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Hormone specific regulation of natural killer cells by cortisol

Direct inactivation of the cytotoxic function of cloned human NK cells without an effect on cellular proliferation

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Corticosteroids have previously been reported to partially inhibit the natural cytotoxic activity of peripheral blood lymphocytes. However, since only a few percent of peripheral lymphocytes are natural killer (NK) cells, it has not been possible to determine whether corticosteroids directly inhibit NK cells or mediate this effect via other cell types. This report documents direct functional inactivation, but unimpeded proliferation, of cloned human NK cells by subphysiologic levels of cortisol. In contrast, high concentrations of testosterone, progesterone or estradiol had no significant effect on proliferation or cytotoxic activity of the cloned NK cells. The kinetics of inhibition of NK function by cortisol are consistent with a transcription-dependent mechanism.

NK cell; Immunoregulation; Cellular cytotoxicity; Cortisol; Corticosteroid

1. INTRODUCTION

Corticosteroids are known to affect a number of immune functions and are used for the treatment of several autoimmune diseases [1–3]. A large body of evidence supports the involvement of natural killer cells in immune surveillance against viruses and tumors, and possibly in the pathogenesis of some autoimmune diseases [4]. Recently, interleukin-2 (IL-2), has been used to generate lymphokine-activated killer (LAK) cells [5,6], which include activated NK cells [4], and which may have significant potential for immunotherapy of cancer and other diseases [7]. A number of previous reports have indicated that the natural cytotoxic activity of human peripheral blood lymphocytes is inhibited in vitro or in vivo by natural [8,9] or synthetic [10] glucocorticoids. A recent study indicates that LAK cells are less sensitive to inhibition by glucocorticoids than are unactivated NK cells with respect to their cytolytic activities [11]. However, due to the low frequency of NK cells in human peripheral blood (about 3%), and the heterogeneity of lymphocytes comprising LAK cell populations, it has not been possible thus far to determine whether glucocorticoids act directly on NK cells or inhibit their function indirectly by inducing another cell type to produce a secondary mediator [10].

The availability of a cloned human NK cell line [12,13] now permits the systematic characterization of NK cells and their mechanism of action [14]. In the present work, the effects of cortisol on the proliferation and cytotoxic activity on this human NK clone were investigated. Additional naturally occurring steroids were also examined to determine the specificity of the observed inhibition. The dependence of NK function on the concentration of cortisol and the kinetics of cortisol-dependent inhibition were also determined. The results obtained strongly support a primary role for cortisol in the regulation of NK cells and demonstrate the utility of this cell line for further characterization of the mechanism of NK regulation by glucocorticoids.

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2. MATERIALS AND METHODS

Cortisol, testosterone, progesterone and estradiol were purchased from Sigma (St. Louis). Fetal bovine serum with low cortisol content (5.52 nM) was obtained from Hyclone (Logan, Utah) and Lymphocult-T (crude T cell growth factor) from BioTest (Dreieich, Germany).

2.1. *Natural killer cells*

The cloned human NK cell line designated NK3.3 was generously provided by Dr J. Kornbluth (U. Arkansas). The NK3.3 clone has a unique cell surface phenotype, $CD2^+$, $CD3^-$, $CD4^-$, $CD5^-$, $CD8^-$ $CD16^+$, $CD38^+$, $CD45^+$, $HLA\text{-}DR^+$, DP^+, DQ^+, $Leu7^-$ that distinguishes it from mature T cells, monocytes and other lymphocyte populations [12]. It has also recently been shown that NK3.3 cells do not produce functional transcripts for the α, β, or γ chains of the T cell receptor [13]. NK3.3 cells were maintained in continuous culture at 3×10^5 to 1×10^6 cells/ml in RPMI-1640 medium supplemented with 20% fetal bovine serum (Hyclone) from a lot selected for low corticosteroid content (less than 1 μg/dl) and 20% IL-2 (Lymphocult-T, BioTest) medium. Viable NK3.3 cells were counted every 48 h, cen-

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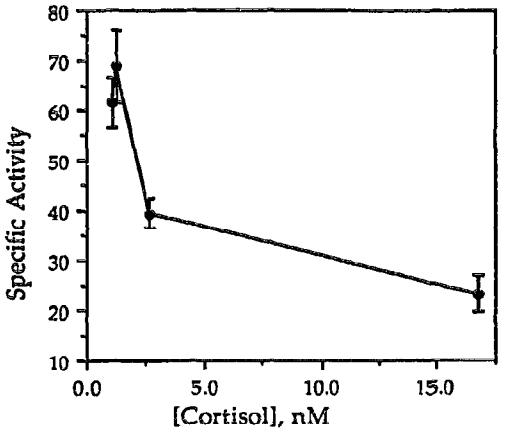

Fig. 1. Dose-response curve for the inactivation of cytotoxic function of NK3.3 cells cultured for 6 days in medium supplemented with cortisol.

