THE SELECTIVE EOSINOPHIL CHEMOTACTIC ACTIVITY OF HISTAMINE

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Histamine is stored predominantly in granules of mast cells (1, 2) and basophils (3) and is released into the milieu after the degranulation of the cells in an immediate hypersensitivity reaction (4, 5). The influx of eosinophils observed in immediate hypersensitivity reactions has been associated with the release of preformed peptides designated eosinophil chemotactic factor of anaphylaxis (ECF_A) (6) which could be distinguished from histamine by both size (7) and charge (6). In vitro studies of histamine as a chemotactic factor have been limited (7–10); however, no effect upon eosinophil migration was observed. In this study we demonstrate that histamine itself is selectively chemotactic for human eosinophils between 3×10^{-7} and 1.25×10^{-6} mol/liter, while higher concentrations of histamine inhibit eosinophil migration.

Materials and Methods

Materials were obtained as follows: Diamine oxidase, atropine sulfate, and L-histidine decarboxylase, type II (Sigma Chemical Co., St. Louis, Mo.), trypsin and chymotrypsin (Worthington Biochemical Corp., Freehold, N. J.), pronase-CB grade B, L-histidine monohydrate monohydrochloride, and histamine diphosphate grade A (Calbiochem, San Diego, Calif.), Sephadex G-25, Sephadex G-100, and Ficoll (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.), Hypaque (Winthrop Laboratories, New York), *Escherichia coli* endotoxin 0111: B4 lipopolysaccharide W (Difco Lab., Detroit, Mich.), metiamide (Smith, Kline & French Laboratories, Philadelphia, Pa.), mepyramine maleate (Merck Sharp & Dohme, Inc., West Point, Pa.), 5-μm nucleopore polycarbonate filters (Neuro Probe, Inc., Bethesda, Md.), 3-μm cellulose nitrate filters (Sartorius, Göttingen, West Germany), $Na_2{}^{51}Cr\ O_4$ in 0.9% saline (New England Nuclear, Boston, Mass.), Hank's tissue culture medium pH 7.2 (National Institutes of Health Media Supply Section, Bethesda, Md.), Gey's tissue culture medium, pH 7.2, containing 2% bovine serum albumin (BSA), 2% penicillin and streptomycin (Microbiological Associates, Bethesda, Md.), triarylmethane and methyl alcohol fixative, xanthene dye, and thiazine dye (Harleco, Philadelphia, Pa.).

Isolation of Human Eosinophils. Eosinophils were obtained from four patients with hypereosinophilia of unknown etiology (11) and differing degrees (30–85%) and one patient with hypereosinophilia secondary to *Strongyloides stercoralis* infestation. Heparinized blood from these patients was diluted 1:4 in phosphate-buffered saline (PBS). 30-ml aliquots of this PBS suspension were layered on 12 ml of 10.7% Hypaque-6.3% Ficoll gradients and spun at 900 *g* for 30 min at 20°C. The serum, mononuclear cell interface, and Hypaque-Ficoll layers were aspirated with a suction and discarded. The pellets that contained neutrophils, eosinophils, and erythrocytes were pooled, suspended in PBS to the original blood volume, and sedimented for 30–45 min in 3% dextran at room temperature. After the erythrocytes had sedimented, the upper granulocyte layer was aspirated and centrifuged at 200 *g* for 10 min at 4°C. The remaining erythrocytes were lysed with 0.2% sodium chloride, agitated for 15 s by vortex, and brought to 0.9% saline by addition of an equal volume of 1.6% saline. The mixture containing only neutrophils and eosinophils was then

washed once with Gey's medium, centrifuged, and resuspended in Gey's medium to a final cell concentration of 2.3 × 10^6 cells/ml for use in the chemotactic assays. Eosinophil preparations responded to histamine in the same fashion when the cells were prepared using dextran sedimentation alone or using Hypaque-Ficoll cushions followed by dextran sedimentation. The latter procedure yielded a higher percentage of eosinophils and was therefore used routinely.

Eosinophil Chemotaxis. For most experiments a modification of the method described by Snyderman et al. (12) for monocyte chemotaxis was used to measure eosinophil chemotaxis. The cells were suspended in Gey's medium at a concentration of 2.3 × 10^6 cells/ml. A modified Boyden chamber was used with the cells in the upper chamber and the stimulus in the lower. A 5-μm pore polycarbonate filter was used to separate the two chamber compartments. The chambers were incubated at 37°C for 1 h in 100% humidity, 5% CO_2, then the filters were fixed in triarylmethane and methylalcohol and stained with a xanthene and thiazine dye mixture followed by six sequential distilled water rinses. Cells that had migrated through the filter were counted in 5–10 high-powered fields (hpf)[1] and expressed as cells/hpf. All samples were done in quadruplicate, the standard error determined, and background (buffer control) counts subtrated. When two samples were compared, the Student's *t* test was used to calculate statistical significance.

A second chemotactic method was employed using a modification of the ^{51}Cr radioassay for neutrophil chemotaxis as described by Gallin et al. (13). Eosinophils were incubated for 1 h at 37°C with 1 μC of ^{51}Cr per 10^6 cells. Only eosinophil preparations of >70% were used for labeling with ^{51}Cr. Instead of two cellulose nitrate filters being placed in the chemotactic chamber, a 5-μm pore, 12-μm thick polycarbonate filter was inserted on top of a 3-μm pore, 145-μm thick cellulose nitrate filter. This allowed the incubation time to be reduced from 3 to 1 h. The polycarbonate filters were removed and stained, as described above, for visual counting. The cellulose nitrate filters were rinsed and placed in individual gamma counter vials for isotope counting, as previously described (13). Chemotactic activity was expressed as corrected counts per minute in the lower cellulose nitrate filter (LF) according to the following formula:

$$\frac{\text{observed cpm LF} \times 10{,}000}{\text{cpm}/10^6 \text{ granulocytes}}.$$

All assays were performed in quadruplicate and the data expressed as the mean ± SEM of the four filters.

