Interleukin 2 Protects Hairy Leukemic Cells from Lymphokine-activated Killer Cell-mediated Cytotoxicity

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ABSTRACT

Interleukin 2 (IL-2) activates natural killer cells and generates lymphokine-activated killer (LAK) cell-mediated cytotoxicity. In “adoptive immunotherapy,” a combination of LAK administration and IL-2 infusion was found to be effective therapy for some tumors and ineffective for others. Here we report a novel function for IL-2, its ability to protect tumor cells (cell lines obtained from hairy cell leukemia patients) against LAK activity. The protective effect induced by IL-2 is similar to that induced by interferon (IFN). Protection by both cytokines requires new mRNA/protein synthesis; both IL-2 and IFN reduce the ability of tumor target cells to trigger LAK effector cells following binding between these two types of cells. However, endogenous IFN is not the mediator of the IL-2 protective effect against LAK activity since monoclonal antibodies against IFN-α and IFN-γ did not abolish the protective effect of IL-2. In addition, IL-2 does not induce the expression of class I major histocompatibility complex antigens on the target cell surface, believed to be the signals for the IFN-induced protection against natural killer and LAK activities. Finally, leukemic cells resistant to IFN-α did respond to IL-2 treatment and became less sensitive to LAK cytotoxicity. Thus the ability of IL-2 to protect tumor cells from LAK activity may explain the lack of response to adoptive immunotherapy in tumors that express the IL-2 receptor.

INTRODUCTION

IL-2 is a lymphokine synthesized and secreted primarily by those T-helper lymphocytes which have been stimulated with mitogens or by the interaction of the T-cell-receptor complex with the antigen/MHC on the surface of antigen-presenting cells. T-helper cells, upon activation, synthesize IL-2 and express IL-2 receptors and subsequently stimulate clonal expansion of antigen-specific T-cells. Large granular lymphocytes which express NK activity are also producers of IL-2. In addition, IL-2 possesses a variety of other immunological activities.

LAK cells are activated following in vitro culturing of peripheral blood lymphocytes for several days with IL-2 (either highly purified homogeneous natural preparations or the recombinant form). This activation is accompanied by the proliferation and expansion of those cytotoxic cells expressing IL-2 receptors. IL-2 induces LAK cell activity by broadening the cytolytic activity of a subset of resting NK cells by increasing both the number and lytic capacity of these cells. Large granular lymphocytes bearing CD3+, CD56+, and CD16+ surface antigens have been shown to be the predominant cell types which become LAK cells (3), but some CD3+ T-cells are also involved in IL-2-activated cytotoxicity (4). LAK cells recognize their targets in a non-MHC-restricted and non-antigen-specific manner. LAK cells demonstrate a broader target cell range than NK cells, killing NK-sensitive, some NK-resistant, and fresh (autologous or allogeneic) noncultured, surgically obtained solid tumor cells (3, 5). Moreover, LAK cells exhibit cytotoxic activity against cells across species barriers. Usually, LAK cells do not recognize and attack normal cells. These findings have led to the establishment of successful therapies (“adoptive immunotherapy”) for treatment of experimental tumors in animal models, and subsequently to clinical trials in the treatment of advanced human malignancies such as renal cancer, melanoma, and acute leukemia, where patient peripheral blood lymphocytes are cultured in vitro with IL-2 and readministered to the patient in combination with systemically administered IL-2 (5).

HCL is a lymphoproliferative disorder of B-lymphocytes, with pathological manifestations usually including splenomegaly and pancytopenia (6). NK cell-mediated cytotoxicity has been reported to be low in the peripheral blood lymphocytes of patients with HCL (7) and increased in those responding to treatment with interferon (IFN)-α (8). NK activity from normal individuals as well as NK activity of peripheral HCL patients’ blood is enhanced by either IFN-α or IL-2 treatment in vitro (9). Receptors specific for IL-2 are expressed both on NK cells and on the leukemic hairy cells (10). In addition, an association between the level of serum-soluble IL-2 receptor and the clinical and pathological disease states in HCL patients has been reported (11). Here we report a novel function for IL-2, its ability to protect HCL cells against LAK activity. The protective effect induced by IL-2 was found in some characteristics to be similar to that of IFN. However, endogenous IFN is not the mediator of the IL-2 effect, but IL-2 has a direct protective effect against LAK activity. The ability of IL-2 to protect tumor cells from LAK activity may explain the lack of response to adoptive immunotherapy in tumors that express the IL-2 receptor.

