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Fcγ and CD11/CD18 receptor expression on normal density and low density human eosinophils

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SUMMARY

We have studied the expression of Fcγ receptors and leucocyte integrins (CD11/CD18 family) on human eosinophils using specific monoclonal antibodies (mAb) and flow cytometric analysis. Peripheral blood eosinophils of normal density and low density were compared with neutrophils and monocytes. Several properties of the human eosinophil were established. These were (i) that the eosinophil expressed FcγRII (CDw32) only (unlike monocytes which bear FcγRI and FcγRII and neutrophils which bear FcγRII and FcγRIII); (ii) that the absence of FcγRIII (CD16) on eosinophils served as a basis for distinguishing eosinophil and neutrophil populations by immunofluorescence; (iii) that the leucocyte adhesion glycoproteins, LFA-1α(CD11a), CR3-α (CD11b), p150, 95-α (CD11c) and the common β-chain (CD18), were expressed on the eosinophil as well as the neutrophil; (iv) that CD18 expression was significantly reduced on low-density eosinophils from the hypereosinophilic syndrome. Thus, our findings emphasize the unique phenotype of the human eosinophil in terms of Fcγ receptor expression, the similarity of the eosinophil and neutrophil with regard to the leucocyte integrins and that eosinophils of low density do not differ greatly from those of normal density in terms of receptor expression.

INTRODUCTION

It is well established that a blood and tissue eosinophilia is associated with helminthic infections and that eosinophils are also a feature of allergic inflammation (Gleich & Adolphson, 1986). Eosinophil-mediated damage might be an important factor in denudation of the bronchial epithelium in asthma (Laitinen *et al.*, 1985). Following adhesion to the vascular endothelium and subsequent diapedesis, eosinophils, like neutrophils, may become activated as a result of changes in the microenvironment. *In vitro*, stimulation of eosinophils with various agonists results in the generation of lipid mediators (Weller *et al.*, 1983; Shaw *et al.*,1985) and partial reductive products of oxygen (Pincus, 1983), together with exocytosis of basic granule proteins (Spry, 1985). The effector function of eosinophils in adaptive immunity, i.e. the extracellular killing of non-phagocytosable targets such as opsonized helminthic larvae, is triggered via complement and/or immunoglobulin receptors present on the cell membrane (Anwar, Smithers & Kay, 1979).

There are a number of specific receptors which may play a role in the recognition of opsonized particles and the activation of cellular responses (Hogg, 1987). Among those well characterized for the neutrophil and the monocyte are the three types of Fcγ receptor (FcγRI/CD64, FcγRII/CDw32, FcγRIII/CD16), complement receptors (CR1/CD35 and CR3/CD11b), and other membrane proteins important for intercellular adhesion (LFA-1α/CD11a, p150,95-α/CD11c and ICAM-1/CD54).

FcγRI is the only receptor that will bind monomeric IgG, while FcγRII and FcγRIII only exhibit detectable binding with multivalent ligand, such as immune complexes (Anderson & Looney, 1986). Complement receptor type 1 (CR1) is the receptor for the third component of complement, C3b. The leucocyte integrins are a family of three receptors, LFA-1, CR3 and p150,95, which are heterodimers composed of two non-covalently linked polypeptide chains. Each receptor has a distinct alpha chain (190,000, 185,000 and 150,000 MW, respectively) which is associated with a common β-chain (95,000 MW) (Springer & Anderson, 1986). Complement receptor type 3 (CR3) is the receptor for C3bi and has binding sites for other molecules (Ross, Cain & Lachmann, 1985); p150,95 binds C3bi and may bind other ligands as well (Myones *et al.*, 1988). Lymphocyte function-associated antigen-1 (LFA-1) interacts with intercellular adhesion molecule-1 (ICAM-1) on other cells to facilitate intercellular adhesion (Dustin *et al.*, 1986).

Abbreviations: BSA, bovine serum albumin; CR1, complement receptor type 1; CR3, complement receptor type 3; ICAM-1, intercellular adhesion molecule-1; IFN-γ, interferon-gamma; LDE, low density eosinophil; LFA-1, lymphocyte function-associated antigen-1; M, monocyte; mAb, monoclonal antibody; MTZ, metrizamide; N, neutrophil; NDE, normal density eosinophil; SMF, specific mean fluorescence.

