

Effect of steroids on immunoglobulin-induced eosinophil degranulation

Hirohito Kita, MD,* ** Randa Abu-Ghazaleh, MD, PhD,*
Colin J. Sanderson, PhD,** and Gerald J. Gleich, MD* **

Rochester, Minn., and London, England

Because glucocorticoids are a mainstay in the treatment of asthma and other allergic diseases, we tested the effect of various steroid hormones on secretory IgA- and IgG-induced eosinophil degranulation in vitro. Human normodense eosinophils were purified by discontinuous Percoll density gradient, and hypodense eosinophils were obtained by culture of normodense cells with recombinant human interleukin (rIL)-5. Eosinophils were incubated with various steroids, including dexamethasone, hydrocortisone, methylprednisolone, estradiol, or dihydrotestosterone at concentrations from 10^{-9} to 10^{-4} mol/L. Sepharose 4B beads coupled to ovalbumin, secretory IgA, or IgG were added as targets of degranulation and incubated at 37° C for 4 hours. In some experiments, rIL-5 was added to eosinophils before addition of beads to activate the cells. The release of eosinophil-derived neurotoxin was measured by radioimmunoassay as an index of degranulation. Dexamethasone (10^{-9} to 10^{-4} mol/L), hydrocortisone (10^{-9} to 10^{-4} mol/L), estradiol (10^{-9} to 10^{-7} mol/L), and dihydrotestosterone (10^{-9} to 10^{-4} mol/L) had no effect on normodense eosinophil degranulation. Methylprednisolone, 10^{-5} mol/L, inhibited degranulation of normodense eosinophils up to 20%, whereas 10^{-4} mol/L inhibited degranulation of hypodense eosinophils, up to 30%. Overall, no difference in inhibition by steroids was observed between normodense and hypodense eosinophils. rIL-5 enhanced immunoglobulin-induced eosinophil degranulation, but this effect of rIL-5 was not blocked by any of the steroids tested. These results suggest that eosinophil degranulation and rIL-5-mediated eosinophil activation are not direct targets of glucocorticoids and that the beneficial effects of glucocorticoids on allergic inflammation in vivo are not likely due to direct effects on eosinophil degranulation. (J ALLERGY CLIN IMMUNOL 1991;87:70-7.)

Eosinophils are known to play a prominent role in many inflammatory and allergic diseases through the release of cationic toxins into tissues,¹ as demonstrated by deposition of eosinophil granule proteins in damaged areas histologically^{2,3} and by measurement of increased concentrations of MBP and Charcot-Leyden crystal protein in sputa of patients with asthma.^{4,5}

Glucocorticoids are steroid hormones with potent anti-inflammatory effects.⁶ For this reason they are used extensively in the treatment of allergic and in-

Abbreviations used

MBP:	Major basic protein
DCS:	Defined calf serum
EDN:	Eosinophil-derived neurotoxin
HPLC:	High-performance liquid chromatography
HSA:	Human serum albumin
OVA:	Ovalbumin
PIPES:	1,4-piperazinediethanesulfonic acid
RIA:	Radioimmunoassay
rIL-5:	Recombinant interleukin-5
sIgA:	Secretory IgA

From the *Department of Immunology, the **Allergic Diseases Research Laboratory, Mayo Clinic and Mayo Foundation, Rochester, Minn., and ***National Institute for Medical Research, Mill Hill, London, England.

Supported by National Institutes of Health Grants AI 15231 and AI 09728, and from the Mayo Foundation.

Received for publication Feb. 20, 1990.

Revised Sept. 11, 1990.

Accepted for publication Sept. 11, 1990.

Reprint requests: Gerald J. Gleich, MD, Department of Immunology, Mayo Clinic, Rochester, MN 55905.

1/1/25466

flammatory disorders.⁷ The targets and the mechanisms of action of their beneficial effects in these allergic diseases are still obscure. Eosinophils possess glucocorticoid receptors⁸; in vivo or in vitro treatment of eosinophils with glucocorticoids causes reduced chemotaxis and adherence.^{9,10} Furthermore, glucocorticoids inhibit antibody-dependent cellular cytotoxicity in rat eosinophils.¹¹ However, some studies

TABLE I. Eosinophil degranulation induced by protein-coated beads

Beads coated with	EDN released	
	(ng/5 × 10 ⁵ eosinophils)	% Of total EDN released
OVA	40.2 ± 3.8 (18)	2.5 ± 0.2 (18)
sIgA	263.3 ± 33.2 (18)*	16.9 ± 1.5 (18)*
IgG	98.1 ± 13.3 (16)*	5.8 ± 0.6 (16)*

Eosinophils from 10 different subjects were tested. Results are presented as mean ± SEM. Purified normodense eosinophils were incubated with medium for 2 to 6 hours, and the target beads were added. Incubation was continued for 4 additional hours at 37° C and 5% CO₂. Total EDN was determined by lysis with Nonidet P-40. All beads were coated with 5 mg/ml of the respective proteins.

*Significantly more EDN was released by incubation with sIgA- or IgG-coated beads than with OVA-coated beads ($p < 0.05$). Numbers in parentheses indicate duplicate experiments.

have used doses of glucocorticoids that likely far exceed the physiologic concentrations of free-plasma cortisol in humans.^{12, 13}

We have reported that IgG, IgA, and especially sIgA, cause the eosinophil to release the granule-associated EDN.¹⁴ In this study, we examined the effect of a variety of steroid hormones on immunoglobulin-induced eosinophil degranulation.