Further delineation of the effect of histamine upon eosinophil migration was performed using the method described by Zigmond and Hirsch (14). The cells were processed and suspended in Gey's tissue culture medium, as previously described. A 3-μm cellulose nitrate filter was placed in modified Boyden chambers, the cells and samples introduced, and the chambers incubated for 30–60 min at 37°C. After incubation the filters were rinsed in normal saline, fixed in methanol, and stained with Mayer's hematoxylin and eosin. The filters were dehydrated with increasing concentrations of ethanol, cleared with xylene, and mounted on glass slides with immersion oil. All samples were assayed in duplicate. The migration front was determined by measuring the micrometers from the top of one filter to the furthest distance traveled by two cells/hpf (14). Five such measurements were made for each filter. The measurements from duplicate filters were pooled and the mean and standard error determined. Such pooling of data was allowed because the variance of migration fronts within a single filter was greater than the variance among duplicate filters.[2] The means of duplicates were compared using the Student's *t* test. Cells were counted at different levels in the filters by focusing with the microscope micrometer to the desired levels. Three high powered fields per filter were counted at each level. The cell counts from duplicate filters were pooled and the mean and standard error determined. Again the variance of cells/hpf at each level within a single filter was greater than the variance among duplicate filters.[2] The means of duplicates were compared using the Student's *t* test.

Chemotactic Agents. Partially purified C5a was prepared as previously described by Gallin and Rosenthal (15). 4 ml of fresh serum were incubated with 0.3 mg/ml of *E. coli* endotoxin for 1 h at 37°C. The reaction was stopped with 15 mg of EDTA. The entire sample was placed on a

[1] *Abbreviations used in this paper:* hpf, high-powered fields; LF, lower cellulose nitrate filter; PBS, phosphate-buffered saline.

[2] Analysis of variance kindly performed by Dr. David Alling, Special Assistant for Biometry, National Institute of Allergy and Infectious Diseases.

Sephadex G-100 column and collected in PBS. Every third fraction was assayed for chemotactic activity. The activity found between 11,000–20,000 daltons was inactivated by rabbit antihuman C5, but not rabbit antihuman C3. Aliquots from this pool were diluted 1:1 with Gey's medium before testing for chemotactic activity.

Histamine was diluted in Gey's or Hank's tissue culture medium using polypropylene tubes or beakers.

Histamine Bioassay. Histamine was assayed by measuring the contraction of an atropinized guinea pig ileum (16). Chromatographically pure histamine was used as standards at concentrations of 5 and 10 ng/ml. All samples were diluted to this range with Tyrode's buffer before application to the bioassay.

Results

The Eosinophil Chemotactic Activity of Histamine. Histamine caused enhanced migration of human eosinophils, when assayed at concentrations of 10^{-5} M, 10^{-6} M, and 10^{-7} M in modified Boyden chambers with nucleopore filters. As shown in Fig. 1, the chemotactic response varied directly with the density of eosinophils used in the upper compartment of the Boyden chamber. No detectable chemotactic response to any dose of histamine was noted with mixed leukocyte populations containing less than 20% eosinophils. The chemotactic dose response of each population of eosinophils was maximum at 10^{-6} M histamine, while concentrations of histamine at 10^{-5} M or 10^{-7} M were less effective.

There was no migration of neutrophils to any dose of histamine utilized regardless of the percentage of eosinophils. The striking dependence of eosinophil chemotaxis upon the percentage of eosinophils in the final preparation might be an inhibiting effect of the contaminating neutrophils upon the eosinophil movement or might merely be a function of the absolute eosinophil count. We therefore assayed a cell preparation, containing 93% eosinophils and 7% neutrophils, with 10^{-6} M histamine and compared the resultant eosinophil migration to the eosinophil migration obtained when these cells were mixed 1:1 with a cell preparation, containing 95% neutrophils and 5% eosinophils, which had no detectable eosinophil chemotaxis. The mixture, which had 49% eosinophils, had half the response of the preparation containing 93% eosinophils. Thus an inhibitory effect of neutrophils upon eosinophil chemotaxis was not observed since the amount of migration was directly proportional to the absolute eosinophil count.

Diminution of eosinophil migration to 10^{-5} M histamine compared to 10^{-6} M histamine was a consistent finding with the preceding chemotactic technique. A possible explanation for the diminished response at higher doses of histamine was that the cells were falling off the filter. A modification of the ^{51}Cr-labeled leukocyte radioassay for chemotaxis employing two filters was designed to test this hypothesis as described in the Materials and Methods section. Fig. 2 demonstrates that the diminution of eosinophil migration to higher doses of histamine is apparent whether counting ^{51}Cr-labeled cells by counts per minute in the lower cellulose nitrate filter or morphologic inspection of the upper polycarbonate filter. Since incubation for 1 h does not allow the cells to migrate through the second filter in this system, the decrease in eosinophil migration seen at higher histamine doses cannot be attributable to cells falling off the filter.

