α-Interferon Activates the Natural Killer System in Patients With Hairy Cell Leukemia

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To elucidate the mechanisms of α-interferon's (α-INF) therapeutic effect on clinical and laboratory findings in hairy cell leukemia, we sequentially monitored different immunologic parameters in three patients treated with recombinant α-INF. The most evident effect of this treatment on the immune system was the recovery of natural killer (NK) cell in vitro activity of peripheral blood lymphocytes, which was severely impaired before therapy. In particular, NK function began to improve after 3 months, and a complete recovery was obtained after 6 months in all cases. This increase parallels the improvement in clinical and laboratory findings.

HAIRY CELL LEUKEMIA (HCL) is a malignant B cell disorder usually characterized by isolated splenomegaly and peripheral pancytopenia associated with the presence in the blood of mononuclear hairy cells that infiltrate the spleen and bone marrow.[1,2] Although splenectomy has been the treatment of choice in this disease for a number of years, a large number of patients fail to respond to this approach.[2] The use of different therapeutic strategies based on chemotherapeutic agents has been unsatisfactory.[3] A highly effective treatment of these patients has been recently offered by α-interferon (α-INF)[4-7]; however, the mechanisms that account for its therapeutic efficacy are unclear. In particular, no information has been provided thus far on the possible modifications of immunologic parameters induced by α-INF treatment, which may correlate with the improved clinical and laboratory findings in HCL-treated patients.

Interferons are powerful stimulants of natural killer (NK) cell activity,[8] and this seems to be the mechanism of INF antitumor action in the animal model.[9] On the other hand, severe deficiency of NK function has been demonstrated in HCL-untreated patients.[10,11] The aim of this study was to monitor different immunologic parameters sequentially in HCL patients treated with recombinant α-INF (rINF-α_2). Special attention was paid to the phenotypic and functional analysis of NK cells, and these findings have been related to the modifications observed on the clinical and laboratory parameters. A 9-month follow-up was taken into account.

MATERIALS AND METHODS

Patients. Three patients (two males and one female aged 64, 44, and 64 years, respectively) with HCL were treated with rINF-α_2 obtained from the Schering Corp (Shering, Italy) as lyophilized powder (5×10^6 IU/vial) and reconstituted with sterile water. Informed consent was obtained from all patients, who had been advised of procedures and attendant risks. The diagnosis of HCL was based on clinical, morphologic, and cytochemical criteria[1,2]; none of the patients had a splenectomy or previous treatment. Before rINF-α_2 therapy, a severe neutropenia (<500/μL) was present in all patients; two of them also had thrombocytopenia and mild anemia (Fig 1). The treatment started in Nov 1984 and consisted of 2×10^6 IU/m^2 of rINF-α_2 injected subcutaneously three times a week. Adverse reactions of moderate intensity consisting mainly of fever that was controlled by acetaminophen were seen in two patients. A severe neutropenia with septic fever developed in one patient during the fourth week of treatment, requiring interruption of rINF-α_2 administration for 40 days.

Separation procedures. Mononuclear cells were obtained from freshly drawn heparinized peripheral blood by centrifugation on Ficoll/Hypaque gradient. The cells were washed three times and resuspended in RPMI 1640 (GIBCO, Grand Island, NY). Adherent cells were removed by two sequential incubations for 45 minutes in plastic Petri dishes at 37 °C in an atmosphere of 95% air and 5% CO_2.

Phenotypic analysis. The following monoclonal antibodies (MoAbs) were used in this study: OKT3 (Ortho Pharmaceutical, Raritan, NJ) reacts with mature T cells[12]; OKT4 and OKT8 (Ortho) selectively bind to T lymphocyte subsets including cells with helper and suppressor/cytotoxic activity, respectively[12]; HNK-1 (Leu-7, Becton-Dickinson, Sunnyvale, Calif) defines a differentiation antigen expressed on NK and K cells[13]; and anti-Tac (kindly provided by Dr T. Uchiyama, Kyoto, Japan) has been found to recognize the membrane receptor for interleukin 2 on T and B cells[14] and on hairy cells.[15]

Nonadherent cells obtained as described were incubated for 30 minutes at 4 °C with the aforementioned reagents. Following incubation, cells were washed three times, and a fluorescein-conjugated F(ab)$_2$ goat antimouse immunoglobulin antiserum (Cappel Laboratories, Cochranville, Pa) was added for 30 minutes at 4 °C. After further washing, 300 cells were examined under a Leitz Orthoplan microscope (Wetzlar, FRG) equipped for epifluorescence.

