Review

Cytokines in immunodeficient patients with invasive fungal infections: an emerging therapy

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Immune response is the major contributor to host defense against opportunistic fungal infections such as candidiasis, aspergillosis and other rare infections. A number of cytokines have been developed and studied in vitro for activity against fungal pathogens. The most studied among them in relation to fungal infections are granulocyte colony-stimulating factor (G-CSF), granulocyte–macrophage colony-stimulating factor (GM-CSF), macrophage colony-stimulating factor (M-CSF) and interferon-γ (IFN-γ). The fields where these cytokines have been predominantly studied or where they may need more study are primary immunodeficiencies of the phagocytic cells, neonatal age, human immunodeficiency virus infection and cancer-related conditions such as neutropenia and hemopoietic cell transplantation. In this review, the in vitro, experimental animal and clinical data of cytokines are summarized in relation to invasive candidiasis, aspergillosis and emerging fungal infections. Cytokine administration to patients together with antifungal agents, as well as transfusion of cytokine-upgraded phagocytes, are promising immunotherapeutic modalities for further research.

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Immune response is the major contributor to host defense against opportunistic fungal infections. This is illustrated by the finding that when immune components are absent or dysfunctional, invasive fungal infections occur. Neutropenia is a particularly major risk factor for the development and outcome of fungal infections. Patients with cancer and/or hemopoietic cell transplantation usually develop neutropenia secondary to their malignancies or to their chemotherapy treatment. Other risk factors are corticosteroid treatment and phagocytic dysfunction occurring in immunosuppressing conditions, including bone marrow transplantation (BMT) and infection with human immunodeficiency virus (HIV).

While infections due to *Candida* spp. and *Aspergillus* spp. are the most common, previously rarely encountered opportunistic fungi have emerged as important pathogens. As examples, *Trichosporon* spp., *Fusarium* spp., *Scedosporium* spp. and *Penicillium marneffei* can cause invasive infections. In the setting of neutropenia, overall mortality of candidiasis exceeds 60%[1] while aspergillosis and fusariosis can have a mortality of >90%.[2,3] Unfortunately, these infections are often only minimally responsive to antifungal therapy, especially in patients with neutropenia, where even the most efficient antifungal agents have limited efficacy. Therefore, new therapeutic approaches are urgently needed.

Rapid advances in understanding the pathogenesis of these infections, combined with the availability of recombinant cytokines, have opened the way for immunotherapeutic approaches. A schematic general view of the effects of cytokines on the antifungal function of phagocytes through transduction of activation signals is shown in Figure 1. The critical role of phagocytic immunity has become evident, and numerical as well as functional reconstitution of effector cells by treatment with cytokines and/or white blood cell transfusions (WBCTx) has been attempted. Cytokines that are of most interest due to their ability to up-regulate the function of phagocytes and the fact that they have no major toxicity, are granulocyte colony-stimulating factor (G-CSF), granulocyte–macrophage colony-stimulating factor (GM-CSF), macrophage colony-stimulating factor (M-CSF) and interferon-γ (IFN-γ). There is a large body of preclinical data as well as recent studies of cytokine use in the prophylactic and therapeutic management of fungal infections. The most important of these data are summarized below.

IN VITRO EFFECTS OF CYTOKINES ON ANTIFUNGAL DEFENSE

Invasive candidiasis

Cytokines modulate the ability of phagocytes to ingest and kill *Candida* spp., thus influencing host defense against infection. Downregulation of Th1-profile responses may allow *Candida* to evade intracellular destruction by phagocytes.[4,5] As well as being critical in the association between fungus and host at the mucosa,

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Th1 cytokines may also be important in invasive infections, when the recruitment of antigen-specific lymphocytes and locally high cytokine concentrations are required to stimulate the anticandidal activities of nonspecific effector cells, including neutrophils (PMNs) and macrophages.[6] The role of cytokines, such as G-CSF, GM-CSF, M-CSF and IFN-γ, appears to be very important in this process. The in vitro effects of hemopoietic cytokines are summarized in Table 1.

