Brief Definitive Report

Widespread Intradermal Accumulation of Mononuclear Leukocytes in Lepromatous Leprosy Patients Treated Systemically with Recombinant Interferon γ

By Carl Nathan,* Kathleen Squires,‡ William Griffo,* William Levis,¶ Matthew Varghese,§ C. K. Job,** Ali R. Nusrat,‡‡ Stephen Sherwin,§§ Samuel Rappoport,‖ Elizabeth Sanchez,* Rochel A. Burkhardt,‡‡ and Gilla Kaplan‡‡

*From the Divisions of *Hematology-Oncology, ‡Infectious Diseases, §Dermatology, and ‖Neurology, Department of Medicine, Cornell University Medical College, New York, New York 10021; the ¶U.S. Public Health Service Hansen's Disease Center, Bayley-Seton Hospital, Staten Island, New York 10304; the **U.S. National Hansen's Disease Center, Carville, Louisiana 70721; the ‡‡Laboratory of Cellular Physiology and Immunology, The Rockefeller University, New York, New York 10021; and §§Genentech, Inc., South San Francisco, California 94080*

Summary

Intradermal administration of recombinant interferon γ (rIFN-γ) to lepromatous leprosy patients has converted the local histology toward a tuberculoid pattern. However, such changes have been confined to the site of injection. In contrast, in the present study, marked, intradermal accumulation of $CD3^+$, $CD4^+$, $CD8^+$, and $CD1a^+$ T cells and Leu-$M5^+$ mononuclear phagocytes was induced at a distance from the sites of administration, in a dose-dependent manner, by 10 daily intramuscular injections of 10–30 μg rIFN-γ/m^2. Mononuclear cell infiltration began within 3 d of onset of rIFN-γ therapy and persisted at least 8 wk. Intramuscular administration of rIFN-γ to lepromatous patients receiving concurrent chemotherapy can safely induce widespread histologic features of an upgrading reaction.

Leprosy, the first disease associated with a specific bacterium (1) and one of the first to be treated with chemotherapy (2), remains one of the most prevalent life-threatening infections. The lepromatous form of the disease is marked by anergy to the causative organism *Mycobacterium leprae*, a paucity of infiltrating lymphocytes, and widespread proliferation of the bacterium within macrophages in the skin. Despite years of multi-drug therapy, cure is not often achieved. In contrast, patients with the tuberculoid form of the disease display vigorous delayed-type hypersensitivity; the lesions are rich in activated T cells and macrophages, which markedly restrict the proliferation of the organism; and cure is attained routinely, sometimes even without chemotherapy (3). In effect, tuberculoid leprosy illustrates what immunotherapy might hope to achieve in lepromatous leprosy.

Over the past 18 yr, injection of at least six different immunologically active agents has been reported to induce tuberculoid features (partial or complete upgrading reactions) in lepromatous patients, either systemically (4, 5) or at the sites of injection (6–12). These agents were allogeneic lymphocytes (4); transfer factor (5); mixtures of *M. leprae* or *M. lepraemurium* with BCG (6); rIFN-γ (7–10); purified protein derivative (11); and IL-2 (12). Of these, rIFN-γ has been studied most widely, based on the rationale that it is preferable to use a defined, standardized agent, and that lepromatous leprosy can be viewed, in part, as an antigen-specific IFN-γ deficiency state, resulting in failure to activate macrophages (7). Four studies involving 96 patients in the United States, Brazil, Africa, and India have given a consistent picture: one to five intradermal injections of 1–20 μg rIFN-γ induced local conversion from a lepromatous to a more tuberculoid histology, with rapid local reduction in apparent bacterial burden in 28 of 42 cases evaluated (67%) (7–10).

Lepromatous disease affects nearly the entire dermis, while the changes induced by intradermal injection of rIFN-γ have been confined to the site of injection (10). Thus, the foregoing results appeared to hold little therapeutic promise, unless more frequent administration and/or higher doses of rIFN-γ can be shown to induce a more widespread response. However, it has been feared that any attempt to achieve bioactive

concentrations of rIFN-γ throughout the dermis might precipitate or exacerbate destructive immunologic reactions that occur frequently in leprosy. The present study demonstrated that a 10-d course of intramuscular rIFN-γ in lepromatous patients had little toxicity, yet induced long-lasting, widespread, intradermal accumulation of mononuclear leukocytes. This opens the door to studies of rIFN-γ in leprosy with curative intent.

