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CHEMOTAXIS OF HUMAN BASOPHIL LEUCOCYTES

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SUMMARY

Human peripheral blood basophils from two patients with unusually high basophil counts in association with chronic myelogenous leukaemia migrated *in vitro* toward various chemotactic agents of human origin. These included supernatants from sensitized lymphocytes challenged with specific antigen, diffusates from lung fragments challenged with pollen antigen E, the enzyme plasma kallikrein, and two complement derived agents, C5a and $C\bar{5}\bar{6}\bar{7}$. Thus, chemotaxis *in vitro*, a previously unreported property of human basophils, has been observed, although none of the agents tested produced a selective chemotactic response.

INTRODUCTION

The ability of neutrophils, eosinophils and mononuclear cells to react in chemotaxis has been reported by several workers (Boyden, 1962; Kay & Austen, 1971; Ward, 1968). The capacity of basophil leucocytes to respond to a chemotactic stimulus has been difficult to study since the cell is not readily available in high concentrations. We have had the opportunity to study the chemotactic response of leucocytes of two patients with chronic myelogenous leukaemia who both had an unusually high percentage of circulating basophils. Known chemotactic agents of human origin, including supernatants from lymphocytes challenged with specific antigen (Ward, Remold & David, 1970), diffusates from lung fragments challenged with antigen E (Kay & Austen, 1971), the plasma enzyme kallikrein (Kaplan, Kay & Austen, 1972), a fragment cleaved from the fifth component of complement (C5a) (Ward & Newman, 1969) and the trimolecular complex of the fifth, sixth and seventh components of complement ($C\bar{5}\bar{6}\bar{7}$) (Lachmann, Kay & Thompson, 1970) were examined for their ability to attract *in vitro* the cells of these two patients.

MATERIALS AND METHODS

Chemotactic factors

The preparation of supernatants from sensitized lymphocytes challenged with specific

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antigen, the source of streptokinase-streptodornase (SKSD) and purified protein derivative of tuberculin (PPD) used as antigens, and the method of testing for macrophage migration inhibitory factor (MIF) using guinea-pig macrophages as target cells have been described (Rocklin, Meyers & David, 1970). The lymphocytes from two normal human donors, A and B, were separated from 60 ml of peripheral blood and cultured for 3 days in the presence or absence of antigen. The cell-free supernatants were removed each day, and fresh medium was added. Each sample gave a final pooled volume of between 12 and 14 ml. After extensive dialysis against saline and then water, the supernatants were lyophilized and reconstituted in Hanks' solution. The supernatants from donor A and donor B were concentrated 2·5 and seven times, respectively. The materials were tested for chemotaxis using 0·5-ml volumes.

A diffusate was prepared from human lung passively sensitized with serum from a ragweed sensitive individual and challenged with ragweed antigen E in Tyrode's solution (Kay & Austen, 1971). The 3-ml diffusate from 300 mg of lung fragments contained histamine, slow reacting substance of anaphylaxis (SRS-A) and eosinophil chemotactic factor of anaphylaxis (ECF-A). Volumes of 0·8 ml were used in the chemotactic chambers.

Preparations of highly purified human prekallikrein and Hageman factor fragments were incubated in phosphate buffered saline to yield kallikrein as described (Kaplan *et al.*, 1972; Kaplan & Austen, 1970), and assayed for chemotaxis in volumes of 0·25 ml.

Human C5 was prepared by the method of Nilsson & Müller-Eberhard (1965). C5a was generated by incubating 50 μg of C5, contained in potassium phosphate buffer with added calcium at pH 7·8, with 10 μg of trypsin (Mann Research Laboratories) for 10 min at 30°C, after which the reaction was terminated by adding 5 μg of soybean trypsin inhibitor (Mann Research Laboratories). The amount of soybean trypsin inhibitor (SBTI) used was that which prevented the action of trypsin on benzoyl DL arginine p-nitroanilide at the same ratio of enzyme to inhibitor under conditions previously described (Eriksson, 1965). For chemotaxis, trypsinized or untreated C5 was tested in 0·15-ml volumes each containing 20 μg of protein. As a further control, equivalent amounts of trypsin and SBTI were used. With the cells from patient 2 partially purified C5a was employed. Two millilitres of trypsinized C5, prepared as described above, were applied to a 3 × 85-cm column of Sephadex G-100, using conditions previously described for the isolation of C3a (Bokisch, Müller-Eberhard & Cochrane, 1969). Three millilitre-fractions were collected. When alternate fractions were tested for chemotaxis of human peripheral blood leucocytes, activity appeared as a single peak shortly after a cytochrome C marker. Two-tenths millilitre from the tube containing the most chemotactic activity was used for basophil chemotaxis studies.

