bjh review

The immune response to fungal infections

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Summary

During the past two decades, invasive fungal infections have emerged as a major threat to immunocompromised hosts. Patients with neoplastic diseases are at significant risk for such infections as a result of their underlying illness and its therapy. *Aspergillus*, *Candida*, *Cryptococcus* and emerging pathogens, such as the zygomycetes, dark walled fungi, *Trichosporon* and *Fusarium*, are largely opportunists, causing infection when host defences are breached. The immune response varies with respect to the fungal species and morphotype encountered. The risk for particular infections differs, depending upon which aspect of immunity is impaired. This article reviews the current understanding of the role and relative importance of innate and adaptive immunity to common and emerging fungal pathogens. An understanding of the host response to these organisms is important in decisions regarding use of currently available antifungal therapies and in the design of new therapeutic modalities.

Keywords: aspergillosis, candida, cryptococcosis, T cells, dendritic cells.

Overview

With the increasing number of immune compromised patients, fungi have emerged as major causes of human disease. These pathogens are largely opportunists, causing infection when host defences are breached. In this regard, patients with neoplasms are particularly at risk due to their immunocompromise, which can be a consequence of either their underlying malignancy and/or the treatment for their disease. Of the over 100 000 fungal species in existence, only a small percentage is known to cause human infection. Risk factors for systemic candidiasis include presence of intravascular catheters, receipt of broad-spectrum antibiotics, injury to the gastrointestinal mucosa and neutropenia. Patients with haematological or solid malignancies and transplant recipients are especially vulnerable. Mucosal candidiasis also occurs in such patients and in those with Acquired Immune Deficiency Syndrome (AIDS). Cryptococcosis predominantly affects persons with advanced AIDS, lymphoid and haematological malignancies and transplant recipients. Filamentous fungi, including species of Aspergillus and Fusarium, the Zygomycetes, and the dark walled fungi, generally cause invasive disease in neutropenic hosts and solid organ transplant recipients. At highest risk are patients with prolonged and profound neutropenia after treatment with highly cytotoxic chemotherapy for haematological malignancies and recipients of haematopoietic stem cell transplantation (HSCT). In the latter group, infections with filamentous fungi are increasingly encountered during the post engraftment period. In this article, we will discuss the host response to the pathogenic fungi most likely to infect oncology patients.

The immune response varies with respect to the fungal species encountered. The relative importance of specific innate and adaptive defence mechanisms differs, depending upon the organism and anatomical site of infection (Table I). Within a species, the fungal morphotype (e.g. yeast, pseudohyphae and hyphae of *Candida albicans*) may be an important determinant of the host response. Whereas yeasts and spores are often effectively phagocytosed, the larger size of hyphae precludes effective ingestion. Pathogenic fungi have developed mechanisms to elude and subvert host defences. Some fungi have evolved as intracellular parasites and can survive within phagocytes by using them to evade fungal killing and to disseminate throughout the host. Major characteristics of the immune response are the interdependence of various arms of the immune system and the interplay between host defences and fungal pathogenic mechanisms.

Several shared defence mechanisms are operative in response to a range of fungi. Neutrophils, macrophages and monocytes are fundamentally important antifungal effector cells. Phagocytes already residing in target organs at the time of infection attempt to kill or damage fungi. Additional effector cells, including neutrophils and monocytes, are recruited to sites of infection by the action of inflammatory signals, such as cytokines, chemokines and complement components. Fungi are killed or damaged by production and/or release of reactive oxygen intermediates and antimicrobial peptides (Diamond

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Table I. Defects in host defences that predispose patients to infections with specific fungi.

Fungal pathogen	Host factor
Candida (mucosal)	Impaired cell mediated immunity
Candida (disseminated)	Impaired mucosa or integument, neutropenia
Aspergillus	Neutropenia, high-dose corticosteroids
Cryptococcus	Impaired cell mediated immunity, corticosteroids
Zygomycetes	Neutropenia, deferoxamine treatment, corticosteroids, diabetic ketoacidosis
Fusarium	Neutropenia, impaired integument, corticosteroids
Scedosporium	Neutropenia
Trichosporon	Neutropenia, impaired integument

et al, 1980; Mambula *et al*, 2000). Whether the cells use intracellular or extracellular antifungal mechanisms depends upon the infecting species, morphotype, and route of exposure (Diamond *et al*, 1978; Schaffner *et al*, 1982; Kan & Bennett, 1988).

Dendritic cells initiate innate and adaptive immunity to a range of microorganisms (Huang *et al*, 2001). These cells capture and process antigens, express lymphocyte co-stimulatory molecules, migrate to lymphoid organs and secrete cytokines to initiate immune responses (Banchereau & Steinman, 1998). Dendritic cells have an instrumental role in linking innate and adaptive responses to a range of pathogenic fungi including *Aspergillus fumigatus*, *Cryptococcus neoformans* and *C. albicans* (Bauman *et al*, 2000; Braedel *et al*, 2004). Signals transmitted by dendritic cells can vary depending upon the encountered fungus or morphotype with resultant differences in the nature of adaptive immune responses elicited.

Differentiation of $CD4^+$ T cells along a T-helper (Th) cell type 1 (Th1) or type 2 (Th2) pathway and development of specific Th responses, is an essential determinant of the host's susceptibility or resistance to invasive fungal infections. Development of Th1 responses is influenced by the concerted action of cytokines, such as interferon (INF)-γ, interleukin (IL)-6, tumour necrosis factor (TNF)-α, and IL-12, in the relative absence of Th2 cytokines, such as IL-4 and IL-10 (Romani, 2002). The predominance of Th1 over Th2 type cytokines correlates with protection against various mycoses (Romani *et al*, 1994; Roilides *et al*, 1999). Within this framework, however, are subtleties relating to quantitative and temporal production of cytokines and the ultimate development of particular T-cell responses, as well as a role for modulation of immunity so as to limit autoimmune injury.

Both human and fungal cells are eukaryotic, as opposed to bacteria, which are prokaryotes. Thus, in addition to anatomic similarities (such as possessing a nucleus surrounded by a nuclear membrane, 80S ribosomes, and Golgi apparati), fungal and human cells have similar mechanisms for DNA, RNA and protein synthesis. This greatly limits the number of potential antifungal drug targets because the vast majority of compounds that inhibit fungi also are toxic to human cells. Therefore, antifungal drugs tend to target the few features of fungal cells that differ from human cells. Most fungal cell membranes contain ergosterol, rather than cholesterol, which is the sterol found in human cell membranes. Amphotericin B directly binds to ergosterol, whereas the 'azoles' and terbinafine target ergosterol synthesis. However, the major distinguishing feature is that fungal cells have a rigid cell wall containing chitin, mannans and glucans (the target of the echinocandin class of antifungal drugs).

