Effects of Corticosteroids on Eosinophil Chemotaxis and Adherence

LEONARD C. ALTMAN, JOHN S. HILL, WILLIAM M. HAIRFIELD, and MICHAEL F. MULLARKEY, *Division of Allergy and Infectious Diseases, Department of Medicine, University of Washington, and The Mason Clinic, Seattle, Washington 98195*

ABSTRACT Therapeutic doses of corticosteroids frequently induce eosinopenia; however, the mechanism(s) involved remain obscure. To investigate this question, we studied the effects of corticosteroids on eosinophil adherence and migration. Eosinophils from normal donors were prepared by dextran sedimentation and Hypaque gradient centrifugation to 45-96% purity. Adherence was measured by filtration of whole blood and isolated eosinophils through nylon wool columns. Before prednisone administration, adherence was 83.8±3.2% for eosinophils in heparinized blood and 82.1±3.2% for isolated eosinophils. 4 h after oral prednisone administration whole blood eosinophil adherence was reduced to 53.9±10.7%; at 24 and 48 h adherence was normal. In contrast, isolated eosinophils showed no decrease in adherence 4, 24, or 48 h after corticosteroid administration. Similarly, in vitro addition of hydrocortisone to isolated eosinophils at 0.01 and 2.0 mg/ml did not reduce adherence. Eosinophil migration was tested in modified Boyden chambers by "lower-surface" and "leading-front" methods, using zymosan-activated serum and buffered saline to assess chemotactic and random migration, respectively. In vitro incubation of eosinophils with hydrocortisone or methylprednisolone produced a dose-dependent inhibition of chemotaxis. Using lower-surface methods the minimal concentration effecting substantial inhibition was 0.01 mg/ml for both drugs. At 2.0 mg/ml hydrocortisone and methylprednisolone inhibited eosinophil chemotaxis 82.6±4.4% and 85.0±3.5%, respectively. Using leading-front chemotaxis techniques significant inhibition was detected at 0.001 mg/ml hydrocortisone. Eosinophils incubated and washed free of corticosteroids responded normally to chemoattractants, indicating that the inhibitory effect of these drugs was reversible. Hydrocortisone at 2 mg/ml inhibited random eosinophil migration, although this effect was not apparent at lower concentrations. Corticosteroids did not act as chemotactic factor inactivators and were not toxic as measured by trypan blue exclusion. Eosinophils obtained from donors who had received 40 mg of prednisone orally for four days showed normal chemotactic responses, probably reflecting the fact that the cells were washed free of plasma before testing. In contrast, incubation of eosinophils in plasma from donors who had received a 300-mg bolus of hydrocortisone induced 46.1±4.5% more inhibition of chemotaxis than did incubation in normal plasma. These results indicate that: (*a*) eosinophil adherence is transiently reduced following in vivo corticosteroid administration, (*b*) eosinophil chemotaxis is inhibited by both in vitro and in vivo administration of corticosteroids, and (*c*) the chemotaxis inhibiting effect is nontoxic, cell-directed, dose-dependent and reversible. Inhibition of eosinophil adherence and chemotaxis may in part explain how corticosteroids produce eosinopenia and decrease the local accumulation of eosinophils.

INTRODUCTION

The mechanism(s) by which corticoisteroids affect eosinophils and induce eosinopenia remain obscure. Many explanations have been proposed, including: (*a*) cessation of bone marrow release of eosinophils (1); (*b*) reduction of bone marrow eosinophil production (2); (*c*) reversible sequestration of eosinophils in extravascular locations (3); and (*d*) eosinophil destruction (4).

Alternatively, corticosteroids may decrease the local accumulation of eosinophils and promote eosinopenia by affecting the adherence and/or chemotaxis of these cells. Eosinophils, after being produced in the bone marrow, appear in the peripheral blood and eventually distribute themselves into the tissues. In humans most eosinophils reside in the extravascular space,

Address reprint requests to Dr. Altman.
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the normal tissue to blood ratio being between 300:1 (5) and 500:1 (6) For this reason, it has been suggested that changes in peripheral blood eosinophil levels, particularly increases (i.e., eosinophilia), reflect recruitment of tissue eosinophils into the circulation by chemoattractant substances. (7). It is perhaps equally possible that these attractants may facilitate the mobilization of eosinophils into the circulation from the bone marrow. Several factors that can attract eosinophils have been described in blood and tissues. These include (*a*) eosinophil chemotatic factor of anaphylaxis (8), (*b*) lymphokine chemotactic factors (including eosinophil chemotactic factor precursor and eosinophil-stimulation promoter (9, 10) (*c*) complement-derived factors (11), and (*d*) histamine that has eosinophil chemotactic activity over a narrow concentration range. (12, 13) Therefore, corticosteroids might produce eosinopenia by rendering eosinophils unresponsive to normally circulating attractants or by inhibiting the ability of these cells to adhere to endothelial surfaces. This would interfere with the normal mobilization of eosinophils into the circulation from the bone marrow or other tissues. In support of this hypothesis are studies that have shown that corticosteroids inhibit the adherence and chemotaxis of eosinophils from patients with the hypereosinophilic syndrome (14) and experiments in a patient with rheumatoid arthritis (15) and in guinea pigs that demonstrate the chemotaxis inhibiting effect of corticosteroids (16). In the present study we investigated the effects of corticosteroids on the adherence and migration of normal human eosinophils to further explore the mechanisms by which these drugs might produce eosinopenia and limit the local accumulation of eosinophils.