To determine whether the enhanced migration was due to increased random

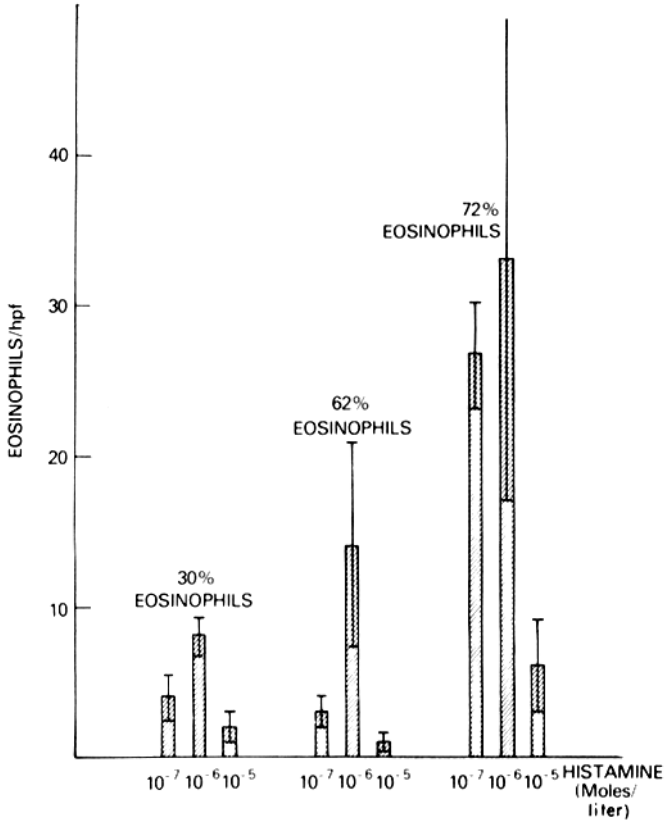

FIG. 1. Eosinophil migration to histamine using Hypaque-Ficoll purified eosinophil-neutrophil mixtures from three patients with differing degrees of eosinophilia. The assay was performed by visual inspection of polycarbonate filters using 2.3×10^6 granulocytes/ml with increasing proportions of eosinophils. The number of neutrophils migrating was never greater than background.

motility or chemotactic activity, equal concentrations of histamine were placed on each side of the Boyden chamber. Both the ^{51}Cr radioassay and the visual assay showed a response strikingly diminished from the response seen with histamine only on the stimulus side. In most experiments this response was no more than fluctuations around or slightly above background (Fig. 2). Furthermore, an experiment was performed in which the cells were stimulated by a 6×10^{-7} M histamine solution and the cells suspended either in buffer alone or buffer containing increasing concentrations of histamine. A single polycarbonate filter was used in the Boyden chamber. A dose of 3×10^{-7} M histamine in the cell side diminished the response by over 50%, while equal concentrations diminished the cell response by over 80%. Thus, a concentration gradient was necessary for maximal cell migration consistent with a chemotactic response to histamine.

Histamine-stimulated eosinophil chemotaxis was confirmed using the assay described by Zigmond and Hirsch (14). Incubation times between 30 and 60 min were chosen so that the cells did not have time to migrate across the entire

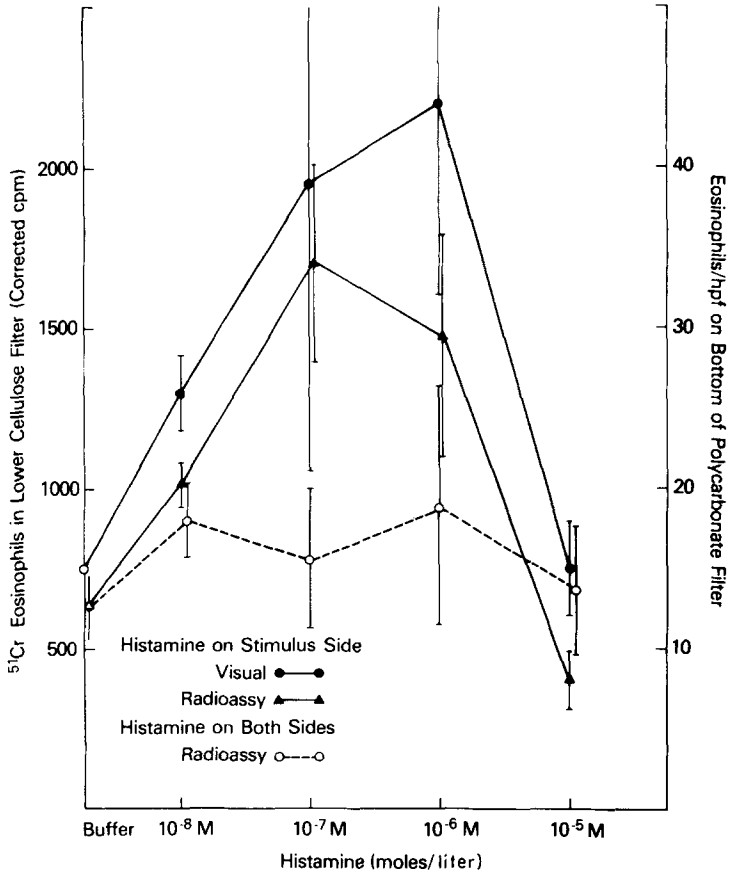

FIG. 2. Chemotactic dose response curves of eosinophils to histamine expressed in counts per minute (cpm) of ^{51}Cr-labeled cells in the lower cellulose nitrate filters and in cells/hpf of eosinophils that had migrated through to the lower surface of the upper polycarbonate filter. A flat response by the ^{51}Cr radioassay was obtained when histamine was placed on both sides of the filters. Points at each concentration were shifted slightly to demonstrate the standard error more clearly.