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Eosinophils exhibit considerable heterogeneity and can be separated into distinct populations by density gradient centrifugation. There is evidence that the lower density cells are activated in terms of metabolic activity and IgG Fc and complement receptor expression (Bass *et al.*, 1980; Pincus *et al.*, 1981; Winqvist *et al.*, 1982). Much of the work characterizing IgG Fc and complement receptors on eosinophils has used the rosette technique (Anwar & Kay, 1977), which is a relatively imprecise method for determining receptor expression. There are few data on the expression of the leucocyte integrins on eosinophils (Fischer *et al.*, 1986; Capron *et al.*, 1987; Kimani, Tonnesen & Henson, 1988).

We have used specific monoclonal antibodies and flow cytometry to investigate the expression of these receptors on normal density and low density eosinophils. These findings have been compared with the neutrophil and the monocyte in order to identify differences which may be relevant to the specialized function of the eosinophil.

MATERIALS AND METHODS

Materials

Materials were obtained as follows: BSA grade V, mouse myeloma proteins MOPC 21, UPC 10, MOPC 141 and TEPC 183, propidium iodide and sodium azide from Sigma Chemical Co., Poole, Dorset; RPMI-1640 from Gibco, Paisley, Renfrewshire; Dextran 110 from Fisons, Loughborough, Leicestershire; metrizamide from Nyegaard Ltd, Birmingham.

Monoclonal antibodies

mAb to FcγRI (CD64) and FcγRII (CDw32) (mAb 32 and mAb IV.3, respectively) were a kind gift from Dr W. Fanger, Dartmouth Medical School, Hanover, NH. Anti-CR1 (CD35), anti-Leu 15 (CD11b) and anti-Leu 11b (CD16) were purchased from Becton-Dickinson UK Ltd, Oxford. mAb to LFA-1α (CD11a), p150,95-α (CD11c), the common β-chain (CD18) and ICAM-1 (CD54) (TS1/22, SHCL3, TS1/18 and RR1/1, respectively) were generously provided by Dr T. Springer, Dana-Farber Cancer Institute, Boston, MA. A panel of anti-CDw32 mAb was obtained from the Fourth International Leucocyte Typing Workshop: KB61, 2E1, CIKM5, IV.3 and 41H16 (the CD nomenclature in this paper conforms to the Fourth International Leucocyte Typing Workshop designations). All of these mAb were of murine origin and all were IgG except for anti-Leu 11b, which was IgM. FITC-conjugated $F(ab')_2$ fragments of rabbit antibody to mouse immunoglobulin (F313) was purchased from Dako Ltd, High Wycombe, Bucks. FITC-conjugated goat anti-mouse IgG and R-phycoerythrin (PE)-conjugated goat anti-mouse IgM were purchased from Southern Biotechnology Associates, Birmingham, AL.

Patients

Peripheral blood leucocytes were obtained from donors with mild eosinophilia (range 7–16%, median 10%) associated with allergic rhinitis or mild asthma. Normal density eosinophils, as well as neutrophils and monocytes, were separated from the blood of these individuals. Eosinophils of low density, and sometimes of normal density also, were obtained from the blood of patients with the hyper-eosinophilic syndrome. Total white cell counts in these individuals were elevated and eosinophils comprised 45–67% (median 53%) of the circulating leucocytes. The majority of the patients with the hyper-eosinophilic syndrome were receiving systemic corticosteroids, whereas the individuals with mild eosinophilia were not.

Cell isolation

Heparinized peripheral blood (10 U heparin/ml) was mixed with dextran in the ratio nine parts blood to two parts dextran and allowed to sediment for 30 min at 37°. The leucocyte-rich plasma was washed twice, resuspended in RPMI-1640 and layered onto discontinuous metrizamide (MTZ) gradients (Vadas *et al.*, 1979). After centrifugation at 1200 *g* for 45 min at 20°, six separate cell bands were recovered and washed once in cold RPMI-1640. The cell pellets were resuspended in cold lysis buffer (NH_4Cl, 8·2 g, and $KHCO_3$, 1·0 g, in 1 litre, pH 7·4), centrifuged at 250 *g* for 10 min, and washed twice in cold RPMI-1640. After resuspending in cold RPMI-1640, the cells were counted using Kimura stain (Kimura, Moritani & Tanizaki, 1973) and kept on ice until used. Cells were pooled from the top two layers of the gradient (18% and 20% MTZ) to give a mononuclear cell-rich preparation, cells at the 20%/22% interface were normally neutrophils of greater than 90% purity, and normal density eosinophils were recovered from the lower three layers, the purity also being greater than 90%. Eosinophils were considered to be low density when they were recovered from the 20%/22% interface and above, and where the purity was less than 90% the preparation was double stained with anti-CD16 to distinguish the contaminating neutrophils.