MATERIAL AND METHODS

Steroids and cytokines

Dexamethasone (No. D1756), hydrocortisone (No. H4001), 6 α -methylprednisolone (No. M0639), β -estradiol (No. E8875), and 5 β -dihydrotestosterone (No. E5396) were purchased from Sigma Chemical Co. (St. Louis, Mo). Steroids were dissolved in absolute ethanol at a concentration of 4×10^{-3} mol/L and diluted to the desired concentration in Hybri-Care medium (American Type Culture Collection, Rockville, Md.). Hybri-Care medium containing 10% (vol/vol) ethanol and its tenfold sequential dilutions were used as solvent controls. The final concentration of ethanol in 1×10^{-4} to 1×10^{-9} mol/L steroids was 2.5% to $2.5 \times 10^{-5}\%$. Analyses of dexamethasone, hydrocortisone, and methylprednisolone by HPLC demonstrated that these compounds eluted similarly to authentic standards and that their concentrations compared favorably to concentrations expected on the basis of weight. Dexamethasone was also tested in vivo and induced eosinopenia in mice. Human rIL-5 was purified from supernatants of cultured COS cells transfected with the expression vector pcEXV-3 encoding the human gene for IL-5. rIL-5 was purified by hydrophobic interaction and ion exchange HPLC.¹⁵ The activity of rIL-5 was measured by the human eosinophil colony-forming assay.¹⁶

Preparation of protein-coated Sepharose beads

Immunoglobulins coupled to cyanogen bromide-activated Sepharose 4B beads (Pharmacia Diagnostics, Piscataway, N.J.) were used as primary stimuli for degranulation. The quantities of proteins bound to beads are expressed as milligrams of protein per 1 ml of packed beads. Purified serum IgG was purchased from Organon Teknika-Cappel (Mal-

vern, Pa.). sIgA from human milk was purchased from Accurate Chemical and Scientific Corp. (Westbury, N.Y.). The beads were coupled to sIgA, serum IgG, and OVA (Sigma Chemical Co.) at 5 mg/ml of packed beads. The coupling procedure was described previously.¹⁴ Briefly, beads were swollen in 1 mmol/L of HCl and washed with borate buffer (0.2 mol/L of H₃BO₃, 0.5 mol/L of NaCl, and 0.02 mol/L of NaOH, pH 8.6). Proteins, dissolved in borate buffer, were added to beads and mixed overnight at 4° C. The beads were washed in borate buffer, blocked with 0.1 mol/L of lysine monohydrochloride, and finally washed alternately in borate and 0.1 mol/L of acetate buffer, pH 4.0. The beads were stored at 4° C in borate buffer until use.

Eosinophil separation

Heparinized (10 U/ml) venous blood was obtained from normal volunteers and a patient with episodic angioedema associated with eosinophilia.¹⁷ The blood was mixed with 6% Gentran 70 (dextran) in 0.9% NaCl (Travenol Laboratories, Deerfield, Ill.) at a 5:1 (vol/vol) ratio and sedimented for 60 minutes at 37° C. The buffy coat was collected and washed three times in PIPES buffer (25 mmol/L of PIPES, 5 mmol/L of KCl, 25 mmol/L of NaOH, 5.4 mmol/L of glucose, and 1% DCS [Hyclone Laboratories, Logan, Utah], which was heat inactivated at 56° C for 30 minutes). The cells were suspended in 1.5 ml of Percoll (Pharmacia Diagnostics) density, 1.070 mg/ml, with 5% DCS, and overlaid on discontinuous Percoll density gradient consisting of the following densities: 1.120, 1.100, 1.090, 1.085, and 1.080. DNAase, 1 mg/ml (Sigma Chemical Co.), was added to each of the fractions. The osmolarity of Percoll ranged from 315 to 290 mOsm/kg, and the pH was 7.4. The gradient was centrifuged at 1500 *g* in a JA-20 fixed angle rotor on a Beckman J2-21 centrifuge (Beckman Instruments, Inc., Palo Alto, Calif.) at 4° C for 45 minutes. One milliliter fractions were collected. Eosinophil density was measured as described previously.^{14, 18} Eosinophils from normal individuals were collected from fractions with density >1.095. In some experiments, eosinophils from a patient with episodic angioedema and eosinophilia¹⁷ were collected from fractions with densities 1.099 to 1.095 mg/ml, 1.093 to 1.087 mg/ml, and 1.085 to 1.080 mg/ml. These eosinophils were termed normodense, interme-

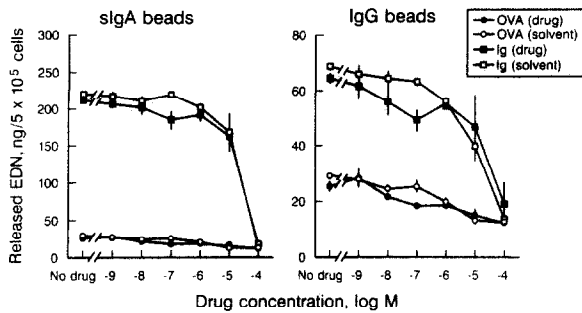


FIG. 1. The effects of dexamethasone on immunoglobulin-induced eosinophil degranulation. Purified normodense eosinophils were incubated with dexamethasone or solvent for 1 hour, and Sepharose beads coated with OVA, sIgA, or serum IgG were added, followed by incubation for an additional 4 hours. Points represent mean $\pm$ SEM of EDN release (nanograms per 5.0×10^5 of eosinophils) of duplicates from a representative experiment.

