Eosinophilopoietin

A CIRCULATING LOW MOLECULAR WEIGHT PEPTIDE-LIKE SUBSTANCE WHICH STIMULATES THE PRODUCTION OF EOSINOPHILS IN MICE

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ABSTRACT In earlier studies, methods were developed to raise specific antibodies in rabbits against purified suspensions of mouse or human eosinophils. On administration of antieosinophil serum (AES) to mice, the mature eosinophils in tissues, peripheral blood, and bone marrow were depleted, while the immature eosinophil pool in the bone marrow was observed to proliferate. The current investigations explore the generation of eosinophilopoietic factors during AES-induced eosinophilopenia. Mice received three injections of AES, one every other day. As the peripheral eosinophil counts started to recover after the last AES injection, the serum was collected and transferred to normal animals. Within 2 days the recipients showed an increase in peripheral blood as well as in bone marrow eosinophils. The rise in bone marrow eosinophils was due to newly formed cells as evidenced by increased uptake of [^{3}H]thymidine. The generation of eosinophilopoietic activity was specifically related to depletion of eosinophils but not neutrophils. The eosinophilopoietic activity was: (*a*) dependent on the volume of serum transferred, (*b*) lost on dialysis, and (*c*) largely heat labile. The activity eluted as a low molecular weight substance on G-25 Sephadex and was digested by pronase but not by trypsin. Active fractions collected from G-25 columns were not chemotactic for the eosinophils in vitro. Thus, specific depletion of mature eosinophils generates a low molecular weight peptide which stimulates eosinophilopoiesis in vivo. It is suggested that this substance be named eosinophilopoietin.

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INTRODUCTION

Eosinophilia is associated particularly with worm infections, allergies, and neoplasms (1–3). The increase of eosinophils in the above conditions is not usually accompanied by changes in the neutrophils or basophils, although all of the granulocytes are thought to develop from a common stem cell (4). This dissociation in the production of different granulocytes suggests the presence of specific regulatory mechanisms for each cell line. With respect to granulopoiesis, a great many factors have been described which stimulate the production of neutrophils (5, 6), but little is known about the regulation of eosinophils. Specific stimuli for eosinophil chemotaxis and migration have been studied by several investigators (7–10). The central role of lymphocytes in the induction of the eosinophilic response after *Trichinella spiralis* infection was reported by Basten and Beeson (11). They postulated that the increased production of eosinophils under these conditions might be mediated by a diffusible factor (11). More recently, a diffusible stimulator of eosinophilopoiesis has been described which is produced by spleen cells maintained in diffusion chambers implanted intraperitoneally (12).

The investigations reported here have utilized the recent development of monospecific antieosinophil serum (AES)[1] (13) to explore the regulatory mechanisms for eosinophilopoiesis. AES, which was raised against mature mouse eosinophils obtained from the peritoneal cavity, reacted only with mature forms of these cells in the peripheral blood and bone marrow. The antiserum had no direct effect on immature bone

[1] *Abbreviations used in this paper*: AES, antieosinophil serum; ANS, antineutrophil serum; HBSS, Hanks' balanced salt solution; NRS, normal rabbit serum; MS, mouse sera.

marrow eosinophils, and these cells were observed to increase in number (1, 13). On the basis of these findings, we have explored the effects of AES-mediated depletion of mature eosinophils on the mechanism stimulating the production of their immature precursor cells. An in vivo assay system for eosinophilopoiesis was developed in which eosinophils in the blood, bone marrow, and those undergoing DNA synthesis were measured. When serum from eosinophil-depleted animals was passively transferred to normal recipients, a dose-dependent increase in bone marrow eosinophils was observed. Gel chromatography and enzyme studies suggested that the eosinophilopoietic activity was due to a low molecular weight peptide-like substance.

METHODS

Animals. Young adult female Swiss albino mice (18–22 g body wt) obtained from Zivic Miller (Allison Park, Pa.) were used in these experiments.

