

# Fungi & Systemic Mycoses

December 2, 2011

Alfred Lewin



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## References

<http://www.doctorfungus.org/>

[\*Klepser, J. Critical Care 26, 225.e1 \(2011\)\*](#)

[\*Romani, Nature Reviews Immunology 11, 275-288 \(2011\)\*](#)

# Why Care?

- Fungi are a leading cause of nosocomial infections.
- Fungal infections are a major problem in immune suppressed people.
- The incidence of fungal infections is increasing.
- Fungal infections are often mistaken for bacterial infections, with fatal consequences.

# Classification of Fungi

Domain

Kingdom

Archea

Bacteria

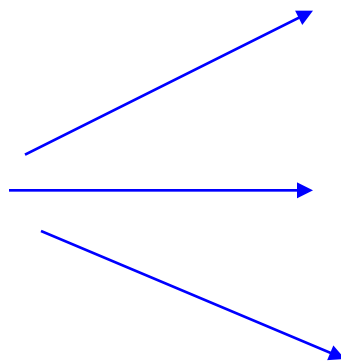
Eukaria

Planta

Animalia

Mycota

(Mycetae)



# Four major phyla of Fungi

**Chytridiomycota**— sexual and asexual spores motile, with posterior flagella

**Zygomycota**— sexual spores are thick walled resting spores called zygospores

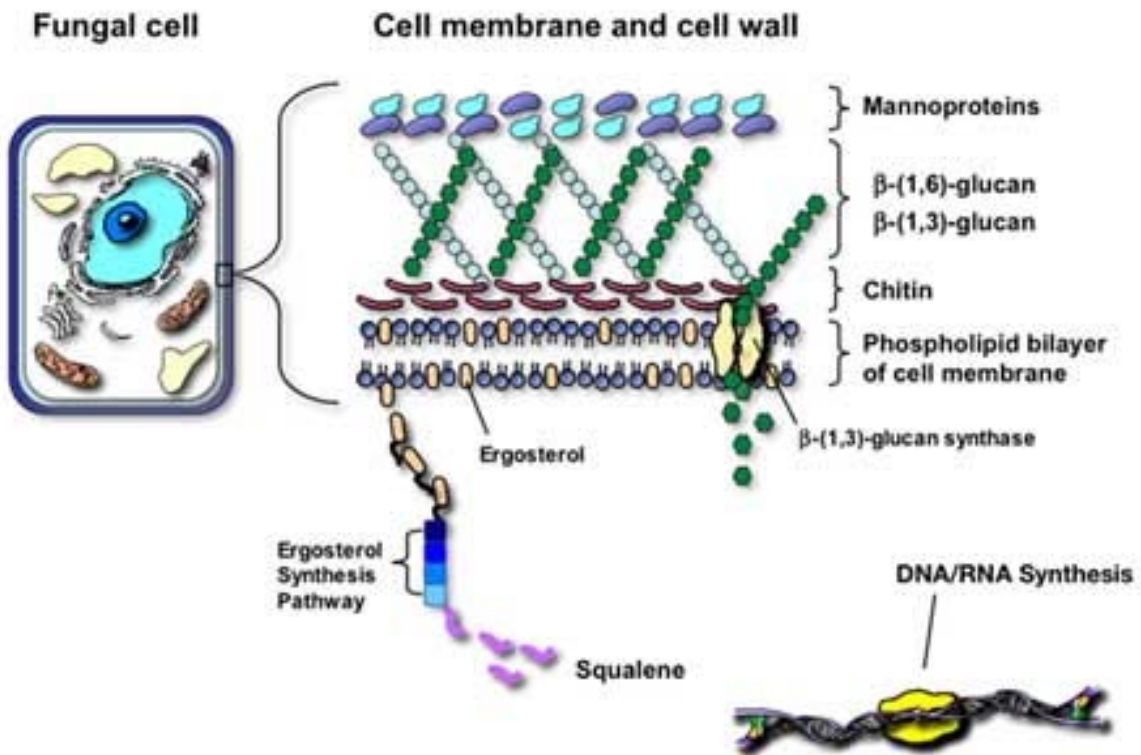
**Ascomycota**—spores borne internally in a sac called an ascus

**Basidiomycota**—spores borne externally on a club-shaped structure called a basidium

**Deuteromycetes** or *fungi imperfecti*, have no known sexual state in their life cycle.

# Characteristics of fungi

A. **eukaryotic**, non-vascular organisms



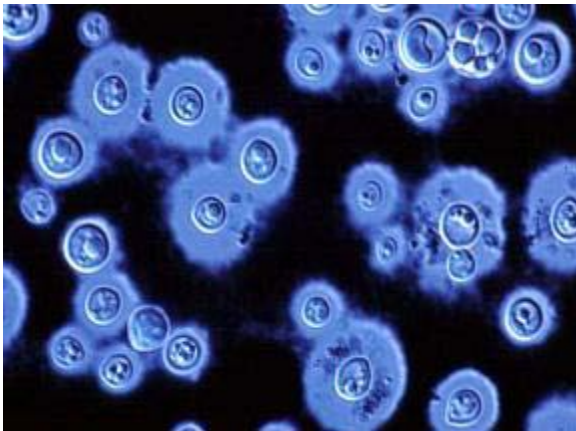
G. **cell walls** composed of mostly of chitin and glucan.

# More Characteristics of Fungi

- H. fungi are **heterotrophic** ( "other feeding," must feed on preformed organic material), not autotrophic ( "self feeding," make their own food by photosynthesis).
  - Unlike animals (also heterotrophic), which ingest then digest, fungi digest then ingest.
  - Fungi produce exoenzymes to accomplish this
- I. Most fungi store their food as **glycogen** (like animals). Plants store food as starch.
- K. Fungal cell membranes have a unique sterol, **ergosterol**, which replaces cholesterol found in mammalian cell membranes
- L. **Tubule protein**—production of a different type in microtubules formed during nuclear division.

