Host Defense and Immunomodulation of mucosal Candidiasis

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Candida albicans

- **Dimorphic** fungal organism
  - Blastoconidia (yeast) – 25°C, low pH (< 5.0)
  - Hyphae – 37°C, high pH (> 5.5)
- **Commensal** organism of mucosal tissues - normal flora
  - Developed *Candida*-specific immunity
  - Blastoconidia
- **Opportunistic pathogen** of mucosal tissues
  - Hyphae
    - Oropharyngeal candidiasis (OPC) (Thrush)
    - Denture stomatitis (DS)
    - Vulvovaginal candidiasis (VVC)
      - Recurrent VVC (RVVC)
Candida albicans (in tissue)
Oral Candidiasis
Epidemiology of Mucosal Candidiasis

- Oropharyngeal candidiasis (OPC)
  - Disease of immuno-compromized persons
    - HIV-infected

- Vulvovaginal candidiasis (VVC)
  - Disease of immuno-competent and otherwise healthy women

- Denture Stomatitis (DS)
  - Disease of immuno-competent otherwise healthy denture wearers

- Host defenses (immunity) responsible for protection are expected to be very different for VVC, DS, OPC
Terms - Immunology

**Host defense**

- **Cells**
  - Leukocytes
    - T cells (CD4, CD8); B cells (adaptive IR)
    - Neutrophils; macrophages (innate IR)
  - Epithelial cells (mucosal) (innate IR)

- **Cytokines** – soluble biological response modifiers
  - Interleukins
  - Chemoattractants (chemokines)

- **Receptors** – bridge the cells and cytokines for biological responses
Terms for today’s talk

- Cells
  - CD4 T cells; CD8 T cells**
  - Neutrophils (PMNs)
  - Epithelial cells

- Receptors
  - E-cadherin
  - Annexin-A1

- Cytokines (biological response modifier)
  - Alarmins
Objectives

- Host defense against *Candida albicans* at different mucosal sites is extremely different.

- Immune factors associated with protection and susceptibility to infection are unique to the anatomical site.

- Role of mucosal biofilm in pathogenesis is likewise different for different mucosal sites.
Host defense against Mucosal Candidiasis

- **Dogma for host defense against mucosal candidiasis**
  - CD4 T cells → Protection/resistance to infection

- **Host defense against OPC → dogma with caveats**
  - HIV disease → lose CD4 T cells → increased susceptibility
    - OPC → recurrent infections, sporadic infections; no infections
    - Do other immune responses function in some capacity for protection?
  - CD8 T cells play a role in protection when CD4 T cells lost

- **Host response against VVC → against dogma**
  - No role for CD4 T cells (or CD8 T cells)
  - Epithelial cells (resistance), neutrophils (susceptibility)

- **Host response against DS → ??**
Oropharyngeal Candidiasis (OPC) in HIV Disease

HIV^+OPC^+ <200 CD4 cells
CD8 T cells in OPC lesions

OPC^- site  Buccal mucosa  OPC^+ site

Epithelium  Lamina propria  Normal activated memory T cells

Myers et al., 2003
Cellular migration into tissues

- Circulating lymphocyte enters the high endothelial venule in the lymph node.
- Binding of L-selectin to GlyCAM-1 and CD34 allows rolling interaction.
- LFA-1 is activated by chemokines bound to extracellular matrix.
- Activated LFA-1 binds tightly to ICAM-1 and diapedesises—lymphocyte migrates into the lymph node.
Adhesion molecules in OPC

McNulty et al., 2005

*Longitudinal study- Quimby et al. 2011

\(\alpha_4\beta_7\) (T cells) - **MAdCAM** (tissue)  \(\rightarrow\) Migration to mucosa  \(\rightarrow\) CD8 T cells migrated into tissue

\(\alpha_6\beta_7\) (T cells) - **E-Cadherin** (tissue)  \(\rightarrow\) Migration of cells through mucosa

Dysfunction!
Host susceptibility to OPC

**Figure 1.** OPC: Protection, susceptibility, and results of treatment with ART/PI or IFN-γ

- **OPC- < 200 CD4⁺**
  - Protected
  - *Primary defense: CD4⁺ T cells*
    - below protective threshold
  - *Candida SAPs - low*
  - *Epithelium E-cadherin - high*
  - *Recruited CD8⁺ T cells migrate to epithelium*

