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Corticosteroid–interleukin 2 interactions: inhibition of binding of interleukin 2 to interleukin 2 receptors

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SUMMARY

PHA activated human peripheral blood mononuclear cells (MNC) were incubated with human interleukin 2 (IL-2) in the absence or in the presence of 10^{-6}–10^{-9} M hydrocortisone (HC). HC suppressed the proliferative response of the activated MNC to highly purified human leucocyte IL-2 in a dose dependent manner. This suppression was in full accordance with an inhibition of the IL-2 binding capacity, whereby the high affinity binding sites were reduced by 85%, while the low affinity sites were less affected. The mechanism by which HC inhibits the binding of IL-2 is still unknown. Evidence is presented that HC binds only weakly to IL-2. We conclude that HC interferes with the IL-2 binding by modulating the binding and/or signal processing function of the IL-2 receptor.

Keywords IL-2 receptor cortisol

INTRODUCTION

The immunosuppressive effect of corticosteroids has been described by a large number of authors. The precise mechanism of action, however, remains poorly understood (Claman, Moorehead & Benner, 1971; Fauci, Dale & Balow, 1976; Gillis, Crabtree & Smith, 1979; Cupps & Fauci, 1982; Ansar-Ahmed, Penhale & Talal, 1985). Some effects seem to be correlated with the binding of the steroids to specific corticosteroid receptors, while others probably are not (Lippman & Barr, 1977; Fauci *et al.*, 1980; Peterson *et al.*, 1981; Katz, Zaytown & Lee, 1985). HC is known to interfere with the mitogen response of human T lymphocytes (Fauci & Dale, 1974). Moreover, HC suppresses the production of IL-2 (Gillis *et al.*, 1979) as well as the proliferative response of T lymphocytes to exogenous IL-2.

It is widely accepted that the ability of IL-2 target cells to proliferate in response to IL-2 is dependent on the expression of specific IL-2 binding sites, or IL-2 receptors (Robb, Munck & Smith, 1981; Robb & Greene, 1983; Robb, Greene & Rusk, 1984). Cells which lack IL-2 receptors will not respond to the growth factor.

We will show in this paper that in the presence of HC not only the expression of the IL-2 receptor is suppressed but that also the already existing receptors become undetectable within minutes after exposure to HC.

MATERIALS AND METHODS

Cells. Mononuclear blood cells (MNC) were obtained either freshly from donors or from pooled human buffycoats by Ficoll-Paque (Pharmacia, Freiburg, FRG) density gradient centrifugation (Böyum, 1968).

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T lymphoblasts. Washed MNC were subjected to iron phagocytosis and magnetism in order to deplete them of monocytes. The non-phagocytic cells were stimulated with PHA-RG (8 μl/ml, Wellcome, Bönningstedt, FRG) and cultivated for 7 days at 37°C and 5% CO_2 in the presence of 5% fetal calf serum (FCS) in RPMI 1640 with 1% L-glutamine and 1% penicillin-streptomycin (all from Biochrom, Frankfurt, FRG). The cells were harvested on day 7 and deep frozen in RPMI 1640 containing 10% FCS and 10% dimethyl sulphoxide (DMSO, Serva, Heidelberg, FRG). After thawing, the cells were checked for the best conditions to show a high response to highly purified human IL-2 and no or negligible response to PHA.

Indicator cells. Cells for the biological detection of IL-2 were PHA induced 7 day cultured T lymphoblasts used at a concentration of $0{\cdot}4 \times 10^6$ per ml RPMI 1640 containing 1% L-glutamine and 1% penicillin-streptomycin solution (Biochrom) and 2% human AB-serum. Fifty microlitres of these cells were added to 50 μl test solution or to a suspension of disrupted IL-2 target cells, respectively.

Production of highly purified IL-2. Human MNC obtained by Ficoll-Paque gradient centrifugation as described above were stimulated with phorbol-12-myristate-13-acetate (PMA, 20 ng per ml) and calcium-ionophore A 23187 (150 ng per ml, both from Sigma, München, FRG). After incubation for 22–24 h at 37°C and 5% CO_2, the supernatant was harvested and separated from the bulk of proteins by Matrex-Gel-Blue-A dye ligand chromatography (Amicon, Witten, FRG). The eluate was extracted with 1% trimethyl silylated controlled pore glass (CPG, Sigma) with a molecular cut-off at approximately 20 kD (Henderson *et al.*, 1983). The CPG eluate was subjected to final purification (Henderson *et al.*, 1983) by reversed phase high performance liquid chromatography (RP HPLC) on Synchropak S-300 (Synchrom, Linden, USA). The final product was 86% pure with a minor contamination of a 24 kD protein. The biological activity corresponded to $1{\cdot}2 \times 10^6$ BRMP units per mg protein.

