Direct Suppression of Natural Killer Activity in Human Peripheral Blood Leukocyte Cultures by Glucocorticoids and Its Modulation by Interferon

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ABSTRACT

In vitro treatment of human peripheral blood leukocytes for 18 to 24 hr with physiological concentrations of glucocorticoids resulted in a marked decrease (up to 90%) in natural killer (NK) activity. The effect on NK activity was both dose and time dependent and was specific for glucocorticoids. Glucocorticoids had no effect when added directly to the 4-hr 51Cr release cytotoxicity assay, nor did they alter the susceptibility of K562 cells to NK-mediated cytolysis. Glucocorticoid-induced inhibition occurred in Percoll-fractionated peripheral blood leukocytes enriched for NK activity. Viabilities of steroid-treated and untreated cultures were similar. Mixing experiments failed to demonstrate the involvement of suppressor activity in the inhibition. Purified cloned human leukocyte interferon subtype A and inducers of interferon enhanced NK activity in the presence of glucocorticoid, although the levels of enhancement were lower than those produced by these agents in the absence of the steroid.

Thus, glucocorticoids appear to suppress human NK activity by interacting directly with the NK effector cell, and our results obtained with physiological concentrations of these steroids suggest that they may play an important role in regulating NK activity in vivo. Additionally, these findings suggest a possible means for overcoming this immunosuppressive side effect of glucocorticoid therapy by simultaneous treatment with interferon.

INTRODUCTION

NK activity appears to be associated with a subpopulation of normal lymphocytes capable of spontaneously lysing certain tumor and normal cell targets (11). There is now considerable evidence supporting the role of NK cells in in vivo resistance to tumor growth in mice (10, 24, 25, 30, 31), and it has been hypothesized that NK activity is a primary mechanism of immune surveillance (11, 12). Although NK activity has been studied extensively in humans and other animals, its regulation is poorly understood. Interferon and substances capable of inducing interferon enhance NK activity (8, 16, 33), while a variety of other immunopharmacological agents inhibit NK activity (4, 13, 14, 20).

In particular, glucocorticoids depress NK activity of human peripheral blood when administered in vivo (21–23). These steroids are used therapeutically for a variety of conditions, including treatment of immunological disorders, various neoplastic diseases, and preservation of renal transplant, many of which display a high incidence of secondary cancers (9, 26, 29, 35). Since deficiencies in NK activity have similarly been correlated with increased susceptibility to tumor development (26, 27, 29), it is important to understand how this immune function is affected by glucocorticoids.

Glucocorticoid administration causes a marked redistribution of lymphocytes in the peripheral circulation (6, 7), and it is possible that glucocorticoids influence levels of NK activity indirectly simply by altering the relative proportion of NK cells in blood. One report suggests that this is the case (21). Whether glucocorticoids can directly influence NK activity is unclear (21–23). In order to understand the mechanism for depression of NK activity by glucocorticoids in vivo, it is important to resolve this question. This need prompted the present study in which we have examined the effect of glucocorticoids on NK activity of isolated human PBL in culture. Our findings indicate that glucocorticoids suppress human NK activity in vitro with dose-response and specificity relations consistent with physiological actions of these steroids. Suppression appears to occur via a direct effect on the NK effector population. In addition, because interferons enhance NK activity and are currently being used therapeutically for treatment of human cancers, we have examined the relationship between the effects of glucocorticoids and purified cloned human leukocyte interferon on NK activity.

MATERIALS AND METHODS

Preparation of Effector Cells. PBL were isolated from heparinized blood of normal healthy donors (male and female; ages 22 to 35) by Ficoll/Hypaque density centrifugation (1). The cells were washed 3 times with serum-free RPMI 1640 (MA Biologicals, Bethesda, Md.) after which they were resuspended to the desired density (5 × 10⁶ cells/ml) in complete RPMI 1640, which is RPMI 1640 supplemented with 10% fetal bovine serum (MA Biologicals), 5 × 10⁻⁵ M 2-mercaptoethanol, 15 μM N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (Research Organics, Cleveland, Ohio), and gentamicin (50 μg/ml; Schering Corp., Kenilworth, N. J.).