METHODS

Eosinophil preparation. Heparinized blood (10 U/ml) was collected from normal donors, and the eosinophils were separated by a modification of the method of Day (17). In brief, erythrocytes were removed by dextran (4.5%) sedimentation (45 min at 20°C) and were isolated by Hypaque (1.152 mg/ml) density gradient centrifugation (20 min at 0°C, 400 *g*). Preliminary studies, using various Hypaque densities, showed that optimal purity and recovery of eosinophils were achieved at a density of 1.152 mg/ml (Fig. 1). Residual erythrocytes were lysed with hypotonic saline, and the eosinophil-enriched preparations were then washed twice in sterile phosphate-buffered saline containing 0.1% gelatin. Eosinophil purity ranged from 45 to 96%. Virtually all contaminating cells were neutrophils. Eosinophil viability determined by trypan blue exclusion was 92–99%. The cells were suspended to a final concentration of 1.5×10^6 viable eosinophils per ml in Gey's balanced salt solution, pH 7.2, with 2% bovine serum albumin (Gey's).[1]

[1] *Abbreviations used in this paper*: Gey's, Gey's balanced salt solution (pH 7.0) supplemented with 2% bovine serum albumin; ZAS, zymosan-activated serum.

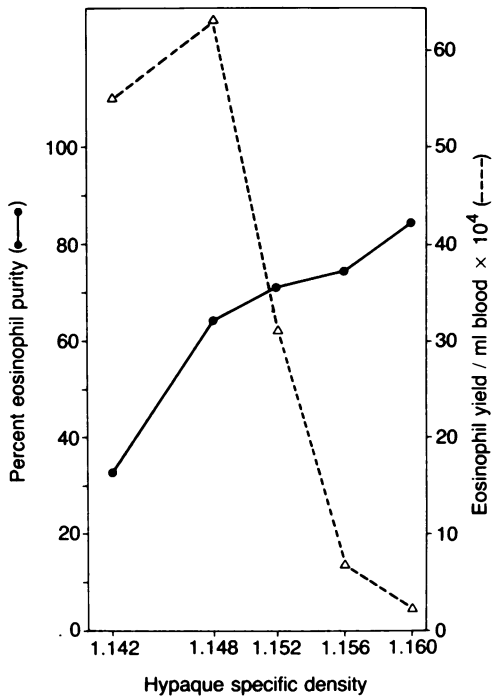

FIGURE 1 Changes in the purity and yield of eosinophils at varying specific densities of Hypaque.

Chemotaxis methods. Chemotaxis was measured in modified Boyden chambers, using micropore filters. Two variations of this technique were employed: (*a*) migration of cells through filters to the lower-surface (18) and (*b*) a leading-front method (19). In both assays 400 μl aliquots of eosinophil cell suspensions (6×10^5 eosinophils) were placed in the upper compartments of chemotaxis chambers and the attractant or control agents placed in the lower compartments. Polycarbonate filters, pore size 8 μm, (Nuclepore Corp., Pleasanton Calif.) were used to separate the two compartments in the "lower-surface" assay and nitrocellulose filters, pore size 3 μm, Sartorius Co. Inc., San Francisco, Calif.) were used in the "leading-front" method. Chemotaxis chambers were incubated for either 30 min (leading-front) or 45 min (lower-surface) at 37°C in a humid atmosphere of 95% air—5% CO_2; the filters then removed, fixed, and Wright-Giemsa stained (Diff-Quick, Harleco, American Hospital Supply Corp., Gibbstown, N. J.). Zymosan-activated serum (ZAS) was used as a chemoattractant since preliminary studies indicated that it was the most potent eosinophil attractant of four agents tested (Table I). Phosphate-buffered saline was used to measure random migration. Determinations were performed in quadruplicate. To investigate the effect of in vitro drug exposure on eosinophil chemotaxis, cells were preincubated with the agent in question for varying periods (1–120 min) before placing them in the chemotaxis chambers. With the lower-surface method, cell migration was quantified by counting the number of eosinophils that had migrated completely through the filters in 20, 5×5-μm microgrid oil-immersion (×1,000) microscopic fields. Results are expressed as the mean number of eosinophils per 20 fields ± 1 SEM. With the leading-front method, migration was quantified by counting the number of cells present in high power (×440) microscopic fields at each 10-μm interval from the starting

