Aspergillosis-the role of the host

Is aspergillus really an opportunistic infection?

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CONFLICT OF INTEREST

• Sponsored symposium: Pfizer, Astellas, MSD, Gilead

• Grants: Gilead, Astellas
• Pathogenesis of invasive aspergillosis

• Clinical risk factors for invasive aspergillosis
• **Pathogenesis of invasive aspergillosis**

• **Clinical risk factors for invasive aspergillosis**
**INNATE RESPONSE IS QUITE SPECIFIC!**

Pathogen-associated molecular patterns (PAMPs): glucan, chitin, and galactomannan

- They are characteristic of pathogens and they are never found in the host.
- They are invariable in different species.
- They are essential for the survival or pathogenicity of the microorganism.

**Cytokine response**
Boychud PY et al. Toll-like receptor 4 polymorphisms and aspergillosis in stem-cell transplantation


**Graph A:**
- **Donor genotype**
- **Recipient genotype**

**Graph B:**
- **Donor genotype**
- **Recipient genotype**
Cunha C et al. Genetic PTX3 Deficiency and Aspergillosis in Stem-Cell transplantation

Table 2. Multivariate Analysis of the Association of Donor PTX3 Variants with the Risk of Invasive Aspergillosis among Transplant Recipients in the Discovery and Confirmation Studies.*

<table>
<thead>
<tr>
<th>Donor PTX3 Variant</th>
<th>Discovery Study (N = 268)</th>
<th>Confirmation Study (N = 330)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adjusted Hazard Ratio</td>
<td>Adjusted Odds Ratio</td>
</tr>
<tr>
<td></td>
<td>(95% CI)†</td>
<td>(95% CI)‡</td>
</tr>
<tr>
<td>+281A/G SNP, GG genotype</td>
<td>2.92 (1.69–5.05)</td>
<td>2.14 (1.20–3.80)</td>
</tr>
<tr>
<td>+734A/C SNP, AA genotype</td>
<td>2.62 (1.52–4.54)</td>
<td>1.92 (0.91–3.04)</td>
</tr>
<tr>
<td>Haplotype h2/h2</td>
<td>3.08 (1.47–6.44)</td>
<td>2.78 (1.22–8.93)</td>
</tr>
</tbody>
</table>

*HLA-maching, total body irradiation, GVHD, antifungal prophilaxis
Cytokine response against *Aspergillus* infection

**Pro-inflammatory cytokines**
- TNF-alpha, INF-gamma
- IL-12, IL-15

**Anti-inflammatory cytokines**
- IL-10, IL-4

**Innate immune system**

**Protection against fungal infections**

**Impaired host defense against fungal infections**

Garcia-Vidal C, et al; *Curr Opin Infect Dis* 2014
De Boer M et al. Influence of polymorphisms in innate immunity genes on susceptibility to invasive aspergillosis after stem cell transplantation
Plos one 2011; 6: e18403

Table 2. Genetic polymorphisms in the innate immune system considered of potential influence on susceptibility to invasive aspergillosis.

<table>
<thead>
<tr>
<th>Gene name</th>
<th>SNPdb id</th>
<th>Position nucleotide change</th>
<th>Reported effect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL1B</td>
<td>rs16944</td>
<td>-511C&gt;T</td>
<td>Negatively influences IL-1β levels. A higher frequency of the IL1B -511TT genotype was found in patients with IA as compared to patients without IA.</td>
<td>Wilkinson et al. [31] Sainz et al. [32]</td>
</tr>
<tr>
<td>IL10</td>
<td>rs1800872</td>
<td>-592A&gt;C</td>
<td>Promotor SNP, protective effect in conjunction with the −1082 and −819 IL10 promoter polymorphisms</td>
<td>Seo et al. [33]</td>
</tr>
<tr>
<td>IL10</td>
<td>rs1800896</td>
<td>-1082G&gt;A</td>
<td>Promotor SNP, conferring a diminished expression of the IL10 gene and a subsequent protective effect with respect to IA</td>
<td>Sainz et al. [22]</td>
</tr>
<tr>
<td>IL12B</td>
<td>rs41292470</td>
<td>GC&gt;CTCTAA</td>
<td>Promotor SNP, reported influence on response to tuberculosis; association with IA unknown</td>
<td>Sahiratmadja et al. [34]</td>
</tr>
<tr>
<td>TLR1</td>
<td>rs5743611</td>
<td>239G&gt;C</td>
<td>Associated with IA in ASCT recipients</td>
<td>Kesh et al. [16]</td>
</tr>
<tr>
<td>TLR1</td>
<td>rs4833095</td>
<td>743A&gt;G</td>
<td>Associated with IA in ASCT recipients when present in combination with the TLR6 745C&gt;T polymorphism</td>
<td>Kesh et al. [16]</td>
</tr>
<tr>
<td>TLR4</td>
<td>rs4986791</td>
<td>1363C&gt;T</td>
<td>Associated with IA when present in donor DNA of ASCT recipients</td>
<td>Bochud et al. [14]</td>
</tr>
<tr>
<td>TLR4</td>
<td>rs4986790</td>
<td>1063A&gt;G</td>
<td>Associated with IA in ASCT when present in recipient DNA. Associated with IA when present in donor DNA of ASCT recipients</td>
<td>Carvalho et al. [15] Bochud et al. [14]</td>
</tr>
<tr>
<td>TLR6</td>
<td>rs5743810</td>
<td>745C&gt;T</td>
<td>Associated with IA in ASCT recipients when present in combination with the TLR1 743A&gt;G polymorphism</td>
<td>Kesh et al. [16]</td>
</tr>
</tbody>
</table>
**Lupiañez CB et al.** Polymorphisms in host immunity-modulating genes and risk of invasive aspergillosis: results from the AspBIOmics consortium

