PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 148, 301-306 (1975)

Chemotactic Deactivation of Human Eosinophils by the Eosinophil Chemotactic Factor of Anaphylaxis[1] (38527)

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The eosinophil chemotactic factor of anaphylaxis (ECF-A) is an acidic peptide (1) mediator of approximately 500 mol wt which is preferentially chemotactic for guinea pig (2, 3) and human (1, 4, 5) eosinophils as assessed with mixed populations of target cells, and is the most active eosinophilotactic factor for purified eosinophils when compared to other factors at concentrations exhibiting similar neutrophil chemotactic activity (1). ECF-A is released by antigen challenge from guinea pig lung fragments passively sensitized with IgG1 (2), and by an IgE dependent mechanism from human lung and nasal polyp fragments (4, 6, 7), isolated human lung cells (8) and purified rat peritoneal mast cells (6). ECF-A is a preformed mediator which has been specifically associated with rat mast cells (6) and human leukemic basophils (9) by its extraction from essentially pure populations of these cell types; in the rat mast cell it is associated with the granules (6).

Rabbit neutrophils preincubated with certain chemotactic factors exhibit a time-and-dose-dependent diminution of the chemotactic response to a subsequent stimulus; this phenomenon has been termed deactivation (10). Deactivation is associated with the activation and decay of an esterase required for the chemotactic response and derived from proesterase one by stimulation with the chemotactic principle (11). In contrast to complement-derived factors, low molecular weight bacterial chemotactic factor neither deactivates the cell nor activates proesterase one (12). In the present study the capacity of human eosinophils to undergo deactivation in response to ECF-A and other principles has been established, and deactivation by ECF-A has been further analyzed for its time course, selectivity and magnitude.

Materials and Methods. Polystyrene disposable chemotactic chambers (Adaps, Inc., Dedham, MA) were assembled with 3 μm or 8 μm pore size micropore filters (Millipore Corp., Bedford, MA) as previously described (6). Hanks' solution and Medium 199 with phenol red (Microbiological Associates, Bethesda, MD), ovalbumin five times recrystallized (Miles Laboratories, Inc., Miles Research Co., Kankakee, IL), dextran, Sephadex and Ficoll (Pharmacia Fine Chemicals, Inc., Piscataway, NJ), sodium diatrizoate (Hypaque, Worthington Laboratories, New York, NY), sodium metrizoate (Triosil-75, Glaxo, Ltd., England), two times recrystallized trypsin and soybean trypsin inhibitor (Worthington Biochemical Corp., Freehold, NJ), (1-^{14}C)-glucose (Amersham-Searle Corp., Arlington Heights, IL), sodium lauryl sulfate (SLS) (Fisher Scientific Co., Medford, MA) and plastic 35 × 10 mm Petri dishes (Falcon Plastics, Div. of B–L Laboratories. Inc., Los Angeles, CA) were obtained from the manufacturers.

Beta radiation from (^{14}C) glucose solution was quantitated with Bray's fluid in a liquid scintillation counter (Nuclear-Chicago Corp., Des Plaines, IL).

Measurement of chemotaxis. Purified populations of eosinophils, mononuclear leukocytes and neutrophils were obtained from peripheral blood of normal donors or individuals with eosinophilia by modifications (6, 13) of previously published methods (14, 15). Chemotaxis of human leukocytes was assayed by a modification (6, 13) of the Boyden micropore filter assay (16) employing 3 μm filters for neutrophils (13) and eosinophils (6), and 8 μm filters for mono-

[1] This work was supported by Grant Nos. AI-07722, AM-05577, and AI-10356 from the National Institutes of Health.

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nuclear leukocytes (16). The medium for all experiments was Hanks' balanced salt solution, 0.5% ovalbumin, pH 7.4. The chemotactic factors in all experiments were ECF-A purified from extracts of human lung by Sephadex G-25 gel filtration at a final concentration of 2 g equivalents of lung/ml (6), human C5a generated by tryptic digestion of 50 μg highly purified C5/ml (17) and human kallikrein generated from purified prekallikrein by activation with Hageman factor fragments and generating 2.5 μg bradykinin/ml from 0.2 ml heat inactivated plasma (18). 2×10^6 cells were incubated for 3 hr at 37° in moist chambers and the leukocyte responses were expressed as the mean of 10 high power fields (hpf), five from each of duplicate chambers, corrected for background counts in filters from chambers without a chemotactic stimulus.

