Interferon-γ and Granulocyte-Macrophage Colony Stimulating Factor Therapy in Three Patients with Pulmonary Aspergillosis

A. Bandera, D. Trabattoni, G. Ferrario, M. Cesari, F. Franzetti, M. Clerici, A. Gori

Abstract

An immune response mediated by type 2 cytokines is thought to contribute to the development and unfavorable outcome of aspergillosis. Adjuvant therapy with interferon-γ (IFN-γ) and granulocyte-macrophage colony stimulating factor (GM-CSF) was added to antifungal treatment in three nonneutropenic patients (one HIV-positive and two HIV-negative patients) with culture proven aspergillosis refractory to classical antifungal therapy. Clinical improvement was observed concomitantly with an increase in peripheral blood leukocyte proliferation and type 1 cytokines production. Our findings suggest an association between the improvement in type 1 cytokine production observed during IFN-γ and GM-CSF administration and a better control of *Aspergillus* infection in patients with progressive disease despite adequate antifungal therapy.

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Introduction

Great progress has recently been made in the development of antifungal therapy. New classes of antifungal drugs have been introduced that show promise for achieving cure from infection and a lower incidence of adverse effects [1–3]. Despite these developments, treatment failure is still a significant problem, occurring in 20%–30% of patients with invasive aspergillosis [3]. Resolution of invasive mycoses is often dependent on recovery from granulocytopenia or restoration of cellular immunity, which indicates that host defense mechanisms are extremely important in the clearance of these pathogens. Thus, the use of cytokines has been proposed as a potentially important strategy for preventing or treating invasive aspergillosis. Indeed some observations have shown that high concentrations of type 2 cytokines are associated with a poor outcome in patients with aspergillosis [4], and that subjects susceptible to fungal infections, such as stem cell transplant recipients, have a Th2-biased cytokine response [5]. IFN-γ and GM-CSF have been shown to play a pivotal role in the host defense against filamentous mold infections, by promoting intracellular antimicrobial activity of effector mononuclear cells and polymorphonuclear phagocytes [6, 7]. Although IFN-γ has been given safely in severely immunosuppressed patients with invasive fungal infections [8, 9] and multiple case reports have suggested successful application of IFN-γ, G-CSF or GM-CSF for patients with refractory mycoses [10–13], prospective studies of cytokine therapy in patients with invasive pulmonary fungal infections have not been yet produced. In this paper, we describe the use of combination GM-CSF and IFN-γ as adjuvant treatment for pulmonary aspergillosis in an HIV-positive patient and in two nonneutropenic patients unresponsive to classical antifungal therapy. We also investigated the immune characteristics of patients before and after the introduction of immunoadjuvant therapy.

Patients and Methods

Three patients with culture-proven, *Aspergillus* pulmonary infection, refractory to chemotherapy were evaluated at the Institute of Infectious Diseases and Tropical Medicine of the University of Milan (Table 1). Isolation of *Aspergillus* spp. from bronchoalveolar lavage fluid associated to host factors and clinical/radiological thoracic presentation in the three cases corresponded to the EORTC criteria of ''probable'' aspergillosis [14]. The extent and severity of *Aspergillus* disease were determined on the basis of a physical examination, radiographs and cultures. The protocol was approved by the Institutional Review Board and an informed consent was obtained from all the study participants.

A. Bandera *(corresponding author)*, **A. Gori**
Division of Infectious Diseases, Dept. of Internal Medicine, ''San Gerardo'' Hospital, University of Milan-Bicocca, Via Solferino 16, 20052, Monza (Milan), Italy; Phone: (+39/039) -2333794, Fax: -2333898, e-mail: a.bandera@hsgerardo.org

D. Trabattoni, M. Clerici
Dept. of Preclinical Sciences, Chair of Immunology, LITA VIALBA, University of Milan, Milan, Italy

M. Clerici
Dept. of Biomedical Sciences and Technologies, Chair of Immunology, University of Milan and Don C. Gnocchi Foundation IRCCS, Milan, Italy

A. Bandera, G. Ferrario, M. Cesari, F. Franzetti, A. Gori
Dept. of Clinical Sciences, Infectious Diseases Section, ''Luigi Sacco'' Hospital, University of Milan, Milan, Italy

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Table 1
Characteristics of the patients and clinical responses to interferon gamma and GM-CSF therapy.

