Fungus Infections: Neglected, Dangerous and Increasing

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Laboratory of Clinical Infectious Diseases
National Institute of Allergy & Infectious Diseases
Lots of Fungi (~5M!)
Not Too Many Are Important To Us

Yeast
  * Candida, Cryptococcus

Filamentous molds
  * Aspergillus, Mucor, Trichophyton

Dimorphic fungi
  * Histoplasma, Coccidioides, Paracoccidioides, Blastomyces
Only a Few Cause Disease in Normal Hosts

**Yeast**
*Candida* (vaginal yeast infections), *Cryptococcus*

**Filamentous molds**
*Aspergillus, Mucor, Trichophyton* (athlete’s foot, nail infections)

**Dimorphic fungi**
*Histoplasma, Coccidioides, Paracoccidioides, Blastomyces*

Beyond these 2 superficial fungal infections, severe or refractory mycoses typically underlie host defects
# The Global Burden of Fungal Infections

Statistics of the 10 most significant invasive fungal infections.

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*Estimated on the basis of available data, subject to error.
†Endemic dimorphic mycoses are restricted to certain areas.
Aspergillus

Conidia

Hyphae
Molds Are Angioinvasive

Aspergillus in the Lung

Halo sign  Non-specific nodule  Air crescent-sign
Aspergillus Dissemination
Candida

- *C. albicans* part of normal human commensal flora
- Multiple morphologies – yeast (commensal), hyphae (pathogenic) and pseudohyphae
- Causes wide range of infections following disruption to immunity
  - Mucosal
  - Systemic
The Burden of *Candida* Infections in Humans

- **Colonized Humans**
  - Infection
  - Commensal State
  - Mucocutaneous Candidiasis
  - Not life-threatening: Approximately 75% of all women → vaginitis; 90% of AIDS patients → oral thrush

- **Non-Colonized Humans**
  - Invasive Candidiasis
  - Life-threatening: Leading cause of nosocomial bloodstream infection; Mortality of 40% despite treatment
Spectrum of *Candida* Infections

A. Cutaneous

B. Mucosal

C. Disseminated

D. Chorioretinitis

Deeply Invasive

Images courtesy of Kenneth Rolston, MDACC

Transplantation and Invasive Fungal Disease

Probability

0.5
0.4
0.3
0.2
0.1
0.0

0 1 2 3 4 5 6 7 8 9 10 11 12

Months after transplantation

IFI: 19%

Mold: 15%

Candida: 5%
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Jochem B. Buil, Eveline Snelders, Laura Bedin Denardi, Willem J.G. Melchers, Paul E. Verweij
Increasing Echinocandin Resistance in Candida glabrata: Clinical Failure Correlates With Presence of FKS Mutations and Elevated Minimum Inhibitory Concentrations

Barbara D. Alexander, Melissa D. Johnson, Christopher D. Pfeiffer, Cristina Jiménez-Ortigosa, Jelena Catania, Rachel Booker, Mariana Castanheira, Shawn A. Messer, David S. Perlin, and Michael A. Pfaller

Simultaneous Emergence of Multidrug-Resistant Candida auris on 3 Continents Confirmed by Whole-Genome Sequencing and Epidemiological Analyses

Novel Iatrogenic Risk Factors for Fungal Disease
Imatinib Changed Everything

Dan L. Longo, M.D.


A Surge of SMKIs in Recent Years

Drug Discovery Today • Volume 21, Number 1 • January 2016

PUBMED RESEARCH: Small Molecule Kinase Inhibitors (SMKIs)

Imatinib (2001)
A Surge of Anti-Cytokine Biologics

- Intracellular kinases inhibitors
- Lxekizumab, Secukinumab, Brodalumab
- ABT-122
- Novel anti-IL-6 agents

OSTEOCLAST
BONE RESORPTION

PANNUS FORMATION

CHONDROCYTE
CARTILAGE DEGRADATION

Trends in Molecular Medicine
Overview

Basic principles of host defense against:
- mucosal fungal disease
- intracellular fungi (endemics, *Cryptococcus*)
- invasive mold and *Candida* infections
Overview

