Response to systemic antifungal therapy alone remains disproportionately less satisfactory in immunosuppressed transplant and oncology patients. As insight in fungal immunopathogenesis forges ahead, interventions for boosting immune functions along with antimicrobial drugs have shown promise in preclinical experiments. The clinical experience with immunotherapy for invasive mold disease is limited. Most studies have involved small numbers of patients at a single institution or data collected retrospectively. An overview of various facts of immune modulatory drug intervention is presented, including major considerations in antifungal immunotherapy in immunosuppressed patients. Patients in whom immunotherapy is being considered must be critically evaluated to identify the underlying immune defects, including treatment-induced immunosuppression. Antifungal immunotherapy is appealing; however, before routine clinical use is recommended, well-designed prospective comparative clinical trials are urgently needed.

**Keywords.** aspergillosis; interferon gamma; myeloid growth factors; antifungal drugs; fungal cytoskeleton.

**INTRODUCTION TO ANTIFUNGAL IMMUNOTHERAPY**

Response to systemic antifungal therapy alone remains disproportionately less satisfactory in immunosuppressed transplant and oncology patients compared with the general population. As the insight in fungal immunopathogenesis forges ahead, interventions for boosting immune functions along with antimicrobial drugs have shown promise in preclinical experiments. The clinical experience with immunotherapy for invasive fungal disease (IFD) is limited. Most studies have involved small numbers of patients at a single institution or data collected retrospectively. Therefore, all such therapies should be considered investigational or be given only in certain settings such as failure of standard antifungal drugs, relapsed or breakthrough IFD, or known predictors of poor outcomes.

Table 1 outlines major considerations in antifungal immunotherapy in immunosuppressed patients. Patients in whom immunotherapy is being considered must be critically evaluated to identify the underlying immune defects, including treatment-induced immunosuppression. If possible, the immune defects should be addressed, and immunosuppressive therapy discontinued. However, in most patients who have hematologic malignancies or have received allogeneic stem cell transplant (ASCT) or organ transplant, discontinuation of corticosteroids and other immunosuppressive drugs is not feasible.

**Myeloid Growth Factors**

The rationale for administering recombinant myeloid growth factors (MGFs) such as granulocyte-colony stimulating factor (G-CSF) and granulocyte macrophage colony-stimulating factor (GM-CSF) to neutropenic patients at risk of or with established IFD is to accelerate myeloid recovery [1–3]. In 2 multicenter studies, the decrease in the duration of neutropenia in patients with acute myelogenous leukemia who were given MGFs diminished the risk for IFD [4, 5]. In fact, the American Society of Clinical Oncology and
National Comprehensive Cancer Network have established guidelines for MGF use in neutropenic patients [6, 7]. However, there are limited clinical trial data to support prophylactic MGF use in nonneutropenic patients undergoing antineoplastic therapy at risk for IFD [8].

**Granulocyte Colony-Stimulating Factor**

As neutrophils are the most important line of defense during the early stages of IFD, G-CSF, which induces granulopoiesis, has been used to treat IFD in immunosuppressed, neutropenic patients. In vitro, G-CSF treatment of neutrophils amplifies oxidative burst by 75% and damage to *Aspergillus* hyphae by 37% compared to no treatment with G-CSF [9]. In one animal study, human G-CSF provided significant protection against aspergillosis in cyclophosphamide-treated neutropenic mice [10]. Another animal study showed that pretreatment of neutrophils with G-CSF prevented corticosteroid-induced suppression of hyphal damage [11]. Furthermore, in experimental models of hematogenous aspergillosis, survival was improved 36% in cyclophosphamide-treated animals given G-CSF in addition to amphotericin B compared to those treated with amphotericin B alone [12]. A similar benefit was noted among 5-fluorouracil-treated mice with pulmonary aspergillosis who were given G-CSF (filgrastim) in addition to an anti-*Aspergillus* triazole; however, a paradoxical increase in fungal burden and large lung abscesses were noted in mice who were pretreated with corticosteroids and then given a similar G-CSF plus antifungal combination [13]. This severe antagonistic effect was not reproduced by others using a G-CSF and posaconazole combination [14]. Nevertheless, the contradictory findings highlight the complexity of patients’ immune/inflammatory dysfunction, and a thoughtful evaluation to establish the presence of an underlying immune defect is needed prior to administration of immunotherapy such as MGFs (Table 2).

