

BIOCHEMICAL ACTIONS OF GLUCOCORTICOIDS ON
MACROPHAGES IN CULTURE
Specific Inhibition of Elastase, Collagenase, and Plasminogen
Activator Secretion
and Effects on Other Metabolic Functions*

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Glucocorticoids have a major role in the therapy of inflammatory and immunologically mediated diseases. The precise mechanism of the suppressive and anti-inflammatory effects of these drugs is still unknown, but the functions of the mononuclear phagocyte system are generally believed to be sensitive to glucocorticoid action (1-3). During glucocorticoid administration in vivo, monocytopenia occurs (4-6) and monocytes fail to accumulate at inflammatory sites (7-10). In the presence of glucocorticoids, macrophages do not respond to macrophage migration inhibitory factor (11), and fail to become "armed" or activated (8, 11-13). In experiments in vivo it is difficult to determine whether glucocorticoids are acting directly on macrophages or indirectly on lymphocytes and other cells that produce mediators of macrophage function (3, 8).

Macrophages may serve as a direct target for the therapeutic actions of anti-inflammatory steroids. In the accompanying paper (14), I establish that mononuclear phagocytes contain specific, high affinity receptors for glucocorticoids. In the present paper, I examine the action of glucocorticoids on biochemical functions of monocytes and macrophages cultured in vitro. Mononuclear phagocytes at all stages of maturation are sensitive to glucocorticoids. Production of monocytic and granulocytic colonies from bone marrow precursors and the secretion of elastase, collagenase, plasminogen activator, and nonspecific neutral proteinases by mature macrophages are inhibited by physiological concentrations of glucocorticoids. I have studied macrophages from glucocorticoid-sensitive (rabbit, mouse) and glucocorticoid-insensitive (human, guinea pig) species, and all show similar glucocorticoid-mediated actions, even though they differ in hormone-mediated effects on lymphocytes (1, 3). Because steroid concentrations comparable to those for saturating the high affinity glucocorti-

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oid binding sites suppress macrophage secretion and colony formation, it is likely that these effects are mediated by specific glucocorticoid receptors.

Materials and Methods

Cells. Human blood monocytes, adjuvant-induced rabbit pulmonary alveolar macrophages, resident mouse peritoneal macrophages, and thioglycollate-elicited mouse peritoneal macrophages were obtained as described in the accompanying paper (14). Pulmonary alveolar macrophages from unstimulated guinea pigs were obtained by lavage procedures similar to those used for rabbits (14, 15).

Cell Culture. Mouse macrophages were cultured in Dulbecco's modified Eagle's medium (DME)¹ supplemented with 10% fetal calf serum (FCS) in 16-mm-diameter multiwell dishes for 24–48 h before experiments. The cultures were washed 4 times with DME. Unless otherwise indicated, the cells were then cultured in DME supplemented with 0.2% lactalbumin hydrolysate (DME-LH) (14, 16). Cortisol and steroids of similar solubility were dissolved at ≈ 0.1 mM in phosphate-buffered saline, and their concentrations were determined spectrophotometrically (14); less soluble steroids were dissolved in 100% ethanol at ≈ 0.1 mM and added to cultures to a final concentration of <1% ethanol. Freshly prepared steroid solutions were added to culture media just before use.

Human monocytes ($0.5\text{--}1.0 \times 10^6/16\text{-mm well}$) were cultured on ¹²⁵I-labeled fibrin plates (14, 17) in DME + 10% FCS containing 60 μg of soybean trypsin inhibitor (STI)/ml for 24–48 h. The cells were then washed 4 times with DME, and the medium was replaced with DME-LH or DME + 5% dog serum that had been acid-treated and plasminogen-depleted (ATDS-P) (17, 18) with steroids and with or without added dog plasminogen (1–30 nM).

Rabbit and guinea pig alveolar macrophages ($0.5\text{--}1.0 \times 10^6/16\text{-mm dish}$) were cultured in DME-LH alone for 24–48 h; the medium was then replaced with fresh DME-LH containing steroids as required.

Determination of Bone Marrow Colony Formation. Formation of monocytic and granulocytic colonies (CFU-C) from bone marrow precursor cells was determined by culturing 5×10^4 bone marrow cells from adult Swiss mice (CF-1; Charles River Breeding Laboratories, Inc., Wilmington, Mass.) in agar with L-cell colony-stimulating factor (19). Half-maximum concentrations of colony-stimulating activity were used in these experiments, and dexamethasone was added when the plates were poured. Colony number and type were determined at 7 and 14 days of culture; colonies contained at least 50 cells, and clusters contained fewer than 50 cells. Colonies were scored as being granulocytic, monocytic, or mixed.

Metabolic Experiments. Phagocytosis of 1.1- μm -diameter latex particles or sheep erythrocytes coated with 7S immunoglobulin G from rabbit anti-serum to sheep erythrocytes was studied as described previously (13, 16, 20). Metabolism of glucose to CO₂ was measured by standard methods (12). For studies of protein synthesis, macrophages were incubated with 5 μCi of [³⁵S]methionine/ml in methionine-free DME, and the amount of cell-associated isotope that was precipitable in 5% trichloroacetic acid was determined.

Enzyme Assays. Lysozyme was assayed with *Micrococcus lysodeikticus* cell walls as substrate (21) and compared with egg white lysozyme standards.

Collagenase was measured with reconstituted ¹⁴C-labeled guinea pig skin collagen fibrils as substrate (16). Samples of conditioned medium were activated before assay by treatment with chloromethyl ketone-treated trypsin (TPCK)-trypsin (10 $\mu\text{g}/\text{ml}$, 30 min at 25°C), followed by addition of a fivefold excess of STI (18). 1 U of collagenase activity hydrolyzed 1 μg of collagen fibrils/min at 37°C.