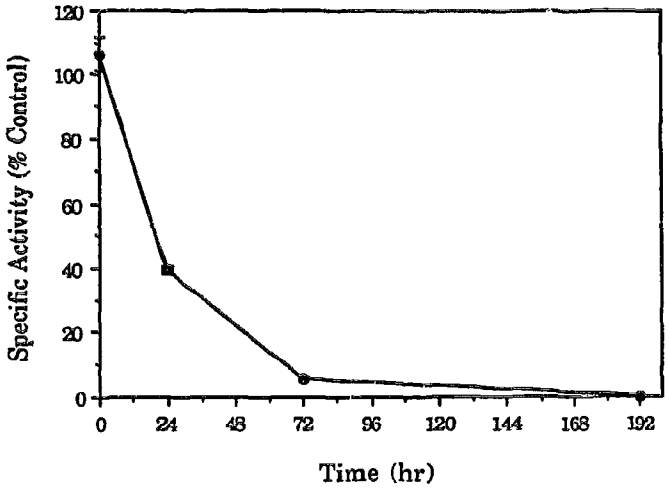

Fig. 2. Kinetics of inactivation of the cytotoxic function of NK3.3 cells by cortisol. The relative cytotoxic activity for K-562 target cells of NK3.3 cells in medium supplemented with 100 μM was compared to control cells cultured in the standard low cortisol medium. Error bars represent 90% confidence intervals.

trifuged, the used medium decanted and the cells resuspended at 1×10^6 cells/ml in fresh medium. Cytotoxicity assays were performed 18–20 h after addition of fresh medium.

2.2. *Target cells*

The K-562 cell line, derived from a patient with myelogenous leukemia [15] was maintained in RPMI-1640 medium containing 25 mM bicarbonate, and 10% fetal bovine serum (RPMI-FBS). On the day of an experiment, an aliquot of K-562 cells was removed from culture, centrifuged (150 × *g* for 10 min) and resuspended in 0.5 ml of medium. Subsequently, 250 μCi of sodium chromate (^{51}Cr, New England Nuclear, Boston, MA) were added. After incubation for 1 h at 37°C, the cells were washed 3 times with cold RPMI-FBS medium and resuspended in that medium. Viable cells were enumerated with Trypan blue, and diluted to the concentrations desired for the experiment.

2.3. *Cellular cytotoxicity assays*

Cytotoxicity assays were performed using a previously described method [16]. Briefly, various concentrations of ^{51}Cr-labeled target cells (1×10^4 to 1×10^5 viable cells in 0.1 ml) and a fixed concentration of NK3.3 cells (generally 1×10^4 in 0.1 ml) were added to wells of conical-bottomed microtiter plates. The plates were then centrifuged (150 × *g* for 5 min at 25°C) and incubated at 37°C in an atmosphere of 95% air and 5% CO_2 for 2 h. Cytotoxicity was terminated by addition of 0.05 ml of ice cold RPMI-FBS to each well and the plates centrifuged (1000 × *g* for 5 min at 5°C). An equal aliquot of supernatant was then removed from each well and counted on a gamma counter. Spontaneous release of ^{51}Cr was determined by incubation of K-562 cells in 0.15 ml of RPMI-FBS without added effector cells, and maximal ^{51}Cr-release was achieved by sonication of an aliquot of K-562 target cells. All reactions and controls were performed in triplicate. Specific activity values (the number of target cells lysed per hour per 100 NK3.3 cells) were calculated by a distribution-free procedure that has been published [17,18].

3. RESULTS

The dose-dependent inactivation of the cytotoxic activity of cloned NK3.3 cells cultured in medium supplemented with cortisol is shown in Fig. 1. Comparable results were obtained in several experiments, and 80–100% inactivation of cytotoxic function was achieved by culture of NK3.3 cells for 8–10 days in medium supplemented with 0.01–1.6 μM cortisol.

