Host-mycobiome interactions in gut homeostasis and pathogenesis

David M. Underhill, Ph.D.
Intestinal Microbiome

10 x more microbial cells in the body than human cells
100 x more microbial genes in the body than in the human genome

Important for digestion, obesity, immunity, ....

... but it’s all about bacteria
Fungal abundance

It has been estimated that as much as 25% of the world’s biomass is fungi.
Human Fungal Diseases

Pneumocystis pneumonia

Blastomycosis affects mainly the lungs and the skin - Blastomyces dermatitidis

Coccidioidomycosis (Coccidioides immitis), San Joaquin Valley fever

Chromoblastomycosis - rhinocladiella species

cryptococcal meningoencephalitis

mucocutaneous candidiasis, thrush, Vaginal candidiasis, or other skin infections – serious systemic infections

Ringworm, Athlete’s foot, etc. - dermathophytes

Sporotrichosis is a fungal disease, usually of the skin, caused by Sporothrix schenckii.

Fungal keratitis - Aspergillus
Lichens are the combination of a fungus with a green alga or cyanobacteria

Black truffle - *Tuber melanosporum* Vittad.- ectomycorrhizal symbiosis on haselnut tree and oaks

Mycorrhizas are associations between fungi and plant roots

Cow Rumen - $10^{11}$ organisms/gram & around 8% are fungi. 
*i.e. Cyllamyces aberensis* - an anaerobic gut fungus

Ants and Termites “farm” fungi for food and to decompose plant matter
So, is there fungus in the gut?

Current “metagenome” studies

Generally report on the order of 0.1% fungal...

Average fungus (1 genome) is 50X larger than an average bacterium and is encased in a cell wall that is harder to extract DNA from than bacterial cell walls.
Detection & Visualization of Murine Intestinal Fungi

PCR with pan-fungus primers

![Graph showing ITS1-2 expression levels relative to β-Actin for different regions of the intestine.](image)

- **Ileum**
- **Caecum**
- **Prox. Colon**
- **Dist. Colon**

**Gene Expression Levels**

- **GREEN** = anti-Fungi
- **Blue** = DAPI

Discussion:

- The expression levels of ITS1-2 in different regions indicate a higher presence in the **Dist. Colon** compared to other regions.
- This suggests a possible association with the intestinal flora.
Intestinal Fungus Occupy the same niche as bacteria

GREEN = anti-Fungi
Blue = DAPI
Does the Immune System See these Fungi?
Fungal Cell Walls Contain β-glucan

Saccharomyces cerevisiae
“ASCA” Anti-Saccharomyces Cerevisiae antibodies

• The first biomarker that was capable of identifying the majority of patients with Crohn’s disease.
• 50-60% of patients with Crohn’s disease have circulating antibodies to fungi (ASCA).
• The antigen is yeast mannan and is part of the cell wall of nearly all fungi.
• More recently, anti-glucan and anti-chitin antibodies have also been measured in serum from IBD patients.

Dectin-1 – An innate immune receptor recognizing fungi

- **Expressed by** Macrophages, Dendritic cells, Neutrophils
- **Recognizes** Fungal Cell Wall β-glucan
- **Dectin-1 deficient mice** are highly susceptible to a variety of pathogenic fungi
- **Dectin-1 deficient people** are similarly susceptible to fungal infections

Goodridge et al. Nature. 2011
Soluble Dectin-1 Binds Fungi

Dectin-1

Soluble Dectin-1 (sDec)

Candida albicans

Yeast

Aspergillus fumigatus

Zymosan

Recognition of Intestinal fungi by Dectin-1

Recombinant, Fluorescently Labeled Soluble Dectin-1

RED = Soluble Dectin-1 Probe
Blue = DAPI
Dectin-1 binding blocked with soluble glucans

Fluconazole treatment of mice depletes fungi

So, there’s lots of fungus there, does it matter?
Dectin-1 knockout mice are highly susceptible to DSS colitis