- **OPC⁺ < 200 CD4⁺**
  - Susceptible condition
  - *Primary defense: CD4⁺ T cells*
    - below protective threshold
  - *Candida SAPs degrade E-cadherin*
  - *Epithelium E-cadherin - low*
  - *Recruited CD8⁺ T cells halted at lamina propria/epithelium interface*

- **OPC⁻ < 200 CD4⁺**
  - Post-therapy: IFN-γ or ART/PI
  - *Primary defense: CD4⁺ T cells*
    - below protective threshold
  - *Candida SAPs inhibited by PI or IFN-γ*
  - *Epithelium E-cadherin restored*
  - *Recruited CD8⁺ T cells migrate to epithelium*

- **Legend**
  - CD8⁺ T cell
  - * Candida SAP - active
  - * Candida SAP - inactive
  - E-cadherin⁺ epi cell
  - Yeast
  - Hyphae
Host defense against vaginal Candidiasis (VVC)

**No protective role for T cells**

**Tolerance**
Immunoregulation by:
- PC dendritic cells
- TGF-β
- γ/δ T-cells
- CD25⁺Treg cells

**Protection**
Inhibition of *Candida* growth by *Epithelial cells* (Non-inflammatory)
↓
Asymptomatic colonization

Fidel and Coworkers; '90-'04
Fidel and Coworkers; '99-'10
Epithelial cell anti-*Candida* activity

**Primary epithelial cells**

- Oral EC
- Vaginal EC

**Clinical relevance**

OPC in HIV ('00), RVVC ('04)

**OPC**

- Infected; N=12
- Colonized; N=10
- Not colonized; N=6

* p<0.0005 between those infected and colonized

* VVC
# Mechanism of epithelial cell anti-*Candida* activity

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Requires cell contact</strong></td>
<td>No soluble factors</td>
</tr>
<tr>
<td></td>
<td>No phagocytosis</td>
</tr>
<tr>
<td></td>
<td>No oxidative/non-oxidative mechanisms</td>
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<tr>
<td></td>
<td>No microtubules/microfilaments</td>
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<td></td>
<td>No intracellular signaling</td>
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<tr>
<td><strong>Requires intact, but not necessarily live epithelial cells</strong></td>
<td>Sensitive to heat/detergent</td>
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<tr>
<td></td>
<td>Resistant to fixation</td>
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<tr>
<td><strong>Static, not cidal</strong></td>
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<tr>
<td><strong>Acid-labile</strong></td>
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- **Annexin-A1***
- **33 kDa acid-labile protein**

Affects signaling cascades within cells that inhibit growth***

Tool for candidate molecule identification

Fidel and Co-workers ’00–’10
Innate immune symbiosis

- **Static activity** - **Symbiotic relationship**
  - Benefit - host: no inflammation, invasion of tissue
    - Maintains commensalism via Annexin-A1 activity on the yeast
  - Benefit - *Candida*: sacrifices growth for protection against other immune responses and killing
    - form of immune evasion – no **Danger** signals

- Annexin-A1 $\rightarrow$ exploited to enhance activity
Host defense against vaginal Candidiasis (VVC)

Cross-sectional clinical studies; animal models

No protective role for T cells

Tolerance
Immunoregulation by:
• PC dendritic cells
• TGF-β
• γ/δ T-cells
• CD25+ Treg cells

Protection
Inhibition of Candida growth by Epithelial cells (Non-inflammatory)
↓
Asymptomatic colonization

Symptomatic infection
Acute inflammatory response
↓
Neutrophils (PMNs) (non-clearing)

Fidel and Coworkers; '90-'04
Fidel and Coworkers; '99-'10
Fidel and Coworkers; '04-'12
Cellular infiltrate = symptomatic infection

- Women with no history – 90% asymptomatic
- Women with infrequent VVC – 55% symptomatic
- Women with infrequent VVC in susceptible state – 90% symptomatic
Vaginal PMN influx in response to Candida

Humans – natural history

Asymptomatic condition

Symptomatic condition

PMNs
- Candida
- Symptoms

Mouse model

uninoculated

inoculated

Low PMN

High PMN

Epithelial cells

Candida condition

PMNs = pathology

Signal

differential early adherence
Chemotactic Signal??

