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Suppression of the Natural Killer Cell Activity of Murine Spleen Cell Cultures by Dexamethasone (41489)

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Abstract. Investigations into the mode of action of glucocorticoids on natural killer (NK) cell activity have been hindered by the lack of an *in vitro* model system. We report that the NK activity of spleen cell cultures of several inbred strains of mice was suppressed by treatment with dexamethasone. The *in vitro* suppression of NK activity was time dependent, requiring at least 5 hr incubation in dexamethasone to achieve maximal levels of suppression, and was dose dependent at pharmacologic concentrations. Thus, based on the results of these studies, an *in vitro* model system for studying glucocorticoid effects on NK activity has been established.

Natural killer (NK) cells have been studied extensively in mice, rats, and man (1–4). The ability of these lymphoid cells to lyse many tumor cell lines without prior immunization implicates NK cells as a first line of defense against neoplasia. Recent evidence demonstrating that thymocytes may serve as targets of mouse NK activity suggest an additional role of NK cells in hemopoietic regulation (5). NK cell activity is enhanced by interferon itself or by substances capable of inducing interferon (6–8). In contrast, NK activity is reported to be suppressed *in vivo* by glucocorticoids. Human peripheral NK activity of normal volunteers and patients with systemic lupus erythematosus is depressed by glucocorticoid treatment (9, 10). Similarly, rat and mouse NK activity is impaired severely 12 to 24 hr after hydrocortisone administration (11, 12). It is not known whether glucocorticoids act directly or indirectly on NK cells and the lack of an *in vitro* model system has hampered such investigations. Although Hochman and Cudkowicz attribute suppressed NK activity in mice after hydrocortisone treatment to stimulation of suppressor cells, more recent evidence demonstrated that the spleen cells of cortisone acetate-treated mice, having depressed NK activity, failed to suppress the NK activity of normal mice (13, 14). To understand the mechanism underlying glucocorticoid action on NK activity, the establishment of an *in vitro* model system is essential. We report here the *in vitro* suppression of NK cell activity of mouse spleen cell cultures by pharmacologic concentrations of dexamethasone.

Materials and Methods. *Mice.* Inbred A/J (H-2^a), BALB/cJ (H-2^d), CBA/J (H-2^k), C3H/HeJ (H-2^k), C57Bl/6J (H-2^b), and DBA/2J (H-2^d) mice were purchased from Jackson Laboratories (Bar Harbor, Maine). All mice used in these studies were males between 5 and 12 weeks of age.

Target cells. YAC-1 lymphoma cells, previously described (15), were maintained in RPMI 1640 (Flow Laboratories, Rockville, Md.) medium supplemented with 10% fetal bovine serum (FLOW), 2 m*M* L-glutamine (Grand Island Biological Co., Grand Island, N.Y.), 100 U/ml penicillin, and 100 μg/ml streptomycin (GIBCO).

Steroid treatment. Dexamethasone (Merck, Sharp and Dohme, West Point, Pa.) was initially prepared as a 10^{-4} *M* stock solution in 95% ethanol and stored at 4°. For use in spleen cell cultures, 0.5 ml of the

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stock solution was evaporated in a 60 × 15-mm plastic tissue culture dish (Falcon, Oxnard, Calif.). The dexamethasone was redissolved in 5.0 ml of complete RPMI 1640 (RPMI supplemented with 10% FBS, 2 m*M* L-glutamine, 15 m*M* HEPES, 5×10^{-5} *M* 2-Mercaptoethanol, and antibiotics) by incubation at 37° for 30 min. Control medium was prepared in the same manner except that dexamethasone was omitted from the ethanol. Thus, both control and steroid preparations were virtually ethanol free. The pooled spleen cells of three to five mice were incubated at 37°, 5% CO_2 in RPMI 1640 containing dexamethasone or control medium. The cells were then washed twice in ice-cold RPMI 1640, counted in trypan blue with a hemacytometer, and adjusted to 10^7 viable cells/ml.