Basic principles of host defense against:
- mucosal fungal disease
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- invasive mold and Candida infections
IL-17 Signaling is Important for Anti-*Candida* Mucosal Host Defense in Mice and Humans

Chronic Mucocutaneous Candidiasis in Humans with Inborn Errors of Interleukin-17 Immunity

Anne Puel,† Sophie Cypowyj,* Jacinta Bustamante, Jill F. Wright, Luyan Liu, Hye Kyung Lim, Mélanie Migaud, Laura Israel, Maya Chrabieh, Magali Audry, Matthew Gumbleton, Antoine Toulon, Christine Bodemer, Jamila El-Baghdadi, Matthew Whitters, Theresa Paradis, Jonathan Brooks, Mary Collins, Neil M. Wolfman, Saleh Al-Muhsen, Miguel Galicchio, Laurent Abel, Capucine Picard, Jean-Laurent Casanova

Inherited IL-17RC deficiency in patients with chronic mucocutaneous candidiasis

Yun Ling, Sophie Cypowyj, Caner Aytekin, Miguel Galicchio, Yildiz Camcioglu, Serdar Nepesov, Aydan Ikincigullari, Figen Dogu, Aziz Belkadi, Romain Levy, Mélanie Migaud, Bertrand Boisson, Alexandre Bolze, Yuval Itan, Nicolas Goudin, Julien Cottineau, Capucine Picard, Laurent Abel, Jacinta Bustamante, Jean-Laurent Casanova, Anne Puel
IL-17–Mediated Protection via Generation of Anti-

Candida Antimicrobial Peptides

Holland, NEJM, 2009; Lionakis & Levitz. Annu Rev Immunol, 2018
IL-17–Targeted Biologics Result in Mucosal Candidiasis

Lionakis & Levitz. Annu Rev Immunol, 2018
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Macrophage-Th1 Cell Crosstalk is Critical for Intracellular Fungal Pathogen Control

Lionakis & Levitz. Annu Rev Immunol, 2018
Macrophage-Th1 Cell Crosstalk is Critical for Intracellular Fungal Pathogen Control

Emapalumab
4 Trials in ClinicalTrials.gov
(HLH [primary, sJIA])

Mavrilimumab
6 Trials in ClinicalTrials.gov
(RA, MS)

>300 Trials in ClinicalTrials.gov
(malignant, autoimmune GvHD)

Lionakis & Levitz. Annu Rev Immunol, 2018
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Red: dTomato- *C. albicans*

Green: Cx3cr1 (macrophages)

Grey: Ly6G (neutrophils)

Jigar Desai unpublished
Chronic granulomatous disease

Winkelstein et al., *Medicine*. 2000

NOX inhibitors: Ongoing clinical trials for diabetic nephropathy
Aspergillus Infection in CGD
Aspergillus Infection in CGD
Aspergillus Infection in CGD
Aspergillus Infection in CGD

PRE-TRANSPLANT

POST-TRANSPLANT

Courtesy of Mark Parta
Overview

Basic principles of host defense against:
- mucosal fungal disease (Th17 cells)
- intracellular fungi (macrophages-Th1 cells)
- invasive mold and *Candida* infections (PMNs/МΦ)
Novel iatrogenic risk factors for systemic fungal disease

- BTK inhibitors
- SYK inhibitors
Novel iatrogenic risk factors for systemic fungal disease

- BTK inhibitors
- SYK inhibitors
Ibrutinib: A Game Changer in B-cell Malignancies
Ibrutinib and Fungi

