only in patients group. Culture and serological examinations results of the sputum and serum with statistical analysis were as follow: 1-Significant differences were observed between the means of IgG & IgM levels , in respect to sera of patients and normal controls(P<0.05).

This differences were significant between patients with positive sputum cultures and patients with negative cultures ,as well. 2-The most frequency of isolated fungi were as follows: Candida albicans (36%),Aspergillus fumigatus(28%),Aspergillus SPP(26%),Aspergillus niger(22%).Negative culture results were obtained from sputum only in 22 percent. 3- This study, showed that double diffusion technique is not a sensitive test,because by this test, positive results in patients were revealed only in 3 of 50 patients.

It is concluded that the patients with pulmonary tuberculosis are susceptible to aspergillosis, and for this reason, these patients should be checked for opportunistic mycoses during treatment against tuberculosis.
P062

EVALUATION OF THE PRESENCE OF SPECIFIC IGG AGAINST ASPERGILLUS FUMIGATUS AND THERMOPHILIC ACTINOMYCETS ANTIGENS IN SERA OF SILO WORKERS

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Saprophytic fungi such as Aspergillus are predominant in the environment and in the concentration of the fungal conidia in food and feed stuff (silos). Farmer’s lung disease is a hypersensitivity pneumonia that is characterized by fever and dispenia caused by respiration of organic dust and moldy grass or grains containing Aspergillus and Thermophilic actinomycets.

In this study, a total of 60 silo workers were sampled for their sera on Mashhad city in Iran, and 60 non-silo workers were sampled for their sera as the control group. Standard Aspergillus and Thermophilic actinomycets antigens (Immy Co.) were used in Counter Electrophoresis method (CIE).

The results of CIE showed that 11 (18.4%) of silo workers’ sera and 2 (3.4%) of the control group were positive for Aspergillus antigens, whereas 16 (27%) of the sera of the silo workers and 5 (8.4%) of the control group were positive for Thermophilic actinomycets antigens. With regard to the positive results, significant differences were observed between the experimental and control groups at p<0.05.
CIRCULATING GLUCAN COMPARED WITH GALACTOFURANOSE-ANTIGENS FOR THE EARLY DIAGNOSIS OF INVASIVE ASPERGILLOSIS

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Background
Several recent studies have compared the beta-1,3-Glucan (BG) assay with the Platelia Aspergillus (PA) ELISA for diagnosis of Invasive Aspergillosis (IA). However, no study has been published in which the kinetics of BG and Galactofuranose (Galf)-antigens are compared. We retrospectively analyzed prospectively collected consecutive serum samples from patients with probable or proven IA.

Methods
170 serum samples were collected from 10 patients with IA, i.e. 5 patients with proven and 5 patients with probable IA based on the EORTC/MSG consensus definitions. These serum samples included series that had consequently negative galf-antigen tests and series that show conversion from negative to positive circulating antigen. All samples were tested in duplicate with the Fungitell BG assay (Associates of Cape Cod) and results were compared with the galf-antigen assay (PA ELISA, BioRad).

Results
Results were compared using a cut-off of 1.0 ng/ml galactomannan (GM) for the PA ELISA and a cut-off of 60 pg/ml BG for the Fungitell assay. Circulating BG was detected on days -13, 0, +2, +4 and +32 compared with circulating galf-antigens in the 5 patients with proven IA. In 4 patients with probable IA, circulating BG was detected on days -6, -11, 0 and +4 compared with circulating galf-antigens. In one patient with probable IA and persistent negative PA ELISA serum reactivity, the Fungitell assay also showed no reactivity.

Conclusions
Circulating BG was detected in 3 of 10 patients earlier than (Galf)-antigens, but later in 4 of 10. This variability might imply that monitoring of both markers simultaneously is required in high-risk patients.
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**Background**
The fungal component 1,3-beta-D-glucan (BG) is increasingly used to diagnose invasive aspergillosis (IA) and other fungal infections in immunocompromised patients. We observed reactivity in serum samples of 2 hematology patients during treatment with intravenous amoxicillin-clavulanic acid (AMC). Samples were negative once treatment had been discontinued. Neither patient had evidence for invasive fungal disease. We aimed to find the cause for this false reactivity.

**Methods**
Using the recently FDA approved BG assay (Fungitell, Associates of Cape Cod), we tested 10 serum samples from 6 hematology patients without evidence for invasive fungal disease that were treated with intravenous AMC. Furthermore, the AMC batches used for treating these patients were also tested for BG reactivity. In addition, the serum of 2 patients was tested before and after completing i.v. administration of AMC. The results were compared with BG reactivity in sera from patients treated with ceftazidime and healthy blood donors.

**Results**
BG was detected in 9 out of 10 serum samples (cutoff value 60 pg/ml). The level of mean reactivity (1339 ± 1798 pg/ml) was significantly higher than found in serum of 10 patients treated with ceftazidime (17.7 ± 26.5 pg/ml) (P=0.002) and healthy blood donors (8.0 ± 13.8 pg/ml) (P=0.001). The serum of two patients tested before i.v. administration of AMC was negative but levels of 805 and 446 pg/ml, respectively, were detected after completing the infusion. Ten batches of AMC infusion fluid used during this period were found positive for BG (9414 ± 7774 pg/g antibiotic) as opposed to 4 batches of ceftazidime (10 ± 21 pg/g antibiotic) (P=0.004). A single dose of 1000/200 mg amoxicillin-clavulanic acid contained 2,856 to 28,016 pg of BG. The serum of patients treated with AMC also contained significantly higher levels of galactofuranose-antigens (Platelia Aspergillus ELISA, BioRad) compared with those of ceftazidime treated patients and healthy blood donors (P=0.003 and P=0.009, respectively).

**Conclusions**
These results are highly suggestive of cross-reactivity of the BG assay with AMC. Physicians should be aware of the possibility of false positive BG in patients treated with this antibacterial agent. The presence of two different fungal components in AMC strongly supports a fungal origin. Given the difficulties encountered in diagnosing invasive fungal disease, it would be desirable to eliminate the fungal material from antibiotic agents.
P065

DETECTION OF GLUCAN IN CEREBROSPINAL FLUID SAMPLES FROM PATIENTS WITH PROVEN CEREBRAL ASPERGILLOSIS

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Background
Detection of the fungal cell wall component beta-1,3-Glucan (BG) is increasingly used for diagnosis of invasive mycoses in immunocompromised patients. Only a few studies have been published that compare the performance of the recently FDA approved Fungitell BG assay (Associates of Cape Cod) with the Platelia Aspergillus (PA) ELISA (BioRad) in sera of patients with Invasive Aspergillosis (IA). In addition to sera, we retrospectively analyzed consecutive CSF samples from patients with proven cerebral IA for BG and galf-antigen levels.

Methods
23 CSF samples were collected from 3 patients with proven cerebral IA. All samples were tested in duplicate with the Fungitell BG assay (Associates of Cape Cod) and results were compared with the galf-antigen assay (PA ELISA, BioRad).

Results
BG and galf-antigens were detected in all 23 CSF samples. BG levels ranged from 370 pg/ml to more than 67,000 pg/ml while galf-antigen levels ranged from 1 to more than 200 ng/ml. In one of the patients (16 CSF samples), galf-antigen level started high but decreased to the cut-off value (1 ng/ml galactomannan) later in time. The BG level also decreased during time of infection but was still about 1000 pg/ml, which is 50 times the cut-off value in serum.

Conclusions
In addition to serum samples, BG can be detected in CSF samples. The BG level did not always correspond with the galf-antigen level. Monitoring of both markers in CSF might be of value in the diagnosis of cerebral mycoses.
P066

DEVELOPMENT OF A NOVEL AND STANDARDISED REAL-TIME PCR ASSAY FOR THE DETECTION OF ASPERGILLUS SPP. IN IMMUNOCOMPROMISED PATIENTS

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Background

Invasive aspergillosis (IA) is a leading cause of morbidity and mortality in the growing immunocompromised patient population which comprises transplant recipients, individuals with hematological malignancies and a range of diseases arising from decreased immune function. The mortality rate is extremely high in these neutropenic patient groupings with reports as high as 90%. This is partly attributable to the lack of standardised molecular tests for the early diagnosis and optimal therapeutic intervention by clinicians to manage disease. Real time polymerase chain reaction (PCR)-based methodologies for the detection of circulating genomic DNA from Aspergillus spp is a promising technology for the diagnosis of invasive aspergillosis since the method can be both extremely specific and detect circulating pathogen nucleic acids with a high degree of sensitivity. However, until now no standardised PCR diagnostic assay was commercially available.

Method

Five ml EDTA blood was collected from BMT and leukaemia patients (n = 33) weekly. In total, 145 specimens were analysed for the presence of circulating Aspergillus genomic DNA. Nucleic acid was prepared by an initial erythrocyte lysis step and subsequent lymphocyte enrichment by employing the HighPure Nucleic Acid Kit (Roche, Germany). An internal control (IC) was introduced at the lysis step of the sample preparation to control for extraction efficiency and the presence of inhibitors. Aspergillus DNA and IC were amplified and detected in parallel using the affigene aspergillus tracer assay (Sangtec Molecular Diagnostics, Sweden) on the MX3000P instrument (Stratagene, USA) according to the Manufacturer’s instructions. The real time-PCR assay was standardised according to the EC IVD directive. At the time of blood collection, patients were clinically assessed for symptoms of aspergillosis.

Results

Of the 33 patients, two were diagnosed as having a proven or probable aspergillosis according to the EORTC criteria. These two patients also showed a positive result when employing the affigene aspergillus tracer kit. None of the other patient samples were found to be positive for Aspergillus using this real-time PCR method.

Conclusion

The affigene aspergillus tracer standardised real-time PCR assay serves as a valuable new molecular tool, in combination with defined EORTC criteria for assessment of clinical symptoms, for the diagnosis of aspergillosis in immunocompromised patients.
ASPERGILLUS GALACTOMANNAN DETECTION IN HAEMATOLOGICAL PATIENTS IN CORDOBA (SPAIN)

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Introduction and objectives
Invasive aspergillosis has become an important death cause among immunodepressed patients, particularly haematological ones. One of the reasons for this is the absence of an early diagnose. In the last few years, a new diagnostic tool is being used, Aspergillus galactomannan antigen enzyme immunoassay detection. Our aim in this work was to study the characteristics of the haematological patients who had positive galactomannan determinations in serum.

Material and methods
Between July 2002 and April 2005, 1366 galactomannan antigen determinations in serum were made in our service to 278 patients; among them, 1167 determinations (156 patients) were from haematological patients. These determinations were performed using Platelia Aspergillus (BioRad, Marnes-la-Coquette, France). Two weekly determinations were performed while patients remained in hospital and a weekly determination in revisions as they were discharged.
We retrospectively studied the evolution and characteristics of those patients who presented two or more consecutive positive galactomannan determinations (defining 0.5 as positive cut off), considering their haematological disease, kind of transplantation if they received it, antibiotic and antifungal treatment and invasive aspergillosis degree according to EORTC/MSG criteria.

Results
Twelve haematological patients presented two or more consecutive positive galactomannan determinations in serum. Among them, eight patients fulfilled EORTC criteria for probable invasive pulmonary aspergillosis, three for a possible one and one of them did not fulfil any criteria or presented clinical symptoms, being considered a false positive. In general, positive determinations coincided in time with the apparition of respiratory symptoms or radiological signs.
Previous disease: six patients presented acute myeloblastic leukaemia (one of them as a myelodysplastic syndrome after a Hodgkin lymphoma), two acute lymphoblastic leukaemia, one bilineal leukaemia (M/LproB), one Burkitt lymphoma, one peripheral T-cell lymphoma and a diffuse large B-cell non Hodgkin lymphoma.
Transplantation: three patients received no transplantation, four received a Stem cells autotransplantation, two received an HLA identical allotransplantation, two umbilical cord transplantation and one reduced intensity conditioning allotransplantation.
Antimicrobial treatment: Due to participation in a study, all the patients were receiving...
antifungal prophylaxis before the positive galactomannan determinations, eleven of them with voriconazole and one with fluconazole. All the patients received antibiotics in the previous month to the positive galactomannan determinations, either as prophylaxis or for treating a bacterial infection.

**Conclusions**
As in previous studies, we have to point out the low apparition of falsepositives, which is due among other reasons to the fact that we study haematological patients, whom the detection is specially indicated for. Serial galactomannan determination in haematological patients has significantly improved their control despite receiving antifungal prophylaxis.
DIFFERENTIAL GENE EXPRESSION IN ASPERGILLUS FUMIGATUS MUTANTS WITH REDUCED SUSCEPTIBILITY TO ECHINOCANDINS

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The echinocandins caspofungin acetate (CAS) and micafungin (MF) belong to a new class of antifungal agents that inhibit the activity of beta-1,3-D-glucan synthase (GS), thus damaging the fungal cell wall. Although no clinical resistance of Aspergillus to CAS or MF has been reported as yet, the development of in vitro reduced susceptibility is presumed to be inevitable. To study the potential for clinical resistance in Aspergillus, two classes of Aspergillus fumigatus mutant strains were previously isolated in our laboratory that exhibited reduced susceptibility to MF and/or CAS. In the first class, a site-directed mutation within the target gene (AfFKS1, encoding the putative catalytic subunit of GS) was introduced and shown to confer low-level (16-fold) reduced susceptibility to both CAS and MF. By contrast, a second class of spontaneous mutants displayed reduced susceptibility to CAS in a dose-dependent manner, yet retained sensitivity to MF. Neither target site mutations, nor changes in target gene expression were found to be present in these strains. Microarray analysis is being carried out with the mutant strains S678Y (class 1) and RG101 (class 2) to investigate gene expression patterns in response to CAS or MF. A subset of differentially expressed genes identified by preliminary microarray results that may be involved in the reduced susceptibility phenotypes of these strains have also been analysed by real-time RT-PCR. The putative genes differentially down-regulated in strain S678Y (in comparison to the wild type strain) in the presence of MF encode a GPI protein, a G-protein membrane sensor, and a golgi transport protein. These genes may be involved in a cell wall integrity pathway which is not activated in the S678Y strain in response to MF, due to the reduced susceptibility of the strain to the drug. Genes differentially up-regulated in the strain RG101 (in comparison to the wild type strain) in response to CAS include a putative ABC transporter and a gene which may be involved in cell cycle regulation. It is purported that the putative ABC transporter may be involved in the reduced susceptibility of strain RG101 to CAS, possibly by hindering access of the drug to the GS target. Further investigation is currently being carried out to further characterize the molecular mechanisms underlying the reduced susceptibilities of these strains to echinocandins.
INTERACTION OF PROPOFOL WITH ANTIFUNGAL AGENTS AGAINST ASPERGILLUS FUMIGATUS AND ASPERGILLUS FLAVUS

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Aspergillus is a growing agent of disseminated fungal infections, particularly among neutropenic and transplant patients admitted in intensive care units. Propofol, administered in a lipid emulsion, is a widely used anaesthetic and sedative agent in critical care patients. The interaction between propofol and the antifungal drugs may change the susceptibility pattern of Aspergillus species.

Materials and methods
Clinical isolates of A. fumigatus (3 strains) and A. flavus (4 strains) were incubated with serial dilutions of two different propofol 1% infusions (®Lipuro and ®Fresenius) in combination with antifungal agents amphotericin B (AMB), itraconazole (ITC), voriconazole (VRC) and posaconazole (PSC), according to the checkerboard dilution method (1). Concentration of the antifungal agents ranged between 0.03 and 16 µg/ml. Propofol dilutions 1/8, 1/4 and 1/2 were tested. After 48 hours at 37ºC, the minimum fungicidal concentration (MFC) was evaluated by culturing 20 µl of each dilution in Sabouraud agar plates (2). The number of colony forming units was determined following 24 hours at 37ºC. All tests were run in duplicate. Data were compared at a significant level of 0.05.

Results
MFC values were lower than 1 µg/ml for all tested strains and antifungals, being exception A. fumigatus strain F09 (4 µg/ml for VRC), A. fumigatus strain F13 (2 µg/ml for ITC and VRC) and A. flavus strain F04 (2 µg/ml for VRC). Both propofol infusions affected significantly (p<0.05) the susceptibility pattern of all tested strains. Almost all strains became resistant (MFC>16 µg/ml) testing propofol and ITC simultaneously. The presence of propofol increased, in a dose-dependent manner, within 1 to 6 dilutions the MFC of both Aspergillus species to AMB, VRC and PSC. ®Fresenius was associated to higher MFC values comparing with ®Lipuro (p<0.05) in both Aspergillus species.

Conclusions
Propofol is a widely used anaesthetic that, administered as a lipid emulsion, may affect seriously the susceptibility pattern of Aspergillus species to antifungal agents changing, in some cases, completely the profile from susceptible to resistant strains. Drug interactions should be fully evaluated and later taken into account when establishing antifungal therapeutic regimens, particularly in patients receiving multiple medications. The diffusion of antifungal agents in different lipid layers should be evaluated in further studies.
References
COMPARISON BETWEEN CLSI M38-A AND E-TEST METHOD FOR EVALUATION OF THE SUSCEPTIBILITY PROFILE OF ASPERGILLUS SPECIES TO POSACONAZOLE

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The susceptibility profile of Aspergillus strains to antifungal agents is an extremely valuable tool to get the correct answer in the clinical treatment. Different in vitro methods have been evaluated in the last years trying to find a good correlation with the clinical trials, in a short period of time. Posaconazole is a recent and promising antifungal agent presenting very good activity in vitro against Aspergillus species. Our objective was to compare the susceptibility profile of different Aspergillus species by CLSI M38-A protocol and E-test method after 24 and 48 hours.

Materials and methods
A set of 44 isolates of A. fumigatus (14 strains), A. flavus (10 strains), A. niger (10 strains) and A. terreus (10 strains) were studied. The susceptibility profile was evaluated by CLSI M38-A protocol and by E-test method following 24 and 48 hours at 37ºC. The percentage of agreement was defined by the percentage of similar MIC values from both methods (one dilution lower or higher were considered similar). All tests were run in duplicate.

Results
According to CLSI method, the minimal inhibitory concentration (MIC) values for posaconazole were lower or equal to 0.5 µg/ml for all tested Aspergillus. Testing E-test method resulted higher MIC values comparing with M38-A protocol (4 strains of A. niger presented a MIC of 2 µg/ml, following 48 hours of incubation). The percentage of agreement between CLSI method and E-test was 75% after 24 hours and 50% after 48 hours for all Aspergillus species. A. fumigatus and A. flavus presented higher agreement values after 24 hours (79% and 80% respectively) comparing with A. niger and A. terreus (70%). However, the agreement between CLSI method and E-test read after 48 hours was extremely low for A. niger (30%), A. terreus (40%) and A. fumigatus (50%), E-test presenting higher values.

Conclusions
It was not found any resistant strain to posaconazole in this study. Following the CLSI M38-A protocol, posaconazole presented low MIC values for all Aspergillus species. E-test results read after 24 hours showed a better correlation with CLSI M38-A results, than after 48 hours. Further comparison with clinical trials is needed for a better evaluation of both methods used for susceptibility testing.
AZOLE RESISTANCE IN ASPERGILLUS NIGER

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Background
Aspergillosis is on the increase, and is now the most common invasive infection caused by filamentous fungi. Furthermore, antifungal drug resistance is a significant clinical problem in some patient settings, as more patients require prolonged antifungal therapy. Itraconazole resistance in Aspergillus fumigatus is well recognised, although incidence varies in the literature. Data for non-fumigatus species however, is limited.

