Diagnostics of Invasive Aspergillosis: From Experimental Models to Clinical Evaluation

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Inhalation Chamber Apparatus for Aspergillosis

- Acrylic chamber for up to 40 mice
- Standard respiratory therapy nebulizer
- Compressed air tank with regulator

Sheppard. AAC 2004;48:1908
Inhalational Models Advantages

- Relatively low inoculum (~1000 conidia) delivered to alveoli
- Recapitulates human disease
- Reproducible outcome between different experiments and laboratories
- Late mortality
  - Allows multiple time point sampling without survivor bias
- Mortality complete by 14 days

Sheppard. AAC 2004;48:1908
Sheppard. AAC 2006;50:3501
Neutropenic vs. Non-neutropenic Model

- **Neutropenic:**
  - Cyclophosphamide at 250 mg/kg SC on day -2 and 200 mg/kg SC on day +3 of infection
  - Cortisone acetate at 5 mg/mouse SC on days -2 and +3 of infection

- **Non-neutropenic:**
  - Cortisone acetate at 10 mg/mouse SC on days -4, -2, 0, +2, +4 of infection
  - Ceftazidime IP while immunosuppressed
Effects of Different Types of Immunosuppression on Mouse Survival

Neutropenic
(Cortisone Acetate + Cyclophosphamide)

Non-neutropenic
(Cortisone Acetate)

Spikes. JID 2008;197:479
More Rapid Development of Disease in the Non-neutropenic Model

Day 4

**Neutropenic**
(Cortisone Acetate + Cyclophosphamide)

**Non-neutropenic**
(Cortisone Acetate)

Chiang. I&I 2008;76:3429
Lung GM Levels are Higher in Non-Neutropenic Mice

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Neutropenic (Cortisone Acetate + Cyclophosphamide)</th>
<th>Non-neutropenic (Cortisone Acetate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected</td>
<td><img src="infected.png" alt="Infected" /></td>
<td><img src="infected.png" alt="Infected" /></td>
</tr>
<tr>
<td>Uninfected</td>
<td><img src="uninfected.png" alt="Uninfected" /></td>
<td><img src="uninfected.png" alt="Uninfected" /></td>
</tr>
<tr>
<td>GM Concentration (U/g lung)</td>
<td><img src="gm_concentration.png" alt="GM Concentration" /></td>
<td><img src="gm_concentration.png" alt="GM Concentration" /></td>
</tr>
</tbody>
</table>
Serum GM Levels are Higher in Neutropenic Mice

**Neutropenic**
(Cortisone Acetate + Cyclophosphamide)

**Non-neutropenic**
(Cortisone Acetate)
The Pulmonary Cytokine Response is Different in Neutropenic vs. Non-neutropenic Mice

Chiang. I&I 2008;76:3429
Effects of Different Types of Immuno-suppression on Virulence Mechanisms

**Neutropenic**

![Graph showing survival rates for AF293 glIP mutant and AF293 in neutropenic conditions.]

**Non-neutropenic**

![Graph showing survival rates for AF293 ace2 mutant and AF293 in non-neutropenic conditions.]

Posaconazole is Highly Efficacious in the Neutropenic Model

N>18 mice/group. All treatments prolonged survival p<0.0150. N=15 for 1h. N>30 mice/group. All treatments reduced lung fungal burden compared to controls p<0.0213.

Najvar LK, et al, ICCAC 2007 (abstract M-1848)
Posaconazole is Less Efficacious in the Non-neutropenic Model

N=10 mice/group. Controls all succumbed by day 4 or 8. The median survival was prolonged for LAMB (p=0.006) in A and both PSC (p=0.007) and LAMB (p=0.009) prolonged survival in B.

No Therapy Reduced Pulmonary Tissue Burden in the Non-neutropenic Model

Inoculum: $7.4 \times 10^7$ Conidia/mL. N= 5-10 mice/group. No therapy reduced the fungal burden.

<table>
<thead>
<tr>
<th></th>
<th>Neutropenic</th>
<th>Non-neutropenic</th>
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</thead>
<tbody>
<tr>
<td>Mortality</td>
<td>Late</td>
<td>Early</td>
</tr>
<tr>
<td>Pulmonary GM</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Serum GM</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Influence of AF virulence factors on mortality</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Response to posaconazole</td>
<td>Good</td>
<td>Poor</td>
</tr>
</tbody>
</table>
Contributors and Collaborators

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