In Vivo Induction of Proteins During Therapy of Hairy Cell Leukemia With Alpha-Interferon

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The use of biological response modifiers is a promising new field in the therapy of malignant disease. One of the most successful applications has been the treatment of hairy cell leukemia (HCL) with α-interferon (α-IFN). The mechanism of the antineoplastic effect of IFN remains unknown, despite extensive investigation of the problem. We investigated the direct biochemical effects of IFN on hairy cells and showed that hairy cells in culture respond to exogenous recombinant α-interferon (r-Hu-IFN-α2) by the induction of synthesis of specific proteins. For some time, IFN has been known to induce the synthesis of specific proteins in cells exposed in vitro. Hairy cells, however, are particularly sensitive to very low IFN concentrations; in addition, more proteins are induced, and for a far longer period than previously reported in other cell types. Most patients with HCL have pancytopenia, and bone marrow fibrosis usually precludes marrow aspiration. For these reasons, it is difficult to obtain samples of hairy cells from patients for in vitro experimentation. A few patients with HCL develop a leukemic phase during their illness, however, during which phase leukocyte counts are >10,000/μL, and >50% of the WBCs are hairy cells. From the large referral base of HCL patients at our institution, we were able to select several such patients who were about to begin therapy with α-IFN. Using samples from these patients, we investigated the in vivo induction of specific proteins in hairy cells by pharmacological doses of r-Hu-IFN-α2.

MATERIALS AND METHODS

Materials

r-Hu-IFN-α2 was kindly provided by Schering Corporation (Bloomfield, NJ). [35S]Methionine 15 Ci/mmol was from Amersham (Arlington Heights, IL). EndHANCE fluorographic solution was purchased from New England Nuclear Corporation (Boston). All other reagents were from commercial sources and were of reagent grade.

Methods

Peripheral blood samples were obtained from seven patients with HCL prior to the initiation of IFN-α-2b therapy. All patients were in the leukemic phase of the disease (Table I). Additional samples were obtained at 2 days (6 patients), and 2 (2 patients) or 4 weeks (6 patients) after IFN therapy was started. Mononuclear cells were isolated on a Ficoll-Hypaque gradient, and brought to a concentration of 2 × 10^6 cells/mL. All patients were advised of the procedures and possible risks, according to institutional guidelines, and all gave informed consent.

Pre-IFN samples. r-Hu-IFN-α2 was added to flasks of the cell suspension at 1,000 IU/mL. Similar flasks without added IFN were used as controls. The flasks were incubated for 24 hours at 37°C in 5% CO_2, after which the cells were transferred to methionine-poor RPMI 1640 Select-Amine medium (GIBCO, Grand Island, NY). [35S]Methionine at 100 μCi/mL was added to each flask. Cells were pulse-labeled for 4 hours and lysed according to the method of Laemmli, with protease inhibitors added to the lysis buffer. Nucleic acids were sheared by serial passage through syringe needles down to 30 gauge. Samples were stored at -80°C until used.

IFN therapy. The patients were subsequently started on therapy with r-Hu-IFN-α2, at a dose of 2 × 10^6 IU/m² subcutaneously (SC) three times weekly for 1 year.

Duration of induction. Flasks of pre-IFN hairy cells were used in these experiments. One set of flasks had no IFN added. The other flasks had r-Hu-IFN-α2 at 1,000 IU/mL added as above. After 24 hour incubation with added IFN, cells from two sets of flasks were washed several times and resuspended in IFN-free medium. At 4 and 9 days, respectively, after being washed free of IFN, cells from these two sets of flasks were transferred to methionine-poor Select-Amine and pulse-labeled with [35S]methionine as described above. Another set of flasks was incubated with added IFN for 9 days before pulse-labeling. The cells that had been incubated without IFN for 9 days were transferred to "spent" medium (prepared by incubating hairy cells in IFN-containing medium for 9 days and then centrifuging to remove the hairy cells, leaving a cell-free spent medium). After 24-hour incubation in the spent medium, the cells were transferred to Select-Amine and pulse-labeled as described above.

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Electrophoresis. Polyacrylamide gels were prepared by a modification of the method of Laemmli, wherein the acrylamide: methylene bis-acrylamide ratio was 30:1:1. Separating gels of 12% polyacrylamide were used. Electrophoresis was carried out at a constant 200 V. Gels were fixed in 30% methanol:10% acetic acid for 30 minutes. The films were exposed to Kodak XAR-5 film at constant 200 V. Gels were fixed in 30% methanol:10% acetic acid for 30 minutes. The cells were pulse labeled for 4 hours.

Whole cell lysates were prepared as described above, and electrophoresis and autoradiography were done. Samples from one patient were not radiolabeled; therefore, after electrophoresis, the protein bands were visualized by silver stain only, and the gels were photographed. Radiolabeled cell lysates from two of the other patients were analyzed after electrophoresis by silver staining as well as by autoradiography.