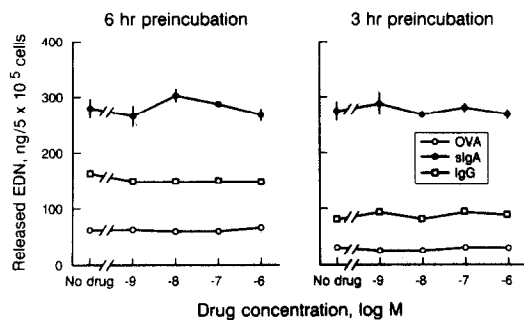


FIG. 2. The effects of dexamethasone on immunoglobulin-induced eosinophil degranulation. Fresh normodense eosinophils were incubated with dexamethasone for 3 or 6 hours before addition of beads. EDN release is described as in the legend to Fig. 1.

diate-dense, and hypodense fractions, respectively. Eosinophils were washed four times in PIPES buffer and counted with Randolph's stain.¹⁴ The cells were cultured immediately or stored overnight in Hybri-Care medium with 10% DCS at 4° C for degranulation experiments on the next day. The purity of the cells used was >85%. The viability of eosinophils was >95% after overnight incubation as measured by fluorescein diacetate.¹⁹ The protocol for eosinophil procurement was approved by the Mayo Institutional Review Board.

Production of hypodense eosinophils by culture with rIL-5

Freshly isolated eosinophils were resuspended at a concentration of 5.0×10^5 cells per milliliter in Hybri-Care medium containing gentamicin, 50 μ g/ml and 0.1% HSA (Sigma Chemical Co.), 10% (vol/vol) of DCS, and rIL-5, 50 U/ml, to generate hypodense cells, as described by Rothenberg et al.²⁰ Twenty milliliters of this suspension was seeded into a 75 cm² culture flask. At 48-hour intervals the

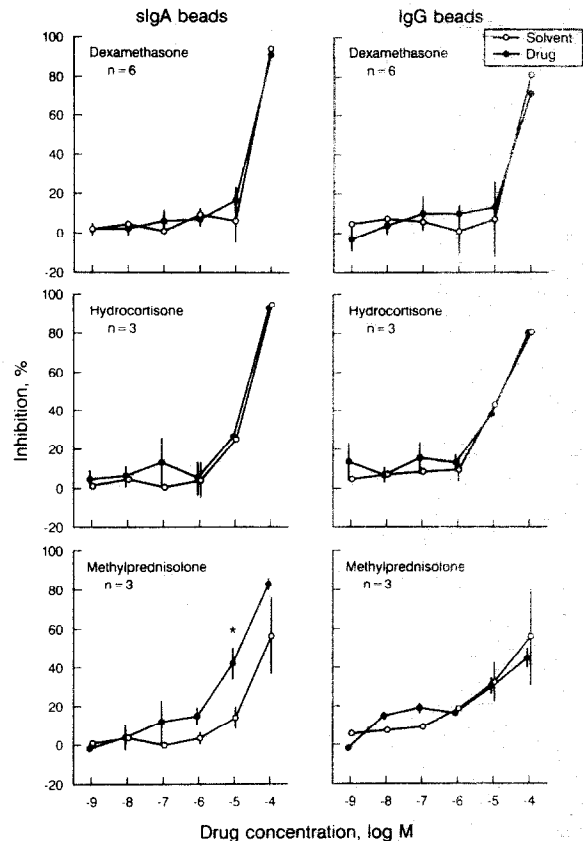


FIG. 3. The effects of glucocorticoids on immunoglobulin-induced eosinophil degranulation. Inhibition of degranulation (percent) is presented as mean $\pm$ SEM of three to six separate experiments from eight normal individuals. Each experiment was done in duplicate. Normodense eosinophils were incubated with various steroids or solvent for 2 to 6 hours, Sepharose beads coated with sIgA or serum IgG were added, and the tubes were incubated for an additional 4 hours at 37° C and 5% CO₂. Significant inhibition (different from the solvent control) was observed with sIgA beads in the presence of methylprednisolone at a concentration of 10^{-5} mol/L (* $p < 0.05$).

culture medium containing the suspension of eosinophils was aspirated and centrifuged at 200 g for 10 minutes at room temperature in 50 ml conical tubes (Falcon Plastics, Becton Dickinson Labware, Lincoln Park, N.J.). The eosinophils were suspended in 20 ml of fresh medium and added back to the original culture flask. The yield of eosinophils was 83% and 87% in two experiments. The purity and viability of eosinophils after 7 days of culture was 100% and >80%, respectively. The median density of eosinophils before and after culture was 1.100 and 1.087, respectively.