Elastase was assayed quantitatively by measuring release of peptides from [³H]NaBH₄-reduced elastin in the presence of the hydrophobic ligand, sodium dodecyl sulfate (SDS) (17). For routine assays, tubes contained 200 μg of elastin and 50 μg of SDS in 100 mM Tris-HCl buffer, pH 7.6,

¹ Abbreviations used in this paper: ATDS-P, dog serum that has been acid-treated and plasminogen-depleted; CFU-C, colony-forming units from bone marrow stem cells; DME, Dulbecco's modified Eagle's medium; DME-LH, DME supplemented with 0.2% lactalbumin hydrolysate; FCS, fetal calf serum; K_{1/2}, half-maximum concentration; SDS, sodium dodecyl sulfate; STI, soybean trypsin inhibitor; TPCK, chloromethyl ketone-treated trypsin.

containing 5 mM CaCl₂, and 25–200 μ l of conditioned culture medium in a final volume of 300 μ l. The release of labeled peptides by elastases was linear to \approx 40% of the total available radioactive elastin determined by complete hydrolysis with porcine pancreatic elastase. Assays were incubated for 4–24 h as required. 1 U of elastase hydrolyzed 1 μ g of elastin/h at 37°C. Elastase was also determined qualitatively by radial diffusion of conditioned medium samples in elastin-SDS-agarose plates (17, 22).

Plasminogen activator was measured in several ways. Cells were grown on plates coated with ¹²⁵I-labeled fibrin, and the release of labeled peptides was monitored (17). Plasminogen activator was also measured in conditioned medium and cell lysates by the fibrin plate assay (17), by direct observation of conversion of ¹²⁵I-plasminogen to plasmin heavy and light chains (9), and by single-cell assays (13). 1 U of plasminogen activator solubilized 5% of the available ¹²⁵I-fibrin/h in the presence of plasminogen (\approx 1 μ g/h at 37°C).

For assay of nonspecific neutral proteinases, conditioned culture medium was incubated on ¹²⁵I-labeled fibrin-coated plates in 50 mM Tris-HCl buffer, pH 7.6, without plasminogen, and the release of radioactive peptides was measured. 1 U of proteolytic activity solubilized 1 μ g of ¹²⁵I-fibrin/h. Protein was determined by the method of Lowry et al. (23).

Unless indicated otherwise, all results are shown as mean \pm SD for determinations on three replicate cultures.

Materials. Steroids were purchased from Sigma Chemical Co., St. Louis, Mo., Steraloids, Inc., Pawling, N. Y., or Calbiochem, San Diego, Calif. Cortisone was recrystallized before use. Tissue culture media and supplements were obtained from Grand Island Biological Co., Grand Island, N. Y. Multi-well tissue culture dishes (Costar) were purchased from Microbiological Associates, Walkersville, Md. Elastin from bovine ligamentum nuchae was obtained from Elastin Products, Inc., St. Louis, Mo. Latex particles were purchased from Dow Diagnostics, Inc., Indianapolis, Ind. Anti-serum to sheep erythrocytes was purchased from Cordis Laboratories Inc., Miami, Fla. TPK-trypsin was purchased from Worthington Biochemical Corp., Freehold, N. J. *M. lysodeikticus*, egg white lysozyme, and bovine fibrinogen were purchased from Sigma Chemical Co. Brewer thioglycollate medium was purchased from Difco Laboratories, Detroit, Mich. [³H]NaBH₄ was obtained from New England Nuclear, Boston, Mass. Na¹²⁵I and [³⁵S]methionine were purchased from Amersham Corp., Arlington Heights, Ill. All other reagents were obtained from standard sources.

Results

General Considerations. In lymphocytes, one of the earliest and most striking effects of glucocorticoids is a change in cellular metabolism; glucose uptake is depressed, ATP economy is affected, and lactate production is decreased (24). During culture of macrophages with glucocorticoids, the cells acidified their culture medium to a reduced extent as a function of glucocorticoid dose. This striking effect was observed in all macrophages (human, mouse, rabbit, guinea pig) cultured at densities high enough to produce a visible pH change in the medium, as monitored by phenol red indicator. The biochemical nature of the inhibition of the pH decrease has not been determined, but this inhibition alone could not account for the decrease in proteinase secretion that occurred. When macrophages are treated with cytochalasin B (3 μ M), glucose uptake decreases and the cells fail to acidify culture medium, but secretion of proteinases is not inhibited (13, 25). A small, but consistent decrease in CO₂ production (15–20%) was detected when stimulated mouse macrophages were treated with 10–1,000 nM dexamethasone.

Even after 72 h of treatment, glucocorticoid concentrations of 1,000 nM or less were not toxic to macrophages; this contrasts with the lytic effects of glucocorticoids on lymphocytes (1, 26, 27). Overall protein synthesis measured by incorporation of [³⁵S]methionine was not affected by glucocorticoids (0.1–1,000

nM dexamethasone), although specific changes in the patterns of secreted and cellular proteins were seen (data not shown). Phagocytosis of either latex particles or IgG-coated sheep erythrocytes was not affected by treatment of mouse macrophages with up to 1,000 nM dexamethasone, as observed previously *in vitro* (13, 28, 29).

Effects of Glucocorticoids on Secretion of Neutral Proteinases by Stimulated Mouse Macrophages

Macrophages elicited *in vivo* by various inflammatory stimuli secrete neutral proteinases and some other macromolecules at a rate 3–100 times that of unstimulated macrophages (16, 20, 22, 30). Lysozyme, a constitutive secretory product of macrophages and a sensitive indicator of macrophage viability (21), is regulated by a different mechanism than elastase and other proteinases, and its secretion was not affected by glucocorticoids. Thioglycollate-elicited mouse peritoneal macrophages secreted lysozyme at the same rate in controls and cultures treated with dexamethasone (Fig. 1).