thickness of the filters. Fig. 3 shows eosinophils/hpf at increasing filter depths in response to different histamine concentrations. Compared to buffer alone, increased numbers of eosinophils/hpf were seen with 10^{-6} M histamine on the stimulus side at all three levels in the filter. No significant increase in eosinophil numbers over background were seen at the three filter levels with histamine on both sides of the filter regardless of the concentration. Since no consistent concentration dependent increase in random motility was observed, the phenomenon of increased eosinophil movement with 10^{-6} M histamine is defined as chemotaxis according to Zigmond and Hirsch (14). 10^{-6} M histamine on the stimulus side increased the number of eosinophils observed throughout the filter, as shown in Fig. 4, while when 10^{-6} M histamine was placed on both sides of the filter the response was not significantly different from buffer alone. When the $\log_e$ eosinophils/hpf was plotted against the square of the distance (μm^2), a nonlinear curve was obtained (Figure 5 A). This is in contrast to the

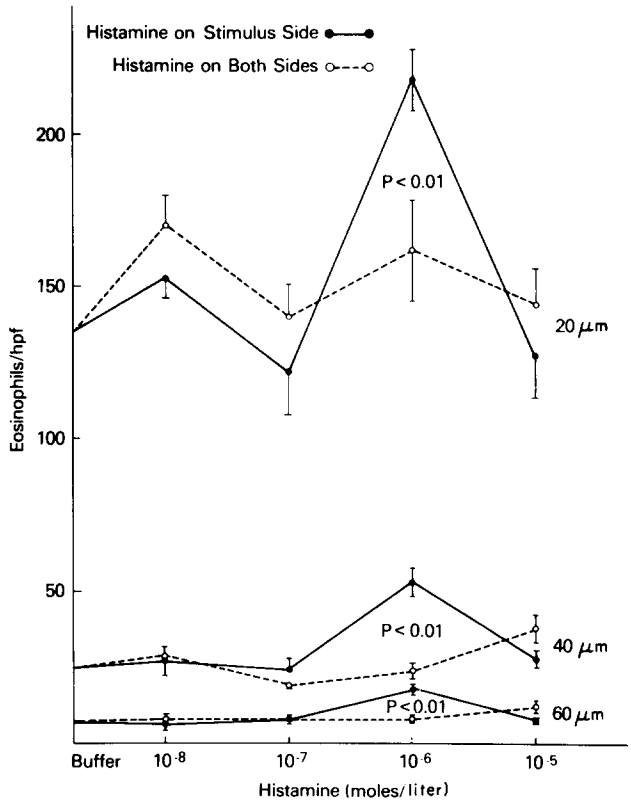

FIG. 3. Chemotactic dose response curves of eosinophils to histamine at 20-μm, 40-μm and 60-μm filter depths expressed in cells/hpf. A flat response was observed when histamine was placed on both sides of the filter. *P* values compare the number of eosinophils migrating with histamine on the stimulus side versus the number migrating with histamine on both sides of the filter.

straight line shown by Zigmond and Hirsch for the migration of horse leukocytes in buffer (14), as for human neutrophil migration in buffer alone or in response to C5a.[3] A similar nonlinear curve was derived whether the eosinophils were being attracted by histamine, randomly migrating in buffer alone, or in histamine and buffer on both sides of the filter. A plot of $\log_e \log_e$ eosinophils/hpf vs. distance squared (μm^2) gives a better approximation of a straight line (Fig. 5 B). This indicates that the eosinophils did not migrate as a homogeneous population (14).

Deactivation of Eosinophil Migration with Histamine. Most substances that are chemotactic attractants deactivate the effector cells when these cells have been preincubated with the chemotactic agent (17). Eosinophils were therefore preincubated with Gey's medium or with 6×10^{-7} M histamine in Gey's medium at 37°C for 30 min. The cells then were centrifuged at 1,000 rpm, 4°C for 5 min, and resuspended in fresh 37°C Gey's medium to their initial concentration. The standard technique for assaying chemotaxis using polycarbonate filters was employed. Fig. 6 demonstrates the results of this experiment using twofold

[3] Unpublished observations.

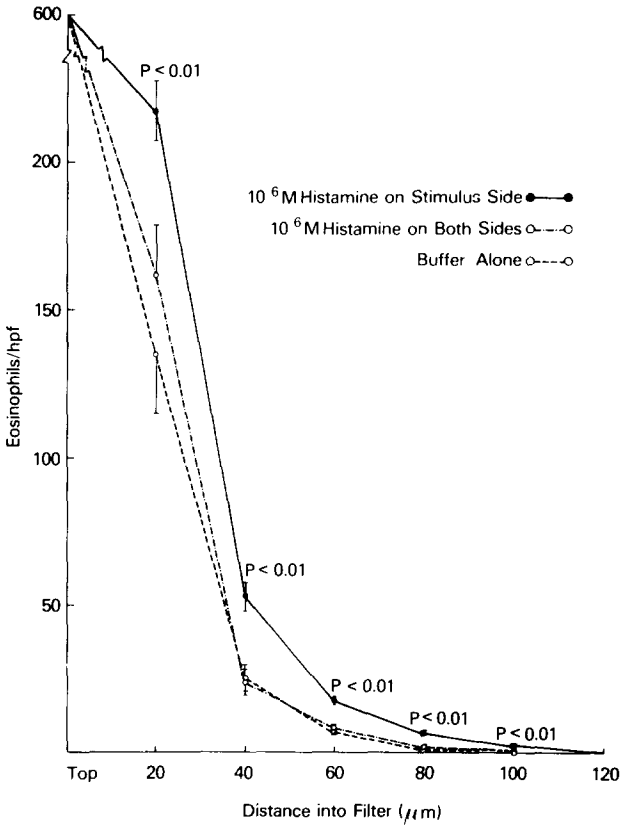

FIG. 4. Eosinophils/hpf at 20-μm distances into a cellulose nitrate filter when incubated for 45 min in buffer alone, 10^{-6} M histamine on both sides of the filter and 10^{-6} M histamine on the stimulus side. *P* values compare the number of eosinophils present with histamine on the stimulus side of the filter to the number present with buffer alone.

