Immunopharmacology of Modern Antifungals

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In addition to their in vitro inhibitory and fungicidal effects, modern antifungal agents interact in vivo with host immune functions involved in defense against fungal pathogens. The nature of such interactions is diverse and depends on the drug, the immunological status of the host, and the fungal pathogen. Given the prominent role of the host’s immune response in controlling invasive fungal infection, immunomodulation by antifungal drugs may prove to be clinically significant. Elucidation of the immunopharmacology of these drugs may aid in designing therapeutic regimens for specific clinical scenarios associated with defined immunological dysfunction.

Invasive fungal infection (IFI) is a leading cause of morbidity and mortality among severely immunocompromised patients. The immune deficits associated with IFI include profound and sustained neutropenia, hematopoietic stem cell or solid organ transplantation, high-dose corticosteroid treatment (particularly for patients with graft-versus-host disease), and HIV infection [1, 2]. The outcome of IFI in highly immunosuppressed patients remains dire, despite the availability of modern antifungal drugs [1, 3]. Therefore, priming of the host immune response as an adjunct to conventional antifungal therapy represents an important direction for future research [4–6].

The past 15 years have seen acceleration in antifungal drug development. Our armamentarium of systemic antifungal agents now includes 5 classes of drugs, of which the predominant ones are amphotericin B (AmB) and its lipid formulations, the azoles, and the echinocandins. Although the direct antifungal mechanisms of these drugs are well characterized (figure 1), antifungals have also indirect, immune system–mediated effects on fungi, some of which have only recently come to light. A better understanding of these immunopharmacological properties may be instrumental in designing rational drug therapy for IFI (table 1).

OVERVIEW OF THE HOST IMMUNE RESPONSE TO FUNGI

Immunocompetent hosts respond to invasion by fungal pathogens by mounting an orchestrated response, consisting of both innate and adaptive components of the immune system [43] (figure 2). The innate immune system comprises professional phagocytic cells (neutrophils, mononuclear leukocytes, and dendritic cells) and soluble factors (complement, collectins, and defensins) [43]. Innate immunity is able to identify and rapidly destroy fungal pathogens by virtue of pattern recognition receptors, which respond to motifs that are shared by groups of pathogenic organisms, collectively named “pathogen-associated molecular patterns” [43]. The pattern recognition receptors implicated in the recognition and clearance of fungal pathogens include the lectin-like receptor dectin-1, which binds β-glucan in the fungal cell wall, and the Toll-like receptors (TLRs) TLR2, TLR4, and others (table 2) [43–58]. Engagement of these receptors on neutrophils and macrophages triggers intracellular signaling cascades that elicit the production of inflammatory cytokines, phagocyte degranulation, and respiratory burst, promoting fungal killing through both oxidative and nonoxidative mechanisms [43, 44]. Alveolar macrophages (AMs) [59, 60] and neutrophils [61] are the first line of innate immunity against inhaled conidia. AMs are able to efficiently phagocytose and destroy large numbers of conidia by producing reactive oxygen intermediates [59, 60]. Conidia that escape phagocytosis by AMs germinate into hyphae and are targeted by neutrophils, which are drawn to the lung by chemotactic signals and release reactive oxygen intermediates and granule content extracellularly [60, 62].
figure 1. mechanisms of action of antifungal drugs. the cellular targets of the main classes of antifungals are shown: polyenes (amphotericin b) disrupt membrane integrity by binding to ergosterol and forming transmembrane pores (1); triazoles inhibit c-14α-demethylase, the key enzyme in the ergosterol synthetic pathway (2); echinocandins inhibit the enzyme 1,3β-d-glucan synthase, thus disrupting the glucan-rich cell wall (3); and 5-flucytosine (5-fc) is converted to 5-fluorouracil (5-fu), which is further converted to metabolites that inhibit fungal dna and rna synthesis (4).