Enzyme histochemistry and immunohistology of bone marrow biopsies were performed according to the method previously described in detail by using a series of markers, including tartrate-resistant acid phophatase, RFB-4, anti-Tac, and anti-HLA-DR MoAbs.[16]

Functional evaluations. NK activity was assessed by lysis of ^{51}Cr-labeled K-562 target cells as previously described.[17] Briefly, target cells were labeled overnight at 37 °C in 5% CO_2 with 100 μCi $Na^{51}CrO_4$ (CEA IRE Sorin, Biomedica, Saluggia, Italy), extensively washed three times before use, and then adjusted to a final concentration of 10^5 cells/mL in RPMI 1640 supplemented with 10% fetal calf serum (FCS) (Microbiological Products, Wakersville, Mass). To perform the test, 100 μL of labeled targets (10^4 cells) were cocultured with effector cells for four hours at 37 °C in 5% CO_2 at different dilutions to final ratios between effectors and target cells (E/T): 5:1, 10:1, 20:1, 40:1, and 80:1 in triplicate round-bottom wells (Limbro plates Limbro, Hamden, Conn). Triplicate wells with

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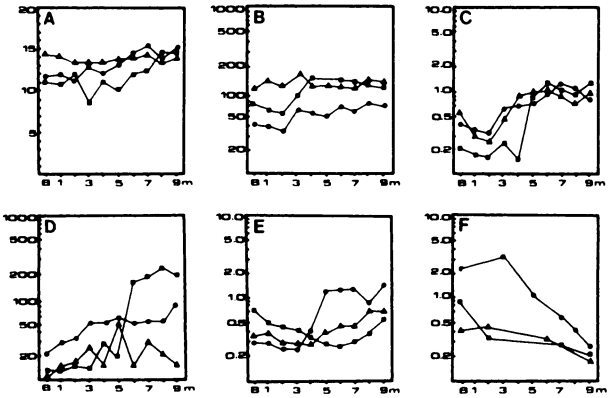

Fig 1. Peripheral blood findings before (B) rINF-α_2 therapy and during the follow-up at 1, 2, 3, 4, 5, 6, 7, 8, and 9 months (m). ▲, ●, and ■ represent individual patients (F.N., M.R., and P.A., respectively). (A) Hemoglobin (gr/dL), (B) Platelets ($\times 10^{-3}$), (C) Neutrophils ($\times 10^{-3}$), (D) Monocytes, (E) Lymphocytes ($\times 10^{-3}$), (F) Hairy Cells ($\times 10^{-3}$).

target cells in medium alone and in detergent were assessed to determine spontaneous and maximum release, respectively. Following this incubation supernatants were harvested and counted in a gamma counter. The mean value of triplicate assays was used to calculate the percentage of cytotoxicity according to the formula: percent cytotoxicity = (cpm release in test − cpm spontaneous release)/(cpm maximum release − cpm spontaneous release) × 100. Spontaneous release from the target cells was always less than 8%.

The proliferative response to phytohemoagglutinin (PHA) was evaluated as previously described.[17]

Twenty age-matched healthy volunteers were used as controls. All data are expressed as the mean ± SE of the mean. Statistical analyses were performed by the Cochran-Cox test.

RESULTS

Figure 1 shows the changes in peripheral blood counts in individual patients following rINF-α_2 treatment. Two patients had an improvement of their anemia and thrombocytopenia. With regard to WBC, the neutropenia dramatically improved in all cases, and the number of monocytes showed a net increase in two cases (M.R., P.A.). Lymphocytes increased in two out of three patients (M.R., F.N.). The number of hairy cells in the peripheral blood, as determined by morphology both on May-Grunwald-Giemsa–stained smears and phase-contrast examination of cells in suspensions, decreased in all patients.

