Glucocorticoids and Lymphocytes.
III. Effects of Glucocorticoid Administration on Lymphocyte Glucocorticoid Receptors

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To determine the effects of glucocorticoid administration on the number of measured lymphocyte glucocorticoid receptor sites and the duration of such effects, seven normal volunteers were studied. Glucocorticoid receptor levels of the lymphocytes circulating in the blood of each volunteer were determined. Glucocorticoid was then administered in a regimen of a total of four doses of dexamethasone 4 mg p.o. every 6 hr. Determinations of the number of receptors were performed at 6 hr and at various subsequent times after the end of dexamethasone administration. When compared to baseline receptor numbers, six volunteers showed a decrease in receptor number after glucocorticoid administration (median maximum decrease 2,046 sites/cell). The fall in receptor number occurred rapidly, reaching a nadir within 30 hr from the end of glucocorticoid administration. The return of receptor number to baseline was more gradual, requiring from 3 to as long as 17 days in one subject. Our results suggest that in order to accurately interpret glucocorticoid receptor numbers in human lymphoid cells, glucocorticoid should not have been administered for 3 wk prior to determinations of receptor levels.

MATERIALS AND METHODS

Six men and one woman ranging in age from 25 to 34 yr were studied. None had any known medical problems or recent illnesses. Each volunteered for the study and gave informed consent. Baseline pretreatment laboratory studies included peripheral blood leukocyte and differential counts, lymphocyte B and T surface marker determinations, and glucocorticoid receptor analyses. After the baseline laboratory studies were obtained, each volunteer began a course of glucocorticoid consisting of four doses of dexamethasone 4 mg p.o. every 6 hr, begun at 0800 hr. Repeat determinations of the initial laboratory studies were obtained at 6, 30, 54, and 78 hr, and at 7, 14, and in one case 17 days from the end of the course of dexamethasone.

Our techniques used for the quantitation of B and T lymphocytes have been previously described. In brief, suspensions of viable lymphocytes were studied for the presence of surface immunoglobulin by immunofluorescence using monospecific antisera against heavy and light chains and for sheep erythrocyte receptors by rosette formation with unsensitized sheep erythrocytes. Those lymphocytes that showed surface immunoglobulin were defined as being of B-cell origin, and those that formed rosettes were defined as being of T-cell origin. The non-T, non-B or “null” cell population of lymphocytes was defined by the absence of surface immunoglobulin and receptors for sheep erythrocytes.

For the glucocorticoid receptor assay, 60 ml of heparinized blood was diluted 1 part in 4 in RPMI 1640 medium and cultured for 24 hr at ambient temperatures. The mononuclear cell population of the suspension was then separated by Ficoll-Hypaque gradient centrifugation and studied for glucocorticoid receptors. The separated blood routinely yielded more than 85% lymphocytes as determined by cytofluorometric analysis. For those occasional samples contaminated with large numbers of monocytes, receptor results were not utilized.

To promote dissociation of endogenously bound dexamethasone, the cells were washed 3 times in serum-free RPMI 1640 medium with a 30-min equilibration at 37°C between each centrifugation. Since we had previously found that the T1/2 is approximately 12 min for dissociation of glucocorticoid from rat thymocyte receptors at 37°C, it was felt that this washing procedure would minimize “masking” of receptors by endogenously bound glucocorticoid. Additionally, in other studies (Longo PA, Munck A, Bloomfield CD,
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Smith KA: unpublished), we have found that such a washing procedure promotes maximal dissociation of glucocorticoid from the human promyelocytic line, HL-60.

The methods used for determining receptor sites per malignant cell have been previously described in detail. Briefly, the cells were incubated with a near saturating concentration (40 nM) of [3H]-dexamethasone (specific activity 35 Ci/mole, New England Nuclear, Boston, MA) with and without an excess of unlabeled dexamethasone (2 μM) for 30 min at 37°C. The cell suspension was then cooled to 3°C. Cyttoplasmic receptors were determined by lysing the cells with a rapid dilution into hypotonic MgCl2 (1.5 mM) containing dextran-coated charcoal to adsorb free glucocorticoid. After centrifugation, an aliquot of the released cytosol was removed and counted by liquid scintillation. Nuclear receptor sites were determined similarly by lysing cells in hypotonic MgCl2. The released nuclei were then pelleted, the cytosol removed, and the nuclear pellet counted. Most of the data in this article are expressed as total glucocorticoid receptor sites per cell (RT), which represents the sum of the measured cytoplasmic (RC) and nuclear (RN) receptor sites per cell.

In previous studies we have evaluated the reproducibility of RT for human peripheral blood mononuclear cells. In seven individuals, duplicate determinations were performed on the same sample. For RT the mean range about the mean was ±18%. Essentially identical results were found when consecutive specimens of peripheral blood were tested. In 20 subjects, 2 consecutive specimens of blood were assayed within 2 wk of each other. For RT, the mean range about the mean was ±18.5%. Because of these results, it was felt that a single baseline RT determination prior to dexamethasone administration was adequate.