if a fungal pathogen manages to breach these early lines of defense, the adaptive immune response is brought into play. fungal antigens are collected and processed by dendritic cells, which transport them to draining lymph nodes where they are presented to t helper and regulatory t (treg) cells [43]. these cells orchestrate antibody-mediated and cellular immunity to fungi [43]. once activated, t helper cells proceed to elaborate either th1 (protective) cytokines (tnf-α, ifn-γ, il-1, il-6, and il-12) or th2 (nonprotective) cytokines (il-10, il-4, and il-5). tnf-α is a key defensive cytokine against fungi. tnf-α stimulates the antifungal activity of neutrophils and macrophages and polarizes t helper cells toward a th1 response [63, 64].

fungi have developed a variety of mechanisms for evading—and, in some cases, down-regulating—the host’s immune response. production of scavenger molecules, such as melanin, mannitol, and catalase, enables fungi to resist damage by reactive oxygen intermediates [65]. several fungi, such as histoplasma capsulatum, have acquired means of surviving within phagocytic cells; these include inhibition of phagosome-lysosome fusion [66], competition with host cells for essential nutrients [66], phenotypic switching [67], and the production of antioxidants [65]. β-glucan in the cell wall of h. capsulatum is concealed under an external coat of α-glucan, which prevents recognition by host phagocytic cells [68]. this successful evasion mechanism has been postulated to underlie the virulence of h. capsulatum.

evasion from innate immunity by phenotypic switching is a strategy employed by several pathogenic fungi. for example, germination of aspergillus fumigatus conidia and generation of tissue-invasive hyphae are associated with loss of tlr4 signaling and a shift from a proinflammatory tlr4-associated response to a predominantly anti-inflammatory tlr2 response [69]. in candida albicans, a multilayered cell wall controls the exposure of pathogen-associated molecular patterns to immune cells. an outer layer of n- and o-linked mannosyl residues is recognized by the mannose receptor and tlr4, respectively [49], and shields an inner layer of β-glucan from recognition by the dectin-1–tlr2 complex, except at budding scars [39]. the importance of this setup is evidenced by the increase in cytokine production evoked by unmasking the β-glucan layer. because budding scars are not formed during filamentous growth, switching from yeast to pseudohyphae phenotype eliminates β-glucan exposure and dectin-1 signaling [70].

finally, fungi may directly damage immune-effector cells. gliotoxin, an immunosuppressive mycotoxin produced in abundance by a. fumigatus hyphae, has been shown to inhibit migration, phagocytosis, and respiratory burst in neutrophils and macrophages and to induce apoptosis of these cells [71–73].

the nature and degree of the underlying immunological defect determine not only susceptibility to ifi but also the clinical presentation, histopathological features, and natural history of infection. a successful immune response to an invading fungal
### Table 1. Summary of immunomodulatory effects of antifungal drugs.

