Relationship of the Clinical Response and Binding of Recombinant Interferon Alpha in Patients With Lymphoproliferative Diseases


Patients with hairy cell leukemia (HCL) and chronic lymphocytic leukemia (CLL) were treated with recombinant interferon alpha A (rIFN-αA). The binding of iodinated recombinant interferon-α to baseline samples of peripheral blood mononuclear cells (PBMCs) from the leukemia patients was compared with clinical responsiveness to rIFN-αA. HCL patients (8/10) responded to rIFN-αA therapy, whereas none (0/10) of the CLL patients studied responded. The PBMCs from the eight responsive HCL patients bound approximately twice as much iodinated interferon as the PBMCs from nonresponsive CLI patients. This difference was due to more high-affinity receptors per cell with no difference in the affinity of the interferon-receptor interaction. However, because PBMCs from HCL patients were larger than PBMCs from CLI patients, the cell surface receptor density was similar. The leukemic cells from one of the two nonresponsive HCL patients bound iodinated interferon similarly to the cells from the responsive HCL patients, whereas the leukemic cells from the other nonresponsive HCL patient bound considerably less. The rapidity of response of the HCL patients did not correlate with the level of binding of iodinated interferon. Our results suggest that the absolute number of interferon receptors per cell may be one of several important parameters in the response to rIFN-αA therapy, and that the responsiveness of a particular lymphoproliferative disease or a particular patient to rIFN-αA therapy cannot be predicted or explained solely by the degree of interaction between IFN and its cell surface receptor.

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PHASE II TRIALS of natural and recombinant interferon alpha preparations have demonstrated clinical efficacy in a number of lymphoproliferative diseases. The most impressive responses have been reported for patients with hairy cell leukemia (HCL),1 low- and intermediate-grade non-Hodgkin’s lymphoma,2,4 and cutaneous T cell lymphoma.5 Although early trials with natural interferon alpha suggested activity for chronic lymphocytic leukemia (CLL) patients,6 a phase II trial with recombinant interferon alpha A (rIFN-αA) demonstrated no appreciable efficacy in CLI patients.7

The initial event in the action of interferon (IFN) is the binding to specific cell surface receptors (reviewed in references 8 and 9). The present study was undertaken to determine whether clinical responsiveness to rIFN-αA therapy can be explained, and therefore predicted, by the degree of binding of IFN to its receptor. In this study, patients with HCL and CLI were treated with rIFN-αA. The binding of iodinated recombinant interferon alpha 2 (125I-rIFNα2) and iodinated recombinant interferon alpha A (125I-rIFN-αA) to pretreatment samples of peripheral blood mononuclear cells was measured. Binding data were compared with clinical responsiveness to rIFN-αA.

MATERIALS AND METHODS

Patients. The study group comprised patients undergoing rIFN-αA therapy at the Biological Response Modifiers Program’s Clinical Unit at the Frederick (Maryland) Memorial Hospital and consisted of ten cases of HCL10 and ten cases of CLI.11 All patients in the study were advised of procedures and attendant risks, in accordance with institutional guidelines, and signed informed consent agreements. With the exception of one nonresponsive patient, only those HCL patients with >70% leukemic cells in their peripheral blood were included in the study. All of the CLI patients had 80% to 100% leukemic cells in their peripheral blood. The patients were diagnosed by examination of bone marrow biopsies. Those with HCL had infiltration of the marrow with mononuclear cells containing filamentous cytoplasmic projections and tartrate-resistant acid phosphatase. Those with CLI had at least 30% mature lymphocytes on a differential count of nucleated marrow cells.

Patients with HCL were treated daily with intramuscular (IM) or subcutaneous injections of rIFN-αA at a dose of 3 × 106 units.10 Responses were defined as a normalization of the peripheral blood cell counts and a >50% reduction in leukemic cells in the bone marrow lasting for at least one month.10 All of the responding patients are still receiving IFN therapy and have had long-lasting responses, with no relapses witnessed to date. Patients with CLI were treated three times weekly with an IM injection of rIFN-αA at doses ranging from 5 to 50 × 106 U/m2 of body surface area.11 Responses were defined as a >50% reduction in the size of measurable lesions for at least one month.11

Cells. Leukemic and normal cells were obtained from peripheral blood by venipuncture. Peripheral blood mononuclear cells (PBMCs) were separated by Ficoll-Hypaque and washed three times with RPMI 1640 medium with 10% fetal calf serum. Cell viability was >95% for all fresh samples. In some cases, frozen cells were used; cell viability was >80%. No difference was observed between the binding of 125I-IFN-α2 to fresh or cryopreserved cells.