Immunofluorescence

Samples of 5×10^5 cells in 50 μl RPMI-1640 were mixed with saturating amounts of each mAb (10–50 μl) and the volume made up to 100 μl, where necessary, with ice-cold PBS containing 0·1% NaN_3 and 0·5% BSA (PAB). After incubation for 30 min at 4°, the cells were washed once with 3 ml of cold PAB and then mixed with an excess of FITC-conjugated $F(ab')_2$ fragments of rabbit antibody to mouse immunoglobulins. After incubation for 15 min at 4°, the cells were washed once more, resuspended in PBS containing 0·1% NaN_3 and kept on ice until analysed. Non-specific binding of the mAb was determined by incubating the same cells with identical concentrations of mouse myeloma proteins of the same antibody isotype but with irrelevant antigen specificity. To identify dead cells, propidium iodide was added at a final concentration of 2 μg/ml 5 min before the cell preparations were analysed. Where cells were double-stained for CD16, i.e. low density eosinophil preparations of less than 90% purity, anti-Leu 11b was also added to the primary incubation mixture and two isotype-specific second antibodies were used, i.e. FITC-conjugated goat anti-mouse IgG and PE-conjugated goat anti-mouse IgM.

Flow cytometry

Flow cytometry was performed on a FACS Analyzer (Becton-Dickinson, Mountain View, CA), equipped with an electronic (Coulter) volume sensor, which was standardized with fluorescent microbeads. Fluorescence intensity was determined on 10,000 cells from each sample using logarithmic amplification, which was converted to the linear equivalent by a Hewlett-Packard Consort 30 computer. Cell volume was also measured, on a linear scale, and 90° light scatter was recorded using logarithmic amplification, which was also converted to the linear equivalent. Cell populations and debris were defined by

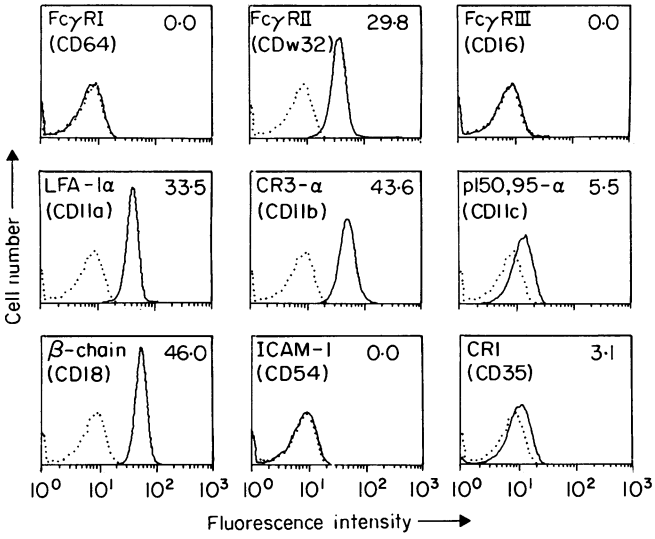

Figure 1. Representative histograms of logarithmic fluorescence intensity of 10,000 normal density peripheral blood eosinophils stained with specific mAb (solid line) or the appropriate control antibody (broken line) and analysed on a FACS Analyzer. The values shown indicate the specific mean fluorescence of the cells.

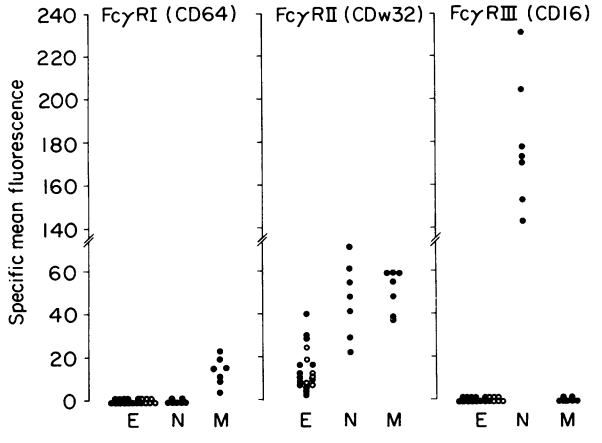

Figure 2. The expression of the Fcγ receptors on eosinophils (E), neutrophils (N), and monocytes (M). Open circles represent low density eosinophils. Receptor expression is represented as specific mean fluorescence of 10,000 cells.

