Immunotherapy in Patients with Systemic Mycoses
A Promising Adjunct

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Abstract

Evidence from several in vitro and animal model studies suggests a modulatory role of haemopoietic, TH1 and TH2 cytokines in host defence against fungi, and highlights their potential utility as adjunctive therapy for management of systemic mycoses (SM). However, there are limited clinical data to support the use of cytokines in prevention and treatment of SM. Thus, at present no adjunctive treatment is justified for routine use in all patients. Potential application of these immunomodulatory agents include the use of granulocyte-macrophage colony-stimulating factor or macrophage colony-stimulating factor in the management of mycoses in neutropenic patients with myelogenous leukaemia or bone marrow transplantation. Interferon-γ may have a useful role against aspergillosis in patients with chronic granulomatous disease. Granulocyte colony-stimulating factor-elicited white blood cell transfusions may be life saving to patients with refractory SM. Better understanding of synergy between cytokines and specific antifungals may provide powerful tools for managing these serious infections.

The management of systemic mycoses (SM) remains a challenge for clinicians, especially those caring for immunocompromised patients. SM have become an important problem that has increased in frequency in recent years, particularly among patients with cancer and those undergoing transplantation, who are often immunocompromised as a result of their disease or chemotherapy. In addition to cancer-related neutropenia, other conditions are associated with a high frequency of SM. Such conditions are corticosteroid treatment and phagocytic dysfunction occurring in patients with chronic granulomatous disease (CGD), HIV infection, graft versus host reaction and birth prematurity. Despite currently available antifungal agents, therapy of SM is associated with considerable morbidity and mortality, underscoring the need for more effective therapeutic strategies.

Undoubtedly the most important factor in the outcome of SM is the underlying immunological status of the host. Therefore, immunotherapy aimed at enhancing host defence mechanisms may prove extremely productive in this regard. The innate host defence against fungi is based on the action of phagocytes, and both the number and the function of these cells can be regulated by the haemopoietic cytokines such as granulocyte, granulocyte-macrophage, and macrophage colony-stimulating factor (G-CSF, GM-CSF, M-CSF). Other cytokines that affect the activity of phagocytes against fungi include TH1 cytokines, i.e interferon (IFN)-γ and interleukin (IL)-15, and TH2 cytokines (IL-4 and
IL-10). Exogenous modulation of some of these agents is now possible, opening new avenues for therapy. Thus, in the past few years, there has been increased interest in the use of immunomodulation as an adjunct to antifungal agents.

1. Preclinical Data

1.1 In Vitro Effects of Cytokines on Antifungal Phagocytic Functions

In recent years, a number of studies have investigated the role of these cytokines in the host defence against fungal infections. Neutrophils (PMNs), mononuclear phagocytes (MNCs), peritoneal macrophages (PMs) and pulmonary alveolar macrophages (PAMs) all exhibit up-regulated antifungal activity by cytokines against Candida spp., Aspergillus spp. and other more rare fungi in vitro. Although they appear to share a number of activities, the modulatory effects of cytokines on phagocytic activity against fungi are variable, being both cytokine-dependent and fungus-specific.[1]

Specifically, G-CSF and GM-CSF increase the number and enhance the antifungal function of both intact and immunosuppressed phagocytes. For example, G-CSF enhances the antifungal activity of PMNs from HIV-infected patients,[2] and both G-CSF and GM-CSF prevent corticosteroid-induced suppression of phagocytic activity against Aspergillus fumigatus.[3,4] Compared with G-CSF, GM-CSF appears to be a less potent inducer of neutrophil production.[5] In addition, GM-CSF promotes the differentiation and proliferation of the cells of the monocyte-macrophage system.[6] Thus it appears to have a theoretical advantage in infections where monocyte-macrophage function is critical. On the other hand, G-CSF has been shown to protect PMNs from the deleterious effects of isolation and irradiation, leading to a renewed interest in neutrophil transfusion therapy.[7] M-CSF has no effect on neutrophil function. However, several antifungal activities of monocytes and macrophages against Candida albicans and A. fumigatus as well as secondary production of IL-1, IFNγ and tumour necrosis factor-α (TNFα) are all enhanced after treatment with M-CSF.[8,9]

