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Human eosinophils and parasitic diseases: light and electron microscopy evidence of interaction with sheep erythrocyte

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SUMMARY

The aim of this study was to investigate the possible formation of spontaneous rosettes between human eosinophils and sheep erythrocytes. In patients with parasitic diseases a percentage of 15·7±6 (range 6–23%), eosinophils were able to form rosettes. Each rosette showed as few as four to eight erythrocytes attached per eosinophil. Two types of interaction were observed at the electron microscope: point attachments and large surface contacts. In patients with non-parasite induced eosinophilia the formation of rosettes is decidedly below the above-mentioned percentage (<2%) or totally absent. It follows that during parasitic infections with blood eosinophilia, whatever the mechanism responsible, the formation of spontaneous rosettes could be of diagnostic significance and might reflect qualitative or quantitative modifications in the eosinophil population.

INTRODUCTION

Bronchial asthma, allergic rhinitis, gastrointestinal allergies and drug reactions may be associated with an increased level of eosinophils in peripheral blood, in secretions and in tissues. Eosinophilia is also a characteristic feature of many parasitic infestations such as schistosomiasis, ascariasis, filariasis and trichinosis (Butterworth, 1977). Previous studies have shown the eosinophil capable of responding to multiple chemotactic stimuli (Ward, 1969; Wissler, Sorkin & Stecher, 1974; Wasserman *et al.*, 1975; Robinson & Miller, 1975; Clark, Gallin & Kaplan, 1975; Turnbull & Kay, 1976; Colley, 1976; Goetzl, 1976) and of phagocytosing bacteria (Mickenberg, Root & Wolff, 1972), mycoplasma (Zucker-Franklin, Davidson & Thomas, 1966), serum coated erythrocytes (Archer & Hirsch, 1963), antigen–antibody complexes (Litt, 1964) and inert particles (Cotran & Litt, 1961; Kostage, Rizzo & Cohen, 1967). Furthermore, eosinophils play a functional role as killer cells in antibody-dependent cellular cytotoxicity against schistosomules (Butterworth *et al.*, 1977a; David *et al.*, 1977; Butterworth *et al.*, 1977b), against human and chicken erythrocytes (Parrillo & Fauci, 1978; Sanderson & Thomas, 1978) and as natural killer cells against nucleated target cells *in vitro* (Parrillo & Fauci, 1978).

Membrane receptors for C4, C3b, C3d and the Fc portion of immunoglobulins (IgG) have already been identified (Anwar & Kay, 1977; Gupta *et al.*, 1976; Parrillo & Fauci, 1978), while the existence of receptors for unsensitized sheep red blood cells appears to be controversial. So far, only Sher and Glover (1976) have observed with the light microscope the formation of spontaneous rosettes between sheep red blood cells and human eosinophils; Hsu and Fell (1974) observed an occasional rosette-forming eosinophil during studies on the interactions between human polymorphonuclear cells and sheep erythrocytes. Furthermore, Yang and Kennedy (1976) reported that dog eosinophils separated from canine transmissible venereal sarcoma were able to form spontaneous rosettes with washed human erythrocytes in a manner similar to that of T lymphocytes.

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The aim of the present study was to verify the real significance of the interactions between sheep erythrocytes and human eosinophils purified from peripheral blood, to examine this phenomenon from an ultrastructural point of view and to determine whether it is related to one or more of the diseases capable of inducing apparent blood eosinophilia.