gating on the volume and 90° light scatter parameters. Dead cells were excluded from analysis by gating out propidium iodide-positive cells. The mean fluorescence of the population was calculated by the computer and the value for the control antibody was subtracted from the value for the specific mAb to give a specific mean fluorescence value.

Statistical significance was assessed by the Student's *t*-test or the *z*-test.

RESULTS

Representative histograms of the fluorescence intensity of normal density peripheral blood eosinophils stained with specific mAb for flow cytometry showed three patterns of receptor expression (Fig. 1). FcγRII, LFA-1α, CR3-α and the common β-chain were all well expressed, p150,95-α and CR1 were expressed in low density and FcRI, FcγRIII and ICAM-1 could not be detected above the background fluorescence.

Table 1. FcγRII (CDw32) expression on peripheral blood leucocytes

Exp. no.	Eosinophils			Neutrophils			Monocytes			
	1	2	3	1	3	4	1	3	4	5
KB61 (Pulford)	29,	32,	15	63,	50,	66	57,	62,	54,	50
2El (Tursz)	36,	38,	18	79,	56,	76	59,	58,	56,	48
CIMK5 (Pilkington)	8,	8,	3	20,	11,	16	18,	16,	11,	9
IV.3 (Anderson)	31,	33,	13	74,	61,	68	60,	68,	61,	48
41H16 (Zipf)	28,	7,	12	65,	52,	40	54,	58,	36,	16

Reactivity of normal density eosinophils, neutrophils and monocytes with a panel of anti-CDw32 mAb. The values given are specific mean fluorescence.

Fcγ receptor expression

FcγRI was not detectable on normal density eosinophils (NDE), low density eosinophils (LDE) or neutrophils (N), but was expressed at low levels on monocytes (M). FcγRII was present on all three cells types, with the order of expression being M = N > NDE = LDE. FcγRIII was very well expressed by neutrophils but was not found on NDE, LDE or monocytes (Fig. 2). Thus, there was no apparent difference in Fcγ receptor expression on NDE and LDE. Since FcγRII (CDw32) was the only Fcγ receptor detected on eosinophils (as well as being present on neutrophils and monocytes), its expression was investigated further using a panel of anti-CDw32 mAb (Table 1). Of the five mAb used, KB61, 2E1 and IV.3 consistently gave bright fluorescence with NDE, neutrophils and monocytes, and CIMK5 consistently stained these cells to give weaker fluorescence. mAb 41H16, however, reacted variably with NDE, neutrophils and monocytes, on most occasions giving bright fluorescence, but in other experiments weaker fluorescence was observed on these cells (Exps 2 and 4).

Expression of adhesion molecules

NDE, LDE and neutrophils exhibited a similar range of expression of the three alpha chains of the leucocyte integrin family (Fig. 3), NDE and LDE only differing significantly from neutrophils in terms of CR3-α expression ($P < 0.02$, Table 2). Monocytes had the greatest expression of all three molecules. NDE, LDE and neutrophils had the brightest fluorescence for CR3-α, expressed about half as much LFA-1α and had low levels of p150,95-α. The specific mean fluorescence of the common β-chain showed a similar pattern as the alpha chains (Fig. 4), monocytes had the highest expression, neutrophils and NDE expressed less and LDE expressed least. ICAM-1 could not be detected on NDE, LDE or neutrophils but was present on monocytes in low density (Fig. 4). CR1 was identified on NDE, LDE, neutrophils and monocytes at relatively low levels which were not significantly different for any of the cell types (Fig. 4). Comparing NDE and LDE, the only significant difference was a