Removal of Adherent Cells and Percoll Fractionation Procedure. Adherent cells were removed using either Sephadex G-10 or nylon wool columns as described in detail by Jerrelds et al. (15). Nonadherent PBL were fractionated on a discontinuous density gradient of Percoll (Pharmacia Fine Chemicals, Uppsala, Sweden) according to the procedure of Timonen et al. (32). Briefly, PBL nonadherent to plastic were passed over a nylon wool column, then layered over a 12-ml Percoll gradient, and centrifuged at room temperature at 550 × g for 30 min. The approximate Percoll concentrations from the top (Fraction 1) to the

1 This research was supported by Research Grants CA 17323 and AM 03535 and by Cancer Center Core Grant CA 23108 from the USPHS.

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3 The abbreviations used are: NK, natural killer; PBL, peripheral blood leukocytes; RPMI 1640, Roswell Park Memorial Institute Tissue Culture Medium 1640; LEIF-A, bacteria-derived human leukocyte interferon subtype A; poly(l-C), alternating copolymer of polyinosinic and polycytidyllic acids; LEIF-D, bacteria-derived human leukocyte interferon subtype D.

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bottom (Fraction 7) of the gradient were 40, 43, 46, 48, 51, 53, and 66%. Greater than 80% of the cells comprising the 40 and 43% Percoll fractions were identified as large granular lymphocytes based on their morphological characteristics in 10% Giemsa-stained preparations (32).

Treatment with Steroids. Dexamethasone (Steroids, Inc., Wilton, N. H.) was prepared as a 200 μM stock solution in 95% ethanol and stored at 4°C. For use in experiments, a 0.25-mL aliquot of the stock solution was added to a 100 x 20-mm culture dish (Falcon Plastics, Oxnard, Calif.), and the ethanol was allowed to evaporate. The glucocorticoid was then redissolved in 10 ml complete RPMI 1640 by incubation at 37°C for 30 min and used after 10-fold dilution (0.5 μM) unless otherwise indicated. Control medium was prepared in the same manner with 0.25 ml 95% ethanol alone. Thus, no ethanol was present in the steroid solutions which were added to the cell suspensions. The other steroids were prepared in a similar manner and used at a final concentration of 1 μM.

Treatment with Interferon and Interferon Inducers. Purified LEIF-A was generously provided by Genentech, Inc. (San Francisco, Calif.). This cloned material which was greater than 95% pure and electrophoretically homogeneous had a specific activity of 1.2 x 10^6 IU/mg protein as determined on human amnion cells (WISH) challenged with vesicular stomatitis virus. The LEIF-A was diluted in complete RPMI 1640 and used at a final concentration of 500 IU/ml. Preliminary experiments showed this concentration to give maximum enhancement of NK activity. Staphylococcal enterotoxin type B (Sigma Chemical Co., St. Louis, Mo.) and poly(I-C) (Sigma) were prepared in complete RPMI 1640 and used at concentrations of 1 and 100 μg/ml, respectively. These concentrations cause maximum enhancement of NK activity.

In Vitro Incubations. PBL cells prepared and treated as described above were incubated in 50-ml conical polypropylene centrifuge tubes (Corning Glass Works, Corning, N. Y.) at 37°C in a humidified atmosphere of 95% air:5% CO2 for the indicated times.

Target Cells. The human cell line K562 was maintained in suspension culture in RPMI 1640 supplemented with 10% fetal bovine serum and gentamycin (50 μg/ml). For use in the cytotoxicity assays, 2 x 10^6 cells were incubated with 50 μCi Na^14CrO4 (New England Nuclear, Boston, Mass.) for 1 hr at 37°C in 95% air:5% CO2. 14Cr-labeled targets were washed 3 times at 4°C in complete RPMI 1640 and resuspended to 1 x 10^6 cells/ml. Viability was assessed by trypan blue dye exclusion before and after the labeling procedure.

Cytotoxicity Assay. After the in vitro incubations, effector cells were washed twice, resuspended in complete RPMI 1640, and examined for viability by trypan blue dye exclusion. No significant differences were observed for viability of effector cells treated with any of the agents above relative to untreated cells. NK activity was measured by a 4-hr 51Cr release assay described previously (3). Percentages of cytotoxicities were computed from the following formula.

\[
\% \text{ of cytotoxicity} = \frac{\text{test cpm} - \text{spontaneous cpm}}{\text{total cpm} - \text{spontaneous cpm}} \times 100
\]

Test cpm, total cpm, and spontaneous cpm represent those in supernatants from target cells incubated with effectors, from target cells lysed with 10% sodium dodecyl sulfate, and from target cells incubated without effector cells, respectively. Spontaneous release never exceeded 10% of the maximum release. Except where indicated, results are expressed as the mean percentage of cytotoxicity ± S.D. of 3 to 6 replicate cultures for representative experiments.

