Immunotherapy of infections caused by rare filamentous fungi

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Abstract

Invasive fungal infections caused by rare filamentous fungi constitute a significant cause of morbidity and mortality in patients with defective immune responses. Despite the advent of new antifungal agents, the problem is escalating as the number of susceptible hosts increases and virulent, more resistant fungal strains emerge. There is evidence that reconstitution of the host immune function is a major contributor to the resolution of these infections. Therapeutic modalities aimed at increasing phagocyte numbers, such as granulocyte transfusions, stimulating the immune response, such as administration of haematopoietic growth factors and other proinflammatory cytokines, or indirectly augmenting immune function have shown promising results in the preclinical setting. Because of the rarity of the infections, multicentre clinical trials are needed to demonstrate the efficacy and safety of the new immunomodulating approaches.

Keywords: Colony-stimulating factors, cytokine, fungal infections, fusariosis, immunotherapy, interleukin, mucormycosis, neutrophils, scedosporiosis

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Introduction

Invasive fungal infections constitute an important cause of morbidity and mortality, especially in immunocompromised patients. Although Candida spp., Aspergillus spp. and Cryptococcus neoformans are responsible for the bulk of invasive fungal infections, other less common but problematic fungal pathogens also cause significant morbidity and mortality [1]. Among the emerging pathogens with worldwide distribution that are appearing with increasing frequency are the group of non-septate Mucormycotina [2], and septate filamentous fungi (such as Fusarium spp. and Scedosporium spp.) [1]. These fungal pathogens mainly affect immunocompromised hosts, such as neutropenic patients with haematological malignancies, recipients of haematopoietic stem cell transplants (HSCTs) or solid organ transplants, patients with diabetes mellitus and ketoacidosis, premature infants, and patients with iron overload [1,2]. Although conventional antifungal therapy remains the treatment of choice, it is becoming more difficult to successfully manage these emerging invasive fungal infections.

Among the factors that complicate the efficacy of antifungal treatment of these pathogens are the inherent resistance of these fungi (Fusarium spp. and Scedosporium spp. are innately resistant to most available antifungal agents), the appearance of resistant fungal strains, the adverse effects associated with antifungal chemotherapy, and the failure of antifungal therapy to sterilize infected organs [3–5]. Therefore, given the limitations of the current antifungal armamentarium, approaches that target the host through manipulations to augment the host immune response could provide a helpful supplement to conventional treatment options.

In this review, we summarize the current state of knowledge about the immunotherapeutic options for mucormycosis, scedosporiosis, and fusariosis.

Mucormycosis (zygomycosis)

Mucormycosis is caused by fungi of the order Mucorales and the family Mucoraceae [6]. In the recent reclassification, all
of the agents of mucormycosis have been placed under the subphylum Mucormycotina [7]. *Rhizopus, Mucor* and *Lichtheimia* (formerly *Absidia*) are the most common genera causing mucormycosis, accounting for 70–80% of all cases, whereas *Cunninghamella*, *Apophysomyces*, *Saksenaea*, *Rhizomucor*, *Cokeromyces*, *Actinomucor* and *Syncephalastrum* are responsible for <1–5% of reported cases [8]. Mucormycosis is a life-threatening infection that typically occurs in immunocompromised hosts, such as those who have received HSCTs or solid organ transplants, patients with neutropenia or malignancies, and patients with diabetes mellitus [6,9,10].

Successful treatment of mucormycosis requires early diagnosis, reversal of underlying predisposing risk factors, surgical debridement, and prompt administration of antifungal agents [11]. Despite aggressive surgical and polyene antifungal treatment, the mortality rate of mucormycosis is exceedingly high (6,9,10); therefore, improvement of the host response to infection appears to be a critical factor influencing prognosis.