Although Th1 cytokines do not increase the number of phagocytes, they have been generally shown to enhance their antifungal activity against fungi. Specifically, IFNγ enhances PMN- and MNC-induced damage of A. fumigatus, and reverses the detrimental effects of immunosuppressive drugs on phagocytes’ activity against A. fumigatus.[13,4,10] Recently, G-CSF, GM-CSF and IFNγ were administered to healthy volunteers and their ex vivo effects on PMNs and MNCs were compared. IFNγ exhibited the broadest antifungal activity and enhanced hyphal damage of A. fumigatus.[11] In contrast, Th2 cytokines have been found to suppress many antifungal activities, with IL-4 and IL-10 having been the most studied. Inhibition of these cytokines prevents suppression.[11,12] More recently identified interleukins such as IL-12 and IL-15 also appear to be important in up-regulation of host defences against opportunistic mycoses.[13,14] In particular, IL-15 augments the anti-candida activity of human phagocytes and may be a very promising cytokine.[14]

1.2 Experimental Animal Studies of Cytokine Therapy

The beneficial effects of cytokines have been translated to favourable outcome of SM in animal models. G-CSF treatment results in significant protection against systemic infection caused by C. albicans, A. fumigatus or other fungi in neutropenic and immunosuppressed animals.[15-17] In non-neutropenic animals, administration of a single dose of G-CSF reduced the mortality of animals and C. albicans growth in organs.[17] Administration of GM-CSF to cyclophosphamide-treated mice enhanced their resistance to lethal challenge with C. albicans.[18] Similarly, treatment with M-CSF has been associated with a favourable outcome in animal models of candidiasis and pulmonary aspergillosis as shown by increased survival, reduced fungal recovery from the organs and decreased pulmonary injury.[19,20]
The results of in vivo studies using IFNγ to enhance immune responses against invading fungi are not consistent. Administration of IFNγ had a beneficial effect on the course of experimental candidiasis in mice. However, it did not reduce the growth of C. albicans in cyclophosphamide-pretreated mice. In another study, administration of IFNγ to naive mice and their subsequent challenge with C. albicans resulted in a higher infectious burden and increased mortality. With regard to Th2 cytokines, while resistance to invasive aspergillosis is correlated with production and responsiveness of TNFα, IFNγ and IL-12, neutralisation of Th2 cytokines enhances the resistance and improves outcome of SM in animal models.

2. Clinical Studies of Cytokine Administration

Several placebo-controlled and/or historically controlled trials, particularly among immunocompromised patients, have shown that cytokines enhance neutrophil recovery rates, lead to fewer hospital admissions for fever and neutropenia and less use of antimicrobial or antifungal agents, and occasionally reduce the incidence and duration of infections. However, the results are not consistent among the studies, and a meta-analysis cannot be performed because of major differences in the design and conduct of these clinical trials. In addition, some of the studies have not been designed to show differences in incidence, duration or outcome of infections. Further, none of these trials has specifically evaluated the role of cytokines in patients with SM. Thus, as there have been few documented SM, no study performed with other objectives has had the statistical power to demonstrate a significant difference. Moreover, since the sensitivity and specificity of diagnostic techniques to identify SM are generally low and subsequently it is difficult to differentiate a patient who has had a fungal infection from one who has not, the role of cytokine therapy in the outcome of SM cannot be established. Thus, few convincing clinical data on the utility of cytokine therapy are available, making the issue of cytokine use in the management of SM controversial. Nevertheless, a number of studies have been reported as either prophylactic or therapeutic for proven fungal infections.

2.1 Cancer-Related Therapy

Malignancies and the related immunodeficiencies constitute the largest group of acquired defects in host defences, and the most significant need for immunomodulation. Thus, most of the clinical studies of adjunctive cytokine therapy have focused on patients with cancer. Available data from some clinical studies suggest that cytokines may be useful in cancer patients with SM. In a prospective, randomised, placebo-controlled phase III study of GM-CSF 250 µg/m²/day (4 hour infusion) in older patients with acute myelogenous leukaemia (AML) conducted by the Eastern Cooperative Oncology Group, 20 of 117 patients had documented SM. Fungal infection-related mortality in the GM-CSF group was 1 of 52 (2%), compared with 9 of 47 (19%; p = 0.006) in the placebo group. Only 1 of 8 patients randomised to receive GM-CSF who developed SM died (13%) in contrast to 9 among 12 patients on placebo (75%; p = 0.02). No apparent difference between aspergillosis and candidiasis was noted with these very small numbers of patients.

When used in the same manner, G-CSF demonstrated a similar but weaker effect. Thus, in a randomised, double-blind, placebo-controlled phase III study of G-CSF 5 µg/kg/day subcutaneously in patients with AML, G-CSF significantly reduced the number of patients requiring antifungal therapy (34 vs 43%; p = 0.04). Although the incidence of infections was identical in both groups, there were fewer fungal infection-related deaths in the G-CSF group (3 vs 6). Although this difference was not statistically significant, G-CSF was also associated with reduced use of amphotericin B.