Objectives
To investigate Aspergillus niger azole resistance in a clinical culture collection. To retest isolates with previously high minimum inhibitory concentrations (MICs) by the most recent susceptibility testing method. To calculate the rate of A. niger azole resistance in the collection.

Methods
The collection contains 39 clinical isolates of which 27 were susceptible on first testing and not retested. 12 A. niger isolates which had elevated MICs to any azole upon isolation were retested for confirmation; from ear (6), respiratory (2), skin (2), mitral value (1) and 1 unknown site. Specimens were subcultured onto Sabouraud glucose agar for 48 hours. Susceptibilities were determined by modified NCCLS M38-A method, using RPMI supplemented with 2% glucose. Isolates were tested against itraconazole, voriconazole, posaconazole, ravuconazole and amphotericin B. Inocula were counted by haemocytometer, and adjusted to give to a final concentration of 5 x 10^4 cfu/mL. ATCC 6258 C. krusei was used as control organism. MICs were read by eye, with a no growth end point at 48 hours. A putative resistant breakpoint of >4mg/L was used. Minimum fungicidal concentrations were determined for all drugs.

Results
31% (12/39) A. niger isolates in the clinical culture collection showed azole resistance. All 12 isolates were resistant to itraconazole, and 5 to ravuconazole, by the current method. One isolate was cross-resistant to all 4 azole drugs. For the purposes of statistical analysis, values of >8mg/L were classed as 16. Geometric means (and range; mg/L) for itraconazole, voriconazole, posaconazole, ravuconazole and amphotericin B MICs were 16.00 (>8), 2.00 (1 - >8), 0.31 (0.125 - >8), 3.00 (1 - >8) and 0.22 (0.06 - 0.5) respectively; those for MFCs were 16.00 (>8), 2.38 (1 - >8), 0.40 (0.125 - >8), 4.24 (2 - >8) and 0.45 (0.125 - 1) respectively.
Conclusions
The rate of A. niger azole resistance in the clinical culture collection was high. Mechanisms of resistance have yet to be determined.

P072

COMPARISON OF TISSUE BURDEN ASSESSMENT BY GALACTOMANNAN EIA AND QUANTITATIVE CULTURE IN A GUINEA PIG MODEL OF INVASIVE PULMONARY ASPERGILLOSIS

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Background
The ability to accurately assess fungal tissue burden in animal models of invasive pulmonary aspergillosis (IPA) is a controversial topic. This led us to examine two different evaluation methodologies for their ability to detect fungal burden in guinea pigs (GP). Methods: Three groups of immunosuppressed GP (n=78) were challenged with nebulized suspensions of Aspergillus fumigatus (AF293) in either an acrylic inhalation chamber (10^9 CFU/ml) or a Madison inhalation chamber (10^10 CFU/ml) for 1 h. Fungal burden was determined for lung, brain, liver, kidney and spleen using standard quantitative culture (CFU) and galactomannan EIA (GM) at selected time points over 8 or 9 d. Selected samples from the acrylic chamber studies were subjected to histological examination. Results: In the acrylic chamber, a 1 log10 drop in lung tissue burden was seen in CFU from d 4 to 8, however, the GM index showed an approximate 1 log10 increase in fungal burden during this time. Additionally, median fungal burden in extra-pulmonary tissues, (brain, liver, kidney and spleen) dropped from 2.5 to <1 (log10) as assessed by CFU. Assessment by GM index revealed persistently elevated GM levels for these same tissues over the 5 day time course as compared to the 1 h threshold level. Similar results were noted in the Madison chamber study: a decrease in lung tissue burden was seen in CFU from challenge to d 9, while the lung GM index showed GM levels increasing from challenge though days 5 to 9. Median fungal burden in the kidney dropped from 2.5 (log10) at d 3 to undetectable levels by d 9 as assessed by CFU. Assessment by GM index revealed steadily elevated GM levels for the kidneys from 7 to 9 d as compared to the 1 h threshold level. Histological examination of lung tissue at d 0 revealed conidia in the alveoli, with no conidia seen in kidney tissue at this time. Histology by d 5 revealed the extensive presence of hyphal tissue invasion in the lungs but with no organisms detected in the kidney.

Conclusions
Assessment of Aspergillus tissue burden by CFU alone may lead to an underestimation of fungal burden in IPA, particularly in the lungs but also in extra-pulmonary tissues. Utilization of multiple tissue burden quantitation methods may provide more precise assessment of fungal load, and may lead to improved assessment of diagnostic and therapeutic strategies in IPA.
DETECTION OF ASPERGILLUS GALACTOMANNAN ASSAY IN HAEMATOLOGICAL AND IMMUNOCOMPROMISED PATIENTS: A SINGLE CENTER EXPERIENCE

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Invasive aspergillosis (IA) is an important cause of mortality in transplant patients (pz) and in immunocompromised subjects. Clinical and microbiological diagnosis is difficult and often therapy is initiated empirically. The detection of Aspergillus galactomannan (GM) has been a major advance for managing patients at risk for invasive aspergillosis, but the test performance has shown significant variations in recent studies.

Objective
To evaluate the potential for false negative and false positive results of Aspergillus enzyme immunoassay for the diagnosis of IA in patients at risk in our Hospital.

Study population and Method
In this retrospective study, the patients included were 89: 33 with haematological malignancies, 26 undergoing an allogeneic hematopoietic stem cell transplantation (allo-SCT), 15 with solid organ transplantation (kidney 7 pz, liver 6 pz, liver and kidney 1 pz), 5 with solid cancer, 4 affected by chronic obstructive pulmonary disease, 3 HIV positive and 3 from Intensive Care Unit. 841 samples were examined for the detection of Aspergillus galactomannan antigen by sandwich enzyme-like immunoadsorbent assay (Bio-Rad Laboratories): 789 sera, 41 bronchoalveolar lavage fluids (BAL), 4 liquor, 4 nasopharyngeal washes, 3 pulmonary biopsies. (mean 9,4 samples/patients; range, 2-63). An index cutoff > 1 was considered to be a positive result; < 0,7 negative. Patients were defined as having proven IA (6), probable IA (9), possible IA (6), or no IA (68) according to the EORTC/Mycoses Study Group criteria.

Results
The galactomannan antigenemia was negative in 717 (90,9%) sera, borderline in 28 (3,5%), positive in 44 (5,6%). Sensitivity for detection of proven IA was 50% (3/6), probable IA was 89% (8/9), possible IA-IFI 83% (5/6). In one of allo-SCT patients, the positive galactomannan antigenemia was detected prior to the onset of clinical symptoms/radiological signs. The specificity was 88,8%. In 6 patients with false-positive results, 3 were receiving piperacillin-tazobactam and 3 had associated autoimmune phenomenon (chronic graft-versus-host-disease). In one patient with pulmonary zygomycosis, all the tests were negative. Utilizing an index cutoff of 0,5, sensitivity increased to 83% (5/6), and 100% (9/9) in patients with proven and probable IA, respectively, while it was the same (83%) in possible IA, but with a loss of specificity that falls to 74%. 6 (5 BAL and 1
biopsy) specimens other than serum, were positives, of these, 5 (4 BAL and 1 biopsy) were true positive and 1 (BAL) was false positive due to piperacillin/tazobactam therapy. The 4 nasopharyngeal washes were negative, 2 of these, were collected in a patient with probable IA during positive antigenemia.

**Conclusion**

The preliminary results in our experience are that Aspergillus galactomannan assays improves ability to diagnose selected cases, sometimes earlier, but by itself is not going to revolutionize diagnosis and it can’t exclude disease in patients at high risk. The indications for testing are: monitoring during immunosuppression; diagnosis of suspected IA; follow-up of positive specimens. In our patients the performance of antigen detection in samples other than serum specimens, is very interesting and could be the aim of well designed perspective studies.
PROSPECTIVE MOLECULAR AND SEROLOGICAL MONITORING FOR EARLY INVASIVE ASPERGILLUS (IA) INFECTION IN HIGH-RISK IMMUNOSUPPRESSED PATIENTS WITH HEMATOLOGIC MALIGNANCIES OR HEMATOPOIETIC STEM-CELL TRANSPLANTATION -A TERTIARY MEDICAL CENTER STUDY IN SINGAPORE

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Diagnosis of *Aspergillus* poses a serious and difficult challenge, as clinical presentation of the patients infected with *Aspergillus* varies and are non-specific. In Singapore, a tropical, year-round high-humidity country, it is not clear whether *Aspergillus* is a major threat to highly immunocompromised patients presented with neutropenic fever under this environment in our tertiary medical center. To test the effectiveness of the *Aspergillus* screening at the onset of admission or neutropenic fever in high-risk patients, we initiated an IRB-approved study to correlate clinical presentation with two validated *Aspergillus* tests – an in-house real-time PCR DNA-based assay using the LightCycler system (Roche, USA) and a commercially available galactomannan assay (Platelia *Aspergillus*; BioRad, USA). This is a prospective molecular and serological monitoring for early invasive *Aspergillus* (IA) Infection in high-risk immunosuppressed patients with hematologic malignancies or hematopoietic stem-cell transplantation. 48 consecutive patients with hematological malignancies [17 acute myeloid leukemia (AML), 13 Lymphoma, 6 myelodysplastic syndrome (MDS), 6 multiple myeloma, 2 acute lymphoblastic leukemia (ALL), 3 chronic myeloid leukemia (CML), 1 other] admitted for transplant or neutropenic fever were recruited and a total of 134 blood samples were collected, with informed consent, for weekly testing. Among them, 18 patients had autologous, and 13, allogeneic transplants. Clinical parameters were collected independently and correlation with *Aspergillus* tests was performed at the conclusion of the study. The median length of hospitalization was 26 days; mean duration of neutropenia was 7.39 days (range: 0-57 days); causes for neutropenia were chemotherapy (n=22), stem cell transplant (n=28). All patients were treated with broad spectrum antibiotics empirically at the time of admission. Only two patients were treated with anti-aspergillus drugs at the time when blood samples were taken. The infection sites included lung (n=9), bacteremia (n=9), urinary (n=4), GI (n=3) and multiple sites (n=10). However, no organism was identified in 34 patients. 41 patients were discharged without complications and 3 expired (disease progression and sepsis, 2 AML, 1 CNS-NHL with HIV). There were no compelling clinical, pathological (positive culture), or subsequent follow up data to suggest clinical fungal infection in these patients. In this representative high-risk patient population, DNA PCR and galactomannan assay screens performed in 44 and 29 patients respectively were all negative. This study suggests our high risk patients were not negatively impacted by the high humidity environment and patients presenting for transplant or with neutropenic fever have a low probability of fungal infection as the aetiology for their fever episodes.
BASOPHIL ACTIVATION TEST: A NOVEL TOOL FOR THE DIAGNOSIS AND FOLLOW UP OF ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS—PRELIMINARY RESULTS

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Allergic Bronchopulmonary Aspergillosis (ABPA) has detrimental effects in Cystic Fibrosis (CF) patients, by deteriorating the lung function and worsening the disease prognosis. The diagnosis of ABPA is based on a combination of clinical, radiological, and immunological criteria (2003 consensus). However, a considerable number of patients only partly fulfil the aforementioned criteria, which would make one eligible for immunoregulatory treatment. Therefore, in these cases, the use of an additional marker would be useful in solving the diagnostic dilemma. Moreover, a laboratory test is needed for the monitoring of the disease severity and, hence, the adjustment of corticosteroid treatment. The Basophil Activation Test (BAT) is the assessment of basophil activation by flow cytometry, after their in vitro stimulation with certain allergens, using CD63 and CD203 surface markers as activation indexes.

The Aim
of this study is to test the value of BAT for the diagnosis and follow up of ABPA in CF patients.

Materials And Methods
Using A. fumigatus extract as allergens, along with negative (PBS solution) and positive (anti-IgE) controls, BAT was applied for a total of 14 CF patients (age: 4-20 years, median: 12y): (a) 10 CF patients with definite ABPA diagnosis, 7 of them already receiving corticosteroids, and (b) 4 CF patients with probable ABPA diagnosis, who partly fulfilled the diagnostic criteria. In all the patients serum total IgE and specific IgE for A. fumigatus were determined by fluoro-enzyme-immunoassay (FEIA-ImmunoCap) and Aspergillus specific IgG by ELISA. Skin prick tests with A. fumigatus extract were performed in all 14 patients.

Results-discussion
Whereas BAT was negative in healthy controls, positive BAT to A. fumigatus extract was a constant finding in all CF patients of this study, with a tendency towards higher activation rates in patients with elevated serum total and specific IgE. However, the fact that total serum IgE levels did not always correlate with BAT results may be attributed to possible allergic reaction to other fungi.

Conclusion
Though in need for further standardization, BAT seems to be a promising complementary test for the diagnosis, early treatment and monitoring of ABPA.
Aspergillus spp are ubiquitous saprophytic fungi that cause a variety of diseases, ranging from hypersensitivity reactions to flu-like pneumonia and life-threatening invasive aspergillosis. As the lung is the primary site of initial infection with airborne conidia, we investigated the innate immune responses of bronchial epithelial cells against different forms of Aspergillus.

Human bronchial epithelial cells were treated with equal numbers of killed spores or germ tubes of either Aspergillus fumigatus or Aspergillus flavus. Analysis by real-time PCR showed that inflammatory cytokines such as IL-8 and IL-6 as well as the proinflammatory protease, caspase-5 were strongly upregulated by both treatments in a dose-dependent manner. Consistently, germ tubes induced a stronger response than spores. TNF-alpha and beta-2-defensin were induced by high a concentration of germ tubes, but not by spores. Taken together, our results show that germ tubes of Aspergillus fumigatus and flavus are potent inducers of innate immune responses in human airway cells. Considering the presence of Aspergillus spores in the air, differentiation between transient spore contact and invasion, as represented by germ tube formation, is important in order to determine proper immunological response. Moreover, these results can also provide additional data in understanding pathophysiology of hypersensitivity reactions due to the aspergilli.
COlonization of Cystic Fibrosis patients with Aspergillus Fumigatus is a Recurrent Phenomenon

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Introduction
Aspergillus fumigatus strains often colonize the respiratory tract of Cystic Fibrosis (CF) patients. Previous low discriminatory molecular typing assays suggested the majority of sequential cultured isolates to be of the same genotype. We used a novel high-resolution fingerprinting assay to analyze multiple A. fumigatus strains from CF patients.

Methods
We collected A. fumigatus strains from nine patients. From 6 patients each, two isolates were collected with a one year interval. From 3 patients, isolates were collected over a period of 3 to 4 years (3, 16 and 13 isolates respectively). All strains were analyzed using the STRAf (Short Tandem Repeats of A. fumigatus) assay.

Results
From 6 patients all intrapatient isolates were of different genotypes. One patient with two isolates was colonized by the same strain over a period of one year. From the patient with 16 isolates, 13 different genotypes were found; two types were isolated more than once within a 5 months period. The patient with 13 isolates harbored four unique isolates and 3 clusters of 3 isolates were from the same type and succeeded each other during the last year.

Conclusion
Over a long period of time, different genotypes of A. fumigatus were found in most of the examined CF-patients. If the same genotype was found more than once, this only occurred in a short time period. Airway colonization of CF patients with A. fumigatus is appear to be a recurrent event. To substantiate this further more isolates from more CF patients should be analyzed.
MATURATION OF ANTIBODY RESPONSE TO NANOGRAM QUANTITIES OF A. FUMIGATUS ALLERGEN

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Proteins originated from different allergens penetrate mucosal surfaces at extremely low concentrations. However, they are recognized by the immune system and can induce IgE production leading to hypersensitivity to the allergen. The maturity of this response is not known. The purpose of this work was to estimate minimal doses of allergen recognized by the immune system and compare affinity of IgG and IgE antibodies induced by immunization with high or low doses of allergens. A major allergen Asp f 2 from A. fumigatus fungi was used to immunize BALB/c mice in the range from 100 µg to 1 pg per mouse. Asp f 2 was injected s.c. 10 times every day in PBS. Specific IgG was found in mice immunized with 50 ng/mouse and higher. Memory response was detected 2 months after immunization without drops in the antibody titer. Asp f 2 specific IgE was found at the dilution 1:20 in all groups immunized with 50 ng/mouse and higher.

To estimate affinity of IgG and IgE antibodies produced during immunization with nanogram quantities of allergens, we have compared dissociation constants (Kd) of antibodies produced in mice immunized with 500 ng of Asp f 2 (total dose) in PBS and in mice immunized with a standard single s.c. immunization of mice with 100 µg of Asp f 2 in CFA. Kd were estimated by inhibitory ELISA. Immunization of mice with high dose of Asp f 2 in CFA induced high affinity IgG (Kd=10 nM) 10 days after immunization. This affinity was the same when measured 2 months later. No IgE was found in this group. In mice immunized with nanogram quantities of Asp f 2 in PBS IgG had low affinity (Kd>10 uM) 7 days after the last injection, but it was increasing steadily during the next month (Kd=200; 100; and 30 nM at weeks 2, 4, and 5, accordingly). High affinity antibodies were still registered 2 months after immunization.

The estimation of IgE affinity has shown that IgE antibodies maturated with the same rate. At week 2, the affinity of IgE was equal to the affinity of IgG (Kd=200 nM) and it increased during the month to the same level (Kd=45 nM) as of IgG. IgE production steadily subsided and no Asp f 2 specific IgE was found at week 7 after immunization. These results demonstrate that i) immune system can recognize effectively nanogram quantities of foreign antigens; ii) immunization with antigen in soluble form is extremely effective when it is injected several times to be displayed to the immune system; iii) immune response to nanogram quantities of Asp f 2 is long-lasting; iv) immunization with soluble antigen induces both IgG and IgE; v) the affinity of IgG and IgE increases steadily in mice immunized with soluble antigen in nanogram quantities while it increases sharply in mice immunized with high dose antigen in CFA; vi) however, finally the affinity of IgG is comparable between groups; vii) the affinity of IgG and IgE is the same.
P080

OPTIMIZATION THE TREATMENT OF PATIENTS WITH INTERMITTENT ALLERGIC RHINITIS TO POLLEN AND ATOPIC BRONCHIAL ASTHMA WITH FUNGAL SENSITIZATION

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The purpose of our study was to estimate the efficacy of complex treatment including standard therapy and immunomodulation in patients with various allergic pathologies. For that purpose we examined 40 children with atopic bronchial asthma with fungal sensitization and 30 adults with pollen allergy. All patients were randomized into experimental and placebo control subgroups. We used various methods: allergological (skin prick test, nasal and bronchial provocation test), laboratory (detection of total and specific IgE), immunological (detection of CD3+, CD4+, CD8+, CD16+, CD20+, HLADR+, Th1, Th2 using Flow Cytometry, IgA, IgM, IgG, in Manchini gel) and clinical examination in all patients for times per year during treatment. In each control subgroup we used traditional therapy plus placebo.

During initial examination we found out deviations in parameters of immune system that are typical for concomitant secondary immunodeficiency. There was reduction of CD3+ (48,6±1,8%), CD4+ (36,38±2,1%), markers of late activation of lymphocytes HLA-DR+ were increased. There was a reduction in quantity of IgA (1,02±0,21g/l) IgG (10,05±0,36g/l). Significant increase of total IgE (804,38±171,36 ng/ml) and allergen specific IgE (8,1±1,34 ng/ml), the amount of circulating immune complexes was increased as well (95,75±2,53).