Determination of the IL-2 binding capacity. IL-2 target cells (T lymphocytes or MNC) were resuspended in Hank's balanced salt solution (HBSS) at a concentration of 4×10^6 cells per ml and incubated with 10 BRMP units of highly purified human IL-2 for 30 min at 0°C according to the technique of Horst & Flad (1985). After incubation, the cells were separated from unbound IL-2 either by filtration (Horst & Flad, 1985) or by gradient centrifugation for 5 min at 0°C, 800 ***g***, over 200 μl of a mixture of 3·4 parts of 0·9% NaCl plus 1 part Ficoll-Paque. The supernatant, containing the unbound IL-2, was sucked off, the cell pellet washed in HBSS, sedimented again and resuspended in 50 μl HBSS. The cells were killed by freezing and thawing and then subjected to the measurement of the total binding capacity. The high affinity binding capacity was determined after an additional step, by which the low affinity bound IL-2 was removed by differential dissociation: the washed cells were exposed to protein absorbing ELISA wells for 10 min at 0°C. During this time, the low affinity bound IL-2 dissociated completely off (dissociation half time less than 1 min), while the high affinity bound IL-2 (dissociation half time 80 min) remained on the cells (Horst & Flad, 1985). These cells were then killed and subjected to the detection of bound IL-2 as above. Low affinity bound IL-2 was calculated by subtraction of the high affinity from the total bound IL-2.

Biological IL-2 detection. Fifty microlitres of indicator cells (see above) were added to 50 μl of IL-2 containing material (e.g. cells killed by freezing and thawing) and incubated for 3 days at 37°C and 5% CO_2. After this time, ^{3}H-labelled thymidine was added, the cells incubated for a further 5 h and harvested with a Skatron cell harvester. Incorporated radioactivity was used as a measure for the IL-2 dependent proliferation. Calibration was done with highly purified human leucocyte IL-2 of known biological activity (see above).

Proliferative response to IL-2. The proliferative response of test cells to IL-2 was determined in the presence of 10 BRMP units of highly purified human leucocyte IL-2. After 3 days the ^{3}H-thymidine incorporation was taken as a measure of the proliferative response. The response was compared to indicator T lymphoblasts and expressed in BRMP units per 10^6 cells.

IL-2 production of test cells. One hundred μl of cells (10^6/ml) in RPMI 1640 containing 2% human AB-serum were stimulated with PHA (8 μl/ml) for 24 h. Fifty microlitres of the clear supernatant were then pipetted into microtitre wells and subjected to the biological IL-2 detection as described above.

Effect of hydrocortisone. Hydrocortisone (Sigma, München, FRG) was dissolved in ethanol

(10^{-3} M) and then diluted with HBSS to 3×10^{-6}–10^{-9} M. After addition of IL-2 and target cells, the final concentration was 10^{-6} M.

The cells were added only after both HC and IL-2 had been previously pipetted into the respective wells. Radioactive HC was obtained from NEN, Dreieich, FRG, specific activity 83·5 Ci/mmole. It was mixed with unlabelled HC in order to obtain a 10^{-6} M solution. This solution was used as described for the unlabelled compound.

Displacement studies with labelled IL-2. ^{125}I-labelled IL-2 was purchased from NEN, specific activity 28·6 μCi per μg. Ten nanocuries were incubated either in the presence or absence of 10^{-6} M HC as in the usual binding assay.

RESULTS

Binding of IL-2 to MNC: effect of PHA and hydrocortisone. Fresh, unstimulated MNC showed only a marginally detectable IL-2 binding, whereby the low affinity was 10-fold higher than the high affinity binding (Table 1). When the same cells were treated with PHA for 7 days, the high affinity binding capacity rose 25-fold, while the low affinity binding capacity was only double that of the prestimulatory value. When hydrocortisone was added to these fully stimulated cells immediately before the binding assay was performed, the high affinity bound IL-2 disappeared almost completely (85%), while the low affinity binding capacity was less affected (Table 1).