Eosinophil degranulation

The degranulation procedure was slightly modified from an earlier study.¹⁴ Purified or cultured eosinophils were washed three times with Hybri-Care medium containing gentamicin and HSA in the absence of DCS. The eosinophils were resuspended in this medium at 1.0×10^6 cell per

milliliter. One hundred microliters of cell suspension was incubated with 50 μ l of various steroids for 2, 3, 6, or 12 hours in a 96-well, round-bottom, tissue-culture plates (Costar Corp., Cambridge, Mass.) in a humidified incubator at 37° C and 5% CO₂, after which 50 μ l of protein-coated beads, suspended in Hybri-Care medium, was added to the cell suspension at a 20:1 ratio of cells to beads. In some experiments, rIL-5 was added to the cell suspension at a final concentration of 50 U/ml 1 hour before addition of beads. Subsequently, steroids, eosinophils, and beads were incubated for 4 hours in a humidified incubator at 37° C and 5% CO₂. The experiment was terminated by centrifugation of the plate at 1200 g for 10 minutes at 4° C. Supernatants from wells were collected and frozen at -20° C until they were assayed (see below). All the experiments were conducted in duplicate.

RIA of EDN

EDN concentrations in the supernatants from incubation of protein-coated Sepharose 4B beads were analyzed by RIA to quantitate eosinophil degranulation as described previously.¹⁴ To measure the total eosinophil content of EDN, eosinophils were lysed with Nonidet P-40 (Sigma Chemical Co.). The RIA is a double-antibody competition assay with radioiodinated EDN, rabbit anti-EDN antibody, and burro antirabbit IgG. Percent inhibition of eosinophil degranulation by drug or solvent was calculated as follows:

$$\% \text{ Inhibition} = \frac{WO - W}{WO} \times 100\%$$

Where WO equals the released EDN when eosinophils were incubated in the absence of drug or solvent, and W, the released EDN when eosinophils were incubated with drug or solvent.

Statistical analysis

Data were analyzed by paired or unpaired Student's *t* test and expressed as mean $\pm$ SEM.

RESULTS

The effect of dexamethasone on immunoglobulin-induced eosinophil degranulation

In the absence of glucocorticoids or solvent, EDN released by incubation with sIgA- or serum IgG-coated beads was significantly higher than with OVA-coated beads (Table I). Dexamethasone was tested for its effect on eosinophil degranulation at doses ranging from 10⁻⁹ to 10⁻⁴ mol/L. Purified normodense eosinophils were incubated with dexamethasone for 1 hour before addition of immunoglobulin-coated beads (Fig. 1). Dexamethasone, 10⁻⁹ to 10⁻⁶ mol/L, or its corresponding solvent had no effect on degranulation induced by sIgA and serum IgG beads. EDN release was inhibited by 10⁻⁵ and 10⁻⁴ mol/L of dexamethasone, but these inhibitory effects were also observed with the solvent control. To test the effect of pro-

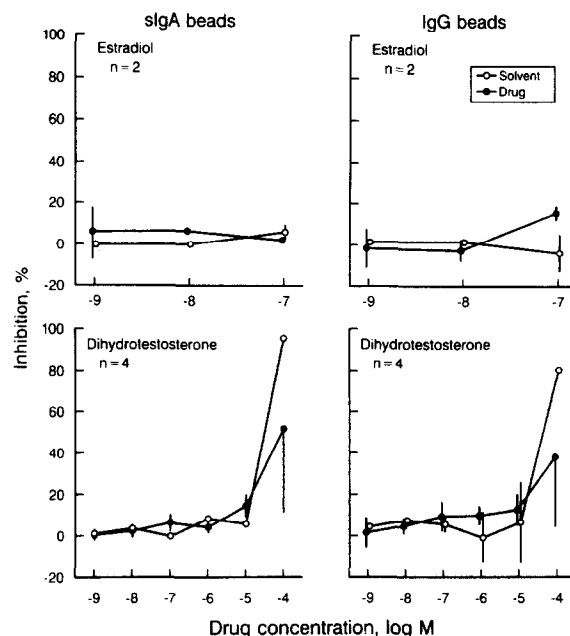


FIG. 4. The effects of estradiol and dihydrotestosterone on immunoglobulin-induced eosinophil degranulation. Fresh normodense eosinophils were incubated with estradiol, dihydrotestosterone, or solvent for 3 or 6 hours before addition of beads. Data demonstrate the percent inhibition of degranulation by drug or solvent. Points represent mean $\pm$ SEM of two to four separate duplicate experiments from five normal individuals.

longing the preincubation time with dexamethasone on immunoglobulin-induced eosinophil degranulation, purified normodense eosinophils were incubated with dexamethasone for 3 or 6 hours before addition of beads (Fig. 2). No effect of dexamethasone was observed at either preincubation period at doses ranging from 10⁻⁹ to 10⁻⁶ mol/L.

The effect of various glucocorticoids on immunoglobulin-induced eosinophil degranulation

The effects of other glucocorticoids on immunoglobulin-induced eosinophil degranulation, as well as a summary of six experiments with dexamethasone, are presented in Fig. 3. Purified normodense eosinophils were incubated with dexamethasone, hydrocortisone, or methylprednisolone for 2, 3, or 6 hours before addition of beads. Dexamethasone, hydrocortisone, and methylprednisolone in the range of 10⁻⁹ to 10⁻⁶ mol/L had no effect. The range of inhibition of sIgA-induced eosinophil degranulation by 1 $\times$ 10⁻⁶ mol/L of dexamethasone and control (Figs. 1 to 3) were -11.3% to 24.3% and 6.9% to 27.3%, respectively; the range of inhibition of

