Host Response to Fungal Infections

- immunology
- immunogenetics
- fungal pathogenicity

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Superficial fungal infections

Systemic fungal infections

<table>
<thead>
<tr>
<th>Mycosis</th>
<th># life-threatening cases/year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillosis</td>
<td>&gt;200,000</td>
</tr>
<tr>
<td>Candidiasis</td>
<td>&gt;400,000</td>
</tr>
<tr>
<td>Cryptococcosis</td>
<td>&gt;1,000,000</td>
</tr>
<tr>
<td>Mucormycosis</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>Pneumocystosis</td>
<td>&gt;400,000</td>
</tr>
<tr>
<td>Dimorphic (endemic) mycoses</td>
<td>~65,000</td>
</tr>
</tbody>
</table>

mainly nosocomial mortality rate: 30-70%
The spectrum of fungal diseases

The organisms causing disease are mainly opportunistic pathogens

Underhill and Iliev, Nat.Rev.Immunol., 2014
$\sim 10^5$ fungal species (only a few hundreds are pathogenic)

mold (*Aspergillus*)  

yeast (*C. albicans*)
Predisposing conditions for opportunistic fungal infections

- **barrier defects**  e.g. severe burn wounds, catheters
- **dysbiosis**  e.g. vulvovaginal candidiasis, systemic candidiasis
- **chemotherapy**  e.g. in leukemia patients
- **immunosuppression**  e.g. in transplant patients
- **immune defects**  - neutropenia  →  e.g. systemic candidiasis
  - functional neutrophil defects  →  e.g. aspergillosis in CGD patients
  - T cell defects  →  e.g. superficial candidiasis
Despite similar clinical risk factors: some patients rapidly develop fungal infections, while others seem to be protected and never do so.

Genetic Differences?
1. Immunogenetics of systemic candidiasis and aspergillosis

2. Congenic defects associated with superficial fungal infections

3. Genetic diversity of the fungus
1. Immunogenetics of systemic candidiasis and aspergillosis

2. Congenic defects associated with superficial fungal infections

3. Genetic diversity of the fungus
SNP array

- Amplification
- Digestion
- Probe labeling

Patient DNA → SNP array → Hybridization

Allele A + Allele B

- Normal
- Deletion
- Duplication

Screen of 200’000 SNPs in a large candidemia cohort

→ Identify novel genetic risk factors for candidaemia

SNPs limited to the SNPs on the array

Kumar et al., Nat. Communications (2014)
SNP array

→ limited to the SNPs on the array

whole genome sequencing

→ the majority (85%) of all disease-causing mutations occur in the exome
→ the exome is 1-2% of the entire genome
→ WES = cost effective

whole exome sequencing
Candidate gene polymorphisms associated with invasive *Aspergillus* and/or *Candida* infections

Wójtowicz and Bochud, Semin Immunopathol (2015)
Caveats:

- sample size
- study design, controls
- statistical testing
- confirmation by independent cohorts
- functional evidence supporting the association
<table>
<thead>
<tr>
<th>Gene</th>
<th>SNPs ID</th>
<th>Genetic association</th>
<th>Replication</th>
<th>Functional evidence</th>
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<tr>
<td></td>
<td></td>
<td>IA</td>
<td>IC</td>
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<td>Pattern recognition receptors</td>
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<tr>
<td>TLR1</td>
<td>rs574361</td>
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<td></td>
<td>rs4833095, rs5743618</td>
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<td>MBL</td>
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<td>Cytokines and related genes</td>
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<td>IL1A</td>
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<td>IL1RN</td>
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<td>TNFR1</td>
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<td>IFNG</td>
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<td>CCL3</td>
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<td>CXCR1</td>
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<td>CX3CR1</td>
<td>rs3732578</td>
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</tbody>
</table>

Caveats:

• sample size
• study design, controls
• statistical testing
• confirmation by independent cohorts
• functional evidence supporting the association

Is the identified mutation the disease-causing mutation?