Antisera. Monospecific rabbit anti-mouse eosinophil serum (AES) was prepared as described previously (13). The antiserum was tested for specificity in vitro by agglutination and cytotoxicity assays and in vivo by its effect on the total and differential leukocyte counts in peripheral blood and bone marrow (1, 13). Rabbit anti-mouse neutrophil serum (ANS) was produced in our laboratories by the method of Simpson and Ross (14).

Groups of normal mice were injected intraperitoneally with 0.25 ml of AES every other day for three doses. The serum of the treated animals was collected 3 days after the last AES injection as the peripheral eosinophil counts started to recover. Control groups consisted of untreated mice or animals that received a similar course of injections using either normal rabbit serum (NRS), AES previously absorbed three times with eosinophils which removed all AES activity, or antineutrophil serum (ANS). Blood collected from each animal group was pooled and was allowed to clot at room temperature for 15 min and at 4°C for 1 h. Sera were separated, pooled, and kept frozen at −20°C until further use. The mouse sera (MS) collected from animals treated with AES, NRS, or ANS were labeled MS-AES, MS-NRS, and MS-ANS, respectively.

Peripheral blood eosinophil counts. Blood was obtained from the retro-orbital plexus using a microhematocrit tube. Absolute eosinophil counts were made using Discombe's fluid as diluent (15). Fresh fluid was prepared each week, and cell counts were performed in bright-line counting chambers at ×400. Total leukocyte counts were obtained by using a Coulter counter model Z_F (Coulter Electronics Inc., Hialeah, Fla.). Smears were also prepared and stained with tetrachrome for differential counts.

Bone marrow eosinophil counts. Counts of the total nucleated cells and eosinophils in the bone marrow were performed by a modification of the method described by van Furth and Cohn (16). Both femurs of the mice were removed and carefully cleaned of attached muscle. The bones were cut at both ends in the region of the metaphyses, and the marrow was flushed out with 2 ml of Hanks' balanced salt solution (HBSS, Grand Island Biological Co., Grand Island, N. Y.) using a 26-gauge needle. For experiments where [^{3}H]thymidine labeling was to be studied, minimal essential medium (Flow Laboratories, Inc., Rockville, Md.) supplemented with 10% fetal calf serum was used. The cell suspension was then dispersed by repeated gentle aspiration in a pipette. The total leukocyte count was determined by a Coulter counter, and the absolute eosinophil count was performed manually (1). The count obtained by the latter method represents all cell stages of the eosinophil series that contain the characteristic eosin-stained granules (1).

Eosinophilopoietic assay: passive transfer of sera. Groups of five–eight animals were used to assay the eosinophilopoietic activity of MS-NRS, MS-AES, and MS-ANS. In the original experiments, 0.3 ml of each serum was injected intravenously, and the peripheral blood and bone marrow eosinophils were quantified at 2, 4, and 6 days. Studies at 2 days were found to give maximal and reproducible results and were used for all subsequent comparisons. The percentage increase of bone marrow eosinophils in experimental animals above the controls was calculated using the formula: (experimental-control)/control × 100. Statistical analysis of all data was done by Student's *t* test.

[^{3}H]Thymidine uptake by bone marrow cells. In vitro incubation of bone marrow cells with [^{3}H]thymidine provided an indication of the number of cells synthesizing DNA and new cell formation. The method employed was similar to that described by Bass (17). Suspensions of bone marrow cells were prepared as above in minimal essential medium supplemented with 10% fetal calf serum. 2-ml aliquots of the cell suspensions were incubated at 37°C in a water bath with 1 μCi of [^{3}H]thymidine (Amersham/Searle Corp., Arlington Heights, Ill.). The reaction was stopped after 1 h with the addition of 15 ml of cold minimal essential medium, and the cells were washed three times by centrifugation at 400 *g* for 10 min. Several cytocentrifuge preparations (Shandon Southern Instruments, Inc., Sewickley, Pa.) on microscope slides were made from each sample and were fixed for 5 min in absolute methanol. The slides were dipped in nuclear emulsion NTB_3 (Eastman Kodak Co., Rochester N. Y.), kept in the dark at 4°C for 3 wk, and developed in Kodak D19 solution. The percentage of labeled eosinophils was determined after staining with May-Grunwald and counting a minimum of 200 cells for each experimental animal. All slides were coded and read blindly.