The fungal cell wall imparts upon the fungus physical protection, making it resistant to certain host defences, such as complement-mediated lysis. Innate immune defences, including β-glucan receptors, mannose receptors, and toll-like receptors (TLRs), have evolved to recognize and respond to components of fungal cell walls. For example, at the phagocytic cell surface are TLRs that identify conserved molecular patterns found on microbial (including fungal) products (Akira *et al*, 2001; Mambula *et al*, 2002; Levitz, 2004). These receptors are composed of an extracellular domain that distinguishes microbial products and a cytoplasmic domain that transmits signals to intracellular adapter proteins. One such adapter, MyD88, initiates a signalling cascade leading to the expression of microbicidal molecules and cytokines. The proportional role of individual receptors, such as TLR2, TLR4, and TLR9, in MyD88 activation varies depending upon the infecting fungus and the site of infection. Specific receptors differentially activate antifungal functions, which may result in dissimilar responses and susceptibility to infection (Bellocchio *et al*, 2004; Braedel *et al*, 2004).

Aspergillus

Aspergillus species are ubiquitous moulds with worldwide distribution. Exposure most commonly occurs when airborne spores are inhaled into the lungs or sinuses. Once inhaled, spores reach distal areas of the lung by virtue of their small size. The most common infecting species is *A. fumigatus*, followed by *A. flavus* and *A. niger* (Marr *et al*, 2002; Husain *et al*, 2003). In normal hosts, isolation of *Aspergillus* generally reflects colonization and not infection (Uffredi *et al*, 2003). The clinical manifestations of aspergillosis vary depending upon the nature of the host. In atopic individuals with an allergic or hypersensitivity response, the fungus triggers immune phenomena including allergic rhinitis, asthma, hypersensitivity pneumonitis and allergic bronchoplumonary aspergillosis (ABPA) (Horner *et al*, 1995). In patients with cavitary pulmonary lesions, saprophytic colonization by *Aspergillus* leads to aspergillomas. Finally, immunocompromised patients may develop invasive aspergillosis (IA). This article will focus on the latter group of patients. The degree of fungal invasion, response to therapy and clinical outcome of IA depends upon the type and depth of immune suppression. In susceptible hosts, *Aspergillus* conidia germinate to form hyphae, the

invasive form of the organism. An essential aspect of the immune response to *Aspergillus* is recognition and killing of conidia and activation of appropriate host defences to confront fungi that have escaped killing and transitioned to the hyphal form. In highly compromised patients, early detection of IA, institution of antifungal therapy and improvement in host immune status are crucial determinants in the outcome of infection (von Eiff *et al*, 1995).

Qualitative and quantitative disorders of phagocyte function are the most important host factors predisposing patients to IA. In patients with neoplasms, virtually all cases are due to the therapy used to treat the malignancy, rather than the underlying disease itself. The major risk factor for IA is chemotherapy-induced neutropenia, with the risk being directly proportional to both the severity and the duration of the neutropenia (Gerson *et al*, 1984). Treatment with high doses of corticosteroids, which depresses neutrophil and macrophage function, also predisposes patients to IA. Other risk factors include chronic granulomatous disease, advanced AIDS and chronic graft-*versus*-host disease. The incidence of invasive fungal infections occurring after resolution of neutropenia is also increasing with the widespread use of highly immunosuppressive regimens, such as CAMPATH1-H or fludarabine for low intensity transplantation. The most frequent sites of involvement are the lungs followed by the sinuses. Infection may disseminate from the respiratory tract to other sites including the eyes, brain, liver, spleen, kidney, skin and bone (Stevens *et al*, 2000). Occasionally, direct inoculation of the fungus during invasive procedures or injection drug use can lead to soft tissue and even disseminated infection. IA in neutropenic hosts is characterized by extensive hyphal infiltration, angioinvasion, coagulative necrosis, intra-alveolar haemorrhage, extra-pulmonary dissemination and high mortality (Berenguer *et al*, 1995). In hosts that are less severely compromised, such as transplant recipients receiving corticosteroids and calcineurin inhibitors, IA tends to be more indolent and is associated with a relatively higher survival rate.

Inhaled *Aspergillus* conidia that are not repelled by respiratory tract mucociliary defences are mostly phagocytosed by macrophages and dendritic cells. These cells constitute the initial line of defence and have a dual role as antifungal effectors and as activators of the immune response (Kan & Bennett, 1988). Resident and monocyte-derived macrophages ingest and kill conidia, thus preventing transition into the invasive hyphal form (Schaffner *et al*, 1982, 1983; Waldorf *et al*, 1984a; Levitz *et al*, 1986; Philippe *et al*, 2003). After recognition and binding of conidia, actin-dependent pseudopodia capture and internalize the fungal particles. Swelling of the conidia inside the macrophage appears to be a prerequisite for fungal killing (Philippe *et al*, 2003). *Aspergillus* containing phagosomes mature by fusion with endocytic compartments. Conidial killing proceeds with acidification of the phagolysosome (Ibrahim-Granet *et al*, 2003). Production of reactive oxidant intermediates within alveolar macrophages is important for conidial killing, although non-oxidative killing mechanisms also play a role. In mice, impairment of NADPH oxidase inhibits killing without impairing phagocytosis. Corticosteroids inhibit production of reactive oxidant intermediates and killing of phagocytosed fungi, which may help to explain the elevated rates of IA in steroid recipients (Philippe *et al*, 2003). Cyclosporin A exerts only a modest effect on phagocytic defences and in the absence of corticosteroids does not increase the progression of IA (Roilides *et al*, 1994; Berenguer *et al*, 1995). Experimental models, as well as reports of IA in patients receiving anti-TNF-α antibodies, suggest cytokine networks are essential for the activation of leucocyte antifungal activity (Warris *et al*, 2001).