GLUCOCORTICOID INHIBIT SECRETION OF NEUTRAL PROTEINASES. In contrast to the observations with lysozyme, secretion of elastase, collagenase, and plasminogen activator was inhibited by addition of glucocorticoids to macrophage cultures. Dexamethasone inhibited secretion of elastase by thioglycollate-elicited macrophages (Fig. 1). The inhibition of elastase secretion into the conditioned culture medium did not result in accumulation of the enzyme within the cells; elastase was present in very low quantities in lysates of both control (22) and steroid-treated cells. The decrease in elastase activity could not be attributed to an increased synthesis of an inhibitor of elastase activity in response to dexamethasone; inhibitor could not be detected in mixing experiments with partially purified macrophage elastase and medium from inhibited cultures, and treatment of the steroid-treated cells or conditioned medium with trypsin (10 μ g/ml, 30 min at 25°C) did not activate a latent form of the elastase. However, these data do not preclude the possibility that an inactive precursor failed to be secreted or converted to active enzyme as a result of steroid interaction with the cells.

TIME COURSE OF GLUCOCORTICOID INHIBITION. The binding of dexamethasone to macrophages is complete within 30 min at 37°C (14). Steroid effects on plasminogen activator production were not detected in the first 2 h of treatment with 1 nM dexamethasone, but began to be seen at 2 h with 100 nM (Table I). The inhibitory effect was both time and dose dependent. The inhibition was more pronounced at 20 h of treatment than at 5 h. Similarly, inhibition of elastase secretion was more pronounced at 12 h than at 6 h (Table I).

DIFFERENTIAL EFFECTS OF GLUCOCORTICOID ON ELASTASE AND PLASMINOGEN ACTIVATOR. Although the secretion of both elastase and plasminogen activator is increased after macrophage stimulation, the two enzymes are regulated independently, as shown by differential effects of colchicine (13, 25). One striking difference was evident when the inhibitory effects of dexamethasone on the secretion of plasminogen activator and elastase were compared (Fig. 2). The concentrations of dexamethasone giving half-maximum inhibition of the secretion of these two enzymes were similar (about 1 nM), but the maximum

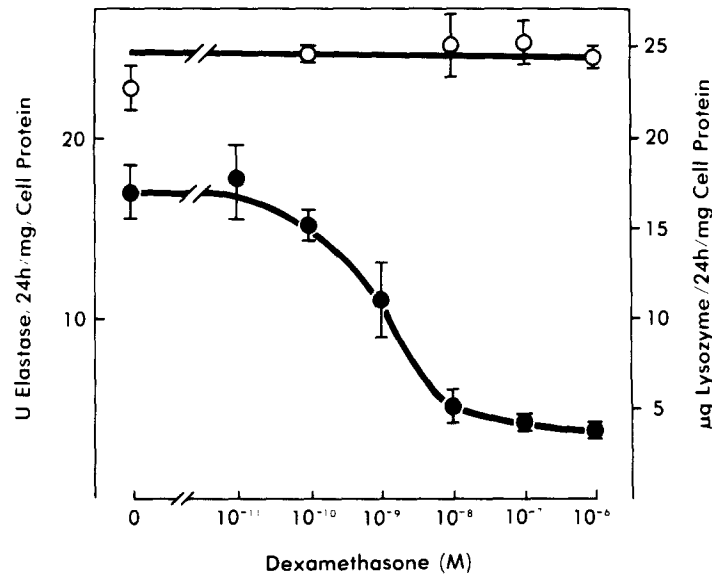


FIG. 1. Comparison of the effects of dexamethasone on the secretion of lysozyme and elastase by thioglycollate-elicited mouse peritoneal macrophages. Macrophages ($0.8 \times 10^6/2\text{-cm}^2$ well; $169 \mu\text{g}$ of cell protein/well) were incubated for 48 h in DME-LH containing various concentrations of dexamethasone. Lysozyme (○) and elastase (●) activities in the conditioned culture media were determined. Results are shown as mean \pm SD ($n = 3$).

TABLE I
Time Course of Dexamethasone Inhibition of Plasminogen Activator and Elastase Secretion by Thioglycollate-Elicited Macrophages

Enzyme	Dexamethasone pretreatment time	Enzyme secreted after dexamethasone treatment	
		1 nM	100 nM
	<i>h</i>	% control	
Plasminogen activator*	0	98	81
	5	68	32
	20	51	8
Elastase‡	6	91	64
	12	71	46

* For plasminogen activator experiments, macrophages (2×10^5) were plated on ^{125}I -fibrin-coated plates for 24 h, then placed in DME + 5% ATDS-P containing $60 \mu\text{g}$ of STI/ml and dexamethasone for the pretreatment time indicated. The cultures were then washed and placed in DME + 5% ATDS-P + 1 nM dog plasminogen with the appropriate dexamethasone concentration. The ^{125}I -fibrin hydrolysis was expressed as percentage of plasminogen-dependent degradation by control cultures. Control cultures secreted 43 U of plasminogen activator/ 10^6 cells/20 h.

‡ For elastase experiments, thioglycollate-elicited macrophages ($2 \times 10^6/35\text{-mm-diameter}$ plate) were placed in 1 ml of DME-LH containing the appropriate concentration of dexamethasone for 6 or 12 h. Elastase secreted into the medium was expressed as percentage of elastase secreted by cultures without dexamethasone. Values are the means of 3 determinations. Control cultures secreted 2.8 U of elastase in 6 h and 6.8 U in 12 h.

