Synergistic Antiproliferative Effect of Recombinant Interferon-γ With Recombinant Interferon-α on Chronic Myelogenous Leukemia Hematopoietic Progenitor Cells (CFU-GEMM, CFU-Mk, BFU-E, and CFU-GM)

By Carmelo Carlo-Stella, Mario Cazzola, Arnold Ganser, Gaetano Bergamaschi, Paolo Pedrazzoli, Dieter Hoelzer, and Edoardo Ascarì

Recombinant interferons, α (rIFN-α) and γ (rIFN-γ), have been demonstrated to have significant antitumor activity as single agents in the treatment of chronic myelogenous leukemia (CML). Due to their possible synergistic efficacy, a combination rIFN therapy in CML has been proposed. To establish a biologic basis for this, we have studied the suppressive effects of rIFN-α and rIFN-γ on the in vitro growth of CML-derived progenitor cells (CFU-GEMM, CFU-Mk, BFU-E, CFU-GM). The optimal conditions for rIFN synergism, and the possible role of hematopoietic accessory cells (T-lymphocytes and monocytes-macrophages) in mediating rIFN-induced growth inhibition. When added to unseparated bone marrow cells, rIFN-α and rIFN-γ significantly reduced colony formation, with 50% inhibition occurring at 71 and 186 U/mL for CFU-GEMM, 40 and 152 U/mL for CFU-Mk, 222 and 1,468 U/mL for BFU-E, and 119 and 442 U/mL for CFU-GM, respectively. A small amount of rIFN-γ (5 U/mL) acted synergistically with increasing doses of rIFN-α, and the values of 50% inhibitory concentrations fell outside the lower limit (10 U/mL) used in our experiments. This synergy was evident even when rIFN-γ was added 72 hours after the initiation of cultures; however, it was completely lost when the target cells were depleted of accessory cells. When a low dose of rIFN-α (5 U/mL) was added to rIFN-γ, the 50% inhibitory concentration values were decreased up to tenfold. These studies (1) confirm that CML-derived hematopoietic progenitors are responsive to the suppressive activity of both rIFN-α and rIFN-γ in vitro, (2) demonstrate that different mechanisms are responsible for the suppressive activity of the two rIFNs, and (3) characterize their synergistic interaction, providing a basis for future clinical trials aimed at investigating combination rIFN therapy in CML.

CHRONIC MYELOGENOUS leukemia (CML) is a clonal myeloproliferative disorder arising from a neoplastic transformation at the level of the pluripotent hematopoietic stem cell.1 The typical clinical course of CML progresses from the chronic through to the accelerated to the blastic phase.2,3 Conventional single-agent chemotherapy that controls the benign phase of the disease does not suppress the Philadelphia (Ph') chromosome or alter the progression toward blast transformation.4 Recombinant interferon-α (rIFN-α), and more recently, recombinant interferon-γ (rIFN-γ) have been reported to induce complete hematologic remission and cytogenetic improvement in about 80% and 25%, respectively, of benign-phase Ph'-positive CML patients.4,5 The possible appearance of resistant clones remains one of the major limitations of treatment with rIFNs.6,7

Interferons (IFNs) are a group of naturally occurring inducible proteins known to exert, in addition to their antiviral activity, several biologic effects, including antiproliferative, immunomodulating, and differentiative actions.8,9 rIFN-α and rIFN-γ markedly suppress the in vitro proliferation of pluripotent hematopoietic progenitor cells (colony-forming unit-granulocyte, erythrocye, macrophage, megakaryocyte [CFU-GEMM]), as well as lineage-restricted progenitor cells (colony-forming unit-megakaryocyte [CFU-Mk]; burst-forming unit-erythroid [BFU-E]; colony-forming unit-granulocyte-macrophage [CFU-GM]) from normal subjects10,14 and patients with myeloproliferative disorders.15-17 Broxmeyer et al18 and Raefsky et al19 demonstrated a synergistic antiproliferative effect of rIFN-γ with rIFN-α on normal human bone marrow-derived progenitor cells, but no information is yet available concerning a possible synergism between rIFNs in suppressing the in vitro growth of CML-derived hematopoietic progenitor cells. Therefore, it was the aim of the present study to evaluate the effect of highly purified rIFN-α and rIFN-γ, alone and in combination, on the bone marrow-derived pluripotent and lineage-restricted progenitors from patients with CML. In addition, the role of hematopoietic accessory cells (T-lymphocytes and monocytes-macrophages) in mediation of the rIFN antiproliferative synergy was investigated.