Cytotoxicity assay. Different effector to target cell ratios were prepared by twofold serial dilutions in complete RPMI 1640. NK activity was measured by a modification of the ^{51}Cr release assay (16). Briefly, 0.1 ml of YAC-1 target cells radiolabeled by incubation in $Na_2{}^{51}CrO_4$ (New England Nuclear, Cambridge, Mass.) were added to the individual wells of 96-well microtiter plates (Nunclon, Roskilde, Denmark) containing 0.1 ml of spleen cell suspension. The plates were centrifuged at 250*g* for 2 min and then incubated for 4 hr at 37° in a humidified atmosphere of 95% air, 5% CO_2. The assays were terminated by centrifugation at 500*g* for 10 min. Released radioactivity in 0.1 ml of the supernatant fluid was measured by liquid scintillation spectrometry. The percentage cytotoxicity was computed from

$$\% \text{ cytotoxicity} = (cpm_{exp} - cpm_{sr})/(cpm_{max} - cpm_{sr}) \times 100.$$

cpm_{exp}, cpm_{max}, and cpm_{sr} represent, respectively, the counts per minute in supernatants from YAC-1 target cells incubated with effector cells, from target cells lysed with 5% sodium dodecyl sulfate, and from target cells incubated without effector cells to give a measure of spontaneous release. Typically, spontaneous isotope release from target cells was less than 4% of the maximum isotope incorporation. Except where indicated, all results are expressed as the mean percentage cytotoxicity of at least three replicate cultures of one of several experiments. Standard deviations of means of the experiments shown were less than 2% unless depicted. Three effector to target cell ratios, 25:1, 50:1, and 100:1 were used in all experiments and gave consistent results.

Experimental Results. *Kinetics of suppression of NK activity by dexamethasone.* Mouse NK activity was measured in 5-hr chromium-51 release assays after preincubation of whole spleen cell preparations in dexamethasone, a potent synthetic glucocorticoid. Cultures that received control medium demonstrated 10 to 11% cytotoxicity. In contrast, NK activity in 10^{-7} *M* dexamethasone-treated cultures decreased from 11 to 9% cytotoxicity after 1 hr of incubation in medium containing the glucocorticoid (Fig. 1). By 6 hr of exposure

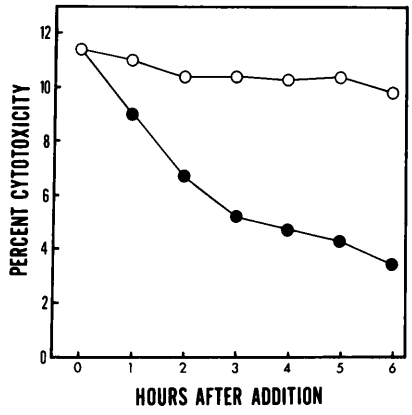

FIG. 1. Kinetics of suppression of mouse NK activity after *in vitro* cultivation in dexamethasone. C57Bl/6 spleen cells were incubated in conical centrifuge tubes at 37°, 5% CO_2. At hourly intervals, control medium (○) or dexamethasone (●) was added to the appropriate cultures in volumes sufficient to yield final concentrations of 10^{-7} *M* dexamethasone. After a total of 6 hr incubation, the spleen cells were washed and assessed for NK activity against ^{51}Cr-labeled YAC-1 cells. Three effector to target cell ratios were used and gave consistent results. Shown here are the results for the 100:1 ratio of a representative experiment. Standard deviations of the means of each point were less than 2% cytotoxicity.

to dexamethasone, NK activity had declined to 3% cytotoxicity, about one-third of the activity in control cultures. In three such experiments, the NK activity in cultures treated for 6 hr with 10^{-7} *M* dexamethasone was significantly decreased compared to the NK activity of control cultures ($P < 0.001$, Student's *t* test).

Experiments in which inhibitory concentrations of dexamethasone were added directly into the cytotoxicity assay wells or in which YAC-1 target cells were cultured for 24 hr prior to assay in medium containing 10^{-7} *M* dexamethasone failed to demonstrate decreased levels of NK mediated cytotoxicity (data not shown). Thus, the suppression of NK activity in dexamethasone-treated cultures is not attributable to the increased resistance of target cells to lysis after contamination of the cytotoxicity assays by possible carryover of dexamethasone from the preincubation cultures, but to a dexamethasone effect on the spleen cells.