The limited clinical data available favor the use of recombinant G-CSF (filgrastim) in neutropenic patients with invasive aspergillosis [15]. In a prospective clinical trial, the addition of G-CSF to amphotericin B was cost-worthwhile compared with amphotericin B therapy alone in the treatment of neutropenic patients with presumed IFD [16]. However, before routine use of G-CSF is recommended in neutropenic patients with IFD, multicenter randomized studies are needed. Administering neutrophils primed with G-CSF during nonneutropenic states that increase the risk for IFD (eg, prolonged therapy with high-dose corticosteroids or graft-vs-host disease [GVHD]) may also prove to be a useful strategy to supplement patients’ antifungal immune defenses; however, further studies are needed.

**Table 1. Principles and Considerations for Immunotherapy in Immunocompromised Patients With Invasive Fungal Disease**

<table>
<thead>
<tr>
<th>Determine patient’s underlying immune defect(s), especially in nonneutropenic cancer patient or transplant recipient</th>
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<tbody>
<tr>
<td>Validate use of immunotherapy and describe validation in medical record</td>
</tr>
<tr>
<td>• Outline justification for proposed intervention(s)</td>
</tr>
<tr>
<td>• Outline immunological basis for intervention(s)</td>
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<tr>
<td>Reserve antifungal immunotherapy for salvage therapy</td>
</tr>
<tr>
<td>• Progressive disease following failure of mold-active therapy alone</td>
</tr>
<tr>
<td>• Disseminated fungal infection in patient with &gt;2 predictors of poor outcome*</td>
</tr>
<tr>
<td>• Patient with refractory profound neutropenia (&lt;500 cells/μL)</td>
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<tr>
<td>Discuss potential adverse events and life-threatening complications of immunotherapy with patient and family, and document discussion in medical record</td>
</tr>
<tr>
<td>Closely monitor patient for efficacy of therapy and toxicity</td>
</tr>
</tbody>
</table>

* Predictors of poor outcome in patients with invasive fungal disease include (a) refractory neutropenia, (b) recent or ongoing high systemic corticosteroid use, (c) severe graft-vs-host disease, (d) critical unit stay, (e) mechanical ventilation, (f) need for renal replacement therapy, (g) relapse of acute leukemia, and (h) discordant initial antifungal therapy.
### Table 2. Recombinant Cytokines Used in Patients With Tissue-Invasive Mold Disease

