Systemic Mycoses

Dave Warshauer, PhD, D(ABMM)
Deputy Director, Communicable Diseases

david.warshauer@slh.wisc.edu
Systemic Mycoses

- *Histoplasma capsulatum*
- *Blastomyces dermatitidis*
- *Coccidioides immitis*
- *Coccidioides posadasii*
- *Paracoccidioides brasiliensis*
- *Penicillium marneffei*
- *Sporothrix schenckii*
- *Aspergillus species*
- *Emmonsia species*
Dimorphism

- Majority systemic mycoses are caused by dimorphic fungi
  - Molds in their normal soil environment, and on laboratory media when kept at 25-30°C.
  - Yeast when the temperature is raised to 37°C (as in the human host).
  - Nutritional factors such as certain amino acids can also enter into dimorphism, but are generally less important than temperature.
Histoplasmosa capsulatum var. capsulatum
Histoplasmosis

- Occurs throughout the world
- Endemic areas
  - Mississippi and Ohio River Valleys in the US.
  - Mexico, Central and South America
- Once thought to be a highly lethal form of pneumonia with up to 90% mortality
- Now known to be a rather common infection in endemic areas.
Histoplasma habitat

- Soil saprobe
- Loves droppings from bats or birds.
  - Requires high levels of creatinine and nitrogen
- Birds not susceptible to infection with Histoplasma
  - Likely related to their high body temperature
    - 40-42C (104-108F)
- Occupational risk for people working with chickens.
- Clearing Starling roosts has been associated with large outbreaks of fatal infections.
Histoplasma capsulatum Disease

- Usually self-limiting flu-like illness and does not require medical intervention.
  - 90% asymptomatic
  - 4:1 male predominance for clinical disease
  - Infants and young children more likely symptomatic
  - Chronic pulmonary disease
  - Disseminated disease in immunocompromised
Pathobiology

• In human infection---small yeast 2-5 μm in diameter.
  - predominantly in macrophages.
• Nonactivated macrophages do not effectively kill *H. capsulatum* and can actually spread the disease.
  - Can multiply intracellularly, kill the phagocyte, and infect additional cells
Pathobiology

- Granulomatous lesions in the lungs
  - Very similar to tuberculosis lesions
  - Lymphocytes, macrophages, Langhans’ giant cells
- Severity of infection directly proportional to the number of conidiospores inhaled.
  - Miliary lesions when large numbers of the spores are inhaled.
- In most infections the cure is spontaneous and lasting immunity occurs.
- Histoplasma may remain viable and recurrence possible with decrease in CMI
Histoplasmosis

- Diffuse pneumonic histoplasmosis
- Radiologic variations
- Calcified miliary histoplasmosis
Calcification
Immunity and Treatment

- Immunity dependent on CMI.
  - Antibody is of little importance
- Healing of lesions leads to calcified granulomas similar to that seen in tuberculosis.
  - Old calcified nodules on chest x-ray not uncommon
- Treatment reserved for life-threatening infections
  - Amphotericin B
  - Itraconazole
Laboratory Diagnosis

• **Histology**
  - GMS stain
  - Wright stain of blood or bone marrow

• **Direct Microscopic Exam**
  - KOH, Calcofluor---2-5um yeast

• **Culture**
  - *Enriched media* (BHI with Blood, Yeast Extract Phosphate, Inhibitory Mold Agar)
    - 2-4 weeks at 30C

• **Antigen Detection**----Urine EIA (miravistalabs.com)

• **Serology**
  - Complement fixation
  - EIA
  - Immunodiffusion

• **Real-time PCR**
Specimens for Fungal Infections

- Respiratory secretions, tissues, blood, CSF, other body fluids
- Discourage swabs
- Transport at room temp
  - Specimens with endogenous flora, refrigerate if >2hr delay
- Blood--Lysis centrifugation, BACTEC MYCO/F Lytic, or BacT ALERT MB
- CSF—large volume (10-20ml)
  - Centrifuge 2000g, 10 minutes
  - Inoculate pellet
Specimens for Fungal Infections

- Urine and other body fluids
  - Centrifuge 2000g, 10 minutes
    - Plate pellet
- Mince tissue, do not grind
  - Place 3-4 pieces on plate and press into agar
  - Exception for Histoplasma—want to grind to release intracellular organisms
Media for Primary Isolation of Systemic Fungi

- **Non-inhibitory media**
  - Sabouraud’s dextrose agar
  - Potato Flake Agar
  - Potato Dextrose Agar

- **Selective Media**
  - Mycobiotic or Mycosel agar---
cyclohexamide and chloramphenicol
Media for Primary Isolation of Systemic Fungi (2)

- **Enriched media w/ or w/o antibiotics**
  - Inhibitory mold agar---chloramphenicol and cyclohexamide
  - BHI with sheep blood w/wo antibiotics
  - Yeast extract phosphate agar with ammonia

- **Incubate plates or tubes at 30°C or 25°C**
  - Hold 4 weeks

- **For Blood Cultures**
  - Lysis Centrifugation
  - BACTEC MYCO/F or BacT ALERT MB
Silver Stain
Culture

Histo at 3 weeks, 30C
Histoplasma capsulatum
H. capsulatum conidia 30C Incubation
H. capsulatum

tuberculate

nethealthbook.com
H. capsulatum—Yeast phase
Differentiation from other Fungi

- Must differentiate from *Seledonium* and *Chrysosporium* species that produce tuberculate macroconidia
  - More rapid growing
  - Not dimorphic
  - Usually will not grow in the presence of cycloheximide
  - Distinguish using DNA probe
Nucleic Acid Probe Identification

