Host immune response against *Scedosporium* species

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*Scedosporium apiospermum* and *Scedosporium prolificans* cause therapy-refractory infections in immuocompromised and immunocompetent hosts. While innate immune response is believed to be critical for the host defense against these fungi, its role has only recently been elucidated. Undefined pathogen-associated molecular patterns on the surface of conidia and hyphae are recognized by pattern-recognition receptors (PRRs) on the membrane of phagocytes, and the signal is transmitted intracellularly. PRRs that are important in the recognition of both fungal species are human Toll-like receptors (or Toll receptors in *Drosophila melanogaster*) and dectin-1. These induce signals responsible for the activation of genes leading to an effective host defense, especially those encoding pro-inflammatory cytokines. Both species are efficiently phagocytosed and elicit an oxidative burst by neutrophils and monocytes. While cytokines, such as interleukin-15, granulocyte-macrophage colony-stimulating factor, granulocyte colony-stimulating factor and interferon-γ, have been found *in vitro* to variably modulate antifungal activity of human phagocytes, cytokines *in vivo* activities are less well documented. Certain antifungal agents exert immunopharmacological effects on phagocytes against *S. apiospermum* and *S. prolificans*. Translation of these *in vitro* findings to appropriate *in vivo* systems and into clinical trials may lead to improved strategies for augmenting innate host defenses in patients infected with these emerging pathogens.

**Keywords** innate immune response, scedosporiosis, cytokines, phagocytes

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**Introduction**

Infections caused by *Scedosporium* species have been reported with increasing frequency in recent years [1,2]. Advances in molecular taxonomy, analyzing partial sequences of the β-tubulin and calmodulin genes, as well as the internal transcribed spacer region of the rRNA gene, have demonstrated that *Pseudallescheria boydii* is best described as the *Scedosporium apiospermum/Pseudallescheria boydii* complex [3].

While the mechanisms of invasion and subsequent propagation of *Scedosporium* species in infected hosts are not well-defined, the host-fungus interactions have recently started to be elucidated. Clinical experience suggests that the respiratory tract is the most probable portal of entry of *Scedosporium* species in the most serious infections [1]. Consistently, pneumonia appears to be the most frequent manifestation of infection [4]. However, conidia may initiate infections in patients through other portals of entry, such as the gastrointestinal tract from ulcerative lesions, or by direct inoculation from areas with trauma or a central venous catheter and eventually disseminate to multiple organs including brain, kidney or heart [5]. Since the conidia enter and infect various parts of the human body, they can challenge different innate immune cells, i.e., microglial cells or Kupffer cells and lead to variable host
responses [6]. Recent data on host defense mechanisms to Scedosporium species are described in this review.

Studies of innate host defenses against filamentous fungi have historically focused on Aspergillus fumigatus and to a lesser extent on Rhizopus oryzae [7-9]. Scedosporium species share some common features with A. fumigatus in terms of utilizing the same portals of entry into human organisms, having similar clinical features and histopathology, as well as causing life-threatening and therapy-refractory infections, especially in immunocompromised patients. Therefore, it is reasonable to presume that analogous immune mechanisms may apply to these two fungal pathogens. However, key differences between Aspergillus and Scedosporium species are that the latter are less common opportunistic pathogens and upon infection, phagocytic cells function less effectively in their eradication and cause an exacerbated immunomodulatory response.

The main line of innate host response to A. fumigatus consists of circulating polymorphonuclear leukocytes (PMNs) and monocytes (MNCs), as well as monocyte-derived macrophages (MDMs), particularly pulmonary alveolar macrophages. A number of pathogen-associated molecular patterns (PAMPs) on the surface of fungal conidia or hyphae bind to pattern-recognition receptors (PRRs) of phagocytes and generate the molecular signal for the pro-inflammatory and anti-fungal activities of phagocytes. Toll-like receptors (TLRs), mannose receptors, dectin-1 and others play critical roles in the recognition of fungal patterns and the intracytoplasmic transduction of the signals [10]. In general, yeasts are recognized by three different recognition systems, namely TLR4, dectin-1 and TLR2, whereas A. fumigatus is recognized by TLR2 and TLR4 [11].

