CONCISE REPORT

Discordant Effect of Interferon on Natural Killer Activity and Tumor Cell Sensitivity to Lysis in Hairy Cell Leukemia

By Nitza Lahat, Esther Aghai, Antonio Kotler, Amalia Kinarty, Ester Sobel, Nachman Gruner, and Paul Froom

We studied the action of α-interferon (IFN) and interleukin-2 (IL-2) on natural killer (NK)-rich fractions and autologous tumor cells from two patients with hairy cell leukemia (HCL). The addition of IFN or IL-2 to the NK-rich fractions resulted in a significant increase in NK activity against the autologous tumor cells. This stimulatory effect was blocked if the target hairy cells (HCs) were preincubated with either IFN or IL-2. Pretreatment of the HCs with ant-Tac antibody entirely prevented the blocking effect of IL-2 and partially the blocking effect of IFN. One patient was treated with recombinant α, IFN. After 2 months there was a dramatic reduction in the number of HCs in the peripheral blood coincident with the loss of the protection effect of IFN against NK lysis of the patient’s HCs. NK activity against autologous tumor cells correlated poorly with that against the K562 cell line. We conclude that there is a discordant effect of IFN and IL-2 on NK activity and HC sensitivity to lysis. The Tac receptor appears to play a role in this sensitivity. Caution should be exercised in extrapolating the effects of NK activity against K562 cells to those on HC targets.

Hairy cell leukemia (HCL) is a lymphoproliferative disease of slowly proliferating B cells that infiltrate the spleen and bone marrow and result in splenomegaly and pancytopenia. Recombinant α-interferon (IFN) has a clear antitumor effect in most patients, with HCL with improvement of hematologic parameters within the first 2 months of treatment.

The mechanism of IFN’s antitumor activity, however, has not been clearly established. IFNs are postulated to have an inhibitory effect on tumor cell growth and to enhance the host’s natural killer (NK) activity. NK cytolytic activity has been found to be low in the peripheral blood of patients with HCL and increased in those responding to treatment with IFN. Normal NK activity is enhanced by IFN and interleukin-2 (IL-2), and hairy cells (HCs) have receptors for IL-2. The relationship, however, between the receptor for IL-2 and the effects of IL-2 and IFN on NK activity is unclear.

Previous authors used the K562 cells as targets to test NK activity in patients with HCL. Lysis of K562 targets, however, may not correlate with specific lysis of HCs by autologous cytotoxic cells. In fact, Oshimi et al. recently showed a poor correlation between the NK activity against K562 cells and that against autologous leukemia or lymphoma cells.

In the following study we investigated the effects of IFN and IL-2 on NK-rich fractions and autologous tumor cells from two patients with HCL.

MATERIALS AND METHODS

The peripheral blood was obtained after informed consent from two patients with HCL. The patients had undergone splenectomy because of transfusion-dependent anemia and had over 98% HCs in the peripheral blood. One patient was tested on two occasions before and three times during treatment with recombinant α,IFN (Inter-Yeda, Rehovot, Israel). He received 3 x 10^9 units daily for 3 months and subsequently three times weekly for an additional 5 months. The other patient was not treated with α, IFN. He was tested on three separate occasions.

Mononuclear cells were obtained from fresh heparinized peripheral blood by Ficol-Hyphaque sedimentation. These cells were >90% SmIg-positive (surface immunoglobulin), Ia-positive, and Tac antigen-positive. They were washed three times with phosphate-buffered saline (PBS) and resuspended in RPMI 1640 (Biological Industries, Beit Hamek, Israel) supplemented with 10% heat-inactivated fetal calf serum (Biological Industries) 100 U/mL penicillin, 100 µg/mL streptomycin, 50 µg/mL neomycin, and 2.5 µg/mL amphotericin B (Sigma Chemical Co, St Louis) (complete medium).

Part of the mononuclear cells, which consisted of 98% HCs, were used as target cells. These cells and K562 cells were labeled with 200 µCi Na25CrO4 (Amersham Corp, Little Chalfont, England). They were cultured overnight at 37°C in a humidified atmosphere at 5% CO2 after the addition of medium alone; 1,000 U/mL α-IFN (IIBR, Ness-Ziona, Israel), 1,000 U/mL γ-IFN (Sigma Chemical Co, St Louis) 30 IU/mL IL-2 (Ness-Ziona, Israel), or IL-2 and IFN after one hour at 4°C with antihuman IL-2 receptor (anti-Tac, Becton Dickinson, Mountain View, CA); or together with neutralizing concentrations of anti-α-IFN antibodies (kindly provided by Professor D. Wallach, Weizmann Institute, Rehovot, Israel). The target cell concentration was 5 x 10^6 cells/mL, and fresh autologous target cells were used for all experiments.