In a nonrandomised study, M-CSF was administered to 46 neutropenic cancer patients with SM. Overall, patients who received M-CSF had a tendency to better survival than did historical controls. The increase in survival was significant in patients...
with candidiasis and Karnofsky score >20% than in historical controls (p < 0.05). Some benefit of the use of M-CSF for patients with aspergillosis has been suggested in individual cases, but an insufficient number of patients has been treated to show a statistical beneficial effect on survival.\[32\]

Several case reports and noncontrolled small studies have shown that G-CSF and GM-CSF may be helpful as part of a combined regimen for SM in selected cancer patients. Recently, the beneficial role of G-CSF in the management of unusual fungal infections caused by *Fusarium*, *Trichosporon* or other rare fungi has been described.\[36-38\] In addition, GM-CSF has been used as adjuvant therapy for various fungal infections in cancer patients with relative success.\[39\]

The discovery that G-CSF improves the killing of fungi by normal PMNs or PMNs from patients infected with HIV has led to explorations of its efficacy as an adjunct to antifungal therapy for SM in non-neutropenic patients. Thus, a multicentre study of administration of G-CSF in non-neutropenic patients with invasive candidiasis has been conducted. Although no significant difference in survival was observed, the patients who had higher numbers of PMNs due to G-CSF had more favourable outcome than those who did not increase their PMN counts.\[40\]

Although few data on utility of cytokines in documented SM are available, several controlled trials have used cytokines as prophylaxis during chemotherapy-induced neutropenia or as adjunctive therapy for febrile neutropenic patients.\[27,28,33-35\] For example, the potential clinical role of G-CSF as prophylaxis has been examined in a randomised trial of 119 neutropenic patients with haematological malignancies and infection after intensive chemotherapy. Patients who received antibacterials plus G-CSF had more clinical responses (82 vs 60%), fewer superinfections, lower mortality, fewer days in hospital and reduced antibacterial use. Although only 4 fungal infections occurred, they were all encountered in the group receiving antibacterials alone. However, this low number of reported infections limits the power of the study to determine a significant difference.\[33\]

In a double-blind, controlled study of M-CSF in patients with AML and febrile neutropenia, a significant decrease in use of systemic antifungals, but no effect on disease-free survival, was observed. Again, it was not clear whether M-CSF was associated with a reduced incidence of fungal infection, since only 5 documented fungal infections occurred in the placebo group and 2 in the M-CSF group.\[34\]

In a retrospective study of patients with autologous bone marrow transplantation for lymphoid cancer, the incidence of infections 28 days after transplantation was compared in patients who had or had not taken GM-CSF. Overall, fewer infections (13 vs 40% of controls; p = 0.001), a trend of fewer SM (4 vs 14%; p = 0.09) and fewer days of amphotericin B use (median 2 vs 8.5 days; p = 0.03) were observed.\[35\]

To date, no randomised trials have compared the efficacy of cytokines in the same clinical setting. However, Peters et al.\[41\] performed an analysis of the relationship between the type of CSF administered and the incidence of SM in a retrospective study of 145 consecutive patients receiving high dose chemotherapy with or without stem cell transplantation. There were no statistical differences in patient characteristics and risk factors for fungal infections. The risk ratio for developing SM in patients treated with G-CSF or no cytokine was 4.20 (p = 0.023) compared with patients who received a monocyte/macrophage-stimulating cytokine (GM-CSF, M-CSF or IL-3). Additionally, there was a trend towards increased mortality in patients treated with G-CSF or no cytokine; however, the difference was not statistically significant. In another study, G-CSF, GM-CSF or no cytokine were administered to 51 oncology patients and their *ex vivo* functional effects on PMNs and MNCs were compared. Monocyte-induced killing of *C. albicans* was enhanced in GM-CSF-treated patients compared with patients receiving G-CSF or no cytokine. No difference in PMN activity was observed between patients receiving either cytokine.\[42\] Although these results are consistent with the promising effect of GM-
CSF in patients with AML, further research is warranted by prospective randomised trials to confirm them and validate the association of the use of monocyte/macrophage-stimulating cytokine and fungal infections.

Much discussion has surrounded the issue of the cost effectiveness of using cytokines in various settings. The cost effectiveness of G-CSF with regard to SM was addressed in a recently published study. Neutropenic patients with presumed SM were randomised to receive either amphotericin B alone or this drug combined with G-CSF. Patients who failed to respond in both groups continued on liposomal amphotericin B. There were 62% responders to the combination versus 33% to amphotericin B alone (p = 0.027). Based on drug acquisition, hospital stay and treatment duration, the combination regimen was more cost effective.