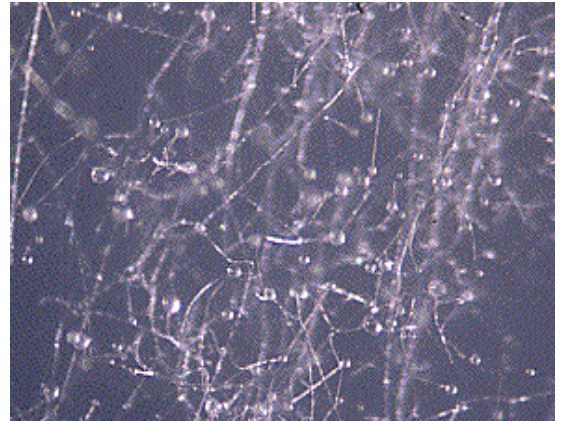
# Fungal Morphology

## Yeast

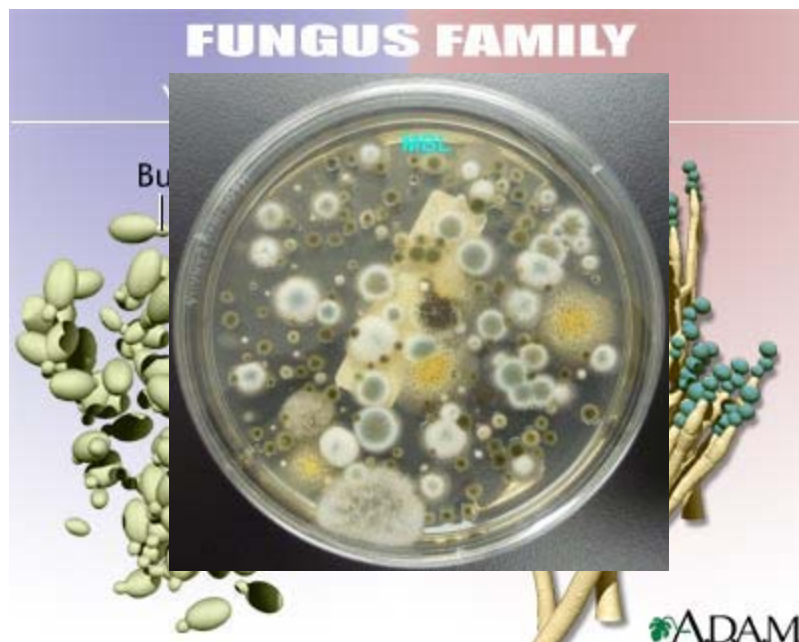


Encapsulated yeast  
*Cryptococcus neoformans*

## Mould


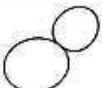
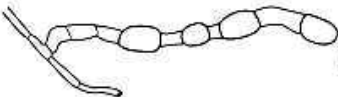
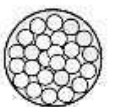
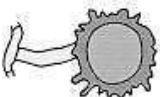
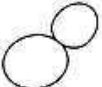
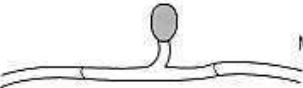


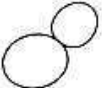


Hyphae (threads)  
making up a mycelium



# Dimorphism

Many pathogenic fungi are **dimorphic**, forming moulds at ambient temperatures but yeasts at body temperature.

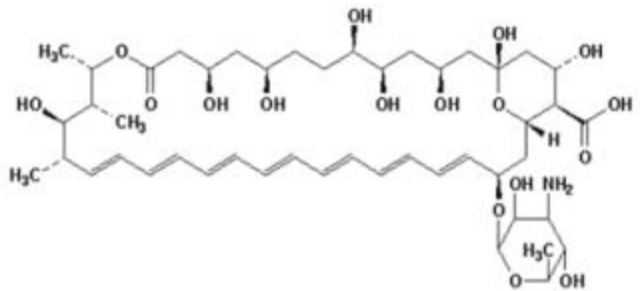
Fungus	In vitro (25° C)	In vivo (37° C)
<i>Blastomyces</i>	 Mold	 Yeast
<i>Coccidioides</i>	 Mold	 Spherule
<i>Histoplasma</i>	 Mold	 Yeast
<i>Paracoccidioides</i>	 Mold	 Yeast
<i>Sporothrix</i>	 Mold	 Yeast

# Antifungal Agents

- Make use of biochemical differences between “us” and “them”
- Target differences in membrane sterol (ergosterol vs. cholesterol)
  - Azoles
  - Polyenes
  - Allylamines
- Target cell wall biosynthesis (caspofungin)
- Target fungal tubulin (grisofulvin)
- Target fungal nucleoside metabolism. (flucytosine)
- Antifungal agents often insoluble and/or toxic.
- Susceptibility testing should be used if available

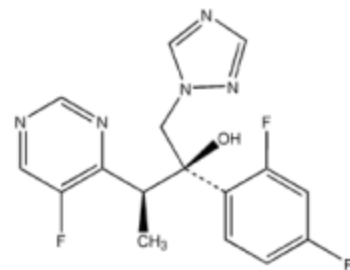
# Antifungal Agents for systemic infections

**Amphotericin** Member of polyene class of antibiotics. Antifungal effect due to interaction with sterols in membrane, making membranes leaky. Has high affinity for ergosterol, but also binds to cholesterol, with severe side effects. Liposomal formulations less toxic



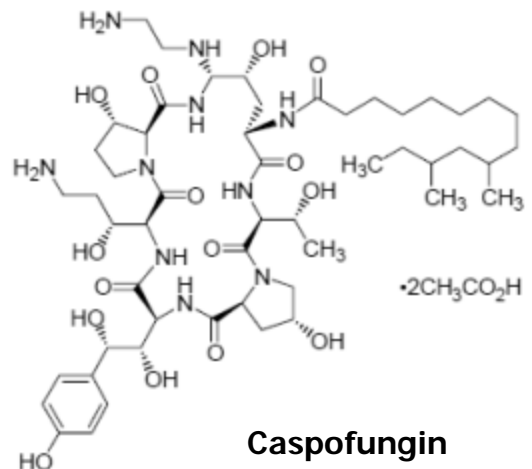
## Azole antifungal agents

Have 5-membered organic rings that contain either two or three nitrogen molecules (the imidazoles and the triazoles respectively).. Two important triazoles are voriconazole and fluconazole. The azole antifungal agents inhibit cytochrome P450-dependent enzymes involved in the biosynthesis of cell membrane sterols. Potential for interaction with other drugs.



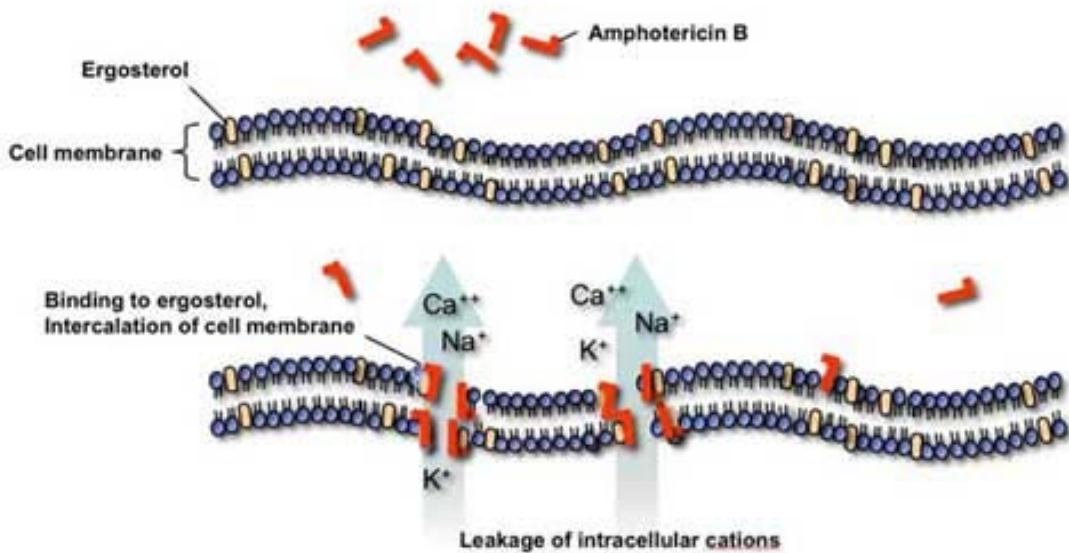
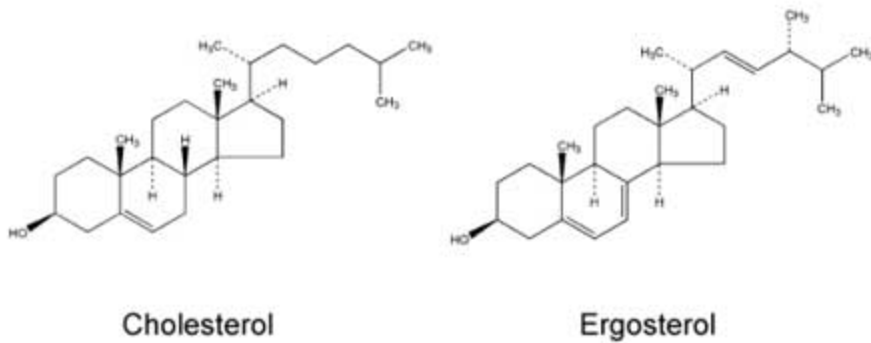
voriconazole

