Comparative Study of Eosinophil and Neutrophil Chemotaxis and Enzyme Release

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It has been well documented that both natural and synthetic chemotactic peptides can induce lysosomal enzyme release from neutrophils treated with cytochalasin B. These same peptides are also potent inducers of unidirectional movement, as demonstrated by the chemotactic response in Boyden chambers. In this study, the ability of another family of leukocytes, eosinophils, to release lysosomal enzymes and exhibit a chemotactic response to both natural and synthetic chemotactic peptides was examined. A striking fundamental difference between neutrophil and eosinophil chemotaxis and enzyme release was shown using C5a, formyl met-leu-phe (FMLP), and ala-gly-ser-glu (AGSG) peptides. The 50% effective doses (ED_{50}) for chemotactic responses to C5a, FMLP, or AGSG by neutrophils and eosinophils were 0.05 μg/ml and 1.0 μg/ml, 10^{-12} M and 10^{-10} M, and 10^{-7} M and 10^{-7} M, respectively. At the same concentrations, these peptides (C5a, f met-leu-phe, and ala-gly-ser-glu) induced the following release of glucosaminidase from neutrophils and eosinophils, respectively: 42% and 2%, 42% and 2%, and 29% and 2%. In striking contrast, immune complexes and opsonized zymosan particles induced the release of 39% and 42% of the total glucosaminidase from neutrophils, while eosinophils released 32% and 43% of the total glucosaminidase from immune complexes and opsinized zymosan particles, respectively. These data indicate fundamental differences between neutrophils and eosinophils in unidirectional movement induced by chemotactic factors and enzyme release mechanism(s). (Am J Pathol 1981, 105:149–155)

IN A NUMBER OF experimental and clinical inflammatory states eosinophils are known to be conspicuous cellular elements in the inflammatory infiltrates. This is especially true in many atopic allergic states,[1] certain allergic drug reactions,[2] and helminthic parasitic infections.[3] However, in spite of the growing field of interest, the specific functions of eosinophils in immune/inflammatory reactions remain to be fully elucidated. Historically, eosinophils were considered to play a minor role in acute inflammatory reactions but a major role in chronic immunologic reactions to antigens.[4] This role contrasts sharply with that of neutrophils, which are the dominant leukocyte in acute inflammatory reactions. Although eosinophils and neutrophils are involved in diverse immune reactions, they do share certain functions common to inflammatory cells. These functions include the ability to respond chemotactically to a given stimulus and phagocytize foreign material.

Since chemotaxis and lysosomal enzyme release appear to be parallel functional responses of neutrophils to chemotactic factors, we were interested in comparing chemotactic and enzyme release responses of eosinophils, in the latter case using both particulate and soluble immune stimuli. In turn, these responses could be compared with the responses in neutrophils. The present paper describes a striking difference between eosinophil and neutrophil chemotactic responses and the release of lysosomal enzymes.

Material and Methods

Preparation of Eosinophils and Neutrophils

Eosinophils from the peritoneum of guinea pigs were obtained by a modification of the procedures of Litt[6] and Pincus.[7] Hartley strain guinea pigs weighing approximately 300 g were given weekly intraperi-

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toneal injections of 10 mg of keyhole limpet hemocyanin (KLH) (Sigma Chemical Co., St. Louis, Mo) in 5 ml of saline. After a minimum of ten injections, cells were collected from the peritoneal cavity by a wash with sterile saline. The eosinophils were then purified by a modification of the procedure of Jong.[8] Cells removed from the peritoneum were suspended in Hanks' balanced salt solution containing 0.1% gelatin; contaminating red blood cells were removed by hypotonic lysis. The cell concentration was adjusted to approximately 2×10^6 cells/ml in the above Hanks' solution and the solution was layered over an equal volume of 45% Percoll (Pharmacia Fine Chemicals, Piscataway, NJ) in phosphate-buffered saline (PBS), pH 7.2. After centrifugation for 25 minutes at 400 *g*, the pellet was washed with PBS twice and finally with Hanks' solution. Prior to use, the cells were resuspended in the Hanks' solution. The final eosinophil preparation contained up to 95% eosinophils. The remaining cells were monocytes. Neutrophils were obtained from the peritoneal exudate of Hartley strain guinea pigs induced by the injection of 20 ml of 0.1% glycogen 5 hours prior to paracentesis. More than 95% of all cells obtained were neutrophils.