MATERIALS AND METHODS

Eosinophil separation. Volumes of blood varying from 20 to 40 ml donated by patients with parasitic diseases (n°7), allergic diatheses (n°10) and other diseases (n°3) with blood eosinophilia (Table 1) were processed according to Sher and Glover's separation procedure (1976) slightly modified as follows: blood diluted 1:1 in HBSS (Hank's Balanced Salt Solution) was layered on Lymphoprep (Nygaard Company, Oslo) at a density of 1078 g/ml and then centrifuged at 400 *g* for 40 min. After resuspension of the sediment in 30 ml of HBSS, 10 ml of 3% gelatin in HBSS were added and the mixture was left to settle for 60 min at 37°C. The supernatant was centrifuged once at 200 *g* for 10 min and the sediment brought to a volume of 9·5 ml with HBSS supplemented with 0·5 ml of foetal calf serum (Gibco) inactivated at 56°C for 30 min, then 200 mg of carbonyl iron (Koch Light, England) were added. After 15 min at 37°C with gentle shaking every 3 min, the tube containing the mixture was placed in an ice bath (4°C) for 2 min. The carbonyl iron was removed with a magnet and the cells, washed once in HBSS, were brought to a volume of 5 ml and then layered on 2 ml of Lymphoprep. After centrifugation at 350 *g* for 20 min the supernatant was aspirated and the pellet, washed twice, was incubated at 37°C with 4·5 ml of tris NH_4Cl for 5 min in order to lysate the contaminating erythrocytes. After two further washings the eosinophils obtained were identified under the optical microscope on May-Grünwald Giemsa stained preparations, then counted and checked for viability by trypan blue dye exclusion.

E rosettes. 0·25 ml of eosinophils (4×10^6/ml) were added to 0·25 ml of a suspension of sheep erythrocytes (0·5%). After incubation for 15 min at 37°C, the preparations were centrifuged at 100 *g* for 5 min and left at 4°C for 2, 4, 6 and 12 hours. After resuspension the cells were further checked for viability and counted. Only those eosinophils interacting with three or more erythrocytes were considered rosettes.

TABLE 1. E-rosetting eosinophils in parasitic and non-parasitic diseases

Subject	Diagnosis	Eosinophils in original WBC differential (%)	Eosinophils after separation (%)	Yield (%)	Viability (%)	E rosettin eosinophils (
CV	Schistosomiasis	29	95	48	98	9
KA	Schistosomiasis	18	97	46	96	6
DAM	Ascariasis	17	88	48	93	18
DEC	Schistosomiasis	32	93	61	99	20
NM	Trichinosis	50	91	71	91	23
DM	Filariasis	26	92	51	99	16
TL	Schistosomiasis, hookworm, amoebiasis	31	98	53	96	18
	Totals (parasitic diseases)	29 (±11)	93 (±3)	54 (±8)	96 (±3)	15 (±
VL	Allergic rhinitis	9	95	33	95	0
DFF	Bronchial asthma	16	93	38	96	0
GT	Chronic ulcerative colitis	8	96	34	91	0
CA	Bronchial asthma	48	97	69	95	0
GG	Allergic rhinitis	28	96	50	97	0
GM	Bronchial asthma	10	89	31	95	0
DCM	Bronchial asthma	48	98	58	98	2
SG	Chronic hepatitis	9	95	48	95	0
CL	Atopic eczema	18	97	46	93	0
MT	Allergic rhinitis	11	97	42	88	2
BA	Bronchial asthma	12	96	34	98	0
SS	Bronchial asthma	44	93	46	93	0
MZ	Eosinophil leukaemia	72	93	74	96	1
	Totals (non-parasitic diseases)	25 (±20)	95 (±2)	46 (±13)	94 (±12)	0, 38

Electron microscopy. Samples were first fixed with 2·5% glutaraldehyde in 0·13 M sodium phosphate buffer pH 7·2 and postfixed in 1% OsO_4 in the same buffer. Cells were then suspended in 2% agar, dehydrated, stained with uranyl acetate in absolute alcohol and finally embedded in Epon 812. Sections were cut on a Porter Blum MT-2 ultramicrotome and after staining with lead citrate, observed at a Siemens Elmiskop 102 electron microscope.

