The Potential Role of Cytokine Therapy for Fungal Infections in Patients with Cancer: Is Recovery from Neutropenia all that is Needed?

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Optimal regimens for the treatment of invasive fungal infections have yet to be defined, and these life-threatening conditions are one of the leading causes of treatment failure in patients with cancer. A substantial body of preclinical work points in the direction of using cytokines as immunomodulators of the multiple deficiencies involved in the progression of fungal infections in neutropenic and nonneutropenic cancer patients. These deficiencies include not only the easily recognized deficiencies in cell quantity but also subtle deficiencies of cell function. Four cytokines (granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, macrophage colony-stimulating factor, and interferon γ) show promise as adjuvant therapy for proven fungal infections in this setting, although clinical experience is still limited. As an additional approach, the concept of white blood cell transfusions has been revived by the use of granulocyte colony-stimulating factor and promises to be helpful in the setting of neutropenia.

While noteworthy progress has been achieved in the treatment of patients with cancer over the past decade, especially in terms of achieving substantial control over bacterial infections by use of potent antibiotics [1–3], infection still remains a frequent cause of treatment failure. In particular, fungal infections have become one of the leading factors contributing to morbidity and mortality in immunosuppressed patients [4–7]. *Candida* and *Aspergillus* species have been consistently noted as the most important fungal pathogens. A large autopsy series of patients with cancer found that 58% of fungal infections were caused by *Candida* species and 30% by *Aspergillus* species [5].

A similar study of bone marrow transplantation (BMT) patients showed that the incidence of invasive aspergillosis among them increased from 5.7% to 11.2% (1987 to 1993) [7], while in a smaller study of patients with hematologic malignancies it increased from 7% to 21% [6]. The mortality associated with invasive aspergillosis in BMT patients, even after engraftment, was close to 90% despite maximal antifungal therapy [8]. Such findings are not encouraging and reflect the severe loss of host defenses in these patients, as well as the relatively poor performance of currently available antifungal agents in such situations. Novel therapeutic strategies are urgently needed.

The most important element of the host response to fungal infection varies in relation to the fungi. For such endemic mycoses as *Cryptococcus neoformans*, *Paracoccidioides brasiliensis*, *Coccidioides immitis*, and *Histoplasma capsulatum* infections, cell-mediated immunity appears to be critical [9–13]. Recent data also suggest at least a partial role for humoral immunity in defense against *coccidioidomycosis* [14] and candidiasis [15–17]. Conversely, neutropenia has long been recognized as the most important risk factor for invasive candidiasis, aspergillosis, and other mold infections such as fusariosis [18–20].

Normal phagocytic cells can ingest and kill *Aspergillus* as well as *Candida* organisms [21–24], but these functions are decreased in patients with acute leukemia because of a decrease in the function of circulating phagocytes [25–27] as well as in the number of cells seen after initiation of chemotherapy. It has recently become clear, however, that neutropenia is not the sole relevant factor; these invasive mycoses have increasingly been noted in patients who have normal polymorphonuclear leukocyte (PMNL) counts. This group includes patients with lymphoma, transplant recipients, those undergoing certain cytotoxic chemotherapy regimens, patients with AIDS, and neonates [28–35].

Secondary risk factors in these nonneutropenic patients involve several different defects. First, lymphoid and hematopoietic malignancies such as Hodgkin’s disease and hairy cell leukemia may be associated with defective cell-mediated immunity related to deficient functioning of monocytes, T cells, and natural killer (NK) cells [36–38]. Second, the use of chemotherapy and radiation therapy directly depletes the immune system. Chemotherapy not only induces aplasia but also impairs cell-mediated immunity and leads to an increased risk of infection with pathogens dependent on cell-mediated immunity [39].

Patients who undergo BMT have the most complex immune deficiency and can be shown to have defective neutrophil che-
motaxis [40–42], altered opsonization [43], lymphopenia [44], defective response of T cells to antigens [45], altered synthesis of IFN-γ [46, 47], and decreased phagocytosis by pulmonary alveolar macrophages. In this population, these multiple defects combine to increase the risk for aspergillosis, and this risk appears to persist, even after successful engraftment. A recent study conducted at M.D. Anderson Cancer Center (Houston) of patients undergoing CD3⁺ T cell–depleted matched unrelated BMT found that 52% of deaths (11 of 21) were caused by refractory fungal infections, particularly aspergillosis, and that most of these infections occurred in patients who had recovered from myelosuppression [8].