Bone marrow biopsies performed after 3 and 7 months of rINF-α_2 therapy revealed an increase of the myeloid component in all patients. A marked reduction of hairy cell infiltration was observed in one case (P.A., from 90% to 20%), whereas in the two other cases, the frequency of hairy cells, although diminished, continued to represent a consistent proportion of the bone marrow cells (70% to 80%).

The evaluation of immunologic markers (Fig 2) shows a slight but not significant decrease of the T4/T8 ratio. Although the frequency of cells with an NK-related phenotype, as determined by the HNK-1 MoAb, increased in all patients (Fig 2), the absolute numbers of these cells were consistently within the normal range (<390/μL) in all cases.

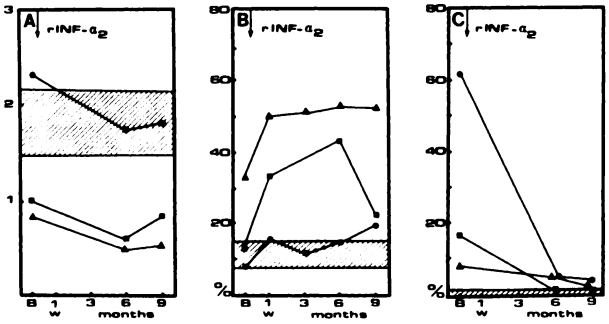

Fig 2. Phenotypic analysis of peripheral blood mononuclear cells with MoAbs before (B) and after 1 week (^1_w) or 3, 6, and 9 months of rINF-α_2 therapy. Shaded areas represent the range values for controls. ▲, ●, and ■ represent individual patients (F.N., M.R., and P.A., respectively). (A) T4/T8 Ratio, (B) HNK-1 Positive Cells, (C) Anti-TAC Positive Cells.

The percentage of Tac-positive cells was reduced in all patients according to the decrease of the number of hairy cells.

Analysis of NK in vitro function (Fig 3) demonstrated an initial decrease, at different E:T ratios, following the first months of rINF-α_2 therapy. NK function began to increase after 3 months, and complete recovery was observed after 6 months in all cases. At the 9-month evaluation, results were comparable with those obtained at the 6-month evaluation. Interestingly, the best response was observed in patient M.R. whose cells expressed the highest frequency of Tac$^+$ cells before treatment.

PHA-induced blastogenesis was reduced with respect to controls and did not reveal significant differences during the follow-up of patients.

DISCUSSION

We have demonstrated that the treatment of HCL with rINF-α_2 restores the NK cell system, which is severely depressed in these patients before α-INF therapy. This parallels the improved clinical and laboratory findings.

α-INF belongs to a large family of biologic response-modifying drugs whose activity includes an antiviral effect, a direct cytotoxic property, and a cell differentiation function.

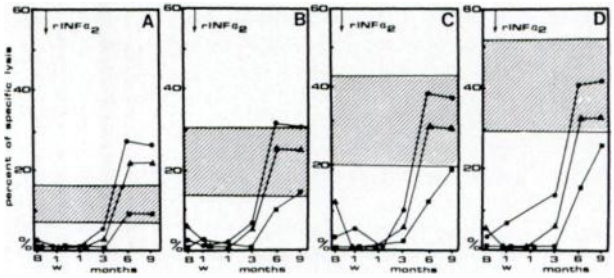

Fig 3. NK in vitro activity before (B) the rINF-α_2 activity and during the follow-up at 1 week (^1_w) and 1, 3, 6, and 9 months. Shaded areas represent the range values for controls. ▲, ●, and ■ represent individual patients (F.N., M.R., and P.A., respectively). (A) 10:1 E:T Ratio, (B) 20:1 E:T Ratio, (C) 40:1 E:T Ratio, (D) 80:1 E:T Ratio.

α-INF has been used in several neoplastic disorders,[18,19] and as a matter of fact, the best response has been observed in HCL patients.[20] However, no complete and longitudinal studies are available that document the effects of α-INF therapy on different immunologic parameters in this disease. For one thing, our data confirmed previous studies demonstrating that the NK activity in these patients is impaired.[10,11] Since the defect of NK function persisted even when these cells were isolated from other cell populations (ref 11 and our unpublished results), the impairment of cytotoxic function cannot be consequent to cellular dilution. Following rINF-α_2 therapy, the NK function initially decreased, but after 3 months the values began to improve and definitely recovered after 6 months.

