Title
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Permalink
https://escholarship.org/uc/item/78w1c40q

Journal
The Journal of experimental medicine, 147(6)

ISSN
0022-1007

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Publication Date
1978-06-01

DOI
10.1084/jem.147.6.1684

Peer reviewed
INTERACTION OF GLUCOCORTICOIDS
WITH MACROPHAGES

Identification of Glucocorticoid Receptors in Monocytes and Macrophages*

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The mononuclear phagocyte system plays a central role in mediating host responses in inflammation (1). Glucocorticoids have anti-inflammatory actions that may be of considerable importance in the therapeutic effects of these agents in chronic inflammation; it is possible that some of these effects are mediated through direct hormonal action on macrophages. Although the site of action of the glucocorticoids on macrophages has not been established, it has been shown that in many other glucocorticoid target systems the effects of glucocorticoids are mediated by specific macromolecular binding proteins, referred to as receptors (2-4).

In this study we have established that monocytes and macrophages contain saturable glucocorticoid-binding proteins, with specificity of binding for cortisol, corticosterone, and related synthetic steroids such as dexamethasone, and that they have dissociation constants for binding within physiological ranges.

Materials and Methods

Cell Culture. Mouse peritoneal macrophages were harvested from female Swiss Webster mice (CD-1 and CF-1; Charles River Breeding Laboratories, Inc., Wilmington, Mass.) weighing 20-25 g, as described previously (5, 6). Resident macrophages were obtained from unstimulated animals, and elicited macrophages were obtained from mice injected 4 days before with 1.0 ml of Brewer thioglycollate medium (5, 6). Cells were plated at 0.5-1 × 10⁶ cells/well in 2-cm²-diameter multiwell plates (Microbiological Associates, Walkersville, Md.) in Dulbecco's modified Eagle's medium high glucose formulation (DME)¹ supplemented with 10% heat-inactivated fetal calf serum (FCS) (Grand Island Biological Co., Grand Island, N. Y.) 1-24 h before binding experiments, cells were placed in serum-free DME supplemented with 0.2% lactalbumin hydrolysate DME-LH.

Rabbit alveolar macrophages were obtained by lung lavage 10-14 days after intravenous

* Supported by grants AM 14780, AM 03535, and CA 17323 from the U. S. Public Health Service, by grants from the National Chapter of the Arthritis Foundation and the Dufault Bequest for Arthritis Research administered by the New Hampshire Chapter of the Arthritis Foundation, and by the U. S. Department of Energy.

¹ Abbreviations used in this paper: DME, Dulbecco's modified Eagle's medium; DME-LH, DME supplemented with 0.2% lactalbumin hydrolysate; FCS, fetal calf serum; K₅₀, half-maximum saturation; KRPg, Krebs-Ringer phosphate buffer containing 5.5 mM glucose.

1684 J. Exp. Med. © The Rockefeller University Press · 0022-1007/78/0601-1684$1.00
injection of 0.15 ml of Freund's complete adjuvant (Difco Laboratories, Detroit, Mich.) (7). The cells were washed 3 times in Hanks' balanced salt solution and resuspended at 2 - 4 x 10^6/ml in DME-LH. The alveolar macrophages were then plated in multiwell dishes at 2 x 10^6 cells/well, or cultured in suspension for 3 h before use in the assays of steroid binding. Cells in suspension were centrifuged, washed in Hanks' balanced salt solution, and resuspended at cytocrits of 0.24 - 0.36 for binding experiments.

Human monocytes were isolated from 300 ml of freshly drawn, citrated blood. Mononuclear cells were concentrated by Ficoll-Hypaque fractionation (8), washed twice in RPMI-1640 medium (Grand Island Biological Co.), and resuspended at 1 x 10^6 cells/ml in RPMI-1640 supplemented with 10% autologous serum. Cells were plated in multiwell plates precoated with 10 μg of fibrin/cm^2 to aid adherence of monocytes. Nonradioactive fibrin layers were prepared by the procedure used for ^125I-labeled fibrin layers (9). After 16 h at 37°C in a CO_2 incubator, nonadherent cells were removed by washing, and adherent cells were placed in serum-free DME-LH for 2 h before measurement of receptors. The mean purity of the monocyte preparation was 93%.

Cells were counted in a Coulter counter (Coulter Electronics Inc., Hialeah, Fla.), and differential cell counts were made on cytocentrifuge preparations stained with Wright's stain.