An additional secondary risk factor concerns the corticosteroids used to treat malignancies, graft-vs-host disease, and other conditions that induce macrophage dysfunction and increase the risk of invasive fungal infections [48]. Finally, immune activation and acquired immunity in response to fungal antigens may be relevant. Deficiencies in either the number or the function of the lymphocytes that produce proinflammatory cytokines might also be relevant in a variety of disease states [49–51]. As exogenous cytokines have the potential for correcting many of these defects, there has been substantial interest in their use as adjuvant therapy for invasive fungal infections in cancer patients.

The Potential Therapeutic Role of Cytokines

The innate host defense against fungal diseases is based on the action of phagocytic cells (PMNLs and macrophages) [51]; both the number and the function of these cells can be regulated by the colony-stimulating factors (CSFs). On the other hand, acquired defense involves cellular and humoral immunity that requires interactions between antigen-presenting cells, T lymphocytes, B lymphocytes, and NK cells that are driven and regulated by cytokines such as IL-2 and IFN-γ [51, 52].

The potential importance of immune activation via cytokines in the host defense against opportunistic fungi has been the subject of several studies [51, 52] and has raised some intriguing questions about novel antifungal strategies for candida and aspergillus infections. Different potential roles for cytokines have been described. First, exposure to fungi and their antigens may induce release of IL-2, IFN-γ, tumor necrosis factor-α (TNF-α), granulocyte colony-stimulating factor (G-CSF), and granulocyte macrophage colony-stimulating factor (GM-CSF) [12, 52–57]. These cytokines may in turn activate or enhance the antifungal function of phagocytes against Candida [58–61] and Aspergillus species [62–64].

In addition, exogenous administration of at least some of these agents is now possible, thus permitting therapeutic bypass of the need for direct host response. As cytokines exert their effect indirectly, via activation of a leukocyte rather than by any direct action on the fungus, they are most effective if there are adequate numbers of circulating leukocytes. Cytokines can be used to ensure adequate numbers of leukocytes by speeding recovery of the patient’s bone marrow function [65, 66] as well as by permitting novel approaches to transfusion therapy. The potential relevance of these concepts for invasive candida and aspergillus infections has only recently begun to be studied [67–70], and a brief review of the relevant cytokines is in order.

IFN-γ

IFN-γ, a polypeptide secreted by T lymphocytes, NK cells, alveolar macrophages, and fibroblasts, is the main cytokine produced during a Th1-type response. IFN-γ is approved for administration to humans and has been shown to enhance the oxidative metabolism and antifungal activity of human macrophages and PMNLs in vitro against a broad range of relevant fungal pathogens: Candida species, Aspergillus fumigatus, C. neoformans, P. brasiliensis, and Blastomyces dermatitidis [23, 59, 63, 71–73]. IFN-γ prevents the detrimental effect of corticosteroids on PMNL activity against A. fumigatus [74]. In addition, IFN-γ activates antigen-presenting cells, stimulates proliferation of T and B lymphocytes, and enhances cytoxicity by T lymphocytes and NK cells [75]; it also appears to be of benefit as an in vivo adjunct when given either prophylactically or after establishment of experimental infections with A. fumigatus [76] and C. neoformans [77].

IFN-γ was also shown to at least partially correct the oxidative burst in a group of patients with chronic granulomatous disease [78, 79], although this in vitro effect was not seen consistently in a subsequent placebo-controlled clinical trial [80] (see below). IFN-γ is generally well tolerated when given to humans, although a transient, dose-dependent flu-like syndrome with fever, chills, headache, fatigue, myalgias, arthralgias, and erythema at the site of injection is frequently seen [80, 81].

The CSFs

Three CSFs relevant to phagocytes are licensed for administration to humans: G-CSF, GM-CSF, and macrophage colony-stimulating factor (M-CSF). CSFs are responsible not only for the replication of bone marrow stem cells but also for the differentiation of granulocytes and monocytes into their mature forms [82]. Cellular sources of these powerful cytokines include monocytes, T lymphocytes, fibroblasts, and endothelial cells [83]. CSFs enhance chemotaxis, phagocytosis, and killing activity of precursor and mature effector cells [84].

