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Isolation of human eosinophils and their lymphocyte-like rosetting properties

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Summary. A method for the isolation of large numbers of almost pure human eosinophils from peripheral blood using hypaque–ficoll density gradients, gelatin sedimentation and carbonyl iron is described. Eosinophils were obtained from patients with parasitic infections, allergic reactions, undiagnosed eosinophilias and normal volunteers. Erythrocyte (E) and erythrocyte–antibody–complement (EAC) rosettes were formed by a number of eosinophils.

INTRODUCTION

Attempts to separate eosinophils from human peripheral blood have been reported (Spiggs & Alexander, 1960; Archer, 1963; Day, 1970). It was observed during lymphocyte purification (Sher, Holm, Kok, Koornhof & Glover, 1976) that the eosinophil was a consistent contaminating cell. Furthermore it was found that these same eosinophils also formed E and EAC rosettes during the estimation of T- and B-lymphocyte rosettes. Hence, it seemed logical that the removal of the lymphocyte population would result in a preparation of pure eosinophils. The purpose of this paper is to describe firstly the procedure adopted to secure large numbers of almost pure eosinophils and secondly their lymphocyte-like rosetting properties.

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MATERIALS AND METHODS

Eosinophil separation

Fifteen patients with eosinophilia due to parasitic infections and allergic diatheses were investigated. Blood was obtained by venipuncture and anticoagulated with preservative free heparin (Heparin. B. P. Evans). The blood was diluted 1:1 with saline and layered on Hypaque–Ficoll S.G. 1·078–1·079 and centrifuged at 400 *g* for 40 min (Böyum, 1968).

The pellet so obtained from 60 ml of diluted blood, consisting of granulocytes and red blood cells (RBC), was resuspended in saline to a volume of 30 ml. Ten ml of 3 per cent gelatin (Difco Laboratories 0143-01) in Hanks Trisma (HT) was then added to the suspension, which was mixed well with a glass rod and allowed to sediment at 37° for 30–45 min.

The granulocyte-rich supernatant was pipetted off, washed three times with HT and resuspended in 9·5 ml of HT in a 150 × 15 mm glass tube. To this was added 0·5 ml of inactivated AB serum and 0·2 mg of carbonyl iron (General Airline and Film Corporation, Linden, New Jersey). The suspension was placed in a water bath at 37° for 10 min shaking gently every 2–3 minutes. Immediately after incubation, the cells were placed in an ice bath for a few minutes followed by the removal of the phagocytosed carbonyl iron with a magnet, contact pull 2 kg, diameter 35 mm, pole-to-pole distance 30 mm (Eclipse). Four washings were done. The residual RBC were removed by ammonium chloride lysis

(Boyle, 1968). After a further three washings with HT the cells were counted.

E rosettes

E rosettes were prepared according to Jondal, Holm & Wigzell (1972). Briefly, 0·25 ml of a 0·5 per cent preparation of SRBC were added to 0·25 ml of eosinophils (4×10^6/ml) and incubated at 37° for 15 mins. These were then centrifuged at 90 *g* for 5 min and left at 4° overnight. After gently resuspending the pellet, a drop was placed on a slide, covered with a coverslip and sealed. Those eosinophils with three or more SRBC were counted as rosettes. Each specimen was done in duplicate and read by two different observers. Two hundred eosinophils were counted and results expressed as a percentage. There was a close correlation between the two observers.

EAC rosettes

EAC rosettes were prepared according to Jondal *et al.*, 1972. Five per cent SRBC were incubated for 1 h at 37° with a 1:2000 dilution of sheep red cell antisera (Burroughs Wellcome). After washing three times with HT the SRBC were incubated for a further 1 h at 37° with a 1:40 dilution of fresh human AB serum (kindly supplied by Dr Shapiro of the South African Blood Transfusion Service) as a source of sublytic complement. These were then washed three times with HT and made up to 0·5 per cent suspension. As for E rosettes, 0·25 ml of SRBC suspension was added to 0·25 ml of eosinophil suspension (4×10^6/ml), centrifuged at 90 *g* for 5 min then incubated at 37° for 15 mins. The preparation was shaken vigorously for 15 s and slides prepared as for E rosettes.

