

## Effective Combination Therapy Using Interferon- $\gamma$ and Interleukin-2 for Disseminated *Mycobacterium avium* Complex Infection in a Pediatric Patient with AIDS

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**A 5-year-old girl presented with disseminated *Mycobacterium avium* complex infection during advanced human immunodeficiency virus infection. Interferon- $\gamma$  or interleukin-2 monotherapy showed only limited effects. Use of a combination of interferon- $\gamma$  and interferon-2 resulted in a remarkable improvement in the patient's condition, accompanied by an increase in circulating CD4<sup>+</sup> T cells.**

In recent years, HAART has produced marked improvements in the morbidity and mortality of patients infected with HIV [1]. Immune reconstitution syndrome (IRS) is an untoward manifestation of the vigorous immune recovery that develops with receipt of potent antiretroviral therapy. In some patients with AIDS, marked inflammatory reactions are directed against pathogens causing latent infection, including *Mycobacterium avium* complex (MAC). Focal pulmonary infection and lymphadenitis (granulomatous inflammation) are representative MAC diseases caused by IRS [2, 3]. The augmentation of cell-mediated immunity toward latent pathogens leads to the clinical manifestations of IRS.

Despite administration of HAART, it is not unusual for CD4<sup>+</sup> T cell counts to not increase even when the patient experiences a marked reduction in HIV load as a result of receiving such therapy. In particular, pediatric patients with AIDS who have defective cell-mediated immunity are likely to remain at risk for serious disseminated infections caused by *Mycobacterium tuberculosis* and MAC [4].

Cytokine administration has had powerful effects on immune function. In the past decade, there has been a great deal of focus on IL-2 therapy [5, 6]. IL-2 therapy for patients with a limited CD4<sup>+</sup> cell count response to HAART has produced remarkable and rapid effects and a dose-dependent increase in CD4<sup>+</sup> T cell counts. In MAC-infected patients with AIDS, the immune system reconstitution explained by the recovery of cell-mediated immunity may be the key for control of opportunistic infections. IFN- $\gamma$  has also been administered to treat MAC infection in some patients with AIDS [7]. Macrophage activation and the production of IFN- $\gamma$  are supposed to play an important role in the eradication of MAC. Therefore, IFN- $\gamma$  treatment is promising, but it has not proven to be safe and effective enough for treatment of MAC infection. Here, we report that combination therapy with IFN- $\gamma$  and IL-2 produced a marked improvement in disseminated severe MAC infection in a pediatric patient with advanced HIV infection.

**Case report.** A 5-year-old girl who was vertically infected with HIV had been receiving zidovudine and indinavir for AIDS since she was 2 years old, but she did not show an increase in CD4<sup>+</sup> T cell count, despite a marked reduction in HIV load (figure 1). The patient's HAART regimen was modified in August 2000 because of an increase in her HIV load. Although the patient's HIV infection was resuppressed successfully with a combination of stavudine, didanosine, and efavirenz, the patient suddenly developed a high fever and serious abdominal pain in October 2000. Findings of abdominal CT scanning suggested that her mesenteric and paraaortic lymph nodes had been so enlarged that ileus symptoms had occurred. A diagnosis of disseminated MAC infection was made in November 2000 on the basis of blood culture and PCR results positive for MAC.

Multiple anti-MAC drugs (clarithromycin 40 mg/kg per day or azithromycin 20 mg/kg per day in combination with ethambutol, rifabutin, ciprofloxacin, and isoniazid) had only a transient effect on her symptoms. The patient developed continuous vomiting, which made her unable to tolerate MAC treatment regimens. Her condition became so severe—with enlargement of the abdominal masses, hepatosplenomegaly, ascites, and malaise—that it was deemed to be fatal.