**Echinocandins** non-competitively inhibit UDP-glucose 1,3- $\beta$ -d glucan synthase, an enzyme that is necessary for the synthesis of 1,3- $\beta$ -d glucan, an essential component of the cell wall of several fungi. Limited toxicity. Not used against *C. neoformans*, *zygomycetes* or *fusarium*.



Caspofungin

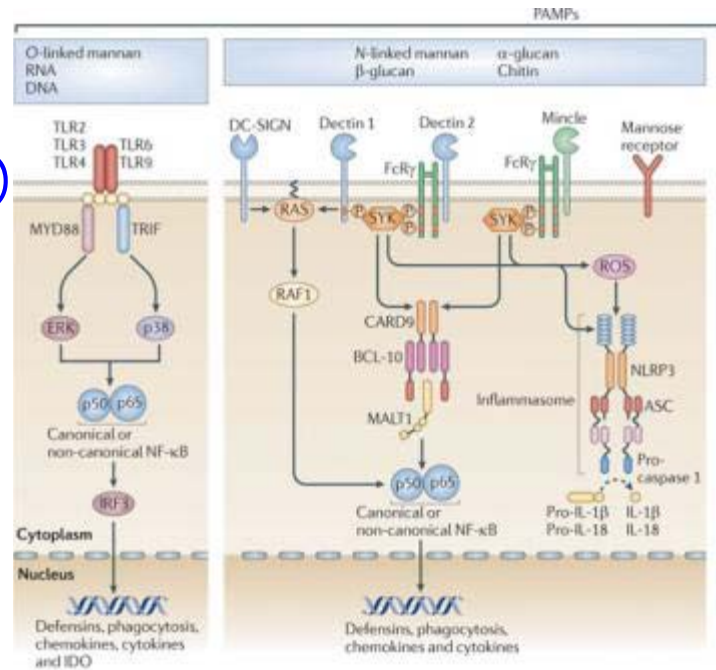
# Amphotericin Binds Ergosterol



# Immune Response to Fungal Infection

Epithelial cells recognize pathogen associated molecular patterns (PAMPs) such as glucans, mannans and chitin.

In response they secrete cytokines and chemokines to attract PMNs which provide a first line of defense.



The uptake of fungi by DCs promotes differentiation of T-helper cells.

A dominant  $T_H1$  response correlates with protective immunity to fungal infections.

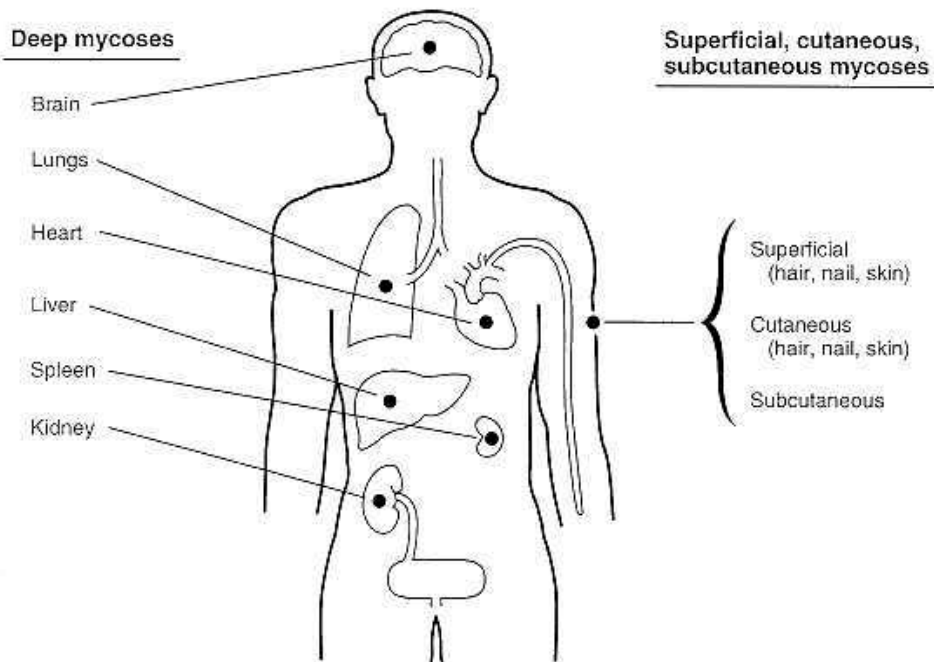
*Evasion of the immune response:* yeast to hyphal transition; changing of surface glycoproteins; hydrophobins; survival in macrophage.

# Lab Diagnoses of Mycoses

- Clinical presentation
  - History (risk factors)
  - Physical Exam (lesions, devices)
- Histopathology
  - Often sufficient
  - Mould or Yeast?
  - Septate hyphae?
- Culture of organism (days to weeks)
  - Problem, contaminating bacteria
- Serology
  - Antibody or Antigen tests
- Molecular Biology
  - RT-PCR

# Mycoses: diseases caused by fungi

- Asthma and allergy
- Skin disease
  - cutaneous
  - subcutaneous
- Recurrent vulvovaginal candidiasis
- Inflammatory Bowel Disease
- Invasive Fungal Disease



# Superficial Mycoses

- Tinea (Pityriasis) versicolor--pigmented lesions on torso
- Tinea nigra--gray to black macular lesions often on palms
- Black piedra--dark gritty deposits on hair
- White piedra--soft whitish granules along hair shaft
- Often associated with organisms of the genus *Malassezia*
- All are diagnosed by microscopy and are easily treated by topical preparations.

## Cutaneous Mycoses

Three genera of dermatophytes, *Microsporum*, *Trichophyton*, and *Epidermophyton* cause infections of skin and its appendages.

