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Binding of immunoglobulin classes and subclasses to human neutrophils and eosinophils

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SUMMARY

A comparative study of membrane expression of immunoglobin (Fc) receptors on human eosinophils and neutrophils has been undertaken using human IgG-1, IgG-2, IgG-3, IgG-4, IgA-1, IgA-2, IgM, IgD and IgE myeloma proteins. Sheep erythrocytes (E) coated with either human IgG-1, IgG-2, IgG-3 or IgG-4 myeloma proteins formed rosettes with human neutrophils and eosinophils. Proportionally more (1½–2 times) rosettes were observed with neutrophils compared to eosinophils. In contrast, E-IgE bound to eosinophils (but not to neutrophils) to a degree that was comparable to E-IgG-1. Although IgG and IgE rosettes were inhibited by aggregates prepared from their corresponding myeloma protein there was no evidence that eosinophils and neutrophils have distinct receptors for IgG subclasses. Cells from four patients with hypereosinophilia were separated on density (metrizamide) gradients. The percentages of E-IgG-1 and E-IgE rosettes with normal- and light-density eosinophils were similar. Neutrophils, but not eosinophils, also bound significantly more E-IgA-1 and E-IgA-2 than the E-human albumin (E-Alb) control. In contrast, neutrophil and eosinophil rosette formation with E-IgM and E-IgD was not significantly different from E-Alb or E alone. These experiments indicate that (i) human neutrophils and eosinophils bind homologous IgG subclass myeloma proteins, (ii) eosinophils, but not neutrophils, bind E-IgE with a similar avidity to that observed with E-IgG1, (iii) neutrophils, but not eosinophils, readily express demonstrable receptors for IgA-1 and IgA-2 and (iv) neither neutrophils nor eosinophils form E-IgM or E-IgD rosettes in greater numbers than the E-Alb controls.

Keywords eosinophils immunoglobulins IgE neutrophils receptors

INTRODUCTION

Although IgG (Fc) receptors have been identified on human neutrophils (Henson, Johnson & Spiegelberg, 1972; Lawrence, Weigle & Spiegelberg, 1975) and eosinophils (Tai & Spry, 1976; Anwar & Kay, 1977; Ottesen *et al.*, 1977; Gupta *et al.*, 1976), the methods used have been either indirect or have employed rosette techniques using heterologous systems, i.e. indicator erythrocytes sensitized with IgG prepared in a variety of animal species. The rosette method described by Gonzalez-Molina & Spiegelberg (1977) in which human myeloma proteins were covalently linked to red cells, allows the identification of human Ig (Fc) receptors using a homologous system and has been employed, for example, to identify IgG (Fc) receptors on subpopulations of lymphocytes (Gonzalez-Molina & Spiegelberg, 1977) and macrophages (Melewicz & Spiegelberg, 1980). This method has also been used to demonstrate IgE (Fc) receptors on human eosinophils (Capron *et al.*,

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1981; Capron *et al.*, 1984). These workers have also identified cytophilic IgE on human blood and tissue eosinophils by flow microfluorometry (Capron *et al.*, 1985).

However, the rosette technique allows a direct comparison of the binding properties of the various human myeloma classes and subclasses to different blood leucocytes contained in the same preparation. For this reason we have used this method in the present study to compare neutrophil and eosinophil rosettes, with particular emphasis on the degree of expression of receptors for IgG (Fc) and IgE (Fc).