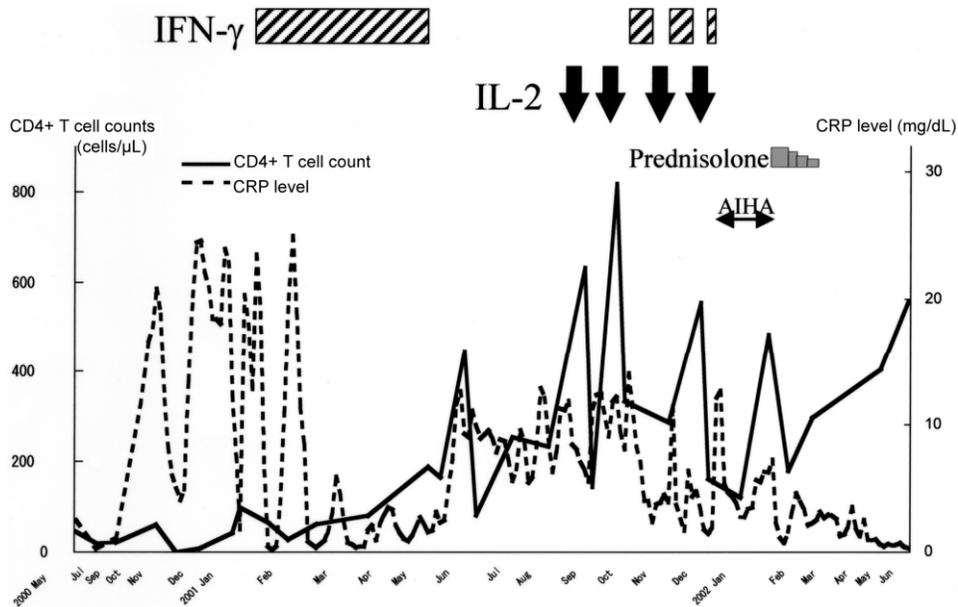
The patient's peripheral mononuclear cells produced very low levels of IFN- $\gamma$  under CD3<sup>+</sup> cell stimulation, which compromised the patient's clearance of MAC infection (figure 2). Therefore, intravenous administration of IFN- $\gamma$  (2 million units/m<sup>2</sup> per day) was started in January 2001 (figure 1). MAC bacteremia disappeared in response to the therapy, but the patient's symptoms persisted after the tapering of IFN- $\gamma$ . On the basis

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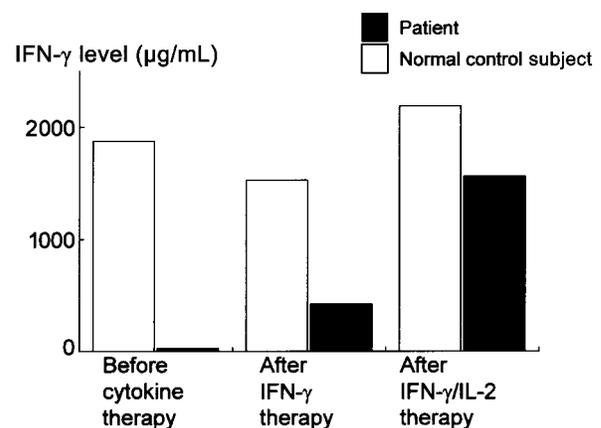
**Figure 1.** Marked improvement in C-reactive protein (CRP) levels and CD4<sup>+</sup> T cell counts as a result of combination therapy with IFN- $\gamma$  and IL-2. After receipt of combination therapy, CD4<sup>+</sup> T cell counts and CRP levels were markedly improved. AIHA, autoimmune hemolytic anemia.

of the conclusion that the low number of circulating CD4<sup>+</sup> T cells (<200 cells/ $\mu$ L) contributed to the poor synthesis of IFN- $\gamma$ , IL-2 was administered twice daily for 5 consecutive days (at a dosage of 1.5 million units/m<sup>2</sup> per day) by subcutaneous injection. Within 1 week after the injection, the circulating CD4<sup>+</sup> T cell count transiently exceeded 500 cells/ $\mu$ L. With the increase in CD4<sup>+</sup> T cell count, the patient's condition improved remarkably, although the cell count decreased gradually to <100 cells/ $\mu$ L 2 weeks after the injection. The patient was able to tolerate MAC treatment (with azithromycin) and 3 cycles of combination therapy with IFN- $\gamma$  and IL-2 (IFN- $\gamma$  administered for 4 weeks and IL-2 administered for 5 days alternately) were also administered, which produced marked improvements in the symptoms and preserved the number of CD4<sup>+</sup> T cells (CD4<sup>+</sup> T cell count, >200 cells/ $\mu$ L). After the last cycle of the combination therapy, autoimmune hemolytic anemia developed in December 2001; this condition was treated successfully with temporary administration of prednisolone (1.5 mg/kg per day administered orally). Although combination therapy with IFN- $\gamma$  and IL-2 was discontinued because of autoimmune hemolytic anemia, the patient's CD4<sup>+</sup> T cell count gradually increased with HAART therapy alone. The MAC symptoms have not resumed after the discontinuation of cytokine therapy.

