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Review

Overview of vertebrate animal models of fungal infection

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ABSTRACT

Fungi represent emerging infectious threats to human populations worldwide. Mice and other laboratory animals have proved invaluable in modeling clinical syndromes associated with superficial and life-threatening invasive mycoses. This review outlines salient features of common vertebrate animal model systems to study fungal pathogenesis, host antifungal immune responses, and antifungal compounds.

Contents

1. Introduction

Fungal pathogens are associated with significant infectious morbidity and mortality in humans and with extinctions in amphibian (i.e. collapse of frog species due to chytridiomycosis) and mammalian (i.e. white nose bat syndrome in the Northeastern United States) populations (Heitman, 2011; Brown et al., 2012; Fisher et al., 2012). The emergence of *Cryptococcus gattii* as a primary pathogen in the Pacific Northwest (Byrnes et al., 2011), the description of a novel opportunistic fungus (*Emmonsia parva*) in South African AIDS patients (Kenyon et al., 2013), and the recent outbreak of fungal meningitis due to contaminated corticosteroid injections (Kainer et al., 2012; Smith et al., 2013); all exemplify novel public health threats posed by fungi.

Superficial and mucosal fungal infections, though rarely life-threatening, affect approximately one-quarter of humans

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worldwide and cause discomfort, disfigurement, diminished reproductive function, and social isolation. Although life-threatening invasive fungal infections are much less frequent, a recent publication estimated that ~2 million such infections occur annually (Brown et al., 2012). Mortality rates associated with invasive infections remain unacceptably high due to lack of access (in the developing world) and limited efficacy of antifungal drugs, and the presence of significant co-morbidities (e.g. AIDS, receipt of immunosuppression for organ transplantation, receipt of myeloablative therapy for cancer) in many patient groups [reviewed in (Brown et al., 2012)]. Furthermore, no vaccines have been licensed for human use to prevent or mitigate fungal infections.

Over the past 4 to 5 decades, researchers have developed a plethora of animal models to investigate fungal pathogenesis, host immune responses, and to examine antifungal properties of chemical and biological compounds. Although inbred strains of laboratory mice are most commonly used to model clinical syndromes associated with pathogenic fungi, other vertebrate hosts (e.g. rats, guinea pigs, rabbits, and zebrafish) have gained popularity, since each model system affords distinct advantages (e.g. repeated body fluid sampling and drug administration in rabbits, availability of genetically defined strains in mice, non-invasive imaging of the infection process in transparent zebrafish larvae) as well as limitations. The purpose of this review is to provide an overview of common syndromes caused by pathogenic fungi and of relevant vertebrate animal models developed to gain insight into fungal pathogenesis process, host immune responses, and the diagnosis and treatment of fungal infections. Due to the broad nature of the topic, it is not possible to emphasize technical detail in all vertebrate animal models discussed herein and the reader is referred to references in Table 1. Several outstanding recent reviews summarize the emergence of fruit flies (*Drosophila melanogaster*) (Lionakis and Kontoyiannis, 2012), wax moths (*Galleria mellonella*) (Achterman et al., 2011; Lionakis, 2011), and nematodes (*Caenorhabditis elegans*) (Muhammed et al., 2012a) as invertebrate mini-hosts that are particularly suited for high-throughput screening of chemical libraries for antifungal drug discovery, high-throughput analysis of fungal mutants, and for host-pathogen studies at ambient temperature (Fuchs and Mylonakis, 2006; Desalermos et al., 2012). Similarly, the review does not aim to summarize the wealth of insight gained from animal models into the pathogenesis, host immune response, diagnosis, and treatment of fungal infections and the reader is referred to recent publications (Sable et al., 2008; Brown, 2011; Steele and Wormley, 2012; Wuthrich et al., 2012; Drew et al., 2013; Lanternier et al., 2013).

2. Human pathogenic fungi and clinical syndromes

The Earth harbors an estimated 5 million species of fungi that prosper in all natural habitats and collectively play a vital role in decomposing organic matter (Blackwell, 2011). Fungi assume different cellular morphologies. Yeasts form round, oval, or spherical cells that divide by budding, or in rare cases, by fission. Molds form branching tubular filaments, termed hyphae, that grow by elongation and propagate by forming conidia (asexual spores), most of which are dispersed in the air. Dimorphic fungi can switch between morphologic states and typically exist as yeast cells in human tissues and as hyphae within natural environments. Medically relevant yeasts, molds, dimorphic fungi, and the most common sites of clinical disease in humans are summarized in Fig. 1A.

Several hundred species of fungi have been documented to cause superficial, deep tissue, and disseminated diseases in humans. Dermatophytes are the primary causes of superficial infections of the skin (in the epidermal layer) and nails, primarily due to species from the genera *Trichophyton*, *Epidermophyton*, and *Microsporum* (Havlickova et al., 2008). Dermatophytes thrive on human keratin and commonly give rise to infections of the scalp (Tinea capitis, a.k.a as ringworm; over 200 million cases in children worlwide), glabrous skin (Tinea corporis), groin (Tinea cruris), feet (Tinea pedis), and nails (Tinea unguium, a.k.a. onychomycosis; up to a 10% prevalence worldwide, particularly in diabetics and the elderly), yet rarely invade deep tissues or enter the circulation (Achterman and White, 2012). In skin areas rich in sebaceous glands, several species of the lipophilic fungus *Malassezia* are a common cause of dandruff and seborrheic dermatitis. Subcutaneous infections that extend into the deeper dermal layer (e.g. chromoblastomycosis or phaeohyphomycosis) are typically acquired by traumatic inoculation of fungal elements into human skin and give rise to chronic, slow-growing infections that can be highly destructive at local sites, yet rarely disseminate systemically. A variety of *Candida* species cause superficial infections of the skin, oral and genital mucosa. For example, 10 million estimated cases of oral or esophageal thrush occur annually, most of which are linked to the HIV pandemic. Vulvovaginal candidiasis is common in adult women, with approximately one-half of women experiencing at least one symptomatic episode during their childbearing years, and an estimated 50 million women that suffer from recurrent disease, defined as four or more episodes annually. Primary immunodeficiency syndromes, particularly defects in the respiratory burst (i.e. chronic granulomatous disease) or in the interleukin-17 (IL-17) cytokine signaling pathway predispose to mucocutaneous candidiasis (Lanternier et al., 2013).

The majority of fungi associated with life-threatening infections are inhaled as infectious propagules, invade sinopulmonary tissues, and in specific instances, disseminate to extrapulmonary sites. *Candida* species are the major exception to this rule and reside in the gastrointestinal tract as commensal organisms in humans. Of the estimated 2 million annual life-threatening invasive infections, 90% are caused by *Candida*, *Cryptococcus*, *Aspergillus*, and *Pneumocystis* species (Brown et al., 2012). Geographically restricted dimorphic fungi (i.e. *Blastomyces dermatitidis*, *Coccidioides immitis*, *Histoplasma capsulatum*, *Paracoccidioides brasiliensis*, and *Penicillium marneffei*) and non-*Aspergillus* molds (i.e. *Mucorales* order; agents of mucormycosis) account for most *remaining life-threatening infections*.

Candida species are the fourth most common cause of nosocomial bloodstream infections in the United States (Wisplinghoff et al., 2004). Candidemia is typically observed in severely ill patients with breaches in gastrointestinal or mucocutaneous barrier function (e.g. indwelling vascular access catheters or following abdominal surgeries). The pathogenesis of invasive candidiasis typically involves colonization of the gastrointestinal tract or mucosal surface,