IFN-γ, a potent immunomodulator of Th1 pattern of response, enhances the phagocytic function against fungi[7] and, in particular, the anti-*Candida* activity of both PMNs and monocytes (MNCs), as has been shown in several in vitro studies.[8,9] Tumor necrosis factor-α (TNF-α), despite its toxicity, is promising because of its potency as an immunoenhancing cytokine. It has been shown to enhance the fungicidal activity of PMNs against *C. albicans* and *C. glabrata* blastoconidia,[8,10] whereas the results with pseudohyphae of *C. albicans* were equivocal.[9] Other cytokines such as interleukin-15 (IL-15)[11] have been shown to have anti-*Candida* activity, but their experimental animal and clinical data are very limited.

Invasive aspergillosis

Cytokines play an important role in the host response and pathogenesis of invasive aspergillosis. Both immunoenhancing and immunoregulatory cytokines have been shown to modulate host defenses against aspergillosis in vitro and in vivo.[12] The most important in vitro effects of hemopoietic cytokines are summarized in Table 2.

In addition, the significance of Th1 and Th2 patterns of response to invasive aspergillosis has been recently demonstrated.[13,14] Incubation of PMNs with IFN-γ increased oxidative burst and PMN-mediated damage to both serum-opsonized and non-opsonized hyphae of *A. fumigatus*.[15] Furthermore, available in vitro data suggest that the effects of IFN-γ on anti-*Aspergillus* activities of human PMNs and MNCs can be combined with the effects of either G-CSF or GM-CSF, and serve as a basis for potential experimental animal and clinical combinational use of these cytokines.[15,16]

IFN-γ was also able to reverse corticosteroid-induced deficiency of certain functions of mononuclear phagocytes.[17–19] While dexamethasone suppressed the fungicidal activity of human MNCs against *A. fumigatus* evidenced as O_2^- production in response to hyphae and as hyphal damage, IFN-γ was able to restore these activities.[20] These effects correlated with in vivo results that IFN-γ had favorable effect on invasive aspergillosis in mice immunosuppressed with corticosteroids[21] as mentioned below. Recently, G-CSF, GM-CSF and IFN-γ were administered to healthy volunteers, and their ex vivo effects on PMNs and MNCs were compared. IFN-γ exhibited the broadest antifungal activity and enhanced hyphal damage to *A. fumigatus*.[22]

The effects of recombinant human TNF-α on antifungal activities of PMNs, MNCs and pulmonary alveolar macrophages (PAMs) against *A. fumigatus* have also been investigated. While the effects on MNC functions were moderate, incubation of PMNs with TNF-α enhanced human PMN O_2^- production in response to PMA, FMLP and non-opsonized *A. fumigatus* hyphae, as well as PMN-induced hyphal damage. Incubation of PAMs with TNF-α increased phagocytosis of *A. fumigatus* conidia but did not affect intracellular killing of conidia.[23]

Table 1. Significant in vitro effects of hemopoietic cytokines on the anti-Candida function of phagocytes

G-CSF
↑ PMN oxidative burst in response to blastoconidia and pseudohyphae of *C. albicans*[85]
↑ PMN fungicidal activity after long incubation with *C. albicans*[86]
↑ PMN hyphal damage of *C. tropicalis, C. parapsilosis* and *C. albicans*[87,88]
GM-CSF
↑ anti-*Candida* activities of PMNs and MNCs[86,89]
M-CSF
↑ MNC-mediated phagocytocis[90,91]
↑ MNC conidial damage[90,91]
↑ MNC O_2^- production in response to FMLP[90,91]

MNC=monocyte.

