MYCOLOGY I

OBJECTIVES

• Identify the important structures of yeasts and molds.
• Describe the types of stains used to identify fungi in primary specimens.
• List the risk factors for acquiring fungal infections.
• Describe the type of infections caused by *Candida albicans*, *Aspergillus*, and *Mucormycetes*.
• Describe the key morphologic characteristics used to identify *Candida albicans*, *Aspergillus*, and *Mucormycetes*.
Fungi = Eukaryotic Cells

- Nucleus with chromosomes and nuclear membrane
- Cytoplasmic organelles
- Rigid cell wall of chitin
- Predominant sterol = ergosterol
  - vs. cholesterol in mammalian cells
- Reproduce sexually or asexually

Fungal Structures: Characteristics and Terms

**Yeast**
- Reproduces through budding or fission
- Unicellular, round cells
- Elongated yeast cell=pseudohyphae
- Germ tube=true hyphae

**Mold**
- Multicellular
- Thread-like structures =hyphae
  - Hyphal mat=mycelium
  - Often described as filamentous or wooly
Fungal Growth and Reproduction

Sexual or asexual reproduction

• Sexual
  – Involves meiosis and fusion of two compatible mating types
  – Teleomorph

• Asexual
  – Involving mitosis only
  – Bits of the hyphae can break off and continue to grow as separate entities
  – Anamorph

Some molds have more than one name=this can be very confusing!

Fungal Structures: Characteristics and Terms

Macro- and Microconidia
Human Mycoses

- Opportunistic mycoses
- Superficial, cutaneous and subcutaneous mycoses
- Systemic mycoses
- Hypersensitivity
- Mycotoxicoses

There are no non-pathogenic fungi

Fungal Habitat and Infection

- Saprobes
  - Most often in soil or decaying vegetation
  - Hyphal phase for molds, production of airborne cells
  - Ex: Dimorphic molds
- Commensal
  - Living in a close relationship where one benefits
  - Ex: Candida sp.
- Symbionts
  - Live together for mutual advantage
- Parasites
  - Living within a host to derive benefits; pathogens cause harm

Fungal Habitat and Infection

Transmission
- Person to person
  - Athletes’ foot
- Contact with environment
  - Inhalation of spores
  - Traumatic inoculation
- Contact with animals
  - Inhalation of bird droppings
  - Touch infected skin/hair of dogs

Route of Infection
- Inhalation of aerosolized yeast or conidia
- Inoculation
Pathogenesis of Fungal Disease

Primary vs. Opportunistic Pathogens
- Colonize, Avoid Host Defense, Multiply
- **Primary**
  - Capable of initiating infection in an immunocompetent host
- **Opportunistic**
  - Most often thrive in patients whose innate or acquired immune defenses are compromised

Primary Fungal Pathogens

Dimorphic Fungi
- Yeast like cells at 35°C
- Mold form at 25°C
- Yeast forms escape the immune system

Examples:
- *Histoplasma capsulatum*
- *Blastomyces dermatitidis*
- *Coccidioides immitis* (not thermally dimorphic)
- *Sporothrix schenckii*
- *Paracoccidioides brasiliensis*
- *Talaromyces (Penicillium) marneffei*

Opportunistic Fungal Pathogens

Generally only cause infection when:
- Disruption to protective barrier of skin or mucous membrane
- Defects in host immune system
- Many have factors that give them advantage over the host

Examples:
- *Candida* sp.
- *Cryptococcus* sp.
- *Aspergillus* sp.
Pathogenesis of Fungi

- Adherence
  - Fungal adhesins that mediate colonization of epithelial surfaces
- Invasion of natural barriers
  - Trauma (rose thorns)
  - Inhalation of spores (Coccidioides in dust)
  - Breakdown of mucosal barriers (Candida through catheters)

Pathogenesis of Fungi

- Avoid phagocytosis
  - Histoplasma yeast multiply in macrophages
  - Cryptococcus has capsule
  - Blastomyces shed cell surface antigens to avoid recognition by macrophages
- Host immunity
  - Humoral immunity not protective
  - Cellular immunity required to eradicate infection

OPPORTUNISTIC MYCOSES
**Candida species**

- Normal flora of GI and GU tracts
- Can colonize skin, fingernails, toenails
- Most common opportunistic fungal pathogen
  - Third most common cause of central line-associated bloodstream infection, exceeding all Gram negative organisms
- Majority of infections are endogenous
  - Due to a lowering of the host’s natural barriers