Materials and Methods

rIFN-γ. rIFN-γ (Genentech, Inc., South San Francisco, CA) was purified from *Escherichia coli* transfected with the cDNA for human rIFN-γ; specific activity was 2×10^7 U/mg. The lyophilate was reconstituted in sterile distilled water to a concentration of 200 μg/ml.

Study Design. Eight men and three women 32–76 yr old were diagnosed by skin biopsy at least 6 mo before the study as having borderline or polar lepromatous leprosy by Ridley-Jopling criteria (3). Their pre-study medications (Dapsone, Rifampin, clofazimine, and/or thalidomide) were continued.

Single Injections. In three subjects, 3-mm punch biopsies were taken from both buttocks, followed by one intra-deltoid injection of 10 or 30 μg of rIFN-γ/m². 2 d later, a third skin biopsy was taken from the buttocks.

Multiple Injections. Four subjects were studied at each of two rIFN-γ dose levels: 10 or 30 μg/m² per day. Control 4-mm punch biopsies were taken from the skin of both buttocks. Beginning the same day, the patients received 10 daily intra-triceps injections of rIFN-γ in alternate arms. Skin biopsies were taken again from the buttocks or lower back on day 4 (one biopsy), day 11 (bilateral biopsies), 3 wk after the end of rIFN-γ injections (one biopsy), and 6 wk after the end of rIFN-γ injections (one biopsy). Of the seven biopsies, three were from one side of the patient and four from the other side. Physical examination, weight, and vital signs were recorded daily. On days 1 and 11, the following were monitored: sensory nerve conduction velocity, chest x-ray, electrocardiogram, urinalysis, hemogram, prothrombin time, partial thromboplastin time, and levels of electrolytes, urea nitrogen, creatinine, calcium, phosphorus, glucose, bilirubin, protein, alkaline phosphatase, lactate dehydrogenase, and transaminases in the serum.

Analysis of Biopsies. Biopsies were divided longitudinally. One portion was processed for Fite's stain and the other for immunocytochemistry using the antibodies and methods described (7, 8, 10–12).

Results

Concern about inducing severe reversal reactions dictated a step-wise approach. First, three subjects with lepromatous disease were given single intramuscular injections of 10 or 30 μg of rIFN-γ/m². There was no toxicity over subsequent months, nor were histologic changes detected 2 d later at a distant site (intradermal injection of rIFN-γ induces local histologic changes within this time [8]). In the next eight subjects, we increased the number of daily intramuscular rIFN-γ injections to 10, giving 10 μg/m² daily to the first four, and 30 μg/m² to the next four. Because the latter dose affected the skin so dramatically, we omitted a planned dose of 100 μg/m². The following results pertain to the last eight subjects.

Clinically Evident Changes in the Skin. Plaques over the entire body of one patient flattened during treatment with rIFN-γ at the lower dose. Two patients at the higher dose level showed faint, widespread erythema, which disappeared when the injections were stopped. The other two patients at the higher dose level displayed rapidly fluctuating cutaneous nodularity. Long-standing nodules shrank; new nodules appeared suddenly but transiently, often with overlying erythema. When rIFN-γ injections were completed, changes in nodularity ceased. There was no accompanying fever, pain, or tenderness.

Immunohistologic Changes in the Skin. The response described below were detected bilaterally, at several time points, in skin remote from rIFN-γ injection sites or their lymphatic drainage.