Preparation of C5 and C5a

The fifth component was isolated and purified as described by Kunkel et al.[9] The C5 preparation was shown to produce one single band on SDS-gel electrophoresis and was shown to be hemolytically active.[10] C5a with potent chemotactic activity was generated by the incubation of serum containing zymosan in the presence of ε-amino caproic acid (EACA) at 37 C for 2 hours followed by the chromatographic procedure of Fernandez et al[11] Protein concentrations were determined by the method of Lowry et al[12] and Warburg et al.[13]

Preparation of Immune Complexes

Heat-inactivated anti-KLH guinea pig serum was kindly supplied by Dr. M. Suko (Dept. of Pathology, University of Connecticut Health Center, Farmington Connecticut). Serum levels of anti-KLH precipitating antibody were determined according to the procedure of Garvey et al.[14] Keyhole limpet hemocyanin at equivalence with serum anti-KLH antibody was incubated at 37 C for 1 hour and then kept at 4 C overnight. After centrifugation at 8000*g* for 10 minutes, the pellet was washed twice and finally resuspended in PBS.

Immune-Complex and Zymosan-Induced Enzyme Release

Leukocytes (2×10^7), suspended in 1 ml of Hanks' solution, were incubated with immune complexes for 15 minutes at 37 C in a shaking water bath. (The immune complexes contained 200 μg KLH and equivalent anti-KLH guinea pig serum.) After incubation, the cells were centrifuged at 1000*g* for 5 minutes, and the supernatant was decanted and assayed for enzyme activity. Leukocytes (2×10^7) were also suspended in Hanks' solution containing 2 mg/ml of zymosan (which had been preopsinized in fresh guinea pig serum and then washed in Hanks' solution) and incubated for 30 minutes at 37 C in a shaking water bath. The cell suspension was then processed as above. For reference values related to enzyme activity, 2×10^7 leukocytes were lysed by repeated passage through a metal needle.

Enzyme Release Induced by Chemotactic Peptide

Lysosomal enzyme release was studied in leukocyte populations according to the procedure of Showell et al.[15] The following chemoattractants were examined for their ability to induce enzyme release: formyl mezhionyl-leucyl-phenylalanine (f met-leu-phe) (Sigma Chemical Co., St. Louis, Mo), alanyl-glycyl-seryl- glutamic acid (ala-gly-ser-glu) (Sigma Chemical Co.), valyl-glycyl-seryl-glutamic acid (val-gly-ser-glu) (Sigma Chemical Co.), and C5a. Purified C5 was used as a negative control. Eosinophils or neutrophils were suspended at 2×10^7 cells/ml in Hanks' solution containing 5 μg/ml cytochalasin B (Aldrich Chemical Co., Milwaukee, Wis). Various concentrations of each of the chemotactic peptides were added to the cells, and the cell suspensions were incubated at 37 C for 5 minutes. After centrifugation at 200*g* for 5 minutes at 4 C, the supernatant was decanted and assayed for enzyme activity. Beta-acetylglucosaminidase was examined in this study because it is an extremely easy enzyme to assay and it is an acid hydrolase found in leukocyte (neutrophil and eosinophil) granules.[16]

Five hundred μl of the supernatant material were removed for measurement of N-acetyl-β-D-glucosaminidase activity according to a modification of the procedure of Fantone et al.[17] The reaction mixtures consisted of 1) 500 μl of a 4 mM solution of P-nitrophenyl-N-acetyl-β-D-glucosamide in 0.05 M sodium citrate (pH 4.5) and 2) 500 μl of the test sample. This sample was incubated for 30 minutes at 37 C and the reaction was terminated by the addition of 500 μl of 0.4 M glycine buffer (pH 10.5). The samples were

read in a spectrophotometer at an absorbance of 410 nm.