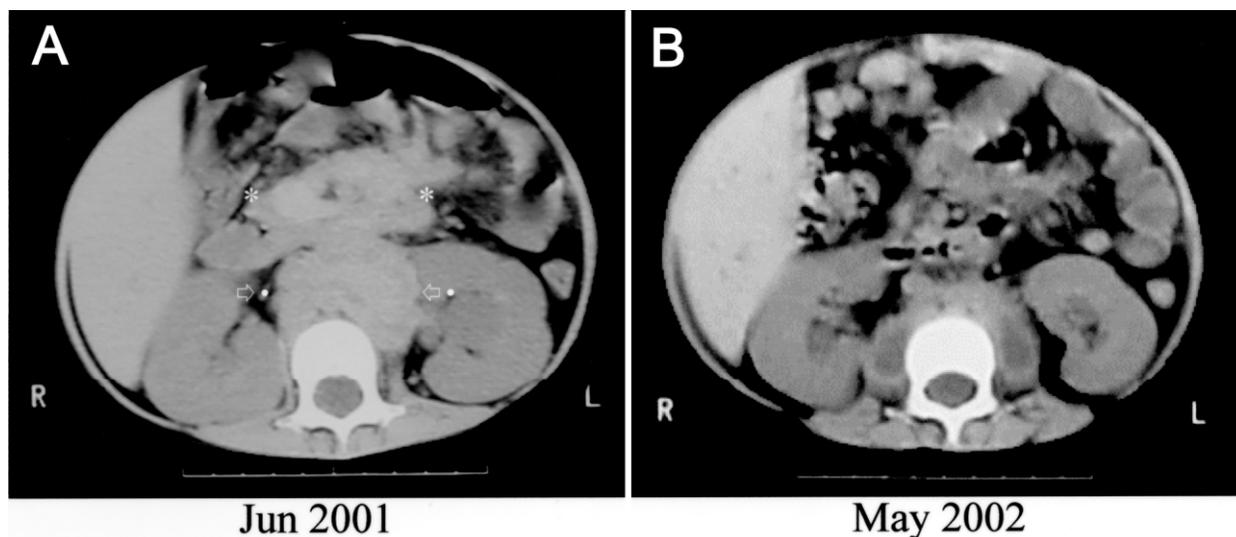
Six months after the initiation of combination therapy, the patient's C-reactive protein levels and erythrocyte sedimentation rate were normalized, and the peritoneal lymph node swelling completely disappeared (figure 3). The patient has been fairly well while receiving the HAART and MAC prophylactic antibiotics (clarithromycin 10 mg/kg daily in 2 divided doses

and rifabutin 5 mg/kg once daily). MAC bacillemia has resolved, and she has maintained an HIV load of <400 copies/mL and a CD4<sup>+</sup> T cell count of >300/ $\mu$ L for 3 years without cytokine therapy.

**Discussion.** Disseminated infection with MAC is likely to be underestimated in children. An increase in the number of MAC infections in adult patients with AIDS has been noted since the 1980s. The number of cases of MAC infection in



**Figure 2.** IFN- $\gamma$  synthesis by mononuclear cells. One million peripheral mononuclear cells were cultured in 1 mL of RPMI 1640 with 10% fetal calf serum at 37°C for 48 h in CD3<sup>+</sup>-mAb coated wells. Then, the supernatants were aspirated and processed for assay of IFN- $\gamma$  by ELISA. The IFN- $\gamma$  synthesis by the mononuclear cells was remarkably restored after combination therapy with IFN- $\gamma$  and IL-2.



**Figure 3.** Abdominal CT scans showing paraaortic (*arrow*) and mesenteric lymph nodes (*\**) of the patient before (*A*) and 6 months after (*B*) initiation of combination therapy with IFN- $\gamma$  and IL-2. The paraaortic and mesenteric lymph nodes observed before combination therapy are enlarged; the lymph nodes were remarkably diminished in size and had degenerated 6 months after initiation of combination therapy with IFN- $\gamma$  and IL-2.

children with AIDS has also increased [8]. MAC is an intracellular parasite that proliferates within macrophages; uncontrolled bacterial replication occurs in children with defective cell-mediated immunity [4].

IFN- $\gamma$  activates macrophages and plays a crucial role in the eradication of MAC infection. It has been reported that patients with mutations of IFN- $\gamma$  receptor genes experience intractable tuberculosis and MAC infections [9]. When our patient was experiencing severe disseminated MAC infection, very little IFN- $\gamma$  was detected in the serum or culture supernatants of the circulating mononuclear cells, which contained negligible numbers of CD4<sup>+</sup> T cells under CD3<sup>+</sup> cell stimulation. The injection of IFN- $\gamma$  improved the patient's condition markedly. Her condition was considered to be almost incurable before administration of IFN- $\gamma$ .

It is noteworthy that a combination of IL-2 and IFN- $\gamma$  markedly increased the number of circulating CD4<sup>+</sup> T cells and resulted in continuous IFN- $\gamma$  production by the mononuclear cells in vitro (figure 2). Reconstruction of the immune systems, including IFN- $\gamma$  production in conjunction with the increase in CD4<sup>+</sup> T cells, may have aided in defense against MAC infection in our patient. These results strongly suggested that CD4<sup>+</sup> T cells are the main source of IFN- $\gamma$  in vivo. Combination therapy with IFN- $\gamma$  and IL-2 could be a useful therapeutic option for the treatment of severe disseminated MAC infection in patients with HIV infection who have reduced CD4<sup>+</sup> T cell counts.

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