Deactivation. Human leukocytes were incubated at room temperature from 1 to 60 min with various chemotactic factors. The cells were then sedimented at room temperature by centrifugation for 5 min at 400 *g* and resuspended in buffer. The wash procedure was performed twice and the cells transferred to the cell compartment of a modified Boyden chamber where they were incubated for a further 3 hr with a chemotactic stimulus. Cells incubated with buffer rather than a chemotactic factor during the deactivation period served as controls. Net chemotaxis reflects a correction for spontaneous migration in buffer alone. Spontaneous migration varied from 0 to 5 cells/hpf for control cells to 0–8 cells/hpf for cells preincubated with chemotactic factors to achieve deactivation. The extent of deactivation was quantitated and expressed as:

chemotaxis (% of control)

= (net chemotaxis of deactivated cells/

net chemotaxis of control cells) × 100

Hexose monophosphate shunt (HMPS) activity of adherent leukocytes. The HMPS activity of purified adherent neutrophils and eosinophils was determined by measuring the extent of conversion of (1-^{14}C) glucose to $^{14}CO_2$ after 80 min at 37° (19). The cpm of $^{14}CO_2$ were standardized by dividing each value by the OD at 280 nm of a 3% SLS solution of the adherent leukocytes and the results expressed as cpm/0.2 absorbency units 280 (AU 280). The effect of chemotactic factors upon HMPS activity was calculated by subtraction of the mean value for duplicate control cells from that of treated cells.

Results. Chemotactic deactivation by ECF-A of human eosinophils. The capacity of human eosinophils preincubated with ECF-A to be rendered unresponsive to subsequent stimulation by the same principle in a chemotactic chamber was analyzed with both purified and unpurified cells. Eosinophils were purified to 77% purity from a donor (M.S.) with a drug reaction, while those from a patient with rheumatoid arthritis (G.R.) with 90% eosinophilia were employed unpurified. 2×10^6 eosinophils were exposed for varying times to a 1:10 dilution of ECF-A and compared to identical populations of eosinophils preincubated for the same duration with buffer alone. The chemotactic response of cells from these two individuals preincubated with ECF-A was diminished at 2 min by 70 and 80%, respectively (Fig. 1).

The effect of varying concentrations of ECF-A on deactivation of 2×10^6 eosinophils purified to 83% purity from a patient (H.B.) with metastatic carcinoma was compared at 2 and 30 min. Incubation of 2×10^6 eosinophils for 30 min with dilutions of ECF-A ranging from 1:10 to 1:160 resulted in nearly complete deactivation. At 2 min deactivation was dose-dependent, with a

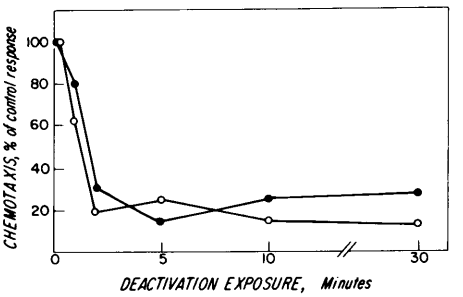

FIG. 1. Time course of chemotactic deactivation of human eosinophils by ECF-A. A 1:10 dilution of ECF-A attracted 49 control eosinophils from patient M.S. (●——●) and 78 from patient G.R. (○——○). Deactivation is depicted as % control chemotactic response.

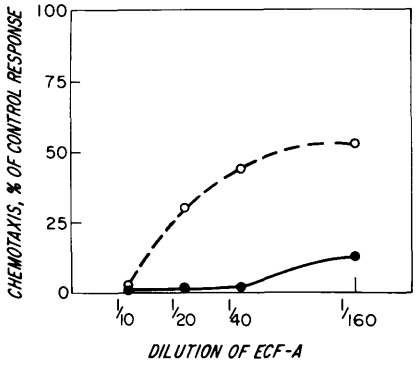

FIG. 2. Dose response of chemotactic deactivation of human eosinophils by ECF-A. A 1:10 dilution of ECF-A attracted 55 control eosinophils/hpf. Deactivation is depicted as % control response of cells exposed to ECF-A for 2 min (○——○) and 30 min (●——●).