MATERIALS AND METHODS

Target Cells. The following HCL cell lines were used in this study: Eskol; IFN-α-resistant Eskol clone (IREs-4); HS-1; and HS-2. The B-lymphoblastoid cell line, Eskol, composed of differentiated cells resembling HCL, has been established from peripheral blood of a HCL patient (12). The IFN-α-resistant Eskol clones were isolated by incubating Eskol cells in the presence of IFN-α (1 × 10^6 units/ml) for 4 months. Every 3 days, cells were centrifuged, washed, and resuspended in media and fresh IFN-α was added. After 4 months, cells were cloned in soft agar containing IFN-α (1 × 10^6 units/ml) for 20 days and four individual clones were isolated and termed IREs-1, IREs-2, IREs-3, and IREs-4. The IREs clones were tested for their response to treatment with IFN-α (300–30,000 units/ml) and found to be completely resistant, on the basis of cell counts, to the antiproliferative effect of IFN-α (13). In the present study we have used the clone IREs-4 as target cells. HS-1 and HS-2 cells were isolated from splenocytes of HCL patients (kindly provided by Dr. H. Ozer, North Carolina University). The chronic myelogenous leukemia cell line, K-562 (ATCC CCL 243) was used as a control target cell. All the HCL cell lines as well as K-562 cells were grown and assayed in RPMI 1640 (GIBCO Inc., Grand Island, NY) supplemented with 10% heat-inactivated fetal calf serum (HyClone Laboratories, Inc., Logan, UT), l-glutamine (2 mM), penicillin (100 units/ml), and streptomycin (100 μg/ml) at 37°C in a humidified 5% CO2 incubator.
**IL-2 Protects Hairy Leukemic Cells from LAK**

**Assay for NKCF Activity.** $^{51}$Cr-labeled target cells, either Eskol or K-562 (1 x 10^5 cells/well, in 50 μl serum-free media), were sedimented in 96-well plates. Supernatants containing various amounts (10, 50, or 100 μl/well) of crude NKCF preparations were added and the total volume was 150 μl/well. The cultures were incubated for 20–24 h. Aliquots of the supernatants were then collected and specific released radioactivity was assayed as described previously.

**Measurement of Class I MHC Antigens on Cell Surface.** Measurement of class I MHC antigen (HLA-A,B,C) expression on Eskol cell surface was done using an indirect immunofluorescence technique and fluorescence activator cell sorter analysis. The entire procedure was performed at 4°C. Target cells (2 x 10^6 cells/tube) were washed with cold PBS containing 0.2% NaN₃ and then incubated with anti-HLA-A,B,C monoclonal antibody (W6/32; diluted 1:100 in PBS + 0.1% bovine serum albumin and 0.02% NaN₃; 45 min; 100 μl). Excess antibody was removed by washing (3 times) with cold PBS and the cell suspension was further incubated with fluorescein isothiocyanate-labeled goat anti-mouse IgG (diluted 1:20 in PBS + 0.1% bovine serum albumin and 0.02% NaN₃; 45 min; 100 μl). The cells were washed with PBS (3 times), fixed with formaldehyde (3%, 1 ml; 18 h), washed again with PBS, passed through glass wool, and then analyzed by FACS II (Becton-Dickinson). At least 10,000 cells were analyzed in each sample for fluorescence intensity and for size at different gains as detected with a photomultiplier tube set at 500–650 nm.

**Statistical Analysis.** All the results are expressed as an arithmetic mean ± SEM. Comparisons were carried out by Student’s t test.

**RESULTS**

IL-2 Protects HCL Cells from LAK Activity. The HCL cell lines were subjected as targets to NK cell assay and were found to be completely resistant (13), as were freshly obtained HCL cells (16). However, Eskol (Fig. 1a), IREs-4 (Fig. 1b), HS-1 (Fig. 1c), and HS-2 (Fig. 1d) cells exhibited a significant sensitivity to LAK cell-mediated cytotoxicity.

