Therapy of Chronic Myelogenous Leukemia With Recombinant Interferon-γ

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Recently, we reported that recombinant interferon-α (rIFN-α) can induce hematologic remissions and cytogenetic improvement in newly diagnosed Philadelphia (Ph)-positive chronic myelogenous leukemia (CML) patients. Although IFN-γ is a structurally distinct molecule, this agent suppresses in vitro hematopoietic progenitor cells in a fashion similar to that of rIFN-α. Therefore, we initiated a study of rIFN-γ at doses of 0.25 to 0.5 mg/m²/d intramuscularly in patients with Ph-positive benign-phase CML. Twenty-six of 30 patients entered in the study were evaluable. Six patients have achieved a complete hematologic response; four, a partial hematologic response. The median follow-up period of patients who are in complete remission is 7.5 months (range, 5 to 12 months). No relapses have occurred among the complete responders.

So far, five patients have had cytogenetic improvement with emergence of 5% to 45% diploid cells in the bone marrow. Fever and flu-like symptoms were the most common side effects, with partial tolerance often developing after about 1 week. The majority of patients tolerated therapy with minimal change in performance status. In conclusion, rIFN-γ has demonstrated clinical activity in CML. On the basis of these observations and the in vitro synergistic growth-inhibitory effects of IFN-α and IFN-γ, we have started trials of combination IFN therapy in CML patients.

INTERFERONS (IFN) ARE A GROUP OF naturally occurring proteins with potent antiviral, antiproliferative, and immunoregulatory activities that have stimulated interest in their evaluation in human malignant, infectious, and immunodeficiency diseases. On the basis of their antigenic and physicochemical properties, the IFNs are divided into three classes: IFN-α (leukocyte), IFN-β (fibroblast), and IFN-γ (immune). IFN-α is a family of at least 14 highly homologous species produced by leukocytes. IFN-β is produced by fibroblasts and epithelial cells. IFN-γ is structurally related to IFN-β, and both are virally induced, acid-stable molecules. In contrast, IFN-γ is a structurally distinct, acid-labile glycoprotein produced primarily by T lymphocytes in response to antigen or mitogen.

α-IFNs have been demonstrated to have significant antitumor activity in hairy cell leukemia and other tumors. A large body of evidence demonstrates significant antacellular effects of IFN-α on normal myeloid stem cells as well as chronic myelogenous leukemia (CML) progenitor cells in vitro. In addition, we have recently reported that recombinant IFN-α (rIFN-α) can induce hematological remissions and, more importantly, cytogenetic improvement in Philadelphia (Ph) chromosome-positive CML patients. These results were of special interest because the invariably fatal course of CML has not been significantly altered by chemotherapy. More recently, we reported that recombinant IFN-α and IFN-γ are combined; this may be related to their binding to distinct human cell surface receptors. These synergistic properties may eventually be exploitable in the treatment of leukemia. Therefore, we initiated an investigation to ascertain the tolerance and efficacy of IFN-γ in benign-phase CML patients.

MATERIALS AND METHODS

IFN. The isolation, manufacture, characterization and purification of human rIFN-γ were performed by Genentech, Inc. The gene for human IFN-γ was cloned and expressed in Escherichia coli by methods previously described. Replication of the IFN gene occurs as the E. coli bacteria divide, thus permitting the production of large amounts of rIFN-γ. The material used in this investigation is identical in sequence to native human IFN-γ except for the presence of a methionine residue at the N-terminal. The final purity of rIFN-γ was 98% as determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The direct inoculation technique was used to confirm sterility. The final product contains 0.5 mg endotoxin/mg protein as determined by the limulus amoebocyte lysate assay. The specific activity of rIFN-γ was approximately 2 x 10⁷ U/mg protein based on antiviral activity assessed by inhibition of encephalomyocarditis virus replication in A549 cells (human lung carcinoma cell line), corrected to the reference IFN standard Gg 23-901-530 of the National Institutes of Health, Bethesda, MD.