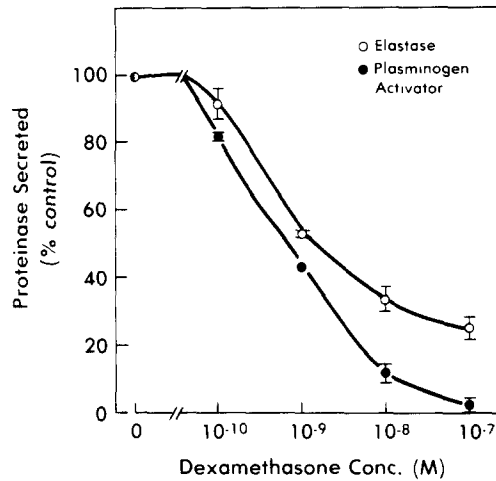


FIG. 2. Comparison of effects of dexamethasone on elastase and plasminogen activator secretion by thioglycollate-elicited mouse macrophages. Macrophages ($2 \times 10^6/35$ -mm-diameter well) were cultured in DME + 10% FCS for 48 h, washed 4 times in DME, then cultured in DME-LH in the presence of various concentrations of dexamethasone for 48 h. The enzyme activities in the conditioned medium were measured. Macrophages cultured without dexamethasone secreted 12.6 ± 1.2 U of elastase and 117 ± 18 U of plasminogen activator in 48 h. Values represent the mean \pm SD ($n = 3$); elastase (\circ); plasminogen activator (\bullet). Conc., concentration.

inhibition of plasminogen activator secretion (97–100%) was greater than that for elastase secretion (65–80%). Inhibition of elastase secretion was not complete, even after 120 h in the presence of $1 \mu\text{M}$ dexamethasone. Although it is possible that the elastase activity was due to the presence of two enzymes, one of which was glucocorticoid sensitive and the other insensitive, this is unlikely. The residual 20–35% of elastase secreted during steroid treatment had the same inhibitor profile as enzyme secreted by control cells. This modulation, rather than total inhibition or induction of cellular proteins, is characteristic of the effects of glucocorticoids in many other systems (1, 6, 24).

GLUCOCORTICOID INHIBITION OF MACROPHAGE SECRETION IS REVERSIBLE. After treatment with dexamethasone for 24 h, the secretion of elastase was restored to at least 80% of control values during the next 24 h. For plasminogen activator, reversal of the inhibitory effect of 10 nM dexamethasone was detected within 2 h after removal of the drug by assay on fibrin plates, by single-cell assays with a casein overlay in the presence of plasminogen, and by conversion of ^{125}I -plasminogen to ^{125}I -plasmin.

INHIBITION OF MACROPHAGE SECRETION OF PROTEINASE IS A SPECIFIC EFFECT OF GLUCOCORTICOID. Only glucocorticoids inhibited secretion of proteinases by macrophages. Dexamethasone, cortisol, and corticosterone inhibited secretion of elastase and collagenase, whereas the anti-glucocorticoids (cortexolone and progesterone), a glucocorticoid analogue (11-epicortisol), and the sex steroids had no inhibitory effects (Table II).

PROGESTERONE ANTAGONIZES THE GLUCOCORTICOID INHIBITION OF MACROPHAGE SECRETION. Progesterone has no glucocorticoid activity, although it competes

TABLE II
*Effects of Various Steroids on Secretion of Elastase and Collagenase by Thioglycollate-Elicited Mouse Macrophages**

Steroid	Concentration	Elastase secreted	Collagenase secreted
	<i>nM</i>	<i>U</i>	<i>mU</i>
None	—	18.3 ± 3.6	119 ± 31
Dexamethasone	10	n.d.	59 ± 7
	1,000	4.1 ± 0.2	0
Cortisol	100	12.2 ± 0.2	77 ± 35
	1,000	8.5 ± 0.7	22 ± 19
Corticosterone	100	9.6 ± 0.1	46 ± 23
	1,000	5.9 ± 0.7	0
Cortexolone	100	15.4 ± 2.0	n.d.
	1,000	12.9 ± 0.2	n.d.
Progesterone	1,000	19.7 ± 1.3	96 ± 26
Cortisone	1,000	n.d.	104 ± 42
11-Epicortisol	100	21.0 ± 4.5	n.d.
Dihydrotestosterone	200	16.3 ± 1.1	n.d.
Estradiol	500	20.2 ± 0.9	127 ± 41

* Macrophages (3.2×10^6) were plated in DME + 10% FCS for 32 h, washed 4 times with DME, then placed in 1.2 ml of DME-LH with steroids for 66 h. Collagenase was assayed after media were dialyzed and lyophilized, reconstituted at 0.1 of original volume, and then activated with trypsin (n.d., not determined). Values are shown as mean ± SD (n = 3).

with glucocorticoids for receptor binding (14). Alone, progesterone had no effect on elastase secretion; in combination with either dexamethasone or cortisol, however, progesterone blocked the glucocorticoid-mediated inhibition of elastase secretion (Fig. 3).

The Effect of Serum on Secretion of Plasminogen Activator and Elastase. Secretion of both plasminogen activator and elastase by thioglycollate-elicited mouse macrophages was detected in the presence of 5–10% ATDS-P. Serum contains endogenous cortisol and progesterone, but there was no significant deviation in dose responsiveness of secretion between dexamethasone with serum and dexamethasone without serum. Therefore, the steroids in dilute serum contributed little to the modulation of macrophage secretion.

Effects of Glucocorticoids on Mouse Monocytic Precursor Cells and on Unstimulated Peritoneal Macrophages

DEXAMETHASONE INHIBITS CELL DIVISION DURING FORMATION OF MONOCYTIC AND GRANULOCYTIC COLONIES FROM MOUSE BONE MARROW PRECURSOR CELLS IN VITRO. The anti-inflammatory effect of glucocorticoids is reflected by a decrease in mononuclear phagocytes in inflammatory exudates, and by a severe monocytopenia (1–6); it has been suggested that monocyte production is diminished by an inhibition of promonocyte division (5). Although glucocorticoid receptors have been studied only in monocytes and in later stages of mononuclear phagocyte differentiation (14), the earliest accessible stage of macrophage differentiation that can be studied in vitro is the formation of monocytic and granulocytic colonies from committed bone marrow precursor cells.