The sensitivity of the HCL cells Eskol, IREs-4, HS-1 and HS-2 to LAK activity was significantly ($P < 0.01$–$P < 0.05$) reduced, in a dose-dependent manner, following treatment of the target cells with IL-2 (Fig. 1). The maximum protection against LAK activity was achieved when all the HCL cells were pretreated with 100 units/ml of IL-2 (Fig. 1). The protective effect of IL-2 against LAK cell-mediated cytotoxicity was also found to be time dependent. At least 6 h of incubation with IL-2 were required to render Eskol cells resistant to LAK activity (Table 1). Maximum protection was achieved following 18–24 h of incubation. Subsequent incubation (36, 48, 72 h) with IL-2 did not increase the level of protection against LAK cells (Table 1).

The protective effect of IFN-α against NK and LAK cell-mediated cytotoxicity is well documented (17). Thus, treatment of Eskol (15), HS-1, and HS-2 (not shown) cells with IFN-α resulted in protection against LAK activity. IREs-4 cells did not respond to IFN-α treatment (not shown), although these cells became less sensitive to LAK cytotoxicity following IL-2 treatment (Fig. 1b). Although K562 cells became resistant to NK and LAK cytotoxicity following treatment with IFN-α, they did not respond to treatment with IL-2 (data not shown). On the other hand incubation of Eskol cells in the presence of other cytokines such as IL-6 or IFN-γ had no effect on their susceptibility to LAK lysis.

IL-2 Protective Effect against LAK Cytotoxicity Is Mediated via Synthesis of New Protein(s). Treatment of Eskol cells with CHI concurrently with IL-2 treatment significantly ($P < 0.01$–$P < 0.05$) reduced its ability to protect Eskol cells against killing by LAK cells. With a relatively higher dose of CHI (40 μg/ml), the target cells became as sensitive to LAK activity as untreated control Eskol cells (Fig. 2). The IFN-α-induced protective effect against LAK cell-mediated cytotoxicity was also abolished by treatment with CHI (not shown).
IL-2 Protects Hairy Leukemic Cells from LAK

Table 1 IL-2 protects Eskol cells from LAK cell-mediated cytotoxicity in a time-dependent manner.

<table>
<thead>
<tr>
<th>IL-2 incubation (h)</th>
<th>50:1</th>
<th>25:1</th>
<th>125:1</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>34.6 ± 4.8</td>
<td>21.8 ± 2.6</td>
<td>14.3 ± 2.4</td>
</tr>
<tr>
<td>2</td>
<td>36.2 ± 4.2</td>
<td>19.4 ± 3.6</td>
<td>13.2 ± 2.0</td>
</tr>
<tr>
<td>6</td>
<td>28.4 ± 3.2</td>
<td>14.8 ± 2.2</td>
<td>11.0 ± 1.4</td>
</tr>
<tr>
<td>12</td>
<td>22.4 ± 2.7</td>
<td>11.8 ± 1.8</td>
<td>8.2 ± 1.4</td>
</tr>
<tr>
<td>18</td>
<td>14.8 ± 1.8</td>
<td>7.4 ± 1.6</td>
<td>4.6 ± 1.0</td>
</tr>
<tr>
<td>24</td>
<td>12.6 ± 1.4</td>
<td>8.6 ± 1.0</td>
<td>5.2 ± 0.6</td>
</tr>
<tr>
<td>36</td>
<td>16.0 ± 2.2</td>
<td>7.7 ± 0.8</td>
<td>5.5 ± 1.3</td>
</tr>
<tr>
<td>48</td>
<td>14.3 ± 2.8</td>
<td>8.4 ± 2.2</td>
<td>6.6 ± 0.6</td>
</tr>
<tr>
<td>72</td>
<td>15.4 ± 1.8</td>
<td>7.2 ± 1.4</td>
<td>5.8 ± 0.4</td>
</tr>
</tbody>
</table>

* Eskol cells (2 x 10⁵ cells/ml) were incubated in the presence of 100 units/ml of IL-2.

