

Available chemotherapies are not entirely successful in treating fungal infections, particularly in the immunocompromised, but **Javier Capilla, Karl Clemons and David Stevens** believe that cytokines can provide a useful adjunct to conventional antifungal drugs.

Through the first half of the 20th century, fungal infections were thought of as exotic diseases. However, coinciding with the greater availability and aggressive use of antibiotics, immunosuppressive treatments, and AIDS, the frequency of severe fungal infections and the diversity of the causal agents has continued to increase until the present day. Most severe fungal infections are caused by saprophytic soil species. With the exception of infections

due to dimorphic fungi such as *Coccidioides immitis* and *Histoplasma capsulatum*, which are primary pathogens, or by commensal species such as *Candida* spp., severe life-threatening infections by fungi are quite rare in healthy patients. In spite of advances in treatment over the last 25 years, currently available antifungal chemotherapies are not optimal, especially for diseases in immunocompromised patients. In addition, fungal resistance to the antifungal therapy, the spectrum of causative agents and the toxicity of prolonged treatments are major difficulties in successful treatment. One desirable treatment strategy consists of immunomodulation to stimulate an adequate host response by the use of a cytokine, as an adjunct to conventional antifungal therapy.

In vitro studies

In the 1980s, substantial experimental data were published that suggested gamma interferon (IFN- γ) might have potential use in the treatment of non-viral infections by enhancing host defences.

Neutrophils, macrophages and dendritic cells are the first effector cells contacting fungal cells. Neutrophils are rapidly recruited to the site of infection and play an essential role in fungal killing. The presence of fungal cells and host effector cells initiates a cascade of events through both non-specific and specific mechanisms of host response. Lymphocytes T helper 1 (Th₁), a CD4+ subset, are the predominant response to infections by invasive fungi, and cytokines associated with the Th₁ phenotype, including interleukin (IL)-12, IL-8 and IFN- γ , are critical to protective responses to the infection. Conversely the Th₂-phenotype cytokines IL-4 and IL-10 contribute to the progression of the infection. Effector mechanisms of IFN- γ and its role in modulating the host response against fungi include stimulation of macrophage and neutrophil killing of fungi by enhancement of both oxidative and non-oxidative mechanisms.

We have shown IFN- γ activity on host cells directed against intracellular and extracellular fungi, against the dimorphic fungi of the endemic mycoses and against fungal opportunists, with murine and human effector cells, with cells of the monocyte-macrophage lineage and with neutrophils, *in vitro*, *ex vivo*, and *in vivo*. *In vitro* studies using macrophages stimulated with recombinant IFN- γ showed an increase up to 44 % of the killing activity against *Candida albicans* (a phagocytatable fungus) and 33 % against the non-phagocytatable fungus *Blastomyces dermatitidis*. In addition, antibodies directed against IFN- γ neutralized the enhanced fungicidal activity of the macrophages or neutrophils.

In vivo studies

Animal models of cryptococcosis, paracoccidioidomycosis, coccidioidomycosis, blastomycosis, histoplasmosis, candidosis and aspergillosis have shown the beneficial effects of IFN- γ therapy in terms of survival and reduction of fungal burdens in infected organs. However, sole IFN- γ therapy has failed to induce complete clearance from infected tissues. In immunocompetent mice the administration of recombinant murine IFN- γ alone or in combination with amphotericin B, an antifungal agent, significantly improved the host's capacity to restrict the proliferation of *Cryptococcus* in tissues. IFN- γ was found to be more dramatic in its therapeutic effects in SCID mice infected with *Cryptococcus*. SCID mice are severely immunodeficient, mimicking the immune status of patients with AIDS that have severe fungal diseases. We

Gamma interferon and fungal infections



◀ Scanning electron micrograph of *Candida* and epithelial cells in the human vagina. D. Phillips / Science Photo Library

Data on the effects of IFN- γ therapy tell us that its activity enhances antifungal activity by activation of cell-mediated immune responses and that combining IFN- γ with an antifungal agent can result in powerful synergy.



◀ Lesion on the back of a patient's hand caused by blastomycosis, a fungal infection caused by *Blastomyces dermatitidis*. It usually affects the lungs after inhalation of fungal spores, but may become disseminated to the skin, or, in extreme cases to the bones, liver, spleen or central nervous system. Scott Camazine / Science Photo Library

Although IFN- γ appears to be a potential broad-spectrum antifungal agent, continued studies are needed to understand how better to modulate the regulation of the necessary part of the immune response during the course of the infection. The accumulated data on the effects of IFN- γ therapy tell us that its activity enhances the antifungal activity by activation of cell-mediated immune responses and that combining IFN- γ with an antifungal agent can result in powerful synergy.