In the first group we treated 20 patients with inhaled glucocorticosteroid - Budesonid 250 mg/kg per day plus immunomodulator - Polyoxidonium per day in rectal suppositories during two weeks each year. After the treatment the attacks of bronchospasm in children were decreased up to 25-35 %. The results of spirometry and peakflowmetry optimized up to 10 - 20%. During immunological examination after treatment we found out normalization of following parameters: CD3+ (72±1,3) immunoregulatory index (1,9±0,2), reduction of total IgE (96±0,1), increase content of IgA (1,7±0,2), reduction in number of circulatory immune complexes (63±0,3).

In the second group we treated 15 patients with 5 component allergen specific immunotherapy during two-year courses and immunomodulator- Polyoxidonium 6 mg per day in rectal suppositories during two weeks each year. After even the firs course of SIT the in the period of pollination we revealed decreasing of symptoms, exacerbations started two weeks later comparing to placebo control group and were not so severe, after the second year of treatment in major part of patients no exacerbations were revealed at all. In those patients who developed exacerbations they were less then in previous year. During immunological examination after treatment normalization of following parameters were found out: immunoregulatory index (1,8±0,1), reduction of common IgE (150,1±31,2), increase content of IgA (1,9±0,2), reduction in number of circulatory immune complexes (101,7±5,3), quantity of CD3+ was normalized up to 19.1%
(66.8±1.5), CD4+ up to 7.2 % (38.6±1.1), CD8+ up to 5.3% (25.4±1.2). Clinical efficacy of allergen specific immunotherapy was 3.1±0.23 points. Statistic analysis of the obtained data reveled that the efficacy of treatment of aspergilla atopic bronchial asthma and pollen allergy optimizes by adding immunomodulator - Polyoxidonium to the traditional therapy.
P081

ISOLATION AND IDENTIFICATION OF SOME INDOOR DUST FUNGI AND THEIR EFFECT ON THE RESPIRATORY SYSTEM

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The study identified 28 fungal genera isolated from house dust samples collected from different areas in both sides of Mosul city during October 2003 and February 2004. 39 species were identified out of the above genera. Candida and related yeast were in abundance (48.71 %) followed by Aspergillus (9.7 %) of which 9 species. (A. fumigatus; A. flavus; A. clavato-nanicus; A. candidus; A. ustus; A. terreus; A. tamaril; A. oryzae and A. niger and 3 telemorph Emericella (2.2 %); Eurotium (0.7 %) and Neosartorya (3.7 %) were identified, among these the species A. niger was the most common (54.5 %), followed by A. flavus (19.1 %). Rhizopus stolonifer was isolated (8.27 %) followed by Cladosporum spp. (6.34 %) which had two species Cl. cladosporioides, Cl. herbarum. Nine species of Penicillium were identified P. aurantiogriseum (6.5 %); P. camembertii (6.5 %); P. chrysogenum (26.0 %); P. citrinum (6.5 %); P. crustosum (10.9 %); P. decumbens (8.7 %); P. expansum (24.0 %); P. purpurogenum (6.5 %) and P. requefiorii (4.4 %) and the total isolates were 46 (3.28 %).
The total Colony Forming Units (C.F.U.) in October 2003 (5.48x10⁴ /gm dust) while in February 2004 they were (8.54x10⁴ /gm dust). As regards locations of samples the C.U.F. were more in house 1.72 X 10⁴ while on kindergartens (1.69 x 10⁴); primary schools (1.41 x 10⁴); intermediate schools (1.44 x 10⁴) and in secondary schools (1.1 x 10⁴).

Antigens were prepared for, A. niger; Candida sp.; CL. herbarum; Fusarium sp.; Mucor sp.; P. chrysogenum and St. herbarum and were tested for a 100 blood samples taken from patients with Respiratory system and skin allergy using agglutination test.
The result showed that for A. niger 40 positive reactions, for Candida sp. 45; CL. herbarum 36; Fusarium sp. 23; Mucor sp. 27; P. chrysogenum 34 and St. herbarum 21, and female were more susceptible than males 135 and 91 respectively.
Also the age-group 20-29 years showed the most positive results 58 from 226 while age-group (1-9 years) and (70-79) showed the least positive results (7 and 5) respectively.
Fungi were the cause of 32.28 % of the respiratory and skin allergies. 50 % of the 26 A. flavus isolates were found to be aflatoxins producing.
Background
Patients of bronchial asthma who fulfill the diagnostic criteria for Allergic Bronchopulmonary Aspergillosis (ABPA) but lacks central bronchiectasis either by bronchography, computerized tomography and / or conventional linear tomography are termed as ABPA-S (ABPA-Serological positive), and others with classical presentation of disease and with central bronchiectasis as ABPA-CB (ABPA with central bronchiectasis). There are many patients who apart from central bronchiectasis have many other radiological features (ORF) like pulmonary fibrosis, bullae, bleb, pneumothorax, etc. and hence these groups of patients should be termed as ABPA-CB-ORF (ABPA with central bronchiectasis and other radiological features).

Method
Sixty patients of ABPA were evaluated. Twenty patients were in each group (Group A : ABPA-S, Group B : ABPA-CB, Group C : ABPA-CB-ORF). These patients were evaluated radiologically, serologically and clinically.

Result
Mean duration of illness in each group was 15 years. The severity of disease was more in ABPA-CB-ORF group with severe obstruction in 70%, whereas in ABPA-CB it was in 25%. 5% of ABPA-S groups were having severe obstruction. There was past history of antitubercular treatment in 70% and 30% in ABPA-CB-ORF and ABPA-CB group respectively. Absolute eosinophil count was raised in each group but was maximum in Group - C. Mean total IgE was 4435 IU, 3071 IU, 797 IU in ABPA-CB-ORF, ABPA-CB and ABPA-S respectively. Specific IgE against A. fumigatus was raised in each group and was maximum in ABPA-CB-ORF (61.9 IU) as compared to ABPA-CB (52.14 IU) and and ABPA-S (10.88 IU). All the parameters were maximum in ABPA-CB-ORF stating it as the most severe form of the disease. Radiological features of ABPA-CB-ORF apart from central bronchiectasis were pulmonary fibrosis, bleb, bulla, paranchymal scaring, multiple cyst, fibrocavitary lesions, etc. The details of the other radiological features will be presented.

Conclusion
ABPA-CB-ORF is most severe form of disease having pulmonary fibrosis, bleb, bulla, paranchymal scaring, cysts other than central bronchiectasis.
Background
Fungal allergens are known to be involved in IgE-mediated allergic diseases and to share cross-reactive structures. Allergic bronchopulmonary aspergillosis (ABPA) is the most severe atopic disease resulting from hypersensitivity to Aspergillus fumigatus (A. fumigatus) proteins which are, however, structurally largely unknown. We aimed to clone and characterise the whole A. fumigatus allergen repertoire in a high-throughput screening program of cDNA libraries displayed on phage surface.

Methods
Inserts from enriched clones displaying potential allergens were subcloned into high level expression vectors, sequenced, and expressed as in Escherichia coli. The resulting recombinant proteins were purified and analyzed by SDS-PAGE, Western blot, inhibition ELISA and skin test. The allergen-specific IgE binding capacity of sera from sensitized patients was determined with an antigen-specific ELISA.

Results
Screening of A. fumigatus cDNA libraries displayed on phage surface yielded an unexpected large number of clones displaying potential IgE-binding proteins. Among these manganese superoxide dismutase (MnSOD), cyclophilins and thioredoxins represent families of phylogenetically conserved pan allergens. Here we show that two cyclophilins (Asp f 11 and Asp f 27) and two thioredoxins (Asp f 28 and Asp f 29) cross-react with the corresponding structures of Malassezia sympodialis (M. sympodialis), a yeast involved in the pathogenesis of atopic eczema, as well as with the corresponding human self antigens. The prevalence of sensitization to the two structures among 40 patients suffering from ABPA is in the range of 70 % thus classifying these structures as major allergens. Moreover, A. fumigatus cyclophilins and thioredoxins show an extended cross-reactivity with the homologous human proteins as demonstrated by their capability to elicit IgE-mediated hypersensitivity reactions in skin tests.

Conclusion
Cyclophilins and thioredoxins represent pan-allergen families spanning phylogenetically conserved structures and show cross-reactivity with the corresponding human proteins both, in vitro and in vivo. Auto-reactivity to human proteins sharing sequence homology with environmental allergens represents an emerging phenomenon, which might explain the perpetuation of clinically severe atopic disorders.

*Work supported by the Swiss National Science Foundation
Airborne moulds are known to cause health hazards such as allergic rhinitis, asthma, extrinsic allergic alveolitis and organic dust toxic syndrome. An extensive survey of the airborne moulds in 94 different poultry houses in Tamil Nadu, India was made using an Andersen-2-stage air sampler. Plates with 2% Malt Extract Agar (MEA) were used for exposure in the air sampler.

A total of 84 species belonging to 37 genera were from cage house atmosphere. Excepting 8 species of Zygomycota and one of Ascomycota all the remaining 75 species belonged to mitosporic fungi. 59 species belonging to 29 genera were recorded from litter house atmosphere, which include 9 species of Zygomycota and the remaining to mitosporic fungi. The Aspergillus spp. was found to be most abundant (97.59 CFU/m³) in the air of cage houses and they represented 32.37% and 120.96 CFU/m³ contributing 43.42% from the air of litter houses to the total CFU respectively. Among the different species of Aspergillus, A. flavus was found to be dominant representing 12.05% and 22.37% from cage houses and litter houses respectively. This is followed by Cladosporium cladosporioides (18.21%), A. niger (8.6%), Scopulariopsis brevicaulis (7.75%), Penicillium citrinum (7.38%) and P. oxalicum (4.82%) in cage houses; and C. cladosporioides (15.27%), A. niger (9.24%), S. brevicaulis (6.42%), C. sphaerospermum (4.93%), A. ochraceus (4.41%) and Drechslera australiensis (4.09%) in litter houses. A total of 50 species belonging to 21 genera were isolated from the feed samples wherein the genus Aspergillus contributed to the maximum (79.57%). A. flavus (45.39%) contributed the highest followed by A. niger (26.37%) and Rhizopus stolonifer (6.96%) respectively to the total CFU. S. brevicaulis contributed 31.85% among the 41 species belonging to 22 genera isolated from the bedding material of the litter houses and 43.01% among the 36 species belonging to 21 genera isolated from the excretal samples from the cage houses.

Exposure to mycotoxins via inhalation of spores and spore-sized fragments of mycelia leads to occupational health hazards. The toxigenic potential of A. flavus (Aflatoxin) is investigated since its predominance in the air mycoflora and in the feed samples.
P085

CLINICAL AND EPIDEMIOLOGICAL CHARACTERISTICS OF ABPA IN GREEK PATIENTS WITH CYSTIC FIBROSIS FOR THE PERIOD 1990 - 2005


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Introduction
Allergic Bronchopulmonary Aspergillosis (ABPA) is a hypersensitivity reaction to Aspergillus fumigatus that colonizes the bronchial tree. It insults patients with cystic fibrosis (2-15%) and bronchial asthma (1-2%). Clinical, radiological, hematological and immunological criteria contribute to the diagnosis (Rosenberg). PURPOSE: Our purpose was the recording of the annual incidence of ABPA to the specific population that is being followed in our department, for the period 1990-2005 and its correlation with age, pulmonary function (FEV1), pulmonary infiltrations, infection with pseudomonas aeruginosa, allergy, meconium ileus and place of residence.

Material-methods
Twenty-four patients, who were evaluated by the same group of doctors, were diagnosed with ABPA during the period 1990-2005, based on specific criteria. 7/24 (29%) of the children were girls. The mean age of presentation of ABPA was 10,2 years. All CF patients were followed annually with IgE, RAST (specific IgE) for Aspergillus and eosinophilia.

Results
15/24 (62%) children were diagnosed during the last four years of our study (2001-2005). 5/24 of the children were aged <6 years old, 8/24 between 6-10 years old, 6/24 between 11-15 years old, 3/24 between 16-20 years old and 2/24 >21 years old. The pulmonary function (FEV1), 1 year before the diagnosis, in 4/18 (22,2%) of the patients was >100% whereas in 12/18 patients (66,7%) it was 71-100% and in 2/18 (11,1%) it was 40-70%. 8/24 children had pulmonary infiltrations on chest X-ray examination and to 50% of them remain permanent damages. 22/24 of the children were colonized with Pseudomonas aeruginosa before the diagnosis of ABPA. Allergies were detected to 7/13 children that were screened for allergies during the period 2000-2005. All of the children had pancreatic insufficiency except one and 4 of the children had a history of meconium ileus. Two of the children with meconium ileus had also documented allergy to cow’s milk (positive RAST), and presented ABPA within a year and eight years after, respectively (coincidence or predisposition). 4/ 24 children lived at a rural area, while the others resided in cities CONCLUSIONS: There is an important bulging of the disease during the last 4 years, especially during the last year. Despite the current opinion and data, five of our patients were aged below 6 years. Most of the patients exhibited very good lung function. The infection of pseudomonas aeruginosa must definitely correlate to the precipitation of ABPA. More than 50% of the patients had allergy (mainly to other fungi).
A STUDY OF THE HYPERSENSITIVITY REACTIONS TO ASPERGILLUS FUMIGATUS ANTIGENS IN PATIENTS WITH ASTHMA

Samaneh Eidi

In this study, 50 asthmatic patients and 50 healthy ones (control group) were enrolled. Levels of total serum IgE, anti-Aspergillus specific IgE and IgG, and also anti-Candida albicans specific IgE and IgG were determined by ELISA technique. There were significant differences between experimental and control groups in respect to means of total serum IgE, and anti-Aspergillus specific IgE and IgG levels. The difference in means of anti-Candida albicans specific IgE levels was statistically significant (P<0.05). The results of variance analysis test indicated a direct correlation between the severity of skin reaction and the titers of anti-Aspergillus and Candida albicans specific IgE levels. In conclusion, our patients showed a significant response, in the forms of an immediate hypersensitivity reaction to fungal allergen, especially those caused by Aspergillus.
Problem of cleanliness and contamination of air is known from earlier ages cultures and
civilization. At the present there are no scientific discipline which not interested in the
airborne investigations. The indoor air and walls contamination of fungi at the different
Neonatal Departments in Bialystok and Lublin were evaluated. The studies were carried
out at the two Neonatal Departments in Bialystok and Lublin. Materials for tests were :
the air samples (in front of the building and the selected rooms) and swabs from the walls.
The air pollution was determined using SAS SUPER 100 (Pbi International). The microbial
flora from walls was assessed using the Count-Tact applicator and the plate Count-Tact
(BioMerieux). Fungi were identified using standard microbial procedures. Mean number
of fungi colonies in the air of rooms of tested departments were following: 193.3 ± 87.3
in Bialystok and Lublin 230 ± 79.7, respectively. Fungi type isolated from the air of the
tested departments were Candida albicans, Penicillium species, Acremonium species,
Aspergillus species, Epicoccum species, and Rhodotorula species. Mean number of fungi
colonies isolated from the walls of rooms of the tested departments were following: 19.3 ±
29.9 in Bialystok and 25.3± 37.03 in Lublin, respectively. Fungi type isolated from the walls
of the tested departments were Candida albicans, Candida species, Penicillium species,
Acremonium species, Aspergillus species, Epicoccum species, Rhodotorula species, and
Cladosporium species The air and walls from the Neonatal Department in Lublin showed a
higher number of fungi colonies compared with Bialystok. The air of Neonatal Department
in Lublin was characterized with larger variety of mycological flora. Almost twice higher
number of the fungi colonies in air outside the hospital building in Lublin in comparison
to Bialystok was determined. Significant differences in occurrence the mycological flora
(in relation to the fungus type and its quantity) in dependence of the place isolation as well
as location of the department were noted. The air outside hospital building in Lublin was
characterized with higher number of fungi colonies than in Bialystok.
Carrots displayed for sale in five markets in Benin City, Nigeria were investigated for their Aspergilli load. The carrots were cut lengthwise and chopped into 5mm diameter pieces. The pieces were divided into three batches; one batch was unwashed, the second washed in distilled water and the third washed in distilled water and left in water to which vinegar had been added, for 15 minutes. Plates of Potato Dextrose Agar were inoculated in replicates with three pieces of carrots from each batch, and the plates were incubated at 28 degrees Celsius for 5 days. At the end of incubation, the culture plates were examined under the x40 power of an optical microscope for Aspergillus. Results revealed 3 species of Aspergillus - A. tamarri, A. niger and A. flavus. Aspergillus tamarri was found in all 5 market samples (100%), A. niger (100%) and A. flavus (60%). Each treatment had over 60% Mean Area Infected. The effectiveness of the 3 treatments on the Aspergilli load was in the order: Vinegar + distilled water > Distilled only water > unwashed. These findings indicate a high level of spore load in the air around the area where the markets are situated, a vital factor in human respiratory tract diseases. Also, carrots should be thoroughly washed before consumption; leaving them for some minutes in water to which vinegar has been added is recommended.
ASPERGILLUS-RELATED DEPRECIATION IN NUTRITIVE VALUE OF STORED COTTON (GOSSYPIUM HIRSUTUM L.) SEEDS IN BENIN CITY, NIGERIA

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Due to its high contents of starch, protein and oil, cotton seed recovered by the removal of the lint in the processing of cotton, is a popular soup ingredient in Nigeria. The fungi present in cotton seeds obtained from the open market and stored at ambient temperature were investigated for their effect on the starch, protein and oil contents. Seeds were surface-sterilized in dilute (1:9) 5.75% sodium hypochlorite solution and rinsed in several changes of sterile distilled water after which they were plated out on Potato Dextrose Agar and incubated at room temperature for six weeks, while observing cultures for fungal growth. Uninoculated plates served as control. Fungi were isolated weekly and identified. In a separate experiment, cotton seeds were inoculated with the isolated fungi. Starch component of seeds was extracted with 80% ethanol, protein with 10% trichloroacetic acid and oil with petroleum spirit, following standard procedures. Three fungi - Rhizopus sp, Aspergillus tamarri and Candida sp were isolated consistently from the seeds. Starch, protein and oil loss in the seeds increased significantly with length of storage, reaching peaks of 93, 37.1 and 66.2%, respectively. There was no significant difference between nutrient loss in cotton seeds inoculated separately with A. tamarri and the three fungi combined. This work has demonstrated that A. tamarri plays a vital role in the loss of starch, protein and oil in cotton seeds during storage. Apart from seed deterioration, toxins might also be produced by the fungus, which could adversely affect man on consumption of such seeds. It is recommended that cotton seeds be protected from this fungus; storing the seeds at lower temperatures will likely reduce the activity of A. tamarri as well as those of the other fungi isolated in this investigation.
ASPERGILLUS NIGER, THE MAJOR DOMINANT FUNGAL SPECIES ASSOCIATED DRY TEA IN SHIRAZ, IRAN

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Introduction
At this time, tea consider as a most popular beverage around the world, especially in Iran. The saprophyte fungi almost could contaminate the tea, during tea processing including: Cultivation, Plucking, Withering, Packing and Storing. Well climate conditions are favorable for fungal development and Mycotoxins production. Hospitalized patients have high risk of fungal contamination with tea-born fungi during tea and food serving. Dry tea could be considered as a source of fungal spore in hospitals and allergic clinics.

Materials & Methods
Fifty six commercial brands of tea including: 41 black tea, 3 green tea and 12 tea bags were purchased from the local markets in shiraz. Up to 280 tea samples were cultivated on Czapex dox agar, Sabouraud dextrose agar and broth, supplemented with antibiotics and incubated in 25 C for five days. Fungal isolates were identified by traditional methods. The load of spore contamination of each samples were evaluated by counting the number of colony forming unite per one gram of dry tea.