MATERIALS AND METHODS

Patients. Ten patients with Ph'-positive CML were studied. The diagnosis was based on peripheral blood leukocytosis, low alkaline phosphatase activity in circulating neutrophils, and the presence of the Ph' chromosome in the metaphases of marrow cells. The CML patients had been diagnosed from 1 month to 1 year prior to the time of the study, and all were still in the chronic phase of the disease. All had 100% Ph'-positive metaphases in the bone marrow on direct cytogenetic analysis. Four patients had received prior treatment with busulphan; however, both had been off treatment at least 1 week before the studies were performed.

Cell separation procedures. After informed consent, bone marrow was obtained from patients by aspiration from the posterior iliac crest at the time of examination for clinical evaluation. Normal bone marrow was obtained from five hematologically healthy donors at

From the Department of Internal Medicine, University of Pavia, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS Policlinico S. Matteo, Italy, and the Department of Hematology, University of Frankfurt, Frankfurt, West Germany. Submitted March 1, 1988; accepted June 7, 1988. Supported in part by Grant No. 87.01,2244 from the Italian National Research Council, Special Project Oncology, and a grant from Associazione Italiana per la Ricerca sul Cancro.

Dr Carlo-Stella is a research fellow of the IRCCS Policlinico San Matteo, Pavia.

Address reprint requests to C. Carlo-Stella, MD, Clinica Medica II, Policlinico S. Matteo, I-27100 Pavia, Italy.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1988 by Grune & Stratton, Inc.


1293
the time of cardiac surgery and studied in parallel. To reduce contamination with peripheral blood, the volume of the aspirate was limited to 1.5 to 2.0 mL. Mononuclear light density bone marrow cells (LDBMC) were separated by centrifugation on a Ficoll-Hypaque gradient (density, 1.077 g/mL) at 400 g for 40 minutes at 20°C. Interface cells were washed three times in RPMI 1640 medium ( Gibco, Grand Island, NY) and suspended in Iscove’s modified Dulbecco’s medium (IMDM) (Seromed, Berlin, FRG). Unless otherwise noted, LDBMC were cultured without further separation procedures.

In three separate experiments, the effect of rIFNα was tested on unseparated LDBMC and nonadherent, T-lymphocyte-depleted LDBMC (LDBMC-AC–T–) derived from the same marrow samples. Cell separation techniques have been described in detail elsewhere. Briefly, removal of the adherent monocyte-macrophage cells (AC) was achieved by incubation (37°C, 5% CO2) of LDBMC (5 x 10^6/mL), suspended in RPMI 1640 medium with 20% fetal calf serum (FCS) (Flow Laboratories, Irvine, UK), for two sequential one-hour periods in 25-cm² plastic tissue culture flasks. T-lymphocytes were removed by rosetting for two hours with 2-ethylisothiouronium bromide-treated (AET) (Sigma, St Louis) sheep red blood cells (SRBC) at 4°C, followed by another density gradient centrifugation on Ficoll-Hypaque (1.077 g/mL). The purity of cell fractions obtained after the removal of adherent cells and T-lymphocytes was checked by an immunoalkaline phosphatase method with monoclonal antibodies CD3-OKT3 (Ortho Pharmaceutical, Raritan, NY) directed against mature T cells, and CD14-MY4 (Coulter, Hialeah, Fla) directed against monocytes-macrophages; the second antibody used was the goat antimouse IgG conjugated with alkaline phosphatase (Sigma). After cell separation procedures, LDBMC-AC–T– were >95% MY4-negative and >99% OKT3-negative. The viability of these cell fractions, as determined by trypan blue dye exclusion, was always >95%.

