

Characterization of the *in Vitro* Effects of Glucocorticosteroids on NK Cell Activity

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The NK cell activity of mononuclear cells as well as monocyte-depleted, Percoll-fractionated, NK cell-enriched effector cells against K 562 target cells was inhibited by methylprednisolone (MP) and hydrocortisone (HC) in a dose-dependent manner. The effector/target cell conjugate formation was studied in a single cell agarose assay, and it was shown that MP and HC partly inhibited the NK cell activity by inhibition of the adhesion of effector cells to target.

Key words: natural killer cell activity; methylprednisolone; hydrocortisone.

Accepted for publication 23 November 1985

In the treatment of patients with immunoinflammatory diseases (9), NK cell activity is inhibited by several drugs, including azathioprine, cyclophosphamide and corticosteroids. In the present communication we show that the performance of NK cell assays in the *in vitro* presence of physiological (10^{-6} M), pharmacological (10^{-5} M) or suprapharmacological (10^{-4} M) concentrations of methylprednisolone (MP) and hydrocortisone (HC) inhibit the NK cell activity. The mechanism by which corticosteroids suppress NK cell function is described.

MATERIAL AND METHODS

Measurement of NK cell activity

Mononuclear cells obtained from heparinized blood of healthy human adults by centrifugation on Ficoll-Hypaque were adjusted to 4×10^6 , 2×10^6 , 10^6 , 5×10^5 and 2.5×10^5 cells/ml in RPMI 1640 with 10% fetal calf serum (FCS). NK cell activity against K 562 target cells, 5×10^4 cells/ml, was determined in a ^{51}Cr release assay, as previously described (12). The incubation period was 4 h, and the NK cell activity was measured in triplicate at effector/target cell ratios 80/1, 40/1, 20/1, 10/1 and 5/1. Some

experiments were carried out only at E/T = 80/1. The experimental release (R) was calculated by the formula:

$$R = \frac{\text{activity released in the supernatants}}{\text{total activity}} \times 100\%$$

The specific release was the difference between experimental release in the presence of effector cells and experimental release when target cells were incubated with medium only. The percentage of ^{51}Cr release was calculated for samples made in triplicate (SD = 0.1–3.0%). The within-assay determinations gave a coefficient of variation of 3.0%. The day-to-day determination on samples from the same donors gave overall coefficients of variation of 20%.

Incubation of effector cells with MP and HC

MP (Solumedrol, Upjohn) and HC (Solucortef, Upjohn) were dissolved and diluted in RPMI. The stock solutions were 10 mg/ml. 4×10^6 effector cells were incubated with MP or HC (1 h, 37°C) at final concentrations of 1 µg/ml or less. In most experiments MP and HC were present during the subsequent incubation with target cells as well, but in one series of experiments the effector cells were washed 3 times in RPMI

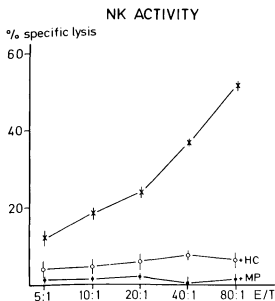


Fig. 1. The effect of MP 1 mg/ml and HC 1 mg/ml on NK cell activity measured at various effector to target (E/T) cell ratios. One representative experiment of three is shown.

before incubation with target cells. Possible toxic effects of MP and HC were tested by staining the effector cells with Nigrosin 0.02 % (w/v). After 5 h incubation with the drugs, cell viability was more than 95 %.

Incubation of effector cells with interferon (IF)

10 μ l of 10^6 IU/ml of α -IF were added to 990 μ l of 4×10^6 effector cells/ml. IF was kindly provided by Dr. Robert Jordal, The Blood Bank, Copenhagen County Hospital, Gentofte, Denmark. The cells were preincubated with IF for 1 h at 37°C and IF was also present during the incubation with target cells.

Single cell agarose assay

The method was performed as described by Grimm et al. (5) and Pedersen et al. (11) with minor modifications (13). The effector cells were NK cell-enriched suspensions prepared on discontinuous Percoll (Pharmacia Chemicals, Uppsala, Sweden) gradients as described previously by Timonen et al. (14). By this method the effector cells contained 60–70 % of large

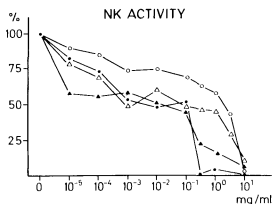


Fig. 2. Mononuclear cells were incubated with various concentrations of MP and HC and used washed or unwashed at testing for NK cell activity. One representative experiment of six is shown.