Inhibition of B Cell Receptor Signaling by Ibrutinib in Primary CNS Lymphoma

Michaël S. Lionakis,1,7 Kieron Dunleavy,2,7 Mark Roschewski,2 Brigitte C. Widemann,3 John A. Butman,4 Roland Schmitz,2 Yandan Yang,2 Diane E. Cole,3 Christopher Melani,2 Christine S. Higham,3 Jigar V. Desai,2 Michele Ceribelli,5 Lu Chen,5 Craig J. Thomas,2,5 Richard F. Little,3 Juan Gea-Banacloche,3 Sucharita Bhaumik,3 Maryalice Stetler-Stevenson,3 Stefania Pittaluga,3 Elaine S. Jaffe,3 John Heiss,3 Nicole Lucas,2 Seth M. Steinberg,3 Louis M. Staudt,2,8,* and Wyndham H. Wilson2,8,*

Lionakis et al., 2017, Cancer Cell 31, 1–11
June 12, 2017 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.ccell.2017.04.012

Disseminated Cryptococcus With Brain Involvement in Patients With Chronic Lymphoid Malignancies on Ibrutinib

Open Forum Infectious Diseases

Atypical Pneumocystis jiroveci pneumonia in previously untreated patients with CLL on single-agent ibrutinib

Inhye E. Ahn,1,* Theresa Jerussi,2,* Mohammed Farooqui,3 Xin Tian,4 Adrian Wiestner,3 and Juan Gea-Banacloche5

Julia A. Messina,1 Eileen K. Maziarz,1 Andrej Spec,2 Dimitrios P. Kontoyiannis,3 and John R. Perfect1

1Department of Medicine, Duke University, Durham, North Carolina; 2Division of Infectious Disease, Washington University, St. Louis, Missouri; 3University of Texas MD Anderson Cancer Center, Houston

BLOOD, 13 OCTOBER 2016 • VOLUME 128, NUMBER 15
BTK Expression in Phagocytes Promotes Protection During Invasive Aspergillosis

Desai and Zarakas et al, in preparation
Novel iatrogenic risk factors for systemic fungal disease

- BTK inhibitors
- SYK inhibitors
CARD9 is centrally positioned in antifungal immune pathways

Human CARD9 deficiency results in fungal disease in the CNS and skin

- Autosomal recessive inheritance
- Patients present with one or more fungal diseases:
  - mucosal candidiasis
  - Fungal brain disease (*Candida, Aspergillus*)
  - Deep subcutaneous fungal disease (*dermatophytes, phaeohyphomycoses*)

Lanternier et al., NEJM, 2014; Rieber et al, JCI Insight, 2016; Drummond et al., JACI, 2018
Fungal meningoencephalitis in CARD9 deficiency

- 10 y/o girl referred to the NIH in 2013
- Mucosal candidiasis since 1st month of life
- Meningoencephalitis
- Persistent infection despite antifungal therapy
Absence of neutrophils in the CSF despite uncontrolled infection

CSF Analysis:
66% lymphocytes
20% monocytes/DCs
10% eosinophils
<1% neutrophils

Drummond and Collar et al., PLoS Pathog, 2015
CARD9 deficiency results in decreased neutrophil accumulation in the CNS

Card9\textsuperscript{--/--} Mice Develop Uncontrolled Fungal Brain Infection

[Graph showing brain cfu/g over hours post-infection for Card9\textsuperscript{++} mice.]
A Model of Microglial Engagement for CNS Protective Antifungal Immunity

Broader implications:
- Neonatal candidiasis
- SYK inhibitors
SYK-CARD9 are centrally positioned in antifungal immune pathways.

Fostamatinib has shown promise in various trials on ClinicalTrials.gov:
- RA
- AML
- GvHD
- Lymphomas
- Solid tumors
- Autoimmune cytopenias

Collaboration with Stephanie Gaillard
Deep Dermatophytosis and Inherited CARD9 Deficiency
Case Presentation

• Age 3: Developed epistaxis and a nodule on her nasal dorsum that progressed into an oronasal fistula
• Biopsy revealed a “mycotic granuloma”
• Started on IV amphotericin – some apparent response
• Surgical resection of mass – removal of inferior turbinates, ethmoidectomy, medial maxillary antrostomy
• Lost to follow-up after discharge
History