Characterization of the eosinophilopoietic activity. The eosinophilopoietic activity of MS-AES was evaluated by a dose-response study using 60, 125, 250, and 500 μl per mouse, by dialysis of 2-ml samples against 2,000 ml of 0.067 M phosphate buffer, pH 7.4 at 4°C for 24 h and by heating the serum at 56°C for 30 min. Gel chromatography was used for the subsequent characterization of molecular size. 1–3-ml samples were applied to 40 × 2.5-cm G-25 Sephadex columns (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J.) equilibrated with 0.001 M phosphate-buffered saline, pH 7.6. Calibration was accomplished with dextran blue, 0.5 mg vitamin B_{12} (Sigma Chemical Co., St. Louis, Mo.), and 1 μCi [^{14}C]histamine (Amersham/Searle Corp.). The column was eluted using the same buffer, and the fractions collected were lyophilized, reconstituted to the original sample volume, and tested for eosinophilopoietic activity as described above. Active fractions were subjected to digestion for 4 h with trypsin (Sigma Chemical Co.) at 37°C in 0.1 M Tris buffer, pH 8, or for 24 h with pronase (Calbiochem, Inc., San Diego, Calif.) at 40°C in 0.02 M potassium phosphate buffer made 0.1 M with sodium chloride, pH 7.4 (18). The reaction mixtures were subsequently fractionated on Sephadex G-25 and assessed for eosinophilopoietic activity.

Eosinophil chemotaxis. Eosinophil-rich peritoneal exudates were induced in mice infected with *Trichinella spiralis* using proteose peptone injections (19). Only those cell sus-

pensions containing >60% eosinophils were used in this assay due to the difficulty in doing differential counts within the filters. The cells were washed in HBSS, and the concentration was adjusted to 6–8 × 10^6 cells/ml. Cell suspensions were then layered on membranes with 3-μm pore size (Millipore Corp., Bedford, Mass.) using a cytocentrifuge as previously described (20). 100-μl aliquots of G-25-fractionated MS-AES and MS-NRS were diluted in HBSS to a total volume of 1 ml and added to the attractant side of the chambers which were incubated for 2 h at 37°C. After incubation, each membrane was dipped in HBSS, stained (20), and mounted on glass slides. The chemotactic index for each membrane was calculated using a πMC particle measurement computer (Millipore Corp.) (20). The average of 10 random fields counted on both sides of the membrane was used to calculate the chemotactic index as follows: chemotactic index = (no. of cells on attractant side)/(no. of cells on starting side) × 500. All samples were done in duplicate, and background (buffer control) counts were subtracted.

Site of production of the eosinophilopoietic activity. The possibility that the eosinophilopoietic activity is generated as a product of eosinophil destruction by AES was evaluated. 1-ml aliquots of AES and NRS were incubated with 15 × 10^6 mouse eosinophils, and fresh rabbit serum was added as a source of complement (21). The cytotoxicity reaction was allowed to proceed for 30 min at 37°C, then the serum and cell mixture was centrifuged (400 *g* at 23°C for 10 min), and the supernate was fractionated on a G-25 Sephadex column. The fractions collected were assayed for their eosinophilopoietic activity in comparison with the corresponding fractions of MS-AES.

RESULTS

Effects of antieosinophil serum injections. Three separate pools of monospecific AES were used in these studies. They were evaluated for agglutinating and cytotoxic effects against eosinophil suspensions in vitro. The antibody agglutinating titers were respectively 1:5,120, 1:2,560, and 1:2,560, and their cytotoxicity titers were 1:5,120, 1:1,280, and 1:2,560.

AES or NRS (0.25 ml/mouse) was administered intraperitoneally to groups of mice every other day for a total of three injections. 48 h after the first injection of AES, the mean eosinophil counts dropped from the pretreatment level of 40±10 to 8±3/mm^3; there was no significant change in animals treated with NRS (Fig. 1). The peripheral eosinophil counts in AES-treated animals remained at a very low level 48 h after the second and third AES injection. 3 days after the third and last AES injection, recovery of peripheral blood eosinophil counts was noted (mean 75±26/mm^3) and at 4 days, it was significantly higher than that of control animals ($P < 0.01$).