MATERIALS AND METHODS

Materials. Materials were obtained as follows: trypsin, soybean trypsin inhibitor, dimethylsuberimidate (DMSI) (Sigma, St. Louis, Missouri, USA); human albumin (Behringwerke, Marburg, West Germany); and sheep red cells (Tissue Culture Services, Slough). The myeloma proteins, all supplied as gifts, were obtained as follows: IgG-1 and IgD (Dr D. Stanworth, The Medical School, Birmingham, England); IgG-2, IgG-3, IgG-4, monomeric IgA-1 and IgM (Dr Erna van Loghem, Netherlands Red Cross Blood Transfusion Service, Amsterdam, Netherlands); monomeric IgA-2 (Dr H. L. Spiegelberg, Scripps Clinic, La Jolla, California, USA) and IgE (Dr Teruko Ishizaka, Johns Hopkins Medical School, Baltimore, USA). Some IgE myeloma for a comparative experiment was also kindly supplied by Dr Monique Capron (Pasteur Institute, Lille, France). The myeloma proteins were purified by DEAE Sephacel chromatography, Sephadex S-300 gel filtration and any contaminating albumin was removed by affinity chromatography using Blue Sepharose CL-6B (Pharmacia, Uppsala, Sweden). The purified proteins gave a single band on immunoelectrophoresis when tested against a polyvalent rabbit antiserum to human serum proteins and monospecific anti-IgG, IgA, IgM, IgD or IgE. Purified proteins were aggregated with DMSI as described (Segal, Taurog & Metzger, 1977).

Preparation of red cells coated with human myeloma proteins (E-Ig). This was performed as described (Gonzalez-Molina & Spiegelberg, 1977). Sheep red blood cells (E) were washed three

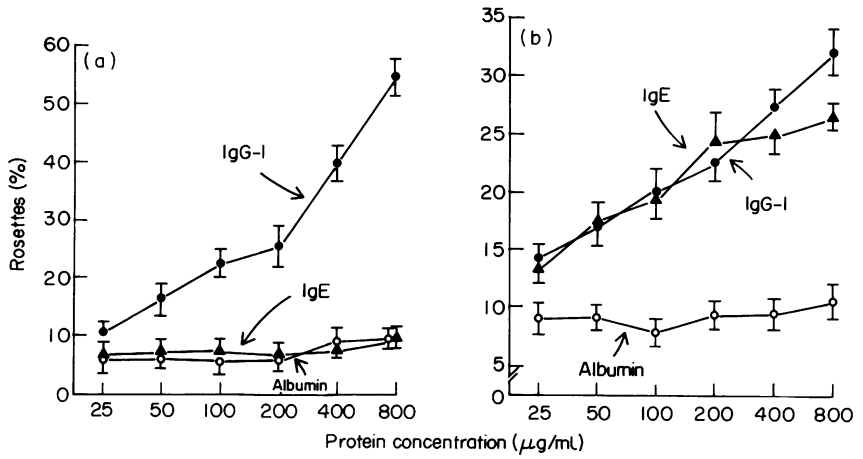

Fig. 1. Neutrophil and eosinophil rosettes by erythrocytes coated with increasing concentrations of IgG-1, IgE or albumin. The points represent the mean ±s.e.m. of (a) six experiments (neutrophils) and (b) eleven experiments (eosinophils). Cells from different subjects were used for each experiment. With neutrophils E-IgG-1 rosettes were significantly higher than E-Albumin at 50 μg/ml ($P<0.01$); 100, 200, 400 and 800 μg/ml (all $P<0.001$). With eosinophils E-IgG-1 rosettes were significantly higher than E-Albumin at 25 μg/ml ($P<0.05$), 50 μg/ml ($P<0.1$) and 100, 200, 400 and 800 μg/ml ($P<0.001$). For eosinophils E-IgE rosettes were significantly higher than E-albumin at 50 μg/ml ($P<0.01$); 100, 200, 400 and 800 μg/ml ($P<0.001$). Eosinophils were obtained from patients with a peripheral blood eosinophil count of between 8 and 20% in association with bronchial asthma, rhinitis and helminthic disease. Statistics were performed using the Student's *t*-test.

times in phosphate buffered saline (PBS). Equal volumes of 10% E and trypsin (0·2 mg/ml) were incubated at 37°C for 60 min with occasional mixing, after which soybean trypsin inhibitor (0·2 mg/ml) was added and the cells were washed a further three times in PBS and resuspended again to 10%. An equal volume of 3% pyruvic aldehyde in PBS (adjusted to pH 7·2 with 10 N NaOH) was added to the red cells and the mixture incubated for 20 h on a rotator at room temperature. The cells were then washed five times with PBS and fixed with 3% formaldehyde in PBS and treated again in the same manner for 20 h on a rotator at room temperature. The erythrocytes were finally washed and stored in PBS at 4°C. E prepared in this fashion were kept for up to 4 weeks.