The IFN was provided in the form of a sterile lyophilized powder. Immediately before administration, the powder was reconstituted with sterile water to a concentration of 1.0 mg/mL.

Patient selection. CML patients whose disease was in the benign phase were entered in the study. Patients were considered to be in benign phase if blasts constituted less than 15% of bone marrow cells, their disease was responsive to hydroxyurea, and in patients whose disease had been diagnosed more than 1 year before the study, cytogenetic analysis showed no evidence of clonal evolution.

Eligibility criteria were as follows: Karnofsky performance status 50%,20 been diagnosed more than 1 year before the study, no antileukemia treatment for at least 2 weeks before initiation of IFN treatment, evidence of relapsing leukemia reflected by increased WBC counts to levels ≥20 x 10⁹/μL, life expectancy of at least 12 months, and bone marrow blasts ≤15%.

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943
least 3 months, and preserved hepatic (bilirubin ≤1.5 mg/dL) and renal function (creatinine ≤2 mg/dL). Patients requiring ongoing therapy with corticosteroids or nonsteroidal antiinflammatory drugs were excluded from the study. Informed consent was obtained from all patients according to institutional policy.

Treatment plan. The patients were followed in the outpatient clinic of The University of Texas M.D. Anderson Hospital and Tumor Institute at Houston for the first 2 weeks and then continued their treatment at home. The treatment regimen was chosen on the basis of our experience in phase I studies, which showed that rIFN-γ is absorbed after intramuscular (IM) administration and demonstrates many biologic properties in vivo including activation of macrophages. The maximum tolerated dose determined in our phase I trial was used in this study. Patients received 0.25 mg/m²/d of IFN administered rIFN-γ for seven days. If this dose was tolerated with a ≥20% decrease in Karnofsky performance status, the dose was increased to 0.5 mg/m²/d thereafter. This dose was reduced by 50% in case of any of the following toxicities: a ≥30% decline in Karnofsky performance status, an increase in serum liver enzymes five or more times the normal values, or an increase in the serum creatinine level to ≥2.5 mg/dL. A further 50% dose reduction was incorporated if toxicities continued. The induction phase of the study lasted 6 weeks. At the end of induction, all patients with evidence of complete or partial hematologic remission, or hematologic improvement, were maintained on the same dose and schedule of rIFN-γ.

Each patient had a physical examination before the study, and the size of the spleen and liver was assessed. Complete blood cell counts, differential, and platelet counts were performed twice in the four days preceding the first injection. Pretreatment assessment included 12-channel blood chemical analysis, serum electrolyte analysis, determination of serum lactate dehydrogenase level, anti-rIFN-γ antibody assay, leukocyte alkaline phosphatase score, urinalysis, ECG, and technecium 99 scan of the liver and spleen. Bone marrow biopsy and aspiration samples were evaluated for cellularity, morphology, and cyto genetic analysis. The cyto genetic techniques used on the bone marrow have been previously described, and ≥20 metaphases were counted.

During the course of the study, the CBCs were repeated two to three times weekly during the first month and two times per month thereafter. Bone marrow aspiration, biopsy and cytogenetics, and liver/spleen scan were repeated every 2 to 3 months.

Criteria for response. A complete hematologic remission required normalization of the peripheral WBC counts to levels of less than 9 × 10⁹/µL with normal differential counts and no immature forms (blasts, promyelocytes, myelocytes, or metamyelocytes) and the disappearance of all clinical symptoms and signs of disease including palpable splenomegaly. A partial hematologic remission was defined as a decrease in the WBC counts to at least 50% of pretreatment levels and to less than 20 × 10⁹/µL. Patients whose peripheral WBC counts had normalized but who had persistent splenomegaly or immature peripheral cells were included in this category. Treatment was considered to have failed for all patients who had less than a partial hematologic remission.

