Stage-specific Innate Immune Recognition of *Aspergillus fumigatus* and Modulation by Echinocandin Drugs

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A. fumigatus Germination

- Swelling
- Germination
- Hyphal Formation

Intact pulmonary immune defense

Conidial clearance

Defective pulmonary immune defense

Tissue-invasive hyphae
Alveolar Macrophages in Host Defense

- Sentinels at the portal of entry
- Conidial Phagocytosis
- Trigger effector cell recruitment through release of chemokines (CXCL1, CXCL2)
- Release inflammatory mediators
- Kill conidia in a phagocyte oxidase-dependent manner in vitro (Philippe et al., Infect. Immun, 2003)


Downloaded 1/09/08 at: http://upload.wikimedia.org/wikipedia/commons/thumb/4/43/S3-Alveolar_Macrophages_with_Conidia_in_Liquid_Medium.ogg/mid-S3-Alveolar_Macrophages_with_Conidia_in_Liquid_Medium.ogg.jpg
Neutrophils in Host Defense

- Mice depleted of neutrophils or with defective neutrophil trafficking are highly susceptible to invasive apergillosis (Mehrad et al., JI, 1999; Bonnett et al., Infect. Immun., 2006)

- **Antifungal effector functions**
  - NADPH oxidase (Morgenstern et al., JEM, 1997)
  - Granule proteins
    - Neutrophil Elastase, Cathepsin G (Tkalcevic et al., Immunity, 2000)
    - Lactoferrin (Zarember et al., J. Immunol., 2007)
    - Pentraxin-3 (Garlanda et al., Nature, 2002)
  - Neutrophil BAL Aggregates (Bonnett et al., Infect. Immun., 2006)
  - Neutrophil Extracellular Traps (Jaillon et al., JEM, 2007)
How do conidia trigger host inflammatory responses?
Live Conidia induce Neutrophil Recruitment into the BAL fluid at 24 hours p.i.
Live Conidia induce TNF/ CXCL2 Secretion by Alveolar Macrophages

**Graphs:**
- **TNF**
  - Live: 45 ng/ml
  - HK: 15 ng/ml
  - Medium: 6 ng/ml
  - LPS: 6 ng/ml

- **CXCL2**
  - Live: 18 ng/ml
  - HK: 15 ng/ml
  - Medium: 6 ng/ml
  - LPS: 6 ng/ml

**Legend:**
- **Live**: Live conidia
- **HK**: Heat-killed conidia
- **Medium**: Culture medium
- **LPS**: Lipopolysaccharide
Killed germinating Conidia are highly inflammatory

[Diagram]
- Swelling
- Germination
- Hyphal Formation

<table>
<thead>
<tr>
<th>Time</th>
<th>TNF</th>
<th>CXCL2</th>
</tr>
</thead>
<tbody>
<tr>
<td>t = 0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5 hours</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7 hours</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>&gt; 12 hours</td>
<td>&gt; 12</td>
<td>&gt; 12</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Time</th>
<th>TNF</th>
<th>CXCL2</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 hours</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5 hours</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>7 hours</td>
<td>0</td>
<td>9</td>
</tr>
</tbody>
</table>

Heat-killed
Killed swollen Conidia induce Neutrophil Influx into the BAL fluid

<table>
<thead>
<tr>
<th></th>
<th>Total Cells</th>
<th>Macrophages</th>
<th>Neutrophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat-killed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>swollen conidia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Live Conidia</td>
<td></td>
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</tbody>
</table>
Swollen Conidia and Germlings expose β-glucan on their surface

Anti β-glucan

Isotype Control Ab
Conidia Stimulate Dectin-1- and MyD88-dependent Pathways

**TNF**

- WT
- MyD88^/-

**CXCL2**

- WT
- MyD88^/-
Modulation of Host Inflammatory Responses by Antifungal Therapy

- Echinocandins target fungal-β-D-glucan synthase
- Echinocandins reduce *A. fumigatus* bulk β-glucan levels
- Echinocandins do not fully inhibit *A. fumigatus* growth, yet induce prominent morphologic changes at or above the MEC

![1 x MEC Caspofungin](image1)
![No Caspofungin](image2)
Echinocandins Alter the *C. albicans* β-glucan Surface Content at sub-MIC Concentrations

C. *albicans* Caspofungin MIC$_{50}$ 2.5 ng/ml

Caspofungin Exposure Decreases Macrophage Inflammatory Responses to *A. fumigatus* Conidia