Steroid Binding

**MEASUREMENT OF STEROID BINDING TO RECEPTORS IN INTACT CELLS.** Monolayers of macrophages in 2-cm^2-diameter wells were washed rapidly 3 times at room temperature with ~2 ml of Krebs-Ringer phosphate buffer containing 5.5 mM glucose (KRPg). Each well then received 100-200 μl of KRPg containing [1,2,4-3H (N)]dexamethasone or [6,7-3H(N)]dexamethasone (New England Nuclear, Boston, Mass.; 30-50 Ci/mmol). In a few experiments, [1,2,3-3H(n)]triamcinolone acetonide (New England Nuclear; 45 Ci/mmol) was used instead of [3H]dexamethasone. The plates were incubated at 37°C for 70 min, then placed on ice. Aliquots of medium from each well were taken to determine the concentration of free [3H]dexamethasone. Each well was rinsed 6 times with 2 ml of ice-cold KRPg to remove free steroid, and then left for 20 min at room temperature with buffer. The treatment at room temperature removed most of the nonsaturable fraction of cell-associated steroid, as previously shown for thymus cells (10, 11). The remaining steroid was extracted with 150 μl of ethanol at room temperature for 20 min, followed by an additional 50 μl to rinse the wells. Samples were counted in Bray's fluid (New England Nuclear) in a Packard Tri-Carb liquid scintillation spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.). Data were analyzed by conventional plots of bound versus free steroid, and by Scatchard plots separated by trial and error into saturable and nonsaturable components (12, 13).

Protein content of monolayers was determined in sham-treated wells which had not been extracted with ethanol, and on residual cellular protein after ethanol extraction by solubilization in 0.5 M NaOH followed by Lowry-Folin procedure with crystalline bovine serum albumin as standard (14).

**COMPETITIVE BINDING STUDIES.** Competition for binding with whole cells was carried out as for binding of [3H]dexamethasone alone, except that the incubation media contained both [3H]dexamethasone and the unlabeled competing steroid at the concentrations indicated in Results. Cortisol and other steroids of similar solubility were dissolved in KRPg, and their concentration was determined spectrophotometrically by measuring adsorption at 250 nm and assuming ε = 1.4 x 10^4 M⁻¹ cm⁻¹ (11). Less soluble steroids such as estradiol were dissolved in ethanol; incubations for these steroids contained up to 1% ethanol, which did not affect dexamethasone binding. Steroids were obtained from Steraloids, Inc., Pawling, N. Y., Calbiochem, San Diego, Calif., or Sigma Chemical Co., St. Louis, Mo.

**DEXAMETHASONE BINDING TO "CYTOPLASMIC" AND NUCLEAR FORMS OF GLUCOCORTICOID RECEPTORS AND TEMPERATURE-SENSITIVE TRANSLLOCATION FROM "CYTOPLASMIC" TO NUCLEAR FORMS.** Thioglycolate-elicited macrophages were either washed out of the peritoneal cavity of mice and used directly, or cultured for 24 h in 100-mm-diameter tissue culture dishes as described above, and scraped off the surface with a rubber policeman before use. The cells were sedimented and washed three times with KRPg at room temperature, and finally incubated at a cytocrit of 0.33 ml packed cells/ml of suspension with [3H]dexamethasone in the absence or presence of 500 nM unlabeled dexamethasone. The values obtained in the presence of unlabeled dexamethasone were for nonsaturable binding. The cells were incubated at 3°C for 120 min, then at 37°C for 30 min. After both incubations, the cells in duplicate 20-μl aliquots of the suspensions were disrupted by
MACROPHAGE GLUCOCORTICOID RECEPTORS