These three CSFs have been shown in multiple studies to enhance the activity of phagocytic cells against Candida species, A. fumigatus, C. neoformans, and H. capsulatum [58, 59, 62–64, 74, 85–88]. In addition, G-CSF has been shown to protect PMNLs from the deleterious effects of irradiation and irradiation [89, 90]. These effects have taken on special importance in recent studies reassessing the utility of WBC transfusion [91].
Despite the many similarities among these cytokines, each also has distinctive features. GM-CSF almost doubles the life span of neutrophils, exerts a stimulatory effect upon them, and enhances their attachment to endothelial cells [92] and epithelial cell membranes [93]. GM-CSF also prolongs the life span and enhances the antibody-dependent cytotoxicity of eosinophils [94]. In addition, GM-CSF promotes the differentiation and proliferation of the cells of the macrophage-monocyte system [95, 96]. This activity is lacking in G-CSF; thus, GM-CSF would have a theoretical advantage in infections where monocyte-macrophage function is critical. Although formal proof is lacking, G-CSF appears to be more potent than GM-CSF in increasing the neutrophil production rate [97, 98]. M-CSF, as expected, stimulates survival, proliferation, differentiation, and activation of progenitor and mature cells of the monocyte/macrophage, and the antibody-dependent cell-mediated activity of eosinophils [101]. Two preparations of GM-CSF are commercially available: a glycosylated yeast-derived form and, in Europe, a nonglycosylated Escherichia coli–derived form. The first dose of the nonglycosylated form has been associated with transient dyspnea, oxygen desaturation, flushing, tachycardia, hypotension, myalgias, nausea, and vomiting [102]. These phenomena are seen less often with the glycosylated form [101, 103–105]. Myalgias and bone pain are the principal side effects of G-CSF [106], while the main toxic effects associated with use of M-CSF have been bone pain and thrombocytopenia [99].

Other Cytokines

While not approved by the U.S. Food and Drug Administration and perhaps too toxic for routine use, TNF-α is a proinflammatory cytokine produced by PMNLs, macrophages, monocytes, keratinocytes, T and B lymphocytes, NK cells, astrocytes, and endothelial cells [107, 108]. It has been shown to have diverse properties, including activation of PMNLs, enhancement of phagocytosis and microbicidal activity, activation of eosinophils, and stimulation of fibroblast growth [61, 62, 109, 110]. The in vitro activity of TNF-α to augment the fungicidal capacity of macrophages and PMNLs against _Candida_ species, _C. immitis_, and _C. neoformans_ has been demonstrated in a variety of experiments [111, 112]. Release of TNF-α by macrophages and peripheral blood mononuclear cells in response to _Candida_ and _Aspergillus_ species organisms has also been demonstrated [113–115].

Another candidate cytokine, IL-2, is a powerful proinflammatory cytokine produced by T cells [116]. Interleukin-2 promotes proliferation of lymphocytes and enhances the function of cytotoxic T lymphocytes and NK cells. IL-2–activated lymphocytes bind _C. neoformans_ and _Candida albicans_ [117, 118]. IL-2 has also been shown to increase PMNL antifungal activity against _Candida_ [119]. Lymphocyte and NK cell production of IL-2 in response to _Aspergillus_ has been shown [55, 120].

IL-12 is a proinflammatory cytokine produced by phagocytic cells, by dendritic cells, and at lower levels by keratinocytes and nonmucosal mast cells. IL-12 induces T and NK cell proliferation and production of Th1-type cytokines (IFN-γ, GM-CSF, TNF, and IL-2) [121]. Several animal models have shown that the neutralization of IL-12 increases the severity of infections produced by _H. capsulatum_ [122], _C. immitis_ [123], and _C. albicans_ [124]. On the other hand, exogenous IL-12 has been shown to be beneficial in mice with cryptococcosis [125], coccidioidomycosis [123], and histoplasmosis [126] but not in cases of candidiasis [127] or aspergillosis [128].

The neutralization of the two major cytokines that characterize a Th2-response, IL-4 and IL-10, may be an additional approach for adjuvant treatment against fungal infections. Both cytokines have been proven to decrease the antifungal activity of macrophages against _C. albicans_ [129] and of IL-10 against _Aspergillus_ species [130]. In animal models the neutralization of these cytokines improved survival of mice infected with _C. albicans_ [127, 131, 132]. A similar model with IL-4 showed the same pattern in invasive aspergillosis [128].

Clinical Applications of Cytokines in Fungal Infections

Cytokines as Adjuvant Therapy

Despite this intriguing body of preclinical data, establishing the clinical utility of cytokines as therapy for fungal infections in cancer patients has been difficult. Three basic strategies have been pursued: use of cytokines at induction of neutropenia in high-risk patient groups, use of cytokines as part of the therapy for febrile neutropenic patients, and use of cytokines in defined fungal infections.