The results of experiments to determine the specificity of inactivation of cytotoxic function by steroids are summarized in Table I. NK3.3 cells were cultured for 10 days at equal concentrations (2 μM) of the indicated steroids. As shown in Table I, cortisol rapidly and completely inactivates NK3.3 function. Culture of NK3.3 cells in the presence of the other steroids, at a concentration that is two orders of magnitude higher than the concentration of cortisol required for 100% inactivation of NK3.3 cells, did not significantly alter the cytotoxic activity of this NK clone.

The kinetics of inactivation of this NK clone by cor-

Table I

Effect of steroid hormones on the cytotoxic activity and proliferation of cloned human NK cells

Steroid	Cytotoxic Activity		Proliferation 8 days
	3 Days	8 Days	
Control	58.9 (56.1–61.9)	38.7 (35.8–42.6)	84 533.47 ± 8222.97
Cortisol	3.3 (1.6–13.2)	0.0 (− 12.1–2.8)	75 814.87 ± 7331.44
Testosterone	40.9 (38.2–44.6)	36.6 (34.2–39.4)	70 524.60 ± 11 474.51
Progesterone	64.2 (60.7–69.8)	28.8 (24.1–34.6)	57 608.53 ± 5562.20
Estradiol	63.5 (60.8–66.9)	28.1 (25.3–31.7)	99 671.57 ± 9722.49

Cytotoxic activity and proliferation of cloned NK3.3 cells were measured at 2 time intervals after subculture in the presence of 0.57 μg/ml of the indicated steroids. The 90% confidence intervals for the specific activity values are given in parentheses. Proliferation was measured as [^{3}H]thymidine uptake in 18 h by 1×10^5 cells. Mean uptake ± SD of triplicate cultures is presented.

tisol are presented in Fig. 2. Although incubation in the presence of cortisol inhibits over 50% of the NK activity within 24 h, complete inactivation requires more than 72 h of continuous exposure to cortisol. Transfer of cortisol-inactivated NK3.3 cells to standard medium (containing 1.1 nM cortisol derived from the FBS) resulted in gradual recovery of cytotoxic function (data not shown).

4. DISCUSSION

The experiments in this report document that continuous culture of cloned human NK cells in the presence cortisol, even at concentrations as low as 20 nM, result in complete inactivation of their cytotoxic activity. This inhibition was specific for cortisol and, since the normal physiologic level of cortisol in human serum ranges from 140 to 400 nM [19], these results indicate that the cytotoxic activity of NK cells may be greatly suppressed by cortisol in normal individuals. This hypothesis is supported by previous reports that fresh human NK cells are inactivated by culture in medium supplemented with human serum [20], but that NK activity generally increases upon culture of human lymphocytes in medium supplemented with fetal bovine serum [21]. The cortisol concentration of media supplemented with 10-20% FBS can range from <1 to 2 nM, which causes little or no inactivation of NK function (see Fig. 1).

In vivo administration of IL-2 produces a transient increase in the activity of cytotoxic cells [22,23], but Il-2 therapy alone is generally ineffective for in vivo therapy of tumors [22–24]. In contrast, ex vivo culture of lymphocytes in media supplemented with Il-2 gives rise to highly active LAK cells [5,6,21] which, in combination with IL-2, have shown some clinical utility (see [25,26]). Based on the results reported herein, it is possible that the relatively poor clinical response to therapy with IL-2 alone is at least partly due to inhibition of cytotoxic function by physiologic levels of cortisol. In fact, IL-2 administration has been reported to increase serum cortisol levels [24]. The recent report by Imir et al. [11] that incubation of human LAK cells for up to 72 h with 10^{-3}–10^{-9} M dexamethasone resulted in little or no inhibition of cytotoxic function is in marked contrast to glucocorticoid inhibition of fresh NK cells [8–10] or cloned human NK cells documented herein, and should be investigated further. Piccollella et al. [27] have proposed that dexamethasone inhibits development of NK cells by inhibiting IL-2 receptor expression. The ability of IL-2 dependent NK3.3 cells to propagate for several weeks in medium containing high concentrations of cortisol does not support an inactivation mechanism based on altered expression of the IL-2 receptor.

The NK3.3 cell line, whose cytotoxic function can be reversibly inactivated by subphysiologic concentrations of cortisol without toxicity or reduction in proliferative capacity, should serve as an excellent model for elucidation of the molecular basis of the cortisol-dependent inactivation of cellular cytotoxic function. Further studies with this cloned human cell line may also form the basis for development of improved protocols for immunotherapy of cancer and other diseases.

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