TABLE I
Response of Human Eosinophils to Various Chemoattractants

Chemoattractant	Chemotactic response*
33% ZAS	56.0±1.5
33% C5a	43.3±7.2
33% B-CTX	11.0±2.1
33% fmlp 10 μM	6.0±1.2
33% fmlp 1 μM	5.8±1.1
33% fmlp 0.1 μM	4.8±1.7
PBS	3.3±0.8

Abbreviations used in this table: C5a, complement-derived chemotactic factor partially purified by G-75 Sephadex chromatography; B-CTX, bacterial chemotactic factor prepared from a culture filtrate of *Escherichia coli* American type culture collection No. 25922; fmlp, formylmethionyl-leucyl-phenylalanine; PBS, phosphate-buffered saline.
* Expressed as eosinophils per 20 oil immersion microscopic fields ±1 SEM.

surface of the filter. Four fields were counted at each interval in all filters. The results are presented as the mean number of eosinophils per microscopic field ±SEM at each 10-μm interval. For leading front assays only eosinophil preparations of more than 80% purity were used as it was difficult to perform differential counts within filters. For lower-surface assays, no cell preparation contained less than 45% eosinophils and most were more than 80% pure. It has previously been reported that eosinophil chemotaxis is unaffected by neutrophil contamination between 15 and 98% (20). In addition, we found that corticosteroid-induced suppression of eosinophil chemotaxis was unaffected by neutrophil contamination between 4 and 55%.

Adherence assay. Adherence was measured by pouring 1-ml samples of heparinized blood or separated eosinophils over nylon wool columns that had been prepared previously by packing 80 μg of nylon fiber into a 0.4-ml vol of a tuberculin syringe (21). After this, the columns were incubated for 15 min at 37°C and the effluents collected. Total leukocyte and differential counts were performed before and after passing the samples over the columns. Adherence was calculated as a percentage based on the number of eosinophils in the effluents and the number in the original samples. The results are expressed as mean±SEM. All studies were performed in quadruplicate. To investigate the effect on adherence of in vitro drug exposure, eosinophils were preincubated with hydrocortisone (0.01 and 2.0 mg/ml) for 20 min before being tested.

Corticosteroid administration in vivo. The in vivo effect of corticosteroids on eosinophil function was examined in two ways: (*a*) Normal subjects were given 40 mg of prednisone orally for either one (adherence studies) or four (chemotaxis studies) consecutive days. At various times after the last dose, blood was collected, eosinophils were prepared, and the adherence and chemotaxis of the cells was then measured. (*b*) Normal volunteers were given 300-mg of hydrocortisone in 50 ml of 5% dextrose and water intravenously over a 10-min period. At 0, 30, 60, and 120 min, blood was taken and plasma samples were prepared. Separate aliquots of normal eosinophils from a different individual were then incubated for 45 min at 37°C in the pre- and posttreatment plasma samples. The chemotactic responses of the various cell aliquots were then measured and compared.

Drugs. Hydrocortisone sodium succinate and methylprednisolone sodium succinate (Upjohn Co., Kalamazoo, Mich.) were dissolved in Gey's or plasma at various concentrations, as outlined in the Results section.

Statistical methods. The data were analyzed with Student's *t* and paired *t* tests.

RESULTS

Effect of corticosteroids added in vitro on eosinophil chemotaxis using the lower-surface technique. Table II shows the inhibitory effect of hydrocortisone and methylprednisolone on eosinophil chemotaxis as measured by the lower-surface chemotaxis technique. In these experiments, 33% ZAS was used as an attractant. Both compounds produced a dose-dependent inhibition of eosinophil chemotaxis; the lowest concentration effecting substantial and consistent inhibition being 0.01 mg/ml for both drugs. At 2 mg/ml, hydrocortisone and methylprednisolone inhibited eosinophil chemotaxis by 82.6±4.4 and 85.0±3.5%, respectively. Maximal inhibition was evident after 1

TABLE II
Inhibition of Eosinophil Chemotaxis by Hydrocortisone and Methylprednisolone

Drug	Concentration	n	Chemotaxis inhibition*
	mg/ml		%
Hydrocortisone	0.001	3	12.0±2.6
	0.01	2	42.5±12.5
	0.05	3	59.6±5.9
	0.1	5	60.5±13.2
	1.0	5	84.0±3.4
	2.0	7	82.6±4.4
	2.0 (after washing)	3	11.6±5.8
Methylprednisolone	0.0001	2	6.0±1.0
	0.001	2	16.5±10.5
	0.01	3	49.3±8.3
	0.1	4	58.0±5.6
	1.0	4	78.0±5.8
	2.0	5	85.0±3.5
	2.0 (after washing)	4	7.7±4.9

Eosinophils were incubated at 37°C for 20 min with either hydrocortisone or methylprednisolone at 2 mg/ml, then washed twice in Gey's, restandardized to 1.5×10^6 eosinophils/ml and chemotaxis tested as described in the Methods section.