Clinical Name	Site	Most frequent organism
Tinea capitis (epidemic)	scalp	Trichophyton tonsurus, Microsporum audouinii
Tinea capitis (non-epidemic)	scalp	Microsporida canis, Trichophyton verrucosum
Tinea favosa	scalp, torso	Trichophyton sp.
Tinea barbae	beard	Trichophyton rubrum, T. verrucosum
Tinea corporis	arms, legs torso	T. rubrum, M. canis, T. mentagrophytes
Tinea cruris	crotch	T. rubrum, T. mentagrophytes, Epidermophyton floccosum
Tinea pedis, manus	feet hands	T. rubrum, T. mentagrophytes
Tinea unguium	nails	T. rubrum, T. mentagrophytes, E. floccosum
Tinea imbricata	torso	T. concentricum

# Tinea corporis



## Subcutaneous mycoses

Subcutaneous infections - over 35 species produce chronic inflammatory disease of subcutaneous tissues and lymphatics. e.g. sporotrichosis - ulcerated lesions at site of inoculation followed by multiple nodules - caused by a dimorphic fungus: *Sporotrix schenckii*.



# Systemic fungal infections are uncommon

Natural immunity is high; physiologic barriers include:

1. Skin and mucus membranes
2. Tissue temperature--fungi grow better at less than 37°C
3. Pattern recognition by TLR and CLR receptors
4. Secretion of antimicrobial peptides

## Infection requires a large inoculum and a susceptible host

1. infection often occurs in *endemic areas*
2. most infections are *asymptomatic* or self-limiting
3. in immune-compromised hosts, infections are more often fatal (AIDS)

## Systemic fungal disease is most often associated with four organisms

1. *Coccidioides immitis*
2. *Histoplasma capsulatum*
3. *Blastomyces dermatitidis*
4. *Paracoccidioides brasiliensis* (*S. America*)

# Coccidioidomycosis

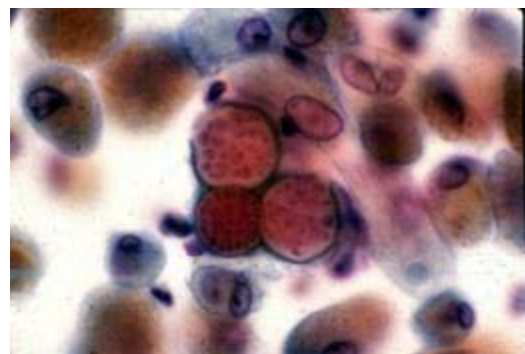
- *Coccidioides immitis* is considered to be the *most virulent* of fungal pathogens.
- Restricted to hot, semi-arid areas of SW USA and Mexico.
- Grows in the soil, but inhalation of a single spore can initiate infection.



Conidia



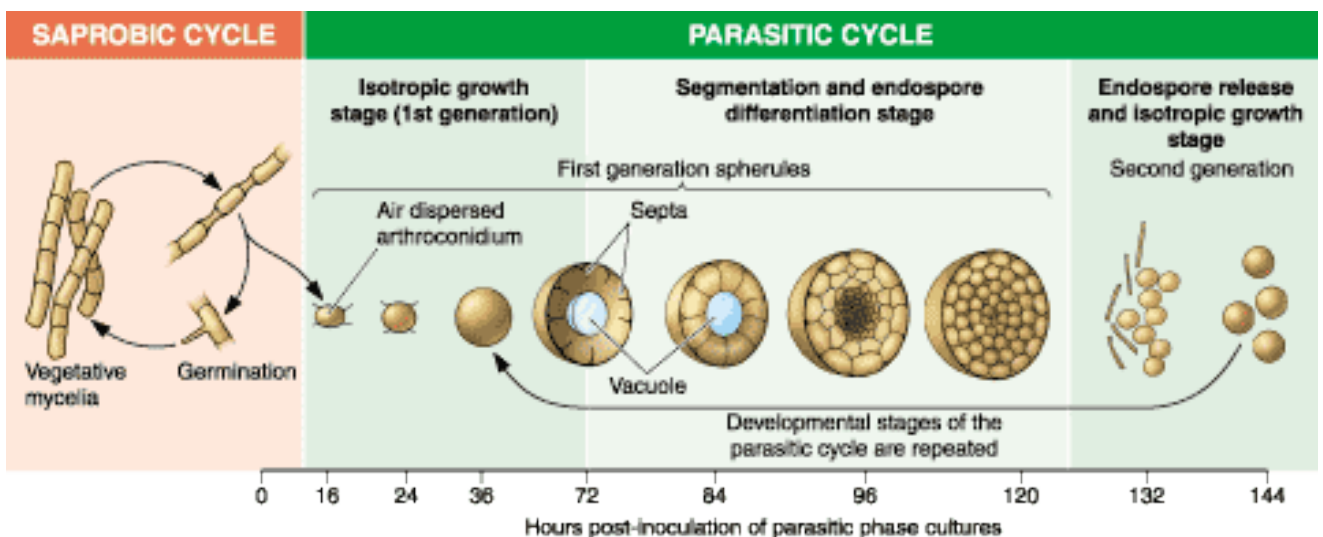
Spherules



In infected tissues, *C. immitis* appears as a mixture of endospores and *spherules*.

# Coccidioidomycosis:

- A. Encounter:** Mycelium found in dry, dusty soil. Contact by **inhalation** of arthroconidia
- B. Spread:** Most commonly an asymptomatic self limited pulmonary disease, but may spread via the blood to skin, soft tissues, bones, joints and meninges.
- C. Immune Response:** T-cell mediated (Th-1): IL-2, IFN- $\gamma$
- D. Evasion of Defenses:** Resistant to killing by phagocytes
- protein rich, hydrophobic outer wall
  - alkaline halo associated with urease
- E. Damage:** secreted proteinases break down collagen, elastin, hemoglobin, IgG & IgA



# Coccidioidomycosis:

## **E. Risk Factors**

1. Ethnicity
2. Age: Extremes more susceptible
3. Sex: Males more susceptible
4. Pregnancy: 3<sup>rd</sup> trimester
5. Immunosuppression

## **F. Symptoms**

1. Fever
2. Arthralgia
3. Erythema nodosum

## **G. Diagnosis**

1. Exam: Suppurative or granulomas inflammation
2. Histopathology: spherules or endospores seen in sputum, exudates or tissue
3. Culture: —danger, highly infectious!
4. Serology: Complement fixation assay (in cerebrospinal fluid), particle agglutination assay

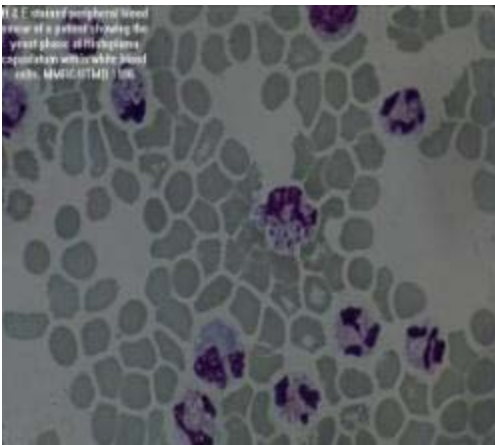
## **H. Treatment**

1. Often none.
2. Amphotericin B followed by an azole

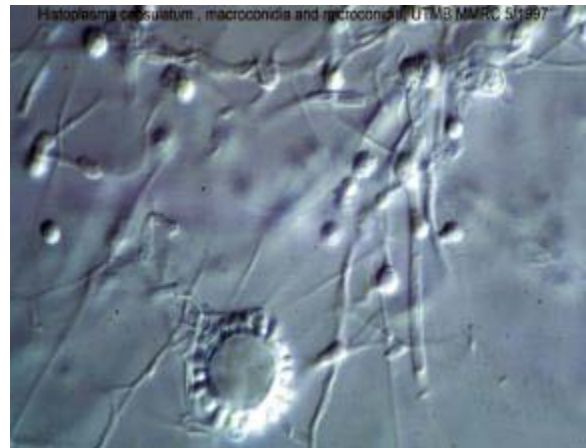
# Histoplasmosis

(also called *cave disease*)