_Prophylaxis during neutropenia._ The most convincing information available comes from controlled trials of G-CSF and GM-CSF administered for amelioration of leukopenia after cytotoxic chemotherapy [65, 105, 133–136]. Both compounds have been shown to reduce the duration of severe neutropenia, which, in turn, has led to fewer hospital admissions for fever and neutropenia, less use of antimicrobial agents, and fewer documented infections [103]. Unfortunately, there were not enough fungal infections in the placebo-treated groups of any of these studies to permit even a limited estimate of the potential of the cytokines in the prevention and/or treatment of fungal infections.

_Empirical therapy for febrile neutropenic patients._ Patients with fever and neutropenia have been enrolled in 5 controlled trials—3 of GM-CSF, 1 of G-CSF, and 1 of both agents—that have been described in the literature [106, 137–140]. While these studies collectively demonstrated that cytokines tend to shorten the duration of severe neutropenia, the effect
on fever and clearance of infection was less obvious. In addition, the use of cytokines neither reduced the need for antimicrobial agents nor affected the outcome of infection. There were too few defined fungal infections to permit any conclusions to be drawn about the utility of CSFs for these infections.

**Therapy for defined fungal infections.** In a pilot trial by Bodey and colleagues, eight patients with documented refractory fungal infections were treated with glycosylated GM-CSF plus amphotericin B [66]. The conditions of 4 of 5 patients with candidiasis, 1 of 2 with aspergillosis, and the 1 with trichosporonosis improved with this therapy. GM-CSF was used at a relatively high dose in this study (400 µg/[m²·d]); thus, it is not surprising that the major side effect was the capillary leak syndrome. Given the limited nature of the study and the lack of a comparison group, no conclusions about efficacy can be drawn.

Similarly, in a 1993 study by Nemunaitis and colleagues, a series of patients with a variety of types of cancer were given M-CSF combined with an antifungal agent as therapy for an invasive fungal infection (30 patients had candida infections, 15 had aspergillus infections, and 1 had a mucor infection) [100]. Several different doses of M-CSF were studied, and all demonstrated a slight trend toward an increased WBC count. Patients who received higher doses of M-CSF developed thrombocytopenia, although this was not clinically significant. Greater survival was observed among patients with candida infections and a Karnofsky score of >20%, compared with the figures for historical controls. As with the study by Bodey and colleagues [66], unfortunately this nonrandomized study provided few data on the effect of the cytokine on the course of fungal infection.

Finally, several case reports have shown that G-CSF and GM-CSF appeared helpful as part of a combined regimen for fusariosis and trichosporonosis in selected cancer patients [141–143].

**Prevention of fungal and other infections.** Significant morbidity and mortality are associated with invasive fungal infections in patients who have apparently adequate neutrophil counts. Cytokines, particularly IFN-γ, GM-CSF, and G-CSF, increase the number and/or stimulate the function of phagocytic cells. It is tempting to speculate that the use of these cytokines as prophylactic agents in this setting might be valuable. More specifically, GM-CSF or G-CSF, alone or in combination with IFN-γ, could be used immediately after engraftment following allogeneic BMT or even autologous BMT in certain settings. These agents could thus be used to stimulate phagocytic cells and hence decrease the morbidity and mortality associated with aspergillosis, which has become the leading cause of death of these patients. A prospective randomized trial evaluating the role of GM-CSF after engraftment is currently in preparation.

As an additional approach, either IL-12 or IL-15 might be administered in combination with crude fungal antigen to induce a protective immune response such as that produced in this manner against *Leishmania major* [144] and *Toxoplasma gondii* [145].

**Use in other settings.** Because of the potential of CSFs to reverse leukopenia, the studies of cancer patients have focused mostly on use of CSFs. Outside of the cancer arena, the broad-based immune activation produced by IFN-γ has attracted substantial interest as well. The usefulness of IFN-γ prophylaxis in patients with chronic granulomatous disease was examined in one of the largest single studies of cytokines to date [80]. In this study, 367 patients were randomized to receive either placebo or IFN-γ at a dose of 50 µg/m² three times a week.

Clinically, systemic IFN-γ prophylaxis produced a 72% reduction in the risk for serious infections, compared with the risk with placebo. While this effect was primarily due to the reduction in the rate of bacterial infection, aspergillus pneumonia was somewhat less common in the patients receiving IFN-γ (4 of 65 placebo-treated patients vs. 1 of 63 IFN-γ-treated patients; OR, 4.1; 95% CI, 0.5–32). Although these numbers are too small to permit strong conclusions, a parallel blinded study that examined the ex vivo function of PMNLs in the same group of patients found that in the placebo-treated patients there was a decrease over time in the ability of PMNLs to damage *Aspergillus* hyphae, while in the IFN-γ-treated patients there was an increase in their function [146]. These results, along with several case reports describing a potential role for adjunctive IFN-γ therapy for refractory invasive fungal infection, suggest that this particular cytokine might play a useful role in some settings [147–150].