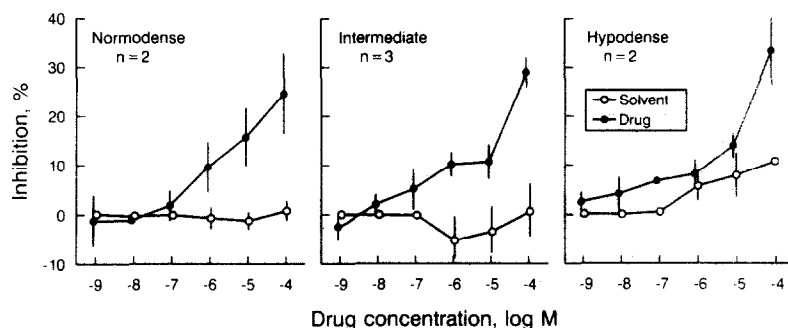


FIG. 5. The effects of methylprednisolone on immunoglobulin-induced degranulation of eosinophils from a patient with episodic angioedema associated with eosinophilia. Percent inhibition is presented as the mean $\pm$ SEM of two separate duplicate experiments. Eosinophils were separated into three subsets by density and incubated with methylprednisolone for 3 hours, Sepharose beads coated with sIgA were added, and incubation was continued for 4 additional hours at 37° C and 5% CO₂. Significant inhibition of degranulation by methylprednisolone was observed at 1×10^{-4} mol/L compared to solvent for intermediate density and hypodense eosinophils ($p < 0.05$).

sIgA-induced eosinophil degranulation by 1×10^{-6} mol/L of methylprednisolone and control were -1.2% to 8.1% and 5.6% to 19.7%, respectively. At the concentrations of 10^{-5} and 10^{-4} mol/L, dexamethasone, hydrocortisone, and the solvent control at equivalent concentrations inhibited degranulation. However, the inhibition curve of the drug and solvent did not differ significantly at any point, suggesting that dexamethasone and hydrocortisone themselves are not exerting an effect on immunoglobulin-induced eosinophil degranulation but rather that the inhibition can be attributed to the ethanol content of the drug and solvent. Among all these comparisons, only methylprednisolone, 1×10^{-5} mol/L, significantly inhibited sIgA-induced degranulation compared to solvent.

The effect of estradiol and dihydrotestosterone on immunoglobulin-induced eosinophil degranulation

Fresh normodense eosinophils were preincubated with estradiol or dihydrotestosterone for 3 or 6 hours before addition of beads (Fig. 4). Dihydrotestosterone, over the range 10^{-9} to 10^{-4} mol/L, and estradiol, over the range 10^{-9} to 10^{-7} mol/L, had no effect on immunoglobulin-induced eosinophil degranulation.

The effect on hypodense eosinophils

Because of a study indicating that eosinophil precursors from patients with eosinophilia are more sensitive to steroids than eosinophil precursors from normal subjects in terms of eosinophil colony growth,²¹ we tested the effect of glucocorticoids on degranulation of eosinophils from a patient with episodic angioedema associated with eosinophilia.¹⁷ Eosinophils

from the patient were purified and separated into three fractions by density, as described in METHODS, and the effect of methylprednisolone was tested by adding methylprednisolone 3 hours before addition of beads into incubation (Fig. 5). No significant inhibition of degranulation was observed with any of the density fractions in the range of 10^{-9} to 10^{-6} mol/L of methylprednisolone. At 1×10^{-4} mol/L of methylprednisolone, degranulation of intermediate and hypodense eosinophils was significantly inhibited, 20% compared to solvent ($p < 0.05$).

To test further the hypothesis that degranulation of hypodense eosinophils is inhibited by glucocorticoids, hypodense eosinophils were induced by culture of normodense eosinophils with rIL-5 for 7 days²⁰ and were incubated with medium, solvent control, or glucocorticoid, 1×10^{-6} mol/L, for 12 hours before addition of beads (Table II). Immunoglobulin-induced hypodense eosinophil degranulation was not inhibited significantly by dexamethasone, hydrocortisone, methylprednisolone, or dihydrotestosterone, all at 1×10^{-6} mol/L.

The effect of steroids on rIL-5 enhancement of degranulation

Preliminary experiments confirmed the finding that rIL-5 enhances immunoglobulin-mediated eosinophil degranulation.²² To investigate the effect of steroids on the rIL-5-induced activation of eosinophil degranulation, purified normodense eosinophils from normal volunteers were preincubated with various steroids at 10^{-6} mol/L for 12 hours, after which rIL-5 was added to eosinophils at a final concentration of 50 U/ml for 1 hour before addition of beads (Table III). rIL-5 significantly enhanced both sIgA-induced eosinophil

TABLE II. The effect of glucocorticoids on hypodense eosinophil degranulation

Drug	EDN released (ng/5 × 10 ⁵ of eosinophils) by beads coated with	
	OVA	sIgA
Medium	68.3 ± 0.4	181.5 ± 3.7
Solvent	64.8 ± 0.8	182.1 ± 3.3
Dexamethasone, 10 ⁻⁶ mol/L	61.7 ± 0.4	193.9 ± 8.9
Hydrocortisone, 10 ⁻⁶ mol/L	63.3 ± 0.3	191.7 ± 4.3
Methylprednisolone, 10 ⁻⁶ mol/L	62.2 ± 1.5	199.8 ± 6.9
Dihydrotestosterone, 10 ⁻⁶ mol/L	73.3 ± 2.7	183.3 ± 12.0

EDN release is presented as mean ± SEM (ng/5.0 × 10⁵ of eosinophils). Cultured hypodense eosinophils were incubated with medium, solvent, or steroid for 12 hours, OVA- and sIgA-coated beads were added, and the cells were incubated for 4 additional hours at 37° C and 5% CO₂. The total EDN content of eosinophils (lysed with Nonidet P-40) was 1234.8 ± 54.0 ng/5.0 × 10⁵ of eosinophils. This experiment was performed twice in duplicate.