<table>
<thead>
<tr>
<th>Drug, immune function</th>
<th>Nature of interaction</th>
<th>Experimental system(s)</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AmB-D</td>
<td>Neutrophils and mononuclear cells</td>
<td>Production and release of inflammatory cytokines, prostaglandins, NO, and ROIs</td>
<td>In vitro [7–12]</td>
</tr>
<tr>
<td></td>
<td>Neutrophils</td>
<td>Additive activity against <em>Aspergillus fumigatus</em> conidia (macrophages) and hyphae (neutrophils)</td>
<td>In vitro [13]</td>
</tr>
<tr>
<td></td>
<td>Neutrophils</td>
<td>Increase in TLR2 expression</td>
<td>In vitro [14]</td>
</tr>
<tr>
<td>B and T lymphocytes</td>
<td>AmB is mitogenic for lymphocytes but suppresses lymphocyte proliferation indirectly through its effects on arachidonic acid and H₂O₂ metabolism in mononuclear cells</td>
<td>In vitro [15–18]</td>
<td></td>
</tr>
<tr>
<td>T helper cells</td>
<td>Polarization towards Th1 immunity and resistance to fungal infection</td>
<td>Murine model of candidiasis [19]</td>
<td></td>
</tr>
<tr>
<td>AmB lipid formulations</td>
<td>ABCD</td>
<td>Neutrophils and mononuclear cells</td>
<td>Up-regulation of inflammatory cytokine genes</td>
</tr>
<tr>
<td></td>
<td>Additive activity against <em>A. fumigatus</em> conidia (macrophages) and hyphae (neutrophils)</td>
<td>In vitro [13]</td>
<td></td>
</tr>
<tr>
<td>Neutrophils</td>
<td>Additive activity against <em>A. fumigatus</em> hyphae</td>
<td>In vitro [13]</td>
<td></td>
</tr>
<tr>
<td>Anti-hsp90 antibodies</td>
<td>Synergistic activity against <em>Candida</em> species</td>
<td>In vitro, murine model of candidiasis, and a randomized clinical trial [21, 22]</td>
<td></td>
</tr>
<tr>
<td>Azoles</td>
<td>Neutrophils and mononuclear cells</td>
<td>Synergistic activity of azoles, cytokines, and immune-effector cells against <em>Candida albicans</em></td>
<td>In vitro [23–30]</td>
</tr>
<tr>
<td></td>
<td>Fungal sensitization to ROI by accumulated 14α-methyl sterols.</td>
<td>In vitro [31]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inhibition of NO degradation by fungal flavohemoglobin renders fungi susceptible to damage by immune-effector cells</td>
<td>In vitro [32–35]</td>
<td></td>
</tr>
<tr>
<td>Monocytes</td>
<td>Increase in TLR2 expression</td>
<td>In vitro [36]</td>
<td></td>
</tr>
<tr>
<td>T helper cells</td>
<td>Polarization towards Th1 immune response and resistance to fungal infection</td>
<td>Murine model of candidiasis [19]</td>
<td></td>
</tr>
<tr>
<td>IL-12</td>
<td>Induction of Th1 response facilitates activity of fluconazole against <em>C. albicans</em> and <em>Cryptococcus neoformans</em></td>
<td>Neutropenic mice with disseminated candidiasis [37, 38]</td>
<td></td>
</tr>
<tr>
<td>Echinocandins</td>
<td>Neutrophils</td>
<td>Unmasking of β-glucan facilitates fungal damage by neutrophils and antibodies</td>
<td>In vitro [39, 40]</td>
</tr>
<tr>
<td></td>
<td>Monocytes and macrophages</td>
<td>Preexposure to echinocandins enhances the activity of these cells against <em>A. fumigatus</em></td>
<td>In vitro [41, 42]</td>
</tr>
</tbody>
</table>

**NOTE.** ABCD, amphotericin B colloidal dispersion; ABLC, amphotericin B lipid complex; AmB, amphotericin B; AmB-D, amphotericin B deoxycholate; hsp90, heat shock protein 90; L-AmB, liposomal amphotericin B; NO, nitric oxide; ROIs, reactive oxygen intermediates; TLR, Toll-like receptor.

The pathogen depends on the ability of the host immune system to clear the pathogen while minimizing collateral tissue damage [74]. Thus, although some immune deficits are associated with a compromised ability to clear invading fungi, others are characterized by a dysregulated, exuberant immune response. An example of the relevance of the host immune status may be seen in the contrasting features of invasive pulmonary aspergillosis (IPA) in the neutropenic host, as opposed to the non-neutropenic, corticosteroid-treated host [75]. In animals and patients with chemotherapy-induced neutropenia, IPA is associated with the presence of numerous hyphae invading blood vessels and tissue, thrombosis, coagulative necrosis, hemorrhage, a scant mononuclear inflammatory exudate, and eventual dissemination [76, 77]. In contrast, IPA in corticosteroid-treated subjects is associated with tissue infiltration by neutrophils, areas of pneumonia and bronchiolitis, inflamm-
Figure 2. The host immune response to invasive fungal pathogens. The innate immune response (top), including phagocytic cells (macrophages and neutrophils) and soluble factors (pentaxins, complement, and other opsonins) is the first line of defense against invasive fungi. Fungal antigens taken up by dendritic cells are processed and presented to T lymphocytes, thus initiating an adaptive immune response (bottom). Stimulated T cells differentiate into subsets (Th1, Th2, and regulatory T cells) under the influence of cytokines. Differentiated T cells affect the function of immune innate cells, as well as specific antibody production by B lymphocytes. MHC, major histocompatibility complex; PRR, pattern recognition receptor; TCR, T cell receptor.