* Expressed as $100 - \left[\frac{\text{chemotactic response of drug treated eosinophils}}{\text{chemotactic response of untreated eosinophils}} \times 100\right]$.

min preincubation and did not increase further with preincubation as long as 120 min. When 5 and 15% ZAS was used as the attractant, hydrocortisone (2 mg/ml) produced 66.6±2.7 and 64.7±5.2% chemotaxis-inhibition, indicating that the suppression produced by this drug is seen over a range of chemoattractant concentrations. The effect of corticosteroids on random eosinophil migration could not be evaluated with the lower-surface technique because so few eosinophils migrated (mean 4.3±0.6/20 fields) that measuring corticosteroid-induced inhibition was not possible. To determine whether the inhibitory effect of corticosteroids on eosinophils was cell-directed or chemotactic-factor directed, hydrocortisone at 2 mg/ml was mixed with ZAS before addition to the chemotaxis chambers. In these studies, inhibition of chemotaxis averaged 35.0±4.0%, whereas the same concentration of hydrocortisone produced 86.0±6.0% inhibition when mixed directly with the cells. These values are significantly different ($P < 0.01$). When hydrocortisone at 2 mg/ml was added with phosphate-buffered saline to the lower compartment of the chemotaxis chamber, eosinophil migration averaged 2.3±1.2 cells/20 fields compared to 1.3±0.2 in the absence of hydrocortisone, indicating that this drug does not have chemotactic activity. These studies suggest that corticosteroids act predominantly as cell-directed inhibitors of chemotaxis, and, do not inhibit eosinophil migration by chemotactic deactivation. The partial inhibition (35.0±4.0%) of eosinophil chemotaxis observed after addition of hydrocortisone to ZAS may reflect diffusion of the drug into the upper compartment of the chemotaxtic chamber and, hence, a cell-directed effect.

To find out whether the inhibitory effect of corticosteroids was reversible, eosinophils were incubated with hydrocortisone and methylprednisone at 2 mg/ml for 20 min at 37°C, then washed twice in Gey's, after which their chemotactic response was measured (Table II). Inhibition averaged 11.6±5.8 and 7.7±4.9% in washed cell preparations compared with 82.6±4.4 and 85.0±3.5% ($P < 0.001$ for both comparisons) for cells not washed free of hydrocortisone and methylprednisolone, indicating that corticosteroid-induced inhibition of chemotaxis is reversible. Eosinophil viability after incubation with corticosteroids was 92–98%.

Effects of corticosteroids added in vitro on eosinophil chemotaxis using the leading-front technique. Figs. 2–4 show the effects of various concentrations of hydrocortisone on eosinophil chemotaxis as measured by the leading front technique. Fig. 2 shows studies in which 5% ZAS was used as an attractant, Figs. 3 and 4 illustrate experiments in which 15 and 33% ZAS was used. At 5 and 15% ZAS, significant inhibition of eosinophil migration was evident at 0.001 mg/ml hydrocortisone ($P < 0.002$ and

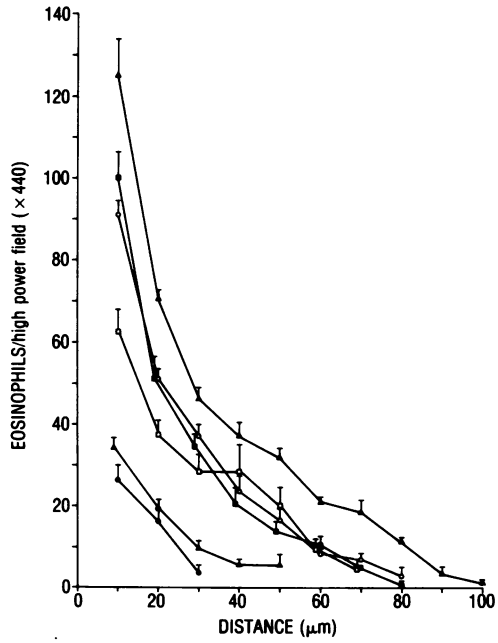

FIGURE 2 In vitro eosinophil chemotaxis to 5% ZAS measured by the leading-front technique. Cells were incubated with no steroid (▲) or with hydrocortisone (○, 0.001; ■, 0.01; □, 0.1; △, 1; ●, 2 mg/ml) at the indicated concentrations for 20 min before testing. The data are the mean±SEM of 48 determinations from three separate experiments.