TABLE III. The effect of steroids on rIL-5 action on eosinophil degranulation

Drug	EDN released (ng/4 × 10 ⁵ of eosinophils) by beads coated with			
	OVA		sIgA	
	- rIL-5	+ rIL-5	- rIL-5	+ rIL-5
Medium	13.7 ± 0.6	18.5 ± 0.1*	186.4 ± 2.3	212.0 ± 0.3*
Solvent	13.0 ± 0.9	13.3 ± 2.1	160.0 ± 5.6	208.0 ± 2.6*
Dexamethasone, 10 ⁻⁶ mol/L	15.7 ± 0.1	18.3 ± 1.6	156.8 ± 12.6	228.4 ± 7.4*
Hydrocortisone, 10 ⁻⁶ mol/L	15.4 ± 0.1	19.0 ± 0.5*	168.4 ± 9.2	222.0 ± 4.4*
Methylprednisolone, 10 ⁻⁶ mol/L	12.3 ± 0.6	17.0 ± 0.7*	147.3 ± 6.2	228.2 ± 9.7*
Dihydrotestosterone, 10 ⁻⁶ mol/L	16.4 ± 0.1	20.4 ± 0.1*	163.5 ± 7.4	190.5 ± 5.9

EDN release is presented as mean ± SEM (ng/4.0 × 10⁵ of eosinophils). Normodense eosinophils were incubated with medium, solvent, or steroid for 12 hours, after which rIL-5 (50 U/ml) or medium was added. After an additional 1-hour incubation, beads were added, and the mixture of beads, cells, and drug were incubated for 4 hours at 37° C and 5% CO₂. The total EDN content of eosinophils (lysed with Nonidet P-40) was 1003.8 ± 72.8 ng/4.0 × 10⁵ eosinophils. All comparisons were performed in duplicate.

*Significantly increased EDN release in the presence of rIL-5 compared to the absence of rIL-5 ($p < 0.05$).

degranulation and nonspecific release in the presence of OVA-coated beads. However, the enhancement was not blocked by 1 × 10⁻⁶ mol/L of dexamethasone, hydrocortisone, methylprednisolone, or dihydrotestosterone.

DISCUSSION

We evaluated the ability of steroids to alter immunoglobulin-induced eosinophil degranulation. None of the glucocorticoids tested had an effect on sIgA- and IgG-induced eosinophil degranulation at concentrations in the pharmacologic range, 10⁻⁹ to 10⁻⁶ mol/L, with either normodense or hypodense eosinophils with incubations up to 17 hours. Indeed, of the various steroids tested, only methylprednisolone inhibited sIgA-induced degranulation by 20% at a very high concentration (10⁻⁵ mol/L). Furthermore,

rIL-5 enhancement of degranulation was not blocked by any of the steroids tested.

To evaluate the ability of steroids to modify eosinophil degranulation, we used human eosinophils, protein-coated beads as targets, and the release of an eosinophil granule protein as an indicator of eosinophil degranulation. First, we examined the effect of various steroids on degranulation of normodense eosinophils because prior studies demonstrated that eosinophils from patients receiving oral glucocorticoids have reduced chemotaxis and reduced adherence in vitro.^{9,10} However, in vitro treatment of eosinophils with glucocorticoids has revealed conflicting results; hydrocortisone, at concentrations up to 10⁻⁴ to 10⁻³ mol/L, demonstrated no effect on chemotaxis in one study²³ and inhibited chemotaxis in another study.²⁴ Methylprednisolone inhibited rat eosinophil-mediated

antibody-dependent cellular cytotoxicity at 10^{-7} to 10^{-3} mol/L.¹¹ In the case of eosinophil granule protein levels in blood, no change in MBP levels, but a small and significant decrease in plasma Charcot-Leyden crystal protein levels was observed in normal volunteers receiving prednisolone.²¹ In an earlier study,²⁵ a high concentration of hydrocortisone, 10^{-5} mol/L, reduced the release of the eosinophil cationic protein from human eosinophils stimulated with serum-treated Sephadex beads. In this study, dexamethasone, hydrocortisone, and methylprednisolone had no effect on eosinophil degranulation induced by sIgA- and IgG-coated beads at pharmacologic concentrations of 10^{-9} to 10^{-6} mol/L. As illustrated in Fig. 3, methylprednisolone inhibited sIgA-induced degranulation 20% over solvent control at 10^{-5} mol/L. However, this concentration is far above the concentration of free-plasma hydrocortisone (2×10^{-9} to 2×10^{-8} mol/L)¹³ and even above high pharmacologic levels. For example, intravenous administration of 100 mg of hydrocortisone yields plasma levels up to 4×10^{-6} mol/L.²⁶ In the treatment of patients with asthma, 200 mg of hydrocortisone might be administered in a single intravenous infusion, followed by a continuous infusion of 500 mg, and finally another intravenous infusion of 200 mg a few hours later. In this situation, the plasma level of hydrocortisone transiently might be as high as 2×10^{-4} mol/L. However, in our experiments, this concentration of hydrocortisone and even dexamethasone did not inhibit sIgA- and IgG-induced degranulation. The other steroids, estradiol and dihydrotestosterone, also had no effect on degranulation at concentrations of 10^{-9} to 10^{-7} mol/L and 10^{-9} to 10^{-4} mol/L, respectively.