Inflammatory necrosis, and few fungal elements [76–79]. Hematopoietic stem cell transplant recipients with IPA develop a pattern of tissue injury similar to that of neutropenic patients, probably in association with impaired migration of neutrophils to the lungs [79, 80]. Thus, it seems that, although a high fungal burden, tissue invasion, and dissemination are the key elements of pathogenesis in patients with absolute or functional neutropenia, in the corticosteroid-treated host, it is the excessive inflammatory response that is responsible for much of the tissue injury and mortality.

**AmB: A PROINFLAMMATORY DRUG**

AmB, a polyene produced by *Streptomyces nodosus*, exerts its rapid fungicidal activity by binding to ergosterol in the fungal membrane and forming oligomers that act as transmembrane pores, through which potassium and other intracellular molecules are lost. AmB acts additively in vitro with AMs and neutrophils against a variety of pathogenic filamentous fungi [13]. Whether AmB increases or attenuates gliotoxin production by *A. fumigatus* is controversial; discordant results have been described in in vitro [81] and in vivo studies [82].

AmB deoxycholate (AmB-D) is a potent proinflammatory stimulant of innate immune cells. Clinically, the proinflammatory activity of AmB-D is associated with acute infusion-related toxicity, manifesting as rigor, chills, myalgia, and fever—effects that have been shown to correlate with increased blood levels of inflammatory cytokines [7]. Mononuclear cells and neutrophils exposed in vitro to AmB-D rapidly release a variety of inflammatory cytokines (TNF-α, IL-6, IL-1RA, and IL-1β), chemokines (IL-8, MCP-1, and MIP-1β), nitric oxide, prostaglandins, reactive oxygen intermediates, and intercellular adhesion molecule 1 [8, 9, 15, 83]. Stimulation of innate immune cells by AmB is mediated through TLR2 and CD14 and involves a signaling cascade that includes the adapter protein MyD88 and nuclear factor κB, culminating in the up-regulation of genes encoding for inflammatory cytokines [10].

There is also evidence that AmB interacts with adaptive humoral immunity. Survivors of invasive candidiasis have high serum levels of antibodies directed against heat shock protein 90, a fungal molecular chaperone, compared with nonsurvivors [84]. Studies in vitro and in animal models have documented synergistic activity of AmB against *Candida* species in combination with anti–heat shock protein 90 antibodies [21]. In a randomized clinical trial, treatment of patients with invasive candidiasis with a combination of monoclonal anti–heat shock protein 90 antibodies and liposomal AmB (L-AmB) resulted in a significantly higher response rate (OR, 5.8; *P* < .001) and lower attributable mortality, compared with L-AmB alone [22]. AmB has also been shown to modulate the T helper cell response in mice with candidiasis by inducing a protective Th1 phenotype [19].

However, recent work suggests that the robust proinflammatory properties of AmB-D might not always be advantageous, at least in nonneutropenic hosts with *Aspergillus* pneu-
Table 2. Fungal ligands and their innate immune receptors.