Humoral factors participate in the host response to *Aspergillus*. Resting conidia, germinating conidia and hyphae are potent activators of the complement cascade and induce deposition of complement components upon the fungal surface. Resting conidia activate the alternative pathway and induce neutrophil chemotaxis. As the fungus matures into swollen conidia and then hyphae there is progressive dependence on the classical pathway (Kozel *et al*, 1989). In alveolar fluid, surfactant proteins A (SP-A) and D (SP-D) enhance chemotaxis, binding, phagocytosis and oxidative killing (Madan *et al*, 1997). These C-type lectins also agglutinate *Aspergillus* conidia, thereby immobilizing the pathogen. However, despite the importance of these humoral factors in experimental systems, the predisposing factor for the vast majority of patients with IA is phagocytic dysfunction and not defects in the humoral immunity.

Macrophages and dendritic cells activate host defences in response to Aspergillus. At the surface of these cells are TLRs that identify microbial products (Akira *et al*, 2001). Signalling pathways associated with each TLR vary and activation of different receptors may result in dissimilar biological responses. For example, TLR 2 and TLR 4 differentially mediate the release of specific cytokines in response to the fungus (Braedel *et al*, 2004). When stimulated by *Aspergillus* conidia, macrophages produce proinflammatory cytokines, including TNF-α, IL-1α and IL-1β through TLR 4-dependent mechanisms. *Aspergillus* hyphae, on the other hand, stimulate production of the anti-inflammatory cytokine IL-10 through TLR 2-dependent mechanisms. Thus, activation of specific cell surface receptors during germination may allow *Aspergillus* to counteract host defences (Netea *et al*, 2003). TNF-α release, initially by resident macrophages and later by recruited immune cells, is associated with chemokine production [including Macrophage Inflammatory Protein (MIP)-1α and MIP-2] and influx of neutrophils and monocytes into infected tissues (Mehrad *et al*, 1999). Mice lacking functional CC chemokine receptor 1(CCR1) are much less efficient at blocking tissue invasion from the blood and have increased susceptibility to infection (Gao *et al*, 1997). *Aspergillus* conidia and hyphae induce nuclear factor (NF)-κB translocation, and release of TNF-α and MIP-2 in a TLR 2- and TLR 4-dependent manner. Neutrophil recruitment is severely impaired in mice lacking both functional TLR 2 and TLR 4, but is less impaired

in single TLR 2 or TLR4 deficient mice, suggesting that both receptors are required for an optimal immune response to *Aspergillus* (Meier *et al*, 2003). In humans, optimal TNF-α signalling in response to the various morphotypes of *Aspergillus* requires TLR 2, CD14 and MyD88 (Mambula *et al*, 2002; Levitz, 2004). Administration of corticosteroids suppresses macrophage production of IL-1α, TNF-α, and MIP-1α, all of which are protective against aspergillosis (Brummer *et al*, 2003).

Dendritic cells modulate the antifungal host response. *Aspergillus* antigens induce activation and maturation of these cells. Ingestion of *Aspergillus* conidia and hyphae proceeds through distinct phagocytic mechanisms and elicited responses differ depending upon the morphotype encountered. Following exposure to *Aspergillus*, dendritic cells migrate to the spleen and draining lymph nodes and induce local and peripheral Th cell reactivity to the fungus (Bozza *et al*, 2002). The development of specific Th responses is an essential determinant of the host's susceptibility or resistance to IA. As with other fungi, production of Th1 cytokines appears to be protective, whereas Th2 responses are not (Cenci *et al*, 1998; Cenci *et al*, 1999, 2001; Del Sero *et al*, 1999). In that regard, proinflammatory signals, including granulocyte-macrophage colony-stimulating factor (GM-CSF), TNF-α, IFN-γ, IL-1, IL-6, and IL-12, IL-18 as well as the chemokines MIP-1, monocyte chemoattractant protein (MCP)-1, and MIP-2, are associated with protection, and IL-4 and IL-10 with invasion. Cell surface and secreted pattern recognition receptors mediate the development of Th responses. TLR-associated MyD88-dependent signalling is crucial for priming antifungal Th1 responses (Bellocchio *et al*, 2004). The secreted pattern recognition receptor PTX3 binds to select microbial products, including *Aspergillus* conidia, and has a non-redundant role in resistance to the fungus. PTX3-deficient mice are susceptible to invasive pulmonary aspergillosis with impaired recognition of the fungus by alveolar macrophages and dendritic cells and inappropriate induction of a Th2 response (Garlanda *et al*, 2002).

When bronchoalveolar macrophages fail to control the fungus, conidia germinate into hyphae, pierce through the cell and grow extracellularly. Neutrophils and monocytes recruited from the circulation phagocytose and damage fungi that have escaped killing and are transitioning (or have transitioned) into hyphae. As described above, proinflammatory signals, including TNF-α, complement, chemokines, and surfactant proteins, recruit neutrophils into sites of infection. Since hyphae are too large to be completely phagocytosed, hyphal damage is achieved via extracellular means. Non-oxidative mechanisms include release of lysozyme and neutrophil cationic peptides. Oxidative killing is mediated by myeloperoxidase (MPO)-dependent and MPO-independent oxidative systems (Washburn *et al*, 1987). The importance of oxidative killing is demonstrated in chronic granulomatous disease. This hereditary disease is characterized by impaired production of oxidative intermediates and elevated rates of invasive infections due to several catalase positive pathogens, including *Aspergillus* (Cohen *et al*, 1981; Diamond & Clark, 1982). Phagocytosis of *Aspergillus* is enhanced by opsonization and proinflammatory molecules (Marr *et al*, 2001). TNF-α augments the capacity of neutrophils to damage hyphae, possibly through enhanced oxidative mechanisms, and increases anticonidial phagocytic activity of resident macrophages (Roilides *et al*, 1998a). Granulocyte colony-stimulating factor (G-CSF), GM-CSF and especially IFN-γ enhance monocyte and neutrophil activity against hyphae (Gaviria *et al*, 1999). IL-15 enhances hyphal damage and IL-8 release by neutrophils challenged with *Aspergillus* (Winn *et al*, 2003a). IL-8 recruits neutrophils to sites of inflammation and mediates release of antimicrobial peptides. By contrast, production of IL-4 by $CD4^+$ T lymphocytes impairs neutrophil antifungal activity (Cenci *et al*, 1997). IL-10 suppresses oxidative burst and antifungal activity of mononuclear cells against hyphae, while increasing their phagocytic activity (Roilides *et al*, 1997). Corticosteroids reduce oxidative burst and superoxide anion release by neutrophils, thereby inhibiting hyphal killing (Roilides *et al*, 1993; Brummer *et al*, 2003). Treatment with G-CSF and IFN-γ may prevent this impairment.