• GenProbe® Assay
  - Rapid
  - Chemiluminescent assay using labeled probes specific for each agent
  - Labeled DNA probe hybridizes with rRNA of the fungus
  - Available for *H. capsulatum*, *Blastomyces dermatitidis*, and *C. immitis*
BLASTO!
Blastomycosis

- *Blastomyces dermatitidis*
- Agent of North American Blastomycosis,
- Geographical distribution is similar to *H. capsulatum*
- More common in Wisconsin than *H. capsulatum*. 
A

Kurt Reed et. al. *PLOSone* 3(4): e2034, 2008

B
Blastomycosis

• The epidemiology is poorly understood
  – Lack of a good skin test reagent
  – Ecologic niche not well established
• Difficult to recover from the soil in endemic areas.
• Eagle River, Wisconsin outbreak 1985
  – First time Blastomyces isolated from the environment at the site of an outbreak
  – Isolated from soil containing decayed vegetative matter and from decomposed wood.
Clinical Manifestations

• Two clinical presentations
  – A **primary cutaneous infection** which usually remains localized to one area of the body
    • May indicate systemic disease
  – **Primary pulmonary infection** with possible secondary dissemination.
    • 30-45 day incubation
    • Mimics flu progressing to cough, weight loss, chest pain, low grade fever
    • 75% with isolated pulmonary disease
    • Infection may involve any organ
    • Secondary cutaneous infection
  – **Asymptomatic in >50% of those infected**
Systemic Disease

- Common sites of infection in systemic disease
  - Bones---long bones, ribs, vertebrae
  - Joints
  - Genitourinary tract----prostate, epididymis
  - CNS----common in AIDS (40%), uncommon in immunocompetent (<5%)
Cutaneous Form

- A chronic suppurative granulomatous lesion.
- The presence of epithelial microabscesses and characteristic yeasts in the tissues is considered diagnostic.
- It is important to obtain urine and sputum samples from a patient with cutaneous blastomycosis since systemic spread may occur.
Histology

- **Body fluids or tissue specimens**
  - Look for the characteristic yeast form.
    - Large (8-15 µm) and thick walled.
    - The wall is prominent; "doubly refractile" on bright field microscopy.
    - A single daughter cell (bud) is present with a broad connection between the two cells (**BROAD-BASED BUDDING**).
Direct Exam

KOH Prep

Calcofluor White
KOH Exam
Calcofluor White
Gram Stain
Culture Characteristics

• On culture:
  - Slow growing gray/white mold
  - Delicate, septate hyphae
  - Conidia usually absent on blood-containing media. May be sparse on PDA and SAB
  - “Lollypop” conidiation
Blastomyces dermatitidis
Blastomyces Mold Phase

• This form of conidia is also found in such fungi as Chrysosporium sp., Pseudallescheria boydii (Scedosporium), and various Trichophyton sp.

• Differentiation from these other species can be made by the following characteristics:
  – Slower growth
  – Growth in the presence of cycloheximide
  – Dimorphism
  – Nucleic acid probes
Blastomyces Yeast Phase
Blastomyces Conversion
Coccidioides immititis and C. posadasii
Coccidiodes immitis

• Coccidiomycosis is sometimes known as “San Joaquin Valley fever”. Up to 95% of the residents of the endemic area are skin test positive (coccidioidin test positive)

• Lower Sonoran Life Zone
  - Arid climate, hot summers, few winter freezes, low altitude, alkaline soil, sparse flora
  - Drought followed by heavy rains---Increased infections
  - 100,000 infected annually in U.S.

• Variety of animals infected
  - Positive cultures around rodent burrows

• Archaeology students discover new “infected” sites
Clinical Manifestations

- The primary disease is pulmonary, secondary to inhalation of small numbers of arthrospores
- Usually resolves spontaneously as an influenza-like infection.
  - 60% asymptomatic
  - 40% influenza-like illness, LRI or systemic illness
    - Cough, sputum, chest pain, malaise, fever, chills, night sweats, arthralgias, anorexia
Clinical Manifestations

• In a minority of cases a more chronic pulmonary infection occurs
  - Granulomatous lesions of the lung
  - Can lead to cavitation

• In rare cases (0.5%) dissemination occurs which can lead to rapidly fatal results.

• Reactivation infection occurs
Coccidiomycosis

Lesions variable:
- Papules
- Pustules
- Plaques
- Nodules
- Ulcers
- Abscesses
Histology

- Histological examination useful in confirming a diagnosis.
  - Spherules
  - 10-60 µm in diameter, but they may be as big as 200 µm.
- Immature spherules can be similar in size to the large yeast cells of *B. dermatitidis*
- The spherules contain **endospores** 2-5 µm in diameter
  - Similar in size to *Histoplasma capsulatum*
  - Will not see budding
Culture

- The organism grows fairly rapidly.
  - Visible growth on Sabouraud's agar within a few days.
Microscopic Features

• **Arthrospores:**
  - Formed by fragmentation of hyphae
  - Very thick walled. Provides them with resistance to drying.
  - “Barrel-Shaped”
    • As a culture ages on media the entire hyphal mass may fragment and form arthrospores.
  - **Spores are extremely infectious - Handle with extreme care.**

• **Remember:** Arthrospores can be made by other fungi.
  - Malbranchea sp., Gymnoascus uncinatus, Auxarthon sp.
  - Geotrichum and Trichosporon can also form arthrospores

• Confirm the identification nucleic acid probe test
Coccidioides---Arthrospores

Disjunctor cells
Select Agent Regulations

• Report to CDC within 7 days of ID
  – Responsibility of lab confirming ID
  – Select Agent APHIS/CDC Form 4
• Secure against loss, theft, or release
• Destroy all subcultures and specimens
• Good News
  – Proposed to remove Coccidioides from SA list
Caution!

WORK SAFELY!