dilutions of histamine between 3×10^{-7} M and 5×10^{-6} M as the chemotactic attractant. Preincubation of eosinophils with histamine markedly diminished the cell responsiveness to a subsequent histamine stimulus. Deactivation of eosinophils with histamine was also demonstrable by preincubating cells with 10^{-6} M histamine and using the Zigmond-Hirsch chemotactic assay.

Since deactivation is thought to be a surface phenomenon (18), the time-course of deactivation should be very brief. Fig. 7 shows that deactivation to histamine occurs within the first 5 min of preincubation. Another characteristic of deactivation of cells is the ability of one chemotactic agent to cross-deactivate the effector cell, that is reduce or eliminate the cell response to a second chemotactic factor (17). Fig. 7 also displays the rapid deactivation of eosinophils preincubated with 6×10^{-7} M histamine when partially purified C5a is used as the chemotactic agent.

Synthesis and Destruction of Histamine-Induced Chemotactic Activity. An unidentified contaminant could have caused the observed eosinophil chemotactic activity. To evaluate this possibility the histamine preparation was incubated with 2.0 mg/ml diamine oxidase (histaminase) as well as 2.0 mg/ml

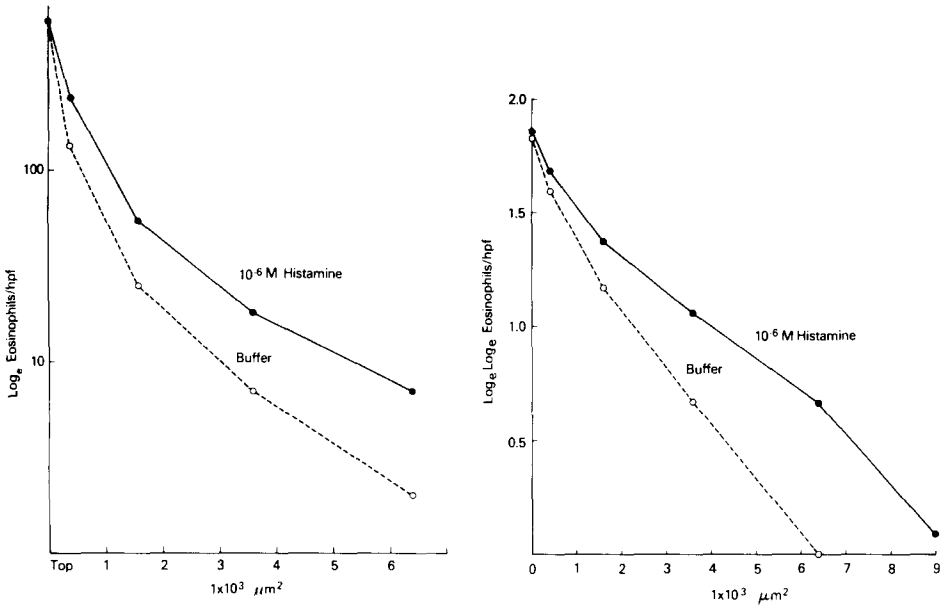

FIG. 5. The data from Fig. 4 re-expressed on log$_e$ eosinophils/hpf against the square of the distance (μm^2) is shown on the left (Fig. 5 A). The data from Fig. 4 re-expressed on log$_e$ log$_e$ eosinophils/hpf against the square of the distance (μm^2) is shown on the right (Fig. 5 B).

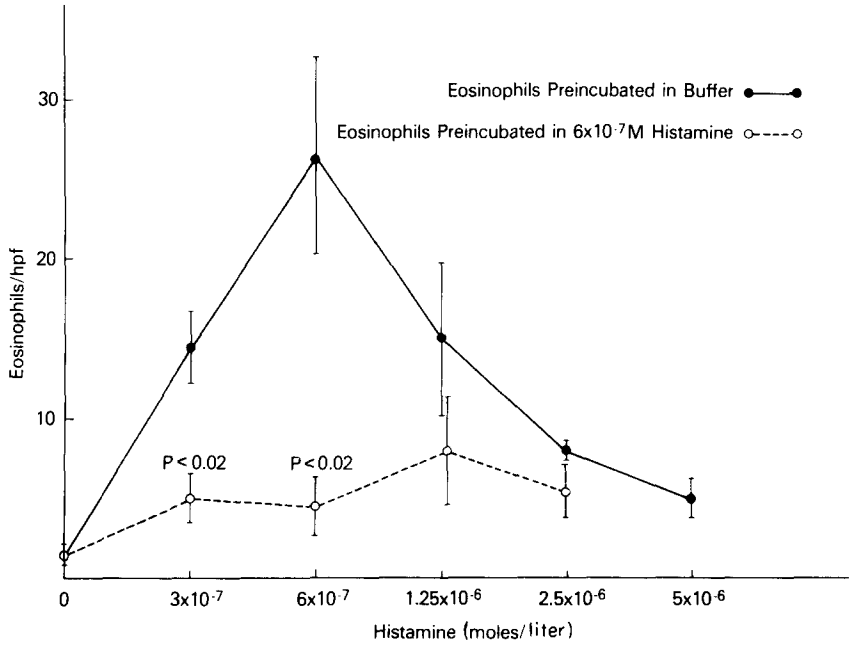

FIG. 6. Chemotactic dose response curves of eosinophils to histamine after the cells had been preincubated with buffer or 6 × 10^{-7} M histamine for 30 min at 37°C.