50% reduction in chemotactic response occurring at a 1:40 dilution (Fig. 2). As the net chemotactic activity, assessed at 3 hr in a modified Boyden chamber, of the dilutions employed was 55, 45, 28 and 3 cells/hpf for the dilutions 1:10, 1:20, 1:40 and 1:160, respectively, deactivation was apparent at both 2 and 30 min with a concentration (1:160) which was not demonstrably chemotactic.

The effect of preincubation with ECF-A on the responses of 2×10^6 eosinophils (88% purity, patient M.S.), neutrophils (93% purity), and mononuclear cells (96% purity), to ECF-A, kallikrein, and C5a, respectively, was determined. Each cell type was incubated with a 1:10 dilution of partially purified ECF-A for varying time intervals, washed twice and placed in a modified Boyden chamber for assessment of the chemotactic response. The fall in chemotactic activity of the mononuclear and neutrophilic leukocytes was 30% and 20%, respectively, at 2 min as compared to 72% for eosinophils, and there was little change thereafter (Fig. 3). The chemotactic activity of the ECF-A employed was 37 control eosinophils/hpf, 12 control mononuclear cells/hpf and 8 control neutrophils/hpf.

Chemotactic deactivation of eosinophils by diverse stimuli. 2×10^6 eosinophils of 72% purity from patient M.S. were exposed to dilutions of ECF-A (1:20) and C5a

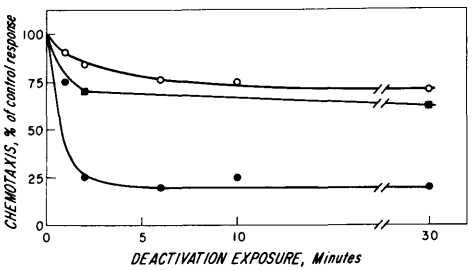

FIG. 3. Time course of deactivation of human leukocytes by ECF-A. Chemotactic stimuli were a 1:10 dilution of ECF-A which attracted 37 control eosinophils/hpf (●——●), a 1:10 dilution of C5a which attracted 56 control mononuclears/hpf (■——■) and a 1:10 dilution of kallikrein which attracted 63 control neutrophils/hpf (○——○). Deactivation is depicted as % control chemotactic response.

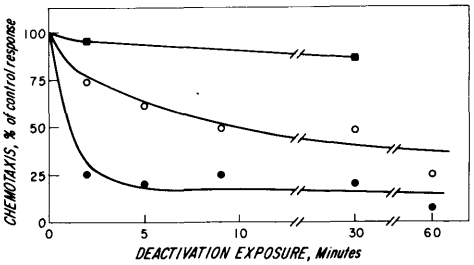

FIG. 4. Eosinophil deactivation by ECF-A (●——●), kallikrein (■——■) and C5a (○——○). Chemotactic response of control eosinophils to a 1:10 dilution of ECF-A was 21 cells/hpf. Deactivation is depicted as % control chemotactic response.

(1:10), which exhibited comparable chemotactic activity for eosinophils, and to a dilution of kallikrein (1:10) which exhibited chemotactic activity similar to C5a for purified neutrophils. After varying time intervals the cells were washed twice and transferred to modified Boyden chambers to assess chemotaxis to a 1:10 dilution of ECF-A. C5a elicited a slower and less profound deactivation of eosinophils than did ECF-A, while kallikrein had no demonstrable effect (Fig. 4).

Deactivation, which had been routinely assessed by diminution in response to ECF-A, was next examined by comparing ECF-A and C5a in a cross deactivation experiment. 2×10^6 eosinophils were exposed for 30 min to dilutions of ECF-A (1:40) and C5a (1:20) exhibiting comparable chemotactic activity for eosinophils or

to a dilution of kallikrein (1:10) which exhibited chemotactic activity similar to C5a for purified neutrophils. The cells were washed twice and portions were transferred to modified Boyden chambers to assess their chemotactic response to C5a and to ECF-A. As shown in Table I, eosinophils deactivated by ECF-A or by C5a were equally limited in their response to the homologous or heterologous stimulus, reflecting full cross deactivation. Kallikrein was not chemotactic for eosinophils and did not deactivate them for either stimulus.