→ Validation!

- synonymous vs. non-synonymous SNPs
- exclude SNPs that are frequent gene variants
- analyse the transcriptome and proteome to find the relevance of the mutation
Future perspectives:

- assign each patient an individual risk score based on his genetic background ('genetic prediction')
- apply personalized prophylaxis and treatment schemes (as in modern oncology)
- Whole-Exon-Sequencing will likely be the method of choice.
Identification of novel mutations also promotes the understanding of protective mechanisms against systemic fungal infection.
1. Immunogenetics of systemic candidiasis and aspergillosis

2. Congenic defects associated with superficial fungal infections

3. Genetic diversity of the fungus
Mendelian defects in the IL-17 pathway

Chronic Mucocutaneous Candidiasis (CMC)

- oroal mucosa
- skin
- nails
- vaginal mucosa

e.g. inherited IL-17RC-deficiency  
(Ling et al., JEM, 2015)

→ IL-17 plays a non-redundant role in protection from superficial Candida infection
The IL-17 cytokine family

Monte et al., Infect. Immun. (2013)

Pappu et al., Trends Immunol (2012)
IL-17 in autoinflammatory disease

targeting IL-17 as a therapeutical approach

<table>
<thead>
<tr>
<th>Agent</th>
<th>Mechanism</th>
<th>Psoriasis</th>
<th>Rheumatoid arthritis</th>
<th>Psoriatic arthritis</th>
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<tr>
<td>Secukinumab</td>
<td>Anti-IL-17A</td>
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<td>Ixekizumab</td>
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<td>Brodalumab</td>
<td>Anti-IL-17RA</td>
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<td>SCH 900117</td>
<td>Anti-IL-17A</td>
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<td>Anti-IL-17A/TNF</td>
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<td>RG7624</td>
<td>Anti-IL-17A/IL-17F</td>
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</tbody>
</table>

*Based on ongoing clinical trials according to clinicaltrials.gov on 7 June 2013.

Phase 2 study completed; no clinical trials in RA are ongoing.

Kirkham et al., Immunology, 2013

response rate:

- high
- moderate-low
- moderate

side effects...

“...localized mucosal or cutaneous candidiasis, primarily oral candidiasis. These infections were mild to moderate in severity and were clinically manageable/treatable with standard therapy without discontinuation.”

FDA-approved in 2015
Mendelian defects in the IL-17 pathway

e.g. inherited IL-17RC-deficiency

immortalized fibroblasts from patients (P1, P2) & controls (C1, C3)

response to IL-17 stimulation

transfectants

(Ling et al., JEM, 2015)
• Regulation of IL-17 induction in response to *C. albicans*

• IL-17-mediated effector mechanisms

• IL-17-independent antifungal defense
Experimental model of oropharyngeal candidiasis (OPC)

C. albicans
(strain SC5314)

C57BL/6 (WT)

IL-17RA-/-
IL-17RC-/-

→ acute inflammatory response

day 7 p.i.

Trautwein-Kirchner et al., Mucosal Immunology 2015
Multiple sources of IL-17 during acute OPC infection

- Complementary and redundant cellular sources for the IL-17 response during murine OPC

Gloadiator et al., J. Immunol (2013)
Sparber et al, in preparation

[in humans?]
IL-17 & neutrophils

**Diagram**
- IL-17A, IL-17F
- IL-17RC, IL-17RA
- TRAF5, TRAF6
- NFκB, MAPK
- Target genes, chemokines, cytokines
- Neutrophils

**Images**
- Control vs. neutrophil-depleted
  - Day 1:
    - Untreated control
    - α-G-CSF + 1A8
  - Day 3:
    - CFU/g
    - 1A8
    - α-G-CSF

**References**
Trautwein-Weidner, Gladiator et al., Mucosal Immunology (2015)
Neutrophil recruitment during OPC is IL-17-independent

Trautwein-Weidner, Gladiator et al., Mucosal Immunology (2015)
IL-17 immunity and neutrophil response are uncoupled and act complementary in the oral mucosa.