RESULTS

Our eosinophil suspension showed a purity of 88–98%, with yields of 31–74% of the total number of eosinophils in the initial blood sample. Viability after purification was 88–99% (Table 1). In the separa-

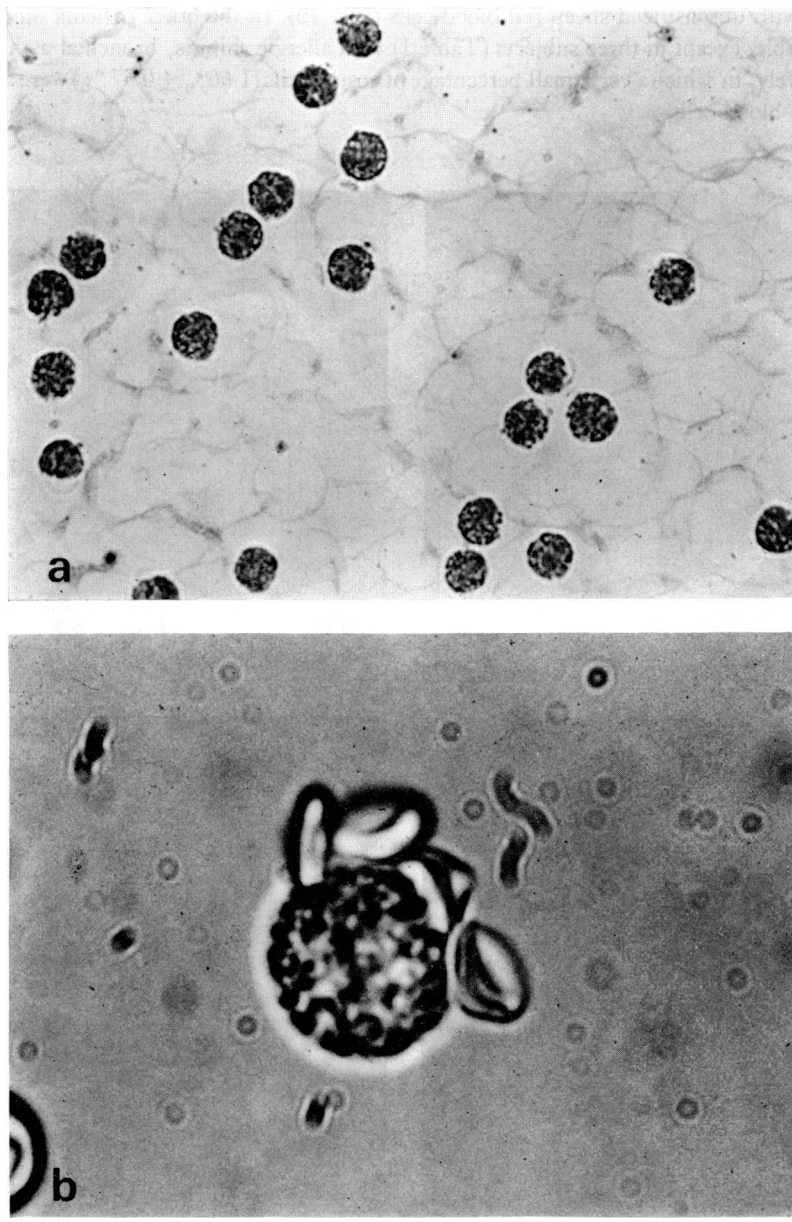

FIG. 1(a) Purified eosinophil suspension observed after May-Grünwald-Giemsa staining. (Magnification × 220.) (b) Rosette-forming eosinophil after 12 hr incubation at 4°C. (Magnification (a) × 220; (b) × 9000.)

tion procedure the following steps were found to be critical in order to obtain a suspension of high purity and viability: (a) the incubation time of the neutrophil–eosinophil suspension in the presence of carbony iron was particularly important. Too long an incubation time resulted in a substantial loss of eosinophils since certain cells evidenced phagocytic properties; (b) a second passage on Lymphoprep yielded a higher degree of purity (Fig. 1a) avoiding any possible interference of residual cells with the spontaneous rosette test.

As regards the observation of rosettes, the samples incubated for 12 hr proved to give better and reproducible results. A shorter incubation time resulted in a reduced number of spontaneous rosettes. On the other hand, viability was progressively reduced after 18 hr incubation.