**Candida species**

- Oval budding yeasts
  - Pseudohyphae and true hyphae
  - Colonies resemble bacteria in culture

**Medically Important Candida Spp.**

- *C. albicans*
  - Predominant isolate
- *C. glabrata*
  - No pseudohyphae
  - Higher rates of resistance to fluconazole
- *C. parapsilosis*
- *C. tropicalis*
- *C. krusei*—fluconazole resistant

- Frequency of isolation depends on
  - Antifungal use
  - Age of patient
  - Immunosuppression
  - Geography
  - Infection control practices
Candidemia in Hospitalized Patients

- Hospitalized patients with candidemia are at a twofold greater risk of death than those with other bloodstream infections.
- Risk factors
  - Hematologic malignancy
  - Neutropenia
  - Undergoing GI surgery
  - Premature infant
  - Patients >70 years of age
- Compounded by antibiotics, central line, colonization or acute hemodialysis

Clinical Syndromes

- Can cause infection in any organ system
- Superficial mucosal and cutaneous to widespread hematogenous dissemination

Candida Skin and Nail Infections

- Onychomycosis and paronychia
  - Change in the texture color and structure of the nail.
  - Sides of the nails are yellowish gray
- Skin has a fairly sharp but irregular erythematous area with easily detachable cigarette paper like scaling.
Mucosal Infections

Pseudomembranous candidiasis=Thrush
- White patches in the mouth and on the tongue
- May be limited to the tongue or extend to esophagus and entire GI tract
- Generally seen in individuals with some level of immunosuppression

Vulvovaginal candidiasis=Yeast Infection
- Candida can overgrow due to:
  - Stress, pregnancy, and illnesses that affect the immune system
  - Certain medicines eg. birth control pills and steroids.
  - Antibiotics can kill "good" bacteria that normally keep the growth of Candida in the vagina in check.
  - Uncontrolled diabetes

Chronic Mucocutaneous Candidiasis
- Rare hereditary immunodeficiency disorder due to malfunction of T cells
- Candida infections develop and persist, usually beginning during infancy
- Severe, unremitting mucocutaneous lesions with a disfiguring granulomatous appearance
- Extensive nail involvement and vaginitis
Candida Endophthalmitis

- Exogenous - trauma or a surgical procedure on eye with direct inoculation of organism into the anterior chamber.
- Endogenous - candidemia with hematogenous seeding of retina and choroid
- If untreated, both forms progress to endophthalmitis, in which the vitreous is infected, and can result in loss of sight

Endocarditis

- Hematogenous seeding of
  - Prosthetic or damaged heart valve
  - Myocardium
  - Pericardial space
- Presenting like bacterial endocarditis with fever and heart murmur
- Embolic events are more common

Systemic Candida Infections

- Fungemia
  - Endogenous—most common; from patient’s GI or genitourinary tract
  - Exogenous—contamination of indwelling vascular catheter
- Hematogenous dissemination
  - Skin lesions
  - Deep tissue
  - Bone and joint
**Candida albicans**

**Laboratory Diagnosis**

- Direct exam by Gram stain or Calcofluor White
  - Microscopy is less sensitive than culture
  - A negative does not rule out fungal infection
- Difficult to implicate Candida as pathogen where it is normal flora
- Isolate from blood cultures and tissue
- Test yeast for production of germ tube
- Perform species level identification

**Candida albicans**

**Direct Microscopic Exam**

Calcofluor White

- Fluorescent brightener that binds to chitin

**Candida albicans**

**Direct Microscopic Exam**

Gram stain

- *Candida albicans* produces clusters of round blastoconidia along the hyphae and particularly at points of septa.
- Pseudohyphae and true hyphae are also observed.

Yeast cells and pseudohyphae in smear of heart tissue. Gram stain, 1000X
Candida albicans
Laboratory Methods

Germ tube
- The beginning of true hyphae
- Observed by microscopic examination upon inoculation of the strain in serum.
- Can also be seen in tissue
- The two Candida spp. that produce germ tube are Candida albicans and Candida dubliensiis.

The Germ Tube
- Buds and pseudo-hyphae can be distinguished from germ tubes by the constricted attachment at the point of origin.
- Germ tubes don’t show constriction at the point of origin.