Statistical Significance. Statistical significance of differences in the percentage of cytotoxicity comparing control and dexamethasone-treated PBL was calculated using the paired sample t test (2 tailed). p < 0.01 was considered significant.

RESULTS

Suppression of NK Activity in Cultured Human PBL by Dexamethasone. Table 1 shows the effect of treatment of human PBL with 0.5 μM dexamethasone on NK activity. The table summarizes data obtained for 6 different donors assayed multiple times in separate experiments. Results are presented for an effector:target cell ratio of 50:1 using K562 cells as targets, but similar results were obtained at other ratios and with other NK-sensitive targets (not shown). In vitro treatment of PBL with dexamethasone for 18 to 24 hr resulted in a significant (p < 0.01) decrease in NK activity for all donors. The magnitude of the effect varied from one donor to another and even for the same donor from one experiment to the next. However, we have not found an individual whose cells appear refractory to glucocorticoids in this regard.

Time Course for Dexamethasone Suppression of NK Activity. In initial experiments, we tested the effect of adding dexamethasone directly to the 4-hr 51Cr release cytotoxicity assay of PBL against K562 cells. We observed no effect of dexamethasone on NK activity under these conditions for concentrations up to 1.0 μM (data not shown). Furthermore, addition of the steroid to K562 cells alone during the 4-hr assay period did not alter their spontaneous release of 51Cr.

In order to determine the time of treatment required for dexamethasone to inhibit NK activity, PBL cultures were incubated for a 24-hr period, throughout which dexamethasone was added at various times. After the incubation, the cells were washed twice and assayed for cytotoxicity against K562 cells. Chart 1 shows the mean of results obtained from 4 separate experiments with different donors. While no inhibition was seen in cultures treated with dexamethasone immediately prior to the 51Cr release cytotoxicity assay (Time 0), some inhibition was apparent as early as 6 hr after exposure to the steroid and continued to increase up to 24 hr. Other experiments in which dexamethasone was added to PBL at the onset of in vitro culture (Time 0) and in which samples were assayed at various times thereafter for NK cytotoxicity showed a similar time course for development of the suppressive effect. Therefore, in the experiments described below, PBL were incubated with steroid for 18 to 24 hr prior to being assayed for cytotoxicity.