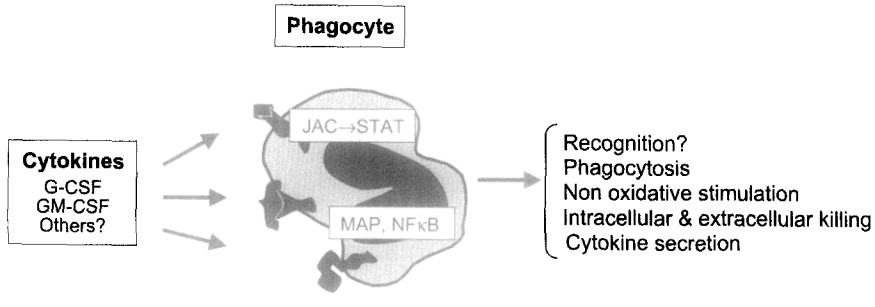

Figure 1. Schematic model of the effect of cytokines on the function of phagocytes through transduction of activation signals. JAK, Janus kinase; STAT, signal transducer and activator of transcription; MAP, mitogen-activated protein kinase; NFκB, nuclear transcription factor κB.

Invasive infections due to rare or emerging fungi

Trichosporon spp. and *Scedosporium* spp., both drug-resistant fungi, as well as *Fusarium* spp. and *P. marneffei* have emerged as important pathogens in immunocompromised patients.[24–33] Risk factors for infection by these rare fungi are prolonged neutropenia and corticosteroid therapy.[25,27,38–41] The modulatory effects of cytokines on phagocytic activity against these fungi are summarized in Table 3.

EXPERIMENTAL ANIMAL AND CLINICAL STUDIES OF CYTOKINE THERAPY

Chronic granulomatous disease (CGD)

Prevention Early studies have shown that IFN-γ enhances the oxidative metabolism of PMNs and MNCs of patients with CGD in vitro and in vivo.[34–36] In a large, prospective, randomized, placebo-controlled trial, the incidence of invasive fungal infections in the group of CGD patients who received 50 μg/m^2 IFN-γ three times a week was reduced from 24% of controls to 4% in 2 years.[37] In this study, IFN-γ administration did not affect oxidative burst production suggesting no causative role of oxidative burst enhancement on microbicidal activity of patients' PMNs. The majority of cases in which oxidative burst was enhanced were patients with variant X-linked forms of CGD, which is the minority form of the disease. In an ex vivo accompanying study of some of the patients from the above investigation, PMNs of IFN-γ-treated patients resulted in more damage to *Aspergillus* hyphae than PMNs of controls.[38] From these and other studies, it appeared that enhancement of antifungal activity against mold hyphae is not closely related to modulation of oxidative burst. Probably, non-oxidative mechanisms are more important than oxidative metabolites in hyphal damage.[39]

In a subsequent double-blind randomized study, however, CGD patients treated with 100 μg/m^2 IFN-γ for 2 consecutive days did exhibit a higher capacity of oxidative burst after stimulation with FMLP or PMA and increased damage to *A. fumigatus* hyphae as compared to patients treated with 50 μg/m^2 IFN-γ or before initiation of IFN-γ administration.[40] These findings may be relevant to the decreased incidence of invasive aspergillosis observed in CGD patients treated with IFN-γ prophylactically.

Therapy Many cases have been reported in the literature of patients with invasive fungal infections, the vast majority of which have been due to *Aspergillus* spp., who were cured with combined antifungal therapy and IFN-γ. Those patients were not receiving IFN-γ prophylactically. Use of other cytokines or other immunotherapies has been rare in CGD patients. One patient with X-linked CGD and invasive multifocal infection due to *A. nidulans* was successfully treated by HLA-genoidentical BMT, G-CSF-elicited PMNs, G-CSF and liposomal amphotericin B. The infection had been unresponsive to treatment with amphotericin B and IFN-γ. At 2 years post-BMT, the patient was well, with full immune reconstitution and no sign of *Aspergillus* infection.[41]

Another interesting case was that of a 15-year-old male with X-linked CGD who developed a lobar pneumonia and tibial osteomyelitis due to the filamentous fungus *Chrysosporium zonatum*, while prophylaxis with IFN-γ was just initiated. The mold was isolated from sputum and affected bone, and hyphae were observed in the bone by histopathology. Therapy with liposomal amphotericin B and IFN-γ eradicated infection from both sites.[42]