Proliferative response of MNC to IL-2: effect of PHA and hydrocortisone. Table 1 shows that fresh MNC exhibited only a weak proliferative response to highly purified IL-2. After 7 days of treatment with PHA, however, a 24-fold increase was seen very similar to the high affinity binding capacity. 10^{-6} M HC, when added after 7 days immediately before the proliferation assay was

Table 1. Binding capacity, proliferative response to IL-2, and IL-2 production of fresh normal human MNC measured before (day 0) or 7 days after stimulation with PHA

	Binding capacity (bound U/10^6 cells)		Proliferative response to 10 U IL-2* (U/10^6 cells)	IL-2 Production† (U/10^6 cells)
	Low affinity	High affinity		
Day 0	0·78 ± 0·27	0·08 ± 0·03	0·67 ± 0·40	2·65 ± 1·45
Day 7	1·60 ± 1·16	2·12 ± 0·78	16·38 ± 8·74	21·40 ± 9·28
Day 7 + HC	1·20 ± 1·24	0·32 ± 0·32	1·04 ± 0·48	3·28 ± 1·77

The cells were isolated by Ficoll density gradient centrifugation and thereafter incubated for 30 min with 10 BRMP units of highly purified human leucocyte IL-2. In the third group (lower line) the cells were incubated additionally with 10^{-6} M hydrocortisone (HC). The binding capacity was determined after 30 min incubation with IL-2 alone or with IL-2 and HC. The proliferative response was measured using the ^{3}H-thymidine incorporation assay in the presence or absence of HC. The IL-2 production was measured in supernatants of cultures incubated for 24 h either with or without HC.

All values are expressed as mean ± s.d. of six healthy donors.

* The proliferative response (R) of the test cells was calculated as:

$$R = \frac{10 \times \text{ct/min (T)}}{\text{ct/min (I)}}$$

whereby 10 is the amount of BMRP units added, T are test cells, and I are indicator cells.

† Production compared to BRMP reference IL-2.

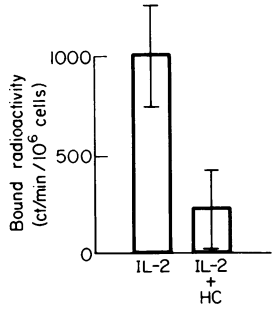

Fig. 1. Effect of 10^{-6} M HC on the binding of ^{125}I-recombinant IL-2 to human PHA stimulated lymphoblasts. Cells were incubated either in the presence or absence of 10^{-6} M HC for 30 min at 0°C. Cell-bound radioactivity was determined after a short washing step. Results are expressed as mean ± s.d. of six samples.

performed, reduced this response to almost prestimulatory values. This again was similar to the alterations seen in the high affinity IL-2 binding capacity.

IL-2 production of MNC: effect of PHA and hydrocortisone. Table 1 demonstrates that the production of IL-2 followed the same general pattern as seen in the IL-2 binding capacity and in the IL-2 response: the PHA stimulated increase in production was almost completely abolished by 10^{-6} M HC.

Inhibition of the binding of ^{125}I-IL-2 to T lymphoblasts. In T lymphoblasts which had been cultivated for 7 days in the presence of PHA, the binding of ^{125}I-labelled IL-2 could be largely abolished (78%) by 10^{-6} M HC, which was very similar to the data obtained with unlabelled IL-2 (Fig. 1).

Influence of increasing amounts of HC on the proliferative response of lymphoblasts to IL-2. The effect of increasing amounts of HC is shown in Fig. 2. HC concentrations between 10^{-9} and 10^{-6} M reduced the response to highly purified IL-2 in a dose dependent manner. Concentrations higher than 10^{-8} M caused a weaker decrease in the response which was, however, likewise dose related.

Binding of ^{3}H-HC to T lymphoblasts. This experiment was undertaken in order to assure that the amount of HC bound to lymphoblasts would not suppress the biological IL-2 response of our indicator cells.

Incubation with 10^{-6} M HC in the presence of IL-2 resulted in a binding of $1{\cdot}3 \times 10^{-12}$ moles per 10^6 cells. This was considered representing the HC contamination in our biological binding assay. It may be recognized that this low level of contamination is far below the level which suppressed the IL-2 response in our indicator T lymphoblasts (compare Fig. 1).