Equal volumes of 4% E were mixed together with varying concentrations of the purified myeloma proteins in 0·15 molar acetate buffer pH 5. The mixtures were incubated at room temperature for 2 h with continuous mixing. The sensitized cells were washed three times in PBS and stored at 4°C in a 1% suspension in PBS for no longer than 3 days. Erythrocytes were also coated with human serum albumin as a control and treated in the same way as E-Ig.

Neutrophils and eosinophils. Venous blood was drawn into 6% dextran and preservative-free heparin and the granulocytes separated either on Ficoll/Hypaque gradients as previously described (Böyum, 1968) or by metrizamide gradients (Vadas *et al.*, 1979). Discontinuous metrizamide gradients were also used for the preparation of light- and normal-density eosinophils (Vadas *et al.*, 1979).

E-Ig rosettes. Equal volumes (0·05 or 0·1 ml) of 1% E-Ig (or E-Alb) and the granulocyte-rich (eosinophils and neutrophils) suspension (3×10^6 cell/ml) contained in Hank's balanced salt

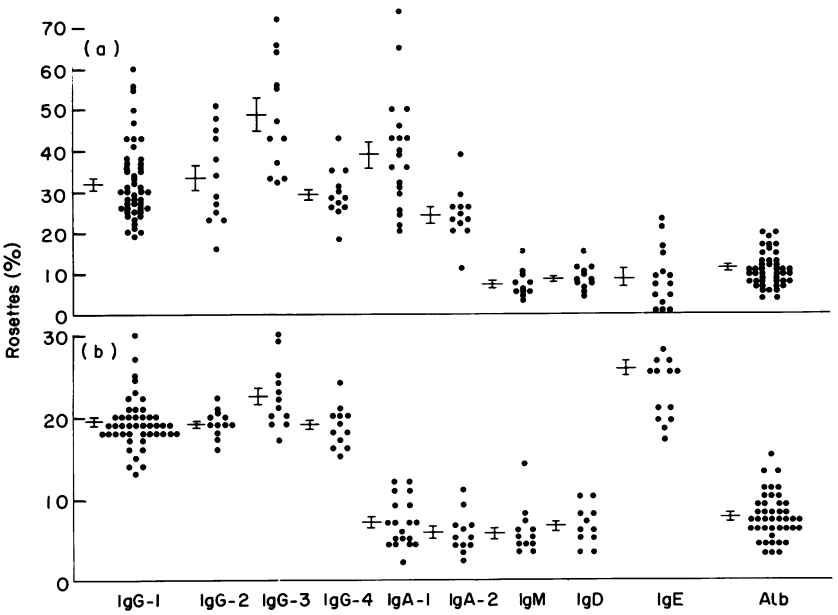

Fig. 2. (a) Neutrophil and (b) eosinophil rosettes by erythrocytes coated with human immunoglobulin classes and subclasses and albumin. Bars represent the mean ±s.e.m.