Phagocytes are capable of damaging Aspergillus and Rhizopus spp. hyphae through oxygen-dependent and -independent mechanisms. While MNCs and macrophages can damage hyphae, the bulk of this role appears to fall upon PMNs [12]. The oxygen-dependent mechanisms consist of a series of reactions starting with the production of superoxide anion (O2\(^{-}\)), which is dismutated into hydrogen peroxide (H\(_2\)O\(_2\)). Myeloperoxidase (MPO) then catalyzes the conversion of H\(_2\)O\(_2\) and halides to generate hypohalides, such as hypochlorite (HOCl) and chloramines, which exert potent antifungal activity [13,14]. Cationic peptides (defensins and cathelicidins) are part of the oxygen-independent pathway of phagocytic cells [15-17]. A variety of cytokines, chemokines and growth factors play an important role in the host response and pathogenesis of filamentous fungal infections [18]. Since the primary objective of these in vitro studies was to elucidate the basic properties of normal host response against fungal pathogens, most of the investigations were performed with immune cells obtained from healthy volunteers rather than from immunocompromised patients. These studies in normal hosts lay the foundation for controlled in vitro and in vivo evaluations of quantitative and qualitative defects of innate host response. However, as effector cell activity from immunocompromised patients may vary greatly as a function of different types and degrees of immunosuppression, careful control of these variables is important in the design of ex vivo experiments in these host populations.

**Signal recognition and response to Scedosporium species**

In the last few years, substantial progress has been achieved in understanding the molecular events of the innate immune response to Scedosporium species. Lamaris et al. developed a model of disseminated fungal infections in Drosophila melanogaster flies and demonstrated the vital role of Toll receptors (equivalent of TLRs in humans) in response to S. apiospermum and S. prolificans [19]. They found that while wild-type flies were resistant to infection by both species, Toll-deficient flies were susceptible and resulted in acute infection and high mortality rates.

Bittencourt et al. reported the isolation and structural characterization of an \(\alpha\)-glucan from the P. boydii cell wall and evaluated its role in the induction of innate immune response [20]. Ex vivo experiments with elicited peritoneal macrophages obtained from mice indicated that soluble \(\alpha\)-glucan, but not \(\beta\)-glucan, led to a dose-dependent inhibition of conidial phagocytosis. Furthermore, a significant decrease in the phagocytic index occurred when \(\alpha\)-glucan from the conidial surface was removed by enzymatic treatment with \(\alpha\)-amyloglucosidase, thus indicating an essential role of \(\alpha\)-glucan in P. boydii internalization by macrophages. \(\alpha\)-Glucan stimulated the secretion of inflammatory cytokines by macrophages and dendritic cells. Once again this effect was abolished by treatment with \(\alpha\)-amyloglucosidase. Finally, \(\alpha\)-glucan induced cytokine secretion by cells of the innate immune system in a signal transduction mechanism involving TLR2, CD14 and the adapter protein of myeloid differentiation primary response gene 88 (MyD88) (Fig. 1). These results might be relevant in the context of infections with P. boydii and other fungi, and \(\alpha\)-glucan could be a potential target for intervention during fungal infections [20].

Furthermore, the PMN-induced hyphal damage of S. apiospermum increased significantly with the addi-
tion of an anti-β-glucan antibody. The combination of caspofungin and anti-β-glucan antibody further increased hyphal damage. For *S. prolificans*, the addition of an anti-β-glucan antibody before incubation with PMNs resulted in a minimal increase in hyphal damage. In addition, exposure to the combination of caspofungin and anti-β-glucan antibody did not result in further increase in PMN-induced hyphal damage [21]. The two complementary studies by Lamaris et al. [21] and Hohl et al. [22] provided evidence that the immunomodulatory effect of caspofungin on the phagocytes was mediated through increased expression of dectin-1 mRNA in PMNs and TNF-α release by macrophages (Fig. 1). Similar results were observed with micafungin [22], suggesting that β-glucan unmasking is an echinocandin class effect [23].