In order to compare among subjects (irrespective of baseline) the amount of change in receptor numbers that occurred after glucocorticoid administration, the percent change in total glucocorticoid receptors (%RT) was calculated for each subject each time RT was measured. The %RT was derived according to the following equation:

\[
\%RT = \frac{R_T(\text{Postdexamethasone}) - R_T(\text{Predexamethasone})}{R_T(\text{Predexamethasone})} \times 100
\]

A negative value represents a decrease in RT following glucocorticoid administration.

In order to quantitate the change in lymphocyte subpopulations that occurred after glucocorticoid administration, the changes in T, B, and "null" lymphocyte percentages (ΔLT, ΔLB, ΔLX, respectively) were calculated for each subject each time lymphocyte surface markers were measured after the end of glucocorticoid. The ΔLX was derived according to the following equation:

\[
\Delta L_X = \%X \text{ Lymphocytes (postdexamethasone)} - \%X \text{ Lymphocytes (predexamethasone)}
\]

A negative value of ΔLX represents a decline in X (i.e., T, B, or "null") lymphocytes following glucocorticoid administration.

RESULTS

Effects of Glucocorticoid Administration on Lymphocyte Glucocorticoid Receptor Levels

The number of RT measured before, compared to those obtained at various times after, glucocorticoid administration is shown in Fig. 1 for each subject. Baseline receptor levels ranged from 2,417 to 5,397 receptor sites per cell (median 4,668). Six of the seven subjects studied had a decline in receptor number in response to dexamethasone. The maximum amount of the decrease in RT ranged from 1,247 to 3,091 sites per cell (median 2,046). One person had an increase in receptor number following dexamethasone.

Among the 6 individuals who showed a decline in receptor number, the nadir RT was reached at 6 hr from the end of the dexamethasone administration in 3, while in the other 3 individuals, the nadir was reached by 30 hr. In 5 of the 6 volunteers, the fall in RT was observed by the first measurement after stopping dexamethasone (Fig. 1). The maximum %RT ranged from -65 to -33 (median -39).

Five of the seven individuals had repeat RT determinations until the receptor levels had returned to normal. In one person, RT had returned to baseline levels by the third day from the end of dexamethasone administration, in an additional person by the seventh day, in two more by the 14th day, and in the final individual by the 17th day. One of the two volunteers in whom RT did not return to baseline did not have a repeat receptor level drawn more than 54 hr from the last dose of dexamethasone. However, for the times repeat receptor levels were determined, a steady return towards initial receptor levels was observed. The other person whose receptor levels never returned to baseline was the one who had an increase in RT following dexamethasone.

The changes in numbers of RT following dexamethasone administration closely paralleled the changes in numbers of nuclear receptor sites per cell. The changes in numbers of cytoplasmic and nuclear glucocorticoid receptors for three representative subjects are shown in Fig. 2.
Effects of Glucocorticoid Administration on Leukocyte Characteristics of the Peripheral Blood

Six of the seven volunteers demonstrated a maximum increase in leukocyte count 6 hr following the last dose of dexamethasone. The amount of increase ranged from 66% to 295% over baseline (median 125%). In one individual, a maximum increase in leukocytes occurred at 30 hr (30% over baseline). In all cases, glucocorticoid administration resulted in an increase in the percentage of circulating polymorphonuclear cells and a decrease in the percentage of circulating lymphocytes. The absolute lymphocyte count (ALC) (leukocyte count \times \text{percent} \ lymphocytes) prior to the initiation of dexamethasone ranged from 0.9 to $2.3 \times 10^3$/cu mm (median 1.4). There was no consistent change noted in the ALC determined when the glucocorticoid receptor levels were at the nadir: in 3 individuals there was an increase in ALC (increases of 18%, 142%, 166%); in 2 individuals there was a fall in ALC (53% and 69% decrease); and in one individual there was no change in ALC.

The relation between change in receptor number ($%R_T$) and the percent change in T, B, and null cells ($\Delta L_T$) following glucocorticoid administration was examined for each patient, and no consistent patterns were found (data not shown). The percent change in lymphocyte subpopulations at the maximum $%R_T$ is shown in Fig. 3. At the time of maximum decline in receptor levels, among the 6 individuals whose receptor levels declined, 3 volunteers demonstrated a decline in percent T cells, 2 showed no change, and 1 showed an increase. The changes in $\Delta L_T$ were never of the magnitude of $%R_T$. Similarly, the direction and magnitude of alterations in circulating B and null cells did not correlate with $%R_T$ (Fig. 3). The one volunteer who demonstrated an increase in receptor number following dexamethasone had a $\Delta L_T$ of -2 at the time of maximal increase in $%R_T$ ($+121$).