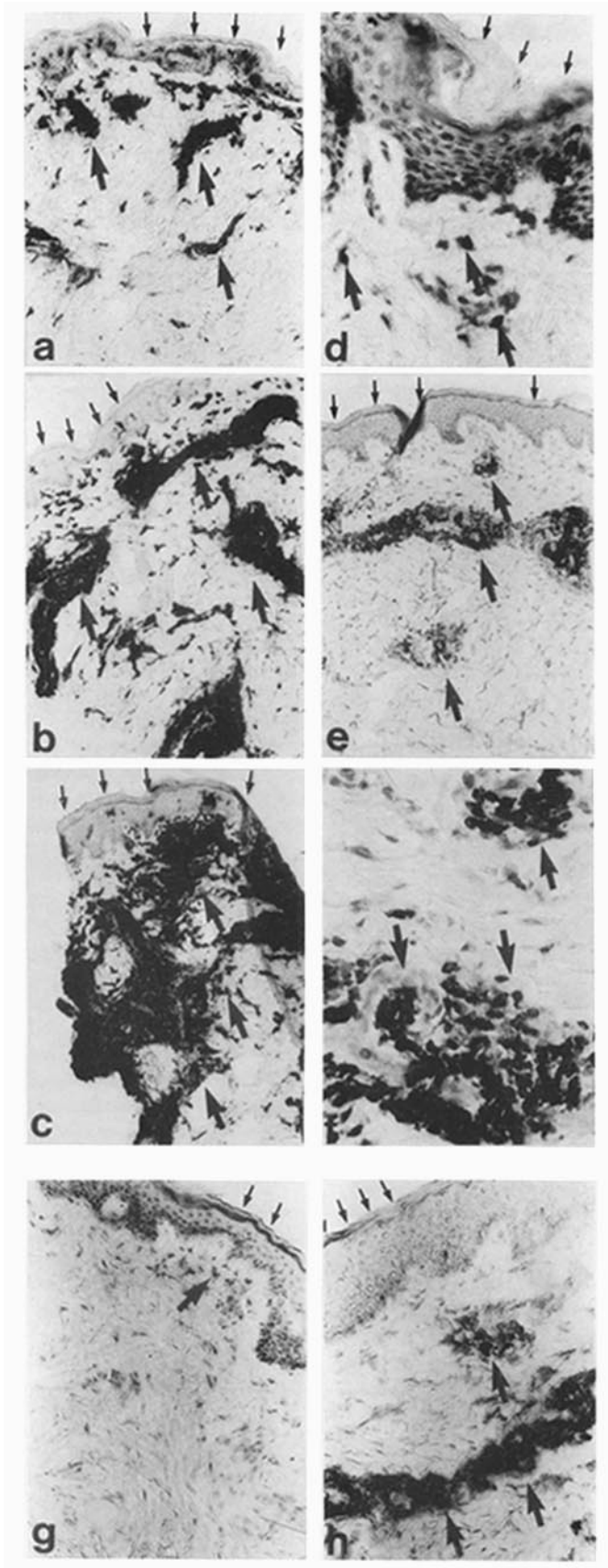
At the lower dose level, the dermis of two subjects showed marked increases in MHC class II–positive cells and T lymphocytes, including cells bearing CD4, CD8, or CD1a (not shown). These changes were evident at day 3, more marked at day 10, and present but diminished by the eighth week. In another patient at the lower dose level, the dermis was already heavily infiltrated with both T cells and macrophages; there was no obvious increase after rIFN-γ administration. One subject showed scant mononuclear cell infiltration both before and after administration of rIFN-γ.

At the higher dose level, three of four subjects showed a massive increase in the area involved by intradermal MHC–positive class II mononuclear cells (Fig. 1, *a–c*). The increased infiltrate included both T cells (Fig. 1, *d–f*) and mononuclear phagocytes (Fig. 1, *g–h*). No granulocytes were observed. Changes were evident by day 3 and progressed thereafter, persisting through the eighth week (Fig. 1 *h*). The one patient in whose skin no histologic changes were noted had begun taking 60 mg of prednisone daily within a few days of completing the rIFN-γ injections (see below).

In contrast to results at the sites of intradermal injection of rIFN-γ (7–10), keratinocytes were not induced to display MHC class II antigens at either dose level (Fig. 1, *a–c*); epithelioid cells became prominent in only one individual (not shown); and the epidermis thickened in only three of the eight subjects (one at the lower dose and two at the higher dose; Fig. 1 *a* vs. *c*, and *g* vs. *h*).

Bacterial Burden. Only two subjects had high initial bacterial indices (3); both received the lower dose of rIFN-γ. Changes in low bacterial indices are difficult to evaluate, especially with small biopsy fragments. No changes were evident over the 8-wk study period.