In contrast, the bone marrow eosinophil count showed a steady increase in the AES-treated animals (Fig. 1). At the time of exsanguination, the mean bone marrow eosinophil count in this group was more than three times the mean in NRS-treated controls: 8±1.0 × 10^5 compared to 2.2±0.6 × 10^5 per femur ($P < 0.005$).

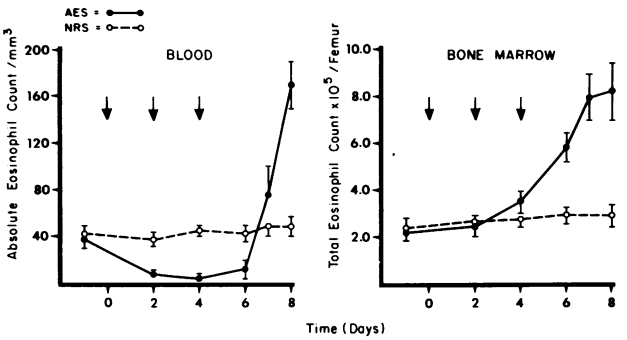

FIGURE 1 Eosinophil counts in the peripheral blood and bone marrow of mice injected intraperitoneally with 0.25 ml of AES or NRS (↓) every other day for a total of three injections. Each point represents the mean±SE of 15 mice. Animals were bled 3 days after the last serum injection.

Eosinophilopoiesis assay. AES- or NRS-treated mice were bled 3 days after the third and last injection. This interval was selected in that it coincided with the recovery of peripheral eosinophil counts after the depletion induced by AES (Fig. 1). Peripheral blood eosinophils at 24 h after the injection of a single dose of 0.3 ml of MS-AES were not significantly different from controls injected with MS-NRS (Fig. 2). At 2 days, the mean peripheral eosinophil count of five MS–AES-treated mice was 246±71/mm^3, compared to a mean of 60±13/mm^3 in the control animals injected with MS-NRS ($P < 0.001$) (Fig. 2). The peripheral blood eosinophils remained significantly higher at 4 days ($P < 0.01$) in MS–AES-treated animals, but at 6 days it decreased to levels not significantly different from the controls. Except for the changes in eosinophil counts, there was no detectable difference in the total or differential leukocyte counts in animals injected with

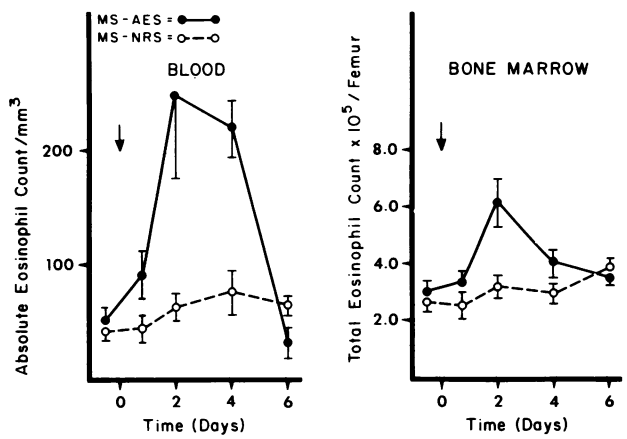

FIGURE 2 Eosinophil counts in the peripheral blood and bone marrow of mice injected intravenously with 0.3 ml of MS-AES or MS-NRS (↓). Each point represents the mean ±SE of five mice.

MS-AES when compared to those which received MS-NRS or normal mouse serum.