In vitro assays. The assay for CFU-GEMM, CFU-Mk, BFU-E, and CFU-GM has been previously described. Briefly, 1 x 10^7 LDBMC or LDBMC-AC–T– obtained after separation procedures were plated in 35-mm petri dishes (Miles Laboratories, Naperville, Ill) in the presence and absence of different concentrations of rIFNα or rIFNγ, alone or in combination, in 1-mL aliquots of IMDM containing 30% human fresh frozen plasma from a single donor; 5% medium conditioned by peripheral blood leukocytes (PHA-LCM) in the presence of 1% phytohemagglutinin (PHA-HA 15) (Burroughs Wellcome, Research Triangle Park, NC); 5 x 10^-3 mol/L 2-mercaptoethanol (Sigma); 1 unit recombinant erythropoietin, provided by Dr J.W. Adanson; and 0.9% (wt/vol) methylcellulose. After incubation for 14 days at 37°C in a humidified atmosphere supplemented with 5% CO2, the cultures were examined with an inverted microscope. Four dishes were set up for each individual data point per experiment. Mixed colonies (CFU-GEMM), defined as containing at least erythroid and granulocytic cells by their in situ appearance, megakaryocytic colonies (CFU-Mk), defined as containing more than eight cells of varying size characterized by a translucent hyaline cytoplasm and highly refractile cytoplasmic border under the inverted microscope, erythroid bursts (BFU-E) with >500 cells, and granulocyte-macrophage colonies (CFU-GM) with >40 cells were all scored from the same plate. In some experiments, a representative number of CFU-Mk was picked up and immunologically analyzed with the monoclonal antibody C 17.28 (provided by Dr P. Lansdorp, Amsterdam) directed against platelet glycoprotein IIIa. Several batches of PHA-LCM were used. These were always prepared by peripheral blood mononuclear cells from the same individual and tested for comparable stimulating activity. Similarly, a single normal donor was used to provide human plasma throughout the entire study.

Recombinant IFNs and monoclonal antibodies against IFNs. rIFNα (specific activity: 3.3 x 10^6 U/mg of protein) was provided by Karl Thomae Co, Biberach, FRG; rIFNγ (specific activity: 2.4 x 10^7 U/mg of protein) was provided by Biogen Research Corp, Cambridge, Mass, and provided by Bioferon, Laupheim, FRG. The interferon bioassay was performed in the HEP-2 cell line/Mengo virus assay. The monoclonal antibodies EB-1 against rIFNα and GZ-4 against rIFNγ, provided by Karl Thomae Co, were used as a 1:200 dilution of mouse ascites. To neutralize the activity of rIFNs, monoclonal antibodies were incubated with rIFN preparations for 90 minutes at room temperature before culture.

Statistical analysis. Four plates per experiment were scored for each data point, and the results were expressed as the mean ± SEM. Statistical analysis was performed with the statistical package Statview (BrainPower Inc, Calabasas, Calif) run on a Macintosh Plus personal computer (Apple Computer Inc, Cupertino, Calif). The student’s t test for paired data was used to test the probability of significant differences between samples. IFN concentrations, resulting in 50% inhibition of colony formation (ID50), were calculated for each experiment by extrapolating from a least square linear regression line relating the logarithm of the IFN concentration to the percentage of colony-formation inhibition. Statistical significance between the geometric means of these concentrations was evaluated by the student’s t test.

RESULTS

Effect of rIFNs on hematopoietic colony formation from LDBMC. When CML-derived LDBMC were exposed throughout the entire culture period to rIFNα or rIFNγ alone, a statistically significant, dose-dependent suppression of colony growth from CFU-GEMM, CFU-Mk, BFU-E, and CFU-GM was observed (Fig 1). The degree of colony growth suppression was not related to the cloning efficiency of the individual samples. Regression analysis with the least squares method revealed that inhibition was linear with the logarithm of the rIFN concentration over the range tested (10 to 10^4 U/mL). The rIFNα and rIFNγ concentrations that produced ID50 were 71 and 186 U/mL for CFU-GEMM, 40 and 152 U/mL for CFU-Mk, 222 and 1,458 U/mL for BFU-E, and 119 and 442 U/mL for CFU-GM, respectively (Fig 1). Although individual patients exhibited variable sensitivity to the inhibitory effect of rIFNs, no one failed to respond to rIFNα, and in only two patients was BFU-E growth, but not that of CFU-GEMM, CFU-Mk, and CFU-GM, unaffected by rIFNγ, with erythroid colony formation being reduced by <40% even at 10^6 U/mL. The inhibitory effect was completely removed when the rIFNs were preincubated with the neutralizing monoclonal antibody specifically directed against the appropriate IFN type (data not shown). In five normal subjects, a dose-dependent suppression of colony growth was also observed. Regression analysis showed that inhibition was linear with the logarithm of the rIFN concentration over the range tested (10 to 10^4 U/mL). IFN concentrations resulting in ID50 were not significantly different from those observed in patients with CML (Table 1).