- **Cytokines/chemokines** → symptomatic vs asymptomatic
  - Pro-inflammatory, activation, T cell
  - Proteomics → identify unique protein in vaginal secretions

- **S100A8/S100A9 proteins** → Alarmins
  - Low MW Calcium-binding proteins
  - Expresses in neutrophils, monocytes, activated macrophages and keratinocytes, and epithelial cells
  - Associated with inflammatory processes and correlates to PMN infiltration
    - Considered a biomarker for inflammation
Identification of PMN chemotactic factors

**S100A8, S100A9 = S100 alarmins**

- Confirmed by ELISA/Western blot (protein) and PCR (mRNA)
- Produced by vaginal epithelial cells in response to *Candida*
- Anti-S100A8 inhibited PMN migration by vaginal lavage fluid in in vitro chemotaxis assay

*Yano et al. Infect Immun 2010*
S100A8 Alarmins can stimulate PMN migration

**In vitro**

- PMN Chemotaxis

**In vivo**

- Vaginal PMNs
Diagnostics/Treatment

- Current diagnostics for VVC/RVVC limited
  - Challenges
    - *Candida* is commensal → present in asymptomatic state
    - Positive diagnosis requires symptoms and culture positive
    - Current diagnostic tests based on organism only
  - Diagnostic test = *Candida* and symptoms
    - Alarmins = symptoms?
      - Diagnostic evidence of symptomatic VVC

- Immunotherapy
  - Block/neutralize alarmins
    - Reduce/eliminate symptoms
    - Relegate *Candida* back to commensal status
**Candida Mucosal Biofilm Formation**

- *Candida* forms biofilms both on oral and vaginal mucosa
  - Kinetics and architecture similar to in vitro biofilm formation
- *Candida* mutants (efg1\(^{-/-}\), bcr1\(^{-/-}\)) colonize both tissues but do **not** form biofilms

DAY185 (WT)  
EFG1\(^{-/-}/BCR1^{-/-}\)
Mucosal biofilm not required for S100 alarmin response.
Candida-associated Denture Stomatitis (DS)

Rat Denture System

Lee et al. 2011
Candida forms a biofilm on the denture and palate in vivo

Denture

Palate

2 4 6 8 weeks post-inoculation

Johnson et al. 2012
Clinical Score

Clinical Score = 1
Pinpoint erythema & edema

Clinical Score = 2
Diffuse erythema & edema

A

Weeks Post-Inoculation

Clinical Score

7% 33% 62%
80% 66% 38%
13%

B  C  D
Clinical Score = 1 Pinpoint
Clinical Score = 2 Diffuse erythema & edema
Clinical Scores

- WT
- bcr1/bcr1
- efg1/efg1
Model of Immunopathogenesis of Denture stomatitis

- Innate Recognition & PMN recruitment
- Amplification of Inflammation

- Oral Epithelium
- Denture Biofilm
- Denture Biofilm + Tissue Colonization
- Denture Biofilm + Tissue Biofilm

- Time
Candida Mucosal Biofilm Formation

- **Denture stomatitis** – biofilm required for disease
  - Disease initiated after biofilm formation
  - Chronic rather than acute disease → biofilm dependent

- **Vaginitis**–biofilm not required for inflammation/symptoms
  - Disease is acute and initiated by adherence/sensitivity to epithelial cells
  - Biofilm likely more critical in treatment/clearance → drugs or immune response (i.e., PMN function, antibody function)
Overall Conclusions

- Host defense against *Candida albicans* is extremely different at different mucosal sites
  - Adaptive immunity – T cells (oral)
  - Innate immunity – PMNs, neutrophils, Epi (vagina)

- Immune factors associated with protection and susceptibility to infection are unique to the site
  - Alarmins → vagina (susceptibility)
  - Annexin-A1 → oral/vaginal Epi cells (protection)
  - E-cadherin → oral (protection)

- Role of mucosal biofilm in pathogenesis is likewise different for different mucosal sites
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