RESULTS

Exposure in vitro to (r-Hu-IFN-α2) resulted in induction of synthesis of several proteins in hairy cells, as analyzed by one-dimensional polyacrylamide gel electrophoresis (PAGE) (Fig 1). The most prominent induced protein was ~80,000 daltons, and was designated p80. Lysates prepared from hairy cells obtained from six patients 2 days after initiation of continuing r-Hu-IFN-α2 therapy also showed induction of p80 synthesis, without addition of IFN during the [35S]methionine labeling period in vitro (Fig 1). The only IFN exposure that these circulating hairy cells received occurred in vivo, as a result of r-Hu-IFN-α2 therapy. In gels of cell lysates obtained from two of these patients 2 weeks after initiation of continuing r-Hu-IFN-α2 therapy, and from five of the patients examined after 4 weeks of therapy, induction of p80 synthesis was not detectable, either by silver stain or by autoradiography. Samples from patient W.M. were analyzed by silver stain only, and did show induction of p80 after 4 weeks of therapy.

When hairy cells were incubated with IFN for 9 days, induction of p80 synthesis was still taking place at 9 days (Fig 2). Previously unexposed hairy cells incubated in IFN-containing spent medium (prepared by incubating hairy cells in IFN-containing medium for 9 days, then centrifuging to remove the hairy cells, leaving a cell-free spent medium), were also induced to synthesize p80 (Fig 2). Thus, hairy cells were still capable of being induced to synthesize p80 after 9 days in culture, and the IFN remained biologically active after 9 days in contact with hairy cells in the medium. Hairy cells incubated in IFN-containing medium for 24 hours and then resuspended in IFN-free medium showed diminished induction of p80 synthesis at 4 days and 9 days after resuspension (Fig 2).

DISCUSSION

The data show that the previously noted induction of synthesis of specific proteins in hairy cells by (r-Hu-IFN-α2) in vitro also occurs in vivo in the cells of patients being treated with this IFN. We believe that the data imply that induction of synthesis occurs in hairy cells since in three of the patients hairy cells constituted 90% to 95% of the circulating mononuclear cells. In support of this interpretation is the fact that when the proportion of circulating hairy cells was reduced by therapy, in most cases to <50%, the induction was no longer detected, although a similar number of mononuclear cells were tested. In vivo induction of protein synthesis in the cells of patients on therapy with IFN has previously been demonstrated in terms of 2'-5' oligoadenylate synthetase activity in circulating mononuclear cells of such patients. Induction of protein synthesis to levels detectable by PAGE has not previously been reported in vivo, and this illustrates the quantitative significance of the amount of p80 induced in the circulating hairy cells of the patients whom we studied. To our knowledge, this is the first description of a
biochemical effect of IFN directly on human neoplastic cells in vivo.

In parallel with our findings from in vitro investigation of the induction phenomenon, the in vivo induction of synthesis of p80 occurred rapidly and was observed within 2 days of the initiation of therapy. Previously, we showed that induction of p80 synthesis in vitro occurred at IFN concentrations ranging from 0.1 to 10,000 IU/mL. At the dose of r-Hu-IFN-α2 used clinically (2 × 10^6 IU/m^2 SC), a serum IFN level of ~100 to 200 IU/mL would be attained at ~4 hours, with elimination over the next 12 hours. 

The current data show that, in vitro, induction of synthesis will continue for at least 9 days in the continued presence of IFN, but that induction is diminished after 4 weeks without exogenous IFN and remains at this level even at 9 days without IFN. In vivo, the interaction of drug elimination and cell turnover might well lead to a more rapid decline in the induction response, so that it is not at a level detectable by PAGE after 2 weeks of IFN therapy. Regulation of the induction of protein synthesis may differ between hairy cells in vivo and in vitro at either a transcriptional, a translational, or a posttranslational level. Down-regulation of the IFN receptor may also occur at different rates in the two situations. The kinetics of induction in vivo may change with time and with turnover of the circulating hairy cell population, so that there is a more rapid decay of the mRNA transcribed and/or a more rapid degradation of the p80 protein (since it was not observed to be induced on silver stain visualization, as well as the autoradiograms). The induction seen by silver stain in one patient (W.M.) after 4 weeks of therapy may indicate some patient-to-patient variability in the parameters of the IFN response in vivo. In addition, the proportion of mononuclear cells that were hairy cells had greatly diminished by 4 weeks of IFN therapy (Table I). (Patient W.M. had the highest residual proportion of hairy cells at 4 weeks, 73%.)

The antitumor effect of IFN remains unexplained. The effect may be mediated indirectly by interaction with intermediary cells such as natural killer (NK) cells, which then act against neoplastic cells. IFN may also have a direct antiproliferative effect on the tumor cells. Alternatively, both mechanisms may play a role. Recent data support older reports that neither NK activity nor an intact immune system is an absolute requirement for inhibition of tumor growth by IFN in vivo in animal models.

IFN therapy does lead to normalization of NK activity, which is characteristically low in patients with HCL. This phenomenon, however, appears to accompany rather than precede the return of all peripheral blood cell counts toward normal. Our data certainly indicate that there is a direct, measurable biochemical effect of IFN on the target hairy cells in vivo when patients are treated with r-Hu-IFN-α2. Whether induction of synthesis of specific proteins such as p80 is involved in the antineoplastic effect of IFN is not known, but it is a marker of direct effects of IFN on a neoplastic cell population, and the extreme sensitivity of hairy cells to IFN in terms of induction of specific protein synthesis, as compared with other cell types, parallels the clinical sensitivity of hairy cell leukemia to IFN therapy.

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REFERENCES


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