*Infect Imm 2016; 84: 643-657*

**TABLE 3** Associations found between immunoregulatory polymorphisms and invasive aspergillosis

<table>
<thead>
<tr>
<th>Variant dbSNP</th>
<th>Gene</th>
<th>Phase 1</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>OR (95% CI)</td>
<td>P value</td>
</tr>
<tr>
<td>rs2243248</td>
<td>IL4</td>
<td>1.19 (0.63-2.26)</td>
<td>0.59</td>
</tr>
<tr>
<td>rs2070874</td>
<td>IL4</td>
<td>0.91 (0.53-1.55)</td>
<td>0.72</td>
</tr>
<tr>
<td>rs2243268</td>
<td>IL4</td>
<td>0.85 (0.52-1.38)</td>
<td>0.50</td>
</tr>
<tr>
<td>rs2243290</td>
<td>IL4</td>
<td>0.67 (0.39-1.16)</td>
<td>0.14</td>
</tr>
<tr>
<td>rs2057768</td>
<td>IL4R</td>
<td>1.20 (0.75-1.92)</td>
<td>0.44</td>
</tr>
<tr>
<td>rs2107356</td>
<td>IL4</td>
<td>2.05 (1.24-3.40)</td>
<td>0.0063</td>
</tr>
<tr>
<td>rs1801275</td>
<td>IL4</td>
<td>1.00 (0.63-1.59)</td>
<td>0.99</td>
</tr>
<tr>
<td>rs4073</td>
<td>IL8</td>
<td>1.02 (0.64-1.61)</td>
<td>0.95</td>
</tr>
<tr>
<td>rs2227307</td>
<td>IL8</td>
<td>1.72 (1.00-2.94)</td>
<td>0.049</td>
</tr>
<tr>
<td>rs2234671</td>
<td>IL8A</td>
<td>1.57 (0.80-3.08)</td>
<td>0.20</td>
</tr>
<tr>
<td>rs1126580</td>
<td>IL8B</td>
<td>1.50 (0.88-2.54)</td>
<td>0.13</td>
</tr>
<tr>
<td>rs3024491</td>
<td>IL10</td>
<td>1.09 (0.67-1.78)</td>
<td>0.72</td>
</tr>
<tr>
<td>rs3024496</td>
<td>IL10</td>
<td>1.16 (0.71-1.90)</td>
<td>0.55</td>
</tr>
<tr>
<td>rs582054</td>
<td>IL12A</td>
<td>1.09 (0.64-1.84)</td>
<td>0.76</td>
</tr>
<tr>
<td>rs3212227</td>
<td>IL12B</td>
<td>0.57 (0.35-0.93)</td>
<td>0.021</td>
</tr>
<tr>
<td>rs20541</td>
<td>IL13</td>
<td>0.76 (0.46-1.24)</td>
<td>0.26</td>
</tr>
<tr>
<td>rs1800925</td>
<td>IL13</td>
<td>0.85 (0.54-1.36)</td>
<td>0.51</td>
</tr>
<tr>
<td>rs1295686</td>
<td>IL13</td>
<td>0.73 (0.45-1.16)</td>
<td>0.18</td>
</tr>
<tr>
<td>rs2069705</td>
<td>IFNγ</td>
<td>0.56 (0.36-0.88)</td>
<td>0.012</td>
</tr>
<tr>
<td>rs1861494</td>
<td>IFNγ</td>
<td>0.74 (0.47-1.17)</td>
<td>0.20</td>
</tr>
<tr>
<td>rs1059293</td>
<td>IFNγR2</td>
<td>0.98 (0.57-1.67)</td>
<td>0.93</td>
</tr>
<tr>
<td>rs9808753</td>
<td>IFNγR2</td>
<td>1.10 (0.65-1.85)</td>
<td>0.72</td>
</tr>
<tr>
<td>rs1799987</td>
<td>CCR5</td>
<td>1.40 (0.83-2.36)</td>
<td>0.20</td>
</tr>
<tr>
<td>rs2734648</td>
<td>CCR5</td>
<td>1.07 (0.67-1.71)</td>
<td>0.76</td>
</tr>
<tr>
<td>rs755622</td>
<td>MIF</td>
<td>1.38 (0.84-2.25)</td>
<td>0.20</td>
</tr>
<tr>
<td>rs25648</td>
<td>VEGFA</td>
<td>1.11 (0.63-1.97)</td>
<td>0.72</td>
</tr>
<tr>
<td>rs6999947</td>
<td>VEGFA</td>
<td>1.28 (0.75-2.18)</td>
<td>0.35</td>
</tr>
<tr>
<td>rs3024994</td>
<td>VEGFA</td>
<td>1.61 (0.86-3.03)</td>
<td>0.15</td>
</tr>
<tr>
<td>rs3025035</td>
<td>VEGFA</td>
<td>1.31 (0.78-2.22)</td>
<td>0.31</td>
</tr>
<tr>
<td>rs2146323</td>
<td>VEGFA</td>
<td>1.63 (1.02-2.61)</td>
<td>0.040</td>
</tr>
<tr>
<td>rs3024997</td>
<td>VEGFA</td>
<td>1.04 (0.67-1.61)</td>
<td>0.87</td>
</tr>
<tr>
<td>rs3025030</td>
<td>VEGFA</td>
<td>1.00 (0.58-1.70)</td>
<td>0.99</td>
</tr>
<tr>
<td>rs998584</td>
<td>VEGFA</td>
<td>0.66 (0.41-1.06)</td>
<td>0.088</td>
</tr>
<tr>
<td>rs6899540</td>
<td>VEGFA</td>
<td>0.84 (0.51-1.40)</td>
<td>0.50</td>
</tr>
<tr>
<td>rs6900017</td>
<td>VEGFA</td>
<td>1.76 (1.02-3.03)</td>
<td>0.046</td>
</tr>
<tr>
<td>rs6905288</td>
<td>VEGFA</td>
<td>0.83 (0.52-1.31)</td>
<td>0.42</td>
</tr>
</tbody>
</table>