The bone marrow eosinophil counts as shown in Fig. 2 rose significantly in animals treated with MS-AES at day 2 ($P < 0.01$) compared to those which received MS-NRS; at days 4 and 6 there were no differences between the two groups. The protocol for this experiment was repeated on six separate occasions using three different pools of AES with similar results. On the basis of these findings, the mean rise in peripheral blood and bone marrow eosinophils 2 days after the passive transfer of sera was used for subsequent comparisons. In five separate experiments with a total of 56 mice divided into experimental and control groups, the increase of peripheral blood eosinophils in MS–AES-treated mice as compared to those treated with MS-NRS was 320±145%; bone marrow eosinophils increased by 77±10%.

The mechanism of the increase in bone marrow eosinophils was investigated by the enumeration of the proportion of the cells synthesizing DNA (Fig. 3). The percentage of bone marrow eosinophils labeled with [^{3}H]-thymidine in normal mice was 13.4±1.0. This did not significantly change in animals receiving MS-NRS, the respective values at 2, 4, and 6 days posttransfusion being 14.0±1.0, 13.4±4.0, and 19.6 ±1.7%. In contrast, animals treated with a single dose of MS-AES showed highly significant increases in the percentage of labeled eosinophils: at 2 days, the mean was 37.0±2.6 ($P < 0.001$), and at 4 days, it was 26.6 ±4.6 ($P < 0.05$), whereas there was no significant difference at 6 days. Thus, the quantitative rise of bone marrow eosinophils produced by the transfer of MS-AES appears to be due to an actual increase in the proliferating pool of eosinophils.

Specificity of the eosinophilopoietic activity. 5 ml of AES was absorbed by incubation with 7×10^8 mouse peritoneal exudate cells containing 62% eosinophils for 1 h at 37°C and for 20 h at 4°C. This procedure was repeated twice and resulted in the loss of AES-agglutinating and cytotoxic activity in vitro. The absorbed AES was then administered to a group of normal mice according to the previously described protocol, and the animals were exsanguinated 3 days after the last injection. Upon injection into normal recipients, no significant changes in the peripheral blood or bone marrow eosinophils were seen at 2 days indicating that the serum obtained from animals treated with the absorbed AES had no eosinophilopoietic activity.

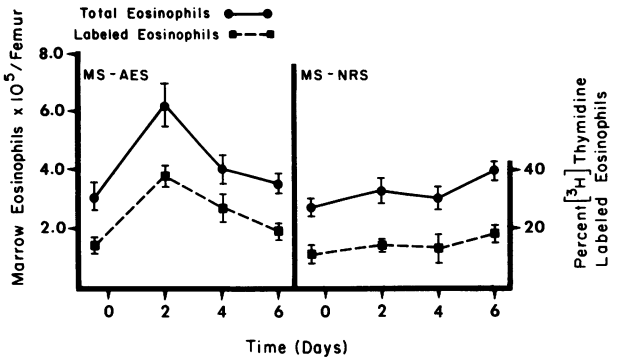

FIGURE 3 Total and percent [^{3}H]thymidine-labeled bone marrow eosinophils in mice injected intravenously with 0.3 ml of MS-AES or MS-NRS on day 0. Each point represents the mean±SEM of five mice.

The specificity of the stimulus for eosinophilopoietin production was also evaluated by studying the effect of neutrophil depletion by ANS. As there was no significant difference in the eosinophil counts of recipients of MS-ANS when compared to those treated with MS-NRS, Table I illustrates the differences in MS–ANS- and MS–AES-treated mice only. 2 days after

TABLE I

Counts of Peripheral Blood, Bone Marrow and [^{3}H]Thymidine-Labeled Eosinophils in Animals Treated with a Single 0.3-ml Intravenous Injection of MS-AES or MS-ANS

	Treatment	Days after transfer of sera		
		2	4	6
			%	
Absolute peripheral blood eosinophil count/mm^3	MS-AES	217±24*	220±27*	42±6
	MS-ANS	60±12	75±8	64±7
Total bone marrow eosinophil count × 10^5/femur	MS-AES	6.2±1.0‡	3.3±1.2	3.5±0.4
	MS-ANS	2.8±0.6	3.8±0.9	3.8±0.4
[^{3}H]Thymidine-labeled bone marrow eosinophils	MS-AES	37±3*	27±3‡	19±4
	MS-ANS	15±2	13±1	19±2