The interaction between chemotactic factors and adherent neutrophils is associated with a stimulation of HMPS activity as assessed by generation of radioactive $^{14}CO_2$ from ($^{14}C_1$) glucose (19). Thus C5a (1:40) and kallikrein (1:40) exhibiting chemotactic activity for purified neutrophils from a normal donor demonstrated augmentation in HMPS activity of these cells; while ECF-A (1:25), at the concentrations employed, lacked chemotactic activity for this human neutrophil population, and caused only a minimal augmentation in HMPS activity. The effect of these same factors upon the HMPS of adherent eosinophils was also examined. Both ECF-A and C5a, with eosinophilotactic properties, increased HMPS activity; while kallikrein was minimally active (Table II).

TABLE I. CHEMOTACTIC CROSS DEACTIVATION OF HUMAN EOSINOPHILS.

	Chemotactic stimulus % deactivation at 30 min	
Deactivating agent	ECF-A	C5a
ECF-A	90	90
C5a	75	70
Kallikrein	<10	<10

Discussion. Deactivation, the time- and dose-dependent diminution of the chemotactic response of neutrophilic leukocytes, rabbit (10) or human (20), induced by preincubation with chemotactic principles is also a characteristic of the eosinophil. At 2 min deactivation of eosinophils by ECF-A could be shown to be dose-dependent, while complete deactivation was noted with a range of doses over a 30 min period (Fig. 2). The deactivation of eosinophils observed with a non-chemotactic dose of ECF-A (1:160, Fig. 2) may be due to the concentration or distribution of ECF-A at the surface of all the cells as compared to the relationships achieved by diffusion of ECF-A through the micropore filter of the chemotactic chamber. Deactivation by ECF-A was demonstrable not only with purified eosinophils but also with eosinophils obtained directly from a patient with 90% eosinophilia (Fig. 1). A structurally unrelated eosinophilotactic factor, C5a, employed at a comparable eosinophilotactic dose also was capable of eliciting deactivation (Fig. 4). Further, there was cross deactivation between ECF-A and C5a, while kallikrein, which was not eosinophilotactic at the concentrations employed, did not deactivate eosinophils to either of the aforementioned stimuli (Table I).

A relationship between chemotaxis and deactivation has been observed both in the relative deactivating activity of diverse chemotactic stimuli on the same cell type, and in the response of different cell types to the same stimulus. Thus ECF-A and C5a,

TABLE II. EFFECT OF CHEMOTACTIC FACTORS ON HMPS ACTIVITY OF HUMAN EOSINOPHILS AND NEUTROPHILS.

	HMPS activity net cpm/0.2 AU_{280}[a]		Chemotaxis net cells/hpf	
Eosinophils	Eosinophils	Neutrophils	Eosinophils	Neutrophils
ECF-A	554	290	6	0
C5a	474	1014	10	27
Kallikrein	154	686	1	19

[a] Reflects correction for the baseline values of control neutrophils and eosinophils which had 897 and 754 cpm/0.2 AU_{280}, respectively.

which are eosinophilotactic, deactivated eosinophils, while kallikrein did not (Fig. 4). On the other hand, kallikrein, which is chemotactic for neutrophils, deactivated this cell type even more rapidly than a comparably chemotactic dose of C5a (20). Further, the modest deactivation of mononuclear cells and neutrophils by a dose of ECF-A which profoundly deactivates eosinophils is consistent with the relatively lesser chemotactic activity of ECF-A for these cells (Fig. 3) (21).

The stimulation of the HMPS of purified adherent neutrophils by chemotactic principles is dependent upon the functional integrity of the principle and is not observed with prekallikrein or kallikrein inactivated by diisopropylfluorophosphate (19). Stimulation of HMPS activity of purified adherent eosinophils was observed with ECF-A and C5a while kallikrein had a minimal effect (Table II). Conversely both kallikrein and C5a, which are chemotactic for neutrophils, stimulate their HMPS activity while ECF-A had a minimal effect compatible with its limited chemotactic activity for this cell type (Table II) (21).

