

Interferon- γ as an Antifungal

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PERSONAL INTRODUCTION

I (D.A.S.) am honored to be able to make this contribution. Although other presenters have dwelled on Tom Merigan's many contributions to both AIDS studies and antiviral chemotherapy generally, I can emphasize another role for him, that of helping to bring interferon (IFN) to the fore of our consciousness [1]. I have the pleasant, unique role of being the only contributor not now identified with virology, and I will address the discipline to which I transitioned after fellowship with Tom—a place where, it turns out, IFN has an important host role [2]. Charles Prober, of the Department of Pediatrics at Stanford, recently made me aware that I have been, with my colleagues, publishing on IFN in 5 different decades now.

THE GROWING PROBLEM OF THE MYCOSES

Fungal infections are sharply increasing in frequency as a component of the infectious disease problem [3–14]. There are a number of reasons for this, including the development of broader-spectrum antibacterials for therapy and prophylaxis, leaving fungi to better compete and to fill the void; increased use of internal metal and plastic prosthetic devices, an hospitable milieu for fungi; increased use of invasive procedures and monitoring, which provide a highway for fungal invasion; increased use of parenteral nutrition, which provides a menstroom for fungal growth; more-aggressive immunosuppression for a variety of diseases, including

autoimmune or rheumatologic diseases; more intensive cancer chemotherapy, with enhanced survival of immunocompromised hosts; greater use of transplantation as therapy for organ dysfunction; AIDS; increased drug addiction; increased survival of premature and low-birth-weight infants in intensive care units; and population shifts of the US population and increased travel and tourism to the Sunbelt and to Latin America, where there is rapid population growth and where endemic mycoses are encountered. The mortality due to invasive fungal infections in some diseases is staggering: for example, mortality due to aspergillosis in some marrow transplant populations is 85%, and mortality due to candidiasis with tissue involvement is 90% [15]. The reasons for the high rate of therapy failure include the lack of specificity of signs and symptoms, unreliability of diagnostic tests, and deficiencies in spectrum and/or killing activity in currently available antifungal drugs. Thus, new treatments are desperately needed.

IMMUNOTHERAPY FOR THE MYCOSES

Therefore, the host's immunity may be considered as a way to improve therapy. The rationale for immunotherapy in mycoses includes the association of cell-mediated immunity and the natural history of disease, such as the observation of mycoses associated with compromised cell-mediated immunity (e.g., cryptococcosis in Hodgkin disease), and, in the healthy host, the correlation in coccidioidomycosis of anergy, disease progression, and increasing antibody titers. There are extensive data from experimental infections emphasizing the importance of cell-mediated immunity in the mycoses, including "subtraction" of cell-mediated immunity, such as with thymectomy, with antilymphocyte serum, or by the nude mouse mutation; subtraction of macrophages, such as with use of silica or by the beige mouse mutation; and reconstitution of cell-mediated immunity, such as with transfer of immune lymphocytes [16–18]. We have

Potential conflicts of interest: none reported.

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The Journal of Infectious Diseases 2006;194:S33–37

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0022-1899/2006/19406S1-0006\$15.00

again become very aware of the importance of cell-mediated immunity in opportunistic mycoses, as a result of AIDS [19–27]. There were initial experiments demonstrating the potential of immunotherapy using modalities such as muramyl dipeptide [28–30], transfer factor [31–34], and other modalities [35]. However, a bigger impetus to the present consideration of immunotherapy was the demonstration of cytokine effects on the antifungal activities of effector cells [36–47].

IFN- γ AND DEFENSE AGAINST FUNGAL INFECTION

We have demonstrated cytokine effects on the antifungal activities of effector cells—for example, in vitro with IFN- γ and tissue macrophage killing of *Blastomyces dermatitidis* [48], *Paracoccidioides brasiliensis* [49], and *Candida albicans* [50] and with pulmonary macrophage killing of *Blastomyces* [51], *Paracoccidioides* [52], and *Histoplasma* [53] species. We then showed that IFN- γ administration in vivo would up-regulate killing of *Blastomyces* and *Paracoccidioides* species by pulmonary macrophages ex vivo [52]. We showed that neutrophils would also be up-regulated by IFN- γ , both in vitro, with increased respiratory burst and killing of *Blastomyces* species [54, 55], and ex vivo, after systemic administration for killing of *Blastomyces* species [56]. We have shown IFN- γ activity directed against intracellular and extracellular fungi, against the dimorphic fungi of the endemic mycoses, and against the fungal opportunists; with murine and human effector cells, with cells of the monocyte-macrophage lineage, and with neutrophils; and in vitro, ex vivo, and in vivo [49–51, 53]. Thus, IFN- γ appears to be, potentially, a broad-spectrum antifungal agent. Our work [49–51, 53] with cytokines in these systems could be summarized as showing that effector cell antifungal activity alone is weak, and antifungal drug activity alone only is fungistatic [57–59]. Combining an effector cell and an antifungal produces synergy. Combining an effector cell and a cytokine results in enhanced antifungal activity (activation). Combining an effector cell, an antifungal, and a cytokine results in powerful synergy [58, 60–67].