Patient no.	Age (year)/ sex	Extent of disease	Steroids	Associated conditions	Dose and duration of IFN-γ and GM-CSF	Concurrent therapy	Clinical response	Adverse events	Current status
1	67/M	Lung, pleural	None	Cystic-bronchiectasic pulmonary dystrophy	50 μg/m^2, 3× week 300 μg daily for 60 days	Liposomal ampho B	Resolution of fever and cough. Clearing of pleural fluid	None	Well, after decortication, antimycotic agents stopped
2	32/M	Lung	For 60 days, stopped 1 month before	HIV infection, bronchiolitis obliterans	50 μg/m^2, 3× week 300 μg daily for 60 days	Liposomal ampho B	Reduction of fever, cough, increase in weight. Clearing of sputum	Mild fever, malaise	Well, antimycotic agents stopped
3	69/F	Lung	None	Cavitary sequelae of TB	50 μg/m^2, 3× week 300 μg daily for 48 days	Itraconazole	Reduction of fever. Negativization of serological test [a]	Fever leading to therapy interruption	Relapse of aspergillosis, antimycotic agents continued

[a] Serological test: precipitins presence

Adjuvant immunomodulant therapy with subcutaneous IFN-γ 50 μg/m^2 (Imukin, Boehringer Ingelheim) three times a week and subcutaneous GM-CSF 300 μg daily (Mielogen, Schering-Plough) was added to antifungal therapy in all patients after obtaining informed consent.

Peripheral blood mononuclear cells (PBMCs) were either unstimulated (medium) or stimulated with phytoemoagglutin (PHA) (2.5 μg/ml), tetanus toxoid (TET) (1:400) or purified protein derivative (PPD) (1 μg/ml) and 10% human AB serum. After 6 days, cultures were pulsed with 1 μCi of [3H]-thymidine and harvested 18 h later. For cytokine production, PBMCs were incubated in the presence/absence of PHA for 48 h.

For cytokine production, tests were performed in duplicate using commercially available enzyme-linked immunosorbent assays (Endogen, Woburn, MA, USA). The values for all the cytokines were calculated from the standard curve of the corresponding recombinant human cytokine in accordance with the instructions of the manufacturer.

Case Reports

Patient 1, a 67-year-old-male with a history of cystic-bronchiectasic pulmonary dystrophy, developed a right pulmonary cavitary infiltrate associated with homolateral hydro-empyema caused by *Aspergillus fumigatus*. Itraconazole therapy (400 mg/die) was stopped after 2 weeks of treatment, as clinical and radiographic findings did not improve, and the patient underwent treatment with liposomal amphotericin B (4 mg/kg/die). The patient then underwent pleural fluid and gas drainage, with intrapleuric povidone–iodine 10% and saline solution washes, due to the rupture of a fungal ball in a subpleuric parenchimal cyst. In consideration of a further progression of the lung infiltrate and persistence of fever and respiratory distress after 20 days of liposomal amphotericin B treatment, adjuvant therapy with IFN-γ and GM-CSF was associated to liposomal amphotericin B for 2 months. After 2 weeks from the introduction of adjuvant treatment, the patient experienced a rapid clearing of pleural fluid with persistent negativization of fungal cultures and great improvement of the clinical conditions, with complete recovery from fever and productive cough. A month after the end of adjuvant immune therapy, clinical and radiological conditions (Figure 1A) improved to the extent that the patient underwent surgical decortication in order to obtain the bronco-pleuric leak closure. Radiological controls after surgical decortication showed only fibrotic tissues and the patient displayed good clinical conditions during the 5 years of follow-up.