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Fungal ligand (PAMP)</th>
<th>Innate PRR</th>
<th>Comments</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida albicans, Aspergillus fumigatus, Pneumocystis jiroveci</td>
<td>β-glucans</td>
<td>Dectin-1</td>
<td>Dectin-1-TLR2 complex involved in Candida recognition</td>
<td>[45]</td>
</tr>
<tr>
<td>C. albicans hyphae</td>
<td>Mannan</td>
<td>Dectin-2</td>
<td></td>
<td>[46, 47]</td>
</tr>
<tr>
<td>C. albicans</td>
<td>Phospholipomannan</td>
<td>TLR2</td>
<td></td>
<td>[48]</td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>Mannan (O-linked)</td>
<td>TLR4</td>
<td></td>
<td>[49]</td>
</tr>
<tr>
<td>C. albicans</td>
<td>Unknown</td>
<td>TLR9</td>
<td></td>
<td>[50]</td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>Unknown</td>
<td>CD14</td>
<td>Coreceptor with TLR2</td>
<td>[51]</td>
</tr>
<tr>
<td>C. albicans</td>
<td>Unknown</td>
<td>Galactomannan</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>Unknown</td>
<td>Galactomannan</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. albicans</td>
<td>β(1,2) oligomannan</td>
<td>Galectin-3 (with TLR2)</td>
<td></td>
<td>[54, 55]</td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>Galactomannan</td>
<td>Pentraxin 3</td>
<td></td>
<td>[56]</td>
</tr>
<tr>
<td>C. albicans</td>
<td>Unknown</td>
<td>Mannan-binding lectin</td>
<td></td>
<td>[57]</td>
</tr>
<tr>
<td>P. jiroveci</td>
<td>Mannan (N-linked) Glycoprotein A</td>
<td>Mannose receptor</td>
<td></td>
<td>[49, 58]</td>
</tr>
</tbody>
</table>

NOTE. DC-SIGN, dendritic cell–specific intercellular adhesion molecule (ICAM)–3-grabbing nonintegrin; PAMP, pathogen-associated molecular pattern; PRR, pattern recognition receptor; TLR, Toll-like receptor.

monia. Specifically, Balloy et al. [76] have shown that treatment with AmB-D reduces mortality in chemotherapy-treated (neutropenic) mice with IPA but has no effect on mortality in corticosteroid-treated mice: 100% of these animals died by day 8 of infection, irrespective of whether they were treated with AmB or the drug vehicle alone. A possible explanation for these findings is that, in the context of corticosteroid treatment, AmB fails to attenuate and may even promote tissue damage from excessive inflammation.

LIPID FORMULATIONS OF AMB: IMMUNOMODULATION BY THE LIPID CARRIER

Three lipid formulations of AmB have been developed, with the aim of delivering higher effective AmB doses while minimizing infusion-related and renal toxicity. The different lipid formulations exhibit distinct effects on inflammatory cytokine production in monocytes. AmB colloidal dispersion (ABCD) is associated with up-regulation of inflammatory cytokine genes, similar to AmB-D. In contrast, AmB lipid complex (ABLC) and L-AmB either down-regulate or have no effect on inflammatory cytokine gene expression in macrophages [7, 11]. These patterns of cytokine production mirror the tendency of these formulations to cause infusion-related toxicity; more severe reactions are elicited by AmB-D and ABCD, whereas severity is attenuated with L-AmB and ABLC [7, 11].

The different immunomodulatory properties of L-AmB, compared with those of AmB-D, may confer an advantage to L-AmB in the treatment of IFI in nonneutropenic, corticosteroid-treated hosts. L-AmB exhibits additive activity with host phagocytic cells against a variety of medically important filamentous fungi [13, 20, 85–87]. Unlike AmB-D, this positive interaction is more pronounced in conjunction with neutrophils than with macrophages. Whether neutrophils are the preferred target of immunomodulation by L-AmB remains unclear. Bellochio et al. [14] found that, in contrast with the TLR2-polarized response to AmB-D, L-AmB induces TLR4-dependent signaling in neutrophils in vitro. These researchers put forward a hypothesis whereby TLR4 activation is associated with a more anti-inflammatory pattern of cytokine production, with IL-10 being produced in preference to TNF-α. By shifting signaling in neutrophils from TLR2 to TLR4, L-AmB may be associated with lesser degrees of inflammatory tissue damage during fungal infection, a property that may be particularly significant in the corticosteroid-exposed host [44].