Second, we tested the effect of steroids on hypodense eosinophils, which are believed to be activated^{24, 27} with eosinophils separated from a patient with episodic angioedema associated with eosinophilia¹⁷ and eosinophils cultured with rIL-5 for 1 week.¹⁹ Our rationale for these experiments was the observation that eosinophil precursors from patients with eosinophilia are more susceptible to effects of glucocorticoids than precursors of eosinophils from normal subjects.²¹ In the present study we found that dexamethasone, hydrocortisone, methylprednisolone, and dihydrotestosterone had no effect on sIgA-induced degranulation by hypodense eosinophils prepared by culture of normodense eosinophils with rIL-5 and that degranulation of hypodense eosinophils from a patient with episodic angioedema associated with eosinophilia¹⁷ was inhibited only by 1×10^{-4} mol/L of methylprednisolone, a markedly elevated concentration. These results discourage belief that degranulation of hypodense eosinophils is more suscep-

tible to glucocorticoids than degranulation of normodense eosinophils.

Third, we examined the effect of steroids on the ability of rIL-5 to enhance degranulation. rIL-5 induces eosinophil differentiation and activation,^{15, 28-30} acts as a chemotactic factor for eosinophils,^{31, 32} causes normodense eosinophils to become hypodense,²⁰ and enhances immunoglobulin-induced eosinophil degranulation.²² Here, rIL-5 enhanced eosinophil degranulation induced by sIgA-coated beads and caused a slight but significant increase in EDN release from control beads. This effect of rIL-5 was not inhibited by any of the steroids, 1×10^{-6} mol/L, even with a 12-hour preincubation.

Finally, we entertained the possibility that some technical reason might explain the lack of effects by steroids, for example, the time necessary for interaction. Normal human eosinophils have steroid receptors with characteristics similar to receptors on neutrophils.⁸ In a system that uses subcutaneous infusion of dexamethasone in mice, the anti-inflammatory effect is apparent at 2 hours, reaches a maximum at 3 hours, and persists 6 hours and longer.^{33, 34} Several of the metabolic effects of glucocorticoids have a latency period of 0.5 to 2 hours,³⁵ and within this time interval, induction of gene expression for anti-inflammatory action presumably is required.³³ In one preliminary experiment, subcutaneous injection of 1 mg/kg dose of our dexamethasone preparation induced eosinopenia in mice that began 3 hours after infusion and persisted for 24 hours (data not presented). We did not observe a difference in the dose response of immunoglobulin-induced eosinophil degranulation between 3 hours and 6 hours preincubation (Fig. 2). Furthermore, the preincubation time of 2 to 12 hours should be sufficient for a glucocorticoid effect on eosinophil degranulation.

These studies lead us to conclude that eosinophil degranulation is not an important direct target of glucocorticoid action in humans. The effect of steroids in vivo could be directed at other functions of eosinophils and/or at other cells that modulate eosinophil function.

We thank Dr. Thomas C. Spelsberg, Dr. Gregory D. Wiseman, and Dr. Kristin M. Leiferman for helpful suggestions, and Ms. Linda H. Arneson for excellent secretarial assistance.

REFERENCES

1. Frigas E, Gleich GJ. The eosinophil and the pathophysiology of asthma. *J ALLERGY CLIN IMMUNOL* 1986;77:527-37.
2. Leiferman KM, Peters MS, Gleich GJ. The eosinophil and cutaneous edema. *J Am Acad Dermatol* 1986;15:513-7.
3. Harlin SL, Ansel DG, Lane SR, Myers J, Kephart GM, Gleich