Results

Physicochemical Interactions of Dexamethasone with Thioglycollate-Elicited Mouse Peritoneal Macrophages. Specific binding of \[^{3}H\]dexamethasone to monolayers of thioglycollate-elicited mouse peritoneal macrophages increased rapidly for 30 min at 37°C, and then reached a plateau (Fig. 1). The rate of binding at 3°C was slower (not shown). Binding could be resolved into two components: a high affinity component, which was saturable and could be displaced by excess nonradioactive steroid, and a low affinity component, which was not saturated up to 100 nM dexamethasone and was not displaced by excess nonradioactive steroid (Fig. 2). The high affinity binding had a half-maximum saturation (K_d) at 3.7 nM dexamethasone. High affinity binding was not influenced by the prolonged washing procedures used, whereas low affinity (nonsaturable) binding usually decreased to <20% of the total binding of radioactive hormone; the 20-min room temperature wash was particularly useful in decreasing nonsaturable binding. High affinity binding was \((80-100)\) fmol of dexamethasone/mg cell protein \((3,500-6,000\) binding sites/cell) (Table I). Binding was enhanced by \(\approx20\%\) when the macrophages were removed from DME + FCS and placed in DME-LH at least 1 h before experiments for hormone binding. High affinity binding did not increase further with culture of the cells in DME-LH for up to 72 h. The number and avidity of the hormone receptors did not change with time. High affinity binding was the same when macrophages that had been explanted 1, 24, 72, or 144 h were used. Binding was proportional to the number of macrophages adhering to the plate. Only viable cells bound the steroid; macrophages killed by heat or acid did not bind dexamethasone.
Fig. 2. [3H]Dexamethasone binding of thioglycollate-elicited macrophages. The binding of dexamethasone to intact cells in monolayer at 37°C was made as described in Materials and Methods. The results of a typical experiment are shown as a Scatchard plot analyzed into saturable (——) and nonsaturable (---) components, and as a conventional plot of bound vs. free dexamethasone (inset). The parameters of the solid lines are: slope = -K, = 0.27 (nM)^{-1}, intercept = 3,500 sites/cell. The line (...) gives the sum of the two components. Original data are shown by (O) and original data after subtraction of the nonsaturable component are shown by (©).

**Table I**

**Characteristics of Glucocorticoid Receptors in Cultured Mononuclear Phagocytes**

| Source                  | Species    | [3H]Labeled ligand | fmol/mg of cell protein | Sites/cell | K_d (nM) |
|-------------------------|------------|--------------------|-------------------------|------------|----------|
| Blood monocytes         | Human      | Dexamethasone      | 470                     | 9,000      | 7.7      |
| Resident peritoneal     | Mouse      | Triamcinolone      | 270                     | 6,100      | 2.1      |
| macrophages             |            | acetonide          |                         |            |          |
| Exudative peritoneal    | Mouse      | Dexamethasone      | 80                      | 4,300      | 3.7      |
| macrophages             |            |                    |                         |            |          |
| P388D1 cells            | Mouse      | Dexamethasone      | 65                      | 4,200      | 4.0      |
| Induced alveolar        | Rabbit     | Triamcinolone      | 3,800*                  | 4,500      | 2.6      |
| macrophages             |            | acetonide          |                         |            |          |
|                         |            | Dexamethasone      | 85                      | 4,600      | 1.8      |

* fmol/ml packed cells.
TABLE II

Competition by Various Steroids for Binding of [3H]Dexamethasone to Glucocorticoid Receptors in Plated Thioglycollate-Elicited Mouse Macrophages

| Competing steroid  | Concentration | [3H]Dexamethasone binding* |
|--------------------|---------------|-----------------------------|
|                    | nM            | % control                   |
| Dexamethasone      | 1,000         | 18 ± 1                      |
| Cortisol           | 100           | 77 ± 1                      |
|                    | 1,000         | 33 ± 1                      |
| Corticosterone     | 100           | 84 ± 6                      |
|                    | 1,000         | 40 ± 2                      |
| Cortexolone        | 90            | 92 ± 3                      |
|                    | 900           | 56 ± 2                      |
| Progesterone       | 120           | 93 ± 3                      |
|                    | 1,200         | 54 ± 3                      |
| Cortisone          | 1,000         | 81 ± 2                      |
| 11-Epicortisol     | 1,000         | 105                         |
| Estradiol          | 100           | 98 ± 8                      |
|                    | 1,000         | 96 ± 10                     |
| Dihydrotestosterone| 100           | 101 ± 15                    |
|                    | 1,000         | 85 ± 11                     |
| Testosterone       | 85            | 85 ± 10                     |
|                    | 850           | 78 ± 3                      |

* Whole cell binding assay; values are shown as mean ± range.

[3H]Dexamethasone was present in the assay at 8 nM.

The binding of dexamethasone to the thioglycollate-elicited macrophages was specific for glucocorticoids. As shown in Table II, dexamethasone, cortisol, and corticosterone competed for receptor-bound [3H]dexamethasone. Progesterone and cortexolone also competed for receptors. These substances sometimes act as antiglucocorticoids (4). Cortisone had a small but reproducible competing effect in macrophages, and it is possible that some limited metabolic conversion to cortisol may have occurred. Estradiol, 11-epicortisol, testosterone, and dihydrotestosterone did not compete for binding.