Dose-Response Relationship for Dexamethasone Suppression of NK Activity. The dose-response relationship for dexamethasone suppression of human NK activity is shown in Chart 2. Some decrease in cytotoxicity was evident at concentrations as low as 0.1 nM, and the maximum suppressive effect was
Considerable inhibition was observed with PBL exposed (18, 19). Results of 2 separate experiments are summarized in cytotoxicity for control and dexamethasone-treated populations at 6, 12, 18, and was that seen for an untreated culture incubated for 24 hr. Control cytotoxicities obtained from 4 separate experiments using different donors. Control cytotoxicity for the indicated times before being tested in a 4-hr *Cr release assay for cultured PBL. All compounds were tested at 1 IÍM, as this to determine which were capable of modulating NK activity of (28). Several classes of steroid hormones were therefore tested to suppress NK activity in vivo (5, 19). Inhibition of peripheral blood NK activity after in vitro treatment with glucocorticoid has been shown to be of short duration. Parrillo and Fauci (23) reported that administration of a single dose of dexamethasone to volunteers resulted in maximum inhibition of peripheral blood NK activity within 24 hr, followed by significant recovery over the next 24-hr period with levels approaching those before dexamethasone treatment by 48 to 56 hr. To determine if in vitro glucocorticoid inhibition of NK activity would be relieved if dexamethasone were removed from cultures, we performed the following experiment. Cultures treated with dexamethasone for 24 hr were divided into 2 groups. The first group was washed free of steroid in the following manner. Cultures were centrifuged, supernatants were removed, and the cell pellets were resuspended in 50 ml serum-free RPMI 1640 at 37° left at that temperature for 10 min. The wash procedure was then repeated, after which the cells were incubated in complete RPMI 1640 for an additional 24 hr. The second group of cultures was washed similarly, except that medium contained 0.5 μM dexamethasone. Samples of each group were assayed for NK activity at 6-hr intervals. The results presented in Chart 3 are representative of 4 separate experiments which demonstrated that, while removal of dexamethasone prevented further decreases in NK activity, there was no reversal of the glucocorticoid-mediated inhibition up to 24 hr after washing. A central question with regard to the mechanism of the suppressive effect of glucocorticoids on NK activity is whether the inhibition by dexamethasone occurs directly at the level of the effector cell mediating NK cytolysis or indirectly through another cell type. To determine what cells are involved in the glucocorticoid-mediated inhibition of NK activity, several enrichment procedures were used. Removal of monocytes from PBL by passage over nylon wool or Sephadex G-10 columns before treatment with dexamethasone did not alter the suppression of NK activity (data not shown).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Untreated control (%)</th>
<th>Untreated control (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dexamethasone</td>
<td>21.0 ± 0.4 (100)</td>
<td>44.8 ± 1.1 (100)</td>
</tr>
<tr>
<td>Triamcinolone acetonide</td>
<td>10.3 ± 1.8 (49.9)</td>
<td>20.5 ± 1.6 (45.7)</td>
</tr>
<tr>
<td>Cortisol</td>
<td>9.0 ± 0.9 (41.4)</td>
<td>24.9 ± 0.5 (55.6)</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>14.0 ± 0.8 (68.5)</td>
<td>30.2 ± 0.2 (67.2)</td>
</tr>
<tr>
<td>Cortexol</td>
<td>14.7 ± 1.5 (70.2)</td>
<td>32.6 ± 1.6 (72.8)</td>
</tr>
<tr>
<td>Cortisol</td>
<td>30.6 ± 0.9 (145.5)</td>
<td>41.0 ± 2.3 (91.5)</td>
</tr>
<tr>
<td>Deoxycorticosterone</td>
<td>26.5 ± 0.9 (126.2)</td>
<td>36.0 ± 0.8 (80.4)</td>
</tr>
<tr>
<td>Progesterone</td>
<td>24.6 ± 0.5 (117.4)</td>
<td>40.1 ± 2.2 (88.9)</td>
</tr>
<tr>
<td>Estradiol</td>
<td>26.0 ± 1.1 (123.9)</td>
<td>40.9 ± 1.5 (91.3)</td>
</tr>
<tr>
<td>Testosterone</td>
<td>26.5 ± 1.5 (129.2)</td>
<td>41.6 ± 0.4 (92.8)</td>
</tr>
</tbody>
</table>

* Mean ± S.D.  
* Numbers in parentheses, percentage of control cytotoxicity.
Further enrichment of NK effector cells was achieved by separation of nylon wool-passed cells on a discontinuous Percoll gradient. The fractions obtained were incubated for 24 hr in the absence or presence of dexamethasone. As shown in Chart 4, dexamethasone inhibition of NK activity was clearly apparent in these fractions which exhibited enriched NK activity. In these enriched fractions, viabilities for both control and dexamethasone-treated cultures were greater than 90%. Although these results are consistent with a direct action of dexamethasone on the cell or cells responsible for NK cytolysis, they do not rule out the possibility that the inhibition occurs indirectly through another cell (or cells) which regulates NK activity and cofractionates with the cytolytic activity on Percoll gradients. This possibility is addressed further in the experiments below.

**Dexamethasone-treated PBL Do Not Suppress NK Activity of Untreated PBL.** Hochman and Cudkowicz (13) have reported that, in the mouse, inhibition of NK activity in vivo results from activation of a suppressor cell. They found that spleen cells from mice treated with hydrocortisone suppressed the NK activity of spleen cells from untreated mice when the 2 populations were combined. We tested the possibility that glucocorticoids inhibit human PBL NK activity by stimulation of suppressor activity in 2 ways.

Initially, PBL cultured overnight in the presence or absence of dexamethasone were mixed in different proportions immediately before being tested in a 4-hr $^{51}$Cr release assay for cytotoxicity against K562 cells. O, control untreated cultures; •, dexamethasone-treated cultures; △, cultures from which dexamethasone was removed. Bars, S.D.