With neutrophils there were significant differences in the rosettes obtained with E-Ig myeloma proteins when compared to E-Albumin. These were as follows: IgG-1, IgG-2, IgG-3, IgG-4, IgA-1 and IgA-2 *v* albumin (all $P < 0{\cdot}001$). IgM, IgD and IgE *v* albumin (not significant). With eosinophils the results were IgG-1, IgG-2, IgG-3, IgG-4 and IgE *v* albumin (all $P < 0{\cdot}001$); IgA-1, IgA-2, IgM and IgD *v* albumin (not significant). The eosinophils were obtained either from normal individuals (having a peripheral blood eosinophilia of between 2 and 5%), or patients with bronchial asthma and rhinitis (eosinophilia between 7 to 15%). Erythrocytes were coated with myeloma proteins or albumin at concentrations of 400 μg/ml. Statistics were performed using the Student's *t*-test. With the exception of three normal subjects, in which the eosinophils were used on two separate occasions, all the data points for each treatment (i.e. E-IgG-1, E-IgG-2 etc.) are from different individuals.

solution were mixed and centrifuged for 5 min at 50 ***g***. The cell pellets with their supernatants were then incubated for a further 60 min at 4°C. The supernatant was then discarded and the pellet gently resuspended in 0·05 or 0·1 ml of Hanks' BSS containing 1% formyl saline. 50 μl of this suspension were then placed on a glass slide and very gently smeared. The slides were dried, fixed in methanol (10 min) and stained in May/Grunwald and Giemsa, dried and mounted. 200 cells were counted and the percentage rosettes calculated as previously described (Anwar & Kay, 1977). The inter-assay coefficient of variation was between 5 and 10%.

RESULTS

Sheep red cells (E) were coated with increasing concentrations of IgG-1, or IgE myeloma protein or albumin (Alb), and incubated with human neutrophils or eosinophils (Fig. 1). There was a dose-dependent increase in neutrophil and eosinophil rosette formation with E-IgG-1 but not with E-Alb. Neutrophils formed approximately twice as many rosettes as eosinophils at each concentration of IgG-1. A concentration-dependent binding of E-IgE to eosinophils, but not to neutrophils, was also observed. The percentage of eosinophil IgG and IgE rosettes was similar for equivalent concentrations of myeloma protein used to coat the red cells.

Neutrophils and eosinophils from normal individuals and patients with eosinophilia (mostly in association with asthma and allergic rhinitis) were then tested for rosette formation with erythrocytes coated with the various human immunoglobulin classes or subclasses (Fig. 2). These were compared with E-Alb. With neutrophils, E-IgG-1, E-IgG-2, E-IgG-3, E-IgG-4, E-IgA-1 and E-IgA-2 rosettes were significantly higher than the E-Alb control, whereas there were no significant differences between E-IgM, IgD, E-IgE and E-Alb. With eosinophils E-IgG-1, E-IgG-2, E-IgG-3, E-IgG-4 and E-IgE were significantly higher than E-Alb. E-IgA-1, E-IgA-2, E-IgM and E-IgD did not form significantly more eosinophil rosettes than E-Alb.

Table 1. Inhibition of neutrophil and eosinophil by aggregated proteins

		Aggregated IgG (μg/ml)		Aggregated IgE (μg/ml)		Aggregated Albumin	
	Control	1000	2000	100	200	1000	2000
Neutrophil rosettes							
E-IgG-1	56·0±8·9	38·0±3·2*	34·0±2·1†	57·0±10·5	58·0±9·7	60·0±9·6	59·0±9·8
E-IgG-2	67·0±8·3	58·0±7·7	52·0±10·2	n.d.	n.d.	72·0±6·4	72·0±10·2
E-IgG-3	80·0±2·1	56·0±5·9‡	48·0±4·7§	n.d.	n.d.	7·9±3·1	82·0±1·1
E-IgG-4	37·0±2·8	n.d.	21·0±2·1§	n.d.	n.d.	n.d.	44·0±5·3
E-IgE	8·5±1·3	n.d.	7·5±3·4	8·6±1·3	7·5±2·0	n.d.	8·5±1·3
E-Alb	8·5±1·0	n.d.	8·5±1·0	7·0±1·5	7·5±2·0	n.d.	8·8±1·9
Eosinophil rosettes							
E-IgG-1	35·0±2·0	24·0±0·6	20·0±0§	32·5±4·2	35·3±3·3	30·0±2·4	31·0±1·9
E-IgG-2	25·0±1·8	19·0±1·7	14·2±1·4‡	n.d.	n.d.	23·0±1·2	22·0±1·2
E-IgG-3	26·0±1·7	17·0±2·3*	17·0±2·3§	n.d.	n.d.	34·0±2·0	35·0±2·2
E-IgG-4	17·0±0·3	n.d.	5·0±0·47§	n.d.	n.d.	n.d.	16·0±2·2
E-IgE	24·5±0·75	n.d.	22·8±0·5	17·3‡±1·1	13·3±0·8§	n.d.	24·5±0·75
E-Alb	8·5±1·6	n.d.	9·8±0·9	9·5±1·9	9·3±0·9	n.d.	9·8±0·9