A variety of factors that may augment virulence by immune evasion have been described in *Aspergillus*, but their contribution *in vivo* is difficult to gauge. Gliotoxin inhibits ciliary, macrophage, neutrophil, and lymphocyte function and induces apoptosis in immune cells (Mullbacher & Eichner, 1984; Pahl *et al*, 1996; Tsunawaki *et al*, 2004). Other *Aspergillus*-derived factors inhibit oxidative killing, interfere with ciliary function, inactivate complement, disrupt production of proinflammatory cytokines and promote adhesion to endothelium (Washburn *et al*, 1986; Jahn *et al*, 2000).

Candida

There are more than 150 species in the genus *Candida*, but only a small number are known to be human pathogens. The most common organisms to cause infection are *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. krusei*. Many clinical isolates of *C. glabrata* and *C. krusei* have reduced susceptibility to fluconazole and itraconazole. Routine azole antifungal prophylaxis in patients receiving intensive chemotherapy, as in HSCT, may facilitate infection with those species. Intravascular catheters may predispose to *C. parapsilosis* infection, and removal of the device is usually necessary for resolution of infection (Pappas *et al*, 2004). Although in nature *Candida* exists mainly in a unicellular yeast phase, in clinical specimens most species are found as yeasts, pseudohyphae and hyphae. *Candida* species, particularly *C. albicans*, commonly colonize the human gastrointestinal, respiratory, reproductive tracts and the skin. A variety of adhesins have been described that facilitate attachment to epithelial and endothelial surfaces. Clinical disease can be divided into two broad categories, mucocutaneous and systemic infection, each with its own risk factors. Transition from colonization to mucosal invasion and/or systemic dissemination depends

upon host and fungal factors (Cole *et al*, 1996). Alterations of normal flora as a result of antibiotic therapy, acquired or primary immune deficiency and breakdown of anatomical barriers predispose to infection.

Oropharyngeal candidiasis (OPC) is among the most common mycotic infections of immunocompromised patients. Clinical manifestations of OPC are diverse, and multiple forms are frequently present concurrently (Dodd *et al*, 1991). Pseudomembranous candidiasis or thrush, the most commonly encountered type, is generally characterized by curd like, white patches on the buccal mucosa, gingiva, palate and tongue that leave a raw, erythematous undersurface when scraped off. Development of infection depends upon both systemic and local determinants. Risk factors for oral candidiasis include extremes in age, diabetes mellitus, particularly when glycemic control is poor, nutritional deficiencies, use of broad spectrum antibiotics and immunosuppression (especially of cell-mediated immunity) (Klein *et al*, 1984; Guggenheimer *et al*, 2000). Local factors that promote infection include dentures, salivary abnormalities, treatment with inhaled steroids, and destruction of mucosal barriers with radiotherapy for head and neck cancers or cytotoxic chemotherapy. Human immunodeficiency virus (HIV) is one of the most important predisposing conditions worldwide. AIDS patients have a particularly high incidence of mucosal candidiasis, which is often recurrent and, when it involves the esophagus, can be disabling (Sangeorzan *et al*, 1994).

Local defence mechanisms against mucosal infection include salivary proteins, such as lactoferrin, beta-defensins, histatins, lysozyme, transferrin, lactoperoxidase, mucins, and secretory immunoglobulin A. These impair adhesion and growth of *Candida* in the oropharyngeal cavity. Healthy oral epithelial cells inhibit blastoconidia and/or hyphal growth of several *Candida* species (Steele *et al*, 2000). Local mucosal immunity appears to be at least as important as systemic cell mediated immunity. Development of OPC has been associated with a salivary Th2-type cytokine profile (Leigh *et al*, 1998).

Cell-mediated immunity plays the dominant role in prevention of candidiasis at the gastrointestinal surfaces. In AIDS, development of oropharyngeal and oesophageal candidiasis correlates with declining $CD4^+$ lymphocyte counts. Therapy with highly active antiretroviral therapy (HAART) is associated with significant decreases in the prevalence of oral and oesophageal candidiasis. This effect may be due to immune reconstitution, decreased viral load and protease inhibitor antagonism of candidal virulence factors (Arribas *et al*, 2000; Bektic *et al*, 2001; Blanco *et al*, 2003). OPC is also associated with T cell immunosuppression from corticosteroid therapy, organ transplantation, cancer chemotherapy and chronic mucocutaneous candidiasis (CMC). Patients with CMC typically present during childhood with candidiasis, which may involve the skin, nails and mucosal surfaces. These patients often have recurrent and persistent infections. Patients with CMC have impaired cell-mediated responses to *Candida* species and subsequently are unable to effectively clear infection (de Moraes-Vasconcelos *et al*, 2001). In normal hosts, neutrophils, monocytes, $CD4^+$ and $CD8^+$ T cells collaborate in mucosal defences. In animal models, selective depletion of neutrophils and monocytes significantly increased the severity of mucosal infection (Farah *et al*, 2001). At the mucosa, activated $CD8^+$ T cells exert a direct effect against *C. albicans* hyphae (Beno *et al*, 1995). Mice that lack functional T and B cells develop ongoing mucosal infection without progression to disseminated disease. However, phagocyte depletion in these animals enhances susceptibility to mucosal and disseminated infection (Balish *et al*, 1993).

Candida species have emerged as an important cause of bloodstream and deep tissue infections. Most cases occur in hospitalized patients and up to half are associated with intensive care units. *Candida* species are the fourth most common cause of nosocomial bloodstream infections. Crude mortality may exceed 50% (Wey *et al*, 1988). Risk factors for candidaemia include breakdown of mucosal barriers due to cytotoxic chemotherapy and surgical procedures, neutropenia, changes in the gut flora due to antibiotics, and invasive interventions that breach the skin, such as intravenous lines and drains (Wey *et al*, 1989). Even with extensive mucocutaneous disease, invasive candidiasis is rare unless such risks are present. Common sites of dissemination include the bloodstream, kidney, liver, spleen, and endovascular structures.