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Rationale for Clinical Use</th>
<th>Clinical Experience</th>
<th>Concerns and Limitations</th>
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<tbody>
<tr>
<td>G-CSF</td>
<td>Leads to proliferation of myeloid precursor cells resulting in accelerated mobilization of newly produced granulocytes from bone marrow to the peripheral blood [56].</td>
<td>Promotes recovery from chemotherapy-induced and primary immune deficiency–associated neutropenia. Relatively ineffective against cancer-related neutropenia.</td>
<td>Leukemia stimulation has been an unsubstantiated hypothetical concern [1].</td>
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<td>Promotes activation of the PMNs indicated by CD11b upregulation [57] and prolongs PMN survival [58].</td>
<td>Some patients with severe chronic neutropenia and most patients with cyclic, congenital, or idiopathic neutropenia experience favorable response to recombinant G-CSF, which is frequently given for prolonged periods.</td>
<td>Prolonged G-CSF therapy has hypothetical risk for de novo myelodysplastic syndromes or transformation to acute myeloid leukemia [59]. Recommended monitoring includes routine peripheral blood analysis with manual differential count and annual bone marrow examination.</td>
</tr>
<tr>
<td>Enhances ex vivo neutrophil oxidative-mediated damage to Aspergillus hyphae [9] and reverses steroid-induced suppression of neutrophil function [16].</td>
<td>Leukocyte integrin activation may occasionally result in transient neutropenia following G-CSF administration [60].</td>
<td>G-CSF mobilized neutrophils in peripheral blood are skewed population of PMNs that are initially less mature and less functional [56].</td>
<td></td>
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<td>GM-CSF</td>
<td>Stimulates proliferation and differentiation of hematopoietic progenitor cells. Increases the production of granulocytes, macrophages, Langerhans cells, dendritic cells, megakaryocytes, and eosinophils. GM-CSF also acts synergistically with erythropoietin to induce proliferation of erythrocyte precursors.</td>
<td>Most clinical experience has emerged in cancer patients with chemotherapy-induced neutropenia. GM-CSF prophylaxis has been associated with significant reduction in severe life-threatening opportunistic infections and invasive fungal disease [1].</td>
<td>Leukemia stimulation has been a hypothetical concern [1].</td>
</tr>
<tr>
<td>Enhances antimicrobial function of mature neutrophils and monocytes against fungal targets, and in experiments shown to reverse steroid-induced cellular suppression [19, 20, 28].</td>
<td>Limited clinical experience with GM-CSF use in patients with established fungal disease has been encouraging [23, 25–27].</td>
<td>Prolonged GM-CSF therapy has hypothetical risk for de novo myelodysplastic syndromes or transformation to acute myeloid leukemia [58]. Recommended monitoring includes routine peripheral blood analysis with manual differential count and annual bone marrow examination.</td>
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<tr>
<td>Promotes neutrophils’ phagocytic ability to clear apoptotic pathogen-loaded PMNs at the site of acute infection [61]. This is important for containment of tissue-damaging inflammation.</td>
<td>GM-CSF use in nonneutropenic patients with invasive fungal disease needs further investigation.</td>
<td>Capillary leak syndrome is a rare and serious adverse event. It requires a high level of awareness and robust supportive care [24].</td>
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<td>IFN-γ</td>
<td>Alters steady-state hematopoiesis. Enhances expression of monopoiesis-inducing transcription factors in myeloid progenitor cells and suppresses G-CSF–driven neutrophil differentiation [62]. The cytokine-induced differentiation of monocytes over neutrophils is critical in containment and clearance of tissue-invasive fungal disease.</td>
<td>Limited clinical experience in neutropenic, nonneutropenic, and steroid-treated cancer patients with invasive fungal disease is encouraging [36, 37].</td>
<td>Potential to induce exacerbation of tissue inflammation, ischemia, and necrosis has been a hypothetical concern but seldom noted in clinical practice [37].</td>
</tr>
<tr>
<td>IFN-γ released by natural killer cells and Th1-primed lymphocytes directly damages Aspergillus hyphae [63]. Addition of this proinflammatory cytokine improves fungal clearance in ex vivo and animal experiments [19, 20, 28, 65].</td>
<td>IFN-γ has been successfully used in allogeneic transplant recipients with difficult-to-treat invasive mold disease [66]. Further studies are warranted.</td>
<td>Potential to induce exacerbation of graft-vs-host disease or graft rejection in ASCT recipients has been a hypothetical concern. Most evidence, however, shows safety of recombinant IFN-γ in transplant recipients with invasive fungal disease [36, 65]. Furthermore, IFN-γ has been demonstrated to promote graft-vs-leukemia effect in ASCT recipients [66].</td>
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Abbreviations: ASCT, allogeneic stem cell transplant; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte macrophage colony-stimulating factor; IFN, interferon; PMN, polymorphonuclear leukocyte; TLR, Toll-like receptor.
For nonneutropenic patients with refractory sinopulmonary or disseminated aspergillosis, rhinocerebral zygomycosis, and other IFDs, it is reasonable to consider adjuvant therapy with GM-CSF to augment macrophage and neutrophil functions [27]. Recently, we reported high-dose corticosteroids prior to GM-CSF (odds ratio [OR], 24; \( P \leq 0.009 \)), GM-CSF started in the intensive care unit (OR, 10; \( P \leq 0.1 \)), or concurrent granulocyte transfusions (OR, 5; \( P \leq 0.02 \)) had significantly higher risk of antifungal treatment failure despite adjuvant GM-CSF use [28]. As with G-CSF, a thoughtful evaluation to establish the presence of an underlying immune defect is needed prior to administration of immunotherapy such as GM-CSF. GM-CSF should generally be reserved for when standard therapy has failed (Table 2).

### Th1 Recombinant Cytokines

#### Interferon-γ

In vitro and in animal studies, exogenous interferon gamma (IFN-γ) improves neutrophil and macrophage handling of *Aspergillus* microconidia and hyphae, alone and in combination with G-CSF [11] or GM-CSF [19, 20]. Improved neutrophil activity against other common fungal pathogens has also been noted following ex vivo exposure to IFN-γ and GM-CSF [29]. Improvements to the scavenger response against filamentous fungi induced by exogenous IFN-γ include (1) augmented neutrophil oxidative killing of *Aspergillus fumigatus* hyphae, (2) prevention of corticosteroid-mediated suppression of neutrophil killing of fungal hyphae, and (3) enhanced killing of *A. fumigatus* hyphae by human monocytes [11, 19, 20, 29, 30]. The favorable data from studies using cytokine depletion, gene knockout mice, and administration of exogenous cytokines have been instrumental in establishing the conceptual basis for IFN-γ immunotherapy for invasive mycoses and in paving the way for early clinical trials [31–33].

