Effect of Phytohemagglutinin on Concentrations of Glucocorticoid Receptors in Lymphocytes from Patients with Connective Tissue Diseases

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We examined the effect of phytohemagglutinin (PHA) on glucocorticoid receptors in lymphocytes, using peripheral mononuclear leukocytes from patients with connective tissue diseases. Glucocorticoid receptor concentrations and dissociation constants (Kd) for [3H]dexamethasone binding to lymphocytes from patients with rheumatoid arthritis, Sjögren's syndrome, and other connective tissue diseases did not differ significantly from values for normal volunteers. Kd values correlated positively with glucocorticoid receptor concentrations after PHA stimulation both in normal volunteers and in patients with connective tissue diseases. The correlations (r values) for the control group and patients were not significantly different.

Additional Keyphrases: rheumatoid arthritis · Sjögren's syndrome · dexamethasone binding · radioassay

Normal lymphocytes are activated by antigens and mitogens (1–3). Addition of phytohemagglutinin (PHA) to cultured lymphocytes produces a two- to threefold increase in glucocorticoid receptor sites per cell (4). Because glucocorticoids are known to be important modulators of gene expression, the increased number of receptor sites per cell may result in a change in glucocorticoid effects on the functions of proliferating lymphocytes after activation.

 Patients with some auto-immune diseases display abnormalities in lymphocyte response to mitogens or specific antigens (5–8). These findings suggest that in these patients the number of glucocorticoid receptor sites per cell after activation may be different from that in normal subjects and that the effect of glucocorticoids on proliferating T-lymphocytes may be altered. In the present study, we examined the effect of in vitro activation by PHA on the concentration of glucocorticoid receptors in mononuclear leukocytes from patients with connective tissue diseases.

Materials and Methods

Patients. We studied 13 patients with rheumatoid arthritis, ages 28 to 66 years; 14 with Sjögren's syndrome, ages 32 to 64 years; nine (ages 28 to 67 years) with other connective tissue diseases, including three with systemic lupus erythematosus, one with polymyositis, two with mixed connective tissue disease, and one each with lupus/polyarthritis/Sjögren's overlap, Sjögren's/arthritis overlap, and lupoid hepatitis/Sjögren's syndrome overlap. Most patients did not take nonsteroidal anti-inflammatory drugs or corticosteroids. The control group consisted of 34 normal volunteers, ages 21 to 43 years.

Cell preparation. Approximately 20 mL of heparinized blood was obtained and layered on a Ficoll-Hypaque gradient (Litton-Bionetics, Inc., Kensington, MD 20786) and centrifuged (400 × g, 40 min, 20 °C). Mononuclear cells at the interface were collected and washed three times with phosphate-buffered saline. Cells were suspended in medium RPMI-1640 (Gibco Laboratories, Grand Island, NY 14072) containing 100 μg of penicillin and 100 units of streptomycin (all from gusco) per milliliter.

Mitogen cultures. One part of fetal calf serum (Flow Laboratories, McLean, VA 22102) and one part of PHA-P (Wellcome Research Laboratories, Beckenham, U.K.) in isotonic saline were added to eight parts of cell suspension prepared as above. We then incubated the cell suspensions in a CO2 incubator as described below.

Time course of [3H]dexamethasone binding and incorporation of [14C]thymidine into activated lymphocytes. After adjusting the volume of the cell suspension to 40–50 mL, we added 1 mL of it to each culture tube. Cell suspensions were cultured at 37 °C under a CO2-enriched atmosphere (50 mL/L) for one to six days. Every 24 h after we initiated the incubation, we washed four tubes of cell suspensions with phosphate-buffered saline and counted the number of viable cells. The cells were resuspended in 1 mL of medium RPMI-1640 containing 5 nmol of [6,7-3H]dexamethasone (46.0 kCi/mmol; New England Nuclear, Boston, MA 02118) per liter, with or without 200-fold molar excess of unlabeled dexamethasone (Sigma Chemical Co., St. Louis, MO 63178), and incubated in a shaking incubator at 20 °C for 3 h in a CO2-enriched atmosphere. After 3 h, we recovered bound [3H]dexamethasone by aspirating cells onto glass microfiber filters (GF/C, Whatman Ltd., Maidstone, Kent, U.K.) and washing with chilled (4 °C) phosphate-buffered saline. The filters were transferred to scintillation vials and 10 mL of "Aquasol" (New England Nuclear) was added. Radioactivities were counted in a Packard Tri-Carb Scintillation Counter.