- GJ. A clinical and pathologic study of chronic sinusitis: the role of the eosinophil. *J ALLERGY CLIN IMMUNOL* 1988;81:867-75.
4. Frigas E, Loegering DA, Solley GO, Farrow GM, Gleich GJ. Elevated levels of the eosinophil granule major basic protein in the sputum of patients with bronchial asthma. *Mayo Clin Proc* 1981;56:345-53.
5. Dor PJ, Ackerman SJ, Gleich GJ. Charcot-Leyden crystal protein and eosinophil granule major basic protein in sputum of patients with respiratory diseases. *Am Rev Respir Dis* 1984;130:1072-7.
6. Schleimer R, Claman HN, Oronsky AL. Anti-inflammatory steroid action: basic and clinical aspects. San Diego: Academic Press, 1989:564P.
7. Fauci AS, Dale DC, Balow JE. Glucocorticosteroid therapy: mechanisms of action and clinical considerations. *Ann Intern Med* 1976;84:304-15.
8. Peterson AP, Altman LC, Hill JS, Gosney K, Kadin ME. Glucocorticoid receptors in normal human eosinophils: comparison with neutrophils. *J ALLERGY CLIN IMMUNOL* 1981;68:212-7.
9. Clark RAF, Gallin JI, Fauci AS. Effects of in vivo prednisone on in vitro eosinophil and neutrophil adherence and chemotaxis. *Blood* 1979;53:633-41.
10. Altman LC, Hill JS, Hairfield WM, Mullarkey MF. Effects of corticosteroids on eosinophil chemotaxis and adherence. *J Clin Invest* 1981;67:28-36.
11. Hallam C, Pritchard DI, Trigg S, Eady RP. Rat eosinophil-mediated antibody-dependent cellular cytotoxicity: investigations of the mechanism of target cell lysis and inhibition by glucocorticoids. *Clin Exp Immunol* 1982;48:641-8.
12. Stevenson RD. Hydrocortisone and the migration of human leukocytes: an indirect effect mediated by mononuclear cells. *Clin Exp Immunol* 1973;14:417-26.
13. Hamaoka Y, Manabe H, Tanaka H, Monden Y, Uozumi T, Matsumoto K. Effects of surgery on plasma levels of cortisol, corticosterone, and nonprotein-bound cortisol. *Acta Endocrinol (Copenh)* 1970;64:439-51.
14. Abu-Ghazaleh RI, Fujisawa T, Mestecky R, Kyle RA, Gleich GJ. IgA-induced eosinophil degranulation. *J Immunol* 1989;142:2393-9.
15. Clutterbuck EJ, Hirst EMA, Sanderson CJ. Human interleukin-5 (IL-5) regulates the production of eosinophils in human bone marrow cultures: comparison and interaction with IL-1, IL-3, IL-6, and GM-CSF. *Blood* 1989;73:1504-12.
16. Campbell HD, Tucker WQJ, Hort Y, et al. Molecular cloning, nucleotide sequence, and expression of the gene encoding human eosinophil differentiation factor (interleukin-5). *Proc Natl Acad Sci USA* 1987;84:6629-33.
17. Gleich GJ, Schroeter AL, Marcoux JP, Sachs MI, O'Connell EJ, Kohler PF. Episodic angioedema associated with eosinophilia. *N Engl J Med* 1984;310:1621-6.
18. Fukuda T, Dunnette SL, Reed CE, Ackerman SJ, Peters MS, Gleich GJ. Increased numbers of hypodense eosinophils in the blood of patients with bronchial asthma. *Am Rev Respir Dis* 1985;132:981-5.
19. Rotman B, Papermaster BW. Membrane properties of living mammalian cells as studied by enzymatic hydrolysis of fluorogenic esters. *Proc Natl Acad Sci USA* 1966;55:134-41.
20. Rothenberg ME, Petersen J, Stevens RL, et al. IL-5-dependent conversion of normodense human eosinophils to the hypodense phenotype uses 3T3 fibroblasts for enhanced viability, accelerated hypodensity, and sustained antibody-dependent cytotoxicity. *J Immunol* 1989;143:2311-6.
21. Butterfield JH, Ackerman SJ, Weiler D, Eisenbrey AB, Gleich GJ. Effects of glucocorticoids on eosinophil colony growth. *J ALLERGY CLIN IMMUNOL* 1986;78:450-7.
22. Fujisawa T, Abu-Ghazaleh R, Kita H, Sanderson CJ, Gleich GJ. Regulatory effect of cytokines on eosinophil degranulation. *J Immunol* 1990;144:642-6.
23. Jones DG, Kay AB. Inhibition of eosinophil chemotaxis by the antagonist of slow-reacting substance of anaphylaxis—compound FPL 55712. *J Pharm Pharmacol* 1974;26:917-8.
24. Gauderer CA, Gleich GJ. Inhibition of eosinophilotaxis by chloroquine and corticosteroids. *Proc Soc Exp Biol Med* 1978;157:129-33.
25. Winqvist I, Olofsson T, Olsson I. Mechanism for eosinophil degranulation: release of eosinophil cationic protein. *Immunology* 1984;51:1-8.
26. Dwyer J, Lazarus L, Hickie JB. A study of cortisol metabolism in patients with chronic asthma. *Aust Ann Med* 1967;16:297-303.
27. Gleich GJ, Adolphson CR. The eosinophilic leukocyte: structure and function. *Adv Immunol* 1986;39:177-253.
28. Sanderson CJ, Warren DJ, Strath M. Identification of a lymphokine that stimulates eosinophil differentiation in vitro. *J Exp Med* 1985;162:60-74.
29. Yamaguchi Y, Suda T, Suda J, et al. Purified interleukin-5 supports terminal differentiation and proliferation of murine eosinophilic precursors. *J Exp Med* 1988;167:43-56.
30. Lopez AF, Sanderson CJ, Gamble JR, Campbell HD, Young IG, Vadas MA. Recombinant human interleukin-5 is a selective activator of human eosinophil function. *J Exp Med* 1988;167:219-24.
31. Yamaguchi Y, Hayashi Y, Sugama Y, et al. Highly purified murine interleukin-5 (IL-5) stimulates eosinophil function and prolongs in vitro survival. *J Exp Med* 1988;167:1737-42.
32. Wang JM, Rambaldi A, Biondi A, Chen ZG, Sanderson CJ, Mantovani A. Recombinant human interleukin-5 is a selective eosinophil chemoattractant. *Eur J Immunol* 1989;19:701-5.
33. Tsurufuji S, Sugio K, Takemasa F. The role of glucocorticoid receptor and gene expression in the anti-inflammatory action of dexamethasone. *Nature* 1979;280:408-10.
34. Church MK, Miller P. Time courses of the anti-anaphylactic and anti-inflammatory effects of dexamethasone in the rat and mouse. *Br J Pharmacol* 1978;62:481-6.
35. Feigelson M, Feigelson P. Relationships between hepatic enzyme induction, glutamate formation, and purine nucleotide biosynthesis in glucocorticoid action. *J Biol Chem* 1966;241:5819-26.