In some experiments, PBMCs were enriched for hairy cells by depletion of contaminant T lymphocytes. PBMCs (0.3 to 1.2 × 108 cells) in 1 mL phosphate-buffered saline with 0.1% sodium azide and 2% human serum albumin (Buffer A) were stained with occasional mixing for 30 minutes at 4 °C with 20 μg fluorescein isothiocyanate (FITC)-conjugated monoclonal anti-Leu-4 antibody (Becton Dickinson, Mountain View, Calif). The cells were washed twice with Buffer A and applied to a 7-mL column of Affi-Gel cell-sorting...
beads with covalently linked antibodies to FITC (Bio-Rad Laboratories, Richmond, Calif). After incubating for 30 minutes at 4°C, the unbound cells were eluted in Buffer A. The unbound cells were washed with RPMI with 10% fetal calf serum. For analysis by flow cytometry, the cells were washed and incubated for two hours at 4°C in RPMI without serum. Flow cytometry was performed as previously described with the following monoclonal antibodies from Becton Dickinson: anti-Leu-4 (pan-T lymphocytes), anti-Leu-12 (B lymphocytes), anti-Leu-11 (natural killer cells), and anti-Leu-M4 (monocytes).

The B lymphoblastoid Daudi cell line was cultured in Dulbecco's modified Eagle's medium with 10% heat-inactivated horse serum, as previously described. Small resting T lymphocytes were separated from total PBMCs of normal donors by nylon wool depletion of contaminant adherent cells followed by centrifugation through Percoll gradients, as described; fractions 5 and 6, which contained >90% T lymphocytes, were used for these studies.

**Interferon.** Recombinant IFN-α-A was prepared by recombinant DNA technology and purified with monoclonal antibodies by Hoffmann-La Roche, Nutley, NJ, in collaboration with Genentech, San Francisco. Purified rIFN-α-A, obtained from Schering-Plough, Bloomfield, NJ, and rIFN-α-A were iodinated, as previously described, with an initial specific activity of 10 μCi/μg and 47 μCi/μg, respectively, based on bindable cpm per microgram of protein active in an antiviral assay. Recombinant IFN-α-A and rIFN-α-A, which are the products of genes cloned by two groups, differ only in a single amino acid, lysine vs arginine, respectively, at position 23, and are considered to be the same IFN subtype. 125I-rIFN-α, 125I-rIFN-α-A, and rIFN-α-A were titered, using an assay for reduction of the cytopathic effect of vesicular stomatitis virus on WISH cells, and activity was expressed as units of IFN by comparison with the international standard GA23-902-530 for IFN-α. Units of biological activity obtained were converted to nanogram IFN, using the specific activity of pure rIFN-α-A (2 x 10^8 U/mg protein).

**Binding assays.** Binding assays and competitive displacement experiments were performed similarly to those previously described, using cultured cell lines. For standard binding assays in the present experiments, 3 x 10^5 to 1.1 x 10^6 cells in 0.5 mL RPMI 1640 medium with 5% fetal calf serum were incubated with 1 ng 125I-rIFN-α-A or 0.3 ng 125I-rIFN-α-A for 1.5 hours at 4°C. In competitive displacement experiments, increasing amounts of unlabeled rIFN-α-A were added to standard binding assays. At the end of the incubation, the samples were layered over 0.2 mL of a 2:1 mixture of di-n-butylphthalate: dinonylphthalate in microfuge tubes at 4°C, as previously described, and centrifuged for two minutes at 10,000 g. The supernatant was aspirated, and the microfuge tube tip, which contained the cell pellet, was counted in a gamma counter (Beckman Fullerton, Calif) with 40% efficiency. A blank reaction, containing no cells, bound less than 50 cpm over the machine background; this blank was subtracted from all bound cpm reported. The binding of 125I-rIFN-α-A in the presence of 133-fold excess of unlabeled rIFN-α-A was defined as nonspecific binding and was subtracted from total binding for Scatchard analysis. Nonspecific binding defined in this way constituted 10% to 20% of the total binding.

**Cell sizing.** Cell volume distributions were measured with a Celsiscope (Particle Data, Elmhurst, Ill), which was standardized with 10.25-μm-diameter polymer latex beads. The data were plotted and analyzed by computer to determine the mean cell volume in each cell preparation.

