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


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.

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analyses data are expressed as the mean ± SEM. The mean value of triplicate assays was used to calculate the percentage of cytotoxicity according to the formula:

\[
\text{percent cytotoxicity} = \left( \frac{\text{cpm release in test} - \text{cpm spontaneous release}}{\text{cpm maximum release} - \text{cpm spontaneous release}} \right) \times 100
\]

The proliferative response to phytohemagglutinin (PHA) was evaluated as previously described. 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-α2 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-α2 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.

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-α2 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-α2 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.

![Figure 1: Peripheral blood findings before (B) rINF-α2 therapy and during the follow-up at 1, 2, 3, 4, 5, 6, 7, 8, and 9 months (m). (A, B, and C) represent individual patients (F.N., M.R., and P.A., respectively). (A) Hemoglobin (gr/dL), (B) Platelets (x 10^3), (C) Neutrophils (x 10^3).](image1)

![Figure 2: Phenotypic analysis of peripheral blood mononuclear cells with MoAbs before (B) and after 1 week (C) or 3, 6, and 9 months of rINF-α2 therapy. Shaded areas represent the range values for controls. (A) T4/T8 Ratio, (B) HNK-1 Positive Cells, (C) Anti-TAC Positive Cells.](image2)

![Figure 3: NK in vitro activity before (B) the rINF-α2 activity and during the follow-up at 1 week (A) and 1, 3, 6, and 9 months. Shaded areas represent the range values for controls. (A, B, and C) 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.](image3)
α-INF has been used in several neoplastic disorders, and as a matter of fact, the best response has been observed in HCL patients. 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. 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-α2 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. Furthermore, several lines of evidence demonstrate that NK cells can directly lyse tumor cells, especially following interferon activation, and can inhibit clonogenic growth of fresh leukemic cells. A lack of production of endogenous α-INF in HCL, possibly resulting from chromosomal abnormalities, could be involved in the defective NK function. Therapy with rINF-α2 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, the severe deficiency of NK activity reported in untreated HCL patients could also be related to the impaired levels of IL 2 consequent to its absorption by hairy cells, which are equipped with IL 2 receptors. In line with this interpretation, other neoplasms, ie, cutaneous T cell lymphomas usually characterized by the expression of IL 2 receptors, are highly responsive to α-INF. The two mechanisms mediated by IL 2 and α-INF might synergistically act on NK boosting. Consistent with this latter interpretation, we found that before starting rINF-α2 therapy the incubation at 37 °C for 18 hours of peripheral blood cells from our patients both with rINF-α2 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. 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. 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 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). 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. 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.

Another effect of α-INF is its cell differentiation property. 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 or via different circuits involving activated cells. 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 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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