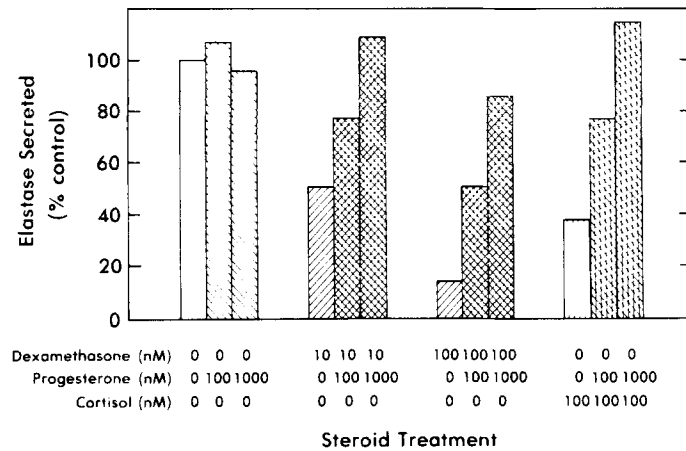


FIG. 3. Antagonism by progesterone of the glucocorticoid inhibition of elastase secretion by thioglycollate-elicited mouse macrophages. Macrophages (2×10^6) were cultured for 24 h in DME + 10% FCS, washed 4 times in DME, then placed in DME-LH containing dexamethasone, progesterone, or cortisol at various concentrations for 48 h; elastase activity of the conditioned media was then measured. Control macrophages incubated in DME-LH alone secreted 16.3 ± 2.7 U of elastase in 48 h. Values are means of three replicate cultures.

Dexamethasone reduced the number of CFU-C that formed in vitro (Table III). After 7 days of incubation, 50% inhibition of CFU-C was seen at 20 nM dexamethasone, and very few colonies were found at 100 nM or higher concentrations. However, many clusters containing fewer than 50 cells were seen at several concentrations of the steroid, suggesting that the decreased number of colonies was due to a diminution in the rate of cell division, rather than to the lysis of the precursor cells. By 14 days of culture, a maximum of 50% inhibition of CFU-C formation was observed at the highest dexamethasone concentration (1,000 nM). The cultures with 1,000 nM dexamethasone contained more clusters of cells than control cultures, suggesting that the number of colonies in the treated cultures would approach the control level after further incubation. Both monocytic and granulocytic colonies were seen in control and dexamethasone-treated cultures, and the glucocorticoid decreased the two populations proportionately (Table III). Because cultures of bone marrow cells were heterogeneous, it could not be determined if the glucocorticoids acted on the stem cells directly or on accessory cells required for differentiation and colony formation in vitro.

GLUCOCORTICOID INHIBIT SECRETION BY RESIDENT MOUSE PERITONEAL MACROPHAGES. Unstimulated macrophages secrete very small quantities of plasminogen activator, collagenase, or elastase (16, 20, 22, 30); their secretion of lysozyme is comparable to that of stimulated macrophages (21) (1×10^6 macrophages secreted $2.2 \mu\text{g}$ of lysozyme, 1.2 U of elastase, and 4.3 U of plasminogen activator per 24 h). The ability of steroids to modulate these basal levels of secretion was examined next. Extracellular secretion of elastase and plasminogen activator by resident macrophages was inhibited 79–97% by glucocorticoids (100 nM dexamethasone, triamcinolone acetonide, and cortisol)

TABLE III
*Effect of Dexamethasone on Monocytic and Granulocytic Colony (CFU-C) Formation in Vitro**

Dexamethasone	Incubation time	CFU-C type		Total CFU-C (per 5×10^4 cells)	Cell clusters (per 5×10^4 cells)
		Monocytic	Granulocytic		
<i>nM</i>	<i>days</i>	%			
0	7	9 ± 2	62 ± 5	54 ± 7	19
	14	8 ± 2	65 ± 6	107 ± 17	22 ± 2
0.1	7	16 ± 5	60 ± 3	52 ± 6	19 ± 2
	14	9 ± 1	61 ± 6	112 ± 13	28 ± 6
1.0	7	12 ± 2	63 ± 6	56 ± 6	24 ± 3
	14	10 ± 2	61 ± 5	108 ± 7	27 ± 4
10	7	11 ± 3	74 ± 9	37 ± 8	44 ± 2
	14	7 ± 2	73 ± 3	90 ± 15	41 ± 4
100	7	8 ± 4	92 ± 4	5 ± 1	20 ± 2
	14	10 ± 3	74 ± 2	71 ± 9	48 ± 10
1,000	7	0	100	1 ± 1	14 ± 8
	14	13 ± 5	73 ± 3	54 ± 9	43 ± 9

* Mean ± SE (3 experiments, each in duplicate). CFU-C were designated as monocytic, granulocytic, or mixed. Percentage of mixed CFC-C is not shown. Colonies and clusters were classified as described in Materials and Methods.

but not by other steroids (100–1,000 nM cortisone, progesterone, cortexolone, dihydrotestosterone, 11-epicortisol), whereas lysozyme secretion was not affected by any of the steroids. The concentration of dexamethasone giving half-maximum inhibition of elastase secretion (2 nM) (Fig. 4) was the same as the concentration that gave half-maximum saturation of the high affinity glucocorticoid receptor in these cells (2.1 nM) (14). Cortisol was less effective as an inhibitor, reflecting its lower binding affinity for the receptor (14). Cortisone, which does not bind to receptors, reproducibly showed a small inhibitory effect on elastase secretion, but it is possible that macrophages convert cortisone to cortisol at a low rate. These results were qualitatively similar to those obtained with stimulated macrophages.

Effects of Glucocorticoids on Mononuclear Phagocytes from Other Species

Significant species differences exist in the responses of cells to glucocorticoids (1, 3) and in secretion of proteinases. Accordingly, macrophages from three other species were examined to study the general applicability of the results of glucocorticoid treatment of mouse cells. Human, rabbit, and guinea pig macrophages all contained specific receptors for glucocorticoids (14).