DISCUSSION

This study indicates that a brief course of glucocorticoid usually results in a fall in measurable glucocorticoid receptors in normal human circulating lymphocytes. Schlechte and Sherman have also recently reported a decrease in receptor number in circulating normal lymphocytes following glucocorticoid administration. In our study, the fall in receptor number occurred rapidly. Among the six individuals whose receptor numbers fell in response to dexamethasone administration, the decline was noted as early as 30 hr after starting the steroid in 4 individuals, and in all instances the decrease in glucocorticoid receptor levels had reached a maximum by 54 hr (i.e., 30 hr from the last dose of dexamethasone). In contrast to this rapid decline in receptor number, the time required to return to preglucocorticoid receptor numbers was prolonged, requiring from 3 to as many as 17 days in one individual following the cessation of dexamethasone administration.

The magnitude and time course of the fall in glucocorticoid receptors in circulating normal lymphocytes following dexamethasone administration are similar to those that we have previously reported in neoplastic cells of patients with lymphoma and leukemia.
patients it was not possible to withhold therapy in order to see how long was required for receptor levels to return to baseline. Since we and others have found that in lymphoid malignancies glucocorticoid receptor number is useful in predicting response to glucocorticoid therapy,7'9 these data regarding prolonged depression of receptor level following cessation of glucocorticoid therapy in normal lymphocytes have obvious therapeutic ramifications. They suggest that in order to accurately interpret receptor numbers, patients should have been off glucocorticoid therapy at least 3 wk at the time of receptor determinations. In patients who have recently received glucocorticoid, falsely low numbers of receptors may be obtained, and the patient may be incorrectly predicted to be a nonresponder to glucocorticoid.

While the cause of the prolonged fall in glucocorticoid receptors remains uncertain, it would not appear to be related to shifting of lymphocyte subpopulations.10 Both the direction and the magnitude of the changes in T- and B-cell populations following glucocorticoid administration varied among volunteers in this study and were of insufficient magnitude to explain the observed decline in receptor number. This does not rule out the possibility, however, that within the different subpopulations of lymphocytes there might be a heterogeneity with respect to glucocorticoid receptor content, and that those lymphocytes with a higher number were selectively removed from the bloodstream.

It does not appear that the decline in receptor number represents a masking of receptor sites by the administered glucocorticoid. Glucocorticoid binds reversibly with its cytoplasmic receptors. The half-time for dissociation of the dexamethasone-receptor complex is approximately 12 min at 37°C.7 The plasma half-life of dexamethasone is on the order of 5 hr.11 Thus, on the basis of known plasma clearance rates and dissociation rates of glucocorticoid from its receptor, exogenous glucocorticoid should not have been present at the times when receptor levels were shown to be depressed (in two of four patients 7 days following the last dose of glucocorticoid). In addition, the laboratory methods that were used for determining receptors promote maximal dissociation of glucocorticoid from the receptors of human cultured promyelocytic cells. Using 3H-dexamethasone, we have found that more than 95% of receptor-bound glucocorticoid is dissociated after the second wash. Our studies, however, do not allow us to distinguish between loss of receptor sites and irreversible masking of receptor sites in hypothetical modified forms of the steroid–receptor complex from which dexamethasone does not dissociate under our conditions.

In various hormone receptor systems, alterations in receptor number have been demonstrated in response to changes in hormone levels. "Down regulation" is the term that has been used to describe a decrease in receptor levels in the presence of high hormone concentrations and "up regulation" the increase in receptor levels when hormone concentration is low. Both down regulation and up regulation have been demonstrated in adrenergic and peptide hormone systems. In addition, studies of HeLa cells12 and normal human T lymphocytes13 in culture, thymocytes and liver cells of adrenalectomized mice in vivo,14 and the malignant cells of patients with leukemia and lymphoma in vivo1 demonstrate a decrease in measured glucocorticoid receptor levels after exposure to exogenous glucocorticoid. Moreover, up regulation of rat kidney aldosterone and glucocorticoid receptors was recently found upon removal of endogenous steroid by adrenalectomy.15 The perfusion of isolated kidneys with aldosterone promoted a down regulation of aldosterone receptor
numbers to the levels found in nonadrenalectomized animals.

The physiologic significance of the decline in glucocorticoid receptor levels has yet to be determined. However, in light of in vitro⁶ and in vivo⁷⁹ studies, which have demonstrated that the magnitude of glucocorticoid inhibitory effects correlates with the number of glucocorticoid receptors in lymphoid cells, it is tempting to speculate that a receptor regulatory mechanism exists that modulates the magnitude of lymphoid cell glucocorticoid suppression upon exposure to high glucocorticoid concentrations.

REFERENCES

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