Neonates

Little is known about the antifungal activity of neonatal phagocytes and its modulation by cytokines. IFN-γ production is defective in neonates,[43] and IFN-γ treatment enhances the antifungal activity of neonatal macrophages against *C. albicans*.[44] Despite enhancing the antifungal activity of adult monocytes and macrophages, M-CSF did not significantly affect the activity of neonatal MNCs against *C. albicans*.[45] As more fungal infections are seen in the neonatal intensive care unit now and more high-risk very-low-birthweight newborns

Table 2. Significant in vitro effects of hemopoietic cytokines on the anti-*Aspergillus* function of phagocytes

G-CSF
↑ PMN O_2^- production in response to hyphae of *A. fumigatus*[15]
↑ PMN hyphal damage[15]
restores the corticosteroid immunosuppression of PMN antifungal activities[47,92]
↑ PMN conidial damage[93]
GM-CSF
↑ PMN hyphal damage[94]
↑ O_2^- production by PMNs and MNCs in response to PMA and hyphae[16,94]
↑ MNC hyphal damage[16]
restores the corticosteroid immunosuppression of MNC antifungal activities[20]
M-CSF
↑ MNC hyphal damage[95]
↑ MNC O_2^- production in response to PMA[95]
↑ MNC conidial damage[95]

Table 3. In vitro effects of cytokines on the antifungal function of phagocytes in response to rare fungi

• GM-CSF, M-CSF and IFN-γ ↑ anti-*T. asahii* activity of MNCs[24]
• G-CSF, GM-CSF and IFN-γ ↑ anti-*F. solani* activity of phagocytes[22]
• GM-CSF and IFN-γ ↑ O_2^- production by PMN in response to *S. prolificans* hyphae[32]
• M-CSF ↑ O_2^- production by PMN in response to *P. marneffei* conidia[33]

survive, the roles of cytokines in the prevention and treatment of invasive fungal infections in the nursery remain to be defined.

HIV infection

Aspergillosis has become an important opportunistic infection in HIV-infected patients.[46] PMNs and MNC-derived macrophages from HIV-infected patients possess decreased ability to damage hyphae and to ingest conidia of *A. fumigatus*, respectively.[47,48] These defects may be related to the well-described $CD4^+$T-helper lymphocyte dysfunction of these patients.[49] Interestingly, the PMN defect is partially correctable by G-CSF in vitro.[47] In addition, in a randomized, multicenter, controlled trial of patients with advanced HIV infection, G-CSF decreased the incidence and duration of severe neutropenia, the incidence of bacterial infections, hospital days, and duration of intravenous antibiotics.[50] No mention, however, of any fungal infections was included in the study. A more recent phase III study of recombinant IFN-γ administration showed a trend of decreased incidence of oral/esophageal candidiasis compared to control subjects.[51]

Cancer-related therapy

Malignancies and the related immunodeficiencies constitute the broadest field of cytokine use. Thus, most of the preclinical and clinical studies have focused on this patient population. The cytokines under review have been studied preclinically, although clinical studies are still missing for most of them.

Experimental animal studies In one of the earliest studies of the use of cytokines in animal models of fungal infections, G-CSF given to cyclophosphamide-treated mice was reported to offer protection from subsequent development of aspergillosis. In that study, steroid-treated mice were protected only when simultaneous antifungal chemotherapy was administered.[52] In a more recent study, mice immunosuppressed with either hydrocortisone or 5-fluorouracil were infected intranasally with *A. fumigatus*. Beginning 3 days before infection, some groups of mice were given recombinant G-CSF, the antifungal triazole SCH56592 (currently named posaconazole), or both. In corticosteroid-pretreated mice, G-CSF strongly antagonized the antifungal activity of SCH56592. While the drug reduced fungal burden in lung tissue and prolonged survival, the combination therapy reversed this effect. In contrast, mice made neutropenic with 5-fluorouracil and then infected with *A. fumigatus* benefited from either G-CSF or triazole, and the effect of the combination was additive. These findings suggest that host factors contribute in different ways to the outcome of cytokine therapy in aspergillosis.[53]