![Chart 3. Effect of dexamethasone removal on NK activity of cultures pretreated with glucocorticoid. PBL were incubated for 24 hr in the presence or absence of 0.5 μM dexamethasone. Glucocorticoid-treated cultures were then divided into 2 groups; one group was washed free of dexamethasone, while the other group was washed with medium containing dexamethasone. Cultures were further incubated for the indicated times before being tested in a 4-hr $^{51}$Cr release assay for cytotoxicity against K562 cells. O, control untreated cultures; •, dexamethasone-treated cultures; △, cultures from which dexamethasone was removed. Bars, S.D.](image)

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Initially, PBL cultured overnight in the presence or absence of dexamethasone were mixed in different proportions immediately before addition to $^{51}$Cr-labeled K562 targets or were incubated together for up to 8 hr before addition to targets. Effector:target cell ratios were kept low (ranging from 5:1 to 20:1) so that cytotoxicity was approximately linear with respect to number of effector cells. Predicted cytotoxicities for the mixed populations were determined by adding the cytotoxicities observed separately for control and dexamethasone-treated PBL at the appropriate effector:target cell ratios. In all cases, the actual cytotoxicities observed using various mixtures of control and dexamethasone-treated cells were close to predicted values, providing no evidence for suppressor activity during the assay (not shown).

These initial findings were corroborated by more rigorous experiments in which Percoll-fractionated populations of dexamethasone-treated PBL were tested for suppressor activity. PBL cultured overnight in the presence of dexamethasone were subsequently separated on Percoll gradients to obtain fractions which contained enriched NK activity (43% Percoll). We reasoned that if dexamethasone inhibited NK activity by stimulation of cells with suppressor activity which fractionated on Percoll similarly to the NK effector cells, then the suppressor activity should also be enriched in the 43% Percoll fraction. To determine if this were the case, dexamethasone-treated PBL comprising the 43% Percoll fraction were added to freshly isolated PBL from the same donor at a 1:1 ratio, and the mixed populations were assayed for cytotoxicity against K562 cells. Freshly isolated PBL were also mixed with cells from NK-depleted Percoll fractions (53% Percoll; see Chart 4) which served as filler cells. The results in Table 3 do not suggest the involvement of a suppressor cell in

![Chart 4. Dexamethasone suppresses cytotoxicity of PBL enriched for NK activity by Percoll density gradient centrifugation. PBL were separated on Percoll gradients, and the various fractions were incubated for 24 hr in the absence (C) or presence (E) of 0.5 μM dexamethasone. Due to a low number of cells recovered in fractions obtained with 40 plus 43% Percoll, they were pooled, as were those fractions with 53 and 66% Percoll. A 4-hr $^{51}$Cr release cytotoxicity assay was performed against K562 cells at an effector:target cell ratio of 5:1. Bars, S.D.](image)

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### Table 3

<table>
<thead>
<tr>
<th>Effector cell combinations</th>
<th>Observed</th>
<th>Predicted</th>
</tr>
</thead>
</table>
| PBL cultured 24 hr        | 32.9 ± 2.8  
| PBL cultured 24 hr with dexamethasone (0.5 μM) | 3.0 ± 0.4  
| 53% Percoll-fractionated PBL cultured with dexamethasone | 0.9 ± 0.1  
| 43% Percoll-fractionated PBL cultured with dexamethasone | 2.2 ± 0.3  
| Freshly isolated PBL     | 21.0 ± 1.8  
| Fresh PBL + unfractionated PBL cultured with dexamethasone | 23.3 ± 1.0  
| Fresh PBL + 53% Percoll-fractionated PBL cultured with dexamethasone | 22.6 ± 1.8  
| Fresh PBL + 43% Percoll-fractionated PBL cultured with dexamethasone | 23.2 ± 1.6  

* Predicted cytotoxicities were determined by adding cytotoxicities obtained separately with fresh PBL and the appropriate group of dexamethasone-treated PBL.  
* Mean ± S.D.  
* NK-depleted fraction.  
* NK-enriched fraction.
dexamethasone-mediated inhibition of NK cytotoxicity. Although the dexamethasone-treated PBL (Percoll fractionated and unfractonated) themselves exhibited depressed NK activity compared to untreated PBL, they did not lower NK activity when added to fresh PBL.

**Supernatants from Dexamethasone-treated PBL Do Not Alter NK Activity of Untreated PBL.** We next determined if treatment of PBL with dexamethasone resulted in the production of a soluble factor capable of suppressing NK activity. Isolated PBL were cultured overnight in the absence or presence of dexamethasone, after which the cell suspensions were centrifuged, and the resulting supernatants were harvested and passed through a 0.45-μm filter to remove any cells or debris. The remaining cell pellets were washed and resuspended in the same supernatant. Spontaneous release of 51Cr from K562 cells was the same for all treatment groups. The results shown in Table 4 rule out the presence of a factor that suppresses lytic activity in the supernatants from cells cultured in dexamethasone. Dilution of the supernatants did not alter the results. Other experiments in which untreated cells were incubated in supernatants of dexamethasone-treated cultures for up to 2 hr before their addition to K562 cells gave similar results (not shown).