NK cells have been thought to play a significant role in the host defense mechanisms involved in the resolution of certain infectious agents and in tumor rejection.[21] Furthermore, several lines of evidence demonstrate that NK cells can directly lyse tumor cells,[22] especially following interferon activation,[23] and can inhibit clonogenic growth of fresh leukemic cells.[24] A lack of production of endogenous α-INF in HCL, possibly resulting from chromosomal abnormalities,[4] could be involved in the defective NK function. Therapy with rINF-α_2 could restore a mechanism that currently takes place normally in the host's defense against foreign insults and/or in the control of tumor growth.

Since interleukin 2 (IL 2) activates NK cells,[25] the severe deficency of NK activity reported in untreated HCL patients[10,11] could also be related to the impaired levels of IL 2 consequent to its absorbtion by hairy cells, which are equipped with IL 2 receptors.[15] In line with this interpretation, other neoplasms, ie, cutaneous T cell lymphomas usually characterized by the expression of IL 2 receptors,[26] are highly responsive to α-INF.[27] The two mechanisms mediated by IL 2 and α-INF might synergistically act on NK boosting.[28] Consistent with this latter interpretation, we found that before starting the rINF-α_2 therapy the incubation at 37 °C for 18 hours of peripheral blood cells from our patients both with rINF-α_2 and with recombinant IL 2 led to an enhancement of the NK in vitro function (data not shown).

The attribution of α-INF effects against tumor growth solely to its enhancement of NK function would be inappropriate. In fact, apart from the activation of NK cells, other mechanisms may be involved in patients affected by different disorders following α-INF treatment.[29] As a matter of fact, the association of other effects could account for the lack of correlation usually observed between clinical improvement and evaluation of the NK in vitro activity in patients other than those with HCL.[30–32] Differences in the type of α-INF, its schedule or route of administration, and especially the dose used could also be a source of discrepancy. In this regard, it has been recently demonstrated[33] that high doses of INF display a direct activity (ie, a cytopathic effect), whereas low doses increase the indirect power of this substance through an enhancement of the antitumor activity of the immune system (eg, the effectiveness of NK cell activation).[34–35] In this regard, evidence recently provided that α-INF enhances the expression of class II HLA antigens on hairy cells has led to speculation that α-INF might exert its antileukemic activity by potentiating a cytotoxic mechanism.[36] The good correlation between the clinical improvement and the NK in vitro function observed in our study suggests that the NK system plays a relevant role in α-INF–treated HCL patients. Thus, in some diseases (notably HCL) the activation of NK activity may be prominent, whereas in other disorders, the cytopathic effect may be the main mechanism involved in tumor growth control. The latter has been clearly demonstrated in some neoplastic conditions, especially using high doses of chemotherapy.[33]

Another effect of α-INF is its cell differentiation property.[37] In this regard, the improvement of neutropenia and bone marrow findings in HCL patients could be consequent to an effect on cellular differentiation either directly[38] or via different circuits involving activated cells.[39] This differentiation ability could also account for the increase in the monocytic component observed in two of our patients, similar to the results described by Territo et al[40] in cancer patients.

We conclude that α-INF therapy leads to a series of effects. Among these, at least in HCL patients, the improvement of NK cell activity might be crucial. Of course, the demonstration of a specific lysis of hairy cells by autologous cytotoxic cells will represent the final proof supporting the relevance of cytotoxic mechanisms in α-INF–treated HCL patients. The small number of patients and the short follow-up preclude firm statements regarding the impact of NK evaluation on the prognosis of these patients. We only want to emphasize that the analysis of immunologic parameters might help to clarify the possible therapeutic action of α-INF in HCL. Further studies on a large series of patients are needed to clarify whether evaluation of NK activity may be useful in predicting the prognosis and/or monitoring α-INF therapy by determining the optimal dose, schedule, and duration of treatment.

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