Results
92.69 Percent of black tea, 91.66 percent of tea bags and 100 percent of green tea were associated with different saprophyte species. Most of the samples had mixed contamination. The fungal species were isolated from tea samples belong to genera Aspergillus, penicillium and mucorales. Aspergillus niger was the most predominant contaminant in tea samples. The highest quantitative mycological examination of tea for spore loads were reveal 200, 50 and 400 spore per gram dry tea in black, green and tea bags, respectively.

Conclusion
The tea bags presented a higher fungal contamination than the regular dry black and green tea, maybe it makes from broken leaves and stems of low quality tea. The level of fungal contamination allows an evaluation of food quality and care must be taken for control of final product quality and storage condition.
ANTIFUNGAL ACTIVITY OF ALLELOPATHIC ASTERACEOUS SPECIES ON GROWTH OF ASPERGILLUS SPECIES

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Effect of aqueous root and shoot extracts of three allelopathic plants of family Asteraceae viz. Helianthus annuus, Ageratum conyzoides and Cirsium arvense on growth of three pathogenic Aspergilli namely Aspergillus niger, A. fumigatus & A. nidulans was studied in Hagem’s liquid nutrient medium. Root extract of H. annuus suppressed the growth of A. niger and A. fumigatus very effectively; A. conyzoides reduced the growth of A. fumigatus to a certain extent. Aqueous extract of C. arvense were least effective against all the test fungal species. A. nidulans exhibited complete resistance against all allelopathic extract tested.
Iran produces 20% of the world date exports, much of that fruit is either damaged or of insufficient quality for human consumption. 35% of the total crops are wastes. This portion of the crop that currently goes to waste can be turned into useful byproducts. Because of the high cost of culture media in Iranan labs, one use of date waste can be as a microbial culture media.

In this project, various solidified date syrups were used as the culture media for some of the date’s natural microflora. The results showed that date syrup is an ideal media for enriching Aspergillus and Mucor growth after extraction by ultrasound waves. This media can also be used as a selective media for these microorganisms because it has inhibitors for some bacteria and fungi. Therefore it is an economical use of extracted date syrup to make enrichment and selective media.
Aspergillosis is primarily a respiratory disease caused by any number of Aspergillus genus. The fungi under suitable conditions can be growth and its spres distributed in environment. The suscepible persons infected by respiratory system. Aflatoxins are highly toxic and carcinogenic mycotoxins produced by Aspergillus spp. some foods are vulnerable to fungal growth and Aflatoxin formation. In present study 70 samples consist of 35 sample of which of soyabean and corn were collected in form of domestic and imported products. Isolation of fungi was made by pour plate method and culturing in saboro dextrose agar and Aflatoxin was extracted by thin layer chromatography (TLC) method. The result of present study showed that 96 percent of soyabean and 88 percent of corn were contaminated with Aspergillus spp with different levels. The highest contamination of Aspergillos was made from imported soyabean. 40 percent of corn and 60 percent of soyabean contain different levels of Aflatoxin . The highest amount of Aflatoxin was 15 ppb detected in imported soyabean and 18 ppb in domestic corn.
Detection and Management of Aflatoxin Contamination in Rice in India

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Rice (Oryza sativa L.) is the most important staple food crop in India. Annually rice is grown in about 40 million hectares during the wet season. The crop is exposed to frequent and heavy rainfall and floods particularly near harvest in coastal areas in eastern, southern and western regions of the country. Often harvested sheaves remain wet and panicles become prone to invasion by fungi. Such grains with moisture content higher than the desired levels enter the storage system. As a result, invasion of both field and storage fungi take place. In general, fungal invasion leads to discoloration, loss in viability and quality of grains as a result of toxin contamination. Hence to detect and manage the aflatoxin contamination, rice samples were collected from all parts of the country and seed mycoflora were isolated. Aspergillus contamination was detected in all the rice samples collected from areas exposed to rain or flood or storage structures or mills. Three different species of Aspergillus were identified which included Aspergillus flavus, A. niger and A. ochraceus. The seeds which were exposed to heavy rains and flooded conditions were dominated by parrot green colored Aspergillus flavus (41 %) and samples collected from storage structures were dominated by A. niger (22 %). A. flavus and A. ochraceous produced both aflatoxins B1 and G1, but A. niger produced only aflatoxin B1. Garlic (Allium sativum) bulb extract (5%) proved significantly more effective with complete inhibition of all test Aspergilli. Pongamia glaberrima kernel extract (20%) showed 57-82 per cent inhibition on mycelial growth of all Aspergilli. Among the biocontrol agents, culture filtrate (15 %) of Trichoderma virens (MTCC 794) inhibited Aspergilli completely while the culture filtrate from T. harzianum (MTCC 2050) showed a moderate inhibition (34-50%). Of the fungicides evaluated, carbendazim 50 WP was effective even at 100 ppm in inhibiting Aspergilli. Tricyclazole 75 WP completely checked the growth of all Aspergillus spp. at 1000 ppm in culture.

Key words: Rice, Aspergillus spp., aflatoxin, plant extracts, Trichoderma spp., fungicides
Visual Identification of Norsoloronic Acid Production by Aspergillus spp. in Rice

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Visual Identification of Norsoloronic Acid Production by Aspergillus spp. in Rice
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The rice crop exposed to frequent and heavy rainfall, and flood is subjected to contamination
by Aspergillus spp. One difficulty in identifying aflatoxin contamination in rice grains is
the limited availability of methods for its detection. Although, the profuse growth of fungus
in and around grains is readily seen, aflatoxin contamination is not visible. To overcome
these limitations, the aflatoxin biosynthetic pathway of Aspergillus spp. was examined. Of
significance is the fact that although aflatoxins (B1, B2, G1 and G2) are not visible in normal
white light, all of the precursors in the pathway from norsoloronic acid (NOR) through to
versicolorin A are highly visible pigmented compounds. This study was therefore aimed to
visually identify the NOR production ability of Aspergillus spp. isolates obtained from stored,
discolored and damaged rice samples collected from several locations in India. Norsoloronic
acid, the first stable bright red-orange colored intermediate in aflatoxin biosynthetic pathway,
is useful in identifying visually the aflatoxin contamination in rice grains. Sixty three isolates
of Aspergillus spp. obtained from discolored and damaged rice grains were tested for NOR
production in yeast extract sucrose or peptone agar with or without ethanol. Three A. niger
isolates produced high intensity red-orange color, four others produced medium orange color
and the rest exhibited low intensity orange-yellow color after incubation of cultures for 7 days
in dark. The successive extracts of medium with acetone and chloroform were analyzed by thin
layer chromatography. In A. niger isolates, golden yellow, green and brown colored spots were
detected on TLC plate at Rf 0.70, 0.90 and 0.97, respectively. In A. flavus, two blue colored
spots with Rf 0.60 and 0.80 were identified. The first blue colored spot, probably a mixture,
showed three peaks at 244, 264 and 360 nm and the second blue colored spot showed a peak at
360 nm. In A. ochraceus also, one blue colored spot with Rf 0.54 was identified which showed
a single peak at 360 nm. The eluted spots in TLC were purified through preparative method.
The UV-vis spectrum of golden yellow colored spot produced by A. niger showed four peaks at
278, 320, 333 and 360 nm while green and brown colored spots showed two peaks, respectively
at 280 and 360 nm. In addition to visual detection of NOR production by Aspergillus spp.
isolated from discolored rice grains, a few other intermediate metabolites in the biosynthetic
pathway of aflatoxin were also detected in TLC. Such identification of NOR can help to detect
economically the suspected aflatoxin contamination in discolored and damaged rice grains
in large number of samples. A calibration of peak absorbance in UV spectrum with aflatoxin
intermediates can provide a preliminary confirmation.

Key words: Rice, norsoloronic acid, aflatoxin, visual identification, Aspergillus spp.
A STUDY OF CORRELATION BETWEEN AFLATOXIN AND GST ACTIVITY IN ASPERGILLUS PARASITICUS BY WESTERN BLOTTING TECHNIQUE

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Aflatoxins are secondary metabolites produced by toxigenic strains of Aspergilli, particularly Aspergillus flavus and Aspergillus parasiticus. There are very little information about correlation between aflatoxin production and glutathione s-transferase (GST). The goal of this study was to establish an accurate method for detection of GST in fungal strains producing aflatoxin. For this purpose, fungal GST was purified and then the affinity purified GST was used in order to preparation antibody GST assays. The fungus (Aspergillus parasiticus wild-14) was cultured on yeast extract sucrose agar (YES), the mycelia processed, for determination of protein content and GST activity, (using CDNB as substrate). The GST showed activity toward 1-chloro 2,4 dinitrobenzene (CDNB). In addition the secretory aflatoxin was measured in culture media and for the mycelia by the thin layer chromatography on silica gel plates. The enzyme was purified from the cytosolic fraction of the fungi using affinity chromatography (Epoxy activated cl-6B conjugated to GSH). Purified enzyme was approved using Sodium dodecyl sulfate polyacrylamide gel electrophoresis. Rabbits were immunised with affinity purified fungal GST and sera was collected for antibody purification. The antibody was applied two different detection assay namely Western blott technique and ELISA, and specificity of the purified antibody against fungal GST was further confirmed by Western blotting technique. ELISA data show that the reaction of antifungal GST is giving to high reaction with cytosol proposed from toxigenic fungi, whereas the reaction was very low when cytosol used from a nontoxigenic Aspergillus niger. It is concluded that GST has a correlation with aflatoxin synthesis by fungi.
PROPHYLACTIC ADMINISTRATION OF AMPHOTERICIN B INHALATION POWDER (ABIP) PROLONGS SURVIVAL OF NEUTROPENIC RABBITS INOCULATED WITH ASPERGILLUS

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Background
ABIP is a novel, highly respirable, dry-powder aerosol formulation of amphotericin B (AmB) in clinical development for the prevention of life-threatening pulmonary fungal infections in immunosuppressed patients. ABIP delivers AmB to the site of fungal invasion in the lung while reducing or eliminating systemic AmB toxicities. Studies of ABIP in laboratory animals show that the half-life in lung parenchyma is ~20d, whereas the half-life in ELF is ~20hr. Using the persistently neutropenic rabbit model, we investigated the prophylactic effect of AmB against an A. fumigatus challenge when dosed 12d or 1d before inoculation to determine whether sustained AmB concentrations in lung parenchyma resulted in sustained antifungal effect.

Methods
Rabbits in 6 groups of 10 received an immunosuppressive regimen starting on Day 1 and throughout the 14d observation period to achieve and maintain neutropenia. Group 1 (Control) received a sham saline solution in place of an A. fumigatus challenge. Groups 2-6 received 50M conidia of A. fumigatus (NIH Strain 4215, ATCC MYA-1163) intratracheally on Day 0. Group 3 inhaled a single 1.5 mg/kg ABIP dose 12d before inoculation with A. fumigatus. Groups 4, 5, and 6 inhaled single 0.15, 0.5, or 1.5 mg/kg ABIP doses 1d before inoculation, respectively. ABIP was spray-dried from feedstock containing AmB and the excipients distearoylphosphatidylcholine (DSPC) and calcium chloride. The powder particle-size was specifically engineered to be highly respirable for deep lung delivery. Doses were selected based on the initial assumption that only free (unbound) AmB is available for prophylaxis (the free fraction of AmB is reported to be <10% in plasma and tissues). Survival during the 14d following inoculation was analyzed using a Log-Rank Test followed by the Holm-Sidak multiple-comparison procedure.

Results
Group: 1, 2, 3, 4, 5, 6  
Inoculated: No, Yes, Yes, Yes, Yes  
AmB Dose(mg/kg): 0, 0, 1.5, 0.15, 0.5, 1.5  
ABIP Dosing Day: NA, NA, -12, -1, -1, -1  
Median Survival: >14, 6, 10*, 9*, 10.5*, 10*  
*Significantly different (improvement) over Group 2 survival (p<0.0001); no differences among Groups 3 to 6 (all equally effective).
The finding that ABIP was as effective when dosed 12d or 1d before inoculation is consistent with observations of long AmB half-life in lung parenchyma, and indicates that AmB in parenchyma is associated with efficacy. This finding has dose and regimen implications in that it appears sufficient to provide AmB concentrations in excess of the MICs of AmB against A. fumigatus in lung parenchyma rather than ELF suggesting that longer, perhaps weekly, dosing intervals could be explored. Based on comparisons to MICs, the lung parenchyma AmB concentrations achieved in this study, and the impressive survival results, total (bound and unbound), rather than free (unbound), AmB may be prophylactic.

**Conclusions**
ABIP, given as a single-dose inhalation 12d prior to challenge, provided a prophylactic effect (prolonged survival) against A. fumigatus induced morbidity/ mortality consistent with the long AmB half-life value in lung parenchyma. Infrequent dosing intervals may be considered in patients. Total lung parenchyma AmB concentration may be best correlated with prophylaxis.
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HISTOLOGICALLY PROVEN CURE OF LUNG ASPERGILLOSIS, RESISTANT TO TREATMENT WITH LIPOSOMAL AMPHOTERICIN B, WITH CASPOFUNGIN

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Aim
To present two cases of successful treatment of systemic aspergillosis with caspofungin, after failure of treatment with liposomal amphotericin B.

1st Case: A 48-year-old white man was admitted to the hospital with acute myeloid leukaemia. While receiving chemotherapy, he developed febrile neutropenia, non-responsive to treatment with broad spectrum antibiotics. Chest CT-scans revealed cavitary lesions, with crescent and halo signs and Aspergillus flavus was isolated from repeated sputum cultures. The patient received liposomal amphotericin B, 4mg/kg, for 45 days, but the radiology findings worsened and A.flavus was once again isolated from sputum cultures. The patient received caspofungin, 70mg on day one and 50mg daily thereafter, for three months. The drug was well tolerated. The patient defervesced and the chest x-rays showed resolution of all lesions, except one. A thoracotomy was performed and a lung biopsy was taken. Histology showed only fibrous tissue and did not have any fungal elements.

2nd Case: A 26-year-old Asian man, from Bangladesh, was transferred to our Department, with chronic myelogenous leukaemia and probable lung aspergillosis. The patient had received liposomal amphotericin B for one month in another hospital. A chest CT-scan revealed multiple cavitary lesions, with crescent and halo signs. One of them seemed to extend to the chest wall. On physical examination he was noticed to have a palpable, painful mass, protruding from the right side of his chest. This was biopsied. Fungal hyphae, compatible with Aspergillus infection, were found on histology. The patient received caspofungin for three months. The mass disappeared and the CT-scan showed only some fibrous lesions. A thoracotomy was performed and complete cure was confirmed by histological examination. Caspofungin was very well tolerated.

Conclusions
In some cases of systemic aspergillosis, where prolonged treatment with amphotericin B has failed, caspofungin may prove to be life-saving.
CLINICAL ISSUES REGARDING RELAPSING ASPERGILLOSIS AND THE EFFICACY OF SECONDARY ANTIFUNGAL PROPHYLAXIS IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

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Introduction
Advancements in early diagnosis and the introduction of effective agents have improved the rates of response of invasive aspergillosis (IA) to primary antifungal therapy. These changes allow the subsequent continuation of cytotoxic chemotherapy and/or performance of hematopoietic stem cell transplantation (HSCT) in an increasing number of patients with hematological malignancies. These developments have increased interest in secondary antifungal prophylaxis (SAP), as the resumption of myelotoxic chemotherapy in these patients is associated with high rates of relapse of aspergillosis in the absence of prophylaxis. However, the risk factors for relapsing IA and the strategies for reducing risk are not well defined.

Methods
We reviewed all English language papers that assessed the risk of relapse of IA and the value of SAP in patients with hematological malignancies receiving further chemotherapy and/or HSCT. Results: We identified 25 such studies that had adequate information. All of the studies were limited by their small, heterogeneous patient populations; uncontrolled retrospective designs; and use of different definitions of relapsing IA and SAP. Importantly, none of the studies differentiated IA relapse from reinfection. The following risk factors for relapse of prior IA were reported: prior documented (versus probable/possible) IA; site of the initial Aspergillus infection (sinuses versus other); partial clinical response of prior IA and incomplete resolution of imaging findings before further chemotherapy; use of corticosteroids; lack of remission of the underlying disease; type of conditioning regimen; use of high doses of cytosine arabinoside; administration of more than three antibiotics; duration of neutropenia > than 28 days; HSCT with stem cells obtained from unrelated donors and/or mismatched family donors; duration of primary antifungal therapy < 1 month; and hematopoietic stem cell source (cord blood versus bone marrow versus peripheral blood). The site of relapse of IA was usually the site of the primary IA. The mortality of relapsing IA was as high as 88% - 100% despite aggressive antifungal treatment. Regarding the efficacy of SAP in reducing the risk of relapsing IA, of the 197 reported patients with prior IA who received further chemotherapy or HSCT while receiving SAP only 31 (15.7%) had relapse of IA in contrast to 26 (62%) of the 42 patients (p<0.0001) who did not receive SAP. In the majority of cases the SAP regimen was consisted of amphotericin B alone or in combination with other fungal agents. The role of other strategies, such as surgery, use of hematopoietic colony stimulating factors, granulocyte transfusions, and adoptive immunotherapy in reducing the risk of relapsing IA is not well defined in the available published experience.
Conclusions
Well-designed, prospective studies assessing the value of old and new antifungal agents administered as SAP as well as co-interventions such surgery and immune enhancement are urgently needed in this important area of Aspergillus clinical research.
Aspergillosis is a frequent risk of complication in immunocompromized patients, specially in lung transplantation. Azole antifungals represent an important option in the management of prophylactic as well as curative treatments against invasive aspergillosis or Aspergillus species colonisations. Itraconazole (ITZ) is used since the early nineties, but pharmacokinetics particularities (long half-life), the lack of IV route in some countries and efficacy concerns encouraged the development of new entities in this class. Both IV and oral voriconazole (VRZ) formulations are now available (2003) and some other azoles are expected.

Azoles are metabolised by P450 cytochromes and known as CYP3A4 inhibitors, with potential clinical implications via numerous pharmacokinetic metabolic drug interactions. Among them, immunosuppressive drugs such as calcineurin inhibitors (cyclosporine CyA, tacrolimus FK) and rapamycins (sirolimus SRL, everolimus RAD) are particularly concerned.

TDM using HPLC or Immunoassay determinations of blood drug levels is conducted in our centre for each of these drugs, in order to manage the relative intensity of drug interactions. Azoles TDM is used to document the achievement of therapeutic concentrations (1-2 mg/L trough level) during the adaptation of the other drugs.

We performed 32 ITZ treatment courses over the 12 past years and 48 VRZ ones during the 3 last years in our lung transplanted population (80% indicated for cystic fibrosis), supporting the following points:

- ITZ acts as a stronger inhibitor (x1.5-2) than VRZ regarding calcineurin inhibitors, resulting in a larger dose reduction for FK (x5) as compared to CyA (x2-3). CyA concentration to dose ratio was respectively 50-75 (n=2) and 20 with or without ITZ, FK one 7.8 +/- 4.5 (n=15) with ITZ and 5.8 +/- 2.6 (n=28) with VRZ versus 1.4 +/- 0.6 (n=19) alone p<0.001
- Dramatic changes in azole concentrations impact the magnitude of the interaction and subsequently the need for adjustment
- The administration route may change the intensity of the interaction. ITZ, highly metabolised by CYP3A4, does not influence IV CyA or FK in such extent than oral routes. It is compatible with the intestinal localisation of metabolic interactions involving CYP3A4. This has to be taken in account when switching routes of administration during this co-prescription, in addition to the correction applied from the relative oral bioavailability. CyA concentration to dose ratio, usually at 100 to 140 during IV administration remained unchanged in 3 cases of co-administration with ITZ.
- In case of very high azole concentrations, switch between antifungals must be conducted with caution, specially regarding the use of a loading dose (one observation of drug interaction between ITZ and VRZ).
- Rapamycins interactions are less documented (n=2), but appear to be very sensitive to azoles.