© by author
Prospective study matching 36 patients with AL or SCT and invasive aspergillosis with 36 patients equal in age, comorbidity, treatment and day of hematologic diseases treatment.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>55.54 (12)</td>
</tr>
<tr>
<td>AL/SCT</td>
<td>63% / 37%</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>55 (70.5%)</td>
</tr>
<tr>
<td>AI proven/probable</td>
<td>15.5%/84.5%</td>
</tr>
</tbody>
</table>
- Waiting for final results

- High levels in patients with IA; marker of worst outcomes

Garcia-Vidal C et al. Cytokine response against invasive aspergillosis in HSTC recipients and AL patients-real life

Unpublished data
FIG. 1. Diagrammatic representation of diseases attributed to *Aspergillus* species as a function of the host’s immune response. ABPA, allergic bronchopulmonary aspergillosis.
Spores (conidias)

A. flavus
A. niger

> 5-10 micras

Aspergillus fumigatus

< 5 micras

10-100 m³
Innate immune system

Adaptative immune system

Macrophages disfunction (steroids, viral infections)

- IL-10
- TNF-alpha
- IL-17

Dendritic cell

- IL-10
- TNF-alpha
- IL-17
Innate immune system

Adaptative immune system

Macrophages

Leucocytes (PMN)

Chronic necrotizing aspergillosis

- **neutropenia** < 100
- **Chronic granulomatous disease**

- Macrophages disfunction (steroids, viral infections)

- **Invasive aspergillosis**
Avascular area
Difficult to access for antifungals

Ischemic necrosis
Ischemic necrosis


Neutropenia: excessive hyphal growth and dissemination
Host immune response and clinical forms of aspergillosis and diagnostic

**Chronic necrotizing aspergillosis**
- Less invasion
- Cavitation forms
- Need for BAL (GMN > 0.8)

**Invasive aspergillosis**
- Angioinvasion
- Halo sign. Air crescent sign
- Serum GMN > 0.5

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Figure from Taylor R, Clin Microbiol Rev 2009: 447-465
• Pathogenesis of invasive aspergillosis

• Clinical risk factors for invasive aspergillosis
Garcia-Vidal C et al. Epidemiology of invasive mold infections in allogenic SCT recipients: biological risk factors for infection according to time after transplantation. 
Clin Infect Dis 2008; 47 1041-50