**RESULTS**

Eight out of ten of the patients with HCL evaluated in the present study responded to rIFN-α-A therapy with dramatic improvement in their hematologic parameters, as shown by normalization of the number of erythrocytes, white cells, and platelets. Surface marker studies with monoclonal antibodies demonstrated a significant decline in the TAC-positive, B cell antigen-positive cells after rIFN-α-A therapy. In contrast, none of the CLL patients included in this study responded to rIFN-α-A.

The binding of 125I-rIFN-α-A was measured to PBMCs from normal donors and to pretreatment samples of PBMCs from nonresponsive CLL patients and from responsive HCL patients having >70% circulating leukemic cells. The mean cpm bound per 10^7 cells in standard binding assays is shown in Table 1. The mean binding of 125I-rIFN-α-A to PBMCs from CLL patients was only slightly higher than the binding to PBMCs from normal donors. However, the mean binding to PBMCs from HCL patients was more than two times higher than the binding to PBMCs from CLL patients (Table 1). The binding of 125I-rIFN-α-A to the lymphoblastoid B cell line Daudi is shown in Table 1 for comparison. This cell line is highly sensitive to the antiproliferative effect of IFN in vitro and expresses high levels of receptors for IFN-α.

Because the binding of IFN at 4°C is a cell surface event, the data can be expressed as cpm bound per equivalent cell surface area. Therefore, cell volume distributions and mean cell volume in the PBMC preparations were measured. Representative sizing profiles of PBMCs from a normal donor, a CLL patient, and a HCL patient are shown in Fig 1. As shown in Table 1, the mean volume of the PBMCs from HCL patients was almost two times greater than the mean volume of the PBMCs from CLL patients and from normal donors. Making the assumption that the cells were perfect spheres, the mean binding per equivalent surface area was 30% lower for PBMCs from patients with CLL than from patients with HCL. However, because hairy cells have many cytoplasmic projections, and thus are not perfect spheres, the surface area of hairy cells is much greater than calculated here. Therefore, the difference in cpm per surface area between CLL and HCL is even less than estimated by this calculation. The mean binding per equivalent surface area

<table>
<thead>
<tr>
<th>Table 1. Binding of 125I-rIFN-α-A to PBMCs From Normal Donors and From HCL and CLL Patients Before Commmencing rIFN-α-A Therapy</th>
<th>Cells</th>
<th>No. of Patients</th>
<th>cpm/10^7 Cells</th>
<th>Cell Volume (μm^3)</th>
<th>cpm/10^7 μm^3</th>
<th>Surface Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBMCs from responsive HCL patients</td>
<td>8</td>
<td>900 ± 163</td>
<td>330</td>
<td>3.90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PBMCs from nonresponsive CLL patients</td>
<td>10</td>
<td>400 ± 96</td>
<td>167</td>
<td>2.72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PBMCs from normal donors</td>
<td>6</td>
<td>340 ± 102</td>
<td>170</td>
<td>2.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daudi cells</td>
<td>4,110</td>
<td>1,216</td>
<td>7.47</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The binding of 125I-rIFN-α-A to PBMCs and Daudi cells and the mean cell volume were measured, as described in Materials and Methods. The cell surface area was calculated from the volume, assuming that the cells were perfect spheres.

*Mean cpm bound ± SD.
with the exception of that obtained with the Daudi cell line, were curvilinear. As previously discussed, many experimental conditions can give rise to artifactual curvilinear Scatchard plots. Therefore, the physiological significance, if any, of these apparent curvilinear Scatchard plots is not presently known.

From the high-affinity portion of the curve, the number of receptors per cell and the affinity of the IFN-receptor interaction were estimated. As shown in Table 2, the number of receptors per PBMC from the HCL patients was approximately two times greater than the number of receptors per PBMC from a representative CLL patient. There were fewer receptors on small resting T lymphocytes than on either of the leukemia cell populations. When the number of receptors was expressed per equivalent cell surface area, these differences were substantially reduced. Indeed, as discussed earlier, because of the morphology of the hairy cells, the difference in receptors per surface area is even less than that shown in Table 2. The affinity of the IFN-receptor interaction was the same on all the cells analyzed (Table 2). These results demonstrated that the differences in the binding of ¹²⁵I-rIFN-α2 to PBMCs from responsive HCL patients compared with those from nonresponsive CLL patients shown in Table 1 could be attributed to differences in the number of receptors per cell with no differences in the affinity of the IFN-receptor interaction.