Candida albicans
Histopathology
- Periodic Acid-Schiff (PAS) stain
  - Note invasion of blood vessel
- Grocott’s methenamine silver (GMS) stain
  - Note blastoconidia and branched pseudohyphae.
Cryptococcosis

_Cryptococcus neoformans_
- Yeast (2-20 μm) with polysaccharide capsule
- Yeast cells are round, not oval
- Found throughout the world
- Associated with pigeon droppings and soil

Cryptococcosis

_C. neoformans and C. gattii_ are the primary pathogens
- Pathogenic encapsulated yeasts

- _C. neoformans_ – worldwide distribution; found in weathered pigeon (and other bird) droppings and in soil, wood, rotting vegetation
- _C. gattii_ – limited global distribution; Tropical/Subtropical climates; Associated with Eucalyptus trees; Endemic in Southern California and Mexico also recovered from animals and humans living in Pacific Northwest. May be endemic to other regions of US

Clinical Syndromes

- Both species can cause disease in immunocompetent hosts
- Primary pulmonary focus—through inhalation
  - Ranges from asymptomatic to fulminant bilateral pneumonia
- More commonly as CNS infection secondary to spread
  - Most patients will present with meningoencephalitis
  - Highly neurotropic
- Other clinical manifestations include:
  - Skin lesions, ocular infection, osseous lesions, prostatic involvement
**C. neoformans**

- More commonly in recovered from immunocompromised hosts
- Cryptococcoma (parenchymal lesion) uncommon
- Risk factors
  - Advanced HIV/AIDS
  - Organ transplant
  - Taking corticosteroids
  - CD4 count<200=high risk for disseminated disease
- Anti-retrovirals and fluconazole result in progressive decline in incidence

**C. gattii**

- More commonly found in immunocompetent hosts
- Cryptococcoma (parenchymal lesion) most common
- Lower associated mortality but more severe neurologic sequelae due to CNS granuloma formation
- Largely under-recognized outside of ‘endemic range’ in US

**Cryptococcosis Laboratory Methods**

- Encapsulated yeast in India ink preparation.
- The small round structure in the center of the white area is the yeast cell.
Cryptococcus on Gram Stain

- Visualized from Blood, CSF, and other specimen types
- Round, encapsulated budding yeast

Cryptococcosis Laboratory Methods

- Colonies are fast growing, soft, glistening to dull, smooth, usually mucoid, and cream to slightly pink or yellowish brown in color.
- The growth rate is somewhat slower than Candida and usually takes 48 to 72 h.
- Produce urease
- Cell wall contains melanin

Cryptococcal Antigen Detection

- Cryptococcal Antigen Latex Agglutination or Lateral Flow Assay
  - Highly sensitive and specific
  - Can detect antigen in CSF and serum
  - Has diagnostic and prognostic value
  - Method of choice for diagnosing patients with cryptococcal meningitis
Cryptococcosis
Histopathology

Methenamine silver (GMS) stained tissue section of lung showing non-encapsulated yeast cells of C. neoformans.

Cryptococcosis
Histopathology

Encapsulated yeast cells in brain tissue. Mucicarmine stains capsule

Aspergillosis
Characteristics of Aspergillus

- Rapidly growing mold
- Found in soil, air, construction dust
- In tissue: dichotomous branching—Y shaped, acute angle
- Septate hyphae
- Most important species: *Aspergillus fumigatus*
- Nosocomial spread may occur
- ID according to color and structures
- Many ‘species’ are actually complexes with indistinguishable cryptic species
  - Implications for antifungal resistance profiles

Clinical Manifestations

- Allergic aspergillosis
  - Spectrum based on degree of hypersensitivity
  - Bronchopulmonary and sinusitis forms
- Obstructive bronchial aspergillosus
  - Underlying pulmonary disease (CF, chronic bronchitis)
- Aspergilloma=Fungus ball
  - Forms in sinus or preformed pulmonary cavity (MTB)

Invasive Aspergillosis

- Ranges from superficial to disseminated infection
- Progression largely due to range of immunosuppression, from mild to severe neutropenia
- Mortality is high (>70%) in invasive pulmonary and disseminated disease
- Risk factors are:
  - Neutropenia <500
  - Cytotoxic chemotherapy
  - Corticosteroid therapy
- Diagnosis is multifactorial and often delayed
- Angioinvasive nature allows dissemination
Aspergillus fumigatus