The effect of L-AmB on neutrophils is due, at least in part, to the anti-inflammatory properties of the liposomes themselves. It has been known for some time that administration of liposomes to endotoxin-challenged mice protects them from endotoxin-induced death in a dose-dependent manner [88]. In serum, unilamellar liposomes adsorb fibrinogen and fibronectin, activate complement, and fix C3bi and are subsequently endocytosed by monocytes and neutrophils by engaging the integrin complement receptor 3 [88, 89]. These events trigger an intracellular signaling cascade linked with inhibition of mitogen-activated protein kinase and subsequent down-regulation of CD18, the ligand for endothelial intercellular adhesion molecule 1 [88]. Thus, liposomes may attenuate the extravasation of neutrophils into sites of inflammation. We have recently shown that pretreatment of corticosteroid-treated mice with empty liposomes improves fungal clearance and survival following intranasal inoculation with A. fumigatus [20]. Remarkably, the protective effect of liposomes approached that of a
AZOLES: SYNERGISTIC ACTIVITY WITH PHAGOCYTES AGAINST FUNGI

Azoles exert antifungal activity by inhibiting the cytochrome P-450 enzyme C-14α demethylase. As a result, ergosterol synthesis is inhibited, while toxic C-14α methylsterols accumulate within the cell. Of the 3 drug classes reviewed here, the azoles are the least active with respect to modulation of the host’s immune system. Nevertheless, azoles have been shown to cooperate with immune-effector cells and cytokines against fungal pathogens.

Both fluconazole and the structurally similar voriconazole act synergistically with phagocytic cells against C. albicans, and this interaction is further enhanced if phagocytes are activated by granulocyte-monocyte colony-stimulating factor, granulocyte colony-stimulating factor, or IFN-γ [23–28]. Although intrinsically fungistic, azoles enhance the in vitro fungicidal activity of monocytes, macrophages, and neutrophils against C. albicans [26–28]. Subinhibitory concentrations of fluconazole also enhance phagocytosis of Cryptococcus neoformans, an effect that may be important in the CNS, where drug levels are often below MIC [90].

The mechanisms forazole-phagocyte interactions are incompletely understood, but there is evidence that ergosterol depletion renders fungal cells more susceptible to both oxidative and nonoxidative damage by phagocytes. A superoxide-sensitive phenotype has been demonstrated in azole-treated C. albicans and in a C-14α demethylase–deficient mutant [31]. In addition, azoles increase the exposure of fungi to nitric oxide, a potent effector molecule produced by IFN-γ–stimulated macrophages [91], by inhibiting nitric oxide degradation by fungal flavohemoglobin [23, 32]. Furthermore, azoles display synergistic activity with nitric oxide against various species of Candida [33]. Lastly, azoles inhibit the transformation of Candida species from yeast to hyphal form, thereby facilitating clearance of this pathogen by phagocytes [92].

Other, more direct immune system effects have recently been described. Voriconazole was found to induce expression of TLR2, nuclear factor κB activity, and TNF-α in human monocytes [36]. In addition, fluconazole induces a protective Th1 response in mice with invasive or mucosal candidiasis [19]. In turn, polarization of T cell immunity toward Th1 by IL-12 administration facilitates the activity of fluconazole against C. albicans [37] and C. neoformans [38].

ECHINOCANDINS: β-GLUCAN UNMASKING ENHANCES FUNGAL KILLING BY NEUTROPHILS AND MACROPHAGES

The echinocandins are lipopeptides that act by inhibiting the synthesis of 1,3-β-D-glucan, a major component in the cell walls of Candida, Aspergillus, and other opportunistic molds. The resultant disruption of fungal cell wall integrity leads to aberrant growth, in the case of filamentous fungi, or cell rupture and osmotic lysis, in the case of Candida species.