IFN antibody assays. The presence of antibodies to rIFN-γ was determined on blood samples obtained before and after completion of the study as well as every month while the patients received IFN. Testing for antibodies was performed by a radioimmunoprecipitation assay. The patient's serum was incubated with ¹²⁵I-labeled rIFN-γ, which was followed by immunoprecipitation with goat antihuman IgG. The radioimmunoprecipitation assay is capable of detecting neutralizing antibody and is approximately ten- to 100-fold more sensitive than the neutralization assay for equivalent epitopes (Genentech, data on file).

RESULTS

Patient characteristics. Thirty CML patients (19 men and 11 women) were entered in the study. Four patients were considered invaluable for response because of a major protocol violation (two patients), noncompliance (one patient), and toxicity requiring discontinuation of therapy on day 10 (one patient). The median age of the evaluable patients was 47 years (range, 22 to 66 years). All patients were in the benign phase of the disease. Their median time from diagnosis was 9 months (range, 1 to 60 months). Twenty of the evaluable patients had prior chemotherapy, usually with hydroxyurea and/or busulphan.

The karyotype of patients was as follows: 23 patients, t(9;22) in 100% of bone marrow metaphases; one patient, t(4;9;22); one patient, t(8;17)(9;22) in 90% of metaphases and t(9;22) in the remaining 10% of metaphases; and one patient, diploid. On Southern blot analysis, the latter patient had the rearrangement in the breakpoint cluster region (bcr) on chromosome 22 characteristic of Ph-positive CML. Such patients have previously been shown to have a clinical course indistinguishable from that of patients in whom the Ph chromosome can be demonstrated by cytogenetic analysis.

Response. Six patients achieved a complete hematologic remission, four, a partial hematologic remission. Pre- and posttherapy hematologic parameters of these patients are shown in Table 1. Complete responders showed a normalization of WBC and platelet counts and the disappearance of immature cells from the peripheral blood. Bone marrow changes included a decrease in the cellularity from a median baseline value of 100% (range, 90% to 100%) to a posttherapy value of 75% (range, 50% to 100%). The bone marrow blasts decreased from a baseline value of 10% (range, 2.6% to 14.6%) to a posttherapy value of 3.2% (range, 1.2% to 5.0%). In the patients failing rIFN-γ therapy, therapy was generally discontinued because of rising or unresponsive WBC counts; no patient developed blast crisis while on study. The median interval to complete hematologic remission was 1 month (range, 0.5 to 5 months). The median follow-up period of patients who achieved a complete hematologic remission is 7.5 months (range, 5 to 12 months). There have been no relapses among the complete responders. To date, five of six
complete responders have shown evidence of cytogenetic improvement, with emergence of normal diploid metaphases in the bone marrow (Table 2). At the time of therapy initiation, the complete responders had favorable disease characteristics in that severe thrombocytosis or splenomegaly was not present. The median time from diagnosis of the complete responders was 10 months (range, 1 to 54 months).

Prior therapy included the following agents: hydroxyurea (three patients), busulphan (three patients), Adriamycin-containing regimen (one patient); and no prior therapy (two patients). The median duration of prior therapy was 2 months (range, 0 to 11 months).

Control of thrombocytosis was achieved in all four patients, with baseline platelet counts >1,000 x 10^3/μL. In these patients, the median (range) pretherapy platelet count was 1,200 x 10^3/μL (range, 1,112 to 1,458 x 10^3/μL); the median (range) posttherapy nadir count was 450 x 10^3/μL (range, 220 to 650 x 10^3/μL). However, the WBC counts of these patients remained elevated. Conversely, hydroxyurea had previously been able to control their WBC counts but not their thrombocytosis.