• Age 9, progressive infection
• Tissue biopsy with granulomas and abscesses with fungal elements thought to be compatible with *Aspergillus*
• Tested negative for HIV, with normal T-cell subsets
• Treated with combination antifungal therapy (voriconazole and caspofungin), followed by voriconazole for 12 more weeks
History

• Returned after 6 months with progressive disease
• Mucosal biopsy PCR positive for *Corynespora cassiicola*; culture negative
• Admitted and treated for 1 month with IV amphotericin and posaconazole with reported improvement

June 2015
History

- On/off antifungal therapy
- Immunodeficiency suspected
  - work-up revealed a CARD9 deficiency

June 2016
Family Medical History

• Mother – unknown; no longer involved in patient’s life
• Father – no known medical problems
• Siblings (3) – all healthy; all negative for CARD9 deficiency
Labs

- BMP: 137/4.5 | 103/22 | 42/0.84 < 85
- LFTs: Alk Phos 167, ALT 7, AST 19, Tbi < 0.2, Alb 3
- CBC: 9.4 > 8.5/27.9 < 349, normal differential
- CRP 59.8, ESR 90
- IgG 2826, IgA 65, IgM 195, IgE 32.1
- Serum galactomannan < 0.500
- Serum beta-D-glucan > 500 (30,000)
Pathology
Biopsy skin - left jaw

Fungal WTMT x500
Fungal culture
5 days at 28 °C
Fungal culture

Microscopic features of mold x200
Corynespora cassiicola

• Dematiaceous mold (distantly related to Alternaria)
• Optimal growth temp: 28-30 °C
• Major plant pathogen causing “target spot”. Infects important crops: rubber tree, soybean, tomato, cucumber, etc
• Rarely a human pathogen

http://www.mycobank.org
https://genome.jgi.doe.gov/Corca1/Corca1.home.html
Corynespora cassiicola

- Most commonly a pathogen of plants/grasses
- Temperature sensitive
- Rare cause of phaeohyphomycosis in humans – follows traumatic inoculation
Development of a Murine Model of *C. cassiicola* Phaeohyphomycosis

- **footpad swelling measurements**
  - week 1
  - week 2
  - week 3
  - week 4

- **day 0**
  - inject *C. cassiicola* subcutaneously into hind footpad

- **day 10**
  - histology
  - ex vivo restimulations
Development of a Murine Model of *C. cassiicola* Phaeohyphomycosis

Card9\(^{+/+}\)

Card9\(^{-/-}\)
Development of a Murine Model of *C. cassiicola* Phaeohyphomycosis

*Card9*+/+
Development of a Murine Model of *C. cassiicola* Phaeohyphomycosis

*Card9-/-*
SYK-CARD9 are centrally positioned in antifungal immune pathways


Fostamatinib
44 trials in ClinicalTrials.gov
- RA
- AML
- GvHD
- Lymphomas
- Solid tumors
- Autoimmune cytopenias

An Oral Spleen Tyrosine Kinase (Syk) Inhibitor for Rheumatoid Arthritis
Fungal Host Defense – Take Home

- Host defense antifungal mechanisms are fungus-specific:
  • mucosal fungal disease (Th17 cells)
  • intracellular fungi (macrophages-Th1 cells)
  • invasive mold and Candida infections (PMNs/Mφ)

- Novel iatrogenic risk factors are expected to significantly increase the spectrum of acquired immunodeficiencies that result in opportunistic fungal disease in humans

- Emergence of resistant fungal strains (fungicides, chronic patient use)
Back to our Patient

• Quadruple antifungal therapy (AMB, triazole, echinocandin, terbinafine)

• Disease status
  • >95% resolution of superficial face lesions
  • Stability of pterygoid muscle and upper arm lesions
  • Slow progression of peri-parotid gland lymph node
  • Slow progression of bi-frontal lobe abscesses s/p debridement on 9/11/2018 with local relapse

• Disease not controllable with medical/surgical management, no oral antifungal regimen for chronic suppressive treatment

• Plan for transplantation. Haploidentical 10-year old brother coming from Colombia on Friday to be harvested

• Future plan for reconstructive surgery of the face
Thank you!