<table>
<thead>
<tr>
<th>Caspo (ng/ml)</th>
<th>0</th>
<th>4</th>
<th>8</th>
<th>16</th>
<th>31</th>
<th>63</th>
<th>125</th>
<th>250</th>
<th>500</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF (ng/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

BMMφ TNF/CXCL2 release (500 ng/ml caspofungin vs. no drug exposure):
- **TNF**  \(0.49 \pm 0.04^*\) (range 0.46-0.54; n=4)
- **CXCL2**  \(0.55 \pm 0.10^*\) (range 0.43-0.62; n=4)
Caspofungin Exposure Decreases Macrophage Inflammatory Responses to *A. fumigatus* germlings

**BMMφ TNF/CXCL2 release (500 ng/ml caspofungin vs. no drug exposure)**

- **TNF**  
  \[0.51 \pm 0.07^* \text{ (range 0.43-0.64; n=7)}\]
- **CXCL2**  
  \[0.61 \pm 0.08^* \text{ (range 0.53-0.74; n=7)}\]
Reduced Inflammatory Responses to Conidia and Germlings Reflect Diminished Dectin-1 Signaling
Caspofungin Exposure enhances Inflammatory Responses to *A. fumigatus* Hyphae

BMMφ TNF/CXCL2 release (500 ng/ml caspofungin vs. no drug exposure)

- **TNF** 4.11 ± 2.39* (range 1.90-7.84; n=8)
- **CXCL2** 2.90 ± 1.40* (range 1.53-5.41; n=8)
Increased Dectin-1 Signaling Accounts for Enhanced Responses to Drug-treated Hyphae

- Dectin-1-dependent TNF release
- Dectin-1-independent TNF release

![Graph showing TNF and Caspo levels](image)
Effects of Echinocandin Drugs on β-glucan Exposure

<table>
<thead>
<tr>
<th>Caspofungin</th>
<th>No Caspofungin</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIC anti-β-glucan</td>
<td>DIC anti-β-glucan</td>
</tr>
<tr>
<td>8 h</td>
<td>10 h</td>
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</tbody>
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[Images showing effects at 8 and 10 hours]
### Effects of echinocandin drugs on fungal β-glucan exposure

<table>
<thead>
<tr>
<th></th>
<th>Caspofungin</th>
<th>No Caspofungin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DIC</td>
<td>anti-β-glucan</td>
</tr>
<tr>
<td>12 h</td>
<td></td>
<td></td>
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<tr>
<td>15 h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 h</td>
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</tr>
</tbody>
</table>

- **Caspofungin**
  - No Caspofungin: DIC and anti-β-glucan images show changes over time.
  - Caspofungin: Images indicate reduced β-glucan exposure compared to non-treated controls.

- **No Caspofungin**
  - Images show consistent β-glucan exposure throughout the time points.

Images depict cellular structures under DIC and anti-β-glucan staining conditions.
Echinocandin drugs increase $\beta$-glucan surface immunoreactivity on hyphae
Quantitative Analysis of β-glucan Immunoreactivity associated with Caspofungin-treated and Untreated Hyphae

<table>
<thead>
<tr>
<th></th>
<th>Integrated Fluorescence Intensity/Fungal Mass (Arbitrary Units)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Caspofungin-treated Hyphae</td>
</tr>
<tr>
<td>Expt. 1</td>
<td>21.4 ± 8.3*</td>
</tr>
<tr>
<td>Expt. 2</td>
<td>43.7 ± 7.0*</td>
</tr>
</tbody>
</table>

Each value represents the average ratio (± SD) of β-glucan immunofluorescence intensity normalized to hyphal mass as calculated from 4-5 fields of view per condition.

* p <0.02 compared to control condition (untreated hyphae).
Summary (Part II)

• Echinocandin drugs alter inflammatory responses to *A. fumigatus*
  - by altering fungal surface β-glucan levels
  - and triggering dectin-1-dependent responses

• Enhanced inflammatory responses to drug-treated hyphae represents a novel mechanism of action that is independent of effects on fungal growth

• This immunopharmacologic mechanism of action may have implications for prophylactic and therapeutic strategies for invasive aspergillosis

• Similar results for Aspergillus and non-Aspergillus molds presented by Lamaris et al., 47th ICAAC, Chicago, IL, September 17-20th, 2007 (Abstract M-1857 and M-1858).
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