Caused by the dimorphic fungus *Histoplasma capsulatum*



Intracellular yeast at 37C



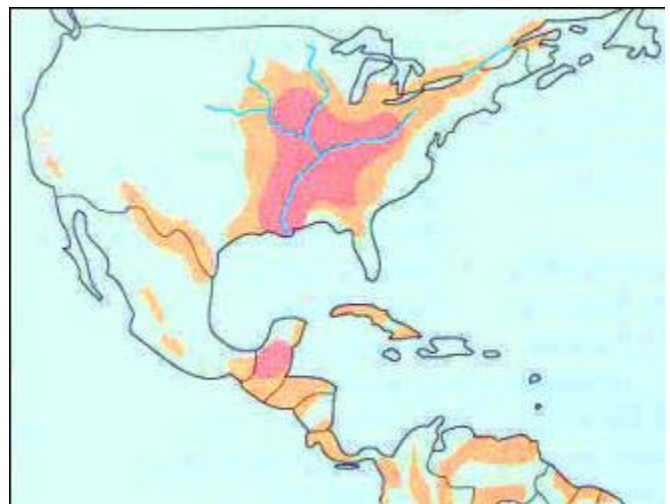
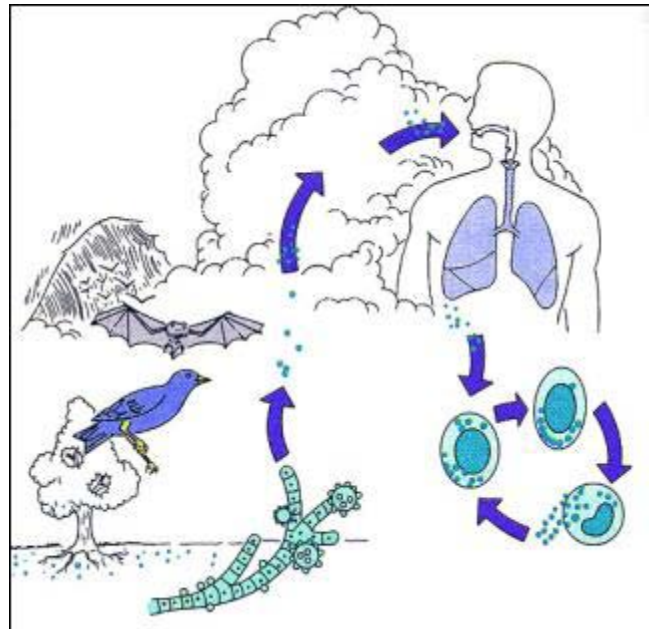
Tuberculated macroconidia, grown at 25C

Histoplasmosis is characterized by intracellular growth of the pathogen in macrophages and a granulomatous reaction in tissue. These granulomatous foci may reactivate and cause dissemination of fungi to other tissues.

# Histoplasmosis

## A. Encounter.

*H. capsulatum* grows in soil, especially soil contaminated by guano. Inhalation of conidia from the environment is source of infection. This is more likely in endemic areas. In U.S. these include the Atlantic Ocean to N. Dakota (500,000 cases/year in U.S.), except New England & Florida. Most cases occur in Ohio Valley and Mississippi Valley)



# More Histoplasmosis

## B. Spread

1. 90% of cases are asymptomatic, but in rare cases flu like respiratory symptoms occur
2. Disseminated histoplasmosis occurs in 1:200 cases and is diagnosed frequently in patients with AIDS living in the central U.S. Other risk factors: being under 2 or receiving massive inoculum
3. In these cases, the organism spreads **via blood** from the lung to involve bone marrow, adrenal glands, heart valves and CNS
4. Spread can also be associated with underlying lung disease (e.g., emphysema).

## C. Immune Response

1. Cell-mediated responses are of primary importance
2. Phagocytic activity of macrophage is considered an important component of resistance to drugs.
3. Activated macrophage can kill yeast cells

## D. Evasion of Defenses

1. Survival in macrophages—elevates pH of phagosomes
2. Yeast cells absorb iron and calcium from host
3. Alteration of cell surface

# Histoplasmosis

## D. Damage

1. Lung--bronchial obstruction and inflammatory sequelae
2. Disseminated histoplasmosis-fulminant disease that may result in toxic shock
3. CNS-fatal if untreated.
4. Mediastinal fibrosis (rare)

## E. Diagnosis

1. Direct histology and culture of blood or bone marrow
2. Serological testing for antibody and histoplasma antigen in blood and urine.
3. Urine test: in HIV-infected patients with disseminated histoplasmosis, histo. antigen detection in urine is at least 90% sensitive.

# Even More Histoplasmosis

## F. Treatment

- Amphotericin still mainstay of therapy vs. disseminated and severe pulmonary histoplasmosis.
- Ketoconazole or itraconazole is effective as therapy for self-limited disease (used in AIDS).

## Ocular Histoplasmosis

A small fraction of individuals form scar tissue in the retina many years after the original histoplasmosis infection. Live organisms cannot be recovered from these specimens. The scarring can obscure the macula and lead to loss of central vision. The first signs are small "histo spots".



# Gene Therapy Death 7/2007



Jolee Mohr, 36, died from widespread histoplasmosis accompanied by a hematoma that ruptured her organs, according John Hart, a pathologist at the University of Chicago. At the time of her death, she had disseminated histoplasmosis in several organs of her body.

Taking Humira  
(adalimumab), a TNF- $\alpha$   
blocker to control RA

Jolee Mohr, 36 enrolled in clinical trial for gene therapy for RA--AAV expressing monoclonal Ab to TNF $\alpha$ .

Feb. 26, 2007: 1<sup>st</sup> shot—no noticeable effect

July 2: Tired and cranky but received 2<sup>nd</sup> shot (temp 99.6)

July 3, woke up feeling ill, vomiting by PM (temp 101)

July 4, feverish and vomiting; family physician “probably a virus.”

July 7<sup>th</sup>, symptoms worsened (temp 104.1). Went to ER—tests indicated liver damage and possible infection—sent home under care of family doctor.

July 12: admitted to hospital. Signs of serious infection, but tests for standard viruses or bacteria were negative.

July 18: Transferred to Univ. of Chicago Hospital

July 24: Dies from massive bleeding and organ failure.