In patients with parasitic diseases a percentage of $15{\cdot}7 \pm 6$ (range (6–23%) eosinophils were able to form rosettes with unsensitized sheep red blood cells (Fig. 1b). In the other patients this phenomenon was not detectable, except in three subjects (Table 1) with allergic rhinitis, bronchial asthma and leukaemia respectively, in which a very small percentage of eosinophils ($1{\cdot}66\% \pm 0{\cdot}57\%$) were able to interact with sheep red blood cells.

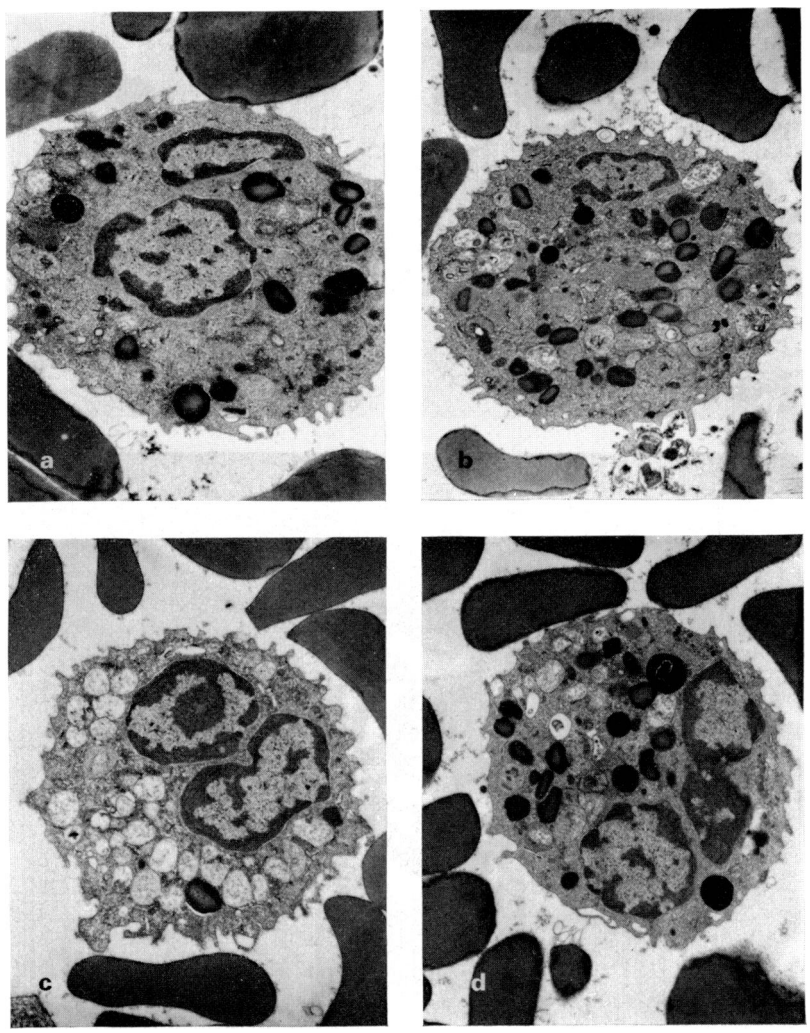

FIG. 2. Ultrathin sections of rosette-forming eosinophils stained with lead citrate. Each rosette shows interaction of as few as four (a) to eight (b) erythrocytes per eosinophil. (Magnification (a) × 7000, (b) × 6500; (c) × 5500; (d) × 5000.)

Electron microscopy

Since no previous work has been carried out at the ultrastructural level on human eosinophils interacting with unsensitized sheep red blood cells, we have studied ultrathin sections of spontaneous rosette-forming eosinophils (Fig. 2).