C. albicans

epithelium

→ confinement of the fungus

neutrophils

IL-17

IL-17-producing cells

C. albicans

IL-17R

IL-17R

IL-17
IL-17 immunity and neutrophil response are uncoupled and act complementary in the oral mucosa

C. albicans

epithelium

tongue, strain SC5314

confined to the fungus 7d

S100a8
S100a9
Lcn2
Defb1
Defb3

IL-17-dependent expression limited to the epithelium

fold induction relative to naive (log2)

-3 0 4 16

IL-17-producing cells

IL-17R

antimicrobial factors

neutrophils

8h 1d 3d 7d

induction relative to naive (log2)

IL-17R

IL-17

IL-17R

IL-17
Identification of genes/pathways that contribute to disease susceptibility

Generation of new hypothesis

Dissection of protective mechanisms at a cellular and molecular level

→ new therapeutic approaches, vaccines
1. Immunogenetics of systemic candidiasis and aspergillosis

2. Congenic defects associated with superficial fungal infections

3. Genetic diversity of the fungus
→ The host immune status determines susceptibility to disease

→ What about the fungus?

- Colonizing population of \textit{C. albicans} is monoclonal
- Vertical transmission
- Large intraspecies diversity (10’000 – 100’000 SNPs)

Impact of the intraspecies diversity on the outcome of infection?

\textit{C. d’Enfert and ME. Bougnoux (Pasteur)}
Very distinct outcomes of infection with different fungal isolates

**SC5314**

**day 1 p.i.**

**day 7 p.i.**

Weight (%)

- **SC5314**: Blue line with squares
- **101**: Red line with circles

**SC5314**

**101**

Log10 (neutrophils/tongue)

Log10 (cfu/g tongue)

**p-value 0.0010**

Pearson r -0.9514

Schönherr et al., Mucosal Immunology, 2017
Pathogenicity correlates with the invasion depth into the epithelium

SC5314

101
day 1 post-inf.

12h post-inf.

stratum corneum
stratum granulosum
stratum spinosum
stratum basale

Schönherr et al., Mucosal Immunology, 2017
The epithelium senses the variation in *C. alicans* and translates it in various degrees of inflammation.

Schönherr et al., Mucosal Immunology, 2017
The epithelium senses the variation in \textit{C. alicans} and translates it in various degrees of inflammation.

\textit{in vivo} (1 day p.i.)

\begin{itemize}
  \item SC5314
  \item IL-1\textalpha
  \item 101
\end{itemize}

Are persistent strains ‘unseen’ by the immune system?

\textit{Schönherr et al., Mucosal Immunology, 2017}
Persistent *C. albicans* isolates do not induce neutrophils

Schönherr et al., Mucosal Immunology, 2017
Delayed induction of the host response with non-pathogenic isolates

kinetic & qualitative differences in the response to different *C. albicans* isolates

Schönherr et al., Mucosal Immunology, 2017
Altmeier et al., unpublished
β-defensins mediate IL-17-dependent fungal control

ALTMEIER, MERTENS AND LeibundGut-LANDMANN., unpublished
IL-17 signaling is essential for fungal control – irrespective of the degree of fungal pathogenicity.

Schönherr et al., Mucosal Immunology, 2017
C. albicans

- The key role of the IL-17 pathway for fungal control is conserved, irrespective of the fungal pathogenicity.
- The neutrophil response and the IL-17 pathway are uncoupled.
- The epithelium translates differences in the fungus into qualitatively distinct host responses.
- Natural variations in C. albicans determine the outcome of fungal-host interactions.
Thank you!

Alumni:
Kerstin Trautwein-Weidner
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Laura Lauener
Patrizia Diethelm
Ana Chavez-Steenbock
Isabelle Zenklusen