Innate immunity is the dominant protective mechanism against disseminated candidiasis. Quantitative and qualitative abnormalities of neutrophils and monocytes are associated with systemic candidiasis. Patients with lymphoma, leukaemia, chronic granulomatous disease, and recipients of intensive cancer chemotherapy with resultant neutropenia are at increased risk for disseminated infection. Neutrophils and monocytes damage and kill yeast cells, hyphae and pseudohyphae (Diamond *et al*, 1980). Similar to the situation with *Aspergillus* hyphae, the large size of *Candida* hyphae and pseudohyphae may preclude phagocytosis. In such cases, several phagocytes collaborate to effect extracellular killing. Neutrophils and monocytes recognize and engulf opsonized and non-opsonized yeast cells via cell-surface pattern recognition receptors, including TLRs, mannose receptors and β-glucan receptors. Binding to individual TLRs and IL-1 receptor (IL-1R) activates specialized antifungal effector functions on neutrophils and other phagocytes (Bellocchio *et al*, 2004). Killing is by oxidative mechanisms, including generation of reactive oxygen and nitrogen intermediates, and by non-oxidative mechanisms. Phagocytosis and killing are augmented by opsonization and proinflammatory cytokines. Invasion of vascular structures facilitates dissemination of *Candida*. Endothelial cells resist vascular invasion by secretion of proinflammatory mediators and expression of leucocyte adhesion molecules, which recruit and bind to activated leucocytes. Mediators of inflammation at the site of damaged endothelial surfaces induce release of antimicrobial peptides from human platelets. *In vitro*, platelet factor (PF)-4, RANTES (regulated upon activation, normal T cell expressed and

secreted) and thrombin-induced microbidical protein (tPMP) are active against *Candida* (Yeaman *et al*, 1996; Tang *et al*, 2002).

Humoral immune mechanisms participate in the host response to *C. albicans*. The fungus activates complement (C) by the classical and alternative pathways with deposition of C3 on the cell fungal surface. Complement activation facilitates the recruitment of phagocytes to infected tissues and enhances their anticandidal activity. Mice deficient in C5 production have an increased propensity to develop disseminated infection. An understanding of the role of antibodies in the host response to *Candida* is evolving. Clinically, B cell deficiency is not associated with increased susceptibility to infection. However, in murine models, protective and non-protective antibodies have been described and monoclonal anticandidal antibodies are currently undergoing clinical trials in humans (Burnie & Matthews, 2004). It has been observed that the protective potential of antibodies with enhanced phagocytosis and killing of the fungus is dependent upon epitope specificity, serum titre, and ability to rapidly and efficiently fix complement to the fungal surface (Han *et al*, 2001). Antibodies directed against mannan antigens activate complement.

Development of Th1 or Th2 responses is an important determinant of the host's ability to contain infection. Th1 responses are correlated with protection. Development of Th1 responses is influenced by the concerted action of several cytokines such as INF-γ, IL-6, TNF-α, and IL-12 in the relative absence of Th2 cytokines, such as IL-4 and IL-10 which inhibit the induction of Th1 responses (Romani, 2002). Progression of infection is associated with predominance of Th2 responses. Within this framework, however, are subtleties relating to the quantitative and temporal production of Th1 and Th2 stimulating cytokines and the ultimate development of a particular T-cell response. Regulation of the immune response appears to be critical in order to elicit protective immunity in the absence of immune pathology for an organism that is normally a commensal of the human gastrointestinal and vaginal tracts (Montagnoli *et al*, 2002). Achieving a balance between Th1 and Th2 cytokines may be important for optimal antifungal protection while minimizing immune-mediated damage. *In vivo* models indicate that T regulatory cells attenuate Th1 antifungal responses, induce tolerance to the fungus and participate in the development of long lasting protective immunity after yeast priming (Montagnoli *et al*, 2002; Romani, 2004).

Dendritic cells play an important role in linking innate with adaptive immunity. The type of response depends in part upon the morphotype of *Candida* encountered. Dendritic cells phagocytose and kill *C. albicans* yeasts and hyphae by a process that utilizes distinct receptors for each morphotypes (Fig 1). Dendritic cells that ingest the yeast form induce differentiation of $CD4^+$ T cells toward a Th1 pathway. In contrast, hyphae induce Th2 responses (d'Ostiani *et al*, 2000). At the molecular level, patrol of the extracellular environment by dendritic cells and neutrophils is mediated by different cell surface receptors. Activation of the TLR-associated MyD88 adapter may occur through signalling by distinct members of the IL-1R/TLR superfamily, with the proportional role of individual receptors varying, depending upon fungal species, morphotype, and route of infection. Activation of MyD88 in dendritic cells is crucial for priming antifungal Th1 response (Bellocchio *et al*, 2004). Neutrophils, macrophages and natural killer (NK) cells also modulate adaptive responses to the fungus. Neutrophils differentially induce Th1 and Th2 responses depending on whether the exposure is to yeast or hyphae. Macrophages activate immune responses by antigen

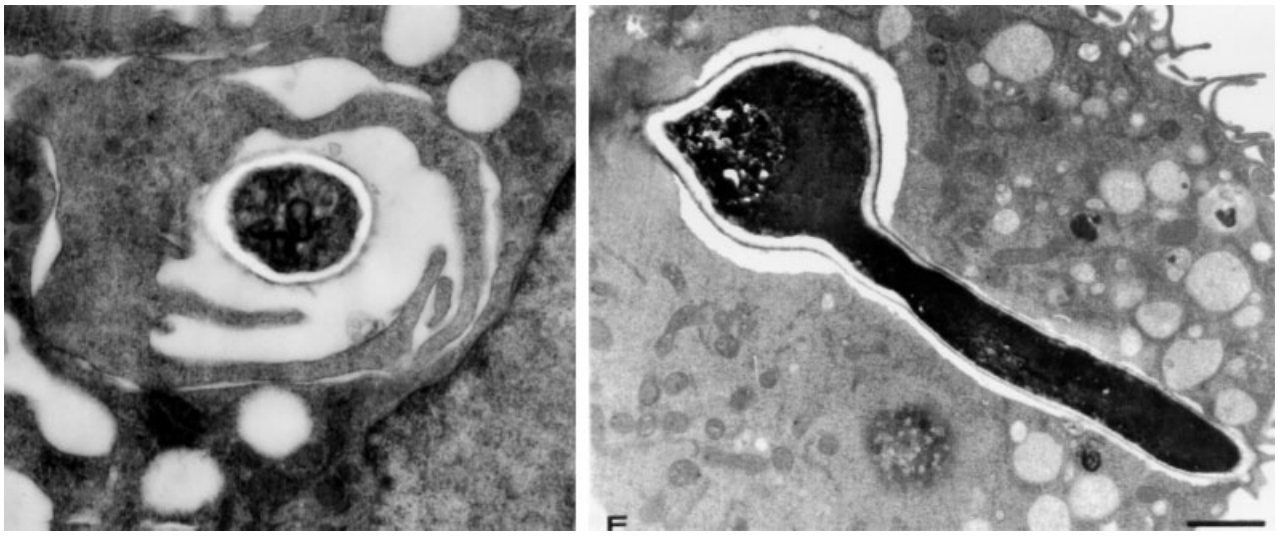

Fig 1. Transmission electron micrograph demonstrating phagocytosis of *C. albicans* yeast (left panel) and hyphal (right panel) morphotypes by dendritic cells. Photomicrographs courtesy of Dr Luigina Romani and are reproduced from d'Ostiani *et al*, (2000), by copyright permission of The Rockefeller University Press.

presentation and secretion of Th1-inducing proinflammatory signals. NK cells induce anticandidal activity in professional phagocytes (Algarra *et al*, 2002).