Chemotaxis Assay

Leukocyte chemotaxis was monitored with modified Boyden chambers according to the procedure of Ward et al.[18] Preliminary experiments revealed an optimal micropore filter pore size of 3 μ for eosinophil and neutrophil chemotaxis. Each assay was performed in triplicate. The chemotaxis index (CI) was computed by counting the number of migrating cells at four consecutive 10-μ depths of field, according to the procedure of Maderazo et al.[19]

Results

Purification of Eosinophils

Since it was essential to know the purity of the eosinophil preparations, a representative low-power electron micrograph of the eosinophil preparations is shown in Figure 1. More than 95% of all cells observed were eosinophils, as determined by cell preparations stained with hematoxylin and eosin.

Chemotaxis of Eosinophils and Neutrophils

The synthetic polypeptides f met-leu-phe, ala-gly-ser-glu, and val-gly-ser-glu and the C5a preparation were shown to be chemotactic for both guinea pig neutrophils and eosinophils. As demonstrated in Figure 2, the neutrophil responses to f met-leu-phe and C5a exceeded the responses of the eosinophils. The optimal concentration of C5a for neutrophil response was 1/50 the concentration required for the maximal response in eosinophils. Also, the chemotactic index in response to C5a was significantly greater for neutrophils than for eosinophils at each leukocyte's respective optimal dose (Figure 2). With f met-leu-phe, the optimal concentration for chemotaxis with guinea pig neutrophils was 10^{-11} M as compared with 10^{-9} M for guinea pig eosinophils. Not only was the concentration optimum for the chemotactic response to f met-leu-phe two logs lower for