RESULTS

Eosinophil separation

Details as to the yield of eosinophils, their purity and viability are presented in Table 1.

The purity varied from 75 to 98 per cent with an average of 90 per cent. This depended solely on the efficiency of the Hypaque–Ficoll separation as the contaminating cell type was usually the lymphocyte. A further separation in Hypaque–Ficoll might yield a higher degree of purity. The viability by vital dye exclusion was very high being almost 100 per cent. Storing eosinophils in RPMI 1640 (Flow Laboratories CAT No. 2·963 m) and 20 per cent AB serum at 4° for 48 h yielded a viability of ± 80 per cent (unpublished observations).

Patients 10, 13 and 15 yielded several million eosinophils in the absence of eosinophilia as assessed by a full blood count picture. This may make it possible to study normal eosinophils.

E and EAC rosettes

The percentage and absolute number of E and EAC-rosetting eosinophils is shown in Table 2. The average percentage of E rosettes was 15·3 with a range of 8–20. Complement receptor positive (CR^+)

Table 1. Total numbers of Eosinophils harvested from Human peripheral blood, their purity and viability

Patient	Volume of blood (ml)	Number of eosinophils per μl	Final no. of eosinophils ($\times 10^6$)	Percentage purity	Percentage viability	Diagnosis
1	100	1881	112·0	93	98	Filariasis
2	100	1080	81·0	93	99	Unknown
3	80	1386	22·0	88	92	Filariasis
4	70	3050	57·0	74	98	Lepromatous Leprosy
5	100	N.D.	155·0	87	98	Lepromatous Leprosy
6	110	4346	134·0	90	97	Filariasis
7	90	858	46·5	87	98	Allergic Eosinophilia
8	100	6664	54·0	88	97	Hookworm
9	65	3980	48·0	98	99	Filariasis
10	90	336	11·0	85	99	Allergy
11	75	1936	12·0	98	98	Filariasis
12	100	732	14·4	92	99	Unknown
13	100	68	4·5	80	96	Strongyloides
14	100	999	43·0	85	97	Unknown
15	87	116	10·0	90	98	Unknown

Table 2. Percentage and absolute numbers of E and EAC-rosetting eosinophils from human peripheral blood

Patient	Percentage E rosettes	Percentage EAC rosettes	Total no. eosinophils per μl	Absolute no. E rosettes per μl of blood	Absolute no. EAC rosettes per μl of blood	Diagnosis
1	20·0	24·5	3980	796	975	Filariasis
2	20·0	21·0	6664	1333	1399	Hookworm
3	19·0	13·0	4346	826	565	Filariasis
4	10·0	23·0	1386	139	319	Filariasis
5	13·5	21·0	1080	146	226	Eosinophilia
6	13·0	16·0	1881	245	301	Filariasis
7	8·0	38·0	1936	155	736	Filariasis
8	12·0	33·0	732	88	242	Unknown
9	21·5	11·5	68	15	8	Strongyloides
10	16·0	21·5	990	158	213	Unknown

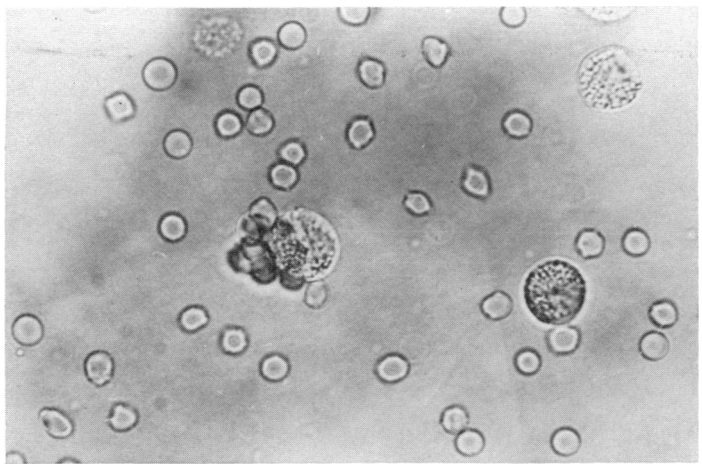

Figure 1. EAC rosettes showing polarization or capping of SRBC × 950.

eosinophils had an average percentage of 22·3 and a range of 11·5–38. Certain rosettes showed a definite polarization or capping of SRBC (Fig. 1). Others showed a uniform distribution (Figs 2 and 3).