HUMAN BLOOD MONOCYTES. Monocytes cultured on ^{125}I -fibrin layers catalyze plasminogen-dependent fibrinolysis at low rates (30). The production of plasminogen activator was inhibited by addition of dexamethasone to the cultures. As shown in Table IV, at 20 h of treatment the release of ^{125}I -labeled peptides was reduced to 42% of control by 1 nM dexamethasone, and to <10% of control by 100 nM; estradiol, dihydrotestosterone, and progesterone had no effect on production of plasminogen activator. This pattern of steroid effects correlates with the observed specificity and avidity of glucocorticoid binding to monocytes (14).

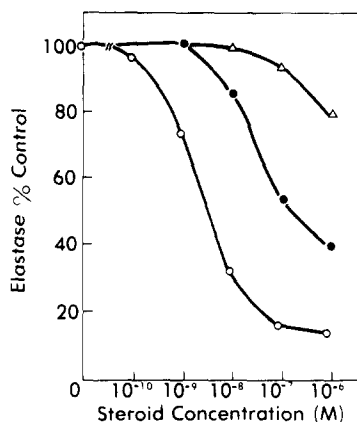


FIG. 4. Dose dependence of glucocorticoid inhibition on elastase secretion by resident mouse peritoneal macrophages. Macrophages ($6 \times 10^5/2\text{-cm}^2$ well) were incubated with the steroids in DME-LH for 48 h, and then elastase activity in the conditioned media was determined. Control cells secreted 288 mU of elastase in 48 h. Wells received either dexamethasone (○), cortisol (●), or cortisone (△). Values are means of three wells.

TABLE IV
Effect of Various Steroids on Production of Plasminogen Activator by Human Monocytes

Added steroid	Concentration	¹²⁵ I-fibrin degradation*
		% control
None	—	100
Dexamethasone	1	41.3
	100	8.1
Estradiol	100	109.9
Dihydrotestosterone	100	94.8
Progesterone	100	105.2

* Monocytes ($\approx 5 \times 10^5$) were cultured on ¹²⁵I-fibrin layers in DME containing 5% ATDS-P with the steroid. The plasminogen-dependent fibrinolysis was measured after 20 h. Values are shown as mean of 3 replicate cultures. Control cultures lysed 17.2% of the available ¹²⁵I-fibrin (≈ 1 U of plasminogen activator).

RABBIT ALVEOLAR MACROPHAGES. Macrophages from the granulomatous lungs of rabbits secreted several proteolytic enzymes that hydrolyzed collagen, fibrin, and elastin, and activated plasminogen at neutral pH. Two key features of dexamethasone inhibition of collagenase, fibrinolytic proteinase, elastase (Fig. 5), and plasminogen activator became apparent. Although the half-maximum concentration (K_d) for dexamethasone binding to the alveolar macrophages was 1–3 nM, the concentrations of dexamethasone producing 50% inhibition of proteinase secretion were as low as 0.05–0.1 nM, and the effects were seen at as little as 0.01 nM. Secondly, the four distinct proteolytic activities had different responses to the inhibitory effects of dexamethasone. The elastase (Fig. 5A) was inhibited to a lesser extent than the collagenase, neutral proteinase (Fig. 5B), and plasminogen activator (data not shown), even at 1

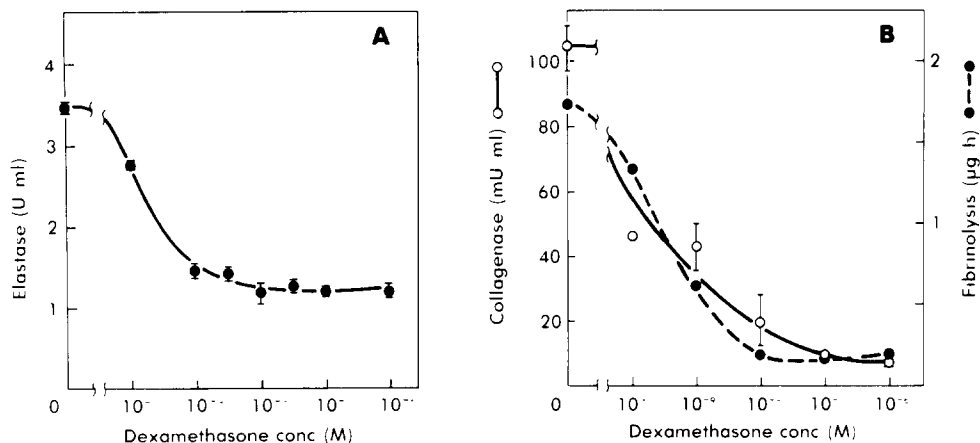


FIG. 5. Effect of dexamethasone on secretion of proteinases by adjuvant-elicited rabbit alveolar macrophages. Macrophages were plated at 1.4×10^6 cells/2-cm² well. (A) Secretion of elastase was studied by incubating macrophages in DME-LH containing dexamethasone for 96 h; conditioned culture media were then assayed for activity with ³H-labeled elastin-SDS as substrate. Values are shown as mean \pm SD ($n = 3$). (B) Secretion of collagenase and fibrinolytic neutral proteinase was examined by performing enzyme assays on conditioned culture media after 48 h incubation with dexamethasone. Values are shown as mean \pm SD ($n = 3$). Collagenase activity (\circ - - - \circ) was determined with radioactive collagen fibrils as substrate. Neutral proteinase activity (\bullet - - - \bullet) was determined with ¹²⁵I-labeled fibrin as substrate. Conc., concentration.

μ M dexamethasone. Similarly, with mouse exudative macrophages, dexamethasone inhibited plasminogen activator secretion to a greater degree than elastase (Fig. 3).

GUINEA PIG MACROPHAGES. An elastase-like proteinase was easily measured in the conditioned medium of guinea pig lung macrophages. Exposure of these macrophages to dexamethasone (10 nM) decreased production of this proteinase to 57% of control values. These inhibitory effects contrast with the relative insensitivity of guinea pig lymphocytes to the catabolic effects of glucocorticoids (1).