**Antifungal phagocytic function against *Scedosporium* species**

Conidia and hyphae of *S. apiospermum* and *S. prolificans* were found, with minor differences, to be susceptible to phagocytes in a manner comparable to *A. fumigatus* [24,25]. Specifically, despite the much larger size of *S. prolificans* conidia (3–7 μm) than those of *Aspergillus* (2.5–3.0 μm), MDMs were able to ingest the conidia of both fungi. However, MDMs inhibited germination of *S. prolificans* conidia less efficiently than those of *A. fumigatus* [25]. Concerning the oxidative burst, *in vitro* studies demonstrated that in the presence of serum, phagocytes were capable of exhibiting sufficient oxidative burst to control *S. prolificans* strains. In the absence of serum, however, the production of O₂⁻ appeared to be lessened (Table 1) [25].

*S. prolificans* isolates were damaged in a dose response manner when challenged with both kinds of phagocytes. Moreover, while phagocytes were able to phagocytose *Scedosporium* conidia similarly to those of *Aspergillus* [25], they tended to induce more damage to the former than to the latter. In these studies with *Scedosporium* species, an effector-to-target ratio of 10:1 has been frequently used. While the true inoculum size of filamentous fungi in human diseases is not known, if we assume that a relatively low inoculum may be involved in immunocompromised hosts, the effector-to-target ratio of many *in vitro* experiments of innate host defenses against filamentous fungi are typically 10:1 or higher. These assays reflect the relatively low inoculum in relation to the number of phagocytic cells.

Further insight into the immunopathogenesis of *Scedosporium* species infections was gained through studies of the phagocytic cell responses to an amphotericin B-resistant *S. apiospermum* isolate and one that was susceptible to this antifungal. This study found that macrophages are capable of phagocytosing *S. apiospermum* conidia, damage hyphae in a concentration-dependent manner and release O₂⁻ in response to serum-opsonized hyphae (Table 1). It also found that hyphae of the two strains had different levels of susceptibility to myeloperoxidase products. This
phenomenon, although not fully elucidated, may be related to the various levels of pathogenicity and antifungal drug resistance of *S. apiospermum/P. boydii* complex [24]. The mechanisms relating α-glucan-mediated opsonization and oxidative burst in response to *S. prolificans* remain unclear and merit further investigation.

Melanins are dark brown or black pigments formed by oxidative polymerization of phenolic compounds and are present in the cell wall of *Scedosporium* species. Despite the fact that cultures of *S. apiospermum* often are grey, brown or almost black due to pigment of the conidia, the fungus has a colourless mycelium [5]. Most fungal melanins are derived from the precursor molecule 1,8-dihydroxynaphthalene through the polyketide biosynthetic pathway and reside in ascomycetes and related deuteromycetes [26]. There are a large number of hypotheses supporting the protective role of melanin against host defense mechanisms and environmental stress. A growing body of evidence supports that melanins function as antioxidants and radioprotectants [27,28]. Indeed, recent studies indicate that melanin in diverse fungi can utilize radiation-transduced energy [28]. Melanin may have a protective effect by scavenging oxygen and nitrogen free radicals produced by phagocytic cells. Additional melanin pathogenetic mechanisms include sequestration of host defensive proteins, cross-linking or shielding cell wall constituents against hydrolytic enzymes or conferring resistance to heat [29,30]. In *Cryptococcus neoformans*, the presence of melanin polymers significantly increased the survival of cells exposed to amphotericin B as compared to non-melanized cells [31]. This suggests an additional protective mechanism by which melanin could bind the drug and/or reduce the permeability of the cell wall to antifungal drugs.

Although the role of melanin in virulence has been amply demonstrated in human pathogens including *A. fumigatus, C. neoformans* or *Exophiala dermatitidis*.

**Table 1** Summarized results of studies investigating the signal recognition of *Scedosporium* species and phagocytic function against these fungi