Each value represents the mean±SE of five animals.
* $P < 0.001$.
‡ $P < 0.05$.

passive transfer of sera, the mean peripheral blood eosinophil counts in the MS–ANS-treated group was 60/mm^3 compared to 217/mm^3 in the group treated with MS-AES, an increase of 261% (Table I). Similarly the bone marrow eosinophils in the MS–AES-treated animals were 162% above those treated with MS-ANS. The proliferative nature of the increase in bone marrow eosinophils was demonstrated by [^{3}H]-thymidine uptake studies. 2 days after treatment with MS-ANS, a mean of 15±2% of bone marrow eosinophils was labeled, whereas the corresponding value for MS-AES-treated animals was 37±3%. Similar statistically significant results were seen at day 4 ($P < 0.05$), but not at day 6 (Table I).

Characterization of the eosinophilopoietic activity. A dose-response curve of the eosinophilopoietic activity on the bone marrow eosinophil counts in groups of five mice each receiving MS-AES is shown in Fig. 4. As assayed at day 2, 250- and 500-μl doses induced highly significant increases in bone marrow eosinophils (each is significant at 0.1% level). 60- and 125-μl doses had no detectable eosinophilopoietic activity in our quantitative in vivo assay system.

A pool of MS-AES which induced 120% increase of bone marrow eosinophils on day 2 was dialyzed against isotonic phosphate NaCl buffer, pH 7.4, for 24 h at 4°C. A control aliquot kept under the same conditions retained the eosinophilopoietic activity whereas no significant effect was detected after injection of the dialyzed sample. Heating another aliquot of the same pool of MS-AES for 30 min at 56°C resulted in a 62% reduction of activity.

Fractions collected from the application of 1–3 ml MS-AES on Sephadex G-25 were read at 280 μm. The profile revealed a peak between 25 and 40% of the bed volume which contained most of the serum proteins; this was followed by elution of low molecular weight materials. Pools of the fractionated materials were assayed for their eosinophilopoietic activity in comparison with the original serum (MS-AES) samples. Significant eosinophilopoietic activity (67% of that induced by MS-AES) was found co-eluting with salts and low molecular weight materials, whereas there was no detectable activity in any other pool. These findings were confirmed by repeating the experiment on three occasions each of which showed similar results (72, 68, and 82% of initial activity) eluting with low molecular weight materials. Subsequently, starting from 65% of the bed volume, 10-ml fractions were collected and assayed individually in groups of five mice (Fig. 5). Fractions eluting between 65 and 77% of the bed volume had no detectable eosinophilopoietic activity, while three 10-ml fractions collected between 78 and 95% of the bed volume induced respective increases in bone marrow eosinophils of 100, 160, and 130% as compared to MS–NRS-treated controls. The activity of MS-AES was eluted in fractions collected between the markers B_{12} (mol wt 1,357) and [^{14}C]histamine (mol wt 186) (Fig. 5).

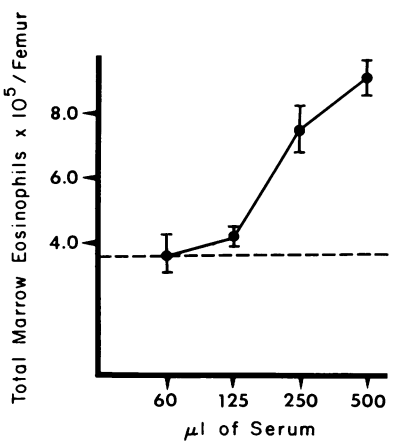

FIGURE 4 Eosinophil counts in the bone marrow of mice 2 days after injection intravenously with increasing doses of MS-AES. Each point represents the mean±SEM of five mice. The mean bone marrow eosinophil counts in a group of 15 normal mice is represented on the graph by the broken line.