Patient 2, a 32-year-old homosexual male, HIV-positive since 1987 with a history of recurrent *Pneumocystis carinii* pneumonia (PCP) and steroid-responsive bronchiolitis obliterans, developed invasive pulmonary *Aspergillus fumigatus* infection. His CD4+ cell count was 87 cells/μl and the HIV-RNA viral load was < 500 copies/ml. The last absolute neutrophil counts had been, respectively, 640 per μl and 970 per μl. The infection failed a 21-day treatment with itraconazole (600 mg/die) and thus, the patient was treated with association of amphotericin B (1 mg/kg/die) and itraconazole (400 mg/die), and subsequently with liposomal amphotericin B (4 mg/kg/die) alone due to renal and liver toxicity. After 2 months of the above-mentioned therapy, worsening of the pulmonary lesions was observed and the patient developed left pneumothorax with pleural effusion, requiring a thoracoscopic procedure of drainage and lung re-expansion. After 3 months, in consideration of the susceptibility tests revealing a strain resistant to itraconazole and amphotericin B and due to the persistence of the septic conditions

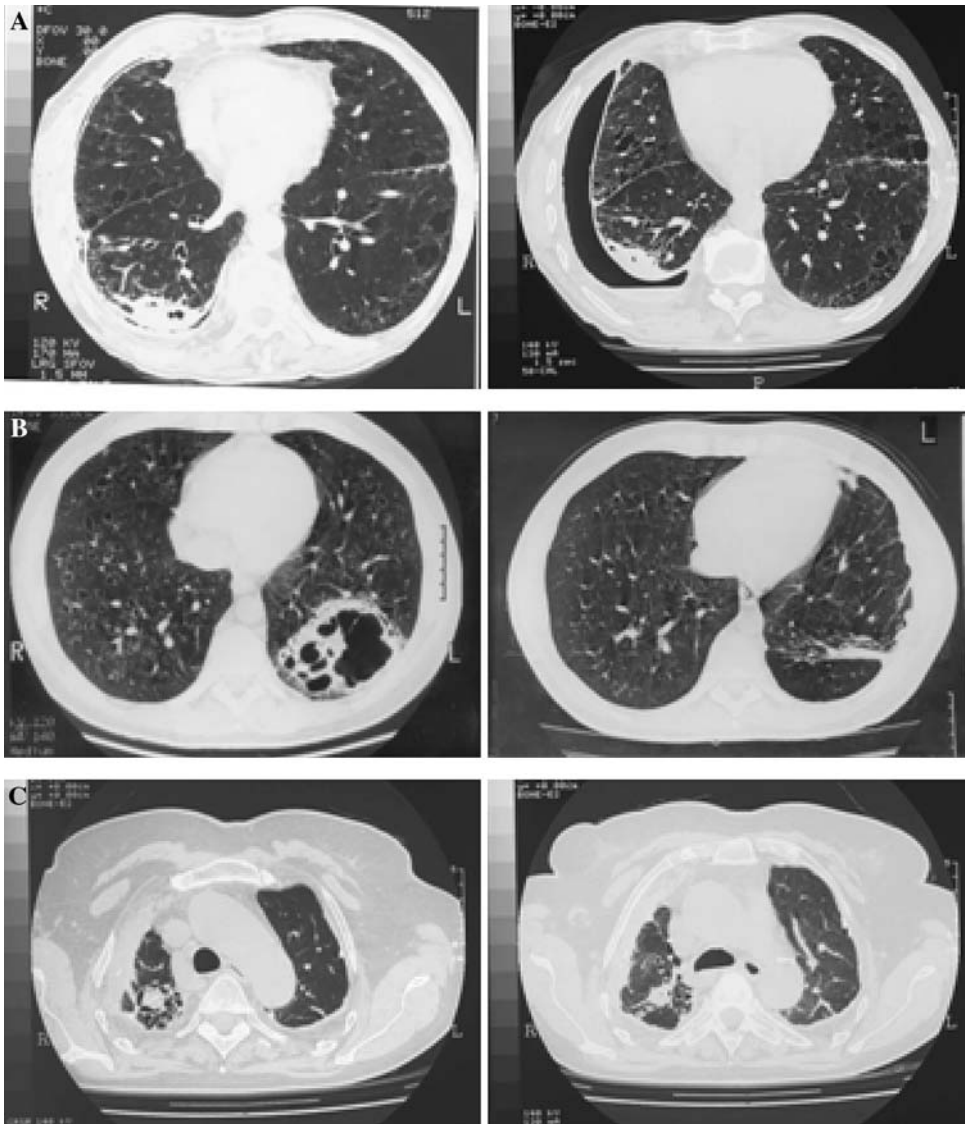

Figure 1. Serial pulmonary CT scans of *Aspergillus* infection in three patients treated with IFN-γ plus GM-CSF adjuvant immune therapy. A. Patient 1: left panel, right lower lobe cavitary infiltrate associated with homolateral pleural hydroempyema after itraconazole and liposomal amphotericin B therapy; right panel, regression of the cavitary infiltrate with persistence of homolateral hydroempyema after 2 months of IFN-γ and GM-CSF therapy. B. Patient 2: left panel, pulmonary lesion after 2 months from the aspergillosis diagnosis and itraconazole therapy; right panel, marked improvement of *Aspergillus* lesions after 2 months of IFN-γ and GM-CSF therapy. C. Patient 3: left panel, large cavitation with solid content at the apex of the right lung after itraconazole and liposomal amphotericin B therapy; right panel, amelioration of the pulmonary lesion after 48 days of IFN-γ and GM-CSF therapy.

with a further increase in the size of the lung lesions, adjuvant immunomodulant therapy with subcutaneous IFN-γ and GM-CSF was added to liposomal amphotericin and the current antiretroviral therapy for 2 months. Two weeks from the introduction of adjuvant therapy the patient showed rapid improvement, as indicated by clearing of sputum cultures, resolution of fever and cough, regression of radiological features and weight gain. The HIV-RNA viral load always remained < 500 copies/ml and no significant changes in CD4+ cell counts were observed. No relapse of aspergillosis was observed during the 6-year follow-up, as shown by the continuously negative sputum cultures and the progressive amelioration of the lung CT scans (Figure 1B).