● — MP; ○ — MP + wash; ▲ — HC; △ — HC + wash.

granular lymphocytes (LGL), identified morphologically in Giemsa stained cytocentrifuge preparations as described (12). 2.5×10^6 effector cells and 2.5×10^5 target cells were mixed in a total volume of 0.25 ml RPMI with 10 % FCS. The cells were centrifuged at 500 g for 5 min and incubated for 5 min, 37°C. During this time some of the lymphocytes were bound to target cells and formed effector/target cell conjugates. Subsequently, the pellet was resuspended by shaking for 3 s on a whirlmixer. The cells were then added to 0.6 ml of the agarose solution, which had been precooled to approx. 40°C, resuspended with a Pasteur pipette and added dropwise to Petri dishes. The percentage of effector/target cell conjugates, referred to as the percentage of target binding cells (%TBC), was determined by counting the number of conjugates and the total number of lymphocytes. At least 400 lymphocytes were counted and the experiments were carried out in duplicate.

RESULTS

MP and HC (1 mg/ml 2.7×10^{-4} M) were preincubated with effector cells and present during the incubation with target cells. The inhibitory effect of MP and HC on the NK cell activity at different E/T ratios is shown in Fig. 1. When MP and HC

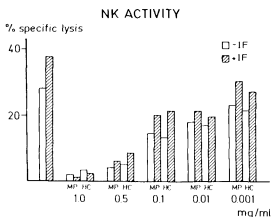


Fig. 3. Mononuclear cells were preincubated with various concentrations of MP and HC, followed by addition of IF. One representative experiment of six is shown.

were incubated with effector cells, washed or unwashed, before incubation with target cells, NK cell activity was significantly inhibited ($P < 0.05$, $n = 6$) at concentrations of 10^{-5} – 10^{-1} mg/ml (2.7×10^{-5} – 2.7×10^{-3} M) (Fig. 2). The inhibition of NK cell activity was significantly higher when MP and HC at concentrations of 1 mg/ml (2.7×10^{-3} M) were present during the incubation with target cells compared to the NK cell activity obtained when effector cells were washed ($P < 0.05$) (Fig. 2).

The effect of IF on the drug-induced inhibition of the NK cell activity is shown in Fig. 3. IF reduced the glucocorticosteroid-induced inhibition of NK cell activity, but the absolute level of the IF-enhanced NK cell activity was below the IF-enhanced NK cell activity of the controls, as IF stimulated both control and glucocorticosteroid-treated cells to the same extent. There was no significant difference in the relative IF-induced increase in NK cell activity between control cells and cells incubated with glucocorticosteroids ($n = 6$). Mean per cent increase in NK cell activity of control effector cells after IF boosting was 33%, while mean per cent increase in NK cell activity of effector cells incubated with MP and HC (0.001–0.5 mg/ml) was 26–40%. Baseline as well as IF-enhanced NK cell activity after incubation with MP and HC 1 mg/ml was almost totally suppressed.

To study whether the incubation of effector cells with MP and HC inhibited cytotoxicity by interference with the recognition and adhesion step, or by blocking the final killing event, the adhesion of effector cells to target cells after incubation with MP and HC 1 mg/ml (2.7×10^{-3} M) was studied in a single cell agarose (Table 1). The data clearly show that MP and HC *partly* inhibit effector/target cell conjugate formation. Washing the effector cells after preincubation with glucocorticosteroids had no effect. The ability of the Percoll-fractionated effector cells used in the single cell agarose assay to mediate NK cell activity was tested in parallel (Table 1).

DISCUSSION

The effect of corticosteroids *in vivo* on NK cell activity has been described by several authors. Parillo & Fauci (8) found 12 mg of the long-acting preparation of oral dexamethasone to decrease NK cell activity at 24 and 48 h, with normalization by 96 h. Onsrud & Thorsby (7), as well as Katz et al. (6), showed increased NK cell activity at 4 h after i.v. hydrocortisone 300 mg and 400 mg, respectively. We found that methylprednisolone pulse therapy (MPPT) (1 g MP intravenously on 4 consecutive days) in patients with rheumatoid arthritis produced a considerable fall in NK cell activity during MPPT, when measured on day 1 after the first and third injection of MP. After 3 months the