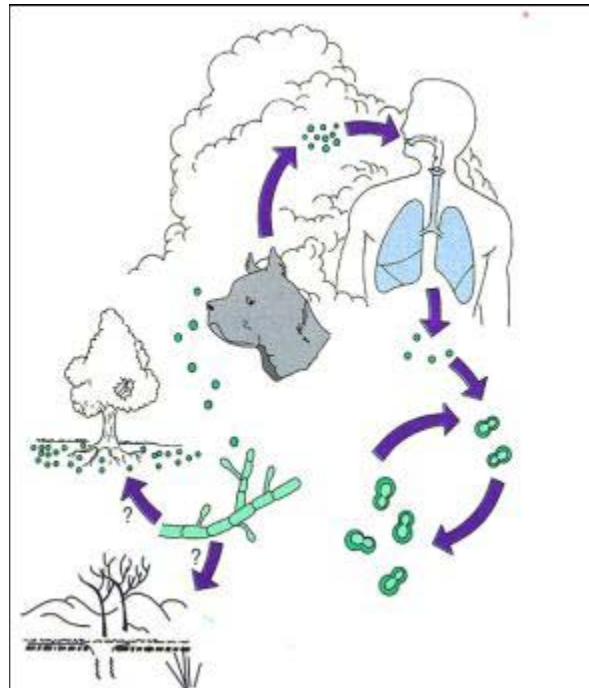
# Blastomycosis

Granulomatous mycotic infection that predominantly involves lungs and skin; but can spread to other organs. Most prevalent in males 40-60 years of age and children.



## *Blastomyces dermatitidis*

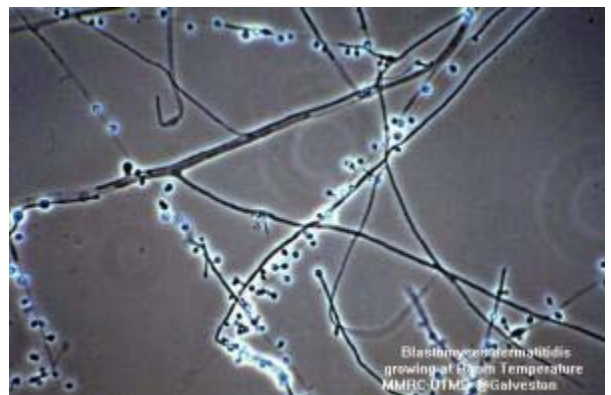
Dimorphic organism originates in the soil and infection ensues by inhalation of spores. Converts to yeast in animal hosts or at 37° *in vitro*.



# Blastomycosis

- **Encounter:** Most cases are in southern, central, and southeastern USA. Infection is by inhalation of spores.
- **Spread:** The pulmonary infection is either self-limited or progressive. Dissemination often occurs to the skin and to the bone - 80% of patients have large skin lesions; a large number also have granulomatous pulmonary lesions.
- **Risk Factors:** Occupational contact with soil; owning a dog. Living in endemic area.
- **Evasion of Defenses:** Escapes phagocytosis by neutrophils and monocytes by shedding its surface antigen after infection
- **Damage:** Consequence of the immune response to the organism—skin lesions respiratory infiltrates.
- **Diagnosis:** based on clinical findings and microscopic detection of organisms in tissue specimens

Molly



# Blastomycosis

## Immune response

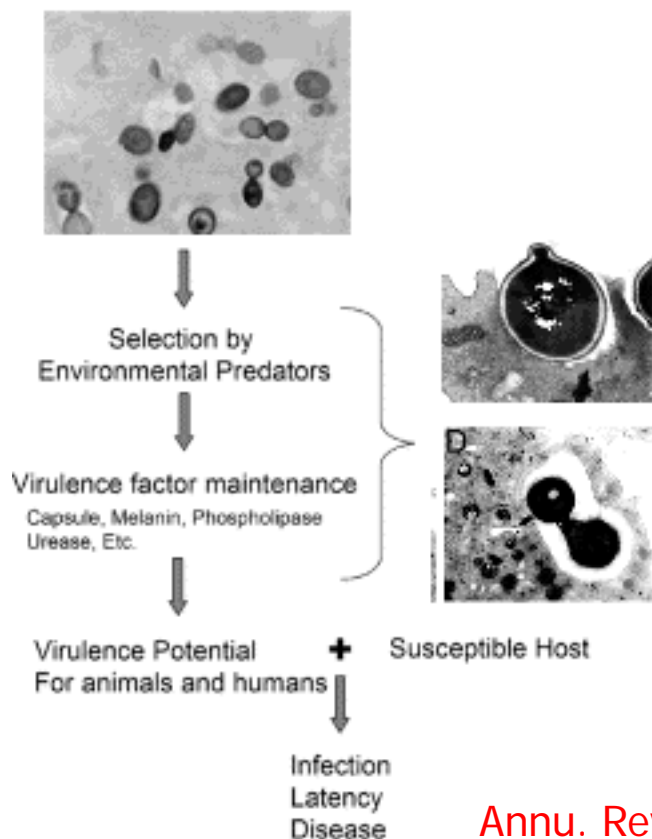
1. Alveolar macrophage provide a first line of defense.
2. T-cell stimulated PMNs kill *Blastomyces* cells (by oxidative mechanisms).
3. Conidia are more sensitive to killing by PMNs because yeast are too big.
4. TH-1 response of primary importance

## Treatment

1. Amphotericin B is the drug of choice for rapidly progressive blastomycosis
2. Itraconazole or Fluconazole for less severe cases

# Question: How did these soil microorganisms evolve traits that enable them to evade the human immune system?

Hypothesis: Predation by soil based organisms such as amoeba and nematodes selected for fungi that can: (1) survive in the phagosome (2) escape from the predator.



[Annu. Rev. Microbiol.](#)  
[2008. 62:19-33](#)

# Opportunistic Mycoses

Opportunistic mycoses are fungal infections that do not normally cause disease in healthy people, but do cause disease in people with weakened immune defenses (immunocompromised people). Weakened immune function may occur due to inherited immunodeficiency diseases, drugs that suppress the immune system (cancer chemotherapy, corticosteroids, drugs to prevent organ transplant rejection), radiation therapy, infections (e.g., HIV), cancer, diabetes, advanced age and malnutrition.

The most common infections are:

Aspergillosis

Candidiasis

Cryptococcosis

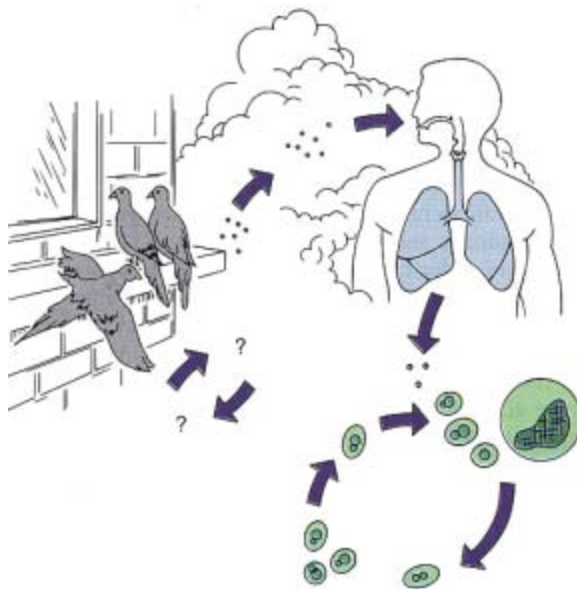
Pneumocystis carinii

Zygomycosis

(more from Dr. Wingard)

# *Cryptococcus neoformans*

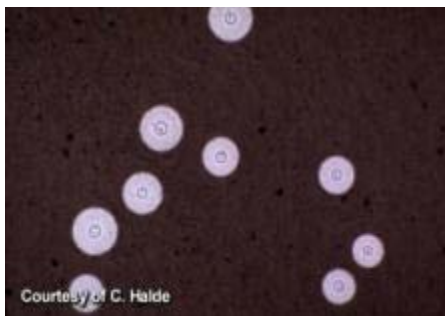
- **Encounter:** Organism is ubiquitous and infections occur worldwide. *C. neoformans* recovered in large amounts in pigeon poop. Does not cause disease in birds. Primary site of human infection is the lungs.