TDM was recommended on efficacy concerns for ITZ only. Despite the lack of recommendation, we suggest the determination of VRZ concentrations also, in order to manage drug-drug interactions of this class in lung transplanted patients. This may be extrapolated to other patients such as HIV, that may receive concomitantly strong CYP3A4 inhibitors (as protease inhibitors) and/or strong inducers (as rifampicin) altogether withazole antifungals.
A COMPARATIVE STUDY OF DIFFERENT DOSES OF LIPOSOMAL AMPHOTERICIN B (L-AMBI) AND MICAFUNGIN (MICA) FOR TREATMENT OF MURINE SYSTEMIC ASPERGILLOSIS DETER

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Background  
Systemic fungal infections caused by Aspergillus species are difficult to treat and determining the appropriate dose and comparative efficacy of different regimens is needed to optimize therapy. In the present study, we used several parameters to compare the efficacy of different doses of L-AmBi and Mica for the treatment of murine systemic aspergillosis caused by Aspergillus fumigatus.

Methods  
Swiss Webster female mice (7 wks old) were suppressed with 100mg/kg cyclophosphamide every 3d, beginning d -3. Day 0, mice were challenged IV with 2.8 X 10ex4 A. fumigatus conidia, and 24h later, treated daily (n=12/group) for 6 days with 5, 10 or 15 mg/kg L-AmBi or Mica, or 5% dextrose (D5W). Survival was monitored for 27 days post-infection. Day +8 (n=5/group) and d+27 (n=5-7/group) mice were euthanized and their kidneys collected for mean Log10 CFU/g, mean galactomannan index (GI), and tissue drug concentrations (mean ug/g) using a zone of inhibition bioassay with Paecilomyces variotii.

Results  
By d+4, all D5W mice had died, and 5, 10 or 15mg/kg of L-AmBi produced a dose-dependent increase in survival: 57%, 71% and 86%, respectively. The dose related increase in survival with L-AmBi was associated with decreasing Log10 CFU/g kidneys (d+8: 2.69, 0.28, 0.17 and d+27: 2.34, 0.94, 0.17) as well as lower GI (d+8: 4.71, 0.56, 0.32 and d+27: 4.34, 2.42, 0.90) for 5, 10 and 15mg/kg, respectively. In comparison, survival with the lower dose of Mica (5 mg/kg) was more effective (86%) than higher dose treatment: 71% (10 mg/kg) and 51% (15mg/kg). The decrease in survival was associated with increasing Log10 CFU/g kidneys (d+8: 2.24, 3.03, 2.49 and d+27: 1.59, 2.87, 2.68) and GI (d+8: 3.53, 4.82, 4.49 and d+27: 2.36, 4.65, 4.44) for 5, 10 and 15mg/kg, respectively. When the D5W mice were moribund, they were euthanized and had 2.79 Log10 CFU/g kidneys and 4.43 GI. Drug levels in the kidneys of L-AmBi mice were about 20 ug/g on d+8 and about 12 ug/g on d+27 for all doses; the Mica levels for 5, 10 and 15 mg/kg dosing were 6, 25 and 22 ug/g on d+8, respectively, with no Mica detectable in the kidneys on d+27.

Conclusions  
The results show that L-AmBi at 10 and 15mg/kg or Mica at 5mg/kg were significantly more effective than D5W in prolonging survival in mice with systemic aspergillosis (P< 0.05). However, L-AmBi at 15mg/kg was significantly more efficacious (P < 0.01) than 5mg/kg Mica in reducing fungal burden and galactomannan content in the target tissue.
AMPHOTERICIN B INHALATION POWDER (ABIP) IS WELL-TOLERATED WITH LOW SYSTEMIC AMPHOTERICIN B EXPOSURE IN HEALTHY SUBJECTS

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Background
Immunosuppression of bone marrow or organ transplant recipients leaves patients vulnerable to life-threatening pulmonary fungal infections. Effective prevention remains elusive. Delivery of sufficient amphotericin B (AmB) concentrations to the lung with little or no therapy-limiting side effects associated with systemic AmB treatment would uniquely improve the prophylactic benefit:risk ratio over existing products. We investigated the tolerability and pharmacokinetics of pulmonary administration of Amphotericin B Inhalation Powder (ABIP), a novel, highly-respirable, dry powder formulation of AmB in normal volunteers.

Methods
Thirty-five healthy subjects were enrolled into a randomized, double-blind, placebo-controlled (5:2; active:placebo), 5-cohort, ascending single-dose trial. Twenty-five received a single-dose of ABIP containing nominally 1, 2.5, 5, 10, or 25 mg AmB, from capsules administered via a small, proprietary dry-powder inhaler. ABIP was spray-dried from feedstock containing AmB and the excipients distearoylphosphatidylcholine (DSPC) and calcium chloride. The powder particle-size was specifically engineered to be highly respirable for deep lung delivery. All capsules contained 10 mg total powder and placebo capsules contained 10 mg of excipients. Venous blood samples were drawn at 0 (predose), 0.5, 1, 2, 4, 8, 12, and 24 hr postdose for pharmacokinetic characterization. Free and total plasma AmB concentrations were below the 1 ng/mL lower limit of quantitation in all but 4 of 280 samples analyzed (all 4 in the 25 mg group; maximum 1.27 ng/mL). Safety parameters, including pulmonary function, were monitored throughout.

Results
All doses were well tolerated. There was no relationship between dose or powder load to the number or severity of adverse events (AEs). Most AEs were transient, mild, and study drug was not implicated. The most common AEs reported within 24 hr of dosing were abnormal mid-flow spirometry (24% ABIP, 30% placebo), cough (12% ABIP, 10% placebo), dizziness (16% ABIP, 10% placebo), headache (20% ABIP, 0% placebo), and dysgeusia (16% ABIP, 0% placebo). The change in mid-flow spirometry was characterized as mild, transient, and not clinically significant. Plasma free AmB concentrations were below the 1 ng/mL lower limit of quantitation in all but 4 of 280 samples analyzed (all 4 in the 25 mg group; maximum 1.27 ng/mL). Mean plasma total AmB concentrations increased with dose, and peaked 8 hr postdose at 21.6 ng/mL (range: 15.7-29.6 ng/mL) in the 25 mg group. AmB was ~95% bound to plasma proteins (plasma free fraction was ~5%). Plasma AmB pharmacokinetics were
consistent with previous animal studies using ABIP and will be used to help characterize AmB pharmacokinetics in human lung via interspecies and physiologically-based pharmacokinetic (PBPK) models to guide therapeutic dose and regimen.

**Conclusions**
A novel, highly respirable, dry powder formulation of AmB was well-tolerated and resulted in low systemic AmB concentrations when administered at doses expected to provide antifungal prophylaxis in the lung. The low AmB exposure observed should reduce or eliminate systemic toxicities and drug-drug interactions in patients. ABIP could provide a new paradigm to safely prevent morbidity and mortality of pulmonary fungal infections in immunosuppressed patients.
P120

CLINICAL RESPONSE IS ASSOCIATED WITH LONGER DURATION TREATMENT WITH AMPHOTERICIN B LIPID COMPLEX (ABLC) IN ASPERGILLOSIS

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Purpose
To examine factors associated with an improved or cured clinical response in Aspergillus fungal infections treated with Amphotericin B Lipid Complex (ABLC).

Methods
A large multicenter patient registry, the Collaborative Exchange of Antifungal Research (CLEAR) was used to retrospectively review patients treated with ABLC. Hospitalized patients must have received at least four doses of ABLC for a documented or suspected fungal infection to be included. Data were collected for patients treated during January 1996 through November 2000 from 160 hospitals in the United States and Canada. Proven or probable Aspergillus infections were included. A multivariable model was used to examine treatment and infection-related factors [first- vs. second-line treatment; presence of underlying hematological malignancy; high dose (>5 mg/kg/day) vs. low dose (<= 5 mg/kg/day) ABLC; long duration (>12 days) ABLC vs. short duration; concomitant nephrotoxins; pulmonary infection site vs. non-pulmonary] associated with clinical response.

Results
There were 388 ABLC-treated Aspergillus infections that were evaluable for clinical response. Duration of ABLC treatment was the only statistically significant predictor of clinical response (Table); long duration ABLC was independently associated with 1.7 times increased odds of an improved or cured clinical response. Based on the multivariable results we compared long vs. short duration ABLC on the proportion of infections achieving clinical response and observed that long duration ABLC was associated with a 48% response rate vs. a 37% response rate for short-duration treated infections, p=0.035. Long- vs. short-duration ABLC patients were similar demographically (mean age 43 yrs. and 47 yrs., for long & short respectively), about 60% male, and >70% Caucasian. Duration of ABLC treatment was the only factor associated with clinical response in proven or probable Aspergillus infections, overriding the effects of high risk status (e.g., hematological malignancy & pulmonary aspergillosis), and treatment-related factors including the timing of ABLC treatment, dose of ABLC and concomitant nephrotoxins.
Conclusion:
Staying the course of ABLC-treatment in Aspergillus infections appears to be an effective treatment strategy. These data offer clinicians empirical verification of the clinical response outcome associated with long duration ABLC.

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>First-line vs. second line ABLC</td>
<td>1.01</td>
<td>0.66 – 1.55</td>
<td>0.947</td>
</tr>
<tr>
<td>Hematological malignancy (yes vs. no)</td>
<td>0.69</td>
<td>0.45 – 1.05</td>
<td>0.081</td>
</tr>
<tr>
<td>High dose (&gt;5 mg/kg/day) ABLC vs. low dose</td>
<td>0.72</td>
<td>0.45 – 1.16</td>
<td>0.177</td>
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<td>Long duration (&gt; 12 days) ABLC vs. short</td>
<td>1.68</td>
<td>1.10 – 2.57</td>
<td>0.017</td>
</tr>
<tr>
<td>Concomitant nephrotoxins (yes vs. no)</td>
<td>0.74</td>
<td>0.49 – 1.13</td>
<td>0.161</td>
</tr>
<tr>
<td>Pulmonary vs. non-pulmonary</td>
<td>1.08</td>
<td>0.70 – 1.68</td>
<td>0.716</td>
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</table>
POSACONAZOLE COMPARED WITH AMPHOTERICIN B LIPID FORMULATIONS IN COMBINATION WITH CASPOFUNGIN AS SALVAGE THERAPY FOR INVASIVE ASPERGILLOSIS IN PATIENTS WITH HEMATOLOGIC MALIGNANCY

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Background
When compared with a historical cohort, posaconazole (POS) was more effective than conventional antifungal therapy as salvage treatment for invasive aspergillosis (IA) (Walsh T et al. Blood. 2003;102:195-196). Anecdotal experience suggested that the combination of lipid formulations of amphotericin B (AmB/LPD) and caspofungin (CSP) may improve the outcome of salvage therapy for IA. We sought to compare POS with the combination of AmB/LPD plus CSP as salvage therapy for IA in patients with hematologic malignancy.

Methods
Between June 2000 and November 2004, 51 patients with hematologic malignancy and proven or probable IA (according to EORTC/MSG criteria) received salvage therapy with POS; 54 similar patients received AmB/LPD plus CSP as salvage treatment. Patients were deemed eligible for salvage treatment if 1) they were intolerant, defined as discontinuing antifungal therapy because of documented organ toxicity (such as persistent elevation of serum creatinine levels or >=2-fold increase in liver function test results) or because of idiosyncratic or major anaphylactoid reactions or 2) if they had refractory IA, defined as failure to improve or progression of disease after at least 7 days of antifungal therapy. Clinical characteristics, including age, gender, underlying malignancy, neutropenia, transplantation, intensive care unit (ICU) status, steroid or tacrolimus use, Aspergillus species, site of infection, and response to salvage therapy, were retrospectively obtained. Complete or partial resolution of clinical, radiographic, and bronchoscopic abnormalities present at baseline was considered a successful outcome; stable or progressive disease or toxicity that required drug discontinuation was considered treatment failure.

Results
Patients in the two groups had comparable risk factors, such as age, underlying malignancy, transplantation history, duration of neutropenia, steroid and tacrolimus use, graft-versus-host disease, Aspergillus species, and disseminated IA. However, patients in the POS group were less frequently in the ICU (26% vs 46%, P < .03) and on mechanical ventilation (14% vs 35%, P < .01). The total response to salvage therapy was 39% for the POS group and 15% for the AmB/LPD plus CSP group (P = .005). When ICU and mechanical ventilator patients were excluded from the analysis, the response to at least 7 days of salvage therapy with POS was 48% (19/40) compared with a 19% (6/31) response rate in patients receiving AmB/LPD plus CSP (P = .01).

Conclusions
In this cohort of patients with hematologic malignancy and IA, POS significantly improved outcome compared with the combination of AmB/LPD plus CSP as salvage therapy.
FACTORS ASSOCIATED WITH MORTALITY IN A TRIAL OF LIPOSOMAL AMPHOTERICIN B (L-AMB) AS INITIAL THERAPY FOR INVASIVE FILAMENTOUS FUNGAL INFECTIONS (IFFI)

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Background
In a recently presented (ASH 2005) randomized, prospective, double-blind trial of 2 dosing regimens of L-AMB for proven and probable invasive aspergillosis and other IFFI, patients treated with 3 or 10mg/kg/d had 12 week survival rates of 72% and 59%, respectively (difference not statistically significant). The study population was further analyzed to evaluate baseline factors associated with survival.

Methods
Patients with proven or probable IFFI by modified EORTC/MSG criteria were randomized to receive L-AMB 3 or 10 mg/kg/d x14d, then 3 mg/kg/d until investigator defined end of study drug treatment. IFFI diagnoses were confirmed by an independent Data Review Board (DRB). Survival was followed to 12 weeks. Multivariate stepwise logistic regression analysis was used to identify baseline factors associated with survival.

Results
201 patients had DRB confirmed IFFI. 93% had underlying hematological malignancies, with 66% having uncontrolled disease; 17% had allogeneic stem cell transplant (allo SCT); 73% were neutropenic at baseline. Survival at week 12 was significantly associated with allo SCT 40% vs. no allo SCT 71%; difference 31% (95% CI 14%, 49%, p<0.001); and hematological malignancy uncontrolled 54% vs. controlled 81%; difference 27% (95% CI 15%, 39%, p<0.001). In addition to treatment regimen received, no differences in survival were found for IFFI site (pulmonary vs. other), age, or baseline values of neutropenia, abnormal hemoglobin, creatinine or liver enzymes.

Conclusions
In a large prospective trial of L-AMB for initial treatment of IFFI, allo SCT and uncontrolled hematological malignancy at baseline, but not neutropenia at baseline, were significantly negatively associated with 12 week survival. Patients entering antifungal efficacy trials should be stratified for both of these factors to avoid confounding biases.
Introduction
In the last few decades, there has been an increase in fungal diseases, especially in those caused by Aspergillus. After formation of fungus ball in pre-existing cavities, antifungal agents are very hard to control. Surgical resection offers the only realistic chance of a permanent cure for aspergilloma. But surgical indication is still a controversy because of the high incidence of postoperative complications. Classically, most complications observed after operations for aspergiloma occurred in patients with sequelae of tuberculosis. The purpose of this study is to evaluate our indications and results in the surgical treatment of aspergilloma, focusing attention on the postoperative complications and risk factors.

Methods
From 1990 to 2004, 60 patients with mean age of 44.6 (range, 20-69) were submitted to pulmonary surgery for excision of aspergilloma. Forty-one patients were male (68%). The most frequent indication for surgery was haemoptysis in 53 patients (88%). Previous tuberculosis were observed in 47 patients (78%) and 26 patients were smokers.

Results
The procedures performed were 46 lobectomies, 7 segmentectomies or wedge resections and 7 pneumonectomies. Postoperative complications occurred in 40% of the patients including: prolonged air leaks (n=12), persistent air space (n=4), empyema (n=3), bleeding (n=3) and bronchopleural fistula (n=2). Risk factor analysis revealed old age, complex aspergilloma, tuberculosis and smoking as significant risk factors for postoperative complications. There were 3 postoperative deaths (5%), related to respiratory failure (n=2) and cardiorythmic disorder (n=1). All surviving patients referred good improvement of symptoms and quality of life. There was no recurrence of disease or hemoptysis.

Conclusions
Surgical resection of pulmonary aspergilloma is effective in preventing recurrence of hemoptysis. It has low risk in asymptomatic patients and in the absence of underlying pulmonary disease. Incomplete reexpansion is frequent when there is underlying lung disease and in smokers. Long-term prognosis is mainly dependent on the general condition of patients. The benefits of the surgery are prevention of hemoptysis and growth of mycetoma, eradication of the pyogenic component, and probable prolongation of life. We recommend early surgical resection of symptomatic aspergilloma and even asymptomatic cases with reasonable complications.
AMPHOTERICIN B LIPID COMPLEX (ABLC) VS LIPOSOMAL AMPHOTERICIN B (L-AMB) MONOTHERAPY OF INVASIVE ASPERGILLOSIS (IA) IN PTS WITH HEMATOLOGICAL MALIGNANCY

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Background
IA is a major cause of morbidity and mortality in patients with HM. There are two lipid formulations that are currently in widespread use, ABLC and L-AMB. There is limited data comparing the efficacy and the safety of these two agents when used as monotherapy of IA in HM.

Methods
Between June 1993 and November 2004, we retrospectively studied 153 consecutive patients with HM and definite or probable IA who received primary antifungal therapy with either L-AMB (n = 103), or ABLC (n = 51) or salvage therapy with L-AMB (n = 50) and ABLC (n = 29). Nephrotoxicity was defined as an increase in creatinine of two times baseline.

Results
For primary and salvage therapy, ABLC and L-AMB had comparable distribution of risk factors such as underlying malignancy, neutropenia, steroid use, admission to intensive care unit, graft-versus-host-disease, Aspergillus species and disseminated IA. Response to primary and salvage antifungal therapy was equally poor in both groups (range 10%-12.5%, P= 0.99). Nephrotoxicity was significantly higher in the ABLC group compared to L-AMB, however, hepatotoxicity was comparable in primary and salvage therapy for both groups. Aspergillosis contribution to death and the frequency of patients who died with aspergillosis at 12 weeks of initiating primary or salvage therapy was also comparable for the two groups.