** The LAK cell-mediated cytotoxicity assay was described in "Materials and Methods." The experiments were done in triplicates. Spontaneous ⁵¹Cr release was 5–12%.

Values represent the data of one of three experiments using different effector cells.

IL-2 Treatment Reduces the Ability of HCL Cells to Activate LAK Cells following Effector Cell-Target Cell Conjugate Formation. The killing process induced by NK and LAK cells consists of several stages. Initially, the target cell is recognized by the effector cell and forms conjugate. The level of conjugate formation between Eskol cells and LAK cells was not affected by pretreatment of Eskol cells with IL-2 (Table 2). Similarly, treatment of Eskol cells with IFN-α did not change the ability of target cells to form conjugates with LAK cells. However, both IL-2 and IFN-α reduce the amount of dead target cells in Eskol cell-LAK cell conjugates (Table 2).

Following binding, the target cell stimulates activation of the conjugated effector cell. The activated killer cells release cytotoxic mediators (NKCF and perforin) which mediate target cell lysis. The sensitivity of Eskol cells to crude preparations of NKCF was assayed. Control, IL-2-treated, and IFN-α-treated Eskol cells exhibited similar susceptibility to NKCF (not shown). Thus, both IL-2 and IFN-α do not change the sensitivity of target cells to the cytotoxic effect of NKCF. However, the ability of Eskol cells to stimulate LAK cell activation and to induce NKCF release from LAK cells was significantly (P < 0.05) reduced following preincubation of Eskol cells with IL-2 (Fig. 3). Similarly, IFN-α reduces the ability of Eskol cells to activate LAK cells following HCL cell-LAK cell conjugate formation (Fig. 3).

IL-2 Protective Effect against LAK Cytotoxicity Is Not Mediated via Endogenous IFN. Because the protective effect against LAK cytotoxicity induced both by IL-2 and IFN-α require synthesis...
The role of the MHC-unrestricted cell mediated cytotoxic arm of the immune system in the remission of hairy cell leukemia is unclear. The HCL cell line, Eskol, like fresh hairy leukemic cells, was found to be completely resistant to NK-cell mediated cytotoxicity (13). However, Eskol cells are sensitive to LAK activity. This result is in agreement with that of others who reported that IFN-α-primed NK cells or LAK cells exerted cytotoxic activity against fresh HCL cells (16). The relatively higher level of cytotoxicity exhibited by the activated effector cells (both IFN-α-primed NK cells and LAK cells) resulted from the high level of NKCF released following binding to the target cells. Patients with HCL have a severe deficiency in NK activity in their peripheral blood (7), in addition to other marked abnormalities in cell-mediated immune functions. The impaired NK-cell mediated cytotoxicity observed in patients with HCL is related to functional defects in NK cells both at the binding and postbinding levels (18). IL-2 alone, or in combination with IFN, is a powerful activator of NK cells. Both cytokines increase NK activity in HCL patients' peripheral blood cells and splenocytes (19, 20). In this study we show an enhancement of killer cell activity by IL-2 against HCL cells; however, pretreatment of the same target cells with the same doses of IL-2 results in resistance to LAK cytotoxicity.

**DISCUSSION**

Adaptive immunotherapy combines administration of LAK cells and coinfusion of IL-2. This type of immunotherapy was relatively successful in some experimental tumors (in animal models) and also in clinical trials in the treatment of advanced human malignancies such as renal cancer, melanoma, and acute leukemia (5). Yet in other tumor types, adaptive immunotherapy was found to be ineffective. Here we report that IL-2 not only stimulates LAK cell-mediated cytotoxicity but also protects HCL cells from LAK activity. This finding may explain the lack of successful adaptive immunotherapy in tumor cells bearing IL-2 receptors.

**Table 2** Conjugate formation between Eskol cells and LAK cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Conjugate formation (% ± SEM)</th>
<th>Conjugated dead cells (% ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment</td>
<td>4.6 ± 0.8</td>
<td>28.6 ± 3.4</td>
</tr>
<tr>
<td>IL-2*</td>
<td>5.2 ± 1.1</td>
<td>14.7 ± 1.2</td>
</tr>
<tr>
<td>IFN-α</td>
<td>4.3 ± 0.6</td>
<td>11.9 ± 1.6</td>
</tr>
</tbody>
</table>

* Eskol cells were treated with IL-2 (100 units/ml) for 18 h.