DISCUSSION

The technique described for separating eosinophils is a simple and uncomplicated one requiring no elaborate laboratory apparatus. It makes use of previously well-established techniques and is hence a reliable and reproducible method. It was found that certain eosinophils phagocytosed carbonyl iron as efficiently as polymorphonuclear leucocytes (PMN) while others did not. To overcome this problem certain manipulations were required. The period of incubation was reduced from 30 min to 10 min followed by immersion in an ice bath. This, together with a reduction in the amount of inactivated serum, resulted in a selective removal of PMN phagocytes, leaving large numbers of eosinophils. An occasional eosinophil was seen to have phagocytosed small particles of carbonyl iron.

The significance of the ability of certain eosinophils to form E and EAC rosettes is not known at present. However, one can deduce from this that the plasma membrane of eosinophils shares certain receptor sites in common with lymphocytes. Using different markers, IgG and IgE has been shown to

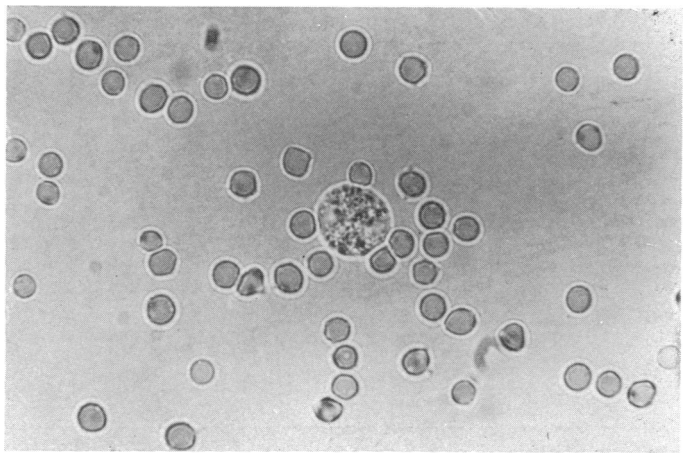

Figure 2. EAC rosettes showing typical distribution of SRBC round the eosinophil plasma membrane (Magnification × 950.

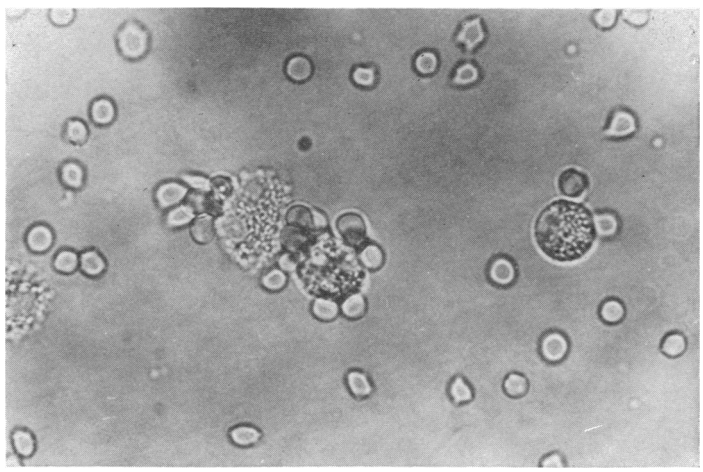

Figure 3. E rosette showing both peripheral distribution of SRBC and capping phenomenon. Note amoeboid shape of the eosinophil with capping.

be present on the plasma membrane of some eosinophils (unpublished data). IgE has been shown to be present on the eosinophil membrane by Hubscher using ferritin labelling (Hubscher, 1975). The presence of IgE on the eosinophil membrane, in different disease states, is being studied at present in our laboratory.

Polymorphism of a number of eosinophils was seen during the preparation of rosettes. The amoeboid forms observed did not interfere with the ability to form rosettes.

In conclusion, it is hoped, that this method for isolating purified suspensions of eosinophils from human peripheral blood will benefit workers in this field of research.

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