To determine whether or not the effect of rIFNα and rIFNγ in combination resulted in synergistic antiproliferative activity on CML-derived hematopoietic progenitors, a
small constant amount of one rIFN, in a concentration (5 U/mL) that in our culture system had no inhibitory effect on progenitor cell growth, was added to increasing amounts of the other rIFN (10 to 10^4 U/mL). The combination of 5 U/mL of rIFN-γ and increasing amounts of rIFN-α was significantly more potent in inhibiting colony formation by CFU-GEMM, CFU-Mk, BFU-E, and CFU-GM than rIFN-α alone (Fig 2). By adding 5 U/mL of rIFN-γ to IFN-α, the concentration of rIFN-α that caused ID₅₀ could be significantly reduced for all classes of hematopoietic progenitors assayed (Fig 2). In fact, these inhibitory concentration levels fell outside the lower limit used in our experiments, thus demonstrating a potent synergistic antiproliferative effect of rIFN-γ with rIFN-α (Table 2). When a small constant amount of rIFN-α (5 U/mL) was added to increasing doses of rIFN-γ, the antiproliferative synergy was less pronounced (Fig 3), with the values of the 50% inhibitory concentrations being reduced two- to tenfold (Table 2). The combination of rIFNs resulted in a synergistic antiproliferative effect in all samples studied. This synergism was evident even in the two patients who were partially unresponsive to rIFN-γ alone. Similar findings were demonstrated in five normal subjects studied in parallel experiments (Table 2).

To investigate whether the antiproliferative synergism of rIFN-γ with rIFN-α was dependent on the presence of both of them in culture from the beginning of the incubation period, a series of experiments was conducted in which LDBMC were exposed throughout the entire culture period to rIFN-α (500 U/mL), whereas either rIFN-γ (5 U/mL in 100 μL) or control medium (IMDM, 100 μL) was added on the first, second, and third day following the initiation of marrow cultures. As shown in Table 3, small amounts of rIFN-γ acted synergistically with rIFN-α in suppressing colony formation even when added 72 hours after the initiation of cultures.

Effect of rIFNs on hematopoietic colony formation from LDBMC--α−β−γ−τ− A possible mechanism of action for this rIFN-γ–related synergistic antiproliferative activity was...
A significant dose-dependent inhibition of accessory cells, ie, monocytes-macrophages and T-lymphocytes, or in combination, on test for paired data (two-tailed values). For paired experiments, each involving a different patient. The percentage of inhibition was calculated by reference to values obtained in control cultures.

The results of these experiments are summarized in Table 4. A significant dose-dependent inhibition of rIFN-a and rIFN-γ on CML-derived pluripotent, erythroid, and granulocyte-macrophage progenitor cell growth from LDBMC-AC-T- was observed when rIFN-α was added in culture. The synergistic inhibitory effect seen by adding small amounts of rIFN-γ (5 U/mL) to rIFN-α was completely lost when the target cells were depleted of accessory cells. The inhibitory activity of rIFN-γ was only evident using LDBMC as target cells, whereas its effect was negligible after depletion of accessory cells (Table 4).

**DISCUSSION**

The recent reports that rIFN-α and rIFN-γ can induce hematologic remissions and cytogenetic improvement in newly diagnosed Ph'-positive CML patients have been of considerable interest. In fact, conventional chemotherapeutic agents that control the benign phase of the disease do not suppress the Ph' chromosome. Therefore, it is hoped that agents able to suppress the abnormal clonal marker could improve the invariably progressive clinical course of CML. Furthermore, since rIFNs are synergistic in vitro, combination IFN therapy may allow even better results than single IFN therapy.