Conclusions
Among patients with HM, primary and salvage therapy of IA with either ABLC or L-AMB as single agent is equally associated with poor outcome. L-AMB appears to be less nephrotoxic than ABLC but there was no significant difference in hepatotoxicity in the two groups.
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EFFICACY AND SAFETY OF POSACONAZOLE IN RENALLY IMPAIRED PATIENTS WITH INVASIVE ASPERGILLOSIS AND OTHER INVASIVE FUNGAL INFECTIONS (IFIS)

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2Winship Cancer Institute, Emory University Hospital, Atlanta, Georgia, USA
3Springfield Clinic, Springfield, Illinois, USA
4University of Texas Health Science Center, San Antonio, Texas, USA
5Schering-Plough Research Institute, Kenilworth, New Jersey, USA

Background
Immunocompromised patients, including solid organ and hematopoietic stem cell transplant recipients and cancer patients with chemotherapy-induced neutropenia, are at increased risk for aspergillosis and other IFIs. However, these patients often have significant underlying renal impairment that can compromise the efficacy or tolerability of antifungal therapy. Posaconazole is an extended-spectrum triazole for the treatment of IFIs. Previous pharmacokinetic studies have demonstrated that posaconazole is not renally excreted to a significant extent, and its exposure is similar in individuals with varying degrees of renal impairment. We report the efficacy and safety of posaconazole in patients with IFI and renal dysfunction from a large, 12-month, multicenter, open-label trial.

Methods
In the study, 330 subjects with IFIs who were refractory to or intolerant of prior antifungal therapy were enrolled and received posaconazole 800 mg/day in divided doses. The primary endpoint was global response in subjects with aspergillosis. Success was defined as complete or partial response; nonsuccess was defined as stable disease, failure, or undetermined outcome. Patients with reduced estimated creatinine clearance (CrCl) or increased serum creatinine (SCr) at baseline were included in this subanalysis.

Results
Of the 330 enrolled subjects, 39 had CrCl <40 mL/min and 20 had CrCl of 40-<50 mL/min. An additional 6 subjects had baseline SCr >2 mg/dL, but their CrCl could not be determined. In subjects with renal impairment and proven or probable aspergillosis, success rates were 46% (11/24) in patients with CrCl <40 mL/min, 31% (4/13) in patients with CrCl of 40-<50 mL/min; and 50% (1/2) in patients with SCr >2 mg/dL. These results were similar to the 42% response seen in all study patients with proven or probable aspergillosis (n=107). In addition, for other IFIs, including candidiasis, fusariosis, and zygomycoses, comparable responses were generally seen in patients with renal dysfunction, regardless of the degree of impairment. The safety profile in subjects with baseline renal dysfunction was similar to that reported for the total study population. In subjects with renal impairment, the most common treatment-related
treatment-emergent adverse events were nausea (6/39 in <40 mL/min group; 3/20 in 40–<50 mL/min group), vomiting (4/39 in <40 mL/min group), increased SCr (4/39 in <40 mL/min group), dizziness (3/39 in <40 mL/min group), increased alkaline phosphatase levels (3/39 in <40 mL/min group), and altered drug level (3/39 in <40 mL/min group).

**Conclusion**
In this study, posaconazole appears to be an effective and well-tolerated treatment in patients with invasive aspergillosis and other IFIs who are refractory to or intolerant of antifungal therapy, regardless of their degree of renal impairment.
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CASPOFUNGIN VS. CASPOFUNGIN COMBINED WITH LIPOSOMAL AMPHOTERICIN B FOR THE TREATMENT OF CEREBRAL ASPERGILLOSIS IN A NURSING RAT MODEL

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Background
Cerebral aspergillosis is associated with an unacceptably high mortality despite antifungal treatment. We compared the efficacy of caspofungin combined with liposomal amphotericin B vs. caspofungin alone for the treatment of cerebral aspergillosis in immunocompetent nursing rats.

Methods
Eleven-day-old non-immunosuppressed Wistar W1 rats were infected by intracisternal injection of 10 microL of a conidial suspension of Aspergillus fumigatus. Treatment started 18 h after infection and was given for 10 days. Regimens were caspofungin 1 mg/kg/d i.p. (n=20) and amphotericin B 5 mg/kg/d + caspofungin 1 mg/kg/d i.p. (n=21). Infected controls (n=14) were given NaCl 0.9% or glucose 5% i.p.

Results
Controls consistently showed symptoms of cerebral Aspergillus infection. Their mean survival time was 4.4 days. Treatment significantly increased survival time to 10.5 and 9.3 days for caspofungin alone and caspofungin combined with amphotericin B, respectively. The difference between treatment regimens was not significant. CNS fungus burden determined by culture of cortical homogenates declined over time, but interestingly there was no significant difference between controls and treated animals. Two animals in each treatment group had sterile brain cultures. Fungal dissemination determined by culture of kidney homogenates was found in 7/14 controls, 0/20 animals treated with caspofungin, and 1/21 animals of the combination group.

Conclusion
Caspofungin alone and in combination with liposomal amphotericin B significantly increases survival time in a lethal model of cerebral aspergillosis. Remarkably, CNS fungus burden did not differ between controls and treated animals. The hypothesis that antifungal treatment may result in a decreased inflammatory reaction is being further investigated.
IN VITRO EFFECT OF TEA CATECHIN ON GROWTH OF ASPERGILLUS NIGER

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Tocklai experimental Station, Jorhat–785008 Assam, India

Molds deteriorate black tea quality during storage and render it unfit for human consumption. During our study on microbiology of black tea in North East India an Aspergillus niger was isolated from black tea samples as contaminants. Black tea quality is largely determined by presence of various oxidised products of polyphenols (Epicatechin gallate, epigallocatechin gallate, Epicatechin) The present study is a part of the investigation on nutrition of Aspergillus niger and the effect of tea catechin in different concentrations on biomass production and germination behaviour was studied in vitro. Catechin exerted considerable inhibitory effect on growth of Aspergillus niger and induced abnormality in conidial germination in vitro.
BIOACTIVE PROTEIN FROM ESCHERICHIA COLI WITH ANTI-ASPERGILLUS POTENTIAL

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Background
Infections caused by Aspergillus species are recognized as emerging cause of morbidity and mortality in a variety of immunocompromised patients, despite profound environmental protection and the widespread prophylactic use of agents with anti-Aspergillus activity. At present, no firm conclusions can be drawn on the use of available antifungal drugs. We had observed the anti-Aspergillus properties in lysate of Escherichia coli BL 21.

Methods
The antifungal protein of E. coli BL 21 was purified using pathogenic isolates of Aspergillus, with a procedure involving ion exchange chromatography on DEAE Cellulose, gel filtration chromatography on Sephadex G 100 and HPLC on C18 reverse phase chromatography. A combination of N-terminal amino acid sequencing, peptide mass fingerprinting using MALDI-TOF and LCMS were used for identification of proteins. The immunogenicity of purified protein was also studied.

Results
The molecular mass of the purified protein, determined by Kodak 1D software, was estimated to be 39.30 kDa. N terminal amino acid sequence analysis revealed that purified protein exhibited 100 percent amino acid identity with alcohol dehydrogenase of yeast. Analysis by peptide mass fingerprinting MALDI-TOF also substantiated these results. The purified protein demonstrated potent antifungal activity against pathogenic species of Aspergillus fumigatus, A. flavus and A. niger. Concentrations of purified protein required for complete inhibition of fungal growth varied from 1.95 to 3.98 microgram per ml. In vitro toxicity tests demonstrated that purified protein had no toxicity against human erythrocytes upto the tested concentrations of 1000 microgram per ml whereas the standard drug Amphotericin B was toxic to 100 percent human erythrocytes at a concentration of 37.50 microgram per ml. The purified protein was immunogenic as it induced the production of the specific antibodies in mice and showed immunoreactivity by ELISA and Western blot.

Conclusions
These results suggested that E. coli BL21 might be important bioresource of lead molecules for developing new therapies for treating fungal infections.
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Background
The antifungal activities of voriconazole (VRC) and micafungin (MICA) alone or in combination therapy were evaluated in a guinea pig model of invasive aspergillosis. Methods: Guinea pigs were immunosuppressed with triamcinolone and made temporarily neutropenic with cyclophosphamide prior to lethal challenge with iv Aspergillus fumigatus. Therapy with oral VRC at 2.5 or 10 mg/kg/bid, ip MICA at 1 or 6 mg/kg/d, or combinations of VRC 2.5 + MICA 1 or VRC10 + MICA 6 was begun 24 hr after challenge and continued for 5 d.

Results
Mortality occurred in 8 of 8 untreated controls vs 7 of 8 treated with MICA 1; 5 of 8 with MICA 6; 4 of 8 with VRC 2.5; 1 of 8 with VRC 10; and with the combinations, in 6 of 8 with VRC2.5+MICA1 and 1 of 8 with VRC10+MICA6. Both doses of VRC reduced CFU counts in all tissues. Combination therapy with VRC2.5+MICA1 reduced only kidney burden vs controls as compared to VRC10+MICA6 therapy which resulted in the lowest tissue burden and was the only therapy to significantly reduce all organs especially lung burden compared to controls:

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Brain</th>
<th>Kidney</th>
<th>Liver</th>
<th>Lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (8)</td>
<td>1.98 ± 0.54</td>
<td>2.87 ± 0.35</td>
<td>3.25 ± 0.34</td>
<td>1.94 ± 0.33</td>
</tr>
<tr>
<td>MICA 1 (8)</td>
<td>2.98 ± 0.47</td>
<td>2.05 ± 0.39*</td>
<td>3.41 ± 0.50</td>
<td>1.33 ± 0.36</td>
</tr>
<tr>
<td>MICA 6 (8)</td>
<td>2.45 ± 0.65</td>
<td>1.59 ± 0.50*</td>
<td>2.39 ± 0.67</td>
<td>1.37 ± 0.44</td>
</tr>
<tr>
<td>VRC2.5 (8)</td>
<td>1.12 ± 0.31</td>
<td>1.05 ± 0.30*</td>
<td>1.06 ± 0.38#</td>
<td>1.17 ± 0.29</td>
</tr>
<tr>
<td>VRC 10 (8)</td>
<td>0.55 ± 0.25*</td>
<td>1.18 ± 0.41#</td>
<td>0.89 ± 0.34#</td>
<td>1.09 ± 0.28</td>
</tr>
<tr>
<td>V2.5+M1 (8)</td>
<td>2.64 ± 0.44</td>
<td>1.67 ± 0.41*</td>
<td>1.89 ± 0.43</td>
<td>1.31 ± 0.35</td>
</tr>
<tr>
<td>V10+M6 (8)</td>
<td>0.72 ± 0.39*</td>
<td>0.40 ± 0.28#</td>
<td>0.39 ± 0.19#</td>
<td>0.31 ± 0.11#</td>
</tr>
</tbody>
</table>

* P<0.05 vs controls; # P<0.005 vs controls, Mann-Whitney

Only VRC 10 and VRC10+MICA6 reduced the number of positive cultures compared to controls. There were positive cultures in 15/32 organs after therapy with the high dose combination (p<0.005) and 24/32 positive organs after VRC10 (p<0.05) therapy, alone vs controls (32/32 positive). The high dose combination reduced the number of positive cultures more than therapy with either VRC 10 or MICA 6 (p<0.02). Conclusions: VRC10 alone or in combination with MICA 6 was more effective than either dose of MICA alone or the low dose combination of VRC2.5+MICA1. Antagonism with either combination therapy did not
occur. High dose combination therapy with VRC + MICA yielded greater reductions in tissue burden including the lung than other regimens and should be evaluated for therapy in this disease.
A PATIENT WITH CHRONIC RENAL FAILURE AND FATAL ASPERGILLUS TERREUS OSTEOMYELITIS OF THE STERNUM

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A 38-year-old, male patient was admitted to the hospital because of a relapsing thoracic abscess involving the sternum and the surrounding soft tissues. He had a history of renal failure since childhood due to vesicoureteral reflux, and had been in dialysis for the last 16 years. He didn’t have diabetes. Four years ago a lesion that was thought to be a furuncle appeared on the chest wall. Since then, he received multiple antibiotic regimens and had two surgical debridement procedures, without definite cure. Pus cultures never revealed a pathogen. Four months before admission involvement of the sternum was noticed on x-rays. He received ceftazidime plus teicoplanin, and ciprofloxacin plus amoxicillin-clavulanate and surgical debridement was performed again.

He was admitted due to incomplete response to these measures. During his stay, he was receiving the former antibiotics. Although afebrile and in a generally good condition, the patient continued to have discharge of hemopurulent material from the chest area above the sternum, and had laboratory indications of disseminated intravascular coagulation. A chest CT scan revealed a hematoma formation that was treated surgically, and Aspergillus sp. was isolated. Liposomal amphotericin B started at a daily dose of 3 mg/kg. Meanwhile the fungus was identified as A.terreus and the antifungal regimen was changed to caspofungin, 50 mg daily. Response was not noticed again and the patient developed overt sepsis and respiratory failure. Finally, he died because of massive hemorrhage of the sternum, after 3 months of hospital stay.

Conclusion
Infections with Aspergillus terreus can be very difficult to diagnose and treat. A high index of suspicion is required, especially in cases without major immunodeficiency, because fatal outcome is likely, even with appropriate treatment.
CHRONIC INVASIVE ASPERGILLOSIS OF THE CNS AND PARANASAL SINUSES IN AN IMMUNOCOMPETENT PATIENT

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A 39-year-old male patient presented with diffuse headache and diplopia. The patient worked as a farmer cultivating tobacco for many years, up to the age of 25, after which he worked as a schoolteacher. His past medical history was remarkable only for nasal congestion during the last fifteen years, for which his physician periodically prescribed intranasal corticoid therapy. Moreover, for many years the patient had lost his smell and taste. He denies use of alcohol or any illicit drugs. A CT scan of the facial skeleton and brain revealed an invasive mass of the sella turcica, clivus, ethmoid, sphenoid and frontal sinuses, with destruction of the adjacent bony structures. Physical examination did not reveal any abnormal findings. In particular, as regards the head and face, no areas of tenderness, swelling or palpable lymph nodes were noted. The patient did not report any fever. Full blood count, erythrocyte sedimentation rate, C-reactive protein, and biochemical tests were unremarkable. Levels of prolactin and ACTH were elevated slightly above normal.

The patient underwent extensive surgical debridement through anterior ethmoidectomy and sphenoidectomy, by intranasal endoscopy. Biopsy of the specimen showed fungal hyphae within necrotizing infiltrates, with septae of aspergillus after Grocott staining. Cultures of the same material revealed growth of Aspergillus fumigatus.

Complementary laboratory examination after the diagnosis excluded the presence of immunodeficiency disorders such as immunoglobulin, complement and T-lymphocyte deficiencies, infection by human immunodeficiency virus and chronic granulomatous disease. The patient was further treated with complimentary therapy with intravenous voriconazole for three weeks with instructions to continue therapy per os.

A CT scan of the facial skeleton and brain after four weeks of treatment showed complete remission of the aforementioned findings, which was confirmed by intranasal endoscopy. Diplopia as well as headaches completely resolved after surgical debridement. Furthermore, after two weeks of antifungal treatment the patient’s nasal congestion improved substantially and his smell and taste returned to normal. Prolactin and ACTH levels also returned to normal.

We report a case of rhinocerebral aspergillosis in an apparently immunocompetent patient. Invasive aspergillosis is very uncommon in immunocompetent patients. It may present in those living in an environment highly contagious for aspergillus. These infections are chronic, slowly progressive and spread to adjacent tissues. Therapy involves extensive
surgical debridement and systemic antifungal therapy. The infection is often difficult to treat, with a high relapsing rate and usually carries a poor prognosis. This patient’s former occupation was probably a major factor for his developing this infection. It is well known that tobacco leaves are very often covered with fungi and is easily inhaled when manipulating the tobacco leaves. It seems probable that patient’s nasal cavity was colonized many years before, and over the years the infection spread to give the symptoms which led him to seek medical advice. The complete remission of the laboratory findings and impressive improvement in the clinical picture after only four weeks of treatment raise the probability of complete and permanent cure.
2nd ADVANCES AGAINST ASPERGILLOSIS
February 22-25, 2006 Athens, Greece

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INVASIVE MOLD INFECTIONS IN A TERTIARY CARE HOSPITAL: 15 – YEAR STUDY

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Objective
Invasive fungal infections are important causes of morbidity and mortality mainly among immunocompromised patients. Their incidence is continuing to rise, partly due to the growing number of patients with impaired host defenses. The infection is transmitted mainly via inhalation of conidia from environmental sources, and the respiratory tract is the major portal of entry and site of infection.

Methods
Between 1991 and 2005 a total of 248 cases of invasive mold infections were diagnosed in the clinical microbiology department from various clinical specimens of patients hospitalized in a 1000 bed adults tertiary hospital. All cases were confirmed by microscopic examination and culture of repeated clinical specimens.

Results
Annual distribution of cases:

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The majority of patients had pulmonary infections and the most frequent isolate was Aspergillus sp. followed by Mucor sp and Fusarium sp. In some cases the culture revealed more than one different pathogens. All the patients were immunosuppressed, and almost half of them had haematological malignancies or were bone marrow transplant recipients.

Conclusion
- Aspergillus sp was the most frequently isolated pathogen.
- A gradual rise in the annual incidence of cases observed during the first decade of the study. The last five years the incidence remained stable.
- Measures to minimize environmental exposure to fungal spores, including use of HEPA filters and laminar air flow in patients rooms and avoidance of areas of hospital construction or renovation are currently emphasised.
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SURGICAL TREATMENT OF RECURRENT ASPERGILLOSIS OF AORTA

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Video and oral presentation of the surgical treatment of recurrent aspergillosis of the aorta in a nine-year-old child.
ACUTE RESPIRATORY FAILURE IN ICU PATIENTS DUE TO ASPERGILLUS TRACHEOBRONCHITIS: REPORT OF THREE CASES

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Aspergillus tracheobronchitis is an uncommon type of invasive pulmonary aspergillosis, in which fungal infection involves exclusively or predominantly the tracheobronchial tree. We describe three patients admitted to the ICU with severe acute respiratory failure due to airway obstruction caused by Aspergillus tracheobronchitis.

The first patient was a 60-yr old male with erytholeukemia after the first course of chemotherapy who developed complete collapse of the left lung due to left main bronchus occlusion by intraluminal fungus growth. Total leukocyte count was 100/mm³. Diagnosis was made by fiberoptic bronchoscopy. The pathologic examination of the intraluminal mass and the bronchial washing culture revealed Aspergillus fumigatus.

The second patient was a 22-yr old female with lymphoma who developed both lobar atelectasis and pulmonary infiltrates leading to severe respiratory failure, 3 months after completing the immunosuppressive therapy. Fiberoptic bronchoscopy revealed extensive endobronchial obstruction with a mass strongly adhered to the bronchial wall. Both histologic examination and culture of that mass revealed Aspergillus terreus.

The third patient was a 42-yr old male with acute lymphoblastic leukemia who developed right lower lobe consolidation and acute respiratory failure during the myelotoxicity phase of his first chemotherapeutic course. Total leukocyte count was 220/mm³. Bronchoscopy revealed hyperemic, friable mucosa with regional ulceration and presence of yellowish pseudomembranes. Histologic examination and bronchial washing culture confirmed the presence of Aspergillus of mixed niger and fumigatus type.

In all 3 patients the diagnosis was made by fiberoptic bronchoscopy after intubation and mechanical ventilation. According to the classification proposed for this entity, (1, 2) in the first 2 patients bronchoscopic findings were consistent with the ‘obstructing’ type of bronchial aspergillosis and in the last patient with the ‘pseudomembranous’ type. All patients had common characteristics representing major risk factors for invasive pulmonary aspergillosis, including underlying disease, persistent and profound granulocytopenia, and previous administration of broad-spectrum antimicrobial agents and corticosteroids. All patients received antifungal therapy includind amphotericin and voriconazole. Despite supportive care, all patients died. We conclude that Aspergillus tracheobronchitis should be considered in immunocompromised ICU patients with suspected lung infection and acute respiratory failure even in presence of atelectasis. Bronchoscopy should be recommended as early as possible to both diagnose and maintain airway patency.