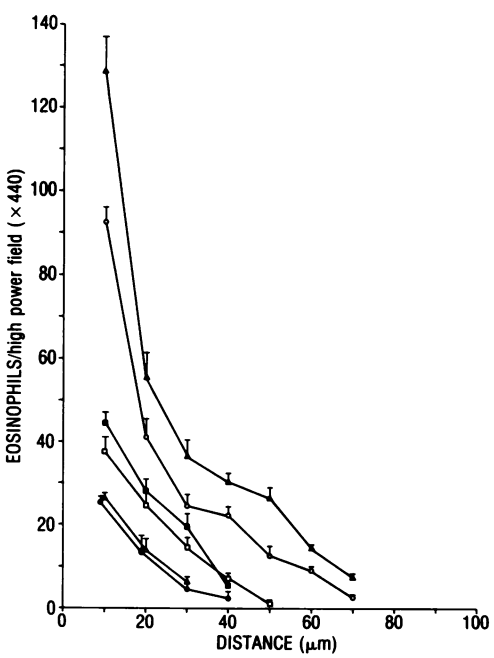

FIGURE 3 In vitro eosinophil chemotaxis to 15% ZAS measured by the leading-front technique. Cells were incubated with no steroid (▲) or with hydrocortisone (○, 0.001; ■, 0.01; □, 0.1; △, 1; ●, 2 mg/ml) at the indicated concentrations for 20 min before testing. The data are the mean±SEM of 48 determinations from three separate experiments.

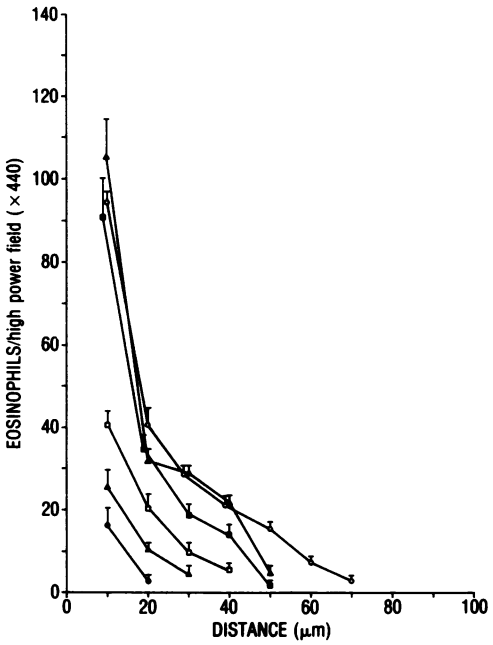

FIGURE 4 In vitro eosinophil chemotaxis to 33% ZAS measured by the leading-front technique. Cells were incubated with no steroid (▲) or with hydrocortisone (○, 0.001; ■, 0.01; □, 0.1; △, 1; ●, 2 mg/ml) at the indicated concentrations for 20 min before testing. The data are the mean±SEM of 48 determinations from three separate experiments.

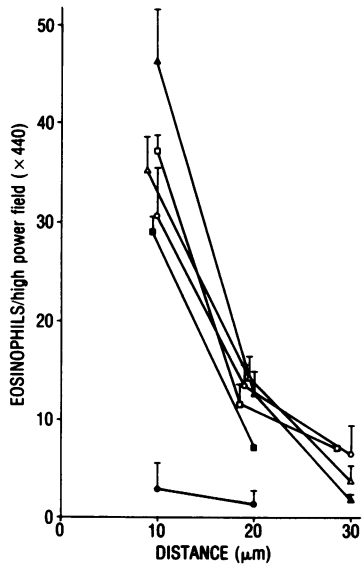

FIGURE 5 In vitro random eosinophil migration measured by the leading front technique. Cells were incubated with no steroid (▲) or with hydrocortisone (○, 0.001; ■, 0.01; □, 0.1; △, 1; ●, 2 mg/ml) at the indicated concentrations for 20 min before testing. The data are the mean±SEM of 48 determinations from three separate experiments.

0.01, respectively, paired t test), whereas substantial and consistent chemotactic inhibition did not occur at concentrations <0.1 mg/ml hydrocortisone with 33% ZAS as an attractant. Fig. 5 shows the effect of hydrocortisone on random eosinophil migration as measured by the leading-front technique. A marked reduction was evident at 2 mg/ml hydrocortisone, however, this effect was not apparent at lower concentrations.