<table>
<thead>
<tr>
<th>Reference</th>
<th>Species</th>
<th>Findings</th>
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<tr>
<td>[19]</td>
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<td><em>S. prolificans</em></td>
<td>α-glucan induces this cytokine secretion</td>
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<tr>
<td>[21]</td>
<td><em>S. apiospermum</em>, <em>S. prolificans</em></td>
<td>Dectin-1 is involved in phagocytic activation</td>
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<td></td>
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<td>Caspofungin has immunomodulatory effect through increased expression of dectin-1 mRNA in PMNs and TNF-α release by macrophages</td>
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<td>[24]</td>
<td><em>S. apiospermum</em></td>
<td>Macrophages are able to phagocytose conidia damage hyphae in a concentration-dependent manner and release O2 in response to serum-opsonized hyphae</td>
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<td>[25]</td>
<td><em>S. prolificans</em>, <em>A. fumigatus</em></td>
<td>MDMs are able to ingest <em>Scedosporium</em> conidia similarly to those of <em>Aspergillus</em> spp.</td>
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<td>[33]</td>
<td><em>S. prolificans</em>, <em>A. fumigatus</em></td>
<td><em>S. prolificans</em> releases more TNF-α and IL-6 by human MNCs than <em>Aspergillus</em> spp.</td>
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<td>[36]</td>
<td><em>S. apiospermum</em>, <em>S. prolificans</em></td>
<td>IL-15 enhances PMN-induced hyphal damage oxidative respiratory burst</td>
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<td>[43]</td>
<td><em>S. apiospermum</em>, <em>S. prolificans</em></td>
<td>IL-8 release of <em>S. prolificans</em> but not of <em>S. apiospermum</em></td>
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<td>[44]</td>
<td><em>S. prolificans</em></td>
<td>Posaconazole or GM-CSF combined with peritoneal PMNs, isolated from immunocompetent healthy mice increased antihyphal activity</td>
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<td>[49]</td>
<td><em>S. prolificans</em></td>
<td>In an immunosuppressed murine model of invasive infection, liposomal amphotericin B at 10 mg/kg/day combined with G-CSF have modest efficacy</td>
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<td>[51]</td>
<td><em>S. apiospermum</em>, <em>S. prolificans</em></td>
<td>Amphotericin B lipid complex displayed additive effect with PMNs against these fungi</td>
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<td>[52]</td>
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<td>Triazoles in combination with PMNs cause additive increase in the hyphal damage</td>
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little data exist on Scedosporium species. A single report on melanin-deficient mutants of S. prolificans showed that melanin did not seem to confer resistance against several important antifungal agents [32].

**Immunomodulation: cytokines and chemotherapeutic agents**

Immunosuppression generally constitutes a significant risk factor for the emergence of invasive fungal infections. In this regard, S. prolificans has been shown in vitro to induce the release of significantly more TNF-α and IL-6 by human MNCs than do Aspergillus spp. This could be attributed to the specific composition of the cell wall of S. prolificans, which may yield more potent stimulatory molecules and speculatively, may be associated with the virulence of the fungus [33]. During inhalation of Scedosporium species elements, the identity and expression of locally released cytokines are not known. In contrast, during inhalation of A. fumigatus by immunocompetent mice, IL-18, IL-12 and TNF-α were found to be released. These cytokines exert their modulatory effects on both intrapulmonary immunoregulatory pathways and on effector cells that inhibit growth of A. fumigatus [34].

A number of studies have aimed to assess the immunomodulatory utility of cytokines in confronting fungal pathogens [35]. IL-15 significantly enhances PMN-induced hyphal damage and oxidative respiratory burst of S. prolificans but not of S. apiospermum. In addition, the presence of IL-15 has been shown to significantly increase IL-8 release from PMNs challenged with S. prolificans, but not the release of TNF-α. The inability of IL-15 to elicit enhanced phagocytic damage of S. apiospermum hyphae is in accord with its greater intrinsic virulence in humans. These findings suggest that IL-15 has species-specific enhancing effects on antifungal activities of PMNs against Scedosporium species. Furthermore, some of the cytokine-induced effects were shown to be the result of direct actions on PMNs’ effector activities. Other effects, related to the increased release of IL-8 acting in an autocrine way on PMNs, resulted in enhanced indirect antifungal actions (Table 1) [36].