As regards the appearance of the eosinophils whether interacting or not with erythrocytes, these

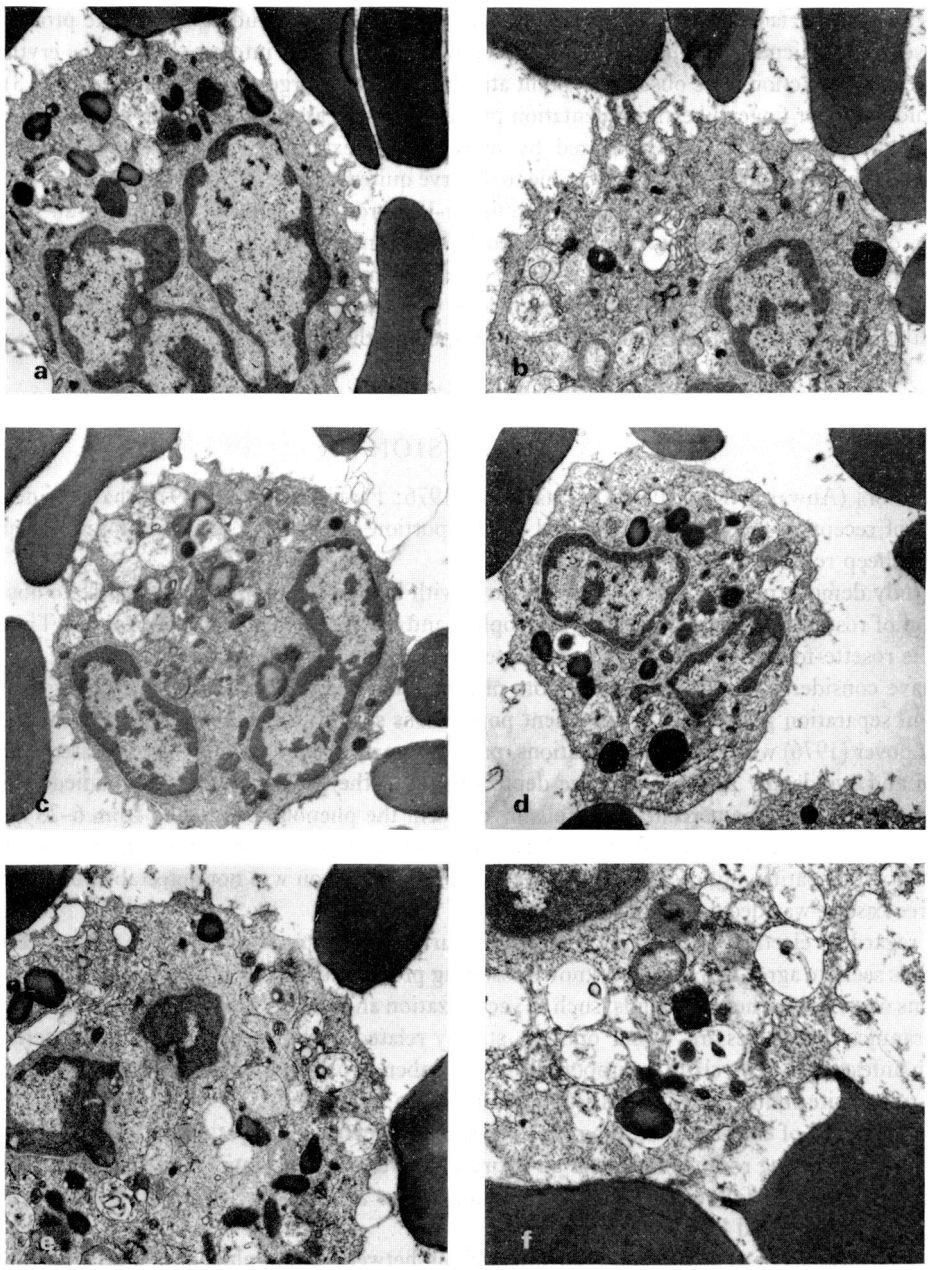

FIG. 3. Different types of interaction between eosinophil and unsensitized sheep erythrocytes. The ultrathin sections show pseudopodia (a, b) and indentation processes (c); large surface contacts (d), also with blebs (e); finger-like processes protruding from the erythrocyte towards the eosinophil surface (f). (Magnification (a) × 6200; (b) × 7000; (c) × 5500; (d) × 6200; (e) × 9000 (f) × 12,000).