Patient 3, a 69-year-old female with a history of pulmonary tuberculosis and subsequent pachipleuritic alterations and persistence of large cavitation at the apex of the right lung, developed recurrent episodes of fever and dyspnea unresponsive to antibacterial therapy. A worsening of the previous pulmonary lesions was observed concomitantly with multiple identification of *A. fumigatus* from cultures of the sputum and broncoalveolar lavage. The patient initially underwent antifungal treatment with itraconazole 400 mg for 20 days and then, due to clinical and radiological failure, with liposomal amphotericin B (4 mg/kg/die). After 6 months of amphotericin B treatment, cultures from broncho-alveolar lavage and sputum were persistently positive for *A. fumigatus* and serological examinations showed invariable high titres of precipitins, despite the ongoing antifungal treatment. Studies of immunological parameters showed no alterations in CD4+ and CD8+ counts (CD4+ 666 per μl, CD8+ 421 per μl). The patient underwent 6 months of itraconazole therapy and due to a further increase in the size of the lung lesions and persistence of cough, fever, and weight loss, adjuvant immunomodulant therapy with IFN-γ and GM-CSF was added to itraconazole. After 2 weeks of adjuvant therapy, the patient experienced a progressive improvement in clinical conditions. Serological test, performed 30 days after adjuvant immunomodulant therapy, showed negativization of precipitins and the immunomodulant treatment was continued for a total of 48 days. After 48 days, the patient interrupted adjuvant therapy because of IFN-γ related fever (grade 2). A pulmonary CT scan, performed after the entire course of adjuvant therapy, showed a

mild amelioration of the pulmonary lesion at the apex of the right lung (Figure 1C). She remained free of disease during the period of IFN-γ and GM-CSF administration. Six months after the adjuvant therapy interruption, despite the ongoing itraconazole therapy, serologic test showed the re-emergence of precipitins and after 7 months the patient experienced a relapse of pulmonary aspergillosis. The patient refused an additional cycle of immunoadjuvant therapy because of the adverse event experienced during the first cycle. At 4 years of follow-up the patient was still being treated with antifungal therapy because of periodic recurrences of pulmonary aspergillosis.

Adverse Events

Fever (grade 2), well controlled by acetaminophen, and fatigue (grade 2) were the two major side effects reported in all three patients during IFN-γ and GM-CSF administration. No changes in hematological laboratory values were observed. No grade 3 or 4 adverse events were observed.