Effects of corticosteroids added in vivo on eosinophil chemotaxis. To test the effect of in vivo corticosteroids on eosinophil chemotaxis, normal volunteers were given 40 mg of prednisone orally for four days. At 1, 3, and 5 h after the last dose blood was taken, eosinophils were prepared, and chemotaxis was tested using the lower-surface technique. Eosinophils in the 1-h samples were 4% less responsive than were cells from normal subjects not receiving corticosteroids (34.3±5.8, $n = 4$ vs. 35.8±3.6 cells/20 fields, $n = 32$). At 3 h, cells from treated subjects were 18% less responsive (29.3±3.9, $n = 4$) and chemotaxis could not be measured in the 5-h samples because of profound eosinopenia. The 1- and 3-h values are not significantly reduced. These results may reflect the fact that eosinophils were washed free of plasma during the isolation and purification procedures. Alternatively, this finding may indicate that the eosinophils remaining in the circulation for up to 3 h after prednisone administration are a subpopulation of cells that resist the chemotaxis-inhibiting effect of corticosteroids. In addition, plasma samples from patients receiving prednisone had no chemotaxis-inhibiting activity and the cortisol concentrations in these samples were normal with one exception (38.7 μg/dl in a 3-h sample, normal range 4–22).

As a second approach to evaluate the chemotaxis-inhibiting effect on eosinophil chemotaxis of corticosteroids added in vivo, four normal subjects were given 300 mg of hydrocortisone i.v. over a 10-min period. At 0, 30, 60, and 120 min blood was taken, plasma samples were prepared, and the potential for chemotaxis inhibition of the 30-, 60-, and 120-min samples was compared with that of the pretreatment samples. (Table III). The mean plasma cortisol concentrations were 14.3, 451.0, 323.0, and 200.1 μg/dl at 0, 30, 60, and 120 min, respectively. Compared with the pretreatment samples, plasma taken after hydrocortisone infusion had a marked inhibitory effect, producing a maximum inhibition of 46.1± 4.5% (60-min sample).

To further examine the inhibitory effect of hydrocortisone, this agent was added in vitro to pretreatment plasma samples and the resulting inhibitory activity compared with that of the postinfusion samples. In other words, the in vivo and in vitro chemotaxis-inhibiting effects of hydrocortisone in plasma

TABLE III

Inhibitory Effect of Hydrocortisone Containing Plasma on Eosinophil Chemotaxis

Cells incubated with:	Chemotaxis inhibition*	n
	%	
Plasma after intravenous infusion of hydrocortisone		
30-min sample	38.3±13.8	2
60-min sample	46.1±4.5	4
120-min sample	36.9±6.1	2
Normal plasma with hydrocortisone added in vitro		
0.001 mg/ml	14.9±1.2	3
0.01 mg/ml	37.5±1.3	3
0.1 mg/ml	42.5±4.3	3
1.0 mg/ml	48.2±1.9	3
2.0 mg/ml	70.2±2.5	3

* Expressed as 100

$$-\left[\frac{\text{chemotactic response of eosinophils in hydrocortisone containing plasma}}{\text{chemotactic response of eosinophils in normal plasma}} \times 100\right].$$

were compared. As shown in Table III, mixing hydrocortisone in vitro with normal plasma produced a dose-dependent inhibitory effect similar to that observed with hydrocortisone in buffer. At 2 mg/ml hydrocortisone-induced inhibition of eosinophil chemotaxis was 70.2±2.5% in plasma compared with 82.6±4.4% in buffer (Table II). Furthermore, these data indicate that hydrocortisone is more effective in inhibiting eosinophil chemotaxis in vivo than in vitro. Plasma taken after infusion of hydrocortisone, with a cortisol concentration of 0.00323 mg/ml (323 μg/dl), produced 46.1% inhibition of chemotaxis, whereas plasma with hydrocortisone added in vitro, produced 14.9% inhibition at 0.001 mg/ml and 35.7% inhibition at 0.01 mg/ml (Table III).

Adherence studies. The adherence of eosinophils in whole blood was significantly ($P < 0.001$) depressed 4 h after oral administration of 40 mg of prednisone. This effect was transient, however, as evidenced by the fact that adherence was normal at 24 and 48 h (Fig. 6). In contrast, the adherence of isolated eosinophils was unaffected by the oral administration of prednisone (Fig. 7). Similarly the adherence of isolated eosinophils was normal in the presence of hydrocortisone added in vitro at either 0.01 mg/ml or 2.0 mg/ml (Fig. 8). These findings are similar to those of Clark and co-workers (14) and are in agreement with our chemotaxis data, which also indicate that corticosteroids are more effective in inhibiting eosinophil function when administered in vivo rather than in vitro.