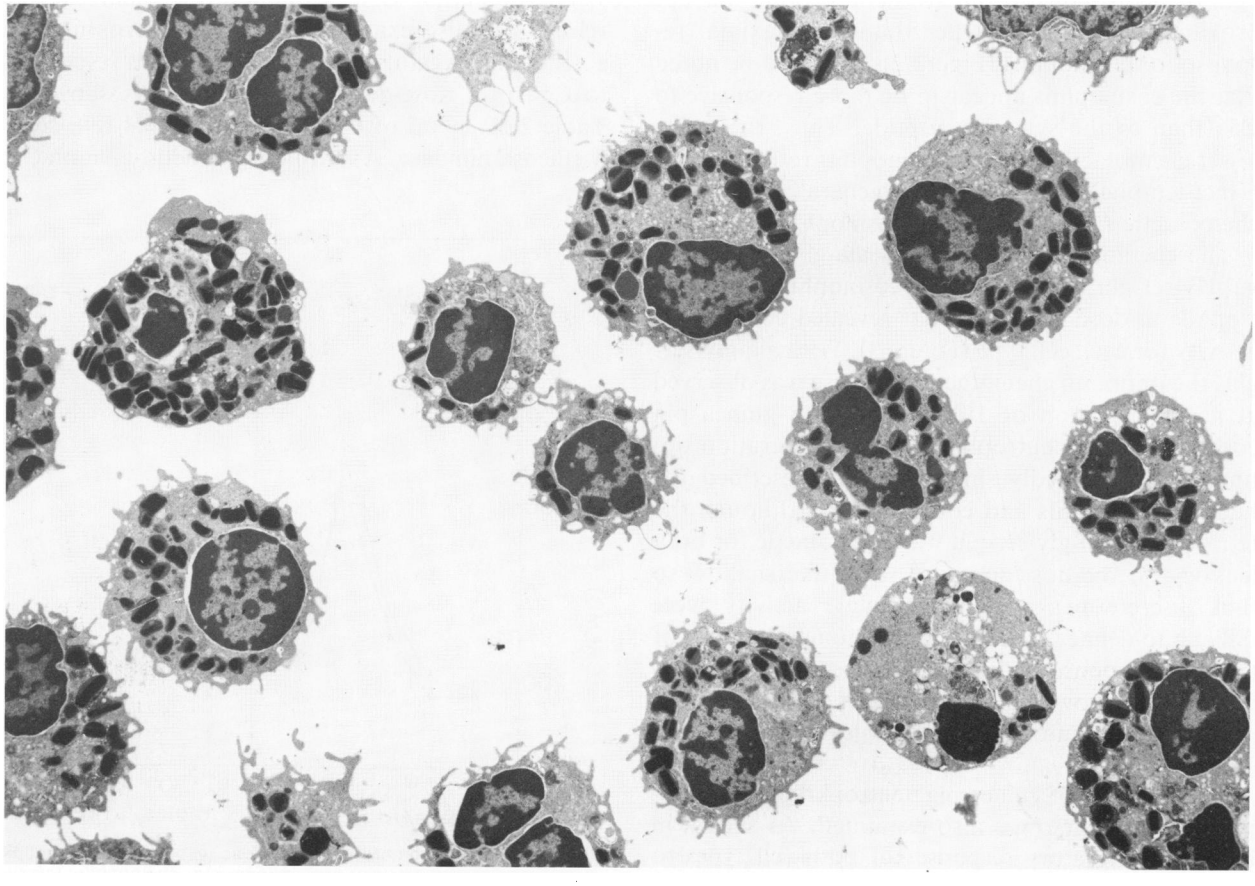

Figure 1—Electron micrograph of guinea pig eosinophils recruited with KLH and purified as described in Materials and Methods. (×3500).

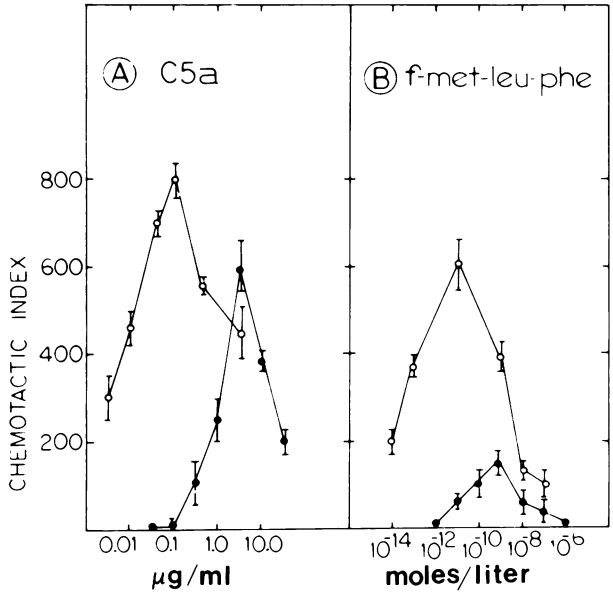

Figure 2—Comparison of the chemotactic responses of the guinea pig neutrophils (*open circles*) and guinea pig eosinophils (*closed circles*) for (**A**) C5a preparation and (**B**) f-met-leu-phe.

neutrophils, but the peak response (chemotactic index) of neutrophils to the synthetic peptide was approximately fourfold higher than the maximal response for eosinophils (Figure 2). It should be noted that the eosinophils appear to be more responsive to C5a than to the synthetic peptide. Thus, the much lower chemotactic index of eosinophils responding to f met-leu-phe is not due to a generalized lack of chemotactic responsiveness of eosinophils.

The chemotactic tetrapeptides ala-gly-ser-glu and val-gly-ser-glu attracted both eosinophils and neutrophils in dose responses that revealed comparable activity for each cell type (Figure 3). With ala-gly-ser-glu, the optimum chemotactic response was observed at a concentration of 10^{-6} M for both guinea pig eosinophils and neutrophils. This concentration optimum is remarkedly similar to that described for human neutrophils and eosinophils.[20] Although the tetrapeptide val-gly-ser-glu was chemotactic for both leukocytes, the dose response was quite shallow so that discrete peaks of chemotactic activity were difficult to define (Figure 3). Even though the slope of the dose response of both leukocytes to val-gly-ser-glu was shallow, it appears that a chemotactic response optimum for both leukocytes occurred at 10^{-6} M.

The response of guinea pig neutrophils and eosinophils to histamine was also examined. As shown in Figure 4, migratory responses of both cell types to histamine were noted, with the optimal responses of neutrophils and eosinophils 10^{-5} M and 10^{-4} M, respectively. The dose response for guinea pig eosinophils is somewhat different than that previously reported for human eosinophils. This may be due to species difference.