**Cytokine-Enhanced WBC Transfusions**

As purely cytokine-based therapies are dependent on the presence of WBCs, profoundly cytopenic patients would be expected to derive little benefit from these therapies. WBC transfusions have long represented a tantalizing solution to this problem. Unfortunately, this concept is beset by many difficulties [151, 152], not the least of which are the dual problems of (1) collecting adequate numbers of cells from the donor and (2) the limited persistence of the transfused cells.

Cytokines do, however, permit at least partial solutions to both of these problems. First, the problem of collecting an adequate number of donor cells has long been addressed by treating the donor with a corticosteroid [153, 154]. However, such therapy has the theoretical disadvantage of suppressing leukocyte function (see above), and this problem might be better solved by administering G-CSF or GM-CSF to the WBC donor. Second, PMNLs are triggered upon isolation to begin the process of programmed cell death, or apoptosis. As apoptotic PMNLs are rapidly removed from the circulation [155], this may account for some of the problems with cell persistence after isolation and transfusion. Here again, recent work has shown that multiple cytokines, including G-CSF and IFN-γ, can reduce apoptosis and preserve PMNL function [156, 157].
The clinical consequences of these ideas have been explored in a recent series of studies. First, a detailed set of in vitro experiments has shown that treatment of normal PMNLs with physiological doses of G-CSF or of IFN-γ plus G-CSF reduced apoptosis, preserved surface-receptor expression, and preserved the ability to kill \(C.\) albicans [89, 90]. As transfused blood products are often irradiated to prevent graft-vs.-host disease, these studies also examined PMNLs after irradiation and found that these cytokine regimens also ameliorated the consequences of exposure to the standard irradiation dose of 30 Gy. Regimens that included GM-CSF instead of G-CSF were not protective.

Of further interest, PMNLs collected from healthy individuals treated with G-CSF were found to behave in much the same fashion as the PMNLs treated in vitro with cytokines. While they could be further protected by additional in vitro cytokine treatment, even untreated PMNLs from these donors demonstrated reduced apoptosis and preserved function despite irradiation. The in vivo consequences of these ideas can be seen in recent studies reported by our group [158, 159] and others [160] in which G-CSF-treated donors were used as the source of cells for WBC transfusions.

In a recent study by Fleming and colleagues, 38 neutropenic patients with refractory invasive fungal infections, including infections due to Aspergillus, Candida, and Fusarium species, were treated with G-CSF-mobilized granulocytes from normal donors [159]. While the strength of the conclusions is limited by the lack of a comparator group, 20 of the patients did have a favorable response to therapy. It is not surprising that patients with better-established infections did not do as well as patients who began therapy soon after the infection was detected. Eight patients were free of infection 3 weeks post-therapy. Adverse reactions included shortness of breath and fever but were uncommon in most of the recipients.

Conclusion: Is Recovery from Neutropenia All That Is Needed?

Four cytokines (G-CSF, GM-CSF, M-CSF, and IFN-γ) show promise as adjuvant therapy for patients with proven fungal infections. Unfortunately, the available data do not clearly document their utility in any particular setting. One consistent theme does emerge, however: it is no longer adequate to think solely in terms of absolute numbers of effector cells. Rather, both number and function should be considered. This concept is illustrated both by situations in which a nonneutropenic patient develops an invasive fungal infection (e.g., is invasive aspergillosis after marrow transplant engraftment related to inadequate function?) and by the intriguing early data on G-CSF-elicted WBC transfusions. In the first situation, the patient has a sufficient quantity of leukocytes but is relatively neutropenic because of their lack of function. In the second situation, the patient appears to still be relatively neutropenic after the transfusion, but the high level of function of these cells is sufficient to produce a strong clinical response.

The next steps in evaluating the cytokines as therapy for fungal infections will require carefully designed comparative studies. As these agents are expensive and have the potential for inducing drug-related side effects as well as possibly worsening graft-vs.-host disease, their use on a broad scale should not proceed without substantial additional investigation. Such trials are going to be difficult, but the initial data are sufficiently promising that every effort to pursue such studies should be made.

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