The rest of the patient’s mononuclear cells were twice adhered to plastic Petri dishes. Nonadherent cells were collected by gently swirling the dishes and slowly pipetting off the culture medium. Five to 7 x 10^6 cells/test tube was layered and centrifuged on Percoll gradients. Lymphocytes from fractions 2 and 3 were collected and used as effector NK cells for the various experiments. By indirect immunofluorescent staining, 54% to 67% of these cells were Leu 11b (anti-Leu 11b, Beckton Dickinson) positive. These effector cells were incubated overnight at a concentration of 5 x 10^6 cells/mL in medium only or with IFN and IL-2 as described for the target HCs. Cytotoxic assays were performed after overnight incubation. Target and effector cells were washed three times with PBS and suspended in complete medium. Target cells (5 x 10^5 cells/well) and effector cells (5 x 10^5 cells/well) were mixed and cultured in triplicate in round-bottomed microtiter plates (Nunc, Roskilde, Denmark).
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RESULTS

The mean baseline NK activity against autologous tumor cells was 1% ± 1% in the two patients studied before treatment with IFN (studied on five separate occasions). The NK activity increased significantly when the NK-enriched fraction was preincubated with either IL-2 or α-IFN (Table 1). The enhancing effect was blocked when the target HCs were preincubated with either α-IFN or IL-2. Pretreatment of the HCs with anti-Tac antibodies abrogated entirely the blocking effect of IL-2 and partially that of α-IFN. Pretreatment with anti-α-IFN antibodies prevented the resistance induced by α-IFN. Results with γ-IFN were similar to those with α-IFN except that the magnitude of response was less for γ-IFN (results not shown). There was no morphological changes seen on phase and light microscopy after incubation with IFN or IL-2.

Patient 1 was retested after treatment with α-IFN for 1, 2, and 8 months. There was no increase in NK activity despite the addition of IFN to the effector cells at 1 month (Table 2). IL-2, however, still had a stimulatory effect that was blocked when the tumor cells were pretreated with IFN in vitro. After pretreatment for 2 months, in vitro preincubation with IFN did not block the enhancing effect of IFN or IL-2 on the NK activity. There was a poor correlation between the NK activity against the HC targets and that against K562 cells. Baseline NK activity against K562 cells was lower than that of normal controls (30% ± 15% in peripheral blood mononuclear cells and 60% ± 15% in enriched NK cell preparations). It was even lower after 1 and 2 months’ treatment with α-IFN. NK activity against K562 cells before treatment and after 1 and 2 months’ treatment with IFN was tested by using an enriched NK cell population. After 8 months of treatment the patient had a normal blood count with no HCs seen on the peripheral smear. NK cytotoxicity toward K562 targets was measured by using peripheral blood mononuclear cells and was found to be normal (baseline, 18% ± 3%, 28% ± 4% with the addition of α-IFN, and 31% ± 4% with IL-2).

Table 1. The Discordant Effect of a-IFN and IL-2 on Effector NK Cells and Target Autologous HCs

<table>
<thead>
<tr>
<th>Cell Treatment</th>
<th>NK-Enriched Lymphocytes</th>
<th>Target Autologous HCs</th>
<th>Percent Lysis (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. None</td>
<td>None</td>
<td>1 ± 1</td>
<td></td>
</tr>
<tr>
<td>B. IFN</td>
<td>None</td>
<td>10 ± 4</td>
<td></td>
</tr>
<tr>
<td>C. IL-2</td>
<td>None</td>
<td>67 ± 4</td>
<td></td>
</tr>
<tr>
<td>D. IFN</td>
<td>IFN + IFN-Ab</td>
<td>24 ± 7</td>
<td></td>
</tr>
<tr>
<td>E. IFN</td>
<td>IFN + anti-Tac</td>
<td>15 ± 6</td>
<td></td>
</tr>
<tr>
<td>F. IL-2</td>
<td>IL-2</td>
<td>2 ± 1</td>
<td></td>
</tr>
<tr>
<td>G. IFN</td>
<td>IL-2 + anti-Tac</td>
<td>23 ± 4</td>
<td></td>
</tr>
<tr>
<td>H. IL-2</td>
<td>None</td>
<td>26 ± 8</td>
<td></td>
</tr>
<tr>
<td>I. IL-2</td>
<td>IFN</td>
<td>5 ± 7</td>
<td></td>
</tr>
<tr>
<td>J. IL-2</td>
<td>IFN + IFN-Ab</td>
<td>25 ± 2</td>
<td></td>
</tr>
<tr>
<td>K. IL-2</td>
<td>IFN + anti-Tac</td>
<td>16 ± 4</td>
<td></td>
</tr>
<tr>
<td>L. IL-2</td>
<td>IL-2</td>
<td>5 ± 6</td>
<td></td>
</tr>
<tr>
<td>M. IL-2</td>
<td>IL-2 + anti-Tac</td>
<td>39 ± 13</td>
<td></td>
</tr>
</tbody>
</table>

The mean values were calculated for the results of five separate experiments done in triplicate except for the results of D, E, J, and K, which represent one experiment done in triplicate.