Recombinant IFN-γ is approved by the US Food and Drug Administration as a prophylactic agent in patients with chronic granulomatous disease on the basis of on a randomized trial in which IFN-γ reduced the number and severity of bacterial and fungal infections by 70%, regardless of antibiotic prophylaxis or genetic subtype of chronic granulomatous disease [34]. In cancer and transplant patients, adjunct immunotherapy with IFN-γ is appealing for the treatment of established IFD. However, recombinant IFN-γ 1b (Actimmune) for IFD treatment in immunocompromised cancer and ASCT patients has been approached with caution, owing to concerns about serious toxicity (including exacerbation of GVHD or graft rejection), the high cost of therapy, and uncertain benefit. Clinical data on adjuvant IFN-γ therapy in patients with IFD remain sparse. In a single-center prospective trial, refractory fungal infections in 4 neutropenic patients with leukemia responded to the addition of IFN-γ [35]. IFN-γ has also been used successfully in a limited number of nonneutropenic patients with treatment-refractory aspergillosis or fusariosis [36].

One concern about IFN-γ in ASCT recipients is whether it could worsen GVHD. My colleagues and I evaluated IFN-γ in 25 ASCT recipients with proven or suspected IFD [37] and found that IFN-γ was tolerated, with no serious toxicity, and did not result in marrow suppression or worsening of GVHD. More than half of the patients with definite pulmonary aspergillosis or disseminated aspergillosis had a response. Interestingly, in some patients, acute and chronic GVHD improved following IFN-γ therapy, making possible a reduction in immunosuppressive therapy [37] (Table 2).

Nevertheless, IFN-γ should be reserved for patients with life-threatening IFD refractory to standard antifungal therapy. Pairing recombinant IFN-γ with G-CSF or GM-CSF is also reasonable in the setting of refractory fungal disease, although it should be emphasized that clinical experience with the combination is anecdotal. The efficacy of either approach is not yet established.

### Immune Modulation With Antifungal Drugs

In patients with treatment-refractory IFD following traditional antifungal drugs, exploiting immune modifying response of drug combination to suit the underlying immune defects is a provocative approach and is also being investigated. Recently, a number of investigators have presented provoking data regarding the auxiliary effect of conventional antifungal drugs on patients’ immune responses and on the susceptibility of fungi to those responses [38–48]. However, the significance of the immunomodulatory effects of antifungal drugs on clinical outcomes remains unknown. Differential Toll-like receptor (TLR) signaling may follow exposure to certain antifungal drugs; this drug-induced differential TLR pathways may result in a pronounced inflammatory response associated with tissue injury or may promote enhanced fungal microconidia and hyphal killing and fungal clearance. The antifungal agents amphotericin B deoxycholate and liposomal amphotericin B have distinct effects on TLR signaling and the antifungal activity of neutrophils in mice. Liposomal amphotericin B diverts TLR2 signaling to TLR4 signaling; this switch activates the oxidative killing mechanisms of phagocytes and reduces the release of proinflammatory cytokines that occurs following exposure to amphotericin B deoxycholate alone [38]. In one study, liposomal amphotericin B enhanced phagocyte-induced hyphal damage of *A. fumigatus* and *Fusarium solani* [39]. In another study, pretreatment with the empty liposomes used in the liposomal amphotericin B preparation attenuated the inflammatory tissue damage of invasive pulmonary aspergillosis in corticosteroid-treated nonneutropenic mice [40].

The azoles also appear to also have immunomodulatory effects. For example, voriconazole has been shown to have such
Voriconazole led to upregulation of TLR2, increased tumor necrosis factor–α expression, and heightened NF-κB translocation to the nuclei; all these ancillary effects strengthen antifungal immune clearance [41].

Drugs such as echinocandins that alter fungal cell wall constituents and expose β-glucan to pattern recognition receptors modulate immune activation of monocytes and macrophages [43]. The echinocandins cause structural changes in the fungal cell wall that lead to cell wall rupture. After exposure to an echinocandin, the fungal cell wall is remodeled, and the wall’s immunogenic β-glucan constituent is unmasked [44], activating macrophages via dectin-1 [45, 46]. Another potential mechanism of echinocandin-mediated modulation of the fungus–host interaction is compensatory cell wall synthesis pathways that are likely to be upregulated following drug-mediated depletion of β-glucan in the fungal cell wall. Furthermore, fungal cytoskeletal remodeling pathways share the regulatory cytoplasmic subunit (Rho-1p) of β-glucan synthase complex, the echinocandin target that also controls cytoskeletal stability in response to various exogenous and endogenous stressful stimuli. The potential influence echinocandins may have on Rho-1 can in turn modify fungal cell wall remodeling that takes place due to drug-induced cell wall injury; this yet-unverified ancillary drug effect may further contribute to configurational changes in cell wall epitopes, perhaps delaying recovery following exposure to cell wall–active drugs and rendering fungi amenable to phagocytosis and death [47]; further studies are needed.