**Combined Effects of Glucocorticoids and LEIF-A on NK Activity.** Interferon augments human NK activity both in vivo and in vitro and has been suggested to be an important endogeneous regulator of NK activity. Thus, it was of interest to determine what effect combined treatment of PBL with cloned LEIF-A and dexamethasone would have on NK activity. Results of experiments using 3 different treatment schedules are summarized in Table 5. Concentrations of LEIF-A (500 units/ml) and dexamethasone (0.5 μM) were both sufficient to produce their maximum respective effects. As seen for Schedule A, in relation to untreated controls, treatment of cells for 24 hr with LEIF-A alone increased NK activity 2- to 3-fold, while treatment with dexamethasone alone decreased activity to less than half. Simultaneous treatment with the 2 agents for 24 hr resulted in enhanced NK activity when compared with dexamethasone treatment alone. Activity was similar or higher than that of untreated controls. Since enhancement of NK activity by interferon occurs rapidly, requiring less than 2 hr of exposure to produce the maximum effect, whereas dexamethasone requires a greater length of time to cause significant suppression, we wondered how varying the schedule of treatment with these 2 agents might affect the results. In Schedule B, PBL were first incubated with dexamethasone for 20 hr to obtain significant suppression of NK cytotoxicity, followed by an additional 4 hr with LEIF-A. In Schedule C, PBL were first pretreated with LEIF-A for 4 hr to maximally activate NK activity, followed by an additional 20-hr incubation with dexamethasone. Results with both these treatment schedules were similar to those with Schedule A. Thus, dexamethasone does not prevent boosting of NK activity in response to LEIF-A regardless of how depressed NK activity is before LEIF-A treatment (Schedule B), nor does LEIF-A under any circumstances prevent the suppression of NK activity by dexamethasone. Instead, the 2 opposing effects appear essentially additive, thereby nullifying each other somewhat.

### Table 4

<table>
<thead>
<tr>
<th>Cells</th>
<th>Resuspension medium for 51Cr release assay</th>
<th>% of cytotoxicity&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (untreated)</td>
<td>Control cell supernatant</td>
<td>44.2 ± 5.3</td>
</tr>
<tr>
<td>Control (untreated)</td>
<td>Control cell supernatant + 0.5 M dexamethasone</td>
<td>41.0 ± 2.5</td>
</tr>
<tr>
<td>Control (untreated)</td>
<td>Dexamethasone-treated cell supernatant</td>
<td>37.0 ± 6.6</td>
</tr>
<tr>
<td>Dexamethasone treated</td>
<td>Control cell supernatant</td>
<td>19.4 ± 1.9</td>
</tr>
<tr>
<td>Dexamethasone treated</td>
<td>Control cell supernatant + 0.5 M dexamethasone</td>
<td>19.5 ± 1.2</td>
</tr>
<tr>
<td>Dexamethasone treated</td>
<td>Dexamethasone-treated cell supernatant</td>
<td>18.3 ± 0.8</td>
</tr>
</tbody>
</table>

<sup>a</sup> Results are shown for an effector:target cell ratio of 25:1. Similar results were obtained with other effector:target cell ratios.

<sup>b</sup> Mean ± S.D.

### Table 5

**Combined effects of dexamethasone and LEIF-A on human PBL NK activity in vitro**

<table>
<thead>
<tr>
<th>Treatment schedule</th>
<th>Treated group</th>
<th>% of cytotoxicity</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (PBL treated separately or simultaneously with both agents for 24 hr)</td>
<td>No treatment</td>
<td>28.6 ± 2.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.0 ± 1.2</td>
<td>58.0 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>Dexamethasone</td>
<td>12.5 ± 0.8</td>
<td>70.5 ± 2.4</td>
<td>70.4 ± 1.1</td>
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<tr>
<td></td>
<td>LEIF-A</td>
<td>41.2 ± 1.9</td>
<td>44.4 ± 0.8</td>
<td>44.4 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>LEIF-A + dexamethasone</td>
<td>28.6 ± 2.4</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
<td>61.0 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>No treatment</td>
<td>12.5 ± 0.8</td>
<td>26.5 ± 1.3</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Dexamethasone</td>
<td>10.5 ± 1.9</td>
<td>69.4 ± 0.8</td>
<td>7.0 ± 0.4</td>
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<tr>
<td></td>
<td>LEIF-A</td>
<td>35.0 ± 1.0</td>
<td>87.5 ± 1.1</td>
<td>40.4 ± 1.2</td>
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<tr>
<td></td>
<td>LEIF-A + dexamethasone</td>
<td>35.0 ± 1.0</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
<td>87.5 ± 1.1</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean ± S.D.