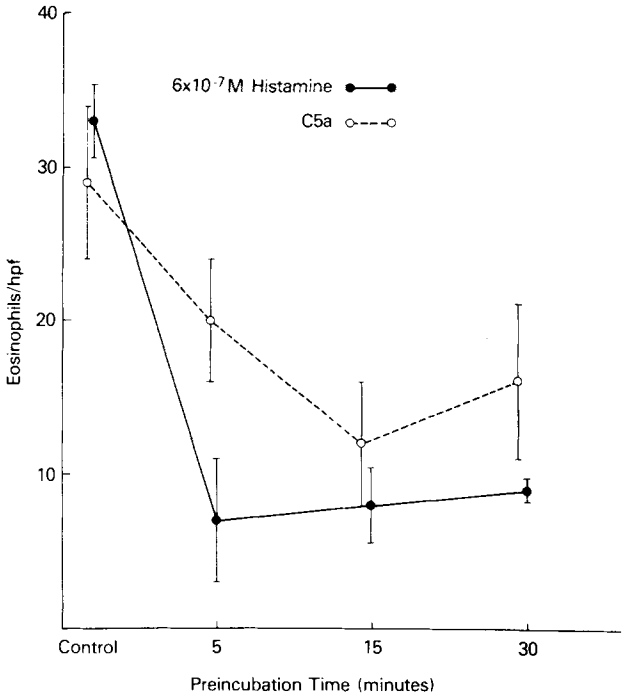

FIG. 7. Deactivation kinetics of eosinophils incubated with buffer or with 6×10^{-7} M histamine for 5, 15, and 30 min before chemotaxis to 6×10^{-7} M histamine or partially purified C5a.

trypsin, chymotrypsin, and pronase in Hank's buffer, pH 7.2, for 2 h at 37°C. The enzyme-substrate solutions were dialyzed overnight against Hank's buffer at 4°C, and the guinea pig ileum contractile activity and eosinophil chemotactic activity of the dialysates were determined. Diamine oxidase completely destroyed both the contractile activity and chemotactic activity of the histamine preparation while trypsin, chymotrypsin, and pronase had no significant effect on either activity. Histamine was next produced enzymatically to determine whether it possessed any associated chemotactic activity. L-histidine hydrochloride monohydrate 10^{-2} M was incubated with 0.2 mg/ml L-histidine decarboxylase for 1½ h at 37°C. By bioassay 5×10^{-4} M histamine was created, approximately a 5% conversion, and the mixture was then dialyzed overnight in Hank's buffer at 4°C, such that the final histamine concentration was 2×10^{-6} M. Controls consisting of either L-histidine hydrochloride or L-histidine decarboxylase incubated with buffer were dialyzed in an identical fashion. The dialysates were assayed for both contractile and chemotactic activities, and the results are illustrated in Fig. 8. The dialysates of L-histidine and L-histidine decarboxylase had no contractile activity and gave minimal eosinophil migration. On the other hand, the dialysate from the L-histidine-L-histidine decarboxylase combination caused an ileal contraction and significant eosinophil migration. Thus, histamine, whether obtained from a commercial source or created by the decarboxylation of L-histidine, is chemotactic for the human eosinophil at 10^{-6} M concentrations.

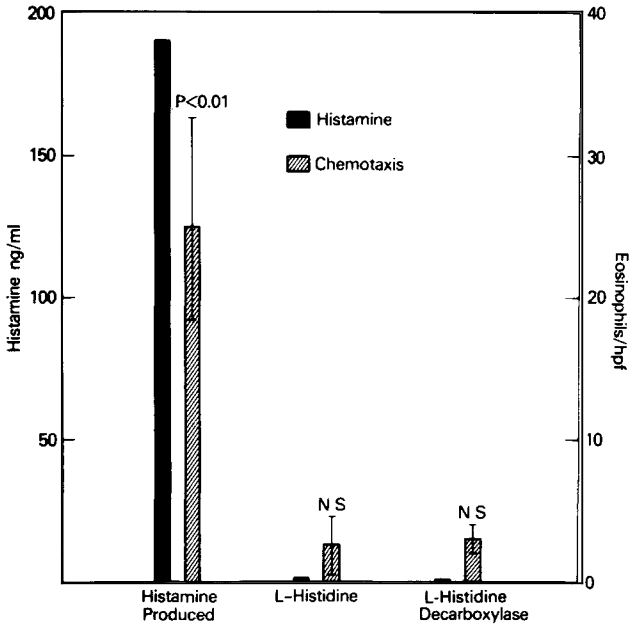

FIG. 8. Histamine and eosinophil chemotactic activity generated by incubation of 10^{-2} M L-histidine with 0.2 mg/ml L-histidine decarboxylase in Hank's buffer, pH 7.2, for 1½ h at 37°C. Histamine produced by the decarboxylation of L-histidine was measured by the guinea pig ileum contractile activity and expressed as nanograms per milliliter from a standard curve. Eosinophil chemotactic activity increased significantly ($P < 0.01$) over control only after incubation of enzyme and substrate together.