The trimolecular complex, $C\bar{5}\bar{6}7$ was made by incubating purified $C\bar{5}\bar{6}$ with C7, as described in the 'reactive lysis' procedure (Thompson & Lachmann, 1970; Goldman, Ruddy & Austen, 1972). For the chemotactic experiments, 0·005 ml of $C\bar{5}\bar{6}$ in veronal buffered saline at pH 7·5 containing 4·7 haemolytic units per ml and 0·05 ml of a 1 in 500 dilution of C7 in 0·1 M acetate buffer at pH 6·0 containing 60,000 haemolytic units per ml were employed (Goldman *et al.*, 1972).

Chemotactic assay

Chemotaxis was measured by a modification of the Millipore technique previously described (Kay, 1970). Blood was drawn into heparin and the red cells were sedimented with 6% Dextran (Kay & Austen, 1971). The leucocyte-rich supernatant was centrifuged to obtain the white cells which were washed twice in Hanks' solution and resuspended in

Hanks' solution containing 0·5% ovalbumin at a final white cell concentration of 2×10^6 per ml. The final volume in the test compartment was brought to 1 ml. After the incubation period, the Millipores were removed, rinsed in Hanks' solution and immersed for 20 sec in a mixture of 1 part absolute ethanol and 1 part saturated mercuric chloride. After washing for 3 min in running tap water, the Millipores were stained for 5 min in 1% aqueous toluidine blue. Following a further 3 min wash in tap water, the Millipores were dehydrated, cleared and mounted as previously described. Basophils were counted using a high power (×90) oil immersion objective; the basophil chemotactic count was expressed as the total count of ten fields.

Patients

Patient 1 is a 57-year-old white male. A diagnosis of chronic myelogenous leukaemia was made in April, 1966. At the time of the investigation he was undergoing an acute blastogenic crisis. His total white cell count was 62,000/mm^3 of which 25% were mature basophils, 35% immature basophils, 10% promyelocytes and 30% mononuclear cells. Apart from busulphan, 8 mg/day, he was receiving no other medication. Following venipuncture for chemotactic studies, he was given, intravenously, cytosine arabinoside, daunomycin and vincristine in doses of 2 mg, 1 mg and 1·5 mg/kg of body weight, respectively. Blood was drawn for further chemotactic studies 18 hr later.

Patient 2 is a 68-year-old white female. A diagnosis of chronic myelogenous leukaemia was made in April, 1969. At the time of the investigation her white cell count was 23,000/mm^3 of which 38% were basophils, 45% neutrophils, 12% blast cells, 1% metamyelocytes, 1% myelocytes, 2% lymphocytes and 1% eosinophils. She was receiving 6-mercaptopurine, 50 mg twice a week, and allopurinol, 100 mg twice a day.

RESULTS

The granulocytes from patient 1 consisted entirely of basophils or their precursors and thus it was possible to study basophil chemotaxis using a filter of 3-μm pore size since the only migrating cells were mature or immature basophils. The circulating white cells in Patient 2 were predominantly neutrophils and using a filter of 3-μm pore size, neutrophils preferentially migrated with no observable basophil chemotaxis. With a filter of 8·0-μm pore size basophil chemotaxis could be demonstrated with the cells from patient 2, although there were also high neutrophil counts.

The chemotactic response of human basophils to human lymphocyte supernatants is shown in Table 1. Supernates from lymphocyte donors A and B were tested against the cells from patients 1 and 2, respectively. Donors A and B both had strongly positive delayed-type skin reactions to 5 units of SKSD but reacted weakly to 0·1 ml of intermediate strength (0·001 mg) of PPD. Lymphocytes from donor B produced MIF following culture with SKSD. No MIF activity was detected when cells from donor B were incubated with PPD. MIF was not assayed with cells from donor A. When tested for chemotaxis, basophils from both patients migrated towards the supernatants of lymphocytes of donors A and B following incubation with SKSD. Lymphocyte supernatants from the PPD culture gave little or no chemotaxis. Samples of SKSD, PPD or medium alone or cells incubated with medium alone did not evoke basophil chemotaxis with cells from either patient.

A human lung diffusate containing ECF-A (Kay & Austen, 1971), 600 ng of histamine and

TABLE 1. Chemotactic activity of human lymphocyte supernatants for human basophils

	Lymphocytes + SKSD	Lymphocytes + PPD
Patient 1*		
Basophil chemotaxis	20	0
Patient 2*		
Basophil chemotaxis	39	12
Macrophage migration (%)†	61	91

* Supernatants from the lymphocytes of donors A and B were used to attract cells from patients 1 and 2, respectively.

† Average value from two populations of guinea-pig macrophages.