Clinical use of IFN- γ

A good deal is known about the safety and use of IFN- γ in humans. Patients with chronic granulomatous disease have an increased risk of developing pulmonary aspergillosis, and IFN- γ treatment is routinely used to increase their resistance to this infection, among others. Recombinant human IFN- γ -1b (rhIFN- γ -1b) has also been given safely to patients with invasive fungal infections, including those with severely impaired immune status. In a small clinical trial on cryptococcal meningitis, those given adjunctive IFN- γ cleared *Cryptococcus* from the CSF more rapidly than those not given IFN- γ . The adjunctive use of IFN- γ did not result in serious adverse effects. The measurable improvement of disease noted following rhIFN- γ -1b therapy indicates the need for large-scale trials. IFN- γ is currently regarded as salvage

therapy for patients with refractory invasive fungal infections that fail conventional antifungal treatment. The recommended dose of rhIFN- γ -1b for adult patients is 50 $\mu\text{g m}^{-2}$ of body surface area, given every other day until resolution of the infection is evident clinically. However, the study of adjunctive IFN- γ therapy should be considered early, especially for severely immunosuppressed patients.

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Further reading

Stevens, D.A. Brummer, E. & Clemons K.V. (2006). Interferon- γ as an antifungal. *J Infect Dis* 194 (Suppl. 1), S33–S37.

demonstrated that intravenous administration of IFN- γ had an even greater beneficial effect than had been shown with the results obtained from immunocompetent mice. One possible explanation for this difference is related to the lower naturally occurring levels of IFN- γ in SCID mice due to T cell defects, and the phagocytic cells of those animals probably respond to a greater degree to exogenous administration of IFN- γ . These severely immunocompromised animals may also lack a variety of other defence mechanisms that might otherwise come into play in response to fungal infection. When IFN- γ was administered to *Cryptococcus*-infected SCID mice in combination with amphotericin B, the rate of cure achieved was significantly higher than using monotherapy, especially in central nervous system infection, demonstrating the synergistic effect of IFN- γ as an adjunct in severe impaired hosts. Similarly, IFN- γ in combination with conventional antifungal therapy was shown to be of benefit in models of systemic histoplasmosis.

However, others have reported IFN- γ to be of benefit or deleterious in murine models of candidosis. In a murine model of orogastrintestinal candidosis, a localized fungal infection, systemic administration of IFN- γ showed low or no beneficial effects – administration of IFN- γ was ineffective and its combination with suboptimal doses of fluconazole

showed no synergism. Similar results have been reported in experimental pulmonary aspergillosis using intraperitoneal administration of IFN- γ , which had no benefit in prolonging survival of mice. Possible caveats to these findings are the importance of the route of administration of IFN- γ (e.g. intravenous IFN- γ , but not subcutaneous administration was beneficial in treating cryptococcal infection), frequency of dosing (e.g. daily dosing was deleterious, but dosing every other day was beneficial in murine cryptococcal disease) and the dosage used (i.e. too high a dose may be deleterious and too low a dose ineffective). Thus, thorough studies must be done before determining the utility of IFN- γ as an adjunctive therapy.

Turning to the importance of the compartmentalized response to IFN- γ , we explored the possibility of using gene therapy for delivering IFN- γ into the central nervous system to combat fungal meningitis by using an adenovirus vector carrying the murine IFN- γ gene under a cytomegalovirus promoter. Intracranial inoculation of the vector resulted in production of high concentrations of IFN- γ ($>30,000 \text{ pg ml}^{-1}$) in the cerebrospinal fluid even 5 days after administration. Shao's group used a recombinant adenovirus vector containing murine IFN- γ cDNA (AdnIFN- γ) given intranasally in a murine model of pulmonary aspergillosis. They showed a

75 % reduction of fungal elements in lung and threefold higher survival than control animals or animals given IFN- γ intraperitoneally. Alveolar macrophages and lung leucocytes isolated from AdnIFN- γ -treated animals displayed enhanced killing of *Aspergillus* organisms *ex vivo*. Studies of this type suggest a potential clinical use for specific IFN- γ gene therapy in the future.

More recently, mice infected via the pulmonary route with a *Cryptococcus* sp. strain engineered to produce murine IFN- γ were able to resolve the primary infection and demonstrated complete protection against a secondary infection with a pathogenic strain, showing the importance of the stimulation of local cell-mediated immune responses and the development of protective host immunity. In contrast, a similar strategy against vaginal candidosis failed in protecting animals against experimental vaginitis.