- **Spread:** *Cryptococcal* meningitis is most common disseminated manifestation. Can spread to skin, bone and prostate.

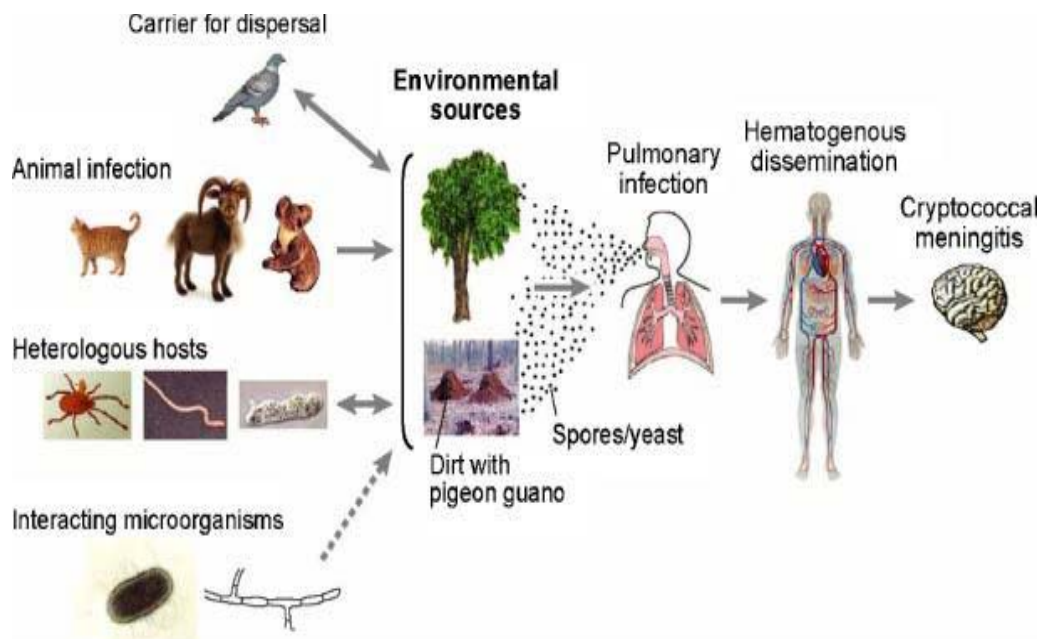
# Cryptococcus neoformans

- **Evasion of defenses:** Yeast cells are resistant to phagocytosis because of capsule. Melanin protects against oxidative injury
- **Immune response:** Activated neutrophils have an increased capacity to phagocytize *C. neoformans*. Cell mediated immunity is our primary defense. About 30% of cryptococcus infections occur in patients with lymphoma (CNS). Major opportunistic infection in patients with AIDS
- **Diagnosis:** Lumbar puncture and microscopic examination of cerebrospinal fluid is diagnostic. (India ink staining). Cryptococcal antigens in CSF and serum. Culture of organisms from blood or CSF



- **Treatment:** Amphotericin B & 5FC. Followed by oral fluconazole.

# Life Cycle of Cryptococcus



[Ann. Rev. Microbiol. 60:69-105; 2006](#)

# Vancouver Island Outbreak

- Largest *Cryptococcus* outbreak recorded
- Caused by *C. gattii*
- Infects immunocompetent and immunocompromised people
- First observed in Vancouver Island in 1999 but now has spread to BC and Pacific Northwest
- Mainly caused by a single genotype of *C. gattii* (VGIIa)
- In U.S. subspecies identification is not routine (need MLST)
- *C. gattii* is usually found in the tropics and subtropics

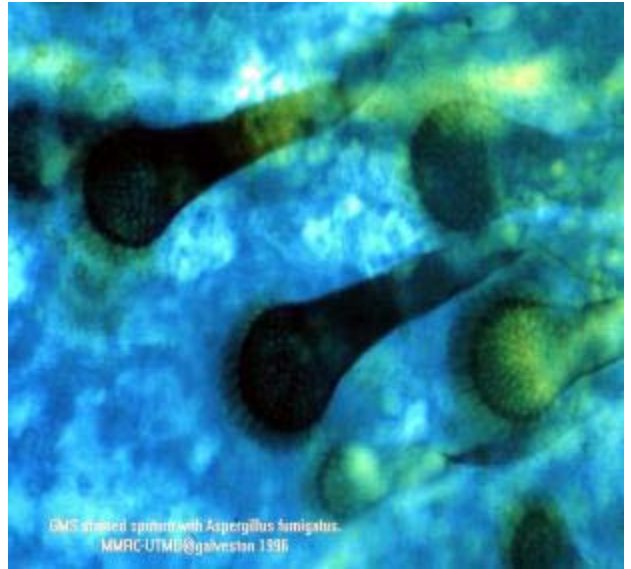
# *C. gatti* outbreak



Map of the Pacific Northwest, comprising parts of British Columbia, Canada, and the states of Washington and Oregon in the United States, showing human and veterinary *Cryptococcus gattii* cases (including marine mammals) by place of residence or detection, and locations of environmental isolation of *C. gattii* during 1999–2008. cf. [Datta et al. \*Emerg. Infect. Disease\* \(2009\)](#)

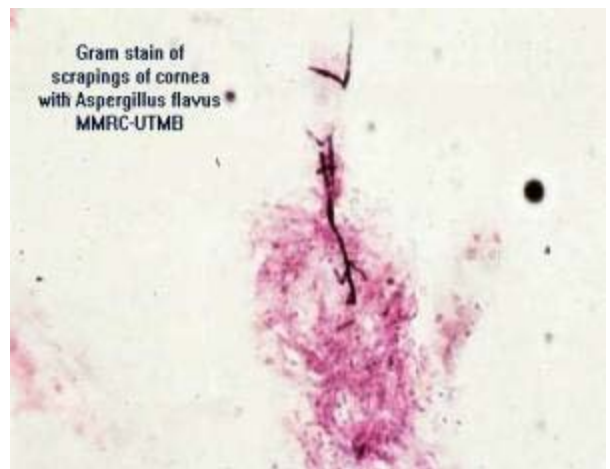
# Aspergillosis

- Genus occurs worldwide and contains hundreds of species.
- These species constitute the most commonly found fungi in any environment



Major portal of entry is the respiratory tract.

Dissemination can occur from the lungs and involve other areas of the lung, the brain, GI tract, and kidney. CNS and nasal-orbital cavities can also occur without lung involvement. Risk factors for invasive disease are neutropenia and high doses of adrenal corticosteroids



# Aspergillosis

- Aspergillosis is the most common fatal infection seen in patients with chronic granulomatous disease of childhood.
- Patients with this condition are unable to form toxic oxygen radicals after phagocytosis.
- Progressive and disseminated disease can complicate neoplastic diseases, especially acute leukemia, bone marrow and organ transplantation (not necessarily AIDS).