Interferon-γ (IFN-γ) and granulocyte-macrophage colony-stimulating factor (GM-CSF) are molecules that primarily induce innate immune response. IFN-γ induces the Th1 response which favours resistance to fungal disease. It regulates the gene expression of NADPH oxidase subunits at the transcriptional level and potentiates the synthesis of antimicrobial peptides in macrophages [37–39]. GM-CSF enhances phagocytosis and oxidative burst, increases expression of several classes of surface receptors on PMNs and inhibits PMN apoptosis [40–42]. Both cytokines enhance PMN antifungal activity against Scedosporium species [43]. Specifically, treatment of PMNs with the combination of IFN-γ and GM-CSF exert broader effects on Scedosporium species, whereas cytokines alone have little effect on enhancing PMN functions. Despite the minor effect of either cytokine alone on the PMN oxidative burst after long-term treatment (22 h), the combined treatment showed enhancement of oxidative burst in response to opsonized S. apiospermum hyphae. Similarly, after short-term (2 h) incubation, the combination significantly enhanced the oxidative burst against opsonized and non-opsonized hyphae of Scedosporium species. This study demonstrated that IFN-γ and GM-CSF exhibited significant time- and species-dependent capabilities to enhance PMN activity against Scedosporium species [43].

When posaconazole and GM-CSF were administered in an immunocompetent murine model of disseminated S. prolificans, they had selective beneficial effects on the fungal burdens in certain organs but offered no additional benefit to survival. However, when posaconazole or GM-CSF were combined with peritoneal PMNs isolated from immunocompetent healthy mice, the PMNs showed significantly increased anti-hyphal activity against S. prolificans [44].

Granulocyte colony-stimulating factor (G-CSF) increases the number of circulating PMNs by stimulating the proliferation and differentiation of myeloid progenitor cells, as well as enhancing their phagocytic activity [45]. Administration of G-CSF in neutropenic animal models of invasive fungal infections including candidiasis, aspergillosis or trichosporonosis was associated with faster recovery from neutropenia and improved survival [46–48]. Consequently, the administration of G-CSF would be potentially beneficial in resolving scedosporiosis. In an immunosuppressed murine model of invasive S. prolificans infection, Ortoneda et al. demonstrated that liposomal amphotericin B (LAMB) at 10 mg/kg/day in combination with G-CSF had a modest effect [49]. However, subsequent studies showed that when LAMB at very high doses (40 mg/kg/day) was combined with G-CSF there was no significant improvement in survival [50]. Of note, administration of G-CSF alone was not more effective as compared to the control group [49,50].
Amphotericin B lipid complex displayed a significant additive effect when used with PMNs against S. prolificans and S. apiospermum [51]. Similarly, in another study, triazoles used in combination with PMNs caused significant additive increase in the damage to S. prolificans and S. apiospermum hyphae. Furthermore, under some conditions, synergy was noted between triazoles and PMNs against S. prolificans hyphae. The synergistic activity was observed at low concentrations of the antifungals. This finding may be of particular importance especially in immunocompromised patients when a triazole reaches its trough level in plasma where such synergy may prevent the regrowth of the etiologic agent [52].

Lamaris et al. [21] found that pre-exposure to caspofungin enhances PMN-mediated hyphal damage of a number of Aspergillus and non-Aspergillus filamentous fungi, with the exception of S. prolificans. In particular, prior exposure of S. apiospermum to caspofungin before the addition of PMNs significantly increased PMN-induced hyphal damage, while incubation of S. prolificans under similar conditions did not significantly affect the damage induced by PMNs. Regardless of the mechanisms underlying these collaborative effects, the findings from these studies would support the concomitant administration of antifungal agents and PMN transfusions to persistently neutropenic patients with invasive scedosporiosis.

**Unmet research needs and future directions**

Future studies on the role of endogenous cytokines in host defense to scedosporiosis should be an important direction of future preclinical research as they may provide new strategies for adjunct therapy in immunocompromised patients. Understanding the normal host response to these pathogens establishes objective targets for immune augmentation in immunocompromised hosts. In the light of the newly identified S. apiospermum/P. boydii complex, it is especially important to investigate to what extent, if any, there exists inter-strain variability in pathogenicity, host-fungus interaction, clinical spectra, or antifungal susceptibilities. Knowledge of the virulence of these species in immunocompetent, as well as immunocompromised subjects and of their response to the host innate immune response and antifungal agents may be useful in order to choose appropriate treatment of the severe and refractory infections caused by Scedosporium species. In summary, advances in understanding the mechanisms of innate host defense against Scedosporium species will lay the experimental foundation for new strategies in management of infections caused by these potentially lethal pathogens. Innovative clinical trial designs will be needed to translate these advances from bench to bedside.

**Declaration of interest:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper

**References**

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This paper was first published online on iFirst on 31 January 2009.