Discussion

Glucocorticoids are allosteric effectors which regulate differentiation and modulate physiological responses. In macrophages and other target cells, the binding of the glucocorticoid to its "cytosol" receptor induces an "activated" state in the steroid receptor, which then acquires affinity for chromosomal sites (14, 31, 32). The steroid-receptor interaction with the genome triggers the biological response characteristic of the target tissue. Although the receptor proteins are present at relatively low intracellular concentrations ($\approx 5,000$ molecules per macrophage [14]), the behavior of the receptors can be followed selectively because of their high specific affinity ($K_d = 1$ nM) for the tritium-labeled hormone. The selectivity of the biological response may occur because productive gene activation is achieved only when receptor molecules occupy multiple sites in the same genetic region (32). In the macrophage, genetic loci regulated by glucocorticoids have not been identified.

In this paper I have explored the action of glucocorticoids on key physiological functions of mononuclear phagocytes in culture. The most accessible effect of glucocorticoids on macrophage functions are inhibitory or catabolic, but glucocorticoids can be anabolic as well (31). In general, the molecular mechanisms for the catabolic effects of glucocorticoids are not as well understood as are the anabolic or inductive effects. Glucocorticoids did not produce measurable changes in overall RNA synthesis of macrophages, although specific effects probably occurred. Overall protein synthesis by mouse macrophages was not affected by glucocorticoids, but some new cellular and secreted proteins appeared, and other proteins decreased with dexamethasone treatment (unpublished data). Low concentrations of dexamethasone (0.4–40 nM) induce angiotensin-converting enzyme in rabbit alveolar macrophages (33).

Steroid-mediated effects occurred at all stages of macrophage development tested, from the differentiation of committed bone marrow stem cells into monocytes to the metabolic and secretory activity of mature macrophages responding to inflammatory stimuli. To assess the general significance of the results described in this paper it is important to distinguish effects of glucocorticoids that are responses of eukaryotic cells in culture from those that are specific to macrophages. First, the proliferation of many cell types is regulated by glucocorticoids (26, 27, 31); however, macrophage differentiation shows some specificity of response both *in vitro* and *in vivo*. The rate of formation of macrophage colonies from bone marrow stem cells was inhibited by dexamethasone. *In vivo* the number of circulating monocytes decreases after steroid treatment (4, 5). Low concentrations of glucocorticoids also inhibit granulopoiesis as shown in this study and previously (34, 35), but potentiate erythropoiesis (36). Second, plasminogen activators are produced by many cells, including monocytes and macrophages (13, 18, 20, 30, 37). Inhibition of plasminogen activator synthesis and secretion by glucocorticoids at physiological concentrations is not a specific response of macrophages, but a common feature of the regulation of this enzyme in many cell types (13, 38, 39). Third, collagenase secretion by macrophages and by synovial cells, fibroblasts, and skin explants is inhibited by low concentrations of dexamethasone and other corticosteroids (18, 40, 41), which probably accounts for the slower rate of collagen degradation in granulomas *in vivo* in the presence of these steroids (42). Thus, the regulation of collagenase production by glucocorticoids is not unique to macrophages. Fourth, secretion of macrophage elastase was inhibited by glucocorticoids. Because secretion of macrophage elastase appears to be a specific property of the mononuclear phagocyte series (unpublished data) its inhibition by glucocorticoids is a specific response of the macrophage. Finally, angiotensin-converting enzyme is induced in macrophages by glucocorticoids (31). This enzyme is produced by a number of cell types and its regulation by glucocorticoids in these cells has not been established. Although few of these responses to glucocorticoids are specific to macrophages, taken together, the spectrum of responsive proteins provides a distinctive profile or domain for the macrophage target cell.

The inhibitory effects on proteinase secretion described could be either direct effects of glucocorticoids on genetic loci that control synthesis and processing of these proteins by macrophages, or indirect actions on loci that determine

effector molecules, which then regulate proteinase secretion by macrophages. Prostaglandins, which are mediators of inflammation, are secreted by stimulated macrophages (43-45), and may modulate secretion of collagenase by guinea pig macrophages (43). In various cell types, production of prostaglandins is inhibited by glucocorticoids (41, 46), which block the release of arachidonic acid from phospholipids (47). It is possible that prostaglandins mediate some of the effects of steroids on macrophages; however, it is unlikely that they account for all of the effects of glucocorticoids. Additions of exogenous prostaglandins or arachidonic acid do not overcome the inhibition by dexamethasone, and indomethacin, an inhibitor of prostaglandin synthetase, does not have the pleomorphic effects of glucocorticoids on physiological functions of macrophages (unpublished data). Other regulatory molecules that influence macrophage functions may also be mediators of glucocorticoid action. Lymphokines (44, 48), cyclic nucleotides (13), or proteinases secreted by the macrophages (48, 49) may mediate the inhibitory effects of glucocorticoids. Further investigation is necessary to assess the relative contribution of these factors to the glucocorticoid responsiveness of macrophages.

Because products of other cells, particularly lymphocytes, may modulate macrophage functions such as secretion (1, 8), it is important to establish whether or not glucocorticoids act directly on mononuclear phagocytes. Although macrophage populations are often considered homogeneous, the primary cultures are contaminated to varying degrees by small numbers of mesothelial cells, fibroblasts, and adherent lymphocytes (50, 51). *In vivo*, lymphocytes may be glucocorticoid target cells for functions expressed by macrophages (1, 8). In the overtly heterogeneous populations of bone marrow, this question remains unresolved. In the more homogeneous cultures of peritoneal and alveolar macrophages, it is likely that the glucocorticoids acted directly, because populations of macrophages contaminated to varying degrees by nonadherent cells did not respond differently to the steroids. This interpretation is supported by the observation of similar inhibitory effects of glucocorticoids on proteinase secretion with a homogeneous line of macrophages (P388D1), derived from a tumor (52).