IgG-1 proteins were used to inhibit E-IgG-1 rosettes, IgG-2 for E-IgG-2, IgG-3 for E-IgG-3, IgG-4 for E-IgG-4. The values represent the % rosettes ±1 s.e.m. (four experiments). E-IgG intermediates were prepared with 2000 μg/ml of myeloma subclass proteins, with the exception of E-IgE which was 800 μg/ml. The aggregation procedure was performed as previously described (Segal *et al.*, 1977).

* $P<0·05$, † $P<0·02$, ‡ $P<0·01$, § $P<0·001$, n.d. Not done.

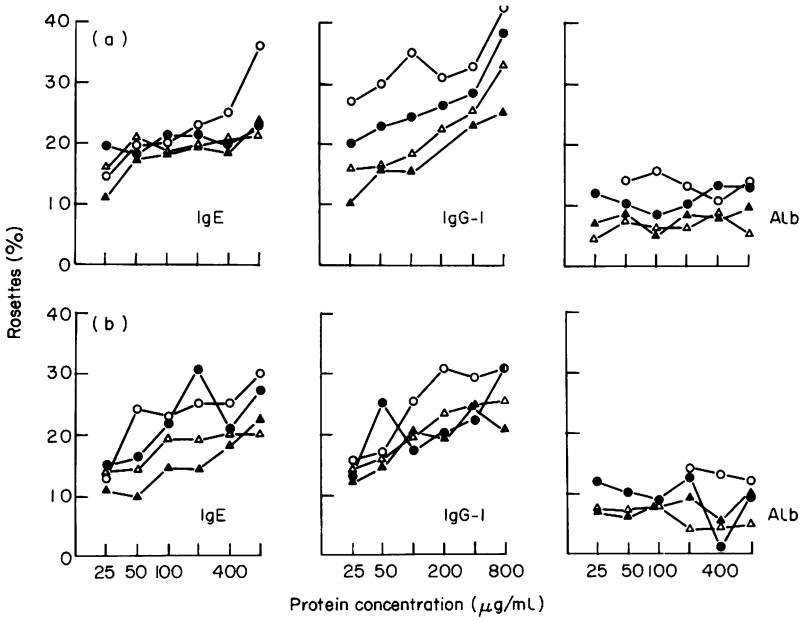

Fig. 3. A comparison of (a) light- and (b) normal-density eosinophils separated by discontinuous metrizamide gradients. Rosettes were performed with increasing concentrations of IgG-1, IgE or albumin. Two patients had an eosinophilia in association with the hypereosinophilic syndrome (○, △), one had the Churg-Strauss syndrome and one patient had eosinophilic leukaemia (▲).