Lysosomal Enzyme Release from Eosinophils and Neutrophils

Upon challenge of cytochalasin-B-treated leukocytes with either of the synthetic peptides (Table 1), the release of significant lysosomal enzyme was demonstrated only with the neutrophils. The eosinophils failed to release β-glucosaminidase in response to any of the synthetic chemotactic peptides. For neutrophil enzyme release, the formylated tripeptide was more potent than the other synthetic peptides examined. Guinea pig neutrophils released β-glucosaminidase only over a narrow concentration range of f-met-leu-phe (10^{-5}–10^{-8} M). The tetrapeptides also induce enzyme release from neutrophils, but the total amount of enzyme released was 1.6–4-fold less with ala-gly-ser-glu and val-gly-ser-glu, respectively, than the release induced by the formylated tripeptide (Table 1).

The ability of C5a to induce lysosomal enzyme release was also examined for both neutrophils and eosinophils. Again, guinea pig neutrophils responded to C5a in a dose-dependent manner. As shown in Table 2, 1 μg/ml of C5a caused maximal release of β-glucosaminidase. As with the synthetic chemotactic

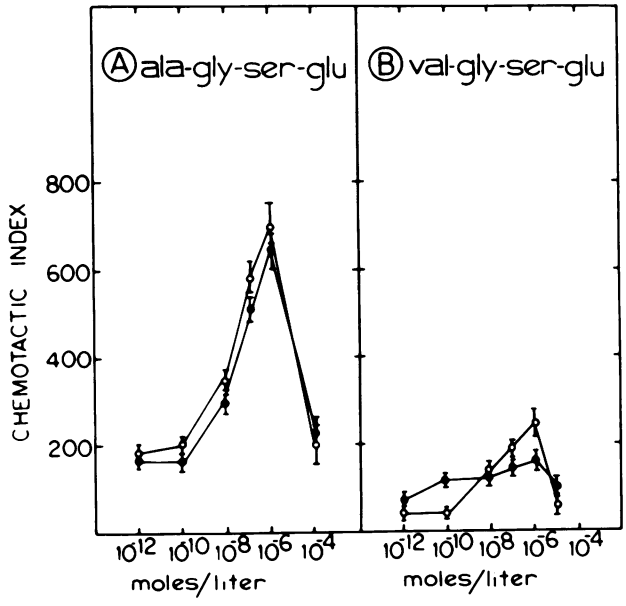

Figure 3—Comparison of the chemotactic responses of guinea pig neutrophils (*open circles*) and guinea pig eosinophils (*closed circles*) for two synthetic tetrapeptides, ala-gly-ser-glu (**A**) and val-gly-ser-glu (**B**).

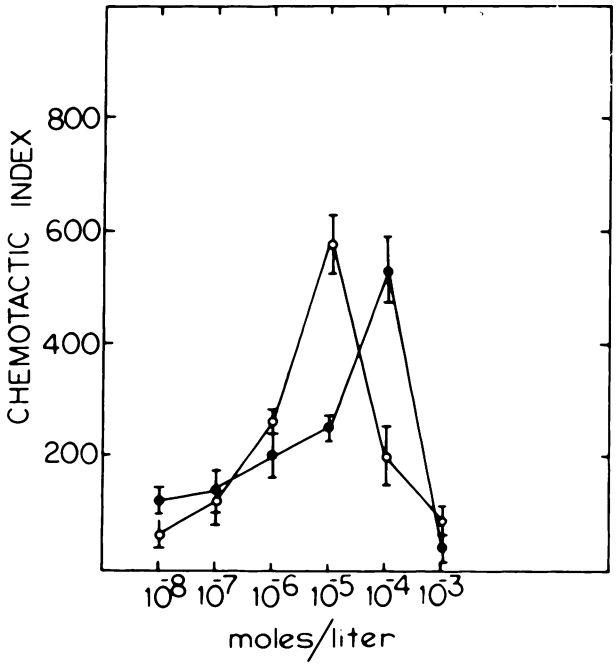

Figure 4—Comparison of the chemotactic responses of guinea pig neutrophils (*open circles*) and guinea pig eosinophils (*closed circles*) for histamine.