Considerable evidence indicates that rIFNs have anticleolar effects on normal and CML-derived hematopoietic progenitor cells in vitro. While the inhibitory effect of rIFNs on CML-derived pluripotent, erythroid, and granulocyte-macrophage progenitor cells has already been demonstrated, the suppressive effect of rIFN-α and rIFN-γ on CML-derived megakaryocytic progenitor cells (CFU-Mk) is described for the first time in this study. Both rIFNs are potent inhibitors of in vitro megakaryocytopenesis in CML patients, with dose dependence being similar to that observed for the other classes of CML-derived progenitor cells as well as for normal progenitors. In our culture system, rIFNs showed no specificity in inhibiting the growth of CML-derived progenitors, ie, the growth of malignant and normal progenitors, as demonstrated.

**Table 2. Concentration of rIFNs Resulting in a 50% Reduction of Colony Formation in Patients With CML and in Normal Subjects**

<table>
<thead>
<tr>
<th>Type of rIFNs</th>
<th>CFU-GEMM</th>
<th>CFU-Mk</th>
<th>BFU-E</th>
<th>CFU-GM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with CML (n = 10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rIFN-α</td>
<td>38</td>
<td>30</td>
<td>241</td>
<td>115</td>
</tr>
<tr>
<td>rIFN-α +</td>
<td>&lt;10†</td>
<td>&lt;10†</td>
<td>&lt;10†</td>
<td>&lt;10†</td>
</tr>
<tr>
<td>rIFN-γ (5 U/mL)*</td>
<td>175</td>
<td>131</td>
<td>1.439</td>
<td>360</td>
</tr>
<tr>
<td>rIFN-γ +</td>
<td>61‡</td>
<td>71‡</td>
<td>122‡</td>
<td>96‡</td>
</tr>
<tr>
<td>rIFN-α (5 U/mL)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal subjects (n = 5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rIFN-α</td>
<td>99</td>
<td>20</td>
<td>70</td>
<td>58</td>
</tr>
<tr>
<td>rIFN-α +</td>
<td>&lt;10†</td>
<td>&lt;10†</td>
<td>&lt;10†</td>
<td>&lt;10†</td>
</tr>
<tr>
<td>rIFN-γ (5 U/mL)*</td>
<td>350</td>
<td>183</td>
<td>200</td>
<td>739</td>
</tr>
<tr>
<td>rIFN-γ +</td>
<td>177‡</td>
<td>41‡</td>
<td>40‡</td>
<td>238‡</td>
</tr>
<tr>
<td>rIFN-α (5 U/mL)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Data concerning the effect of rIFNs in combination with one another were obtained from experiments performed concomitantly with those exploring the effect of each rIFN singly.

†Significantly different (P value at least <.05) when compared with rIFN-α alone. Inhibiting doses resulting in 50% colony formation suppression were estimated for each experiment and compared by student's t test for paired data (two-tailed values).

‡Not significantly different when compared with rIFN-γ alone.
normal progenitor cells was equally inhibited, at least when this specificity was evaluated quantitatively as the rIFN concentration resulting in an ID_{50}. However, MacGlash et al., evaluating specificity at the cyogenetic level, reported that rIFN-γ might selectively suppress Ph1-positive hematopoietic cells.

Synergy between rIFN-γ and rIFN-α in antiviral and antiproliferative assays has been reported. Our data show that a low dose of rIFN-γ acts synergistically with increasing amounts of rIFN-α and induces a strong inhibition of colony formation by CML-derived bone marrow, with a similar degree of inhibitory synergism as that reported by Broxmeyer et al.\(^\text{11}\) for normal bone marrow progenitors. The antiproliferative synergy on CML-derived progenitors is less evident when a low dose of rIFN-α is added to increasing doses of rIFN-γ, as demonstrated by Raefsky et al.\(^\text{19}\) for normal bone marrow CFU-GM and BFU-E. Since we tested only one concentration of rIFN-α (5 U/mL) in combination with increasing doses of rIFN-γ, we cannot rule out the possibility that higher doses of rIFN-α (10 or 50 U/mL) might have given a marked antiproliferative synergism with rIFN-γ. However, the less powerful synergistic activity of rIFN-α might be related to the particular subtype used in our study, since the multiple subtypes of rIFN-α might differ in