In our serial studies of the immune response to experimental *Blastomyces* infection in vivo, we noted a brisk IFN- γ response initially but, with progression, an increase in IgE and interleukin (IL)-4 production [68]. This was consistent with a shift from a Th1 to a Th2 response with progressive disease [68]. In our model of paracoccidioidomycosis, in the nonprogressive setting, we saw dual production of IFN- γ and IL-4 by antigen-stimulated lymph node cells, and, in the chronic form of the disease, administering IFN- γ with antifungal therapy produced a synergistic effect [69]. We found IFN- γ to be a prominent part of the local immune response in brain in experimental cryptococcosis [70]. Cryptococcosis was more severe in IFN- γ -knockout mice or in mice given antibody to IFN- γ [62]. Other researchers showed potentiation of antifungal chemo-

therapy with IFN- γ in cryptococcosis [71], with regard to survival, and we showed a modest effect of IFN- γ alone on reduction of infectious burden and a 40-fold improvement over amphotericin B alone when the 2 modalities were combined [72]. IFN- γ alone was found to be more dramatic in its therapeutic effects in SCID mice and could result in cures of the central nervous system when combined with amphotericin B [73]. In a pilot placebo-controlled trial of IFN- γ as adjunct therapy to conventional chemotherapy in cryptococcosis in HIV-positive humans, there were trends toward more rapid sterilization of the cerebrospinal fluid, a decline in antigen titer in cerebrospinal fluid, and mycological-clinical responses [74]. In mice given antibody to IFN- γ , histoplasmosis became more severe [75]. When mice with histoplasmosis were given IFN- γ with amphotericin, the survival rate was improved over that in mice given amphotericin alone [76]. Others showed that paracoccidioidomycosis worsened in mice given antibody to IFN- γ [77]. IFN- γ therapy was found to reduce systemic candidiasis [78]. IFN- γ is a prominent component of the host immune response in rabbit coccidioidal meningitis [79]. IFN- γ responses in vitro were found to be significantly blunted in patients with coccidioidomycosis who had disseminated disease, whereas Th2 cytokines were not up-regulated [80–82].

Young animals (and humans) are more susceptible to invasive mycoses [83]. We showed that this related to depressed killing of fungal targets (*Blastomyces*) by neutrophils in young animals [84], that spleen cells of young animals produce less IFN- γ in response to nonspecific stimuli, and that the efficacy of the neutrophils could be restored in vitro to the level of that in adults by using IFN- γ [85]. Finally, IFN- γ administration in vivo increases survival of young mice to the levels of resistance seen in mature animals [85].

In addition to parenteral administration as a way to deliver IFN- γ , we have explored the possibility of gene therapy, with the idea of delivering IFN into the central nervous system to combat fungal meningitides, such as cryptococcosis, and to overcome blood-brain barriers to entry of the cytokine. A vector delivered into the central nervous system once could result in prolonged production of IFN- γ by host cells. We studied this with an adenovirus with the murine IFN- γ gene, a cytomegalovirus promoter, and SV40 polyA inserts [86]. We determined that a dose of virus could produce >30,000 pg/mL IFN- γ in cerebrospinal fluid even 5 days after administration, the best route for administration of the vector, and safe doses of virus [86].

PARTNERS FOR IFN- γ

Administration of IL-12 is another modality that appealed to us for immunotherapy of mycoses, largely because IL-12 turns the Th response toward the Th1 pathway, with IFN- γ production by Th1 cells, and, via IL-12 direct action on NK cells as well as by IL-12 induction of IL-2 and subsequent IL-2 effect

on NK cells, the induction of IFN- γ production by NK cells [87]. We showed that administration of IL-12 in vivo could significantly improve resistance to experimental cryptococcosis and, when given together with fluconazole, could significantly enhance fluconazole's effect [88]. Others showed that anti-IL-12 treatment of mice accelerated mortality in histoplasmosis and that IL-12 given to SCID or immunocompetent mice was efficacious and stimulated IFN- γ [89, 90]. Anti-IL-12 was found to exacerbate disease in resistant mice infected with *Coccidioides* species, and IL-12 administered to susceptible mice increased resistance and stimulated IFN- γ [91]. Recently, we have shown that IL-12 production is reduced in response to *Blastomyces* infection in a susceptible mouse strain and that resistance to infection could be conferred by IL-12 therapy [92]. However, optimizing the IL-12 regimen is of key importance, because excessively high doses induced resistance but were not tolerated.

PROPHYLAXIS

Prophylactic IFN- γ is a proven modality to reduce infections in patients with the congenital neutrophil deficiency of the respiratory burst, chronic granulomatous disease [93–95]. Randomized trials have shown a reduction in the number of lethal *Aspergillus* infections in these patients [94].

POSSIBLE MECHANISMS

The positive interactions reviewed above between cytokines and host defenses could have a number of explanations. These positive interactions could occur through synergistic interactions between effector cell products and antifungals, with effector cell production up-regulated by cytokines; antifungal therapy decreasing the antigen load, thus reversing the suppression of type 1 immunity; antifungals priming effector cells for a second signal from cytokines, thus increasing the respiratory burst; antifungals increasing proinflammatory cytokines at a transcriptional level and inhibiting anti-inflammatory cytokines; a synergistic interaction between chitinase, a mammalian gene product, and antifungals; effector cell stimulation by cytokines enhancing antimicrobial uptake by the cells; cytokines restoring a depression of host defenses by an antifungal; or increased susceptibility of fungi, with membranes altered by antifungals, to oxidative products of the effectors. There is published experimental evidence to support each of these mechanisms [16, 17, 62, 96, 97], and it is likely that more than one is operative.

CONCLUSION

In summary, in vitro data show that certain recombinant cytokines are potent activators of specific functions of antimicrobial host defenses against fungi. Experimental animal models have shown protective and therapeutic cytokine activity against fungal infection. These experimental findings provide a foundation for

clinical investigation of recombinant cytokines in the prevention and treatment of fungal infections in various patient populations [98–101]. Clinical trials are needed now to define a place for IFN- γ and other cytokines in antifungal chemotherapy [102].

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