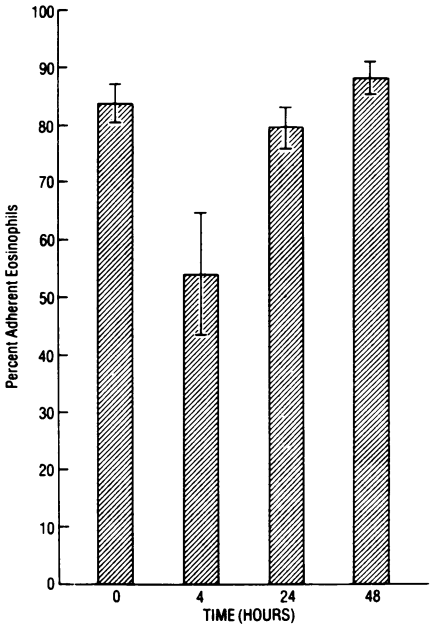

FIGURE 6 Adherence to nylon wool columns of eosinophils in peripheral blood samples taken before, 4, 24, and 48 h after in vivo administration of 40 mg of prednisone. The data are the mean±SEM of 18 determinations from six separate experiments. The 4-h value is significantly different from the 0-h value, $P < 0.001$.

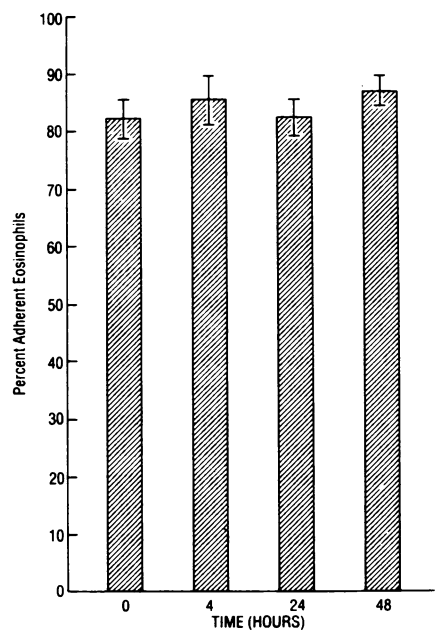

FIGURE 7 Adherence to nylon wool columns of isolated eosinophils taken before, 4, 24, and 48 h after in vivo administration of 40 mg of prednisone. The data are the mean±SEM of 18 determinations from six separate experiments.

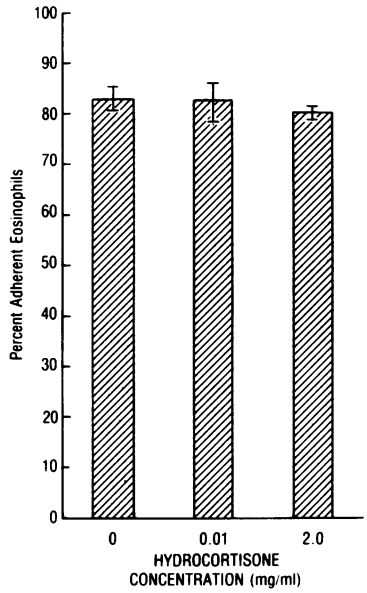

FIGURE 8 Adherence of isolated eosinophils to nylon wool columns. Cells were incubated in vitro with hydrocortisone at the stated concentrations for 20 min before being tested. The data are the mean±SEM of 16 determinations from four separate experiments.

DISCUSSION

In this study, we have shown that (*a*) corticosteroids added in vitro inhibit the chemotaxis response of normal human eosinophils; (*b*) this effect is dose-dependent, cell-directed, nontoxic, and reversible; (*c*) plasma collected after administration of therapeutic doses of corticosteroids inhibits directed eosinophil migration; and (*d*) in vivo administration of corticosteroids transiently decreases eosinophil adherence. In our in vitro studies, relatively high concentrations of hydrocortisone and methylprednisolone, 0.01 mg/ml in lower-surface assays and 0.001 mg/ml in leading-front assays, were required to induce substantial and consistent inhibition of chemotaxis. However, these levels are significantly lower than those used by previous investigators. Moreover, the experiments in which we administered corticosteroids in vivo showed that cortisol at a concentration of 0.00323 mg/ml was sufficient to inhibit eosinophil chemotaxis by 46.1%. Furthermore, our studies indicate that the adherence and chemotaxis-inhibiting effects of corticosteroids are greater when these drugs are administered in vivo rather than in vitro. The reason for this observation is uncertain, but one possibility is that corticosteroids given in vivo cause the release of a circulating mediator which can inhibit eosinophil functions. In support of this hypothesis, are data from Kownatzki et al. (22) that show that mononuclear cells can release a factor that immobilizes eosinophils. This factor is reported to affect eosinophils directly and is not a chemotactic-factor inactivator, nor does it inhibit eosinophil motility by deactivation. Alternatively, it is possible that in vivo administration of corticosteroids causes only certain eosinophils to marginate or leave the circulation so that a functional subpopulation of cells is sampled by venipuncture.