200 units of SRS-A per ml was chemotactic for the basophils from both patients. The cells from patients 1 and 2 gave chemotactic counts of 18 and 26 respectively, whereas no response was observed to antigen E alone or to diffusate from tissue not antigen challenged.

Neither the fragments derived from active Hageman factor or highly purified prekallikrein were chemotactic for basophils when tested alone (Table 2). When these factors were incubated together bradykinin was generated from heat-inactivated plasma, indicating conversion of prekallikrein to kallikrein, and the mixture was chemotactic for basophils from both patients 1 and 2.

Trypsinized C5, to which SBTI had been added, was chemotactic for basophils from patient 1 whereas no migration was seen with untreated C5 or trypsin and SBTI alone. The basophils of patient 2 migrated toward partially purified C5a but not toward untreated C5 (Table 3).

TABLE 2. Generation of chemotactic activity for human basophils by activation of prekallikrein

	Prekallikrein	Hageman factor fragments	Prekallikrein and Hageman factor fragments
Patient 1			
Basophil chemotaxis	0	0	36
Total bradykinin generated from heat inactivated plasma (μg)	0	0	2·10
Patient 2			
Basophil chemotaxis	N.D.	N.D.	35
Total bradykinin generated from heat-inactivated plasma (μg)	0	0	1·18

N.D. = not done.

TABLE 3. The chemotactic activity of trypsinized C5 for human basophils

	Basophil chemotaxis
Patient 1	
Trypsin treated C5 (with SBTI)	24
Untreated C5	0
Trypsin+SBTI	0
Patient 2	
Partially purified C5a	19
Untreated C5	2

TABLE 4. Generation of chemotactic activity for human basophils following interaction of $C\bar{5}\bar{6}$ with C7

	Basophil chemotaxis		
	$C\bar{5}\bar{6}$	C7	$C\bar{5}\bar{6}$+C7
Patient 1	10	0	20
Patient 2	4	0	20

Preparations of $C\bar{5}\bar{6}$ and C7 had little chemotactic activity when tested alone. When these factors were incubated together to form $C\bar{5}\bar{6}7$, the complex prepared unsensitized sheep red blood cells for lysis by C8 and C9 (Thompson & Lachmann, 1970; Goldman *et al.*, 1972) and was chemotactic for the basophils from both patients (Table 4).

DISCUSSION

The capacity of basophils to respond chemotactically to supernatants from lymphocytes incubated with specific antigen (Table 1), a diffusate from sensitized lung incubated with ragweed antigen E, the plasma enzyme kallikrein (Table 2) and two complement-derived factors, C5a (Table 3) and $C\bar{5}\bar{6}7$ (Table 4), has been demonstrated using cells from two patients with unusually high basophil counts. Although directional basophil migration through the entire thickness of the Millipore was observed, the overall chemotactic counts were low. In previous studies (Kay & Austen, 1971; Kaplan *et al.*, 1972), chemotaxis was expressed as mean cell counts per high power field whereas in the present report the data are presented as the total count of ten high power fields. Of interest is that a few hours after blood was drawn from patient 1, therapy was commenced with several antimitotic agents; the following day his cells did not respond in chemotaxis.

No evidence was found for a selective basophil chemotactic agent since these cells responded to all the agents tested. The preparations of kallikrein, C5a and $C\bar{5}\bar{6}7$ were puri-

fied whereas the lymphocyte supernatants and the lung diffusate contained multiple biological agents which may have influenced the identification of a specific basophil chemotactic agent.

When attempting to relate the *in vitro* observations described with clinical situations, it may be noted that, apart from neoplastic disorders, there have been few reports of conditions associated with an increase in the number of tissue basophils. High basophil counts have been observed in contact allergy (Dvorak & Mihm, 1972), the histological picture being similar to cutaneous basophil hypersensitivity described earlier in the guinea-pig (Dvorak *et al.*, 1970). This phenomenon is thought to be a form of delayed-type hypersensitivity produced under special conditions of immunization. The ability of basophils to migrate towards an anaphylactic diffusate is of interest in view of the recent report that basophil infiltration is a late event in immediate-type skin reactions in certain atopic individuals (Felarca & Lowell, 1971).

The significance of the capacity of kallikrein, C5a and $C\bar{5}\bar{6}\bar{7}$ to attract basophils is not known. It should be noted that with the cells from both patients the *in vitro* conditions were optimal for basophil migration since the basophil was only recognizable granulocyte in patient 1 and in patient 2 a large pore size was used. Our recent data on cell selectivity with the eosinophil suggest that ECF-A, present in a high concentration, will attract the neutrophil when eosinophils are absent or comprise a very small percentage of the total cell population. Thus, it is suggested that a broad specificity of chemotactic factors can be shown when conditions for migration are optimal and when other cells which may be preferentially attracted are absent.

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