Clinical data have consistently underscored the importance of neutrophils in the development and outcome of mucormycosis, especially in haematological patients [12]. In all of the reported mucormycosis cases, resolution of neutropenia was correlated with significant improvement [13–17]. In this regard, granulocyte transfusion therapy (GTT) represents a logical approach, being aimed at correcting quantitative defects in patients with prolonged neutropenia or defects in neutrophil function and severe fungal infections [18]. With the advent of human recombinant myeloid colony-stimulating factors (CSFs), GTT showed better clinical efficacy in the therapeutic and prophylactic settings [19]. GTT in patients with invasive fungal infections has not been evaluated in prospective randomized clinical trials; however, clinical data from small case series and case reports have demonstrated good clinical efficacy and safety in difficult-to-treat infections [20]. Particularly in patients with mucormycosis, the current experience with GTT is limited to sporadic case reports [21–24]. It is of note that, in a series of patients with haematological malignancies and life-threatening infections during neutropenia, including five mucormycosis cases, not a single reactivation occurred under prophylactic GTT [22].

Specific proinflammatory cytokines, such as granulocyte CSF (G-CSF), granulocyte–macrophage CSF (GM-CSF), and interferon (IFN)-γ, are critical components of the host defence, promoting upregulation of chemotaxis, phagocytosis, respiratory burst, and/or degranulation of neutrophils, monocytes, and macrophages [25]. *In vitro* immunopharmacological studies and experiments in animal models have shown that neutrophil antifungal activity against different Zygomycetes species can be augmented by IFN-γ or GM-CSF alone or in combination with certain antifungal agents (liposomal amphotericin B or voriconazole) [26–28]. Despite the promising preclinical evidence that IFN-γ and CSFs could enhance the host immune response against mucormycosis, administration of these cytokines as adjunctive therapy has only been reported in case reports and small uncontrolled series [29–33]. In a literature review of 925 reported mucormycosis cases, 15 of 18 (83%) patients who received adjunctive treatment with G-CSF showed a favourable clinical response [9]. Recent reviews of paediatric cases with mucormycosis revealed that cytokine administration could be considered as adjunctive therapy; however, there was no evidence-based clinical effect [34–36]. Overall, cytokine support appeared to shorten the duration of neutropenia and, in concert with liposomal amphotericin B, provided benefit to immunocompromised patients with mucormycosis. However, with the currently available data, it is impossible to define precisely the contribution of cytokine therapy to the successful outcome of these cases, as the findings are limited by publication bias and the multiple medical and/or surgical interventions instituted concomitantly in each case.

Another adjunctive therapeutic modality with an immunomodulating effect that is used for mucormycosis is hyperbaric oxygen (HBO). Administration of HBO for mucormycosis has been described since the 1970s [37,38]. HBO has been shown to have direct fungicidal activity, owing to the production of oxygen-based free radicals and, thus, indirect immunomodulating antimicrobial properties, including correction of lactic acidosis, restoration of phagocytosis, augmentation of the oxidative burst by neutrophils, and enhancement of the antifungal action of amphotericin B. Additionally, HBO increases the rate of tissue healing, by increasing the levels of growth factors that promote angiogenesis [39,40]. A review of the literature that identified 28 mucormycosis cases adjunctively treated with HBO showed that HBO was particularly beneficial in diabetic patients (94% survival), whereas the effectiveness of this treatment was doubtful in patients with haematological malignancies or bone marrow transplants [39]. Clinical data from single centres showed that HBO treatment was a beneficial adjunct in the treatment of invasive fungal infections, including mucormycosis [38,41]. In conclusion, considering the pathophysiology of mucormycosis, HBO appears to be a potentially attractive treatment adjunct. However, current evidence does not support HBO use as the standard of care in mucormycosis cases; therefore, its use should be considered on an individual basis.

Given the central role of iron metabolism in microbial pathogenesis, the use of effective iron chelators has been
proven to be an effective adjunctive antifungal therapy against mucormycosis [42]. Whereas deferoxamine predisposes patients to Rhizopus infection [43] by acting as a siderophore [44], deferasirox is fungicidal in vitro, significantly improves survival and decreases tissue fungal burden in diabetic ketoacidotic or neutropenic mouse models [45]. In addition, deferasirox undergoes a synergistic interaction with liposomal amphotericin B and enhances the host inflammatory response against zygomycetes [45]. In an open-label study, five of eight patients with mucormycosis treated with deferasirox in combination with other antifungal therapy showed improvement by the end of deferasirox therapy, without experiencing serious adverse events [46]. There are case reports with either success [47,48] or failure [49] after salvage deferasirox treatment. Nevertheless, the precise clinical effects of deferasirox are confounded by concurrent treatments and various underlying comorbidities in each patient [47–49]. A prospective, phase II, randomized, double-blind, placebo-controlled study of the safety and tolerability of adjunctive deferasirox therapy in patients being treated with liposomal amphotericin B for mucormycosis was completed in December 2010, and the results are pending (DEFEAT-Mucor trial, ClinicalTrials.gov NCT00419770).