Five of 26 evaluable patients had prior treatment with IFN-α: three patients with rIFN-α (IFN-α-2a, Hoffmann-LaRoche, Inc, Nutley, NJ) and two patients with partially purified IFN-α (Cantell Finnish Red Cross, Helsinki). Of these five patients, one has achieved a complete hematologic remission with rIFN-γ therapy; two, a partial hematologic remission; five patients who failed rIFN-γ therapy were subsequently treated with rIFN-α. Two of these patients have had a complete hematologic response; one, a partial hematologic response.

**Side effects.** Most patients tolerated rIFN-γ treatment with minimal change in performance status. Although fever, night sweats, and flu-like symptoms occurred in the majority of patients (Table 4), partial tachycardias generally developed within 1 to 2 weeks. About one third of patients experienced mild myalgias. One patient who had a history of confusion after rIFN-α therapy became confused on day 10 of rIFN-γ treatment. Computerized tomographic scan of the brain was normal, and an EEG showed only diffuse brain wave slowing. Recovery occurred within 2 weeks after discontinuation of rIFN-γ injections. Elevation in liver enzymes to two to three times baseline levels occurred in approximately one third of patients. Consistent with the results of our previous studies, rIFN-γ had a marked effect on lipid metabolism, with hypertriglyceridemia occurring in virtually all patients.26 These changes were reversible within 2 weeks after stopping IFN administration. A >30% reduction in platelet counts was noted in all patients. Even so, neither neutropenia (granulocyte count ≤1 x 10^9/μL) nor thrombocytopenia (platelet count ≤50 x 10^3/μL) was observed.

**DISCUSSION**

Single-agent chemotherapy can control the proliferative thrust of benign-phase CML but does not suppress the Ph chromosome or alter the inevitable progression towards blast transformation.27 Aggressive combination chemotherapy results in a reproducible but transient decrease in the Ph chromosome level.28-30 The majority of patients are ineligible for the only potentially curative therapy: supralethal chemotherapy and allogeneic bone marrow transplantation.31,32 Therefore, exploration of other possible therapeutic avenues is important.

Previously, we have demonstrated that rIFN-α can induce hematologic remissions and cytogenetic improvement in newly diagnosed benign-phase CML patients.12 Although IFN-γ does not bear significant homology to IFN-α, the in vitro suppressive effects of these two agents on myeloid colonies are similar.13,14,33 Furthermore, McGlave et al33 have recently demonstrated that in parallel studies of CML progenitor cells cultured with and without IFN-γ the presence of interferon resulted in a substantial increase in the

<table>
<thead>
<tr>
<th>Sequence</th>
<th>No. of Patients</th>
<th>Response to Interferon</th>
</tr>
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<tbody>
<tr>
<td>α → γ</td>
<td>5</td>
<td>1* 2* 2*</td>
</tr>
<tr>
<td>γ → α</td>
<td>5</td>
<td>2† 1† 2†</td>
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</tbody>
</table>

**Table 2. Changes in the Percentage of Ph-Positive Bone Marrow Cells Among CML Patients Who Achieved Complete Hematologic Remission**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Ph-Positive Metaphases (%)</th>
<th>3 mo</th>
<th>6 mo</th>
<th>9 mo</th>
<th>12 mo</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>80</td>
<td>50</td>
<td>55</td>
<td></td>
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<tr>
<td>2</td>
<td>100</td>
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<td>3</td>
<td>100</td>
<td>90</td>
<td>95</td>
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<td>100</td>
<td>95</td>
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<td>6</td>
<td>100</td>
<td>100</td>
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**Table 3. Response of CML Patients Who Are Resistant to One IFN to an Alternate IFN**

**Table 4. Side Effects Experienced by 30 CML Patients Treated With rIFN-γ**

<table>
<thead>
<tr>
<th>Side Effect</th>
<th>No. Patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever (&gt;1.3°C)</td>
<td>30 (100) [4]</td>
</tr>
<tr>
<td>Night sweats</td>
<td>25 (83)</td>
</tr>
<tr>
<td>Chills</td>
<td>20 (66)</td>
</tr>
<tr>
<td>Myalgias</td>
<td>20 (66)</td>
</tr>
<tr>
<td>Headaches</td>
<td>14 (46)</td>
</tr>
<tr>
<td>Confusion</td>
<td>13 (43)</td>
</tr>
<tr>
<td>Hypertriglyceridemia*</td>
<td>22 (73)</td>
</tr>
<tr>
<td>Hepatic toxicity†</td>
<td>10 (33)</td>
</tr>
</tbody>
</table>

Numbers in brackets indicate the numbers of patients in whom side effects were severe.