Recently, it was interesting to note that animals treated with amphotericin B for pulmonary aspergillosis showed improved handling of invasive fungal disease when recombinant fibroblast growth factor was given concomitantly, whereas a lesser ability in reversing ed immature neovascularization in the infected lung and its growth factor [48]. Vascular endothelial growth factor promotion for better control of opportunistic fungal lung disease. Pathways may emerge as yet another immune modifying intervention for better control of opportunistic fungal lung disease.

**Dendritic Cell Immunotherapy**

*Aspergillus fumigatus* has the ability to activate, suppress, or subvert the host immune response through protein secretion and cell wall structure changes that occur during its life cycle [49]. Different fungal components have the capacity to activate distinct immune responses; for example, secreted proteins induce mostly a nonprotective Th2 response, membrane proteins elicit protective Th1/regulatory T cells, glycolipids yield Th17, and fungal polysaccharides induce interleukin 10 [49, 50]. Strategies that elicit protective antifungal immunity via immunostimulatory fungal antigen(s) have been explored by a number of investigators.

Antigen-exposed dendritic cells (DCs) provide an intriguing antifungal vaccine delivery platform [51–55]. Most investigative work has focused on pathogen-specific vaccines. Cross-immunogenic proteins combined with an appropriate fungal polysaccharide moiety have the potential for stimulating pan-fungal immunity but are in the exploration phase [52].

Preliminary pathogen-specific and/or cross-immunogenic DC vaccine experiments are encouraging [53]. Human and murine DCs pulsed with live fungi or transfected with fungal nucleic acid resulted in functionally mature DCs that had evivo capability to produce a robust antigen-specific, antifungal IFN-γ response [54]. In animals who received hematopoietic transplantation, DC vaccine generated ex vivo antigen-specific, IFN-γ–producing T lymphocytes resulting in transferable antifungal immunity; however, protection elicited by DC immunotherapy was superior in animals who received the vaccine compared with passive transfer of adaptive pathogen-specific T lymphocytes [54]. Antifungal DC vaccine research remains in the domain of preclinical investigation.

Gene therapy has enhanced the field of DC immunotherapy. For example, animals treated with interleukin 12 (IL-12)–engineered DCs loaded with fungal microconidia exhibited a robust anti-*Aspergillus* IFN-γ response [55]. In mice given 2 doses of heat-inactivated *A. fumigatus* (HAF)–pulsed, IL-12–transduced DCs, no *Aspergillus* could be detected in the lung, whereas in mice vaccinated with HAF-pulsed DCs or those given vector-transduced DCs, abundant pulmonary *Aspergillus* could be detected following a respiratory fungal challenge [55]. Furthermore, a higher survival rate was reported in mice immunized with HAF-pulsed, IL-12–transduced DCs compared with animals given HAF-pulsed DCs or vector-transduced DCs [55]. Further studies in higher mammals and primates are warranted before exploratory human trials.

**CONCLUSIONS**

The understanding for suitability and appropriate clinical application of various aforementioned immune modifying interventions continue to evolve. At present, combined cytokine immunotherapy with GM-CSF plus IFN-γ has been shown to improve outcomes in patients with progressive, antifungal drug-refractory IFDs. In patients with a higher probability for failure to antifungal therapy alone, combined recombinant cytokines may alter disease course, if considered prior to multigener failure and need for critical care unit stay. Furthermore, selection of antifungal drug combinations should also include consideration for their potential nonantimicrobial immune modifying ancillary effects. These considerations need to be based on hosts’ underlying condition(s) and disease(s), nature
of immune dysfunction (ie, prolonged, severe neutropenia) vs innate and/or adaptive immune defects resulting from high-dose corticosteroid therapy, antineoplastic or immune modifying biologics, transplantation, and/or graft-vs-host disease. Antifungal immunization and adaptive immunotherapy require further preclinical research and at present cannot be recommended even for salvage therapy.

Note

Potential conflicts of interest. Author certifies no potential conflicts.

The author has submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References