Littermate controls

Increased production of inflammatory cytokines in intestinal tissue

4 days DSS
Increased Th1 & Th17 mediated immune activation in Dectin-1 knockout mice

CD4+ T cells from lamina propria (LI-LP) and mesenteric lymph nodes (MLN) 4 days after the 7 days of DSS treatment

Anti-CD3/CD28 restimulation of LI-LP and MLN cells.
Commensal fungi include over 200 species representing over 50 genera

Phylogenetic diversity in gut fungi
**A couple highlights...**

**Candida spp.:**
- *Candida albicans*
- *Candida tropicalis*
- *Candida glabrata*
- *Candida parapsilosis*

- Normal microflora: organism commonly found on mucosal surfaces and on skin
- Pathogenic infection especially common in immunosuppressed patients such as in AIDS or cancer chemotherapy

**Saccharomycopsis fibuligera**
- Common environmental fungus
- Especially good at degrading starches
- Industrially used in the fermentation of starches to produce ethanol
- Rice wine
... and there are changes in the community through the length of the intestines that we’ve not yet explored...
Fungal Populations Change During DSS Colitis

- **Candida**
- **Trichosporon**
- **Saccharomyces**
- **Others**

**Specific fungal genus (%)**

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<th>Naive</th>
<th>DSS</th>
<th>Naive</th>
<th>DSS</th>
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<tr>
<td><strong>WT</strong></td>
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<tr>
<td><strong>Clec7a</strong></td>
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**Graphs**

- **Candida** (%): Naive vs. DSS, WT vs. Clec7a
- **Saccharomyces** (%): Naive vs. DSS, WT vs. Clec7a
- **Trichosporon** (%): Naive vs. DSS, WT vs. Clec7a

- **ns**: Not significant
- *****: Significant at p < 0.05
- ****: Significant at p < 0.01

**Legend**

- Red: Candida
- Green: Trichosporon
- Blue: Saccharomyces
- Purple: Others
Tissue Invasion by Fungi in Dectin-1 Knockout Animal
Fluconazole Treatment Abrogates Intestinal Inflammation Specifically in Dectin-1-deficient mice
Specific Fungi Alter the Inflammatory Response to Intestinal Injury

+/- C. trop or +/- S. fib

2.5% DSS (7 days)  water (4 days)

d1 d3 d5 d9

(NT) Collect feces (C. trop or S. fib) Collect feces (DSS/C. trop or DSS/S. fib)

Histology score

WT Clec7a−/− WT Clec7a−/− WT Clec7a−/−
DSS C. trop/DSS S. fib/DSS

IL-17 (ng/ml)

WT Clec7a−/− WT Clec7a−/− WT Clec7a−/−
DSS C. trop/DSS S. fib/DSS

IFN-γ (ng/ml)

WT Clec7a−/− WT Clec7a−/− WT Clec7a−/−
DSS C. trop/DSS S. fib/DSS
So... does this matter in humans?

Where to start?
IBD is a complex disease with many flavors
Undoubtedly many causes of disease and many variables modifying severity
So... does this matter in humans?

Our mouse data implicate Dectin-1 in a DSS model – a model of ulcerative colitis. Our Dectin-1 mice do not spontaneously develop colitis, they simply have a more severe disease.

So, perhaps we’re interested in UC disease severity...

Fortunately, our colleagues at Cedars-Sinai have collected just such a patient cohort.
### Dectin-1 Association with MR-UC

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<th>Haplotype</th>
<th>Frequency In:</th>
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<table>
<thead>
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<td>G G</td>
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<td>G G</td>
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<td>A G</td>
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<td>Other Combinations</td>
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<td>0.14</td>
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\[ P_{\text{logistic regression}} = 0.000027 \]

Dectin-1 Association with MR-UC

Over 20 years, folks with this Dectin-1 variant progress to surgery faster.
And finally, the people who did the work...

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