References
INVASIVE ASPERGILLOSIS (IA) IN AUTOPSY OF PATIENTS WITH HEMATOLOGICAL MALIGNANCIES (2000-2004)

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Despite the expansion of antifungal armamentarium, mortality of invasive aspergillosis (IA) remains high. We analyzed risk factors, clinical characteristics, and sites of involvement in patients with hematological malignancies and autopsy-proven IA (1/2000-12/2004). We evaluated 38 patients with IA (one third were hematopoietic stem cell transplantation [HSCT] recipients). The vast majority of patients (74%) had active hematologic malignancy at autopsy. Significant dose of corticosteroids (58%) and severe neutropenia (55%) were the predominant risk factors for IA. However, a significant proportion of patients (61%) had multiple (> 4) risk factors for IA such as CMV co-infection (13%), treatment with purine analogs (32%) or TNFa inhibitors (11%); and/or other co-morbidities such as bacterial sepsis (58%), renal failure (39%), liver dysfunction (34%), hypoalbuminemia (<2.5mg/dl; 32%), diabetes mellitus (11%), and splenectomy (11%). In addition, 10/14 of the allogeneic HSCT recipients (71%) had severe, corticosteroid-refractory graft-versus-host disease (GVHD) at autopsy.

Half of IA cases (53%) were breakthrough to antifungal agents. Most (24/38, 63%) of IA cases were diagnosed antemortem (EORTC/MSG criteria). The vast majority (32/38, 84%) of Aspergillus infections contributed significantly to the patient death. Disseminated IA was found in one third (13/38, 34%) of cases, with equal rates in neutropenic patients and non-neutropenic patients who had received a high dose of corticosteroids. GI tract (16%), heart (16%), CNS (13%), and liver (8%) comprised the most common sites of Aspergillus dissemination. Dissemination was suspected antemortem in only 4/13 (31%) of cases.

Our study demonstrates that suboptimal diagnosis by conventional methods along with profound and often irreversible impairment of host immune responses represent formidable challenges in managing this devastating opportunistic mycosis.
EFFECT OF ANTIFUNGAL AGENTS ON GALACTOMANNAN RELEASE

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Background
Detection of serum galactomannan by EIA is a useful clinical tool in the diagnosis of invasive aspergillosis (IA). Enhancing the sensitivity of this test could allow for earlier diagnosis and treatment of IA. Since galactomannan is anchored to the A. fumigatus cell wall via 1-3Beta-glucan linkages, we hypothesized that caspofungin, which inhibits the formation of these linkages, may enhance galactomannan release, potentially enhancing the sensitivity and specificity of serum galactomannan determinations.

Methods
To determine the effects of antifungal agents on galactomannan release in vitro, A. fumigatus strain AF293 was grown overnight in YPD medium. The resulting hyphae were resuspended in RPMI media supplemented with varying concentrations of caspofungin acetate, amphotericin B desoxycholate or voriconazole. Serial aliquots of the supernatant were removed and assayed for galactomannan concentration using the Biorad Platelia EIA. Galactomannan concentration was interpolated from a standard curve generated using serial dilutions of the sample that contained the highest concentration of galactomannan.

Results
Exposure of A fumigatus hyphae to 0.5 x MEC of caspofungin for 8 hr resulted in an average 5.8-fold increase in galactomannan release compared to hyphae grown in the absence of drug. Exposure to voriconazole increased the release of galactomannan, by 3.1-fold. Interestingly, amphotericin B was associated with an overall reduction galactomannan release (0.73-fold at 8 hours of exposure). To test the effects of differing doses of caspofungin acetate on the release of galactomannan we compared the effects of 0.25, 0.5 and 1 x MIC of caspofungin on galactomannan release. All three drug concentrations resulted in the same increase in free galactomannan levels at all time points tested.

Conclusion
Caspofungin, and to a lesser extent voriconazole exposure acutely increases galactomannan release in vitro. This effect was not seen with amphotericin B. Further, this effect was evident at even 0.25 x MEC levels of caspofungin. These results suggest that administration of a single dose of caspofungin may be a useful strategy for transiently increasing circulating galactomannan levels for the purpose of diagnosis. Testing of this strategy in vivo is currently underway.
FUNGAL PATHOGENES IN AIR OF INTENSIVE CARE UNITS. PRELIMINARY REPORT

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Rooms of medical staff usually has got a great number of the pathogenic microorganisms. In case of the immunocompromised hospitalized patients this increase a potential risk of nosocomial infections. Aim of study: assessment of the incidence of fungal pathogens in air of the intensive care unit at a one of hospital in Kavala (Greece). Material and methods: Investigations were conducted in the selected intensive care unit rooms from a one of hospital in Kavala. Material into mycological studies was air sampled at the entrance of hospital building, the entrance into operating room, hall and the selected rooms of operating department. The microbial flora from the walls was detected using the Count-Tact applicator and the plate Count-Tact (BioMerieux). Fungi were identified using the standard microbial procedures. The monitoring of airborne fungi pollution was done using a SAS SUPER 100 (pbi international). Classification of isolated fungi was made with an accordance to the current procedures. Humidity and temperature were evaluated by a termohigrometr.

Results

In the air of operating rooms was isolated a significant number of the fungal colonies 0 to 110. In the morning the highest number of fungi was isolated at the hall of entrance into the operating department and afternoon in the central sterilization. The following fungal pathogens isolated from air were Candida albicans, non-Candida albicans, Mucor species, Penicillium species and Aspergillus species. Mean number of fungi colonies isolated from air was 180 ± 140.3, mean temperature 25.3 ± 0.7 and humidity 57.5 ± 2.1.

Conclusions

1. We noted decreasing tendency of number of the fungal pathogenes in the operating rooms and their increase in the other rooms. 2. The main fungal pathogen isolated from the air samples was Candida albicans. 3. No significant differences between number of fungal colonies, temperature and humidity of air were found. 4. Further investigations on isolation of the fungal pathogenes from the air samples of operating rooms are needed.
CENTRAL NERVOUS SYSTEM ASPERGILLOSIS IN CHILDHOOD: A SYSTEMATIC REVIEW

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Invasive aspergillosis is a continuously increasing cause of excessive mortality in immunocompromised children. The brain and the subarachnoid space are anatomic and functional barriers against fungal infections. However, under special conditions, such as trauma, surgery, and immune system abnormalities, fungal pathogens from areas outside the brain breach these anatomic barriers. In particular, CNS infections due to Aspergillus spp. have been considered as devastating infections with very limited treatment options and extremely poor outcome. No comprehensive analysis of CNS aspergillosis cases in children has been published to date.

Methods
The PUBMED database was searched for English publications of pediatric cases (0-18 years) of CNS aspergillosis. The references cited in the above articles were screened for additional cases of CNS infections due to Aspergillus spp. A p value of <0.05 indicated statistical significance.

Results
Seventy-four cases of CNS aspergillosis in patients less than 18yr have been recorded up to June 2005. The median age of the patients was 7.5yr (range 16d-18yr), 23% were infants (<1yr) and 53.4% were males. The most common type of CNS aspergillosis was that of single or multiple brain abscesses and was found in 43/67 (64.2%), followed by vasculitis or meningoencephalitis/encephalitis in 7 cases (10.4%) each. The most frequent Aspergillus species isolated, in cases where species identity was reported, was Aspergillus fumigatus in 83.3%. Among 16 infants, prematurity was the predominant underlying condition (in 4 of them or 25%). Among 54 patients of >1yr, leukemia was the underlying disease in 21 (38.9%) of them. Specifically, acute lymphoblastic leukemia was reported in 15/21 (71.4%), and acute myelogenous leukemia in 6/21 cases (28.6%, p=0.017). Less frequent underlying conditions were liver transplantation in 6 (11.1%), chronic granulomatous disease in 5 (9.3%), solid tumors in 4 patients (7.4%), and various other conditions in 27.8%. The antifungal treatment of the published cases consisted of deoxycholate amphotericin B (DAMB) alone in 13/31 (41.9%) cases, and DAMB with flucytosine, itraconazole or rifampicin in 10/31 (32.3%) cases. Lipid formulations of amphotericin B alone were used in 2/31 (6.5%) cases or together with other antifungals in 4/31 (12.9%) especially as treatment of the relatively more recently reported cases. Voriconazole was used as monotherapy in only one recently published case. Surgery as an intralesional debridement was used in 23/52 (44.2%) cases. Of a total of 61 patients with reported outcome, it was favorable in 28 (45.9%) of them. Specifically, among 36 patients reported after 1990, 22 (61.1%) survived as compared to only 6 (24%) among 25 patients before 1990 (p<0.01).
Conclusions
While CNS aspergillosis in childhood has been associated with high mortality (54%), this has been considerably lower than that reported for adult patients until recently (>80%). Premature birth and leukemia are the predominant conditions underlying CNS aspergillosis in pediatric age. The survival rate has significantly increased after 1990 due to probably earlier diagnosis and introduction of amphotericin B lipid formulations and active triazoles.
Invasive fungal infections represent a great threat in immunocompromised patients and are a significant cause of morbidity and mortality worldwide. We represent a case of a 49 years old lady who admitted our hospital on August 21, 2005 with low grade fever, dyspnea, hyperleucocytosis, anemia and severe thrombocytopenia. The blood smear, bone marrow aspiration and immunophenotype put the diagnosis of T-cell acute lymphoblastic leukemia. The patient received the first cycle of induction chemotherapy according to Hyper-CVAD regimen [HM. Kantarjian et al, JCO 2000;18(3):547-561] and achieved complete hematologic remission on day 17 of the first cycle. She returned home having a performance status of grade ‘0’ (ECOG).

During her second admission on September 17, 2005 while on hematologic plus immunophenotypic complete remission, she received the second cycle of induction chemotherapy (high dose of Cytarabine and Methotrexate). On day 9 of the second cycle she developed malaise with high grade fever while she was on severe neutropenia. She was treated with combination iv antibiotic therapy with tazobactam + piperacillin for the first three febrile days of neutropenia. The fever did not regress and the next day the patient developed septic shock and medication shift to meropenem plus linezolid plus voriconazole. The chest X-Ray and the CT-scan of the thorax revealed inflammatory infiltration of the upper and lower areas of both lungs. The blood cultures were negative whereas Vancomycin Resistant Enterococcus (VRE) and Pseudomonas aeruginosa were eliminated by stool culture and skin lesion respectively. Colistin was added against Ps. aeruginosa according to skin lesion and urine culture results. She recovered from septic shock two days later, the fever declined and obtained a low grade pattern, her neutrophil count was increased but her clinical state did not improve and patient started to complain of headache and nausea. A restaging of leukemia status on day 20 of the second cycle, while on hematologic recovery, with bone marrow aspiration plus immunophenotype and a CT-scan of the upper and lower abdomen was compatible with continuous complete remission. A CT-scan of the face and the brain on day 21 because of consistent headache were negative for lesions. A lumbar puncture on day 25 revealed cerebrospinal infiltration (CSF) by neutrophils, while Fontana-Masson staining of the CSF revealed hyphae of Aspergillus fumigatus. Galactomannan and PCR of both CSF and blood were positive for Aspergillus as well. Liposomal amphotericin was added to voriconazole and a lumbar puncture was repeated fourteen days later. The CSF examination was still positive for Aspergillus infiltration, although MRI of the brain was negative for brain lesions. On day 44 she became lethargic and developed cervical rigidity (meningism).
A new CT-scan of the brain put the diagnosis of subarachnoid hemorrhage. As she became unconscious and further deteriorated she was intubated and moved to the Intensive Care Unit, where she remained febrile, comatose and finally developed severe cerebral oedema, and died. CNS fungal infections are rare complications with high mortality rate. This case is very rare because proven systemic aspergillosis involves blood and CSF without any brain lesions.
In this study, 263 cases of birds were examined at the center of Mycology, Faculty of Veterinary Medicine, University of Tehran. Two hundred and seventeen birds suspected having pulmonary aspergillosis, 29 cases had ocular lesions and 17 cases were affected with skin lesions. Laboratory positive results of lung, ocular and skin lesions were obtained in 185 cases (85.3%), 23 cases (79.3%) and 12 cases (70.6%) respectively. The most frequent aspergillus isolated were A. fumigatus (70.4%) and A. flavus (22.7%). Also, A. niger (3.6%), A. terreus (2.3%) and A. ustus (0.9%) were isolated in this study. In respect to the frequency of A. fumigatus with other isolates, significant differences were observed. Chickens were the most affected birds (85%). In this study, all kinds of the birds showed pulmonary form of aspergillosis. Also, ocular aspergillosis were observed in Canary (3 cases) and chickens (19 cases), and in pigeon, ocular and skin aspergillosis (1 case) were confirmed. Aspergillus fumigatus was the most frequent isolated in all kinds of birds, but in canary, the most isolate was A. flavus (61.5%). Young birds were most involved with pulmonary lesions (96.1%), whereas the ocular and skin lesions were mainly observed in adults.
ASPERGILLOSIS IN GOLDFISH (CARASSIUS AURATUS) IN IRAN

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In this study about 20 goldfish with integumentary fungal infections were examined. In clinical examination, white, grey or green cotton mass in skin (slimy glistening mass when fish is out of water) of goldfish were observed. Injuries were showed on the back of operculum, trunk, the front and back of peduncle. Methods of diagnosis included: Wet mount of skin, culture from injuries, using SDA (Sabouro Dexterose Agar), CMA (Corn Meal Agar), GPA (Glucose Pepton Agar), then incubating in 25 degree centigrade and routine mycological study were done. Obtained results included: In wet mount in most cases, septate hyphae and in some cases, aseptate hyphae. In mycological study, Aspergillus species (Aspergillus flavus, Aspergillus niger and Aspergillus sp.) were the most isolated fungi. Mucur and Fusarium sp isolated from some cases too.
FUNGAL SINUSITIS AMONG THE PATIENTS PRESENTING IN A NEWLY ESTABLISHED TERTIARY CARE HOSPITAL IN NORTHERN INDIA

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The fungal sinusitis is one of the emerging infections and if it is not treated in time it is invariably leading to substantial complications in the surrounding vicinity, particularly the orbit. A total of seventy cases of fungal sinusitis presented in the Government Medical College Hospital, Chandigarh during the last five years. All these patients were having either unilateral or bilateral polypoidal masses in the paranasal sinuses. The male patients were 31 and females were 39. They were belonging to almost all ages, the youngest patient was 4 years old and eldest was 66 years, however, most of the patients were from the active age group of their life. All these patients were thoroughly investigated for any of the underlying disease and it was established that none was having any significant immunodeficiency disorder including HIV. None of the patients was having diabetes mellitus as well. The extent of lesions was assessed by radiological techniques like CT scan and MRI. The excision biopsy was done in all these patients and biopsy material was homogenized and examined in 10% potassium hydroxide wet mount. A portion of the biopsy was kept in a tube in 20% potassium hydroxide at 37°C for an overnight period and then examined under the microscope for presence of fungal elements in the biopsy material. On detection of septate hyphae with acute angle branching, the case was taken to be positive for a fungal etiology. Simultaneously histopathological examination was also done to supplement the fungal etiology by performing hematoxylin and eosin (H&E), periodic acid Schiff (PAS) and Gomori’s methenamine silver (GMS) stains. The biopsy material was grown on the Sabouraud dextrose agar (SDA) and Brain Heart Infusion (BHI) agar and kept at 37°C and simultaneously at 25°C for a period of four weeks. If there was no growth upto four weeks the culture was taken as sterile. This study revealed that 41 patients had Aspergillus flavus, 4 patients had Aspergillus fumigatus, 1 patient Aspergillus niger and 10 patients had Aspergillus species. The other fungal agents like Curvularia geniculata and Candida albicans were found in one each. The fungal cultures in rest of the 12 patients were found to be sterile despite their direct wet mount preparations and histopathological examinations were found to be positive. The patients were treated with amphotericin B, 0.6mg/kg of body weight. A follow up dose of itraconazole was also give as 200 mg twice a day for a period of two weeks. The patients improved substantially and there was no death reported in any of the patients due to this underlying pathology of the sinuses. Most of these patients are still on regular follow up in the Department of oto-rhino-laryngology with no significant complaint. Therefore, fungal sinusitis should be kept as one of the differential diagnosis in such type of patients so that diagnosis can be made in time and complications due to this disease can be avoided.
SYNCHRONOUS OCCURRENCE OF ASPERGILLOSIS AND SQUAMOUS CELL CARCINOMA OF THE MAXILLARY SINUS

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Background
Synchronous occurrence of colonizing aspergillosis and squamous cell carcinoma of the maxillary sinus has only rarely been reported. Therefore we want to demonstrate our experience in four of these patients.

Patients and method
Among 80 patients with colonizing aspergillosis of the antrum (diagnosed by histopathology) there were 4 patients (2 male, 2 female) that were diagnosed at the same time with squamous cell cancer in the same location. The bony walls were affected in all patients. No lymphadenopathy was observed (T4 N0). The two male patients were smokers. All were operated by maxillectomy. All patients received postop. radiotherapy.

Results
Patient age ranged from 51 to 71 years (mean 59 yrs.). No recurrence of aspergillosis was noted during the observation period of 1,6 to 8,5 years (mean 4 yrs.). One patient died after 20 months due to bronchial cancer, all other patients are alive. One female patient developed two recurrences and is free of tumor at present. Histopathology revealed the typical hyphae of aspergillus lying close to the cancer but not invading it. In one female patient a positive fungus culture revealed A. fumigatus. By chromatography it could be demonstrated that this fungus yielded a high amount of the mycotoxin gliotoxin.

Discussion and conclusion
Although intriguing it is difficult to establish a causal relationship between the two lesions. As gliotoxin (proven in just one case) is known to be an immunosuppressant but not a carcinogenic mycotoxin, other local or environmental factors have to be considered as well. Until more cases have been carefully investigated it has to be assumed that these observations are coincidental. In our opinion the most likely explanation is that necrotic parts of the carcinoma become colonized by fungal spores inhaled via the nose.
Invasive aspergillosis is a rare disease that is usually limited to immunocompromised patients. It occurs more commonly in patients with acute leukemia and prolonged neutropenia. We report four cases of disseminated aspergillus infection in children with confirmed chronic granulomatosis disease (CGD), a primary immunodeficiency disorder in which phagocytic cells are defective in generating microbicidal reactive oxidants. Pulmonary infection followed by osteomyelitis and dermal or hepatic abscesses were the most common findings in these patients. Aspergillus fumigatus has been isolated as causative agent in all cases. Only one of the patients responded to the therapy (Deoxycholate Amphotericin B and interferon-gamma) and the rest died regardless of antifungal and surgical therapy. With respect to the high frequency of aspergillosis in the CGD patient, immune deficiency should be investigated in patients with invasive aspergillosis. Moreover, using antifungal drugs as prophylaxis can improve the quality of life and lessen the risk of re-infection in these patients.
A SUCCESSFUL, FIVE MONTHS LASTING MULTI-DRUG THERAPY FOR INVASIVE ASPERGILLOSIS IN A CHRONIC GRANULOMATOUS DISEASE PATIENT

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Chronic granulomatous disease (CGD) is a rare, inherited, genetically heterogeneous disorder with impaired intracellular killing of microorganisms due to ineffective respiratory burst. The disease is characterized by severe, recurrent or persistent infections caused by catalase-positive bacteria and fungi and diffused granulomata. Aspergillus, especially Aspergillus fumigatus and Aspergillus nidulans, is a common cause of pneumonias and abscesses of brain and lungs. It is the most common cause of mortality in CGD patients. Particularly severe course with poor outcome has been observed in cases of pneumonia with involvement of the chest wall and vertebræ and dissemination to the central nervous system.