Table 1
The effect of MP 1 mg/ml and HC 1 mg/ml on target cell binding capacity and NK cell activity

	% TBC	NK cell activity % specific lysis
Control	14.5	45.9
MP + wash	6.5	0.9
MP + wash	8.0	32.9
HC + wash	7.0	1.5
HC + wash	8.0	9.0

Monocyte depleted, NK cell enriched, Percoll fractionated effector cells were used in the single cell agarose assay. The percentage of target binding cells (% TBC) was determined by counting the number of lymphocytes bound to target cells and the total number of lymphocytes. One representative experiment of five is shown and NK cell activity of one experiment made in parallel.

NK cell activity was less than half that before MPPT (10). These results indicated that MPPT induced a strong irreversible effect on NK cells. The results of the different studies summarized here are not directly comparable because the NK cell activity was measured at different times after corticosteroid administration and different doses and different corticosteroid preparations were used.

It has previously been shown that corticosteroids given intravenously to humans, cause lymphocytopenia, monocytopenia, eosinopenia and neutrophilia which reach their maximum 4 h after administration. The changes in NK activity after *in vivo* corticosteroid reported here might be related to the fact that corticosteroids in therapeutic doses cause a reversible redistribution of certain subpopulations from the circulation to other body compartments.

Otherwise the performance of cytotoxic assays in the *in vivo* presence of corticosteroids allows a study of the direct effect of corticosteroids on the NK activity without fluctuations in leucocyte populations. Hoffman et al. (5) demonstrated *in vitro* that the presence of pharmacologic concentrations of hydrocortisone inhibited NK cell activity, whereas Katz et al. (6) were unable to demonstrate this. The duration of lymphocyte preincubation with the drug, and the effect on lymphocyte viability, are not reported.

The *in vitro* results presented in this communication showed that pharmacologic concentrations of MP (10^{-5} M $\sim$ 3.7 μ g/ml) and HC (10^{-5} M $\sim$ 3.7 μ g/ml) (2) decreased the NK cell activity. When MP and HC were removed from effector cells, before incubation with target cells, the glucocorticosteroids still induced inhibition of NK cell activity. These *in vitro* results are in accordance with the *in vivo* findings that high doses of MP induce irreversible suppression of the NK cell function (10).

As addition of IF was not able to abolish the drug-induced suppressed NK cell activity, we conclude that the mechanism by which corticosteroids inhibit NK cell function is not due to a blockage of IF production. Furthermore, the finding that MP and HC inhibit the NK cell activity of monocyte-depleted, Percoll frac-

tionated, NK cell-enriched effector cells, indicates that corticosteroids inhibit the NK cell activity through a direct effect on the NK cells.

Immune cytotoxicity mediated by both cytotoxic T cells, NK cells and K cells is a consequence of two separate events. The first step involves recognition and binding of the cytolytic cells to target cells, the second step is the lytic reaction which leads to the destruction of the target cells. The binding step is known to be dependent on the supply of energy, and on the integrity of microfilaments. The lytic step is dependent on energy supply, the presence of divalent cations, the temperature and the integrity of both microfilaments and microtubuli in the effector cells. In addition, the lytic step is dependent on an intact secretory system (11). The single cell agarose assay allows the study of the effector cell binding to target. A wide variety of agents or treatments have been shown to inhibit NK cell activity. With many of these, it is already possible to state whether they affect initial recognition or whether they only inhibit some post-binding events. Trypsin, pronase, EDTA, Cytochalasin B and immune complexes were thus shown to inhibit the adhesion step (4, 13). This report shows that preincubation of effector cells with MP and HC inhibits the adhesion step as well. The presence of glucocorticosteroids during the formation of conjugates had no influence. The inhibition of effector/target cell conjugate formation was not total, while the presence of MP and HC with target cells induced complete suppression of NK cell activity. This indicates that glucocorticosteroids, while present in the NK cell assay, also inhibit some post-binding events.

In conclusion, the results presented in this report suggest that NK cells are strongly corticosteroid-sensitive, and that MP and HC inhibit NK cell activity by inhibition of the adherence of effector cell binding to target.

ACKNOWLEDGEMENTS

We thank Dr. V. Andersen for valuable discussions. The excellent technical assistance of Anne Rasmussen and Kirsten Ankerse is gratefully acknowledged. The work was supported by the Danish National Association against Rheumatic Diseases.

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