### Table 3. Effect of Delayed In Vitro Addition of rIFN-γ on the Synergistic Antiproliferative Effect of rIFN-γ With rIFN-α

<table>
<thead>
<tr>
<th>rIFN (500 U/mL)</th>
<th>Delayed Addition</th>
<th>Time (h) of Addition</th>
<th>CFU-GEMM % Inhibition* (mean ± 1 SEM)</th>
<th>CFU-Mk % Inhibition* (mean ± 1 SEM)</th>
<th>BFU-E % Inhibition* (mean ± 1 SEM)</th>
<th>CFU-GM % Inhibition* (mean ± 1 SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α</td>
<td>Medium</td>
<td>0</td>
<td>58 ± 11</td>
<td>56 ± 13</td>
<td>59 ± 15</td>
<td>60 ± 9</td>
</tr>
<tr>
<td>α</td>
<td>rIFN-γ</td>
<td>0</td>
<td>86 ± 6</td>
<td>81 ± 9</td>
<td>93 ± 2</td>
<td>85 ± 8</td>
</tr>
<tr>
<td>α</td>
<td>Medium</td>
<td>24</td>
<td>62 ± 13</td>
<td>55 ± 10</td>
<td>63 ± 11</td>
<td>41 ± 19</td>
</tr>
<tr>
<td>α</td>
<td>rIFN-γ</td>
<td>24</td>
<td>88 ± 5</td>
<td>76 ± 5</td>
<td>93 ± 6</td>
<td>76 ± 13</td>
</tr>
<tr>
<td>α</td>
<td>Medium</td>
<td>48</td>
<td>61 ± 11</td>
<td>54 ± 3</td>
<td>67 ± 14</td>
<td>52 ± 18</td>
</tr>
<tr>
<td>α</td>
<td>rIFN-γ</td>
<td>48</td>
<td>92 ± 6</td>
<td>89 ± 5</td>
<td>99 ± 1</td>
<td>77 ± 11</td>
</tr>
<tr>
<td>α</td>
<td>Medium</td>
<td>72</td>
<td>63 ± 12</td>
<td>54 ± 15</td>
<td>69 ± 16</td>
<td>58 ± 15</td>
</tr>
<tr>
<td>α</td>
<td>rIFN-γ</td>
<td>72</td>
<td>90 ± 8</td>
<td>82 ± 14</td>
<td>100</td>
<td>81 ± 13</td>
</tr>
</tbody>
</table>

rIFN-α (500 U/mL) was added at the initiation of culture. Five units per milliliter of rIFN-γ in 100 μL or an equivalent volume of control medium were added to the 1-ml dishes at various times after the onset of the incubation period.

*The results are expressed as the percentage of inhibition evaluated as the mean ± 1 SEM with respect to the control cultures (IMDM) from three separate experiments, each involving a different CML patient. Separate control cultures, in which 100 μL of IMDM were added, were plated for each addition time and used as references to calculate the percentage of colony formation inhibition. The number of colonies per 1 × 10^6 LDBMC ranged from 7 to 28 for CFU-GEMM, from 21 to 121 for CFU-Mk, from 45 to 179 for BFU-E, and from 54 to 184 for CFU-GM-

### Table 4. Influence of Removing Monocytes and T-Lymphocytes From CML Bone Marrow on Inhibition of Hemopoietic Progenitor Cell Growth by rIFNs Alone or in Combination