The syndrome of chronic disseminated candidiasis (CDC, also known as hepatosplenic candidiasis) predominantly affects patients with haematological malignancies upon recovery from neutropenia. Multiple organs, including the liver, spleen, lungs and kidneys may be involved. Clinical and laboratory features include fever, abdominal pain, hepatomegaly and elevated serum alkaline phosphatase levels. The lesions may be visualized on abdominal ultrasound, computed tomography or magnetic resonance imaging. On biopsy, the histopathology shows large granulomas with yeasts and pseudohyphae, although cultures are often negative. CDC is characterized by increased serum levels of IL-10 and local production of Th2-inducing cytokines by hepatocytes and by infected mononuclear cells (Roilides *et al*, 1998b; Letterio *et al*, 2001). Thus, although neutropenia is a major predisposing factor, the propensity for persistence of the fungus in infected tissues may be a consequence of cell-mediated immune dysregulation with suppression of Th1 and overexpression of Th2 responses.

Cryptococcus neoformans

Virtually all cases of cryptococcosis are secondary to *C. neoformans*. Although any organ system may be involved, major sites of infection are the central nervous system and lungs. The fungus has a worldwide distribution and has been recovered from multiple environmental sources including soil and avian excreta. *Cryptococcus neoformans* is the only medically important fungus to possess a capsule, which, as discussed in more detail below, is the organism's major virulence factor. The vast majority of cases occur in immune compromised hosts. In a recent survey of two cities in the USA, over 90% of cases were associated with AIDS (Mirza *et al*, 2003). In the USA and other Western countries, the availability of highly effective antiretroviral therapy has resulted in a decline in the rate of cryptococcosis in the HIV-infected population. However, persons in resource-poor settings and those with limited access to health care continue to be at substantial risk (Mirza *et al*, 2003). Also at risk are patients with diabetes mellitus, and recipients of medications (e.g. corticosteroids) that depress cell-mediated immunity. The latter group includes those receiving immunosuppressive therapy for organ transplants. There is a predilection for cryptococcosis among patients with lymphoproliferative diseases including chronic lymphocytic leukaemia (CLL), multiple myeloma, Hodgkin's disease, chronic myeloid leukaemia (CML), and lymphosarcoma (Kaplan *et al*, 1977). The prognosis for disseminated cryptococcosis in patients with neoplastic diseases may be worse than in patients with AIDS (White *et al*, 1992).

Initial exposure to *C. neoformans* is thought to most often occur with inhalation of aerosolized fungus. Airway turbulence and proper ciliary function prevent most of the yeast from reaching the distal lung (Casadevall & Perfect, 1998). Normally, fungus that reaches the lung parenchyma is controlled by the host response without extra-pulmonary dissemination. *Cryptococcus neoformans* is either eliminated completely or restricted to the lung within granulomas, where it may persist in a latent state indefinitely. When infection is not sequestered, as in cases of defective cell-mediated immunity, pneumonitis and/or dissemination may ensue. The predilection for *C. neoformans* to infect the central nervous system is not well understood. Impaired opsonophagocytosis probably plays a contributing role as has been suggested with other encapsulated pathogens.

Macrophages and dendritic cells are central to an effective response to *C. neoformans*. These cells participate in fungal recognition, phagocytosis, antigen presentation and activation of the host response. Macrophages and dendritic cells efficiently phagocytose opsonized fungus. *Cryptococcus neoformans* and its secreted products are recognized by cellular receptors including mannose receptors, CD14, TLR-2, TLR-4, and CD18 (Shoham *et al*, 2001; Mansour *et al*, 2002). The TLR-associated adapter protein, MyD88 has a major role in the response to *C. neoformans*. MyD88-deficient mice have increased susceptibility to cryptococcosis (Yauch *et al*, 2004). In dendritic cells FcγRII and mannose receptors are essential for fungal uptake and antigen presentation to T cells (Syme *et al*, 2002). These cells react to *C. neoformans* by expressing lymphocyte co-stimulatory molecules, migrating to lymphoid tissue and secreting cytokines (Banchereau & Steinman, 1998). Development of protective cell-mediated immunity depends in part on which subset of dendritic cells is activated by the fungus (Bauman *et al*, 2000). Macrophages respond to *C. neoformans* with release of proinflammatory signals, such as chemokines and IL-1. Secretion of IL-1 regulates proliferation and activation of T lymphocytes, which are important in mediating pulmonary clearance (Vecchiarelli *et al*, 1994).

Beyond phagocytosis, killing of *C. neoformans* is crucial for a successful host response. Internalization with intracellular survival may actually facilitate dissemination of the pathogen. Macrophages become efficient effector cells upon stimulation by proinflammatory cytokines. Intracellular killing mechanisms include lysosomal fusion, phagosomal acidification, sequestration of iron, and enzymatic degradation of fungal proteins. Extracellular killing is mediated by release of antifungal peptides, nitric oxide and possibly reactive oxygen intermediates (Flesch *et al*, 1989; Lovchik *et al*, 1995). Neutrophils and macrophages also contain and slowly kill *C. neoformans* by formation of histiocytic rings. Fungus can be sequestered in tissues by maturation of macrophages into multinucleated giant cells and granuloma formation.