**Table 3** Anti-IFN monoclonal antibodies do not abolish the IL-2-induced protective effect against LAK cytotoxicity

<table>
<thead>
<tr>
<th>Cytokine treatment</th>
<th>LAK cytotoxicity (% ± SEM) against Eskol cells*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>32.8 ± 3.2</td>
</tr>
<tr>
<td>IFN-α-treated</td>
<td>34.4 ± 2.4</td>
</tr>
<tr>
<td>IL-2-treated</td>
<td>29.6 ± 2.0</td>
</tr>
</tbody>
</table>

* The LAK cell cytotoxicity assay was described in "Materials and Methods." Effector cell/target cell ratio was 50:1. The experiments were done in triplicates. Values represent the data of one of three experiments using different donors. Spontaneous 51Cr release was 14–22% and SEM was 0.8–4.6.
The protective effect of IL-2 against LAK activity resembles the protective effect of IFN. Both lymphokines require synthesis of new protein(s) to mediate their activity. Moreover, IL-2 as well as IFN-α has no effect on the ability of Eskol cells to form conjugates with LAK cells. Neither lymphokine has any effect on the susceptibility of the hairy leukemic cells to NKCF. IL-2-induced inhibition of Eskol cell susceptibility to lysis by LAK cells was shown to result from a defect in their ability to stimulate activation of conjugated LAK cells and release of NKCF. This is also true for IFN (21). Although there are similarities between the protective effects against LAK cytotoxicity induced by IL-2 and IFN, it is clear that endogenous IFN is not the mediator of the protective effect induced by IL-2 against LAK activity.

Five sets of evidence indicate that IL-2 directly mediates protection against LAK activity: (a) monoclonal antibodies against IFN-α and IFN-γ do not block the protective effect induced by IL-2, while anti-IFN-α antibodies block the protection induced by IFN-α; (b) exogenous IFN-γ does not reduce the sensitivity of Eskol cells to LAK activity; (c) IFN-α has a cell growth-inhibitory effect both on fresh HCL cells and on Eskol cells (22). No changes were found in Eskol cell viability or cell proliferation following treatment with IL-2. This observation indicates that IL-2 does not induce IFN-α production by either the effector LAK cells or by the leukemic hairy cells; (d) the IFN-resistant clone, IRE-α is less sensitive to LAK cytotoxicity following treatment with IL-2. IFN-α treatment did not affect the sensitivity of IRE-α cells to LAK cell-mediated cytotoxicity, (e) the final piece of evidence against a role for IFN as a mediator of the IL-2-protective effect against LAK activity is the critical one. IFN-α increases the expression of class I MHC antigens in Eskol cells, while IL-2 lacks this ability.

Several observations and experimental manipulations suggest that class I MHC antigens expressed on target cell surface render the cells resistant to NK and LAK activities. There is often an inverse relationship between target cell class I MHC expression and NK/LAK sensitivity (22). Moreover, transfection of class I MHC genomic clones into class I MHC-deficient NK/LAK-sensitive cells generates transfectants which are both high expressors of class I MHC antigens and NK/LAK resistant (23–26). Target cells which are treated in vitro with IFN become refractory to NK and LAK cell-mediated cytotoxicity and express high level of class I MHC antigens. Thus, class I MHC antigens appear to act as an IFN-inducible signal preventing attack by NK and LAK cells. IL-2 mediates its protective effect against LAK cytotoxicity without induction of class I MHC antigens. This finding supports the previous evidence that IFN does not mediate the IL-2-induced protective effect against LAK cytotoxicity.

Adoptive immunotherapy (LAK therapy and IL-2 infusion) has been found to be successful in some malignancies but ineffective in other. The limitation of this type of immunotherapy may be explained, in part, by the fact that IL-2 not only induces LAK activity but also protects some tumor cells from NK and LAK activity. This hypothesis should be studied on IL-2-receptor-bearing, fresh tumor cells and tumor cell lines from patients resistant to adoptive immunotherapy.

REFERENCES

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