factor, eosinophils failed to respond to C5a with enzyme secretion over a 3-log concentration range of C5a. These results contrast to those obtained when particulate material (immune complexes or opsonized zymosan particles) was employed. As demonstrated in Table 2, immune complexes as well as opsonized zymosan particles stimulated the release of cellular β-glucosaminidase from both neutrophils and eosinophils. Immune complexes and opsonized zymosan particles induced the release of 39% and 42% of the total glucosaminidase from neutrophils, respectively, and 32% and 43% of the total glucosaminidase from eosinophils. The release of cytoplasmic enzymes through cell lysis was negligible, since less than 2% of the total cellular content of lactic dehydrogenase was released. Thus, eosinophils do have the ability to release lysosomal enzymes, but they do so only when challenged with a particulate stimulus.

The abilities of eosinophils and neutrophils to release lysosomal enzymes and respond chemotactically to the above stimuli are summarized in Table 3. Compared with guinea pig eosinophils, guinea pig neutrophils respond chemotactically to lower molar concentrations of all the stimuli examined except the tetrapeptide val-gly-ser-glu. The most potent synthetic chemoattractant for neutrophils is f met-leu-phe (ED_{50}, 10^{-12} M), while the most chemotactically active synthetic peptides for eosinophils were f met-leu-phe (ED^{50}, 10^{-10} M) and val-gly-ser-glu (ED_{50}, 10^{-9} M). Particulate stimuli caused the release of lysosomal enzymes from both eosinophils and neutrophils, but only neutrophils released lysosomal enzymes in response to the soluble chemotactic stimuli (synthetic peptides and C5a). Upon stimulation with immune complexes or opsinized zymosan particles, the eosinophils released 32% and 43% of the total glucosaminidase to each of the stimuli, respectively. The most active synthetic secreatogue for the guinea pig neutrophil proved to be the formylated met-leu-phe.

Discussion

In this study we have made a comparative analysis of the response of guinea pig eosinophils and neutrophils to various stimuli, involving chemotactic factors and particular material. Both neutrophils and eosinophils respond chemotactically, in a dose-dependent manner, to the synthetic tetrapeptides ala-gly-ser-glu and val-gly-ser-glu. As shown in Figure 3, there is no statistical difference in the chemotactic indices of eosinophil and neutrophil responses to these two peptides. Even though both f met-leu-phe and C5a could serve as a chemoattractant for neutrophils as well as eosinophils, the optimal response of neutrophils to the synthetic tripeptide and to C5a occurred at concentrations 2 and 1.5 logs lower, respec-

Table 1—Enzyme Release from Neutrophils or Eosinophils Challenged with Chemotactic Peptides*

Concentration (M)	f-met-leu-phe		ala-gly-ser-glu		val-gly-ser-glu	
	Neutrophil (%)	Eosinophil (%)	Neutrophil (%)	Eosinophil (%)	Neutrophil (%)	Eosinophil (%)
10^{-10}	< 2	< 2	< 2	< 2	< 2	< 2
10^{-9}	3 ± 2	< 2	5 ± 3	< 2	< 2	< 2
10^{-8}	15 ± 5	< 2	12 ± 8	< 2	< 2	< 2
10^{-7}	25 ± 6	< 2	16 ± 7	< 2	< 2	< 2
10^{-6}	31 ± 6	< 2	29 ± 10	< 2	< 2	< 2
10^{-5}	42 ± 8	< 2	26 ± 3	< 2	10 ± 4	< 2

* Numbers indicate % of total release of N-acetyl-β-D-glucosaminidase, $\frac{OD_{410}\text{ experimental} - OD_{410}\text{ control}}{OD_{410}\text{ total cell lysate} - OD_{410}\text{ control}}$

Table 2—Enzyme* Release from Guinea Pig Eosinophils or Neutrophils Induced by Immune Complexes, Opsinized Zymosan, and C5a

Stimulus	Neutrophils (%)	Eosinophils (%)
C5a—0.001 g†	< 2‡	< 2
C5a—0.01 g	5 ± 3	< 2
C5a—0.1 g	17 ± 4	< 2
C5a—1.0 g	42 ± 9	3 ± 2
Immune complexes	39 ± 6	32 ± 8
Opsinized zymosan	42 ± 10	43 ± 9

* N-acetyl-β-D-glucosaminidase

† C5a preparation was purified as described in Materials and Methods.