<table>
<thead>
<tr>
<th>Type of rIFNs (U/mL)</th>
<th>CFU-GEMM</th>
<th>CFU-Mk</th>
<th>BFU-E</th>
<th>CFU-GM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uns Depl</td>
<td>Uns Depl</td>
<td>Uns Depl</td>
<td>Uns Depl</td>
</tr>
<tr>
<td>rIFN-α</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10^1</td>
<td>29 ± 6*</td>
<td>23 ± 4*</td>
<td>28 ± 3*</td>
<td>29 ± 3, ns</td>
</tr>
<tr>
<td>10^2</td>
<td>55 ± 5*</td>
<td>50 ± 2*</td>
<td>61 ± 1*</td>
<td>47 ± 9*</td>
</tr>
<tr>
<td>10^3</td>
<td>80 ± 3*</td>
<td>94 ± 1*</td>
<td>76 ± 1*</td>
<td>86 ± 1*</td>
</tr>
<tr>
<td>rIFN-γ + rIFN-α (5 U/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10^1</td>
<td>60 ± 5*</td>
<td>31 ± 4*</td>
<td>68 ± 4*</td>
<td>32 ± 1*</td>
</tr>
<tr>
<td>10^2</td>
<td>89 ± 3*</td>
<td>48 ± 3*</td>
<td>90 ± 7*</td>
<td>50 ± 5*</td>
</tr>
<tr>
<td>10^3</td>
<td>100*</td>
<td>88 ± 1*</td>
<td>97 ± 3*</td>
<td>77 ± 1*</td>
</tr>
<tr>
<td>rIFN-γ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10^1</td>
<td>28 ± 6*</td>
<td>0, ns</td>
<td>40 ± 6*</td>
<td>11 ± 6, ns</td>
</tr>
<tr>
<td>10^2</td>
<td>46 ± 4*</td>
<td>6 ± 1, ns</td>
<td>67 ± 3*</td>
<td>0, ns</td>
</tr>
<tr>
<td>10^3</td>
<td>68 ± 5*</td>
<td>2 ± 1, ns</td>
<td>76 ± 1*</td>
<td>3 ± 3, ns</td>
</tr>
<tr>
<td>rIFN-γ + rIFN-α (5 U/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10^1</td>
<td>30 ± 4*</td>
<td>6 ± 1, ns</td>
<td>20 ± 6, ns</td>
<td>4 ± 2, ns</td>
</tr>
<tr>
<td>10^2</td>
<td>54 ± 2*</td>
<td>3 ± 2, ns</td>
<td>57 ± 3*</td>
<td>11 ± 5, ns</td>
</tr>
<tr>
<td>10^3</td>
<td>63 ± 3*</td>
<td>11 ± 4, ns</td>
<td>77 ± 2*</td>
<td>14 ± 1, ns</td>
</tr>
</tbody>
</table>

The results are expressed as percentage of inhibition ± 1 SEM with respect to the control medium (IMDM) from three separate sets of experiments performed concomitantly on unseparated and accessory cell-depleted marrow cell fractions derived from the same marrow sample. Control numbers of CFU-GEMM ranged from 3 to 15 for LDBMC and from 4 to 12 for LDBMC-AC --T-. Control numbers of CFU-Mk ranged from 12 to 59 for LDBMC and from 18 to 64 for LDBMC-AC --T-. Control numbers of BFU-E ranged from 45 to 179 for LDBMC and from 54 to 184 for LDBMC-AC --T-. Control numbers of CFU-GM ranged from 98 to 234 for LDBMC and from 76 to 185 for LDBMC-AC --T--.

Abbreviations: Uns, light density bone marrow cells; Depl, adherent cell, T-lymphocyte depleted bone marrow cells; ns, no significant change from control (P > .05).

*Significant percentage change from control; P value at least <.05 (two-tailed t test).
inhibitory activity,\(^1\) or to the loss of cell receptors or other surface antigens necessary for inducing an antiproliferative synergism. However, it probably reflects differences in accessory cell activation by the two types of rIFNs. This is further supported by the lack of synergy when accessory cell-depleted bone marrow cells were plated (Table 4).