Effect of H-1 and H-2 Receptor Antagonists on Eosinophil Migration. As previously indicated the chemotactic dose response of eosinophils to histamine consistently showed a maximum response between 6×10^{-7} M and 1.25×10^{-6} M. In Fig. 9, using the single polycarbonate filter chemotactic assay, a linear dose response was seen from 3×10^{-7} M to 1.25×10^{-6} M; however, at higher histamine concentrations the chemotactic response was markedly depressed. To evaluate whether histamine effects eosinophil migration through the well defined histamine receptors (*H-1* and *H-2*) the effects of these receptor antagonists were evaluated. When 10^{-5} M mepyramine maleate, an *H-1* antagonist, was placed on the cell side of the chamber, the chemotactic activity was not significantly altered. However, when 10^{-5} M metiamide, an *H-2* antagonist, was added to the cells immediately before loading the chemotactic chambers the dose response was linear from 3×10^{-7} to 5×10^{-6} M histamine.

Discussion

In this paper we have shown that low doses of histamine attract human eosinophils in proportion to their absolute number present in the cell suspension, while no effect was observed upon human neutrophil migration. A positive chemotactic response was obtained from 3×10^{-7} M through 1.25×10^{-6} M histamine. The response was depressed at higher concentrations and at 1×10^{-5} M histamine, migration was not significantly different from background. This

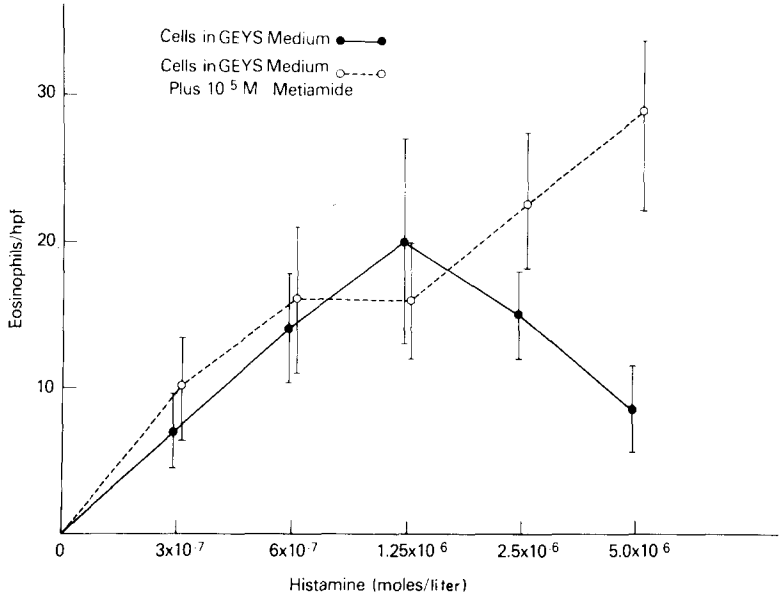

FIG. 9. Chemotactic dose response curves of eosinophils to histamine in the presence and absence of 10^{-5} M metiamide.

type of response has been described previously for horse leukocytes in response to a cell-derived chemotactic factor (14), rabbit peritoneal exudate cells in response to formylated peptides (19), rabbit peritoneal exudate cells in response to chemotactically active peptides from *E. coli* (20), and rabbit neutrophils responding to rabbit serum activated with antigen-antibody complexes (21). When using a single filter morphologic assay in which cells that have migrated through the filter are counted, one possible explanation of the diminished response at higher doses is that the cells may be falling off the filter. This phenomenon has been documented in several instances (14, 21). We have demonstrated that the diminution of eosinophil migration seen when using 10^{-5} M concentrations of histamine cannot be attributable to cells falling off the filter by using two other methods (13, 14). Thus, the ability of histamine to attract eosinophils at 10^{-6} molar concentrations, but not at 10^{-5} molar concentrations, was not an artifact unique to any one assay system. Other investigators (7–10) have not observed histamine-induced eosinophil migration; however, they have routinely used incubation periods of 3 h compared to our incubation time of 1 h. During the long incubation time, the histamine gradient may disperse, since histamine is a small (111 mol wt) molecule which would diffuse rapidly.

The ability of histamine to attract eosinophils was also examined in the three assay systems noted above in order to distinguish increased random motility from chemotaxis. Studies with polycarbonate filters suggested that a concentration gradient was necessary because increasing the concentration of the agent on the cell side led to progressive diminution in eosinophil migration. Furthermore, when equal concentrations of histamine were placed on each side of the Boyden chamber, a flat dose response, only slightly above background, was seen whether using the routine visual assay, the radioassay (Fig. 2), or the Zigmond-

Hirsch assay (Fig. 3). The only exception was a small percentage of cells in the migration front using the Zigmond-Hirsch assay. Increased migration of these leading cells was seen consistently when 10^{-5} M histamine was placed on both sides, but it is not clear why such heterogeneity of eosinophil response was observed. The majority of eosinophils in the filter (cells not in the leading front) had no dose-dependent increase in random motility in response to histamine. These observations indicate that histamine is a bona fide chemotactic factor (14). Furthermore, the ability of histamine to deactivate the cell to either histamine or C5a supports the conclusion that histamine is a chemotactic stimulus (18), since an agent that only enhances random motility should not deactivate the cell.