‡ % of total cellular enzyme release,

$$\frac{OD_{410}\text{ experimental} - OD_{410}\text{ control}}{OD_{410}\text{ total cell lysate} - OD_{410}\text{ control}}$$

tively, than the optimal concentrations for eosinophils (Figure 2). This difference between eosinophils and neutrophils in responsiveness to C5a has also been documented by Kay et al[21] who have also reported a similar difference in neutrophil and eosinophil responsiveness to the eosinophil chemotactic factor of anaphylaxis.

It has been known for some time that the vasoactive mediator histamine can serve as a chemoattractant for eosinophils,[22] but the ability of histamine to recruit neutrophils is not well documented. In our studies, we have shown that histamine can cause, in a dose-dependent manner, chemotaxis of both neutrophils and eosinophils. As shown in Figure 4, there appears to be little statistical difference between the chemotactic indices of neutrophils and eosinophils to the histamine stimulus.

An interesting observation noted in this study is that eosinophils are able to respond by migration to both the synthetic peptides and C5a but are unable to release lysosomal enzymes when challenged with the same stimuli. In this respect, eosinophils contrast sharply with neutrophils, which respond chemotactically in a dose-related manner and in a structure-function relation that duplicates the response in enzyme release.[15] In our studies, only when eosinophils are actively involved in phagocytosis of immune complexes or opsinized zymosan particles does lysosomal enzyme release occur. Perhaps the enzyme released from eosinophils is related to the formation of phagolysosomes with ensuing enzyme regurgitation. It would appear that the neutrophil active secretory mechanism and the biochemical mechanism underlying chemotaxis and enzyme release, as described by Becker and Showell,[5] are not completely represented to the same extent in eosinophils. It has been well established in neutrophils that the interaction of specific receptors on the cell membrane with chemotactic factors is paramount for any subsequent cell response.[23,24] The inability of eosinophils treated with cytochalasin B to release lysosomal enzymes is probably not related to a lack of cell surface receptors for chemotactic factors, but is probably dependent on some other factors beyond this primary interaction at the cell surface level.

Table 3—Optimal Responses of Guinea Pig Eosinophils and Neutrophils in Chemotaxis and Enzyme* Release

Stimulus	Chemotaxis (ED_{50}) Neutrophil (M)	Chemotaxis (ED_{50}) Eosinophil (M)	Enzyme Release† Neutrophil (%)	Enzyme Release† Eosinophil (%)
C5a preparation	0.05 μg/ml	1 μg/ml	42 ± 10	< 2
f-met-leu-phe	10^{-12}	10^{-10}	42 ± 8	< 2
ala-gly-ser-glu	10^{-7}	10^{-7}	29 ± 10	< 2
val-gly-ser-glu	10^{-7}	10^{-9}	10 ± 4	< 2
Immune complexes	—	—	39 ± 6	32 ± 8
Opsinized zymosan	—	—	42 ± 10	43 ± 9

* N-acetyl-β-D-glucosaminidase

† Enzyme release was induced by use of the optimal concentration of each stimulus, not the ED_{50} concentration. Numbers indicate % of total cellular enzyme release,

$$\frac{OD_{410}\text{ experimental} - OD_{410}\text{ control}}{OD_{410}\text{ total cell lysate} - OD_{410}\text{ control}}$$

The results presented here demonstrate the diverse nature of cell responses within the granulocyte spectrum. Although both neutrophils and eosinophils share certain cardinal inflammatory functions, such as chemotaxis and extrusion of lysosomal enzymes, specific aspects within each of these functions remain quite different. Neutrophils, which dominate most acute inflammatory reactions, can respond to synthetic chemoattractants and C5a with migratory responses and by releasing lysosomal enzymes (in the presence of cytochalasin B). Eosinophils, whose ultimate immune function remains an enigma, respond to the above stimuli exclusively through chemotaxis.

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