The effect of G-CSF was also investigated on acute disseminated *C. albicans* infection in non-neutropenic mice. Mice treated with a single dose of G-CSF showed a significantly reduced mortality and fungal growth from kidneys, spleen and liver.[54]

On the other hand, in a neutropenic murine model of disseminated trichosporonosis, G-CSF (30–100 μg/kg per day) administered either before or after infection improved the survival rate from less than 25% up to 100% and led to organ clearance. However, GM-CSF (0.8–2 μg/kg per day) decreased the survival rate. GM-CSF increased PMN counts less significantly than did G-CSF and increased the lethality, with a high level of TNF-α in bronchoalveolar lavage fluid. These results suggested that other host defense mechanisms, such as TNF-α overproduction in the lungs, have an important role in the prognosis of trichosporonosis.[55]

In acute murine candidiasis, Cenci et al found that M-CSF administration protected mice from a subsequent lethal challenge with *C. albicans*. In this model, increased survival and reduced recovery of fungi from the organs was observed.[56]

Furthermore, the effects of M-CSF (100–600 μg/kg per day) in augmenting pulmonary host defense against *A. fumigatus* were studied in neutropenic rabbits with pulmonary aspergillosis, starting 3 days pre-inoculation and then throughout neutropenia. Rabbits receiving prophylactic M-CSF had significantly increased survival and decreased pulmonary injury, as evidenced by computerized tomographic scanning and histopathology. Microscopic studies demonstrated greater numbers and more activated PAMs in lung tissue of rabbits receiving M-CSF in comparison to controls. PAMs harvested from M-CSF-treated rabbits exhibited significantly greater phagocytosis of *A. fumigatus* conidia as compared to PAMs from control rabbits.[57]

The effect of recombinant rat IFN-γ on acute disseminated *C. albicans* infection was investigated in mice. One intravenous dose of IFN-γ shortly before or simultaneously with the inoculation of non-immunosuppressed mice decreased the growth of *C. albicans* from kidneys, spleen and liver. However, it did not reduce the fungal growth of *C. albicans* in cyclophosphamide-pretreated mice. These findings suggest that IFN-γ enhances host resistance against acute disseminated *C. albicans* infection in mice through activation of PMNs.[58]

In corticosteroid-treated mice with invasive aspergillosis, TNF-α succeeded in decreasing mortality (from 40–60% to 0%) and recovery of fungi from organs as demonstrated by culture and histology.[21] In another study, intratracheal challenge of both neutropenic and non-neutropenic mice with *A. fumigatus* conidia resulted in an increase in lung TNF-α levels, which correlated with the histologic development of a peribronchial infiltration of PMNs and MNCs. Neutralization of TNF-α resulted in an increase in mortality in both normal and cyclophosphamide-treated animals,

which was associated with increased lung fungal burden. Depletion of TNF-α resulted in a reduced lung PMN influx in both normal and cyclophosphamide-treated animals, which occurred in association with a decrease in lung levels of the chemokines macrophage inflammatory protein-2 and macrophage inflammatory protein-1α. In cyclophosphamide-treated animals, intratracheal administration of a TNF-α agonist peptide 3 days before the administration of *Aspergillus* conidia resulted in improved survival. This study indicated that TNF-α is a critical component of innate immunity in both immunocompromised and immunocompetent hosts, and that pretreatment with a TNF-α agonist peptide in a compartmentalized fashion can significantly enhance resistance to *A. fumigatus* in neutropenic animals.[59]

Clinical studies In cancer patients, cytokines, mostly G-CSF and GM-CSF, have been used to shorten the duration of therapy-induced leukopenia and as adjunctive therapy in patients with febrile neutropenia. However, their role in the prevention of fungal infections has not been established, as very few of these studies have attempted to address this issue.