EARLY INFECTION

- > 40 years old
- Underlying disease
- Transplant related factors
- Cytopenias
- Respiratory viruses

LATE INFECTION

- CMV diseases
- Graft versus host diseases
- Corticosteroids
- Blood transfusion
Garcia-Vidal C et al. Epidemiology of invasive mold infections in allogenic SCT recipients: biological risk factors for infection according to time after transplantation
Clin Infect Dis 2008; 47 1041-50

> 40 years old

Underlying disease

Transplant related factors

Cytopenias

Respiratory viruses

CMV diseases

Graft versus host diseases

Corticosteroids

Blood transfusion

Dorshkind K et al, Nature reviews immunology 2009
Garcia-Vidal C et al. Epidemiology of invasive mold infections in allogenic SCT recipients: biological risk factors for infection according to time after transplantation
Clin Infect Dis 2008; 47 1041-50

> 40 years old

Underlying disease

Transplant related factors

Cytopenias

Respiratory viruses

CMV diseases

Graft versus host diseases

Corticosteroids

Blood transfusion
Garcia-Vidal C et al. Epidemiology of invasive mold infections in allogenic SCT recipients: biological risk factors for infection according to time after transplantation

Clin Infect Dis 2008; 47 1041-50

> 40 years old

Underlying disease

Transplant related factors

Cytopenias

Respiratory viruses

CMV diseases

Graft versus host diseases

Corticosteroids

Blood transfusion

dissemination → angioinvasion
Epidemiology of invasive mold infections in allogenic SCT recipients: biological risk factors for infection according to time after transplantation

Clin Infect Dis 2008; 47 1041-50

> 40 years old

Underlying disease

Transplant related factors

Cytopenias

Respiratory viruses

CMV diseases

Graft versus host diseases

Corticosteroids

Blood transfusion
Invasive aspergilosis
The presence of environmental airborne mould spores increased the risk of IA admission 6 times after the third 2-week period.
• The presence of environmental airborne mould spores increased the risk of IA admission 55 times after the third 2-week period during circulating viral period.

> 40 years old

Underlying disease

Transplant related factors

Cytopenias

Respiratory viruses

CMV diseases

Graft versus host diseases

Corticosteroids

Blood transfusion

The overall acute GVHD cascade

Crucial factors in the development of chronic GVHD
> 40 years old

Underlying disease

Transplant related factors

Cytopenias

Respiratory viruses

CMV diseases

Graft versus host diseases

Corticosteroids

Blood transfusion
Impaired phagocytosis, degranulation and oxidative burst
Impaired formation of nitric oxide
Inhibition of apoptosis

Lymphopenia
Decreased proliferation and migration of lymphocytes
Decreased cytokine production
Decreased Th1 and increased Th2 cytokine production

Impaired phagocitosis and oxidative killing
Impaired maturation of macrophages
Inhibition of pro-inflammatory cytokine production

Decreased counts of alveolar dendritic cells
Impaired antigen-presenting capacity of dendritic cells
Decreased cell-killing capacity
Fig. 1. Increase in total hyphal length (µm) with time (h) in germlings of isolate AF-6 grown at 37 °C with (●) or without (○) 10^{-6} M hydrocortisone. At each time point the mean ± SEM of five replicate germlings was plotted.
Garcia-Vidal C et al. Epidemiology of invasive mold infections in allogenic SCT recipients: biological risk factors for infection according to time after transplantation

Clin Infect Dis 2008; 47 1041-50

> 40 years old

Underlying disease

Transplant related factors

Cytopenias

Respiratory viruses

CMV diseases

Graft versus host diseases

Corticosteroids

Blood transfusion

- Decreased Th1 and increased Th2 cytokine production in vitro
- Reduced responses in mixed lymphocyte culture
- Decreased proliferative response to mitogens or soluble antigens in vitro, thus causing impaired delayed-type hypersensitivity skin responses
- Increased CD8 T cells or suppressor function in vitro
- Decreased natural killer cells and activity in vitro
- Decreased CD4 helper T cells
- Decreased monocyte/macrophage function in vitro and in vivo
- Enhanced production of anti-idiotypic antibodies suppressive of mixed lymphocyte response in vitro
- Decreased cell-mediated cytotoxicity against target cells in vitro
- Humoral alloimmunization to cell-associated and soluble antigens
- Increased T-regulatory cells and function

Iron Overload

In addition to patients with hemoglobinopathies (eg, thalassemia, sickle cell disease), those with
HOW CAN WE APPLY ALL THIS KNOWLEDGE TO HELP THE HOST TO FIGHT TO ASPERGILLUS AND TO IMPROVE THE OUTCOMES OF OUR PATIENTS?
Thanks for your attention

Contact: cgarcia@clinic.cat