In many other glucocorticoid-responsive systems, the hormone effect depends upon glucocorticoid binding to a receptor protein in the "cytoplasm" followed by intracellular transport into the nucleus (4). In a modified cytosol and nuclear pellet preparation at 3°C, the receptor-bound dexamethasone in thioglycollate-elicited macrophages was largely found in a soluble "cytoplasmic" form, which was rapidly transferred to a nuclear form when the temperature was raised to 37°C (Fig. 3). Thus, these results support the presence of a functional receptor system for dexamethasone in elicited mouse macrophages.

Glucocorticoid Receptors in Resident Mouse Peritoneal Macrophages. Macrophages normally residing in the peritoneal cavity of mice also bound glucocorticoids with high affinity (for triamcinolone acetonide, $K_d = 2.1$ nM) and contained $\approx 6,000$ sites/cell (Table I). As in elicited peritoneal macrophages, steroids with glucocorticoid activity (triamcinolone acetonide and dexamethasone) and progesterone competed with dexamethasone for binding to receptors (Table III).
Cytoplasmic Nuclear

Cytoplasmic Nuclear

Fig. 3. Dexamethasone binding to "cytoplasmic" and nuclear forms of glucocorticoid receptors in thioglycollate-elicited mouse peritoneal macrophages and temperature-sensitive translocation from "cytoplasmic" to nuclear forms. Cell suspensions were incubated with [3H]dexamethasone as described in Materials and Methods. Saturable binding sites in "cytoplasmic" fractions and nuclear pellets for cells incubated at 3°C for 120 min are shown on the left; sites for cells incubated at 3°C for 120 min followed by 37°C for 30 min are shown on the right. Values are expressed as mean and range obtained for binding at 4 nM [3H]dexamethasone.

Mouse Macrophages from Other Sources. High affinity saturable binding of glucocorticoids to macrophages elicited in the peritoneal cavity by pristane (data not shown), resident lung macrophages (data not shown), and P388D1 cells from a macrophage-like continuous line2 (Table I) was also demonstrated.

Rabbit Alveolar Macrophages. Pulmonary alveolar macrophages obtained from rabbits 2 wk after intravenous injection of complete Freund's adjuvant bound dexamethasone and triamcinolone acetonide to high affinity receptor sites (Table I). Cells adhering to plastic surfaces and cells in suspension bound the glucocorticoid equally well. The number and affinity of the rabbit macrophage glucocorticoid receptors were similar to that observed for mouse macrophages. For optimal binding, the macrophages were cultured in serum-free DME-LH for 2-24 h after removal from the lungs. Dexamethasone bound specifically to receptors with high affinity for glucocorticoids. As shown in Fig. 4, dexamethasone, triamcinolone acetonide, and hydrocortisone competed with [3H]dexamethasone for binding to receptors, whereas cortisone, estradiol, and dihydrotestosterone did not.

Human Blood Monocytes. The rabbit and the mouse are glucocorticoid-sensitive species, whereas man is relatively glucocorticoid resistant (2). It was thus of particular interest to examine the glucocorticoid receptors in human mononuclear phagocytes. Blood monocytes in monolayer culture bound dexamethasone (Fig. 5). The high affinity component was saturated by 30 min of incubation at 37°C with 10 nM dexamethasone, and it had a K_d of ~7 nM. The low affinity component was nonsaturable. The human monocytes had ~9,000

2 Werb, Z., R. Foley, and A. Munck. 1978. Glucocorticoid receptors and glucocorticoid-sensitive secretion of neutral proteinases in a macrophage line. J. Immunol. In press.
TABLE III

| Competing steroid        | Concentration | [3H]Dexamethasone binding* |
|--------------------------|---------------|----------------------------|
| None                     | -             | 100 ± 3                    |
| Triamcinolone acetonide  | 10            | 34 ± 0                     |
|                          | 100           | 29 ± 1                     |
| Dexamethasone            | 100           | 32 ± 1                     |
|                          | 1,000         | 18 ± 1                     |
| Cortisol                 | 100           | 63 ± 12                    |
|                          | 1,000         | 30 ± 8                     |
| Cortisone                | 1,000         | 82 ± 6                     |
| Progesterone             | 100           | 73 ± 6                     |
|                          | 1,000         | 38 ± 9                     |
| 11-Epicortisol           | 1,000         | 107 ± 8                    |
| Dihydrotestosterone      | 1,000         | 100 ± 2                    |
| Estradiol                | 1,000         | 95 ± 4                     |