Neurologic Responses and Other Clinical Effects. Examination by a neurologist, and measurement of conduction velocities in afflicted sensory nerves, were carried out immediately before the first injection and on the day after the last injection of rIFN-γ. No changes were detected. Before and after entry to the study, five patients took clofazimine and/or thalidomide to control nerve tenderness, dysesthesia, and/or sensory impairment attributed to erythema nodosum leprosum. Within 4 mo of rIFN-γ treatment, a patient who had recently been on prednisone resumed it; another subject started it. In each case, signs and symptoms of neuritis resolved



without further loss of function. Mild headaches and myalgia after the initial injection of rIFN-γ at the higher dose were forestalled by administration of acetaminophen. No persistent changes were noted in the blood tests listed in Materials and Methods.

Discussion

Intradermal administration of rIFN-γ to patients with lepromatous leprosy (7) was, to our knowledge, the first attempt to treat a nonviral infectious disease of man with a recombinant product of the immune system. Subsequent trials of rIFN-γ in cutaneous leishmaniasis (13), visceral leishmaniasis (14), and chronic granulomatous disease (15) have achieved therapeutic or prophylactic success. In leprosy, however, no evidence has yet emerged that benefit from cytokine therapy can extend beyond the site of injection.

The present trial, though small, suggests that treatment of lepromatous subjects with 10 daily intramuscular injections of rIFN-γ at 10 or 30 $\mu g/m^2$ per day can lead in a dose-dependent manner to generalized, intradermal mononuclear cell infiltration. It seems remarkable that intramuscular injection of a single cytokine in amounts too small to produce detectable serum levels can elicit complex histologic changes that are remote from the sites of injection and that persist for weeks after clearance of the injected agent. In vitro, IFN-γ can induce its own production (16). Perhaps brief exposure to small amounts of exogenous rIFN-γ triggers sustained production of endogenous IFN-γ by cells in the lesions. The observed changes may also reflect the ability of rIFN-γ to induce and/or synergize with other cytokines. Finally, rIFN-γ is pleiotropic, and may have served as a T or NK cell differentiation factor, endothelial cell activator, and/or monocyte chemotactin, as well as a macrophage activator (17).

Treatment with rIFN-γ by the regimen used here appeared to be safe for patients with lepromatous leprosy receiving standard chemotherapy. This was the case even for patients requiring therapy for erythema nodosum leprosum at the time of admission to the study. rIFN-γ may have exacerbated neuritis in some patients, but given the natural history of the disease, a cause-and-effect relationship was difficult to establish. More important, neuritis remained manageable after administration of rIFN-γ, and no further loss of function was encountered in any of the 11 subjects. Follow-up has lasted 4.5 yr for patients receiving one injection, and 15.6 ± 3.5 mo (mean ± SEM; range, 7–27 mo) for those receiving 10 injections.

Figure 1. Immunocytochemical analysis of skin biopsies from the lower back of an individual with lepromatous leprosy receiving a 10-d course of 30 $\mu g/m^2$ rIFN-γ daily in the triceps muscles. The epidermis is marked by small arrows, and antigen-positive cells by large arrows. (*a–c*) Accumulation of MHC class II–positive cells. (*a*) Pre-treatment; (*b*) 10 d; (*c*) 28 d after onset of rIFN-γ injections. (*d–f*) Accumulation of CD3+ T cells. (*d*) Pretreatment; (*e*) day 28; (*f*) higher power view of *E* to demonstrate the association of reaction product with individual cells. (*g, h*) Accumulation of Leu-M5+ mononuclear phagocytes. (*g*) Pretreatment; (*h*) day 51. (*a–c, e, g, h*) ×46; (*d* and *f*) ×183.

In conclusion, rIFN-γ can induce a partial but widespread reversal reaction of the upgrading variety in individuals with lepromatous leprosy, without prohibitive toxicity. It should now be tested if systemic administration of rIFN-γ over prolonged periods can increase the efficiency of antileprotic chemotherapy.

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Address correspondence to C. Nathan, Box 57, Cornell University Medical College, 1300 York Avenue, New York, NY 10021.

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