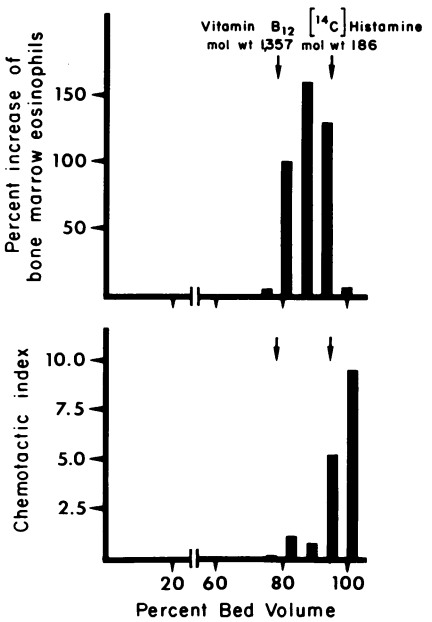

FIGURE 5 Gel-filtration of MS-AES on Sephadex G-25. 10-ml fractions were collected, lyophilized, and reconstituted to original sample volume. For eosinophilopoietic activity, 0.3 ml was injected intravenously in each mouse, and groups of five mice were used to assay each fraction. For chemotactic activity, 100 μl from each fraction were added to the attractant side and the chemotactic index was measured after a 2-h incubation (each value represents the average of duplicate determinations).

Fractions eluting after 95% of the bed volume had no eosinophilopoietic activity.

Enzymatic digestions. Active fractions collected from G-25 columns and exposed to pronase showed marked loss of activity. The mean bone marrow eosinophil count in five mice injected with active fractions of MS-AES was $4.8 \pm 0.8 \times 10^5$/femur on day 2; in those injected with pronase-treated fractions, the corresponding value was $2.4 \pm 0.6 \times 10^5$/femur ($P < 0.01$). Pronase treatment thus resulted in 80% reduction of the original activity of MS-AES. Animals treated with trypsin-digested fractions, however, had a mean count of $6.0 \pm 0.8 \times 10^5$/femur indicating no loss of activity.

Eosinophil chemotaxis. The chemotactic index of the different fractions eluted by G-25 is shown in Fig. 5. No significant chemotactic activity was demonstrated for fractions which were eosinophilopoietic, but significant chemotaxis occurred with fractions eluted between 92 and 104% of the bed volume.

Effect of eosinophil destruction in vitro. The supernatant fluid of eosinophils destroyed in vitro by AES were fractionated on G-25 columns. The eosinophilopoietic activity of the different fractions of MS-AES and of the supernate of the eosinophil-AES mixture is shown in Table II. Whereas fractions of MS-AES which eluted between 78 and 95% of the bed volume showed eosinophilopoietic activity, the corresponding fractions obtained from the cytotoxicity reaction had no detectable activity.

DISCUSSION

In earlier studies, methods were developed to raise antibodies in rabbits against purified suspensions of mouse or human eosinophils (13, 22, 23). The AES contained agglutinating and cytotoxic antibodies which reacted specifically with eosinophils but did not cross-react with any other formed blood elements (13, 23). Of particular importance was the specificity of these antisera for the mature eosinophil; on administration of AES to living animals, mature eosinophils in the tissues, peripheral blood, and bone marrow were depleted without affecting the immature bone marrow pool of eosinophils (1). The prolonged eosinophilopenia in the presence of normal total leukocyte levels made it possible to study the role of the eosinophil in different host responses. For example, eosinophils were demonstrated to be the major effector cell in the resistance to worm infections, in that their depletion abrogated the acquired immunity to *Schistosoma mansoni* in mice (24) and resulted in a significant increase in numbers of muscle stage larvae in *T. spiralis* infections (25). Eosinophils have also been shown to be the effector cell in an in vitro system involving antibody-dependent damage of the maturing forms of *S. mansoni* (26–28). Monospecific AES were originally developed to study the role of eosinophils, but it was quickly realized that they might be a good means of elucidating the regulation of eosinophil production.

Although there is extensive literature on the possible existence of "granulopoietin" (29–31) and "leukocytosis-inducing factor" (32, 33) both of these substances effect only neutrophils. Physiologic and pathologic fluctuations in the counts of different granulocytes occur independently and suggest there may be specific control mechanism for the individual cell lines. The availability of AES, a specific immunologic tool capable of depleting mature eosinophils, has provided an opportunity to study the generation of factors stimulating the multiplication of eosinophil precursors.