Immunological Results

To evaluate the cytokine profile and the modification of the cytokine pattern during the IFN-γ and GM-CSF treatment, the production of IFN-γ, IL-2, and IL-4 by stimulated and unstimulated PBMCs was determined in patients 2 and 3 at baseline (before the introduction of adjuvant immune-therapy) and after 2 months of adjuvant therapy (Figure 2A). After therapy, mitogen (PHA)-stimulated production of IL-2 was greatly increased (30 pg/ml vs 120 pg/ml for patient 2; 0 pg/ml vs 852 pg/ml for patient 3). A threefold increase in mean mitogen-stimulated IFN-γ levels was also observed after IFN-γ and GM-CSF therapy (150 pg/ml vs 1,250 pg/ml for patient 2; 741 pg/ml vs 1,587 pg/ml for patient 3), whereas mean mitogen stimulated IL-4 levels were threefold lower than at baseline in both individuals (18.6 pg/ml vs 7.2 pg/ml for patient 2; 13.8 pg/ml vs 3.4 pg/ml for patient 3). In patients 2 and 3, we also measured antigen-stimulated proliferation before and after the immune adjuvant treatment (Figure 2B). A marked improvement in TET (5,312 cpm vs 24,611 cpm for patient 2; 7,339 cpm vs 18,587 cpm for patient 3), PPD (821 cpm vs 8,463 cpm for patient 2; 315 cpm vs 1,874 cpm for patient 3) and PHA (48,210 cpm vs 68,977 cpm for patient 2; 31,402 cpm vs 45,157 cpm for patient 3) stimulated proliferation was observed after therapy in both cases.

Discussion

Even if new therapeutic options have recently become available, treatment of *Aspergillus* infections can pose difficult problems in clinical management, because of intolerance, regimen failure and possible development of