normally showed a nucleus with 2–3 lobes, a number of granules varying in dimension and protein crystalline content, mitochondria, ribosomes and vacuoles. As far as the granules were concerned we found the following three types: (a) typical granules with a crystalline core surrounded by a more electron dense matrix (Miller, De Harven & Palade, 1966); (b)m embrane-bound granules, smaller in diameter, and without a crystalline core, similar to those described by Parmley & Spicer (1974); (c) large granules are recently reported by Gleich (1977), characterized by the absence of crystalloid and seemingly of the enclosing membrane.

As regards the surface appearance, a relatively large number of protruding finger-like processes have been observed, particularly in the zones more directly involved in the interaction with the erythrocytes. Two types of interaction were observed: 'point attachments' and 'large surface contacts' (Fig. 3). Either true pseudopodia or finger-like and indentation processes can be assigned to the former category and to the latter those interactions characterized by more or less extended surfaces of contact which are sometimes blebbed. In addition it was possible to observe quite frequently 'point attachments' established by the erythrocytes themselves which protrude finger-like processes towards the eosinophil surface.

Under our conditions, the morphology of eosinophils interacting with erythrocytes or those not forming rosettes was slightly different as compared with normal eosinophils. In fact the cells were sometimes vacuolated and the granules often devoid of their content (Fig. 2c). The remaining granules showed density reversal of the crystalline core in respect to the surrounding matrix.

DISCUSSION

Other authors (Anwar & Kay, 1977; Gupta *et al.*, 1976; Parrillo & Fauci, 1978) have evidenced the presence of receptors for C4, C3b, C3d and the Fc portion of IgG, but have not found receptors for uncoated sheep red blood cells.

Our study demonstrates how in parasitic diseases with blood eosinophilia it is possible to observe the formation of rosettes between circulating eosinophils and unsensitized sheep erythrocytes. The singling out of the rosette-forming eosinophils was effected by light and electron microscopy.

We have considered the possibility that the phenomenon could be ascribed to differences in either eosinophil separation procedures or in patient populations studied. Our method of purification, that of Sher & Glover (1976) with minor modifications, provides unstimulated eosinophils separated by negative selection and avoids any possible selective depletion. As to the patient population studied, in subjects with parasitic diseases the percentage of cells involved in the phenomenon varied from 6–23% with an average of 13%. In patients with bronchial asthma, allergic rhinitis, atopic eczema, chronic ulcerative colitis, chronic hepatitis and eosinophil leukaemia, the phenomenon was not detectable, or, if present—as in three cases—was decidedly limited (1·66%).

With regard to electron microscopy results, the surface characteristics observed on rosette-forming eosinophils seem to agree with the well-known rosetting properties of T lymphocytes. The morphological alterations observed in the eosinophils, such as vacuolization and density reversal of the main components of the granules themselves are, in our opinion, strictly related to the specific conditions determined by helminth infestation. The claim is supported by a number of observations reported in the literature in relation to parasite-induced eosinophilia (Zucker-Franklin *et al.*, 1974).

The significance of eosinophil binding with sheep erythrocytes is not yet clear. It is possible that the cells capable of forming rosettes represent an entirely different subpopulation of eosinophils or the basic population itself which following parasite infestation shows an increased ability to interact with sheep erythrocytes as evidenced by rosette formation.

One could also argue that the observed interactions between eosinophils and sheep red blood cells simply correspond to the first stage of a cytotoxic mechanism against foreign cells in the absence of antibody. Eosinophils have been shown *in vitro* to have low levels of cytotoxicity against Chang liver cells and human heart cells (Parrillo & Fauci, 1978).

From the clinical point of view our studies indicate that in the presence of parasitic infestations

with apparent blood eosinophilia, the formation of spontaneous rosettes may be employed as a diagnostic aid and considered as an indicator of qualitative and quantitative alterations in the eosinophil population.

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