In order to test the specificity of the IgG binding, aggregated IgG subclasses and IgE myeloma protein were compared with aggregated albumin for their capacity to inhibit neutrophil and eosinophil IgG-1, IgG-2, IgG-3 and IgG-4 rosettes (Table 1). With the aggregated IgG subclasses myeloma proteins there was a dose-dependent, and statistically significant, inhibition of rosette formation with IgG-1, IgG-2, IgG-3 and IgG-4 respectively but not of E-IgE. However, in data not shown aggregated IgG-1 inhibited eosinophil and neutrophil E-IgG-4 rosettes. Other cross-over experiments were not undertaken. Aggregated IgE inhibited E-IgE rosettes but not E-IgG-1 rosettes.

Eosinophils from four patients with hypereosinophilia were separated with light- and normal-density cells on metrizamide gradients. The percentages of E-IgG-1 and E-IgE rosettes, over a wide dose-range, were similar (Fig. 3).

DISCUSSION

In a previous report it was shown that aggregated IgG-1, IgG-2, IgG-3, IgG-4, IgA-1 and IgA-2 (but not IgD, IgM or IgE) human myeloma proteins released beta-glucuronidase from human neutrophils (Henson *et al.*, 1972). Subsequently, similar findings were obtained by direct binding studies using ^{125}I-labelled immunoglobulins (Lawrence *et al.*, 1975). We have now, (i) confirmed these findings for the human neutrophils using the rosette technique, and (ii) demonstrated that human eosinophils bind, to a similar degree, E-IgG-1, E-IgG-2, E-IgG-3, E-IgG-4 and E-IgE.

The technique employed in these experiments, that is the covalent linkage of human myeloma proteins to erythrocytes (Gonzalez-Molina & Spiegelberg, 1977), has the advantage of being species homologous in terms of the granulocyte Ig (Fc) receptor being studied and also uses a monoclonal immunoglobulin. Although we have used one myeloma protein only, from each of the classes and subclasses, the previous study with ^{125}I-labelled proteins indicated that myelomas of the same class

from different sources had similar neutrophil binding properties (Lawrence *et al.*, 1975). As previously reported with EA_G^{rabbit}, rosette formation with neutrophils was $1\frac{1}{2}$–2 times greater than with eosinophils (Fig. 1).

Although, in preliminary studies, we were unable to confirm the findings of Capron *et al.* (1981, 1984) in regard to E-IgE rosettes (Walsh & Kay, 1984), it is now clear that our original batch of myeloma proteins was inactive in IgE rosette assays. The reasons for this are unclear but may have been related to problems with transport and storage. It is important to have confirmed the presence of IgE receptors on eosinophils because of the potential importance of this observation in our understanding of IgE-mediated disease processes and eosinophil-dependent cytotoxicity reactions. We were unable to show differences between E-IgG and E-IgE binding to light and normal density eosinophils (Fig. 3), which might have been expected as light density cells are generally thought to be more 'activated' (Capron *et al.*, 1984). However, the possibility that they represent a stage in maturity has not been excluded.

It is now appreciated that IgE receptors on eosinophils have a lower affinity than comparable receptors on basophils and mast cells (Capron *et al.*, 1984). The rosette technique is clearly very sensitive in its ability to detect these low-affinity receptors and has the advantage of being relatively inexpensive and suitable for routine clinical work, without the requirement for pure cell populations.

We have been able to confirm and extend previous studies on IgG receptors on neutrophils (Henson *et al.*, 1972; Lawrence *et al.*, 1975), eosinophils (Tai & Spry, 1976; Gupta *et al.*, 1976) by showing that these cells bind erythrocytes coated with myeloma proteins from the four human IgG subclasses (Fig. 2). These rosette reactions were inhibited, in a dose-dependent fashion, by aggregated IgG subclasses proteins (Table 1). It appears that IgG subclasses share the same Fc receptor on neutrophils (Henson *et al.*, 1972) and that this is presumably true for the eosinophil as well. We were also able to show that E-IgA-1 and E-IgA-2 bind to neutrophils (but not to eosinophils), thus confirming the findings of Fanger, Goldstine & Shen (1983) and others (Henson *et al.*, 1972; Lawrence *et al.*, 1975).

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