2.2 Use of Cytokines in Other Settings

Outside the cancer area, CGD is the best studied condition for which cytokines have been used as adjunctive antifungal therapy. Treatment of phagocytes from CGD patients with IFNγ enhances their oxidative burst as well as PMN ability to damage A. fumigatus hyphae in vitro. The beneficial effect of IFNγ prophylaxis in patients with CGD has been shown in a prospective, randomised, placebo-controlled trial. In that study, the incidence of serious infections in the group of patients who received IFNγ was significantly reduced from 24 to 2% in 2 years, and aspergillus pneumonia was somewhat less common. Those results, along with several case reports describing a potential role for adjunctive IFNγ treatment for refractory SM, have prompted prophylactic use of IFNγ in CGD patients.

Finally, results of a limited number of studies using GM-CSF or G-CSF in neonates and patients with HIV infection have shown a reduced incidence of infections. However, no mention of fungal infections was included in these studies. As such patients are at high risk for SM, the role of cytokines against fungal infections in these patients remains to be investigated.

3. Additional Strategies

The optimal use of cytokines in the management of SM is unclear and remains to be defined. Transfusion of cytokine-elicited phagocytes may have the greatest potential for improving the outcome of SM. Similarly, the synergy among cytokines or between cytokines and specific antifungals may also be of great importance.

Recently, a renewed interest in neutrophil transfusion therapy has evolved, based largely on the ability to obtain high yields of better quality PMNs by leukapheresis after stimulation of donors with G-CSF. In addition, the use of G-CSF in healthy donors protects and enhances PMN-mediated activity against fungal hyphae and pseudohyphae. Although there has been no prospective randomised study dealing with the administration of neutrophil transfusion in patients with neutropenia-related fungal infections, the preliminary results in neutropenic patients with refractory fungal infections are encouraging. Data from these uncontrolled studies have shown a >50% favourable outcome of these infections after transfusion. In addition, neutrophil transfusions after stimulation of donors with G-CSF are well tolerated by both donors and patients. Furthermore, transfusion recipients on average exhibit high post-transfusion neutrophil increments that are sustained for more than 24 hours. However, more investigations are needed before this promising technique becomes a standard of care for life-threatening SM in neutropenic patients.

Synergistic activity of phagocytes, cytokines and antifungal agents is an area of active research with potential clinical usefulness. In vitro studies have shown additive effects of G-CSF or GM-CSF and azoles (fluconazole and voriconazole) against either C. albicans or A. fumigatus when added to PMNs or MNCs. Additive effects have also been found between M-CSF-treated PAMs and amphotericin B lipid complex. In murine models of SM, synergism has been shown between G-CSF and posaconazole in neutropenic mice with aspergillosis, and between M-CSF and amphotericin B against candidiasis.
Further, available in vitro data suggest that combinations of G-CSF or GM-CSF with IFNγ have more activity on phagocytes against *A. fumigatus* hyphae than each cytokine separately and serve as a basis for potential experimental animal and clinical combinational use of these cytokines.\[61,62\] However, no animal or clinical studies have examined this and only case reports of treatment with GM-CSF and IFNγ have been reported.\[63\]

4. Conclusions

Many years of research have led to a better understanding of antifungal host defences and cytokine involvement in immunopathogenesis of mycoses. The role of cytokines alone or in combination is better understood and data on the use of cytokines as prevention or combined with an antifungal agent as adjunct to therapy are accumulating. Sufficient clinical trials and guidelines concerning appropriate dose, duration and timing of therapy are not yet available. Thus, at present no adjunctive cytokine treatment should be used routinely in all cases. Nevertheless, reconstitution of immune response by exogenous modulation of enhancing/regulatory cytokines appears to be a promising adjunct to antifungal chemotherapy for these life-threatening diseases. Potential application of these immunomodulatory agents could be:

- use of GM-CSF or M-CSF in prevention and treatment of mycoses in neutropenic patients with myelogenous leukaemia or bone marrow transplantation
- IFNγ in patients with CGD
- G-CSF-elicted white blood cell transfusions to neutropenic patients with refractory SM.

A better understanding of synergy among cytokines and with specific antifungal agents may provide additional powerful tools for managing these serious infections. More investigation of the safety and efficacy of these immunotherapeutic modalities is an urgent priority for current research. In addition, further studies should be conducted to identify patients most likely to benefit from cytokine therapy and to determine the optimal clinical utility of these cytokines in different clinical settings. In order to conduct appropriate clinical trials to prove the beneficial role of cytokine modulation, only selected and very high risk patients need to be targeted in randomised studies as potential beneficiaries of immunomodulation.

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