* Whole cell binding assay with [3H]dexamethasone present in the assay at 3 nM; values are shown as mean ± SD of triplicates.

sites/cell (Table I), well within the range for rabbits and mice. There were reproducibly more glucocorticoid receptors in blood monocytes than in the lymphocytes isolated from the same preparations (≈3,000 sites/cell), and platelets contaminating the monocyte preparations contained no demonstrable receptors (G. Crabtree, K. Smith, and A. Munck, unpublished observations). Cortisol and dexamethasone competed for binding of [3H]dexamethasone to the monocytes, whereas steroids without glucocorticoid activity did not (Table IV).
Thus, the monocyte contained specific binding sites consistent with glucocorticoid action at physiological concentrations.

**Discussion**

The studies reported here show that glucocorticoid receptors are present in intact mononuclear phagocytes from mouse, rabbit, and man. The dissociation constants found (Table I) were \( =2-8 \) nM for dexamethasone; the competition for binding by cortisol at about 10-fold higher concentrations suggests that
glucocorticoid receptor-mediated functions in macrophages would be operative at physiological concentrations.

The number of receptor sites and their affinity were similar for resident macrophages, macrophages elicited by inflammatory stimuli, and blood monocytes, and they were similar to those reported for lymphoid cells (3, 4, 16). Although unstimulated monocytes and macrophages had higher concentrations of steroid receptors than did elicited macrophages, this does not necessarily signify a difference in sensitivity to glucocorticoid action. Mitogen-stimulated lymphocytes contain several times more receptors than unstimulated lymphocytes, and yet both populations are equally steroid sensitive (16).

In the present study, glucocorticoid binding was examined with dexamethasone and triamcinolone acetonide as the ligands. These synthetic glucocorticoids have the advantage of not binding to transcortin, a serum protein that binds cortisol (17); thus, accurate glucocorticoid concentrations could be determined. Also, experiments were made in the absence of serum to decrease interference from exogenous steroids. Serum cortisol and progesterone both compete with dexamethasone for binding, and could influence the free steroid concentrations present in the experiments.

In other work we have shown that P388D1 macrophages, a continuous cell line, contain glucocorticoid receptors indistinguishable from those of normal mouse macrophages.2 We have demonstrated temperature-sensitive transfer of glucocorticoid receptors to macrophage nuclei. With P388D1 cells we have been able to demonstrate glucocorticoid binding directly to proteins isolated from cytoplasm, temperature-dependent activation of the hormone-receptor complex, and translocation of only activated complexes into isolated nuclei.2

In virtually every target tissue investigated, the first step in steroid hormone action is the binding of steroid to specific receptor proteins. Because their specificity and affinity correlate with biological functions, they may mediate the hormone action. Some effects of glucocorticoids on macrophages have been described previously; in most experiments, however, pharmacological concentrations of steroids were used (2, 18). These effects are unlikely to be mediated by the high affinity receptors described in this paper. In the accompanying paper (18) hormone effects at glucocorticoid concentrations similar in specificity and affinity to those for receptor characteristics are shown for macrophage secretion and growth.

Summary

Glucocorticoid binding was measured in resident and thioglycollate-elicited mouse peritoneal macrophages, rabbit alveolar macrophages, and human monocytes. Two assays of binding were used—an assay with intact cells in suspension or monolayers, and an assay of cytosol and nuclear forms of glucocorticoid receptors. The mononuclear phagocytes contained $\approx 4-10 \times 10^3$ high affinity receptor sites per cell, with dissociation constants of $\approx 2-8$ nM dexamethasone. The binding to the saturable sites was specific for steroids with glucocorticoid or antiglucocorticoid activity. Cortisol, corticosterone, and progesterone competed with dexamethasone for binding, whereas estradiol, dihydrotestosterone, and 11-epicortisol competed very little.
Binding of dexamethasone to cytosol and nuclear forms of the receptor complex and temperature-sensitive translocation of cytosol forms to nuclear forms were shown. At 37°C the predominant form of the hormone-receptor complex was nuclear. These results demonstrate that corticosteroids interact with macrophages at physiological concentrations.

We thank Ms. I. Vander Heiden and Ms. Nancy Cidlowski for their technical assistance. These studies were initiated while Z. W. was a visiting professor in the laboratory of Dr. E. D. Harris, Jr., Dartmouth Medical School.

Received for publication 27 January 1978.

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