Dexamethasone dose response. In order to assess the dose response relationship of the dexamethasone-induced *in vitro* suppression of NK cell activity, unfractionated spleen cells were incubated for 5 hr in various concentrations of dexamethasone before assessment of NK cytotoxicity. The results of a representative experiment shown in Table I clearly demonstrate that incubation of unfractionated spleen cells in concentrations as low as 10^{-9} *M* dexamethasone was sufficient to induce dramatic decreases in target cell killing. Suppression of NK cytotoxicity was dose dependent from 10^{-11} *M* to 10^{-8} *M* dexamethasone. Increasing the concentration of dexamethasone to 10^{-7} *M* or 10^{-6} *M* did not increase the degree of suppression of NK activity. Thus, *in vitro* suppression of NK activity by dexamethasone is dose dependent and occurs at pharmacologic concentrations. Similar results also were obtained from spleen cell suspensions of adrenalectomized mice (data not shown).

Dexamethasone suppression of NK activity of different mouse strains. The NK activity of inbred mice of different genetic backgrounds was tested for sensitivity to *in vitro* dexamethasone treatment. The results of a representative experiment, Fig. 2, demonstrate that the NK activity of all but one strain tested was strikingly suppressed after 5 hr incubation in medium containing 10^{-7} *M* dexamethasone. The exception, the spleen cells of A/J mice, had very little NK activity. Consequently, the suppressive effect of dexamethasone may not have been as apparent as that seen in the other strains that expressed much higher levels of NK activity. Nonetheless, it is clear that *in vitro* dexamethasone treatment suppresses

TABLE I. DOSE-DEPENDENT *IN VITRO* SUPPRESSION OF NK ACTIVITY BY DEXAMETHASONE

	Percentage cytotoxicity		
DEX (M)[a]	25[b]	50	100
0	6.1	9.4	12.7
10^{-11}	5.9	8.4	13.2
10^{-10}	4.4	7.0	10.2
10^{-9}	1.4	3.4	4.6
10^{-8}	1.4	2.0	3.1
10^{-7}	0.8	1.5	2.0
10^{-6}	0.8	1.4	2.5

[a] C57BL/6J spleen cells (10^7/ml) were incubated 5 hr in complete RPMI 1640 containing the indicated concentrations of dexamethasone, washed, and assayed for NK activity against YAC-1 target cells.

[b] Effector cell to target cell ratio.

FIG. 2. Dexamethasone-induced suppression of NK activity of various mouse strains. The spleen cells of inbred mice of different genetic backgrounds were incubated 5 hr in control medium (□) or medium containing 10^{-7} *M* dexamethasone (■). After washing, the NK activity of each culture was determined against YAC-1 cells. Although tested at three different effector to target cell ratios, only the mean percentage cytotoxicity (±SD) of the 100:1 cultures are shown in this representative experiment.

murine NK activity regardless of genetic background.

The results of this study establish an *in vitro* model system for investigating the action of glucocorticoids on NK activity. The reduction of NK activity in mouse spleen cell cultures after dexamethasone treatment is time dependent and dose dependent at pharmacologic concentrations. Since the proportion of cells possessing NK activity is thought to be less than 5% of the total splenic population, viability studies are of little value in determining whether DEX is toxic for NK effector cells (17). Nonetheless, the viabilities of treated and control cultures are similar. Additional studies employing this *in vitro* system do not demonstrate the presence of suppressor cell activity after DEX treatment although NK activity is depressed (manuscript in preparation). This would suggest that glucocorticoids suppress NK activity by acting directly on the NK effector cell.

In vitro suppression of NK activity by meaningful concentrations of glucocorticoids, to our knowledge, has not been previously demonstrated although Parillo and Fauci reported that human NK activity is decreased by the addition of very high concentrations of DEX (10^{-5} *M* and 10^{-4} *M*, termed "pharmacologic" and "supraphar-macologic") to 18-hr cytotoxicity assays (9). Since these concentrations are known to produce nonspecific effects, the relevance of these results are questionable (18).

We have obtained very similar *in vitro* glucocorticoid effects on human NK activity (submitted for publication). Incubation of Ficoll–Hypaque-enriched mononuclear cells of normal volunteers for 24 hr in 5×10^{-7} *M* dexamethasone suppressed the NK activity to only 30% of the activity in cultures that had received control medium. Thus, the *in vitro* model system we describe here appears to closely simulate not only the *in vivo* glucocorticoid-induced suppression of NK cytotoxicity in mice but also in humans.

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