Host and fungal factors can inhibit phagocytosis and killing. The polysaccharide capsule is the major cryptococcal virulence factor that impairs phagocytosis, although recent studies also suggest a role for the cytoplasmic protein, antiphagocytic protein 1(App1) (Luberto *et al*, 2003). The outcome of

infection may hinge upon the ability of the host to mobilize defences before fungal virulence factors interfere with a successful immune response (Vecchiarelli & Casadevall, 1998). The capsule masks potential ligands on the cell wall and presents an antiphagocytic surface. Furthermore, capsule reduces the phagocyte respiratory burst, down-regulates protective cytokine production (including TNF-α) and up-regulates production of the Th2 cytokine, IL-10. Mutant strains of *C. neoformans* that lack a capsule are avirulent in animal models of infection. HIV-1 infection impairs intracellular killing but not phagocytosis of *C. neoformans* (Ieong *et al*, 2000). Killing is also inhibited by fungal antioxidant molecules, including superoxide dismutase, mannitol and melanin. Melanin scavenges oxygen intermediates generated by leucocytes, impairs antibody formation, decreases lymphoproliferation, and down-regulates TNF-α production (Jacobson & Tinnell, 1993). Organisms deficient in laccase, the enzyme responsible for melanin production, are relatively avirulent in some mouse models of cryptococcosis (Wang *et al*, 1995).

Clinical and experimental evidence convincingly demonstrates the critical importance of cell-mediated immunity in anti-cryptococcal defences. Most cases of disseminated cryptococcosis are associated with the $CD4^+$ T cell deficiency of AIDS. Infections have also been reported in patients with non-HIV associated $CD4^+$ T cell lymphopenia (Duncan *et al*, 1993). Mice with congenital or acquired T cell deficiency have impaired anti-cryptococcal responses (Graybill & Mitchell, 1979; Graybill *et al*, 1979). Protective immunity is associated with the Th1 responses. $CD4^+$ T cells with a Th1 profile activate the effector arm of cell-mediated immunity to kill *C. neoformans*. In mice, both IFN-γ and IL-12 are required for the initiation of such a response (Hoag *et al*, 1997). Presence of protease urokinase-type plasminogen activator (uPA) correlates with enhanced immunity. In its absence IFN-γ and IL-12 levels are decreased, lymphocyte proliferative responses are diminished, and mice fail to generate protective Th1 cytokines (Gyetko *et al*, 2002).

Both $CD4^+$ and $CD8^+$ T cells combined to mediate cellular infiltration of infected tissue (Huffnagle *et al*, 1994). T cells and NK cells have direct anti-cryptococcal activity. *In vitro*, $CD4^+$ and $CD8^+$ T lymphocytes directly inhibit fungal growth by attaching to the cryptococcal cell surface (Murphy *et al*, 1993; Levitz *et al*, 1994). Binding of NK cells to the fungus and release of cytolytic compounds leads to direct killing. NK cells may further contribute to host defences by secreting IFN-γ and stimulating macrophages to kill the yeast (Kawakami *et al*, 2000). However, *C. neoformans* also decreases NK cell production of GM-CSF and TNF-α, which has the potential to affect phagocyte activity and dampen the immune response (Murphy *et al*, 1997).

Neutrophils are important particularly prior to development of acquired immunity. Early in infection there is an influx of neutrophils into the infected tissues. Enhancement of neutrophil number and function with G-CSF is protective *in vitro* and *in vivo*. A chief function of neutrophils is to kill fungus by oxidative and non-oxidative means. Oxidative killing is achieved with production of reactive oxygen intermediates. Non-oxidative mechanisms utilize antimicrobial peptides, such as defensins and calprotectins (Mambula *et al*, 2000). Neutrophils modulate the immune response by producing proinflammatory cytokines, including TNF-α, IL-1β, IL-6 and IL-8 (Retini *et al*, 1996). In murine models, early production of TNF-α is critical for an effective protective Th1 immunity. A major role for TNF-α is to prime cells for increased chemokine secretion, with resultant leucocyte migration to infected tissue. Pathogen-derived factors can impair neutrophil function. For example, capsular material inhibits rolling and fixed binding of neutrophils on the endothelium and thus limits their influx into infected tissues(Dong & Murphy, 1997; Ellerbroek *et al*, 2004).

Humoral factors contribute to antifungal defences. Human serum and saliva inhibit *C. neoformans* growth. Encapsulated *C. neoformans* are potent activators of the alternative complement system with resultant deposition of C3 breakdown product on the capsular surface. Unopsonized *C. neoformans* is not well recognized by phagocytes. The presence of C3 on the cryptococcal capsule facilitates binding by multiple complement receptors (Levitz & Tabuni, 1991). Complement activation also generates C5a and C3a, which assist in opsonization and leucocyte trafficking. As with other fungi, C5a-deficient mice are particularly susceptible to disseminated infection (Rhodes, 1985).

The role of antibodies in cryptococcosis has been the subject of intense study but their role remains controversial. Anticapsular antibodies are found in most human sera, but they are generally non-opsonic or only weakly opsonic. In mouse models of cryptococcosis, administration of anticapsular monoclonal antibodies may be protective, non-protective, or deleterious, depending upon the type and concentration of immunoglobulin used and the immune status of the host. For example, the same monoclonal antibody can be protective in an immune competent mouse but deleterious in a $CD4^+$ T cell-deficient mouse. The effect of an individual antibody can vary depending on its concentration relative to challenge inocula (Taborda *et al*, 2003). Moreover, isotype switching can convert a protective antibody into a deleterious one. Naturally formed opsonic capsule-binding antibody is not consistently present during infection. A candidate monoclonal antibody has been developed for testing in humans with cryptococcosis (Casadevall *et al*, 1998). *In vitro*, monoclonal antibodies enhance internalization and killing of *C. neoformans* by macrophages and neutrophils. NK cells inhibit *C. neoformans* growth by an antibody-dependent cellular cytotoxicity mechanism (Miller *et al*, 1990). Moreover, by promoting phagocytosis, anticapsular antibody enhances antigen presentation to T cells. *In vivo*, antibody reduces the level of circulating capsule by inhibiting its release from the fungus and by enhancing the clearance of capsular material from the circulation (Martinez *et al*, 2004).

Zygomycosis

Defects in host immunity that predispose to mycoses do not always fall into traditional categories, such as neutropenia, humoral dysfunction or impaired cell-mediated immunity. For example, a diverse group of moulds of the order Mucorales are the aetiological agents of zygomycosis (also known as mucormycosis). These fungi are common components of decaying matter and exposure by inhalation of airborne spores presumably is a frequent occurrence. The most common genera that cause human infection are *Mucor*, *Rhizopus*, *Absidia*, and *Rhizomucor*. Importantly, most of these fungi are resistant to the currently available azole and echinocandin antifungals.