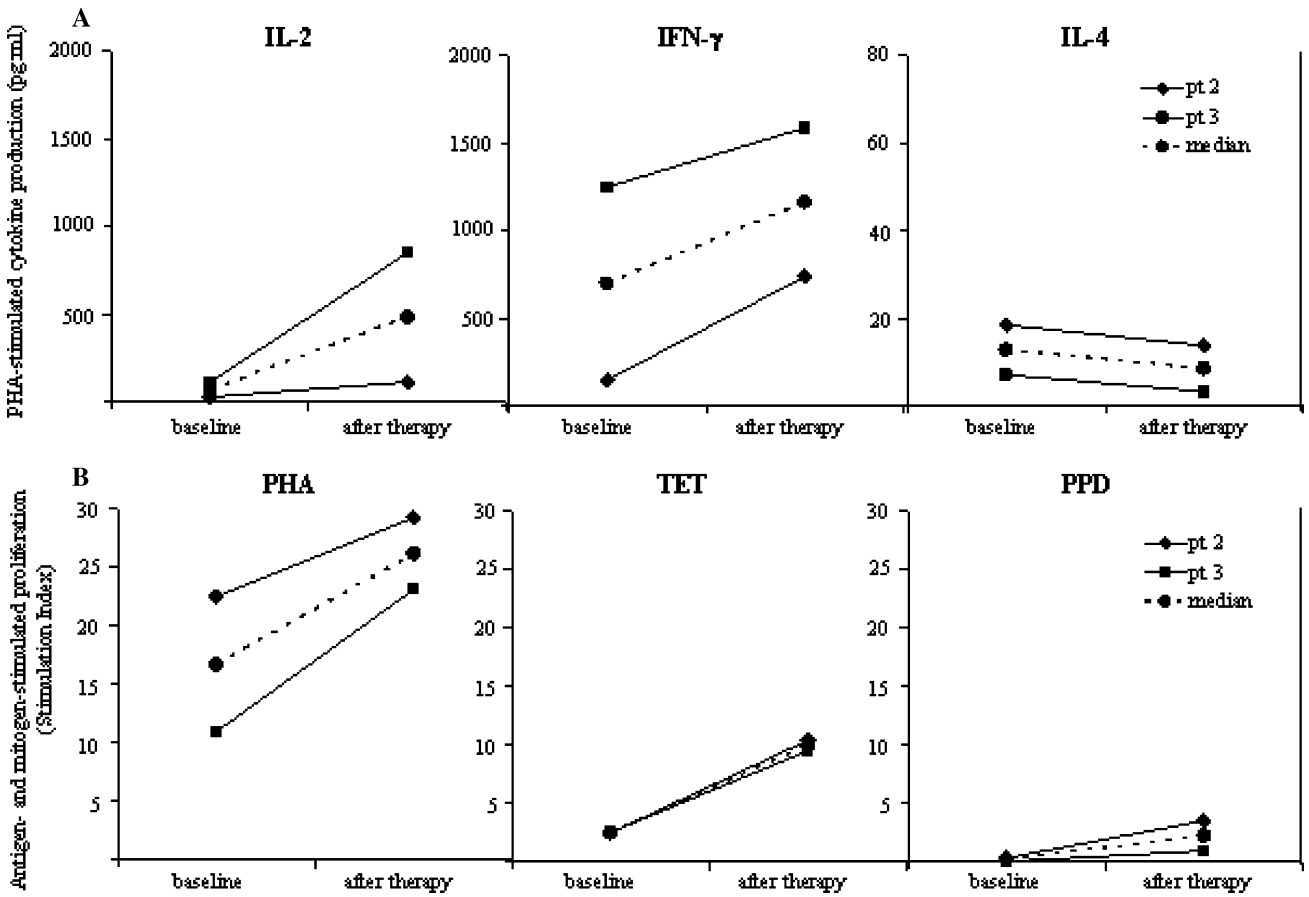

Figure 2. A. Mytogen-stimulated cytokine production at baseline and after therapy with IFN-γ and GM-CSF in patient 2 and in patient 3. B. Antigen- and mitogen-stimulated peripheral blood leukocyte proliferation at baseline and after therapy with IFN-γ and GM-CSF in patient 2 and in patient 3.

resistance [2, 15, 16]. Immunotherapies aimed at enhancement of host defense mechanisms may be extremely useful.

Our study is the first to describe the clinical and immunological effect of adjuvant IFN-γ and GM-CSF immunotherapy in association with antifungal chemotherapy in an HIV-infected patient affected by invasive aspergillosis. Adjuvant immune therapy was associated with the healing of the pleuropulmonary aspergillosis lesions, and with a marked improvement in the signs and symptoms of the disease. The patient did not register significant adverse events and had no clinical relapse during a 6-year follow-up, which is a markedly longer survival time than that of the other aspergillosis cases reported in HIV infection [17].

We also investigated IFN-γ and GM-CSF immunotherapy in association to antifungal treatment in two HIV-negative nonneutropenic patients unresponsive to chemotherapy. In both patients a prompt resolution of clinical symptoms and a progressive amelioration of radiological findings were observed following immunotherapy, in absence of major adverse events, allowing for surgical decortication with successive complete resolution of pulmonary alterations in one of the patients.

Our study shows the immunologic effect of IFN-γ and GM-CSF administration during adjuvant treatment of pulmonary aspergillosis. In two patients studied, after IFN-γ and GM-CSF immunoadjuvant therapy, an important increase in mitogen-stimulated production of type 1 cytokines and a concomitant decrease in IL-4 production were observed. Moreover, an important improvement in the ability of soluble antigens to stimulate peripheral blood mononuclear cell proliferation was observed in both patients after the completion of adjuvant immune therapy. These findings demonstrate an in vivo shift toward a type-1 cytokine immune response subsequent to IFN-γ and GM-CSF immunotherapy, which ultimately coincided with a significant amelioration of the clinical course of *Aspergillus* infection, also confirming previous in vitro and in vivo data demonstrating an association between type 2 cytokine immune response and worsened prognosis of invasive aspergillosis [4, 5].

Limitations of this study include the use of amphotericin B and itraconazole, which were the best antifungal available drugs at the time the patients were treated, before the license of voriconazole and caspofungin. The unavailability of echinocandins and voriconazole, which could have been used successfully in the cases of pulmonary aspergillosis refractory to amphotericin B and itraconazole we describe, thus represents a possible bias of this study.

However, even in the present era of highly active anti-*Aspergillus* drugs, response to treatment in severely immunosuppressed patients remains mostly unfavorable. In addition, the improved understanding of molecular pathogenesis of fungal infections and the complexity of antifungal immune responses of the host has provided the critical information to further evaluate immunity enhancement strategies.

Even if with limited data, our findings suggest an association between the improvement in type 1 cytokine production observed during IFN-γ and GM-CSF administration and a better control of *Aspergillus* infection, in absence of other therapeutic modifications, and warrant further evaluation of the role of cytokine therapy, especially in the early course of life-threatening invasive aspergillosis.

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