<sup>b</sup> ND, not determined.
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Enhancement of human NK activity by interferon inducers in the presence of dexamethasone in vitro

Freshly isolated PBL were incubated for 24 hr with staphylococcal enterotoxin type B (1.0 μg/ml) or poly(I-C) (100 μg/ml) in the presence or absence of dexamethasone (0.5 μM). Cytotoxicity was assayed in a 4-hr 51Cr release assay against K562 cells.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Treatment</th>
<th>% of cytotoxicity at effector:target cell ratios of</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>6:0:1</td>
</tr>
<tr>
<td></td>
<td>Dexamethasone</td>
<td>3.2 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>poly(I-C)</td>
<td>41.4 ± 3.1</td>
</tr>
<tr>
<td></td>
<td>poly(I-C) + dexa-</td>
<td>27.2 ± 7.4</td>
</tr>
<tr>
<td></td>
<td>methasone</td>
<td>37.6 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>poly(I-C) + dexa-</td>
<td>11.0 ± 2.1</td>
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<tr>
<td>2</td>
<td>Control</td>
<td>12.5:1</td>
</tr>
<tr>
<td></td>
<td>Dexamethasone</td>
<td>4.6 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>poly(I-C)</td>
<td>10.0 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>poly(I-C) + dexa-</td>
<td>16.8 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>methasone</td>
<td>40.0 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>poly(I-C) + dexa-</td>
<td>14.1 ± 0.1</td>
</tr>
</tbody>
</table>

* Mean ± S.D.
b SEB, staphylococcal enterotoxin type B.

Although dexamethasone does not prevent boosting of cytoxicity by addition of exogeneous LEIF-A, it is possible that glucocorticoids could interfere with endogeneous production of interferon necessary for maintenance of NK activity. Therefore, we tested the ability of 2 inducers of interferon, staphylococcal enterotoxin type B and poly(I-C), to enhance NK activity of PBL treated simultaneously with dexamethasone. As shown in Table 6, both interferon inducers caused enhancement of NK activity despite the presence of dexamethasone. The results were essentially identical to those obtained with the purified LEIF-A, further supporting the notion that dexamethasone and interferon act independently of each other to modulate NK activity.

**DISCUSSION**

Therapeutic doses of glucocorticoids depress the level of NK activity seen in peripheral blood of treated individuals (21–23). However, whether the lowered NK activity results indirectly because of a redistribution of lymphocytes in circulation caused by these steroids (6–21) or whether glucocorticoids can directly affect NK activity has been unclear (21–23). In this paper, we demonstrate that physiological concentrations of glucocorticoids severely suppress NK activity of cultured PBL. While these results differ from previous reports, there are a number of possible explanations for the differences. Parrillo and Fauci (23) first reported that dexamethasone inhibits human NK activity in vitro, but only at concentrations of 10^{-5} to 10^{-3} M. However, since these concentrations are 10- to 100-fold higher than those seen in vivo and are known to produce nonspecific and even toxic effects (18, 19), these findings are unlikely to have any physiological relevance. With lower concentrations (1 μM or less), they observed no inhibitory effect. However, in their studies, Parrillo and Fauci added dexamethasone directly to the cytotoxicity assay without prior treatment of the effector cells. We obtained similar results when adding dexamethasone to the 51Cr release assay. Our results indicate that inhibition of NK activity is a time-dependent process, requiring treatment of PBL for at least 6 hr to observe any suppression. Since Parrillo and Fauci did not pretreat the effector populations before the incubation with targets, no inhibition of cytotoxicity would be expected, as during the assay, considerable cytotoxicity (i.e., release of 51Cr) would occur before the induction of suppression by dexamethasone. In another study, Onsrud and Thorsby (21) reported that hydrocortisone succinate (0.2 to 20 μM) had no effect on PBL NK activity in vitro, despite the fact that similar concentrations significantly altered levels of NK activity in vivo. They therefore concluded that NK cells were resistant to glucocorticoids. The problem with these studies is that they used hydrocortisone succinate for the in vitro experiments without testing free hydrocortisone to see if it acted similarly. There is no evidence that hydrocortisone succinate is active as a glucocorticoid until it has been hydrolyzed to free hydrocortisone. While hydrolysis readily occurs in vivo, it is possible that in the in vitro system of Onsrud and Thorsby such hydrolysis does not take place. In their studies, this compound also failed to inhibit phytohemagglutinin responsiveness in cultured PBL, a well-recognized effect of glucocorticoids (5, 7). As shown in Table 2, we found that hydrocortisone (cortisol) did, like the other glucocorticoids tested, inhibit NK activity in vitro.