A randomized trial of G-CSF was carried out to investigate the efficacy and toxicity of G-CSF in 119 severely neutropenic patients with hematologic malignancies after intensive chemotherapy and infection. Patients were assigned randomly to receive either antibiotics alone (ceftazidime plus amikacin) or the same antimicrobial regimen plus G-CSF (5 μg/kg per day). Patients who received antibiotics plus G-CSF had more clinical responses (82% versus 60%), less superinfections as well as improved mortality, fewer days in hospital, and reduced antibiotic use. Fungal infections occurred only in the group treated with antibiotics alone. Toxicity secondary to G-CSF was absent.[60]

A prospective, randomized, placebo-controlled phase III study of GM-CSF in acute myelogenous leukemia was conducted in 124 patients 55–70 years of age by the Eastern Cooperative Oncology Group. GM-CSF or placebo were administered from day 11 post-induction. A higher rate of complete response was observed among the patients treated with GM-CSF and longer overall survival. In addition, lower infection-related toxicity was observed. Among patients with pneumonia, mortality was as follows: 2/14 patients (14%) in the GM-CSF group, and 7/13 (54%) in the placebo group (P=0.046). Fungal infection-related mortality in the GM-CSF group was 1/52 (2%) as compared to the placebo group, in which was 9/47 (19%, P=0.006).[61,62]

The mortality of the patients who received GM-CSF or placebo in this study according to the etiologic agent was mentioned in a more recent publication.[63] There were 11 patients with aspergillosis, 7 with candidiasis and 2 with infections due to other fungi. Only 1 of 8 patients who had been randomized to receive GM-CSF and developed fungal infection died (13%). This contrasted with 9 of 12 patients on placebo (75%, P=0.02). No apparent difference between aspergillosis and candidiasis was noted with these very small numbers of patients.

A non-randomized study of M-CSF administration to neutropenic cancer patients was reported. Among them, 30 patients suffered from invasive candidiasis, 15 from invasive aspergillosis, and 1 from mucormycosis. Overall, there was a trend of decreased incidence of fungal infections. There was, however, significantly greater survival in patients with candidiasis and Karnofsky score >20% compared with historical controls (P<0.05).[64]

A double-blind controlled study of M-CSF was conducted in patients with acute myeloid leukemia and febrile neutropenia. Although decreased incidence and duration of febrile neutropenia and significant decrease of use of systemic antifungals was found, no impact on disease-free survival or on incidence of specific infections was observed.[65]

CYTOKINES IN PROVEN FUNGAL INFECTIONS

Cytokine administration to patients

Several case reports have suggested the value of hemopoietic cytokines in treating invasive fungal infections. Some of them are mentioned briefly in this review. G-CSF has been administered together with amphotericin B in a patient with acute myelogenous leukemia and allogeneic BMT suffering from disseminated infection due to *T. asahii* with a successful outcome. Two other patients with acute leukemia and progressive chronic disseminated candidiasis were treated with a combination of IFN-γ and GM-CSF in addition to antifungal therapy and they were cured.[67]

A pilot study of eight neutropenic patients with invasive fungal infections was conducted with GM-CSF as adjuvant treatment. There were six responses, with four complete responses among two patients with *Aspergillus* infection (one responded), five *Candida* spp. cases (four responded) and one *Trichosporon* case (responded).[68] The dose of GM-CSF used in this study was excessive ranging up to 700 μg/m^2, and was followed by increased toxicity.

A subsequent open study of GM-CSF in proven invasive fungal infections in neutropenic cancer patients conducted in 17 patients did not show similar favorable results. Eight of the patients suffered from candidemia, eight from pulmonary aspergillosis, and one from fusariosis. They were treated with GM-CSF 5 μg/kg and amphotericin B 1 μg/kg. There were six deaths, and the authors felt that the regimen failed to improve outcome.[69]

A retrospective analysis of 123 cases of invasive aspergillosis from 20 hospitals in eight countries was conducted by European Organization for Research and Treatment of Cancer Invasive Fungal Infection Cooper-

ative Group in hematologic patients. Although not an objective of the study, 33% of the patients had taken a growth factor (mostly G-CSF) in addition to antifungal agent(s). No significant influence on outcome of the growth factor was observed.[70]

Finally, a multicenter study of administration of G-CSF in non-neutropenic patients with invasive candidiasis has been conducted and recently completed in Europe. The patients who had higher numbers of PMNs due to G-CSF had more favorable outcome than the patients who did not increase their PMN counts. The results of this study have been presented in meetings but not published yet.