We present the boy with CGD diagnosed based on NBT slide negative test at the age of 29 months. The boy was admitted to Department of Immunology due to six weeks lasting fever, coughing and vomiting, lost of his weight and asymmetry of the precordial area. On examination the dyspnoe, tachycardia, lymphadenopathy, hepatomegaly and asymmetry of the chest wall were found. The elevated parameters of the inflammation were detected by the laboratory tests. The diagnosis of invasive aspergillosis was confirmed by chest HRCT scan, bronchoalveolar fluid culture and histopathology examination. The monotherapy with voriconasol was introduced but after 12 days no improvement was noted. Addition of caspofungin caused transient worsening followed by improvement in general condition, laboratory and imaging examination. Voriconasol had to be stopped due to hematuria and renal insufficiency in the further course of treatment. During caspofungin monotherapy worsening was observed again and liposomal amphoterycyn B was added. Double-drug caspofungin and amphoterycyn B therapy was conducted for 16 days. After this time the recurrence of fever was observed. The caspofungin therapy was stopped and liposomal amphoterycyn B in the higher doses was continued for 76 days with the significant clinical improvement. After this time the patient was put on voriconasol treatment with good tolerance.

Conclusion
Aspergillus infection is a very difficult clinical problem in CGD patients. It requires long, sometimes multi-drug therapy.
MOLECULAR EPIDEMIOLOGY OF ASPERGILLUS USTUS STRAINS RECOVERED FROM PATIENTS WITH INVASIVE EYE INFECTIONS AND ASPERGILLEMIA IN A TERTIARY CARE HOSPITAL

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Aspergillus species are commonly isolated from the soil, plant debris, and the indoor environment, including the hospital. Aspergillosis, primary or secondary, or invasive or noninvasive, occurs worldwide and the clinical manifestations are largely determined by the local or general immunologic state of the host. Here, we report the molecular epidemiology of eight A. ustus isolates from four patients, of them, three with fungal endophthalmitis occurring after cataract surgery and one aspergillemic pediatric patient with osteopetrosis. Genotyping study of the strains by random amplification of polymorphic DNA (RAPD) method showed that seven A. ustus isolates from ophthalmologic patients had similar banding pattern. The remaining strain was different than others in five electrophoretic loci by RAPD. Environmental sampling performed in the Department of Ophthalmology yielded the growth of Alternaria spp., Penicillium spp., and Aspergillus fumigatus. Although A. ustus was not isolated environmentally, the observation of similar banding pattern for all ophthalmologic strains suggested us existence of an unidentifiable common source. The ongoing renovation in that department may contribute to development of cases in cluster. Molecular studies are an important necessity of epidemiological investigation as shown in this study.
ANTERIOR CHEST WALL PROTRUSION DUE TO ASPERGILLOSIS MASS AS INITIAL PRESENTATION OF CHRONIC GRANULOMATOUS DISEASE (CGD): REPORT OF A CASE

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Background/Objectives
Chronic granulomatous disease (CGD) is a rare primary immunodeficiency disease that characterized by recurrent life-threatening bacterial and fungal infections and abnormal tissue granuloma formation. Infections in CGD present most commonly as pneumonia, liver abscess, skin abscess, perianal abscess, and osteomyelitis.

Case report
We report a 4-month-old female infant who was admitted with difficult breathing and tachypnea associated with anterior chest wall protrusion. First doubt was a spindle cell tumor such as rhabdomyosarcoma, but Computed Tomography (CT) scan showed a mediastinal abscess in the chest. Culture of the aspirated fluid was positive for Aspergillus. Furthermore, neutrophil leucocytosis, hypochromic microcytic anaemia and elevated ESR found merely reflect response to chronic infection. Finally in evaluation of immune system, NitroBlue-Tetrazolium (NBT) test revealed defect in neutrophil respiratory burst pathway so CGD was diagnosed for her. The patient died despite of intensive antifungal treatment.

Conclusions
The early descriptions of children with CGD characterized them as presenting with lymphadenopathy, hepatosplenomegaly, growth failure, and stigmata of chronic skin infections. Our patient had no family history of immunodeficiency and no these signs expect hepatosplenomegaly. So what makes this case interesting is that she had chest wall mass in the absence of pulmonary involvement and as the first presentation of CGD. In the other hand, typical physical findings may be less commonly observed now, probably due to inappropriate antibiotic therapy in early infancy or childhood. Delay in diagnosis may be result in death. Therefore, we tried to make clinical physician keep in mind the diagnosis of CGD by atypical presentation of this cases with evaluate immunity state especially for neutrophil dysfunction and attention to correct fallow up of these patients particularly in first year of life.
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PREVALENCE OF BACTERIAL AND FUNGAL AGENTS CAUSING CHRONIC RESPIRATORY TRACT INFECTIONS IN HIV/AIDS PATIENTS

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Aims and Objectives
This study was designed to document the prevalence of HIV associated respiratory infections with locally and/or systemically Immunocompromised conditions and correlate the role of clinical and radiological parameters of these patients with laboratory investigations in the early diagnosis and management.

Materials and Methods
130 patients with complaints suggestive of lower respiratory tract infection. Among them 100 were HIV reactive and 30 were HIV nonreactive. Thorough clinical, hematological and radiological examination were carried out. Sputum, bronchial aspirate/BAL and serum samples were collected. Microscopy and culture for Aspergillus spp in addition to detection of specific anti-A.fumigatus IgG and IgE by ELISA and skin sensitivity tests against Aspergillus spp. Both the expectorated as well as samples were collected and processed to examine for the bacterial and fungal pathogens. The data were edited and analysed by EPI info program.

Results
Sputum samples from 63% of HIV reactive and 33.3% of HIV nonreactive patients were culture positive. In all, there were 70 pathogens isolated from the HIV reactive subjects, 44.3% were bacteria, 42.9% were Mycobacteria and 12.8% were diagnosed as Invasive Aspergillosis (IA), IA was found to be most common in <20 and >60 years of age with male: female ratio of 2.5:1.

Conclusions
Lower respiratory tract infection is a common problem among HIV reactive patients and majority are bacterial infections. There is a strong clinical suspicion, a rigorous laboratory work up in the form of microscopy, culture and immunological studies is warranted for an early diagnosis and management of IA in order to avoid serious and fatal complications in the developing country where TB prevalence is high.
FUNGAL ENDOPHTHALMITIS CAUSED BY ASPERGILLUS USTUS IN A PATIENT FOLLOWING CATARACT SURGERY

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Ophthalmic mycoses are being increasingly recognized as an important cause of morbidity and blindness. Many fungal species including aspergilli are recovered as a cause of ocular infections. Although postoperative endophthalmitis due to various Aspergillus species has been previously described, to our knowledge, the first case of postoperative endophthalmitis after cataract surgery caused by Aspergillus ustus, a species that has only rarely been implicated in human disease, is reported here. A 67-year-old medically controlled diabetic patient presented with uveitis, mild ciliary injection and ocular discomfort six weeks after cataract surgery. Anterior chamber paracentesis, vitreous tap and finally complete vitrectomy with removal of the capsular bag including the intraocular lens were performed and culture of several specimens yielded Aspergillus ustus. Despite vigorous systemic (itraconazole and caspofungin) and intravitreal (amphotericin B and caspofungin) antifungal therapy, the endophthalmitis did not improve. The painful eye with marked inflammation was finally enucleated. In vitro susceptibility testing of the isolate showed that it appeared resistant to amphotericin B, caspofungin, itraconazole, voriconazole, and posaconazole, and susceptible to terbinafine.
INVESTIGATIONS OF PLATELETS AND CONIDIA OF ASPERGILLUS SPECIES

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Platelets exert antifungal effects against hyphae of Aspergillus spp. and are capable of binding, aggregation and internalizing microorganisms, which enhances the clearance of pathogens from the bloodstream; platelets play key and multifaceted roles in antimicrobial host defence. In this study we investigated whether platelets are capable of phagocytosis of Aspergillus conidia and examined the release of galactomannan and the germination rate in platelet treated fungi. The in vitro tests were performed on clinical isolates of Aspergillus fumigatus (n=2) and Aspergillus terreus (n=1). Platelet rich plasma from trisodium citrate-anticoagulated blood was prepared by centrifugation. For phagocytosis platelets were labelled with anti-CD42b FITC-conjugated antibody (40µl/10^6 cells, 10 min) and conidia were stained with calcofluor white (10 min). Labelled platelets were coincubated with conidia (10:1, platelets:conidia) for 15 and 30 min shaking at room temperature. Conjugate formation and phagocytosis were visualised with a confocal laser scanning microscope (LSM) using appropriate filters. In addition transmission electron microscopy (TEM) investigations were performed.

Galactomannan production was determined by a commercial kit (Platelia Aspergillus; Bio-Rad) according to the manufacturer’s instructions. 100µl each of 1x10^4 cfu/ml conidial suspension and 1x10^8/ml platelets were incubated for 4, 6, 8 and 12 h. Untreated fungi served as controls. The morphology of A. fumigatus and A. terreus conidia treated with or without platelets was investigated by assessing germination and hyphal elongation. Therefore, 100µl of 2 x 10^4 cfu/ml conidial suspension were inoculated into microwell plates and incubated with 100µl of 1 x 10^8 platelets at 37°C for 12 h. Untreated conidia served as controls. The morphology of the organisms was determined microscopically at the time point indicated.

Platelets were not able to phagocytose conidia but showed extensive conidia-associated-platelets aggregation as shown by LSM and TEM. Inhibition of conidial germination was decreased under platelet treatment (P<0.05). 69% and 98% of conidia did not germinate in A. fumigatus and A. terreus, germination in untreated controls were 92% and 90%, respectively. The decreased germination rate correlated with reduced production of galactomannan (P<0.05). Taken together, our findings suggest that platelets offer antifungal properties against conidia of Aspergillus spp.. The in vivo role of platelets attracts growing attention and some clinical studies clearly point to such function of platelets in man.
FUNGAL KERATITIS IN CORNEAL ULCER'S PATIENTS AT BOO ALI SINA HOSPITAL, SARI, IRAN

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Purpose
Fungal keratitis is a suppurative, ulcerative and sight-threatening infection of the cornea that sometime leads to loss of the eye. The aims of this study were to improve facilities for laboratory diagnosis, to determine the predominant causative micro-organisms, to identify the predisposing factor, and encourage rapid referral of mycotic keratitis patients.

Materials and Methods
A prospective study of corneal ulcer was conducted in Sari between May 2004 and March 2005. Patients who presented with clinically suspected corneal ulcer at the eye unit of Boo-Ali Sina University Hospital in Sari were included in the study. Each patient was examined at the slit lamp. Data were collected by examining and questioning patients. Using standard techniques, a corneal scraping was performed by an ophthalmologist. Corneal materials obtained from scraping the ulcer was smeared onto two slides which were stained with one Gram stain (for bacterial keratitis) and 10% potassium hydroxide (KOH) with or without calcofluor white (KOH+CFW) stain (for fungal keratitis) for microscopic examination. Material was inoculated directly onto blood agar, Sabouraud dextrose agar, and Potato dextrose agar in C-shaped streaks.

Results
A total of 22 patients met the inclusion criteria of this study, among which 10 (45.5%) were female and 12 (54.5%) were male. The average age of the patients was 61.5 ± 17.7 years (with a range of 15-83 years). In direct microscopy branching septate hyphae was identified in 7 patients (31.8%). Two (28.6%) fungi (Aspergillus fumigatus and Fusarium Spp) isolated. Five Patients (71.4%) with fungal keratitis were male and 2 (28.6%) female. The average age of the patients with fungal keratitis was 60.4 ± 12.1 years (with a range of 39 to 73 years). Three (42.85%) of patients with fungal keratitis were farmer. The mean interval between the onset of symptom and diagnosis was 26.4 with a range of 1 to 93 days. Trauma with plant debris and straws were noted in two patients (28.6%) with fungal keratitis. Five patients (71.4%) received topical antibiotics. Analyses using KOH + CFW as the gold standard revealed individual sensitivities for detection of fungi for KOH, Gram stain were 71.4% and 42.9%, respectively.
Conclusion
Infections of the cornea due to filamentous fungi are frequent. Causes of corneal damage and should always be kept in mind. Direct microscopy method is an essential tool in the diagnosis of fungal keratitis. Thus wet mount with KOH+CFW or only KOH can be relied upon as the single most important screening for rapid diagnosis of fungal corneal ulcer and treatment should be prescribed on its bases.

Key word
Corneal ulceration, fungal keratitis, keratomycosis, Diagnosis, KOH+CFW
SUCCESSFUL TREATMENT OF ASPERGILLUS ENDOCARDITIS IN A NATIVE VALVE WITHOUT PRIOR CARDIAC SURGERY

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Aspergillus infective endocarditis in patients without prior cardiac surgery is extremely rare, and is very difficult to cure with few cases of survival reported previously. We describe a patient with native valve endocarditis due to Aspergillus species, who was successfully treated. A 68-year-old man with sigmoid colon cancer developed multiple organ failure with liver abscess due to Klebsiella pneumoniae, brain abscess, sepsis, ARDS, and renal failure, and was transferred to our institute. During his ICU stay, Aspergillus endocarditis was confirmed by mitral valve vegetation revealed by echocardiogram and serologic test. Blood culture grew methicillin-resistant Staphylococcus aureus. Although surgical debridement of vegetation was not believed to be feasible at that time because of his poor general condition, intensive management, resection of colon cancer, and treatment with voriconazole and vancomycin enabled him to undergo the cardiac surgery consisting of debridement of vegetation from the posterior leaflet of the mitral valve and mitral valve repair. He currently continues to receive treatment of oral voriconazole and is doing well.

Intensive management including nutritional support to improve general condition, leading to immunological improvement, treatment with voriconazole, and subsequent surgical debridement seemed to be the key for this patient’s survival.
Thromboembolic episodes after open heart surgery is not uncommon, especially after valve replacement. The source of the embolus is usually prosthetic valve or aortic suture line. Bacterial infective endocarditis is another cause of thromboembolic episodes. Cardiac fungal infections however are rare and are usually associated with disseminated fungemia. Immunosuppression, especially with corticosteroids is a major factor in developing infection. The most common fungal organism to cause endocarditis is candida (62%), which is followed by aspergillus at 18%. Fungal endocarditis results in large bulky vegetations and they often embolise. Destruction of the involved tissue can often lead to devastating results. Signs and symptoms are non-specific and diagnosis is difficult owing to negative blood cultures. Treatment options are antifungal drug therapy, surgery or combination. Outcome is nearly always fatal and recurrence is not uncommon.

A 73-year-old man underwent an uneventful aortic valve replacement and coronary artery bypass grafting. He was readmitted three months later with two episodes of embolic occlusion of his lower limb vessels, which were treated with embolectomy. Trans-oesophageal echocardiography (TOE) and CT scan revealed a ‘thrombus’ in the ascending aorta and arch. Patient was subsequently operated due to unresponsive nature of the thrombus to the anticoagulation treatment. During the operation the ‘thrombus’ was found to be bulky, white and friable with evidence of aortic wall destruction. Aortic prosthesis was free from vegetations. Microbiology and histology confirmed the diagnosis of Aspergillosis. Ascending aorta and proximal arch was then excised and replaced with a prosthetic graft. In spite of aggressive medical treatment postoperatively, the patient died due to multiorgan failure.
Objective
We prospectively evaluated the efficacy of itraconazole intravenous/oral solution-based therapy for refractory central nervous system (CNS) fungal infection, and its safety of combination with amphotericin B, flucytosine and/or fluconazole.

Materials and methods
The study was conducted as a non-randomized open-label trial between June 2004 and September 2005. A total of 17 patients admitted to our hospital with definite (n=14) or probable (n=3) refractory CNS fungal infection were enrolled. In this study, patients treated for a minimum of 7 days were included in the efficacy evaluation. Treatment was individualized based on the patient’s clinical/microbiological conditions. In general, intravenous itraconazole was given 200 mg every 12 hours for the first 48 hours, followed by 200 mg once daily from days 3 to 14 and then oral itraconazole solution (200mg q12h) thereafter. Three patients (one each with cerebral aspergilloma, *Aspergillus* meningitis, and cryptococcal meningitis) were treated with itraconazole alone, the all others were treated with a combination of amphotericin B, flucytosine and/or fluconazole with the itraconazole. Efficacy was assessed based on clinical (resolution of symptoms/signs and microbiological) response. The safety of itraconazole was evaluated by determining the presence of adverse events. Patients receiving one dose of itraconazole were evaluated for safety.

Results
All patients had headache, fever and their intracranial pressures were higher than 300mmH$_2$O. Seven patients had various underlying diseases such as hematopoietic stem cell transplantation, decompensated hepatocirrhosis, diabetes mellitus, etc. Seven patients were complicated with coma and ten had cerebral herniation. There were two episodes of intolerance to amphotericin B (hepatocirrhosis, nephropathy, etc) and three foci of intracranial granulomatous lesions. Six patients underwent Ommaya-reservoir and ventricular shunt procedures. Four patients failed treatment with amphotericine B with or without flucytosine. Three of seventeen patients were considered unevaluable for efficacy because they died when treated with itraconazole after only 2, 6, 2 days respectively. The identified fungi were *Cryptococcus neoformans*, *Aspergillus* spp., and *Prototheca wickerhamii*. Eleven patients (11/14, 78.6%) had an excellent clinical response, including one patient each with cerebral aspergilloma, *Aspergillus* meningitis, *Prototheca wickerhamii* meningitis, two patients with cerebral cryptococcoma, and six patients with cryptococcal meningitis (including three patients treated with itraconazole alone). The remaining three patients with cryptococcosis...
had partial responses. All patients tolerated the itraconazole. Five patients developed non-serious elevated liver function tests possibly or probably due to intravenous itraconazole, and three patients had mild to moderate nausea during two months of oral itraconazole.

**Conclusion**
Itraconazole intravenous/oral solution shows impressive activity on refractory CNS fungal infection in this series of patients who can be treated safely with either itraconazole alone or combined with other antifungal drugs.
SUCCESSFUL TREATMENT OF CEREBRAL ASPERGILLOSIS WITH SEQUENTIAL INTRAVENOUS AND ORAL ITRACONAZOLE IN IMMUNOCOMPETENT PATIENTS: CASE REPORT AND LITERATURE REVIEW

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Central nervous system aspergillosis is the most serious infection in invasive aspergillosis. Conventional antifungal therapy mainly based on amphotericin B has shown unsatisfied results. We report two cases of cerebral aspergillosis treated with sequential intravenous and oral itraconazole solution over a relatively long-term period (>3 months). The patients were free from neurological infection symptoms and signs with the single antifungal drug. A series of CSF recovered and/or cranial MRI showed that the lesions decreased dramatically. To our knowledge, this is the first report of cerebral aspergillosis treated successfully with sequential itraconazole new for
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Making a Difference in Infectious Diseases

Astellas’ involvement in developing medical treatment of infectious diseases has been ongoing since the 1970s and continues today. Our commitment to the future development of new medications has been demonstrated by:

- Pioneering the conduct of empiric antifungal clinical trials
- Initiating some of the world’s largest clinical trials in fungal infections
- Implementing pediatric and neonatal trials
- Collaborating with the medical community
- Sponsoring a clinical surveillance program to examine therapeutic management and patient outcomes for invasive fungal infections

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