- Most common species
- ID according to color and structures

Aspergillus species

A. niger

A. terreus

Aspergillosis Laboratory Diagnosis

- Recovery from sterile sites accompanied by histopathology should be considered significant
- Recovery from ‘contaminated’ sites (e.g. respiratory) should be scrutinized
- Not recovered from blood culture (A. terreus may be exception but VERY RARE)
- Not hard to cultivate (grows on sheep blood agar) but culture is not 100% sensitive
- Use multiple tools to diagnose invasive disease
- Galactomannan antigen detection assay
Mucormycosis

Mucormycosis

- Mucormycosis refers to angiotropic (blood vessel-invading) infection produced by various fungi in the orders Mucorales and Entomophthorales.
- Previously, the term zygomycosis was used to denote invasive fungal infections caused by fungi belonging to phylum Zygomycota, class Zygomycetes, orders Mucorales and Entomophthorales.
- The Mucorales order contains 2 families—Mucoraceae and Cunninghamellaceae. Since majority of human infections are caused by Mucorales fungi, the term Mucormycosis is now used to designate this infection.

Mucormycosis: Definition

- Agents of mucormycosis commonly found on fruit, bread, and in soil and are common components of decaying organic debris.
- Ubiquitous and generally saprophytic, rarely causing disease in immunocompetent hosts.
- 3rd most common cause of invasive fungal infection in immunocompromised patients, especially stem cell transplant recipients and patients with underlying hematologic malignancies.
**Mucomycetes**

- Rhizopus species from the Mucoraceae family are the most commonly identified etiologic agents of mucormycosis in humans.
- The species most commonly associated with mucormycosis is *Rhizopus arrhizus*.

Other organisms causing mucormycosis include:
- *Mucor*
- *Cunninghamella*
- *Apophysomyces*
- *Lichtheimia (Absidia)*
- *Saksenaea*
- *Rhizomucor*
- *Entomophthora*
- *Conidiobolus*
- *Basidiobolus*

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**Mucormycosis**

**Risk Factors**

- Diabetic ketoacidosis
- Renal failure
- Solid organ transplant
- Severely burned patients
- Immunosuppressive disorders (leukemia, lymphoma, AIDS)
- Use of corticosteroids, myelosuppression
- Hematologic malignancy

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**Mucormycosis**

- Generally acute and rapidly developing in debilitated patients.
- Predilection for vessel (arterial) invasion resulting in embolization and necrosis of surrounding tissue.
- Infections are typically acute and fulminant.
- High mortality
  - Overall mortality approx 70-100%
  - Hematologic malignancy 65%
  - Hematopoietic stem cell transplant 90%

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Mucormycosis

- Rhinocerebral mucormycosis
  - Most frequent presentation overall and classically affects diabetics with ketoacidosis, hematologic malignancies
  - Usually presents with facial and/or eye pain, proptosis and progressive signs of involvement of orbital structures (muscles, nerves and vessels)
  - Usually results in death, often within a few days. Mortality rate is approximately 85%.
- Pulmonary
  - Occurs most frequently among neutropenic patients
  - Presents with nonspecific symptoms such as fever, cough and dyspnea; hemoptysis may occur with vascular invasion
  - May mimic and be diagnosed as aspergillosis

Periorbital Mucormycosis

Dangerous fungal infection usually occurring in immuno-compromised patient, affecting the regions of the eye, nose, and through its growth and destruction of the periorbital tissues, it will eventually invade the brain cavity.

Mucormycosis

- Cutaneous
  - May be superficial and extend rapidly into subcutaneous tissues
  - Reported with minor trauma, insect bites, non sterile dressing, burns, wounds from natural disaster
  - Hematogenous spread
- Disseminated disease
  - Angioinvasion
  - Ischemia, necrosis of tissue
Treatment Strategies

• Surgical management
  – Debridement of necrotic tissue for complete eradication
  – Often extensive and extremely disfiguring
  – Far higher mortality in those who do not undergo debridement than those who do

Mucormycosis

Histopathology

• Cytologic or tissue confirmation important
• Hyphae
  – Broad, vary from 6-50 um in diameter
  – Aseptate or sparsely septate
  – Irregularly branched
  – Ribbon-like
  – Right angle branching

Mucormycosis

Laboratory Diagnosis

• Microscopic examination of tissues for broad, non-septated hyphae
• Growth is woolly white to grey
• Examination of culture for characteristic sporangia
• Look for rhizoids
Mucormycosis
Dichotomous branching—Y shaped, acute angle w/septate hyphae

Aspergillosis
Right angle branching—wide angle
Broad, ribbon-like, irregularly branched aseptate or sparsely septate hyphae