Immunoreconstitution through cytokine-elicited white blood cell transfusions

Based on encouraging results of several experimental animal studies that demonstrated the efficacy of WBCTx therapy for the eradication of infection, a series of clinical trials were conducted in patients with neutropenia-related infections. Most of the studies showed that WBCTx could be life-saving among those patients with prolonged neutropenia[71–74] or could improve overall survival.[75] However, methodological problems as well as issues related to the relatively high cost of the procedure, significant adverse effects to the recipient (fever, chills, hypotension, pulmonary infiltrates, respiratory distress, transmission of cytomegalovirus, graft-versus-host disease, alloimmunization and hemolytic reactions), insufficient quantities of PMNs used for transfusion[76] and the development of newer and more effective antibiotics, made WBCTx less attractive.

A new need for WBCTx therapy became apparent due to the increased incidence of neutropenia-related fungal infections and the limited efficacy of antifungal agents in this setting. Recombinant G-CSF has been shown to stimulate the proliferation and differentiation of PMNs in vitro and to increase the PMN count safely in patients with neutropenia.[77] These results raised the possibility that administration of G-CSF to white blood cell (WBC) donors would increase their PMN counts to levels that would lead to a higher yield of better-quality PMNs.

Thus, in one study, 10 profoundly neutropenic patients (<100/μL) having refractory fungal infections were treated with daily WBCTx obtained from donors treated daily with 5 μg/kg of G-CSF subcutaneously.[78] Half of the recipients showed a favorable outcome of the infection after WBCTx. Donors achieved a 4–10-fold increase of their PMN count and the mean PMN yield was 3.7×10^{10}. Recipients achieved median 1- and 24-h post-transfusion PMN counts of 780 and 426/μL, respectively. In another study, G-CSF-primed PMN transfusions were administered to two neutropenic patients with *C. tropicalis* fungemia in combination with amphotericin B, resulting in elimination of the infection.[79]

More recently, G-CSF-elicited WBCTx were used to treat 15 adult neutropenic patients (<500/μL) with documented and refractory fungal infections in a pilot study.[80] All 15 adult patients had hematologic malignancies and 7 had been treated with BMT. The cases included 11 mold infections (7 aspergillosis, 3 fusariosis, 1 unidentified) and 4 yeast infections (3 candidiasis, 1 trichosporonosis). Eight of the patients had evidence for widely disseminated infection. At the time of institution of WBCTx, all of the infections had been progressive despite appropriate antifungal treatment for a median of 10 days, and the patients had been neutropenic for a median of 23 days. At the end of WBCTx therapy, 11 of 15 patients had a favorable response (9 improved and 2 stabilized), and in 7 of them the favorable response appeared to be substantially due to WBCTx. Those patients in whom WBCTx therapy was started early during neutropenia and shortly after diagnosis of fungal infection were more likely to respond. The favorable responses were still seen 3 weeks after the end of WBCTx therapy in 8 of 11 responders. Owing to special efforts to select only blood-related donors and non-alloimmunized recipients, the frequency of adverse reactions was <5%. Donors tolerated the treatment well and achieved a median PMN count of 29.4×10^3/μL that led to a mean PMN yield of 4.1×10^{10}, and mean 1-h and 24-h post-transfusion PMN counts of 594 and 396/μL, respectively. Although this was a small pilot study, it demonstrated that G-CSF-enhanced WBCTx therapy can be life-saving for patients with refractory neutropenia-related fungal infections. More information about the long-term safety of donors' treatment with G-CSF is needed before firm recommendations on the use of G-CSF-elicited WBCTx are made.[81]

PHARMACOLOGY, TOXICOLOGY AND ECONOMICS

Two forms of G-CSF are commercially available. One is a recombinant non-glycosylated protein expressed in *Escherichia coli* (filgrastim). The other is a glycosylated form expressed in Chinese hamster ovarian cells in vitro (lenograstim). Both products have the same net effect, acceleration of myelopoiesis and enhancement of functional responses. As an immediate effect, G-CSF causes an actual decrease in PMN count, which is followed by a sustained dose-dependent rise in PMN counts.