*Triglyceride levels increased to ≥ 1.5-fold baseline levels.
†SGOT levels increased to twofold baseline levels.
emergence of Ph-negative metaphases. These data prompted us to investigate the clinical effects of rIFN-γ in CML patients.

The present study indicates that approximately 25% of benign-phase, Ph-positive CML patients will attain a complete hematologic remission when receiving rIFN-γ therapy. Our experience with rIFN-α indicates that approximately 40% of complete responders will show cytogenetic improvement.12 However, reemergence of diploid cells in the bone marrow requires at least 3 to 12 months of continuous treatment. Furthermore, the reduction in Ph-positive metaphases is ongoing, with maximal responses often observed in the second year of therapy.14 Therefore, it was of interest that all five complete responders treated with rIFN-γ for ≥6 months demonstrated the reappearance of normal diploid metaphases in the bone marrow. Because at present the median duration of treatment is only 7.5 months, a longer follow-up of these patients will be essential to ascertain whether ongoing improvement will occur, as in the case of IFN-α.

Thrombocytosis occurs frequently among CML patients.15 The morbid nature of severe thrombocytosis can be manifested clinically as thromboembolic and hemorrhagic phenomena.35,36 Although control of the WBC count can be achieved with chemotherapeutic agents such as hydroxyurea, the concomitant control of platelet counts often becomes increasingly difficult. One to 5 weeks of rIFN-γ therapy resulted in normalization of platelet counts in all four patients with baseline counts ≥1,000 × 10^9/L. However, significant cytoreduction of peripheral blood leukocytes was not achieved in these patients. We have previously described a similar phenomenon in CML patients treated with rIFN-α.37 An increase in the autonomous proliferative capacity of platelet precursors has been suggested as an indicator of disease progression in CML.38 Therefore, the discrepancy in control of platelets and WBC counts in these patients may reflect the association of more advanced disease with emergence of a resistant malignant clone. Interestingly, these patients’ leukocyte and platelet counts responded differently to chemotherapy and IFN; hydroxyurea controlled the WBC count but not the platelet count. These data suggest that a combination of IFN and hydroxyurea may be beneficial in CML patients with severe thrombocytosis.

Side effects of rIFN-γ usually consisted of fever, chills, night sweats, and myalgias, with tachyphylaxis often occurring after the first week of therapy. These symptoms were qualitatively similar to those experienced by patients taking IFN-α. However, the severe neuromuscular pain occasionally described in IFN-α–treated CML patients37 was not observed after rIFN-γ therapy. Most patients were able to tolerate 0.25 to 0.5 mg/m²/d of IM rIFN-γ with minimal disruption of their usual daily activities.

Neutralizing antibodies to rIFN-α occur in approximately 10% of patients treated with this agent and may be associated with abrogation of some of its biologic activities.39 In contrast, antibody to rIFN-γ was not detected in any patient in this study. Further, anti-rIFN-γ antibody has not developed in any of the approximately 300 patients treated with this agent and tested for antibody (Genentech, Inc, data on file).

In conclusion, the present study indicates that rIFN-γ has clinical antitumor activity in CML. Furthermore, it is possible for patients who are resistant to IFN-α to respond to IFN-γ, and vice versa. On the basis of these results and the in vitro synergistic antiproliferative effects of the two IFNs,13 we have initiated studies of combination IFN therapy in CML.

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