Immunosuppressive agents such as cyclosporin, tacrolimus and sirolimus show synergy in vitro with various azoles (posaconazole, itraconazole, and ravuconazole), amphotericin B or caspofungin against zygomycetes [50,51]. Whether these findings are of clinical relevance remains to be verified in clinical trials [5].

Statins, in addition to their lipid-lowering properties, have pleiotropic immunomodulating functions that, in regard to sepsis or infection, have a beneficial effect for the host. Although the mechanisms through which statins modulate immune and inflammatory responses are largely unknown, it is believed that they reduce the overall magnitude of the systemic response rather than targeting individual inflammatory mediators [52]. Statins have fungicidal activity against zygomycetes, and supporting this is the observation that while the prevalence of diabetic patients increased, the number of mucormycosis cases in patients with diabetes decreased, a phenomenon that coincided with the widespread use of statins in these patients [53]. In fact, statins induce apoptosis-like cell death in Mucor racemosus [54], and in vitro data have shown that lovastatin has activity against a range of zygomycetes and a synergistic effect with voriconazole [55]. It seems that each statin has different effect against zygomycetes, and that different zygomycetes might have different susceptibilities to the same statin [56–58]. These findings provide the rationale for further work on the effects of statins in the clinical setting.

**Scedosporiosis**

The genus *Scedosporium* includes two hyphomycetes of emerging medical importance, *Scedosporium prolificans* and *Scedosporium apiospermum*. *Pseudallescheria boydii* is the teleomorph (sexual state), distinguished from its anamorph (asexual state) *S. apiospermum* [1]. Over the years, both states have undergone several sequential name changes, having been referred to as *Petriellidium boydii*, *Allescheria boydii*, *Pseudallescheria sheari*, and *Monosporium apiospermum* [59].

Treatment of *Scedosporium* infections is particularly challenging, because of the resistance of the fungus to many antifungal agents; thus, on many occasions, salvage treatment options are required. The extremely poor prognosis of scedosporiosis, especially in immunocompromised patients, underlines the importance of reconstitution of the patient’s immune status [4].

*In vitro* studies have shown that antifungal agents collaborate with host defences against *Scedosporium* spp. For example, amphotericin B lipid complex displays a significant additive effect with neutrophils against *S. prolificans* and *S. apiospermum in vitro* [60]. Similarly, triazoles in combination with neutrophils cause a significant additive increase in the damage to *S. prolificans* and *S. apiospermum* hyphae. Furthermore, under certain conditions, synergism has been noted between triazoles and neutrophils against *S. prolificans* hyphae. It is of note that the synergistic activity has been observed at low concentrations of the antifungal agents used. This finding may be of particular importance, especially in immunocompromised patients, when a triazole reaches its trough level in plasma, when such synergy may prevent fungal regrowth [61]. Regardless of the mechanisms behind these collaborative effects, the findings from these studies support the concomitant administration of antifungal agents and granulocyte transfusions to persistently neutropenic patients with invasive scedosporiosis. However, to date, no cases of scedosporiosis have been reported that have been treated with granulocyte transfusions.

Among the cytokines studied that enhance neutrophil antifungal activity against *Scedosporium* spp. are IFN-γ and GM-CSF [62]. Treatment of neutrophils with the combination of IFN-γ and GM-CSF enhanced neutrophil activity against *Scedosporium* spp., whereas each cytokine alone had no effect. Despite the poor effect of either cytokine alone on the neutrophil oxidative burst after 22 h, the combined treatment showed enhancement of the oxidative burst in response to opsonized *S. apiospermum* hyphae. Similarly, after incubation with cytokines for 2 h, only the combination significantly enhanced the oxidative burst against serum-opsonized and
non-opsonized hyphae of Scedosporium spp. Thus, in this study it was demonstrated that IFN-γ and GM-CSF exhibit a significant time-dependent and species-dependent ability to enhance neutrophil activity against Scedosporium spp. [62].