The apparent histamine-induced increase in random motility observed only in the leading front of migrating eosinophils may reflect heterogenity of the eosinophil population. When eosinophils/hpf were counted at 20-μm intervals for the entire filter thickness, a sigmoid curve was observed for buffer alone, histamine on the stimulus side, or histamine on both sides. The sigmoid curve obtained with histamine on both sides of the filter was not different from the sigmoid curve observed with buffer alone, while histamine on one side shifted the curve to the right; however, the movement of eosinophils en masse into the filter was never observed even when prolonged incubation times were used. This latter phenomenon was observed when neutrophils were stimulated with chemotactic factors[3] (14), but neutrophils behave as a homogeneous population of cells while eosinophils have not previously been assessed in this manner. When we plotted the $\log_e$ of the eosinophil/hpf vs. (distance)^2 for the buffer control and histamine, a curve was obtained again suggesting that the population does not behave in a homogeneous fashion, while assays performed using C5a as the stimulus and neutrophils as the effector cell yielded the expected straight line. Since the line observed in Fig. 5 A appeared similar to an exponential curve we plotted $\log_e \log_e$ eosinophils/hpf vs. distance squared. This line (Fig. 5 B) came closer to being linear, suggesting that the cells responded as a continuous population similar to erythrocytes (22). This would be consistent with previous observations that the eosinophil is a long-lived recirculating cell (23), while the neutrophil is a short-lived cell which does not recirculate.

Because of the conflicting evidence in the literature concerning the effect of histamine upon eosinophil migration, studies were performed to document that the effects observed were in fact mediated by histamine. The histamine diphosphate utilized was digested with diamine oxidase (histaminase) which destroyed its contractile activity upon the bioassay as well as its chemotactic activity. However, the diamine oxidase preparation was found to be contaminated with a carboxypeptidase B-like enzyme since it also inactivated bradykinin. It was therefore possible that inactivation of a chemotactic contaminant in the histamine preparation could account for the observed loss of activity. We then obtained a purified preparation of L-histidine hydrochloride and incubated it with histidine decarboxylase to generate histamine. The formation of histamine paralleled the generation of chemotactic activity for eosinophils in the same dose range observed for commercial histamine preparations, confirming that histamine is the active moiety. The latter experiment also demonstrated that the

same result is obtained whether the histamine is associated with phosphate or hydrochloride ion.

Histamine exerts physiologic and pharmacologic effects by interaction with at least two different groups of receptors. The *H-1* receptors mediate the action of histamine on smooth muscle of the gut and bronchi, and these effects are reversibly blocked by classic antihistamines (24), e.g., diphenhydramine, mepyramine, and their analogues. In contrast the action of histamine on the gastric parietal cell, on the guinea pig atria and on the rat uterus, are not inhibited by the classic antihistamines, but they are reversibly blocked by the thiourea analogues of histamine, burimamide, and metiamide (25). Black et al. (25) have designated this second series of histamine receptors the *H-2* receptor. More recently, histamine was found to inhibit T-cell mediated cytolysis and to increase lymphoid cell cyclic AMP levels. Both of these histamine activities were reversed by burimamide and metiamide, but not by mepyramine, indicating that T cells also have an *H-2* receptor (26).

Although the stimulation of cell motility by histamine was not dependent upon either *H-1* or *H-2* receptors, the diminution of cell migration observed at higher histamine doses was dependent upon the *H-2* receptor. Deactivation obtained when histamine was preincubated with the cells could not be reversed by *H-1* or *H-2* antagonists (unpublished data), suggesting that this interaction was mediated through some undefined site as was observed with chemotactic stimulation of the cells. When an inhibiting dose of histamine was used on the stimulus side, the concentration reaching the cells, although obviously greater than that which stimulates migration, must not deactivate the cell since reversibility with metiamide would otherwise not be possible. Further elucidation of the histamine inhibition of chemotaxis mediated through the *H-2* receptor is in progress.

When a chemotactic factor which preferentially attracted eosinophils was shown to be released from human mast cells (8) and basophils (27, 28), the activity was found to reside in peptides that could be distinguished from histamine by size, charge, and susceptibility to enzymes (6). This factor was designated eosinophil chemotactic factor of anaphylaxis (ECF_A). Our results, however, demonstrate that histamine is absolutely selective in its ability to attract eosinophils. Thus, the eosinophil accumulation in immediate hypersensitivity reactions may depend upon the quantity of histamine released, the quantity of chemotactic peptides released, their relative activities upon eosinophils, and their ability to subsequently immobilize cells by inhibiting their responsiveness or deactivating them.

Summary

Histamine diphosphate was shown to selectively attract human eosinophils from mixed granulocyte populations when over 20% eosinophils were used in a modified Boyden chamber chemotactic assay system. This effect of histamine is abolished by incubation with diamine oxidase (histaminase) and was generated by decarboxylation of L-histidine. A linear dose dependent increase in eosinophil migration was observed between 3×10^{-7} M and 1.25×10^{-6} M, while higher concentrations of histamine inhibited the migration of eosinophils. The attrac-

tant activity of histamine was not inhibited by *H-1* or *H-2* receptor antagonists, however, the inhibition of migration observed at higher histamine concentrations was reversed by metiamine, an *H-2* receptor antagonist. The effects of histamine upon eosinophil migration were demonstrable using three different assays: (*a*) counting cells that had traversed 5-μm pore, 12-μm thick polycarbonate filters, (*b*) counting cells that had migrated various distances into a 3-μm pore, 145-μm cellulose nitrate filters, or (*c*) measuring the number of cells that had traversed an upper polycarbonate filter and migrated into a lower cellulose nitrate filter using ^{51}Cr-labeled cells. The ability of histamine to enhance eosinophil migration was shown to be dependent upon the presence of a concentration gradient; histamine did not cause a dose-dependent increase in random motility. Furthermore, preincubation of the eosinophils with histamine deactivate the cells to further stimulation by histamine or by C5a. It is concluded that in low doses histamine is a chemoattractant for human eosinophils, while in higher doses histamine inhibits eosinophil migration. These observations may relate to the influx and localization of eosinophils in immediate hypersensitivity reactions.

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