Because filamentous fungi are inhibited but not killed by echinocandins, the efficacy of these drugs in the treatment and prevention of aspergillosis suggests a role for other, indirect effects of echinocandins in vivo. Recently, echinocandins have been found to have immunostimulatory properties. Preexposure of monocytes and macrophages—but not of neutrophils—to an echinocandin has resulted in enhanced in vitro inhibition of A. fumigatus [41, 42]. The fungistatic activity of caspofungin-exposed mononuclear cells was greater than that of either caspofungin or unexposed mononuclear cells [41].

β-Glucans, a major component of the cell wall of Candida and Aspergillus species, are recognized by dectin-1 on AMs, neutrophils, and dendritic cells [93]. However, under normal conditions, β-glucan epitopes are masked by other cell wall constituents, rendering them undetectable by host immune cells [39]. Exposure of C. albicans to sublethal concentrations of caspofungin resulted in unmasking of β-glucan, causing the exposed fungus to illicit proinflammatory cytokine release from macrophages [40]. Similar enhancement of the host inflammatory response and fungal killing resulted from disruption of the genetic network responsible for β-glucan masking in C. albicans. It is therefore hypothesized that β-glucan unmasking by caspofungin and subsequent enhancement in fungal recognition by host immune cells is a consequence of modulation of this genetic network (figure 3). Recent studies suggest a similar activity of caspofungin in filamentous fungi. Caspofungin increased cell wall β-glucan exposure in hyphal of a variety of pathogenic molds, including A. fumigatus, Scedosporium apiospermum, and Rhizopus oryzae [40, 94]. Enhanced hyphal killing in these studies was associated with increased expression of dectin-1 mRNA in neutrophils [40] and TNF-α release by macrophages [94]. Furthermore, caspofungin-mediated exposure of immunoreactive β-glucan epitopes facilitated fungal destruction by monoclonal antibodies directed against β-glucan. Similar results were observed with micafungin [94], suggesting that β-glucan unmasking is probably an echinocandin class effect.

Of note, recent studies have documented surprising in vivo benefits from the use of caspofungin in the treatment of infections caused by molds that are typically resistant to this drug. Diabetic ketoacidotic mice with disseminated R. oryzae infection had an improved rate of survival when treated with a 10-mg/kg dose of L-AmB and was significantly greater than that of a 1-mg/kg dose of AmB-D [20]. L-AmB may strike a favorable balance in corticosteroid-treated animals; in addition to optimizing AmB delivery to the site of infection, liposomes protect the host from overzealous immune activation and subsequent tissue damage associated with corticosteroid exposure and the proinflammatory properties of AmB itself [20].
combination of ABLC and caspofungin, compared with mice treated with ABLC alone [95]. In addition, caspofungin has shown activity in murine models against the emerging molds *Fusarium solani* [96, 97] and *Scedosporium prolificans* [98]. These findings suggest that echinocandins, by virtue of their unique immunomodulatory activity, may have a role in the treatment of infections caused by fungi that are intrinsically resistant to this class of drugs.

SUMMARY

Manipulation of the immune response as an adjunct to antifungal treatment is likely to become an important component in the management of IFIs. Clinicians dealing with fungal infections are required to look beyond standard in vitro measures of antifungal activity, such as the MIC or minimum effective concentration. A more complete understanding of the immunological lesions underlying IFI and the immunomodulatory activity of antifungal drugs may guide the selection of appropriate regimens for given clinical situations. At present, most data on the effect of immunomodulation on pathogenic fungi are derived from in vitro or animal studies. However, promising results have emerged from clinical trials that evaluated the use of monoclonal antibodies [22, 99] and cytokines [100]. While we await more-definitive results from larger trials, making creative use of the immunomodulatory properties of available antifungals may present a practical alternative when treating these potentially devastating infections.

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**Histoplasma capsulatum**