The finding that deactivation follows interaction of rabbit or human leukocytes with chemotactic principles derived from human plasma such as kallikrein and C5a, or derived from mast cells (ECF-A) may imply a trapping mechanism for migrating cells. Thus cells attracted to a site of chemotactic factor generation might remain after the initial gradient had subsided or shifted elsewhere. The release of ECF-A by mast cells participating in IgE-dependent reactions would selectively attract and then hold eosinophils at the site of the reaction. A recently recognized regulatory function which could then be expressed by the eosinophil would be the release of arylsulfatase, possibly consequent to phagocytosis (1, 22) of mast cell granules (23) or the action of ECF-A itself, with consequent inactivation of SRS-A (22, 24).

Summary. Purified human eosinophils demonstrate diminished chemotactic responsiveness (deactivation) after incubation with the eosinophil chemotactic factor of anaphylaxis (ECF-A). The deactivation is rapid, and selective in that ECF-A deactivated human eosinophils more markedly than neutrophilic or mononuclear leukocytes. The eosinophil can also be deactivated by C5a which is eosinophilotactic, and there is cross deactivation between C5a and ECF-A. Deactivation may be an important physiologic control mechanism enabling the eosinophil to remain at sites of ECF-A release in order to manifest its regulatory functions in immediate hypersensitivity reactions.

1. Goetzl, E. J., Wasserman, S. I., and Austen, K. F., *in* "Proceedings of the Second International Congress of Immunology" (L. Brent and J. Holborow, eds.), North-Holland Publishing Co., Amsterdam, p. 41 (1974).
2. Kay, A. B., Stechschulte, D. J., and Austen, K. F., J. Exp. Med. **133,** 602 (1971).
3. Kay, A. B., Shin, H. S., and Austen, K. F., Immunology **24,** 969 (1973).
4. Kay, A. B., and Austen, K. F., J. Immunol. **107,** 899 (1971).
5. Wasserman, S. I., Goetzl, E. J., Ellman, L., and Austen, K. F., New Eng. J. Med. **290,** 420 (1974).
6. Wasserman, S. I., Goetzl, E. J., and Austen, K. F., J. Immunol. **112,** 351 (1974).
7. Kaliner, M., Wasserman, S. I., and Austen, K. F., New Eng. J. Med. **289,** 277 (1973).
8. Lewis, R. A., Wasserman, S. I., Goetzl, E. J., and Austen, K. F., J. Exp. Med. **140,** 1133 (1974)
9. Lewis, R. A., Goetzl, E. J., Wasserman, S. I., Valone, F., and Austen, K. F., J. Immunol., in press.
10. Ward, P. A., and Becker, E. L., J. Exp. Med. **127,** 693 (1968).
11. Ward, P. A., and Becker, E. L., J. Immunol. **105,** 1057 (1970).
12. Becker, E. L., J. Exp. Med. **135,** 376 (1972).
13. Goetzl, E. J., and Austen, K. F., J. Exp. Med. **136,** 1564 (1972).
14. Boyum, A., Scand. J. Lab. Clin. Invest. **21** (Suppl. 97), 31 (1968).
15. Day, R. P., Immunology **18,** 955 (1970).
16. Boyden, S., J. Exp. Med. **115,** 453 (1962).
17. Ward, P. A., and Newman, L. J., J. Immunol. **102,** 93 (1969).
18. Kaplan, A. P., Kay, A. B., and Austen, K. F., J. Exp. Med. **135,** 81 (1972).
19. Goetzl, E. J., and Austen, K. F., J. Clin. Invest. **53,** 591 (1974).
20. Goetzl, E. J., and Austen, K. F., *in* "Chemotaxis: Its Biology and Biochemistry" (E. Sorkin, Ed.), S. Karger, Basel, in press.

21. Kay, A. B., *in* "Biochemistry of the Acute Allergic Reactions" (K. F. Austen and E. L. Becker, eds.), p. 237. Blackwell Scientific Publications, Oxford (1971).
22. Wasserman, S. I., Goetzl, E. J., and Austen, K. F., J. Immunol., in press.
23. Mann, P. R., J. Pathol. **98,** 183 (1969).
24. Orange, R. P., Murphy, R. C., and Austen, K. F., J. Immunol. **113,** 316 (1974).

Received September 6, 1974. P.S.E.B.M. 1975, Vol. 148.