Introduction

- Fungal infections of mammals are relatively rare
- Suggests that mammals have developed complex defense mechanisms against fungi
- Casadevall (2005) suggested that the evolutionary emergence of mammals may reflect their ability to survive massive exposure to fungal pathogens, and may explain the demise of the dinosaurs

Introduction (cont.)

- How are mammals resistant to fungal infection?
  - Core body temperature of 37-39°C is higher than the temperature at which most fungi thrive
  - Possess alkaline body fluids; fungi prefer acidic or neutral pH
  - Evolution of complex innate and adaptive immune systems

Virulence and Pathogenesis

- Pathogenesis – the ability of an organism to cause disease
- Virulence – the relative ability/degree of an organism to cause disease
- All fungal pathogens can cause disease (i.e., pathogenesis), but some are more virulent, i.e., have a greater ability to initiate disease
- Why the difference? Answer: Virulence Factors

Virulence and Pathogenesis (cont.)

- Virulence factor – attribute/component of a pathogen that permits it to cause disease
  - Typically genetically encoded
  - Mutations or evolutionary changes reduce virulence of a pathogen, but not its viability
  - Key concept: virulence factors are not essential for the organism to exist
  - e.g., the Krebs Cycle is not usually considered virulence factor; its absence is likely to affect virulence, but viability is as likely to be reduced
Virulence and Pathogenesis (cont.)

- Because many fungi only cause disease in immunocompromised individuals, it is difficult to identify true virulence factors.
- A more accurate concept might be "virulence attributes" – components of a pathogen that have arisen through selective pressures to allow fungi to adapt to their environment and to host conditions, the latter being a critical step in pathogenesis.

Aspergillus as a Pathogen

- Aspergillus fumigatus and related species are highly-significant opportunistic pathogens.
  - No classical virulence factors have been discovered.
  - Mutations in 20+ genes have been demonstrated to reduce virulence.
  - All these genes have primary roles in basic fungal biology, e.g., growth, metabolism, etc.
  - Hence, their secondary roles are as "virulence attributes" that promote survival in vivo.

Aspergillus as a Pathogen (cont.)

- Growth and development
  - Hypothesis: pathogenesis of Aspergillus is dependent upon increased in vivo growth rates.
  - Tested with calcineurin mutants (cnaA) – mediates signaling pathways essential to growth and cellular development.
  - Inhibitors of calcineurin already exist and could potentially be used in combination therapy.
Aspergillus as a Pathogen (cont.)

- Secondary metabolism of Aspergillus appears to be a virulence attribute
  - Gliotoxin is an immunosuppressant produced by *A. fumigatus* in vivo
  - Gliotoxin mutants
    - Still viable
    - Reduced virulence
- Thermotolerance – can grow up to 70°C

Cryptococcus as a Pathogen

- *Cryptococcus neoformans* is a significant pathogen of AIDS patients
  - Associated with soil, trees, and avian excretions
  - Recent outbreak of disease in Vancouver, Canada and upper West Coast of the United States caused by a new species, *Cryptococcus gattii*
    - Unusual in that victims were **not** immunosuppressed
    - *C. gattii* possesses true classic virulence factors

Cryptococcus as a Pathogen (cont.)

- Virulence attributes of *C. neoformans*:
  - Growth at 37-39°C
  - Capsule formation
  - Melanin production
- Capsules help prevent phagocytosis
- Melanin helps protect against oxidative radicals produced by the host

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Lecture 3: Fungal Pathogenesis
Cryptococcus as a Pathogen (cont.)

• How did these virulence attributes of \( C. \) \( \text{neoformans} \) arise?
• Answer may lay in interactions of \( C. \) \( \text{neoformans} \) with other organisms in its saprobic environment
• Amoeba and macrophages share many of the same biological processes, including phagocytosis, phagosome processing, and lysosomal digestion of engulfed particles

Hypothesis: \( C. \) \( \text{neoformans} \) evolved virulence attributes to survive attack by amoeba

- Wild type strains (with capsules) replicated and killed the amoebic host when phagocytized
- Capsule mutants were destroyed by amoeba following phagocytosis
- By comparison, the non-pathogenic yeast \( \text{Saccharomyces cerevisiae} \) and the pathogenic yeast \( \text{Candida albicans} \), both having no capsules, were also destroyed by amoeba

Similar results were obtained with other soil-dwelling eukaryotic microbes

Collective results suggest that soil-dwelling microbes can aid in the rapid alteration/evolution of \( C. \) \( \text{neoformans} \)

Perhaps similar mechanisms account for the evolution of the new species, \( C. \) \( \text{gattii} \)

Candida as a Pathogen

• \( \text{Candida} \) species are the fourth most common cause of nosocomial infections
  - Infections mainly occur in immunocomprised persons
  - Very common commensal organism of the skin and lower gastrointestinal tract
  - Many of the infections are from endogenous sources
• Two major virulence attributes: biofilm formation and environmental adaptation

• Biofilm formation
  - Three stages
    - Attachment and colonization
    - Cell growth and proliferation with a basal layer of yeast cells
    - Pseudohyphae and hyphal development and concomitant extracellular matrix production
  - Immediate recognition of specific surface features causes immediate transcriptional program response, including genes for drug resistance
Fungal Dimorphism

- Many fungi have the ability to exist in two or more morphologies
- Only in the “true” dimorphic fungi is this property highly associated with virulence
- Dimorphism is often associated with the specific production of virulence factors, e.g., CBP1 gene of *Histoplasma capsulatum*