We added 10 μL of isotonic saline containing 0.5 μCi of [2-14C]thymidine (6.7 kCi/mmol; New England Nuclear) to three tubes after every 24 h of incubation for six days, to study thymidine incorporation; 24 h later, we counted the radioactivity of [14C]thymidine incorporated into the trichloroacetic acid-insoluble fraction.

Glucocorticoid binding study. We diluted the cell suspensions to 10 mL and transferred them to culture flasks to incubate for four days as described above. After incubation we washed the cells with phosphate-buffered saline and suspended them in RPMI-1640 medium containing 10 g of human serum albumin (Miles Laboratories, Inc., Elkhart, IN 46514) per liter. [3H]Dexamethasone binding was studied for 3 h at 20 °C as previously described (9). Bound steroid was recovered onto glass microfiber filters by aspiration (see above). The receptor concentration (maximum binding capacity) per 10⁸ cells and the dissociation constant (Kd) were determined for each sample.

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Statistical analysis. We used Student's t-test to assess statistical significance. Linear regression of the relationship between glucocorticoid receptor sites per $10^6$ cells and the $K_d$ values was by the least-squares method.

Results

Time course of $[^3H]$dexamethasone binding and $[^3H]$thy- midine incorporation. Specific binding was calculated as the difference between total (without unlabeled dexamethasone) and nonspecific (with unlabeled dexamethasone) bindings. Figure 1A shows the typical time course of $[^3H]$dexamethasone binding to $10^6$ cells incubated with 5 nmol/L $[^3H]$dexamethasone solution. The number of glucocorticoid binding sites per $10^6$ cells became constant after four days. On the basis of this result, we determined the receptor concentration per $10^6$ cells four days after incubation with PHA-P. Figure 1B shows the typical time course of $[^3H]$thymidine incorporation. Thymidine incorporation was maximum from 48 to 72 h of incubation with PHA-P.

Glucocorticoid receptor sites in PHA-stimulated mononuclear leukocytes. Calculations of $K_d$ and glucocorticoid receptors per $10^6$ stimulated mononuclear leukocytes from patients and normal volunteers did not differ significantly among the four groups (Table 1). To clarify the nature of the relationship between glucocorticoid receptor concentration and $K_d$, we have graphed individual values of $K_d$ as a function of glucocorticoid receptor sites in control subjects (A) and patients with connective tissue diseases (B) in Figure 2. In each group, there was a positive correlation between $K_d$ and glucocorticoid receptor sites: $r = 0.672$ ($p < 0.001$) for the controls and $r = 0.515$ ($p < 0.01$) for the patients. These $r$ values are not significantly different.

Discussion

Glucocorticoids are immunosuppressive and anti-inflammatory agents (10), and have immunoregulatory effects at physiological concentration (11). Nowell (12) has reported that PHA-induced mitosis of human peripheral lymphocytes is inhibited by prednisolone-21-phosphate during the early stage of PHA stimulation, and Tormey et al. (13) have reported that prednisolone-21-phosphate inhibits incorporation of thymidine and cytidine into PHA-stimulated lymphocytes such that delayed addition of the steroid results in diminished inhibition. However, Munck et al. (14) found that activated lymphocytes are still highly sensitive 72 h after stimulation with respect to inhibition of thymidine and uridine incorporation and glucose uptake. Recently, Arya et al. (15) have reported that synthesis of T-cell growth factor messenger RNA is inhibited by dexamethasone. Thus a lymphocyte is one of the targets of glucocorticoids, and immunologic response is subject to the inhibitory effects of glucocorticoids (1). Because glucocorticoid actions are generally considered to be based on the binding to specific receptor protein, increased receptor sites per cell after PHA stimulation may have some physiological significance in the function of activated lymphocytes.

In the present study, the mean values of the four groups were not significantly different. However, the range of individual values of glucocorticoid receptor concentration in activated lymphocytes is broad, both in normal volunteers and patients (Figure 2). On the whole, the $K_d$ is high when the increase in PHA-stimulated receptor sites per cell is high. Plotting receptor concentrations in activated lympho-
cells as receptor sites per cell vs $K_d$ shows similar distributions of points for normal subjects and for patients (Figure 2). The fact that the change of glucocorticoid receptor in patients is similar to that of the normal group suggests that the glucocorticoid receptor function of lymphocytes of patients with connective tissue disease is normal.

References