GM-CSF is a recombinant non-glycosylated protein expressed in *E. coli* (molgramostim) and glycosylated protein expressed in *Saccharomyces cerevisiae* (sargramostim) or in mammalian cells (regramostim). GM-CSF transiently decreases leukocyte counts immediately after administration and causes sustained rises in PMN, eosinophil and MNC counts afterwards.

GM-CSF has been described in some cases as inducing pleuritic pain, pulmonary edema, and a capillary leak syndrome. Although bone pain is described in 20%

of patients receiving G-CSF, the other adverse effects associated with GM-CSF are not commonly observed in G-CSF-treated patients. The toxicity of GM-CSF appears to be related to the non-glycosylated preparations expressed in *E. coli*. By comparison, the glycosylated form of GM-CSF is not associated with these adverse effects.

The toxicity profile of recombinant GM-CSF is consistent with priming of macrophages for increased formation and release of inflammatory cytokines, whereas G-CSF induces production of anti-inflammatory factors, such as IL-1 receptor antagonist and soluble TNF receptors, and is protective against endotoxin- and sepsis- induced organ injury. Owing to its increased toxicity, TNF-α has not been studied in clinical trials, despite its highly promising immunomodulatory nature.

Although administration of G-CSF to patients with acute myeloid leukemia (AML) carries the theoretical risk of accelerating the leukemic blast cells, this has not been observed. Indeed, G-CSF has been used safely in patients with AML and myelodysplasia.[82] The adverse effect of GM-CSF accelerating HIV replication in MNCs may be offset by simultaneous administration of antiretroviral agents.

The most common adverse experiences occurring with parenteral IFN-γ therapy are 'flu-like' or constitutional symptoms such as fever, headache, chills, myalgia or fatigue, which may decrease in severity as treatment continues.

Clinical studies of cytokine use in prevention or adjunctive therapy in combination with antifungal agents are limited and do not allow specific recommendations for their cost-effective use. The issue of cost-effectiveness of G-CSF with regard to invasive fungal infections was addressed in a recently published study. Neutropenic patients with presumed invasive fungal infection were randomized to receive amphotericin B either alone or in combination with G-CSF (3–5 μg/kg daily). There were 62% responders to combination versus 33% to amphotericin B alone (P=0.027). Of note, all non-responders received liposomal amphotericin B. Based on drug acquisition, hospital stay and treatment duration, the combination regimen was more cost effective.[83] There is an urgent need for well-structured, randomized clinical trials to determine optimal dose, duration and timing for different combinations of immunotherapy and antifungal agents in high risk patients.

FUTURE DIRECTIONS

The increasing incidence of invasive fungal infections and the emergence of previously rare opportunistic fungal pathogens is of major importance in the management of immunocompromised patients. Host defense normalization and antifungal therapy form the cornerstone of successful treatment. The advances in understanding the pathogenesis of fungal infections and cytokine involvement combined with the availability of a number of cytokines during the last decade have opened new avenues of potential applications. In tandem with destroying fungi using potent antifungal agents, reconstitution and upregulation of immune response by either exogenous administration of cytokines or transfusion of cytokine-elicited allogeneic phagocytes appear to be promising adjuncts to antifungal chemotherapy for these difficult-to-treat and often fatal diseases. Further evaluation of the safety and efficacy of these immunotherapeutic modalities is an urgent priority for research during the start of the new decade. It might be that one has to distinguish between patients with no PMNs or with dysfunctional PMNs when deciding which kind of cytokines to use.[84] Well-controlled studies in patients at very high risk of developing fungal infections, such as profoundly neutropenic or BMT patients should be the scope of future studies.

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