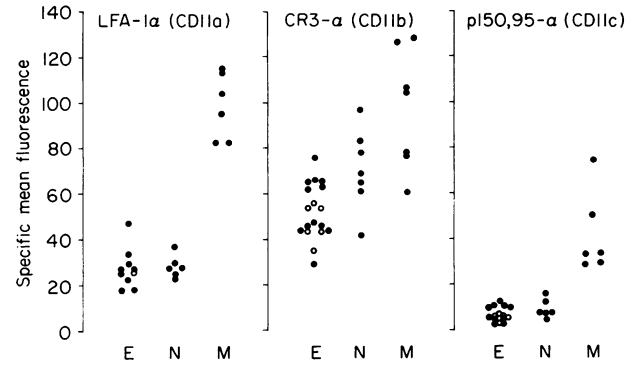

Figure 3. The expression of the three alpha chains of the leucocyte integrin family on eosinophils (E), neutrophils (N) and monocytes (M). Open circles represent low density eosinophils. Receptor expression is represented as specific mean fluorescence of 10,000 cells.

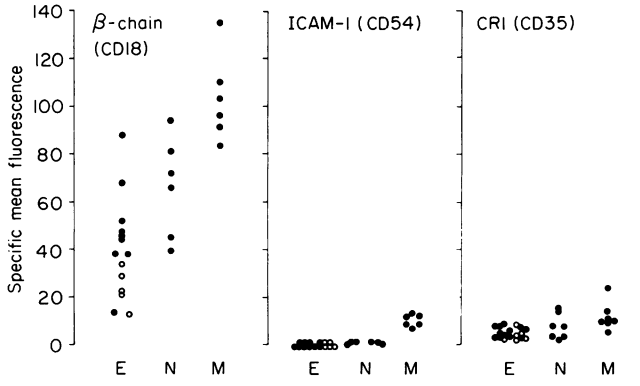

Figure 4. The expression of the common β-chain, ICAM-1 and CR1 on eosinophils (E), neutrophils (N) and monocytes (M). Open circles represent low density eosinophils. Receptor expression is represented as specific mean fluorescence of 10,000 cells.

reduction in the expression of the common β-chain by LDE ($P < 0.01$, Table 2).

Receptor expression, cell volume and 90° and light scatter

The mean values for receptor expression (specific mean fluorescence) were calculated for NDE, LDE, neutrophils and monocytes to allow comparisons to be made between the cell types (Table 2). It can be seen that NDE and LDE had very similar receptor expression, except for the common β-chain, which was less well expressed by low-density cells ($P < 0.01$). Neutrophils and monocytes bound the specific mAb as expected. Mean values for cell volume and 90° light scatter (both quantified on standardized arbitrary scales) were also calculated for NDE, LDE and neutrophils (Table 3). The volume of NDE from donors with a mild eosinophilia was less than the volume of NDE from patients with the hyper-eosinophilic syndrome ($P < 0.05$), but there was no statistically significant difference between NDE and LDE. Neutrophils were significantly smaller than both NDE and LDE ($P < 0.05$). Interestingly, the 90° light scatter was significantly less for LDE compared with NDE from donors with mild eosinophilia and the hyper-eosinophilic syndrome ($P < 0.05$ and $P < 0.01$, respectively), suggesting that the granularity of LDE was reduced. In addition, neutrophils were less granular than eosinophils ($P < 0.001$).

Use of FcγRIII (CD16) to distinguish eosinophils from neutrophils

Preparations of eosinophils, particularly low density eosinophils, are often contaminated with neutrophils and it can be difficult to obtain large numbers of eosinophils of high purity. Eosinophils and neutrophils have very similar volume and 90° light scatter characteristics, as measured by the FACS Analyzer, and they cannot be distinguished from each other using these parameters as, for example, can mononuclear cells. We investigated the differential staining of eosinophils and neutrophils with an anti-CD16 mAb as a method for distinguishing these two cell types in flow cytometry. Since neutrophils had a high density of CD16 expression (in contrast to eosinophils which did not express CD16) there was clear separation between the fluorescence of the neutrophils and eosinophils after staining with an anti-CD16 mAb (Fig. 5a). A mixture of eosinophils and neutrophils can be double stained with anti-CD16 (detected with a PE-conjugated second antibody) together with another antibody of interest, such as anti-CD11b (detected with a FITC-conjugated second antibody). Figure 5b shows that the fluorescence intensity of anti-CD11b on eosinophils and neutrophils overlapped, and by double staining the cells the neutrophil fluorescence was found to be slightly greater (Fig. 5c).