Abbreviation: IFN-Ab, antibody to IFN.

*Significantly increased, P < .01; B and H > A; D and E > C; G > F; J and K > I; M > L.

Denmark) at 37°C in a humidified atmosphere of 5% CO2 (18 hours incubation for HCs and four hours for K562 cells). Triplicate wells with target cells only, for each treatment combination, and in detergent (5% Triton X-100) were assessed to determine spontaneous and maximum release, respectively. Supernatants were harvested and counted in a gamma counter. Cytotoxicity was calculated according to the following formula: percent specific lysis = ((counts per minute released in the presence of effector cells – counts per minute spontaneously released)/(counts per minute maximally released – counts per minute spontaneously released)) × 100. Results were expressed as means ± 1 SD, and possible significant differences were determined by using Student’s t test. In addition, HC targets were examined before and after incubation with or without IFN or IL-2 by phase and light microscopy after Geimsa staining.

Table 2. NK-Mediated Lysis of HCs and K562 Cells: In Vitro Effect of α-IFN Before and After Treatment of Patient 1 With α-IFN

<table>
<thead>
<tr>
<th>Cell Treatment</th>
<th>Targets at Baseline (Percent Lysis)</th>
<th>Targets at 1 mo (Percent Lysis)</th>
<th>Targets at 2 mo (Percent Lysis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. None</td>
<td>1 ± 1</td>
<td>0 ± 0</td>
<td>12 ± 2</td>
</tr>
<tr>
<td>B. IFN</td>
<td>22 ± 9*</td>
<td>4 ± 2*</td>
<td>32 ± 2*</td>
</tr>
<tr>
<td>C. IL-2</td>
<td>24 ± 12*</td>
<td>37 ± 8*</td>
<td>43 ± 3*</td>
</tr>
<tr>
<td>D. IFN</td>
<td>1 ± 1†</td>
<td>0 ± 0†</td>
<td>26 ± 2†</td>
</tr>
<tr>
<td>E. IL-2</td>
<td>1 ± 1†</td>
<td>0 ± 0†</td>
<td>38 ± 4</td>
</tr>
</tbody>
</table>

Laboratory parameters

- HCs (μL): 40,000
- PMNs (μL): 400
- Platelets (μL): 37,000
- Hemoglobin (g/dL): 7.4

Abbreviations: Effector, NK-enriched lymphocytes; HC, hairy cell target cells; K562, K562 targets; PMNs, polymorphonuclear cells; ND, not done.

*Significant increase, P < .05; B and C > A.
†Significant decrease, P < .05; D < B, and E < C.
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DISCUSSION

The main finding of our study was that although IFN increased NK activity it reduced HC sensitivity to NK cytolyis. We are unaware of other studies of the direct effects of IFNs on the sensitivity of HCs to lysis by autologous NK cells. Pretreatment of other tumor cells with IFN, however, has been shown to reduce their sensitivity to NK cell–mediated cytotoxicity.14,15 After treatment with α-IFN for 1 month, the patient’s HCs were not sensitive to in vitro IFN-induced lysis which suggests a protective in vivo effect of α-IFN. Such a temporarily induced resistance may explain a delayed clinical response to treatment. A dramatic decrease in the number of HCs occurred after 2 months of treatment, coincident with the loss of the protective effect of IFN. At that time, however, only minimal NK activity could be demonstrated against K562 targets, even when enriched NK cell preparations were used. This suggests that caution should be exercised in extrapolating the effects of NK activity against K562 cells to those against HC targets.

IL-2 alone and in collaboration with IFN is a powerful activator of NK cells.16 We found enhancement of NK activity by IL-2, but pretreatment of the HCs with IL-2 resulted in resistance to NK cell–mediated cytolyis, similar to the effect of IFN. The Tac antigen represents the IL-2α low-affinity receptor, which becomes a high-affinity receptor in association with the 70-kd protein called the IL-2β receptor.17 The protective effect of IL-2 and that of IFN was at least in part abrogated by pretreatment with anti-Tac antibodies. This suggests that the Tac receptor plays a role in the HC susceptibility to lysis. Further studies of patients with HCL are needed to confirm our findings.

REFERENCES

17. Wright SC, Bonavida B: Studies on the mechanism of natural killer mediated cytotoxicity. IV. Interferon-induced inhibition of NK target cell susceptibility to lysis is due to a defect in their ability to stimulate release of natural killer cytotoxic factors (NKCF). J Immunol 130:2965, 1983

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