Few attempts have been made to explore the mechanism by which glucocorticoids inhibit NK activity. Evidence for the involvement of a suppressor cell in mediating glucocorticoid inhibition of mouse NK activity in vivo has been reported by Hochman and Cudkowicz (13), but Lotzova and Savary (17) found no evidence for suppressor activity and suggested that glucocorticoids affected NK cytotoxicity directly. We have also obtained in vitro evidence to suggest that glucocorticoids can directly inhibit mouse splenic NK activity. Our data presented here for human PBL are likewise consistent with the notion that glucocorticoids act directly on NK effector cells to inhibit their cytolytic function. Using enrichment procedures to eliminate certain cell populations and mixing experiments to detect suppressor activity, we could not demonstrate a requirement for any other particular cell type in the inhibitory process. However, until purified homogeneous populations of NK cells are available, this possibility cannot be ruled out.

Little is known of how NK activity is regulated in vivo, but it has been suggested that interferon produced endogenously may play a key role in modulating NK activity (12). Our data obtained using physiological concentrations of these steroids suggest that glucocorticoids also may function as an endogenous modulator of NK activity. Support for this hypothesis is provided by the fact that animals, including humans, when stressed in a variety of ways (environmentally, psychologically, or physically), display lowered NK activity associated with increased serum glucocorticoid levels (26, 34). This is particularly interesting in view of the increasing evidence suggesting a direct correlation between levels of stress and increased tendencies towards tumor development (26, 29).

Since glucocorticoids and interferon have opposing effects on NK activity, we examined the effect of combined treatment with these agents on PBL NK activity. Although only cloned LEIF-A was used in the present study, we have obtained similar results with a different subtype, LEIF-D, and with cloned human γ-interferon. Our finding that LEIF-A and inducers of interferon...
enhance human NK activity in vitro in the presence of dexamethasone is consistent with in vivo studies with both rats and mice in which enhancement of splenic NK activity by poly(I-C) occurred in the presence of suppressive concentrations of glucocorticoids (4, 20). However, the extent of boosting by LEIF-A or interferon inducers in the presence of glucocorticoids varies somewhat in these systems. Simultaneous in vitro treatment of human PBL with dexamethasone and interferon or interferon inducers resulted in near-normal or higher-than-control levels of NK activity but did not augment NK activity to the level seen with interferon alone. In the in vivo studies with rats and mice, poly(I-C) abolished the glucocorticoid effect on splenic NK activity. This difference may reflect the availability of NK precursor or extrasplenic NK cells which are able to repopulate the spleen after interferon treatment. This possibility is excluded in in vitro studies. From in vivo studies, it has been suggested that there are pools of steroid-resistant precursor NK cells which are stimulated to activate NK cells (presumably now steroid sensitive) by interferon (20). According to this hypothesis, in vitro pretreatment of PBL with interferon before exposure to dexamethasone should result in a greater population of active NK cells with increased sensitivity to dexamethasone. We did not find this to be the case. Similar results were obtained whether cells were treated simultaneously with these agents, with interferon pretreatment followed by glucocorticoid, or first treated with glucocorticoid and then with interferon.

While there is still much to be learned about the precise mechanisms by which glucocorticoids and interferons modulate NK activity, the fact that interferon can enhance NK activity in the presence of glucocorticoids could be of considerable therapeutic importance. Although glucocorticoids are used therapeutically for a variety of purposes, their immunosuppressive side effects (including suppression of NK activity) constitute a major concern in patient management. Our data suggest that the severe suppression of NK activity which occurs in glucocorticoid-treated patients could be overcome by simultaneous treatment with interferon. If NK activity does prove to be an important immunosurveillance mechanism as has been hypothesized, then the capability to maintain this immune function while still deriving the benefits of glucocorticoid therapy would certainly be of value.

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REFERENCES


Glucocorticoid Suppression of Human NK Activity

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Direct Suppression of Natural Killer Activity in Human Peripheral Blood Leukocyte Cultures by Glucocorticoids and Its Modulation by Interferon

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