Our finding, that corticosteroids reduce whole blood eosinophil adherence 4 h after oral prednisone administration confirms the work of Clark and co-workers (14). Of interest, our data also shows that following oral prednisone administration, isolated eosinophils have normal adherence and that the adherence of eosinophils is not effected by the in vitro addition of hydrocortisone. These questions were not examined by Clark et al., however our results suggest that washing cells (in vivo administration, isolated eosinophil experiment) reverses the adherence-inhibiting effect of corticosteroids and that as noted above, corticosteroids are more effective when administered in vivo rather than in vitro. In their paper Clark et al. also reported that eosinophil chemotaxis in vitro was suppressed at 24 h after one dose of oral prednisone and that migration returned to normal by 48 h. We found no chemotaxis-inhibiting effect after oral prednisone ingestion. However, the design of the two studies was somewhat different; our subjects took 40 mg of prednisone for 4 d, and we tested the chemotaxic responsiveness of their eosinophils 1 and 3 h after the last dose. Clark et al. gave one 60 mg dose of prednisone and measured eosinophil chemotaxis at 0, 24, and 48 h. In spite of this discrepancy, both papers present evidence that corticosteroids suppress eosinophil adherence and chemotaxis. In addition to the aforementioned work, two other groups of investigators have studied the effects of corticosteroids on eosinophils, although neither used normal human cells. Gauderer and Gleich (16) demonstrated that corticosteroids inhibited the chemotaxic response of guinea pig eosinophils, whereas Goetzl et al. (15) found that hydrocortisone inhibited both the directed and random migration of eosinophils from a patient with rheumatoid arthritis. In these studies extremely high concentrations of corticosteroids (0.15 mg/ml) were required to demonstrate chemotaxis inhibition. Furthermore, Gauderer et al. (16) concluded, as we did, that the chemotaxis-inhibiting activity of corticosteroids was primarily cell-directed. However Goetzl and co-workers (15) reported that the inhibitory effect of corticosteroids on chemotaxis was not reversible, which is at variance with our conclusion. This may reflect methodologic differences or the type of cells studied because Goetzl et al. examined eosinophils from a patient with rheumatoid arthritis and we used normal cells.

Corticosteroids have been known for many years to induce peripheral eosinopenia (23) and have also been shown to reduce the local accumulation of eosinophils at sites of immediate hypersensitivity reactions as measured by skin window (24–28) and dermal biopsy (29) techniques. Nonetheless, the mechanism(s) of corticosteroid-induced eosinopenia and reduced tissue accumulation remains uncertain. Many studies indicate that eosinophils are not lysed by corticosteroids (30, 31) and there is little evidence to suggest that short-term treatment with these hormones decreases bone marrow eosinopoiesis (32, 33). There are, however, animal studies which indicate that the number of mature eosinophils in the bone marrow increases with corticosteroid administration, suggesting that these hormones delay the release of eosinophils from the marrow (1). Another postulated mechanism of corticosteroid-induced eosinopenia is reversible sequestration in extravascular locations. This hypothesis is supported by autoradiographic studies which indicate that after a brief period of steroid-induced eosinopenia, these cells return to the circulation essentially unchanged (34). Postulated sites of sequestration include the spleen (35), the lungs (30), and margination within the vascular compartment (36), although there are also contradictory studies that suggest that sequestration does not occur in either the spleen (37) or the lungs (38). Alternatively, as suggested by our data and that of others, corticosteroids may produce eosinopenia and decrease the local accumulation of eosinophils by inhibiting adherence and chemotaxis. In fact, the delay in the release of eosinophils from the bone marrow and the sequestration of these cells in the marginal pool or other tissues may result from impaired chemotaxis or adherence. In other words, corticosteroids by affecting these functions may disrupt the normal mobilization of eosinophils from these compartments into the circulation.

Finally, one must ask why patients with neutrophil chemotaxis defects often have a leukocytosis (39) while we are suggesting that impairment of eosinophil chemotaxis leads, contrastingly, to eosinopenia. Again, the answer is not known. It may be that neutrophils that normally circulate in large numbers are unable to leave the vascular space if they are chemotactically impaired, whereas eosinophils, which are present in the circulation in small numbers, are not able to enter the circulation normally if their chemotactic function is reduced.

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