Different mechanisms of action of rIFNs with respect to their antiviral, immunoregulatory, and antiproliferative abilities\(^{32,33}\) might explain the synergistic effect of rIFN-\(\gamma\) with rIFN-\(\alpha\), such as, the existence of separate cell surface receptors for the rIFNs\(^3\) and the intracellular induction by rIFN-\(\alpha\) and rIFN-\(\gamma\) of distinct groups of polypeptides that mediate their antiproliferative effects.\(^{34}\) In addition, rIFN-\(\gamma\) (but not \(\alpha\)) induces cell responsiveness to inhibitory molecules, such as tumor necrosis factor (TNF), by increasing both TNF receptors\(^3\) and TNF secretion.\(^3\) activates cytotoxic accessory cells which in turn inhibit progenitor cell growth,\(^{1,2,27,29}\) and exerts a cell differentiation induction activity at relatively low doses.\(^{40}\)

In benign-phase CML patients, both rIFN-\(\alpha\) and rIFN-\(\gamma\) might induce hematologic remission and cytogenetic improvement.\(^{42}\) Recombinant rIFN-\(\alpha\) has been reported to decrease leukocyte doubling time in vivo and to reduce the percentage of CFU-GEMM in S phase, thus it might delay the onset of the blast phase.\(^{41}\) The disappearance of rearranged restriction fragments of the breakpoint cluster region gene has been demonstrated.\(^{42}\) However, dose-limiting side effects occur in rIFN-receiving CML patients,\(^4\) who might also develop a resistance to these substances despite dose escalation.\(^{4,5}\)

The antiproliferative synergy of rIFN-\(\gamma\) with rIFN-\(\alpha\) on CML-derived progenitor cells could be of particular interest in clinical trials. Although in vitro findings are difficult to transpose in vivo, the synergistic interaction of these two rIFNs could allow a significant reduction in the necessary dose to attain an antiproliferative effect. Kurzrock et al.\(^{44}\) observed that the combined intramuscular administration of the same dose of rIFN-\(\alpha\) and rIFN-\(\gamma\) on a daily schedule resulted in cumulative toxicity. However, timing might be critical, as has been reported for other combination IFN therapies.\(^{45}\) Our data show that synergy between rIFNs is still evident when rIFN-\(\gamma\) is added in culture within 72 hours after the onset of the incubation period; thus, different scheduling regimens could avoid cumulative toxicity while allowing synergistic antitumor activity. Dose ratio, route of administration, and the immune status of the patients—the synergy is largely mediated by accessory cells—are additional important factors in determining the nature of the response.

The combination of rIFNs could overcome unresponsiveness to a single rIFN preparation, as was observed in vitro in two of our patients who failed to respond to rIFN-\(\gamma\) alone (\(ID_{50} > 3 \times 10^4 \text{U/mL}\)), but who did react to the combination. Similar findings have been observed recently in vivo by Kloke et al.\(^7\) The in vivo use of rIFN-\(\gamma\) should improve the rate of cytogenetic remission, since immune IFN might contribute to the selective restoration of nonclonal hematopoiesis in CML patients.\(^{16}\) Due to its differentiation-induction activity,\(^{40}\) rIFN-\(\gamma\) might counteract the progressive increase in the growth rate of the leukemic population and in the loss of the leukemic cells' ability to differentiate into functional cells that mark the passage of CML to blastic phase.\(^3\) Evidence has shown that rIFN-\(\gamma\) can down-regulate collagen synthesis in mouse\(^{46}\) as well as human\(^47\) systems, thus suggesting the possibility that this lymphokine might be useful in the treatment of cases characterized by excessive fibrosis.

In conclusion, our preclinical data strongly support a role for combination rIFN therapy in CML. The pleiotropic effects of rIFNs might lead to a more effective and durable control of CML at the clinical, cytogenetic, and molecular levels.

ACKNOWLEDGMENT

We wish to thank Professor J.W. Adamson for his generous gift of recombinant human erythropoietin.

REFERENCES

13. Mams SW, Oken MM, Zanjani ED: Suppression of normal...
INF SYNERGISM IN CML

1299

33. Branca A, Baglioni C: Evidence that type I and II IFNs have different receptors. Nature 294:768, 1981
Synergistic antiproliferative effect of recombinant interferon-gamma with recombinant interferon-alpha on chronic myelogenous leukemia hematopoietic progenitor cells (CFU-GEMM, CFU-Mk, BFU-E, and CFU-GM)

C Carlo-Stella, M Cazzola, A Ganser, G Bergamaschi, P Pedrazzoli, D Hoelzer and E Ascari