Binding of ^{3}H-HC to IL-2. Highly purified human leucocyte IL-2 was pipetted into dialysis sacs which it could not penetrate: the molecular cut-off of the tubing was 7–8 kD, while the molecular

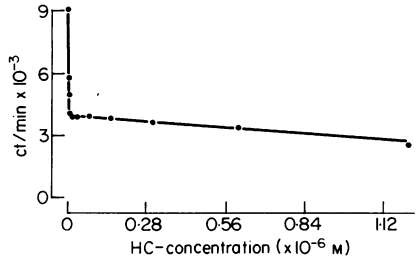

Fig. 2. Effect of increasing amounts of HC on the proliferative response of PHA stimulated T lymphoblasts. The cells were incubated with 10 BRMP units of highly purified human lymphocyte IL-2 on day 7 and checked for ^{3}H-thymidine incorporation after an additional 3 days in the presence of varying concentrations of HC. Each point represents the mean of duplicates.

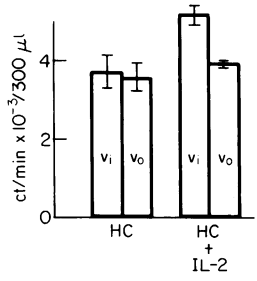

Fig. 3. Binding of ^{3}H-HC to IL-2 as measured by equilibrium dialysis. 10 BRMP units/ml highly purified human leucocyte IL-2 in Hepes buffer, or as control, Hepes buffer alone, were pipetted into the inner volume (v_i) together with 10^{-6} M ^{3}H-HC. The outer volume (v_o) contained only Hepes buffer. The samples were dialysed to equilibrium (24 h) before aliquots (300 µl) were measured. The two left hand columns show that equilibrium was achieved when no IL-2 was present. In the presence of IL-2 a marked accumulation of IL-2 was measured (right columns). The results are expressed as mean ± s.d. of six samples measured.

weight of IL-2 is 15,000 kD. ^{3}H-HC, which can penetrate the tubing, was added to the inner volume of the dialysis system, which was then rotated for 24 h at room temperature.

After 24 h, an accumulation of ^{3}H-HC was found in the IL-2 containing inner volume (V_i, Fig. 3). Even in the presence of 17% human AB-serum in both V_i and the V_o, HC accumulated in the IL-2 containing V_i. There was, however, no dose relationship, and no binding of a high affinity type could be detected (data not shown).

DISCUSSION

The data shown here indicate that HC is capable of interfering with the binding of IL-2 to the IL-2 receptor, with the response of target cells to IL-2, and with the production of IL-2 from stimulated T lymphocytes or peripheral blood mononuclear cells, respectively. It is generally accepted that the IL-2 responsive cells have specific IL-2 receptors and vice versa, that receptor bearing cells are responsive to IL-2. It is in accordance with this model that the response of mitogen activated T lymphoblasts to IL-2 is diminished when the receptor expression is impaired or when the already existing receptor is blocked by a drug like hydrocortisone. The presence of dexamethasone can obviously suppress the expression of IL-2 receptors (Reem & Yeh, 1984). This finding could be confirmed by the present study, in which incubation with HC for 7 days almost completely suppressed the receptor expression. It is interesting, however, that not only the expression can be inhibited but that also the ability to bind IL-2 to already existing receptors can be blocked by HC. This is shown in our study by the fact that incubation of target cells with 10^{-6} M HC for 30 min at 0°C reduced the IL-2 binding to the same degree as did the permanent presence for 7 days at 37°C. Within this time profound metabolic events initiated by the binding of HC to the corticosteroid receptor are not very probable. On the other hand, binding of HC to IL-2 was low so that the formation of an IL-2-HC complex could not explain the strong inhibition of the IL-2 binding.

It was conspicuous that the action of HC in our system was almost exclusively restricted to the high affinity binding sites. The low affinity IL-2 binding capacity was not or only little affected. This is in agreement with the observation (Robb *et al.*, 1984) that high affinity bound ^{125}I-IL-2 was easily displaced by unlabelled IL-2, while the displacement of low affinity bound IL-2 required a much larger excess. It would be tempting to speculate that the high affinity IL-2 binding sites may be also more sensitive to the effect of HC and that IL-2 and HC may have a common acceptor site at which they exert at least part of their specific action. We have been , however, unsuccessful in demonstrating a true displacement of IL-2 by HC. No biological activity was measured in the supernatant of cells incubated first with IL-2, carefully washed, and then incubated with HC for 30 min at 0°C (data not shown). The same negative result with ^{125}I-IL-2 indicated that IL-2

displacement obviously plays no dominant role in the mode of action of HC on the inhibition of the IL-2 receptor. We, therefore, put forward the hypothesis that HC may cause a physicochemical alteration of the receptor structure or of structures in the vicinity of the receptor. This 'modulation' of the function of the IL-2 receptor requires further study.

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