In view of the inhibitory effects of glucocorticoids on macrophages at subphysiological concentrations (e.g., Fig. 5), the degree to which glucocorticoid-sensitive functions are expressed *in vivo* in the presence of potentially inhibitory concentrations of steroids is of interest. Effective cortisol concentrations *in vivo* are modulated by the plasma-binding globulin, transcortin (53), and by progesterone, an antiglucocorticoid (31, 54). In low concentrations of serum, secretion of plasminogen activator and elastase by thioglycollate-elicited macrophages and formation of monocytic CFU-C were not affected, and the serum steroids did not interfere with the inhibitory effects of dexamethasone. Effective inhibition of collagenase secretion by 1 nM dexamethasone in 10% FCS has been reported in another cell system (41). Progesterone antagonized the inhibitory effects of exogenous glucocorticoids on proteinase secretion, as reported here, and on granulopoiesis (34). It is probable that physiological concentrations of glucocorticoids, in the presence of physiological concentrations of progesterone, may not suppress these macrophage functions effectively.

Although receptor proteins in macrophages have a binding affinity for dexamethasone of ≈ 1 nM, glucocorticoid effects were observed at concentrations as low as 0.01 nM in the present work, and other glucocorticoid responses at subphysiological concentrations have reported in macrophages (33, 34) and in other cells (40, 41). Thus, undetected specific sites of even higher affinity for glucocorticoids may exist, as proposed for estradiol receptors (55). An alternative explanation is that the specific effects are initiated when only a few hormone-receptor complexes have bound to the genome at these low concentrations. In most early studies of glucocorticoid effects on macrophages, pharmacological concentrations of steroids were used to achieve effects (4, 5, 8, 9, 11, 29, 35, 56, 57). Because analysis of steroid receptors reveals no low affinity receptors specific for glucocorticoids (14), many of these effects are probably not receptor-mediated.

Products of macrophage secretion are regulated by diverse mechanisms. Lysozyme, a product that is secreted by macrophages in all states of stimulation (21), was not affected by glucocorticoids. Plasminogen activator, collagenase, and elastase are apparently secreted coordinately after macrophage stimulation in vivo and in vitro (13, 16, 20-22) but are, in fact, regulated by independent mechanisms. Plasminogen activator secretion is inhibited by colchicine (13) and prostaglandins (unpublished data), whereas elastase and collagenase secretion is stimulated by these drugs (25, 43, and unpublished data). Although secretion of these three proteinases was inhibited by glucocorticoids, differential sensitivities were observed. In general, plasminogen activator secretion was inhibited to a greater extent (>95%) than was elastase secretion (60-80%). These data support classification of these proteinases into separate regulatory groups sharing a few common pathways. Macrophages have been shown to secrete several products in addition to lysozyme and the neutral proteinases. Secretion of endogenous pyrogen (57) and lysosomal hydrolases (58) may also be inhibited by corticosteroids. The pathways for secretion of some complement components and proteinases have some similar regulatory sequences (59), and it is possible that complement proteins are also modulated by glucocorticoids. Macrophages secrete both proteinases and proteinase inhibitors, e.g., α_2 -macroglobulin (60). The regulation of α_2 -macroglobulin production is not established, but it is unlikely that changes in its secretion by glucocorticoids could produce the observed inhibition of proteinase secretion.

Some metabolic functions were affected by glucocorticoids in the mononuclear phagocytes tested from four species (human, mouse, rabbit, guinea pig); it is not clear, however, whether the spectrum of macrophage response is similar in all species and in all tissues. Although the concentration of glucocorticoid receptors decreased with increasing state of macrophage differentiation or activation (14), monocytes, resident macrophages, and exudative macrophages were all sensitive to glucocorticoids. Similar observations in lymphocytes suggest that increases in glucocorticoid receptor concentrations do not increase steroid sensitivity (61). Mononuclear phagocytes treated with glucocorticoids phenotypically appear to be less differentiated or stimulated than untreated macrophages in their secretion of proteinases and their metabolism. Further studies with glucocorticoids as probes may allow us to define the pathways of macrophage differentiation.

Summary

The effects of glucocorticoids on biochemical functions of macrophages from man, mouse, rabbit, and guinea pig were examined. Secretion of plasminogen activator by human peripheral blood monocytes was decreased by 50% with 1 nM dexamethasone. Differentiation of murine monocytic and granulocytic colonies in agar from bone marrow precursors was decreased by 50% at 7 days with 20 nM dexamethasone. Secretion of elastase, collagenase, and plasminogen activator by resident and thioglycollate-elicited mouse peritoneal macrophages was decreased by dexamethasone, cortisol, and triamcinolone acetonide (1-1,000 nM), but not by progesterone, estradiol, and dihydrotestosterone (1,000 nM); in contrast, secretion of lysozyme was not affected by glucocorticoids or other steroids. The inhibition of macrophage secretion by dexamethasone was both time and dose dependent. Effects were detected within 1-6 h after addition of the glucocorticoids, became maximum by 24 h, and were reversed during a similar time period after removal of the hormones. The extent of inhibition of macrophage secretion increased with increasing glucocorticoid concentration.

Half-maximum inhibition of secretion of elastase, collagenase, and plasminogen activator was seen at dexamethasone concentrations (1-10 nM) similar to those that half-saturated the specific glucocorticoid receptors in these cells. At high concentrations of dexamethasone (100-1,000 nM) the secretion of plasminogen activator was inhibited to a greater extent (>95%) than the secretion of elastase (60-80%). Progesterone alone had no effect on secretion, but it blocked the inhibitory effects of dexamethasone and cortisol. Secretion of collagenase, neutral proteinases, and plasminogen activator by elicited rabbit alveolar macrophages was inhibited with glucocorticoids (0.1-100 nM) but not with progesterone or sex steroids. Secretion of a neutral elastinolytic proteinase by guinea pig alveolar macrophages was also inhibited by dexamethasone. These data support the regulatory role of glucocorticoids on macrophage functions at physiological concentrations.

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