Ortoneda et al. [63], in an immunosuppressed murine model of invasive infection by S. prolificans, demonstrated modest efficacy of liposomal amphotericin B at 10 mg/kg per day combined with G-CSF. Subsequent studies showed that liposomal amphotericin B at very high doses (40 mg/kg per day) combined with G-CSF did not significantly improve survival [64]. Interestingly, administration of G-CSF alone showed no benefit as compared with the control group [63,64]. In an immunocompetent murine model of disseminated S. prolificans infection, it has been shown that posaconazole and GM-CSF have a combined effect in damaging S. prolificans hyphae ex vivo. However, when posaconazole and GM-CSF were administered to mice with invasive infection caused by S. prolificans, they had selective beneficial effects on the burdens in certain organs but gave no additional survival benefit [65]. Despite the limited clinical experience [66], administration of proinflammatory cytokines, especially in neutropenic patients with scedosporiosis, may be worthy of consideration.

**Fusariosis**

Fusarium spp. are common soil saprophytes to which humans are frequently exposed. Fusarium spp. that are frequently implicated in human disease are Fusarium solani, Fusarium oxysporum, and Fusarium moniliforme [3]. Fusarium infections in immunocompetent patients are usually superficial or limited to a single organ, whereas in immunosuppressed hosts (HSCT recipients, and patients with severe or prolonged neutropenia) fusariosis is invasive and disseminated [3,67]. The latter form of fusariosis can be life-threatening, as Fusarium spp. are among the most drug-resistant fungi. Treatment of disseminated infection requires systemic antifungal therapy, surgical debridement of infected tissues, if possible, and immunotherapy [67].

Although the use of immunotherapeutic agents is not established in fusariosis management, there are preclinical data supporting their utility. Amphotericin B formulations (deoxycholate and lipid formulations) at clinically relevant concentrations have been shown in ex vivo studies to have immunomodulatory effects on human neutrophils and monocytes [68] and on rabbit pulmonary macrophages [69] in response to F. solani. Similar ex vivo studies have demonstrated that interleukin (IL)-15 plays an important role in the immunomodulation of host responses against Fusarium spp.

In particular, IL-15 significantly enhanced neutrophil-mediated damage of Fusarium spp., and this effect was attributed, at least in part, to the increased release of IL-8 [70]. The utility of IL-15 as an adjunct to conventional treatment of fusariosis has not been evaluated in the clinical setting; however, considering the low incidence of fusariosis and the marginal efficacy of conventional antifungal treatment, these findings could be of value.

In a retrospective study of invasive Fusarium infections in patients with haematological malignancies, a favourable response was associated with CSF-elicted GTT [71]. Similarly, in a prospective study of neutropenia-related fungal infections, including three Fusarium infections, GTT was proven to be safe and effective. However, as the authors comment, this is a temporary measure to be used only in patients who are likely to recover from neutropenia, and it is essential to start GTT soon after the onset of infection [72]. There is also a case report where the combined use of amphotericin B, GTT and GM-CSF was proven to be safe and life-saving in a neutropenic patient with disseminated fusarial infection [73]. GTT has been administered in paediatric patients to augment the host response against disseminated fusariosis, with a successful outcome [74].

**Conclusions**

The up-to-date knowledge on immunotherapy for mucormycosis, scedosporiosis and fusariosis is limited to ex vivo experiments, animal studies, case reports, and small case series. Randomized controlled clinical trials to provide evidence of the efficacy and safety of immunomodulatory factors are lacking, in all probability because of the relative rarity of these infections. Some controversial issues, such as dosing, timing of immunomodulatory intervention, and the patient groups that would benefit most, need to be clarified. Until randomized multicentre trials are performed, adjunctive therapy with cytokines such as IFN-γ, GM-CSF, and G-CSF, or GTT, can be considered for severe invasive infections caused by rare filamentous fungi, especially when they are refractory to conventional antifungal treatment and the patient is unable to mount an immune response.

**Transparency Declaration**

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