The data reported thus far compared the binding of ¹²⁵I-rIFN-α2 to PBMCs from responsive HCL patients with those from nonresponsive CLL patients. However, not all the HCL patients responded to rIFN-αA therapy and the rate of response varied. We compared the responsiveness and the rate of response to therapy with the binding of ¹²⁵I-rIFN-α2 to PBMCs from HCL patients having >70% circulating leukemic cells. As shown in Table 3, the binding of ¹²⁵I-rIFN-α2 to PBMCs from these HCL patients ranged from 694 to 1,218 cpm per 10⁷ cells, with the exception of patient I, whose cells bound only 207 cpm. After 20 weeks of therapy, HCL patient I did not show any response. The hairy cell count did not decline, which was usually the first sign of response seen within the first week of therapy. In addition, there was no improvement in granulocytes, platelets, or

Table 2. Scatchard Analysis of Competitive Displacement Binding Experiments

<table>
<thead>
<tr>
<th>Cells</th>
<th>R/Cell</th>
<th>R/Surface Area</th>
<th>Kd (mol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBMCs from HCL patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>1.300</td>
<td>510</td>
<td>1.2 x 10⁻¹⁰</td>
</tr>
<tr>
<td>H</td>
<td>1.000</td>
<td>450</td>
<td>1.3 x 10⁻¹⁰</td>
</tr>
<tr>
<td>PBMCs from CLL patient</td>
<td>530</td>
<td>350</td>
<td>1.0 x 10⁻¹⁰</td>
</tr>
<tr>
<td>Resting T lymphocytes</td>
<td>380</td>
<td>300</td>
<td>1.5 x 10⁻¹⁰</td>
</tr>
<tr>
<td>Daudi cells</td>
<td>6,900</td>
<td>1,250</td>
<td>1.3 x 10⁻¹⁰</td>
</tr>
</tbody>
</table>

The number of receptors per cell and the affinity of the IFN-receptor interaction were estimated from the high-affinity portion of Scatchard plots constructed from competitive displacement binding data. A representative experiment is shown in Fig 2.

* Number of receptors per cell.
† Estimated number of receptors per 100 μm² cell surface area.
‡ Dissociation constant.
hemoglobin. Interestingly, this patient had massive splenomegaly, which may have contributed to the lack of detectable response. With the other HCL patients there was no correlation between the cpm bound to PBMCs and the length of therapy before a >90% reduction in circulating hairy cells was observed (Table 3).

After this initial study was completed, PBMCs from a second nonresponsive HCL patient were evaluated for IFN-α receptors. However, because only 28% of the circulating PBMCs from this patient were leukemic, it was necessary to enrich for the hairy cells. As described in Materials and Methods, PBMCs were partially depleted of T lymphocytes by cell-sorting affinity chromatography. As shown in Table 4, this procedure reduced the level of Leu-4-positive T lymphocytes and enriched for Leu-12-positive cells; Leu-12, a B cell marker, correlates with the percentage of hairy cells in these patient’s PBMCs. This procedure did not appreciably affect the ability of the cells to bind iodinated IFN, as shown for patients A and I. In these experiments, 125I-rIFN-αA rather than 125I-rIFN-α2 was used. Although the absolute number of cpm bound was different, the ratio of binding of 125I-rIFN-αA to PBMCs from patients A and I shown in Table 4 was similar to that previously observed with 125I-rIFN-α2 (Table 3). With both types of iodinated rIFN-α, cells from the nonresponsive patient (I) bound considerably less iodinated rIFN-α than the cells from the responsive patient (A). As shown in Table 4, PBMCs from the other nonresponsive HCL patient (J), when enriched for hairy cells, bound intermediate levels of 125I-rIFN-αA. However, this enriched population of cells was still only 48% Leu-12 positive. Analysis by flow cytometry of unfractionated PBMCs from this patient showed that besides the Leu-4+ and Leu-12-positive cells, 12% of the cells were positive for Leu-11, whereas none were positive for Leu-M3, suggesting that there were natural killer cells but no monocytes in the PBMCs of patient J. Therefore, the affinity column-enriched preparation from patient J probably contained large granular lymphocytes (LGLs) in addition to the hairy cells and T lymphocytes. When assayed for the binding of 125I-rIFN-αA under the same conditions as in Table 4, highly enriched preparations of T lymphocytes bound 530 cpm per 10⁶ cells and LGLs bound 810 cpm per 10⁶ cells. The calculated binding of 125I-rIFN-αA to a cell population consisting of 50% hairy cells (using the cpm bound to affinity column-purified cells from patient A), 25% T lymphocytes, and 25% LGLs was 1,860 cpm per 10⁶ cells, which was similar to the binding observed to the hairy cell-enriched preparation from patient J (Table 4). These results suggest that the leukemic cells from nonresponsive patient J bound iodinated IFN at the same high level as the responsive HCL patients, rather than at the low level observed with the other nonresponsive HCL patient I.