In immunosuppressed hosts: invasive pulmonary infection, usually with fever, cough, and chest pain. May disseminate to other organs, including brain, skin and bone. In immunocompetent hosts: localized pulmonary infection in persons with underlying lung disease. Also causes allergic sinusitis and allergic bronchopulmonary disease.

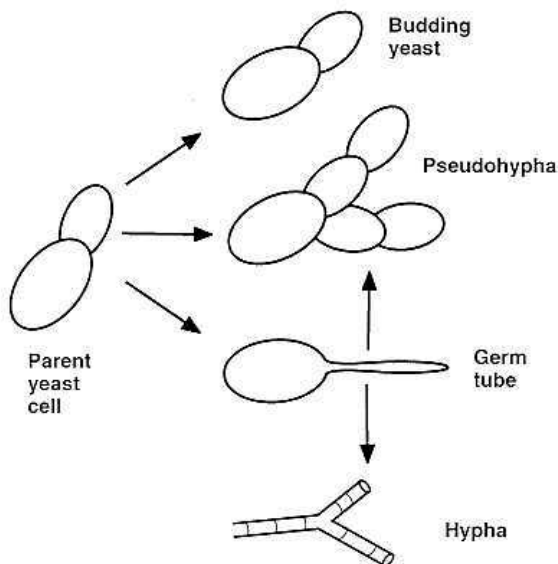


**Agent:** *Aspergillus fumigatus*, *A. flavus*.

# Candidiasis

*C. albicans* is a member of the indigenous microbial flora of humans.

1. Found in the gastrointestinal tract, upper respiratory tract, buccal cavity, and vaginal tract.
2. Growth is normally suppressed by other microorganisms found in these areas.
3. Alterations of gastrointestinal flora by broad spectrum antibiotics or mucosal injury can lead to gastrointestinal tract invasion.
4. Skin and mucus membranes are normally an effective barrier but damage by introduction of catheters or intravascular devices can permit *Candida* to enter the bloodstream.



*In vitro* (25° C):  
mostly yeast;

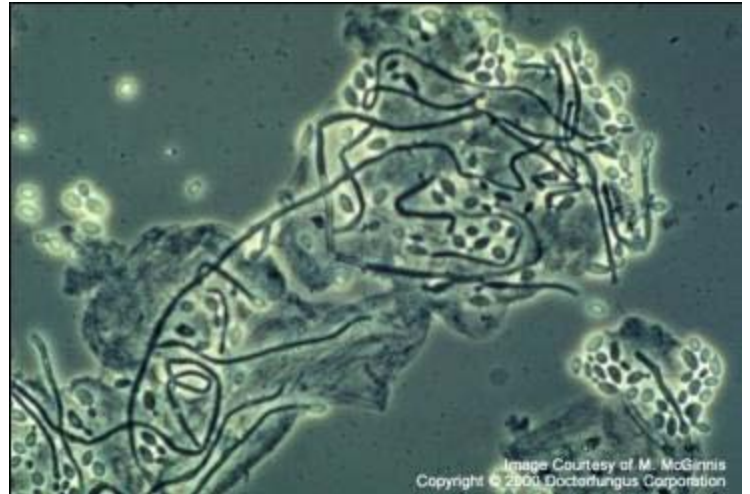
*In vivo* (37° C):  
Yeast, hyphae and  
pseudohyphae



Note difference  
from other fungi

# Candidiasis

Vaginal candidiasis is the most common clinical infection. Local factors such as pH and glucose concentration (under hormonal control) are of prime importance in the occurrence of vaginal candidiasis. In mouth: normal saliva reduces adhesion (lactoferrin is also protective).



Candidal hyphae in mucosal scraping

## Immune Response

Hyphae are too big for phagocytosis but are damaged by PMNs and by extracellular mechanisms (myeloperoxidase and  $\beta$ -glucuronidase). Cytokine activated lymphocytes can inhibit growth of *C. albicans*. Resistance to invasive infection by *Candida* is mediated by phagocytes, complement and antibody, and T cell mediated immunity plays a major role. Patients with defects in phagocytosis function and myeloperoxidase deficiency are at risk for disseminated (even fatal) *Candidiasis*.

# Candidiasis

**TABLE 87-5. CLINICAL CLASSIFICATION OF CANDIDIASIS (ABRIDGED)**

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Cutaneous and subcutaneous candidiasis
Thrush (oral, vaginal)
Stomatitis
Intertriginous candidiasis (groin, axillary, interdigital)
Onychomycosis
Esophagitis
Severe diaper rash
Balanitis
Systemic candidiasis
Esophagitis
Intestinitis
Infant diarrhea
Bronchopulmonary candidiasis
Pyelonephritis
Cystitis
Endocarditis
Myocarditis
Endophthalmitis
Meningitis
Arthritis
Osteomyelitis
Peritonitis
Macronodular skin lesions
Chronic mucocutaneous candidiasis

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Thrush



Cutaneous

## Risk factors for candidiasis

- Post-operative status
- Cytotoxic cancer
- Chemotherapy
- Antibiotic therapy
- Burns
- Drug abuse
- Gastrointestinal damage

# Chronic mucocutaneous candidiasis

Chronic mucocutaneous candidiasis (CMC) is the label given to a group of overlapping syndromes that have in common a clinical pattern of persistent, severe, and diffuse cutaneous candidal infections.

These infections affect the skin, nails and mucous membranes.

CMC patients often develop endocrine and inflammatory disorders, suggesting that immune responses are dysregulated.



CMC can be controlled by oral azole antibiotics.

# Environmental species kill neutropenic patients.

**Zygomycosis.** Zygomycosis due to *Rhizopus*, *Rhizomucor*, *Absidia*, *Mucor* species, or other members of the class of Zygomycetes, also causes invasive sinopulmonary infections.

Category	Features
<b>Rhinocerebral</b>	The most frequent presentation overall and classically affects diabetics with ketoacidosis. Presents with facial and/or eye pain. Common complications include cavernous sinus (rhinocerebral syndrome) and internal carotid artery thrombosis.
<b>Pulmonary</b>	It occurs most frequently among neutropenic patients and presents with nonspecific symptoms such as fever, cough and dyspnea; hemoptysis may occur with vascular invasion.
<b>Gastrointestinal</b>	Usually affects patients with severe malnutrition; clinical picture mimics intra-abdominal abscess. The diagnosis is often made at autopsy.
<b>Cutaneous</b>	Reported with minor trauma, insect bites, no sterile dressing, wounds, and burns. Necrotic lesions progressively evolve from the epidermis into dermis and even muscle.

# Conclusions

- Most fungal infections affect our surface not our contents
- A few dimorphic fungi can cause systemic infections in otherwise healthy people.
  - Endemic areas
  - Contact by inhalation
- Candida species inhabit our guts and usually stay there, but, given the right (wrong) conditions can disseminate to infect almost any organ.
  - Important nosocomial infection
- In immune compromised people, any fungus can be a deadly pathogen