Primary disease is usually initiated in the upper or lower airways and is associated with sinusitis, rhinocerebral mucormycosis, or pulmonary infection, which mimics IA. Extension to contiguous sites occurs if patients do not receive aggressive surgical and medical therapy. Less commonly, infection disseminates to skin, brain, and other sites. Major risk factors for mucormycosis are diabetic ketoacidosis, neutropenia, protein-calorie malnutrition, and iron overload, with or without the concomitant use of deferoxamine. These infections are increasingly encountered in compromised hosts (Kontoyiannis *et al*, 2000). The majority of patients with neutropenia have pulmonary disease, while sinus infection predominates in diabetics. Outcome is greatly dependent upon the site and extent of infection, the use of antifungal agents, and the underlying condition. Mortality of zygomycosis exceeds 70% in bone marrow transplant recipients and neutropenic patients (Roden *et al*, 2004).

Broncholaveolar macrophages encounter inhaled zygomycetes soon after exposure. These cells attach to and ingest the fungal spores to inhibit germination (Waldorf *et al*, 1984b). *In vitro*, serum is required for macrophage-mediated fungistatic activity (Jorens *et al*, 1995). Intracellular killing is mediated by oxidative mechanisms. Treatment with corticosteroids and diabetes mellitus impairs the ability of murine macrophages to prevent germination (Waldorf *et al*, 1984a). In diabetics, serum factors impair the attachment of macrophages to spores and promote germination (Waldorf *et al*, 1984b). Macrophages do not completely ingest, but still damage hyphae by extracellular mechanisms. Neutrophils damage the fungus by attaching to, and spreading over, the surfaces of hyphae similar to their interactions with *Aspergillus* (Diamond *et al*, 1978). Extracellular killing is mediated by oxidative and non-oxidative mechanisms.

Iron deprivation is a major host defence mechanism against mucormycosis. Normal human serum is naturally fungistatic. Serum transferrin contributes to fungistasis by rendering iron inaccessible to the fungus. Fungistasis is reversed if the serum is iron saturated or obtained from patients with diabetic ketoacidosis. Patients with iron overload states, particularly those undergoing deferoxamine chelation therapy, are at increased risk for infection. The iron chelate of deferoxamine, abolishes the fungistatic effect of serum and increases the *in vitro* growth of the fungus of by acting as a siderophore for the pathogen (Boelaert *et al*, 1993). Additional serum factors are operative in the defence against zygomycetes. Zygomcyetes activate the alternative complement system and induce neutrophil chemotaxis (Marx *et al*, 1982; Waldorf & Diamond, 1985).

Miscellaneous fungi

In addition to the infections discussed above, immunocompromised patients are at risk for mycoses due to fungi endemic to specific geographic locales. The dimorphic fungus *Histoplasma capsulatum* is found in soil contaminated by avian excreta. In some endemic areas, the majority of the population becomes infected during childhood. Most individuals have a self-limited illness with lifelong dormant infection in the lung and extrapulmonary sites. Disseminated disease involving the lungs and organs of the reticuloendothelial system is seen with greatly increased frequency in those with abnormal cell-mediated immunity (such as in AIDS). Increased rates of dissemination are seen in patients with malignancies including Hodgkin's disease, CLL, and acute lymphocytic leukaemia (Kauffman *et al*, 1978). *Blastomyces dermatitidis* and *Coccidioides immitis* are dimorphic fungi found in soil. Persons living in endemic areas are at increased risk for blastomycosis and coccidioidomycosis, which often take on an aggressive course in immunocompromised patients. *Pneumocystis jirovecii* (formally known as *P. carinii*), the causative agent of *Pneumocystis* pneumonia (PCP), has emerged as a major pathogen in patients with AIDS. T-cell immunity is critical for host defences to this uncultivable fungus. Not surprisingly then, there is a predilection for developing PCP among patients receiving cytotoxic drugs, corticosteroids or bone marrow transplantation (Roblot *et al*, 2003).

Persons with prolonged neutropenia, graft-*versus*-host disease and corticosteroid use are at increased risk for fusariosis. Patients often present with skin lesions and sino-pulmonary infections. Mortality exceeds 50%, and immune status is the critical determinant for predicting outcome (Dignani & Anaissie, 2004). Neutrophils have a critical role in controlling this mould. Neutropenic mice inoculated with *Fusarium solani* do not mount an inflammatory cellular reaction and develop disseminated disease (Legrand *et al*, 1991). Neutrophils and monocytes damage hyphae of *Fusarium*. Phagocyte activity against *Fusarium* is enhanced by IFN-γ or G-CSF (Gaviria *et al*, 1999; Winn *et al*, 2003b). Patients with severe neutropenia are also at risk for trichosporonosis. In highly compromised hosts, this fungus disseminates to multiple organs including the skin, eye, brain and kidney. Neutrophils and macrophages contribute to the immune response to *Trichosporon* (Lyman *et al*, 1994). Virtually any fungus that can grow at body temperature can act as an opportunist if there is sufficient immunocompromise. Uncommon fungi, including *Scedosporium*, *Paecilomyces*, *Acremonium* and dark walled

fungi, are increasingly encountered in highly compromised hosts. Survival from infections caused by these fungi is highly dependent upon the host immune status.

Concluding remarks

As the worlds' immunodeficient population grows as a result of the HIV pandemic and increased use of highly immune suppressive regimens to treat a variety of illnesses, the challenges of mycotic infections are expected to continue. The increase in the population of compromised hosts, coupled with exciting biotechnology advances has spurred on research into the immune response to fungi. The relative importance and interconnected responses of innate and adaptive immune in protection are actively being investigated. Concomitantly, methods of immune manipulation and reconstitution have become promising areas of research activity.

Future therapies for invasive fungal may include agents that augment antifungal activity of effector cells and alter Th balance. While there is some clinical experience with the use of recombinant cytokines as an adjunct to antifungal drug therapy (Roilides & Walsh, 2004), clinical trials in highly compromised hosts are needed, as many questions remain regarding safety, efficacy, and optimal use. Another potential approach is manipulation of cellular signalling cascades. The finding that innate and adaptive immunity to common fungal pathogens is mediated by the coordinated action of members of the TLR family acting through MyD88 raises the possibility that TLR manipulation may be a useful modality for induction of antifungal resistance (Bellocchio *et al*, 2004). Antifungal vaccines may constitute yet another strategy and, as discussed above, are starting to be studied in patients. Transfusion of dendritic cells that have been exposed to fungi or fungal products and adoptive transfer of T cells has been successfully attempted in animals (Bacci *et al*, 2002; Farah *et al*, 2002; Bozza *et al*, 2003). A better understanding of the role of the host/pathogen interaction will probably lead to further development of these and other potential novel antifungal therapies.

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