**DISCUSSION**

In the work described here, we have examined whether the clinical responsiveness or nonresponsiveness of lymphoproliferative diseases to rIFN-αA therapy can be explained, and therefore predicted, by the level of binding of IFN to its cell surface receptor. Patients with HCL and CLL were studied. Eight out of the ten HCL patients included in the study have responded to rIFN-αA therapy as previously described. Similar responses for HCL patients treated with rIFN-α2 have been reported. The patients with CLL showed a poor response.

PBMCs from responsive HCL patients bound more than twice as much 125I-rIFN-α2 as the PBMCs from nonresponsive CLL patients. It should be mentioned that in these studies, PBMCs from HCL patients were more than 70% leukemic, whereas PBMCs from CLL patients were nearly 100% leukemic. The major contaminating cells in the peripheral blood from CLL patients were neutrophils, which were removed by Ficol-Hypaque treatment. The major contaminating cells in the PBMCs from HCL patients were T lymphocytes and LGLs. Because, as discussed in Results, these contaminating cells bound considerably less iodinated IFN-α than did hairy cells, their presence resulted in an underestimation of the binding to hairy cells, making the differences in binding between pure populations of HCL and CLL even greater than shown in Table 1. The more than twofold difference between

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**Table 3.** Comparison of Rapidity of Response of HCL Patients to rIFN-αA Therapy and the Binding of 125I-rIFN-α2 to PBMCs

<table>
<thead>
<tr>
<th>Patient</th>
<th>Weeks Before Response</th>
<th>cpm/10⁶ Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>807</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>694</td>
</tr>
<tr>
<td>C</td>
<td>2</td>
<td>852</td>
</tr>
<tr>
<td>D</td>
<td>2</td>
<td>1,064</td>
</tr>
<tr>
<td>E</td>
<td>3</td>
<td>784</td>
</tr>
<tr>
<td>F</td>
<td>4</td>
<td>1,218</td>
</tr>
<tr>
<td>G</td>
<td>7</td>
<td>1,063</td>
</tr>
<tr>
<td>H</td>
<td>12</td>
<td>746</td>
</tr>
<tr>
<td>I</td>
<td></td>
<td>207</td>
</tr>
</tbody>
</table>

*In this comparison, a positive response is defined as a >90% decrease in the absolute number of hairy cells in the peripheral blood. All of these patients, except patient I, had a normalization of erythrocytes, granulocytes, and platelets within eight to 35 weeks of initiating therapy. The binding of 125I-rIFN-α2 to PBMCs was measured as described in Materials and Methods.

*No reduction in circulating hairy cells or improved blood counts after 20 weeks.

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**Table 4.** Binding of 125I-rIFN-αA to PBMCs Enriched for Hairy Cells From Responsive and Nonresponsive HCL Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Response to IFN Therapy</th>
<th>% HC*</th>
<th>Enrichment</th>
<th>Flow Cytometry†</th>
<th>cpm/10⁶ Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Leu-4</td>
<td>Leu-12</td>
</tr>
<tr>
<td>A</td>
<td>+</td>
<td>88</td>
<td>+</td>
<td>8</td>
<td>80</td>
</tr>
<tr>
<td>I</td>
<td>–</td>
<td>96</td>
<td>3</td>
<td>6</td>
<td>90</td>
</tr>
<tr>
<td>J</td>
<td>–</td>
<td>28</td>
<td>3</td>
<td>54</td>
<td>19</td>
</tr>
</tbody>
</table>

*Positive and negative responses are as defined in Materials and Methods. PBMCs were analyzed before (–) and after (+) enrichment for hairy cells by T cell depletion as described in Materials and Methods. The binding of 125I-rIFN-αA to the cell populations was measured as described in Materials and Methods. NT, not tested.