Repeated injections of AES into mice depleted the mature eosinophils while the immature bone marrow eosinophils proliferated. In earlier studies we demonstrated that the increase of bone marrow eosinophils after AES treatment is due to an increase in the promyelocyte-myelocyte stage of eosinophil development (1). In the present investigation, transfer to normal mice of sera or their low molecular weight fractions from animals treated with AES induced a significant increase in the eosoinophil population. The rise in bone marrow eosinophils was associated with an increase of [^{3}H]thymidine-labeled cells, reflecting proliferation of the mitotic pool (17). Inasmuch as the eosinophilopoietic response occurred after a delay of 2–4 days, it also indicated new cell formation and not a release effect (34). Subsequently, the response was shown to be related to the presence of specific antibodies against the eosinophils

TABLE II

Bone Marrow Eosinophil Counts 2 Days after Treatment of Mice with Active Fractions of MS-AES Collected from G-25 Sephadex Columns Compared to the Corresponding Fractions of the Supernate of Eosinophil Destruction In Vitro

	Bone marrow eosinophils ($\times 10^5$/femur) on day 2		
Bed volume	MS-AES	Supernate of eosinophil destruction	*P*
%			
74–79	3.6±0.7	4.1±0.7	NS*
80–85	6.1±0.6	3.2±0.8	<0.05
86–91	8.2±1.0	3.6±0.9	<0.05
92–97	5.0±0.4	2.6±0.2	<0.05
98–103	3.1±0.8	3.2±1.0	NS*

Each value represents the mean±SE of five animals.
* No significant difference.

in that AES previously absorbed with eosinophils generated no detectable eosinophilopoietic activity. Moreover, treatment of mice with ANS, followed by transfer of their sera to normal recipients, produced no effect on the eosinophil population in the peripheral blood or the bone marrow, and the percent [^{3}H]-thymidine labeling of bone marrow eosinophils was unchanged.

Further characterization of the eosinophilopoietic activity was then undertaken. The activity was found to be: (*a*) dependent on the volume of serum transferred, (*b*) lost on dialysis, and (*c*) largely heat labile. Gel chromatography revealed that the eosinophilopoietic activity was due to a low molecular weight substance in the range of 186–1,357. Digestion by pronase suggested that the material was a peptide; trypsin had no effect indicating a lack of lysine or arginine residues (18). The eosinophilopoietic fractions collected from gel chromatography were not chemotactic to mouse eosinophils in vitro. Chemotactic activity, however, was demonstrated in subsequent fractions which may be due to previously described chemotactic factors (7) or histamine (35).

These results indicate that a low molecular weight, peptide-like material is generated after depletion of mature eosinophils. This substance which we propose to call "eosinophilopoietin" is a specific stimulator of eosinophilopoiesis in vivo. The kinetics of the response resemble those previously reported on the eosinophilia in the marrow of animals infected with *T. spiralis* which began to develop after a delay of 23 h and doubled at 49 h (36). Our characterization studies suggest that eosinophilopoietin is different from some other factors known to influence the eosinophil leukocyte. The eosinophil stimulation promoter described by Colley is a lymphokine secreted by sensitized T lymphocytes and has a molecular weight of 24,000–56,000 (10, 37). The eosinophil chemotactic mediators described in Austen's laboratory (7, 38) have a range of molecular weight similar to eosinophilopoietin, yet the latter has no chemotactic activity. Another chemotactic factor described by Cohen and Ward is probably also different inasmuch as it is dependent for its activity on both immune complexes and substances released into lymphocyte cultures (8).

In conclusion, we have demonstrated that specific acute depletion of eosinophils generates an eosinophilopoietic substance that is of low molecular weight and is capable of inducing a quantitative and proliferative increase in bone marrow eosinophils. Preliminary investigations indicate that this substance has a molecular weight in the range of 186–1,357, is digested by pronase but not by trypsin, and is partially inactivated by heat. It is suggested that this substance be named eosinophilopoietin.

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