†Percentage of positive cells.
the binding of iodinated IFN-α to PBMCs from HCL and CLL patients was attributable to more high-affinity receptors per cell with no difference in the affinity of the IFN-receptor interaction. However, we found that the cell volume of the PBMCs from HCL patients was approximately twice that of the PBMCs from CLL patients. Moreover, the hairy leukemia cells had many cytoplasmic projections, which further increased their cell surface area. Therefore, our results indicate that the PBMCs from HCL patients have a greater absolute number of high-affinity IFN-α receptors per cell than do PBMCs from CLL patients, but that they have a similar cell surface high-affinity IFN-α receptor density due to differences in cell size and morphology.

We asked whether the twofold difference between HCL and CLL patients in the absolute number of high-affinity IFN-α receptors per PBMC could account for the responsiveness of the HCL patients and nonresponsiveness of the CLL patients to rIFN-αA therapy. It is possible that if a threshold number of interactions between IFN and its receptor is required for an antiproliferative response, the absolute number of receptors per cell, rather than receptor density, is an important parameter in determining a response. Although in vitro antiproliferative responsiveness may not necessarily correlate with clinical responses, the available data on the Daudi cell line is consistent with the above hypothesis, since these cells express a high number of IFN-α receptors and are sensitive to the antiproliferative effects of this IFN. Moreover, in some cases, a reduced responsiveness or lack of response to the antiproliferative effects of IFN has been correlated with a low level of IFN receptors. With other cell lines there is no direct relationship between the number of receptors per cell and the responsiveness to IFN.

Although the data with HCL and CLL patients suggested that the absolute number of IFN receptors per cell may be an important parameter, the following observations provided evidence that this is not a sufficient criterion to explain or predict a clinical response or the rapidity of response to rIFN-αA therapy. First, among the responding HCL patients, there was no correlation between the level of 121I-rIFNα2 binding and the rapidity of response to rIFN-αA therapy. Second, an enriched preparation of hairy leukemic cells from one of the two nonresponsive HCL patients, when normalized for percentage of leukemic cells, bound iodinated IFN-α similarly to the leukemic cells from the responsive patients. This patient was removed from study after eight weeks due to progressive disease. Although the leukemic cells from the other nonresponsive HCL patient bound the lowest amount of iodinated IFN-α, it is premature to conclude that the lack of response is due solely to the low IFN binding; however, it may be a contributing factor. This patient showed no improvement after rIFN-αA therapy and was removed from study because of increasing pain from splenic infarcts.

Interestingly, both of the nonresponding patients had massive splenomegaly. One hypothesis is that the tumor-infiltrated spleens prevented IFN from saturating receptors. Although higher doses of rIFN-αA might have been effective, we felt that they might have led to unacceptable toxicity and instead elected to treat these two patients with 2-deoxycoformycin. Both patients initially responded to deoxycoformycin therapy with improved blood counts and significant reduction in the size of their spleens. Taken together, the data presented here indicate that the responsiveness of a particular patient to rIFNαA therapy cannot be predicted or explained solely by the degree of interaction between IFN and its cell surface receptor.

At the present time, the molecular mechanism for the antiproliferative state induced by IFN is poorly understood. Using cultured cell lines, it has been shown that after the binding of IFN to its receptor, the IFN–receptor complex is internalized by the cells resulting in down-regulation or reduced number of IFN receptors at the cell surface. Whether internalization of IFN or its receptor is required for the biological responses to IFN has not been resolved. After several hours, new proteins induced by IFN treatment can be detected in the cells. Two of these proteins, 2′-oligo(A) synthetase and a protein kinase, are important in the IFN-induced antiviral state. Whether either of these proteins also plays a role in the antiproliferative response to IFN has not been established. Likewise, the function of the other induced proteins is not known.

Because the molecular mechanism for the IFN-induced antiproliferative state in vitro is so poorly understood, it is difficult to explain why some malignancies have responded well to IFN therapy in vivo and why others have not. It may relate to the fact that some malignancies have a viral etiology, and thus the antiviral action of IFN could play a role. In this regard, a T cell variant of HCL was found to contain HTLV-II, a human retrovirus associated with disease. It is also possible that the responsiveness of a particular malignancy or a particular patient to IFN therapy may lie not only with an effect of IFN on the malignant cells, but also with an effect of IFN on the immune system. Further research is required to elucidate molecular mechanisms of the antiproliferative action of IFN in vitro and in vivo.

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