DISCUSSION

Previous studies of IgG Fc and complement receptors on inflammatory cells have used imprecise methods, such as rosetting with opsonized erythrocytes. Such studies have shown differences in IgG Fc receptor numbers on eosinophils and neutrophils and have provided information about overall complement receptor expression (Anwar & Kay, 1978; Walsh & Kay, 1986). However, with the characterization of Fcγ receptors into three types, with different molecular weights and affinities and possibly different functions (Anderson & Looney, 1986), and the discovery of cell-surface adhesion molecules with potential binding sites for a number of ligands (Hogg, 1987), receptor expression can only be meaningfully assessd by more sensitive and specific methods.

Using specific mAb and flow cytometry, we have shown that eosinophils express about one-third as many FcγRII/CDw32 as neutrophils, and neutrophils have a very high density of FcγRIII/CD16 while eosinophils do not have detectable expression of this receptor (Fig. 2). Neither cell expresses FcγRI/CD64, although this receptor can be induced on neutrophils by incubation with IFN-γ (Shen, Guyre & Fanger, 1987). Therefore, it is probable that neutrophils have a greater IgG-binding capacity than eosinophils (Tai, 1980; Yazdanbakhsh, Eckmann & Roos, 1985) due to the very high expression of CD16.

There was a different pattern of Fcγ receptor expression on unstimulated eosinophils, neutrophils and monocytes (Fig. 2); eosinophils expressed FcγRII only, neutrophils expressed FcγRII and FcγRIII and monocytes expressed FcγRI and FcγRII. The panel of anti-FcγRII mAb reacted similarly with eosinophils, neutrophils and monocytes, suggesting that this receptor is the same on all three cells. mAb 41H16 showed variable binding to these cells and may be detecting the

Table 2. Receptor expression on eosinophils, neutrophils and monocytes

	Normal density eosinophils (NDE)	Low density eosinophils (LDE)	Neutrophils (N)	Monocytes (M)
FcγRI	0·0	0·0	0·0	13·7±2·5
FcγRII	15·3±3·1	14·2±2·6	46·6±6·6*	50·7±3·6*
FcγRIII	0·0	0·0	178·8±11·4	0·0
LFA-1	27·5±2·9	(24·3)†	28·5±2·0	98·7±6·0*
CR3	52·9±3·4	51·3±5·8	70·8±6·7****	97·4±9·9***
p150,95	7·9±1·1	6·3±0·7	9·9±1·7	42·4±7·3**
β-chain	48·3±6·9	24·0±3·6***	66·2±8·6	103·0±7·5*
ICAM-1	0·0	0·0	0·0	10·3±1·1
CR1	5·7±0·7	3·9±1·0	8·2±1·9	12·0±2·2

Receptor expression (specific mean fluorescence) presented as the mean ± SEM ($n=9$–13 for normal density eosinophils, $n=5$–6 for low density eosinophils and $n=6$–7 for neutrophils and monocytes; † $n=1$).

All cell types were compared with NDE: * $P<0·001$; ** $P<0·002$; *** $P<0·01$; **** $P<0·02$.

Table 3. Cell volume and 90° light scatter

	Normal density eosinophils ('mild eosinophilia') ($n=9$)	Normal density eosinophils ('HES') ($n=5$)	Low density eosinophils ('HES') ($n=6$)	Neutrophils ($n=7$)
Cell volume	94·1±2·0	102·4±2·8*	99·3±3·9	87·0±1·8*
90° light scatter	186·9±8·8	203·4±9·0**†	155·9±10·3*	118·4±6·0***

Cell volume and 90° light scatter values presented as the mean ± SEM (standardized arbitrary scale).

All cell types were compared with NDE (mild eosinophilia) except †NDE (HES) versus LDE (HES); * $P<0·05$; ** $P<0·01$; *** $P<0·001$.

polymorphism of FcγRII previously described for monocytes (Anderson *et al.*, 1987).

On neutrophils FcγRI and FcγRII are linked to the cytoplasm via a polypeptide tail, while FcγRIII has a phosphatidylinositol glycan membrane anchor (Selvaraj *et al.*, 1988; Huizinga *et al.*, 1988). FcγRIII is shed from neutrophils on stimulation with the bacterial tripeptide formyl-methionyl-leucyl-phenylalanine (Huizinga *et al.*, 1988). It is not clear how a receptor such as FcγRIII, which does not span the membrane and has no cytoplasmic domain, can mediate cellular functions such as phagocytosis. The work of Tosi & Berger (1988) and Huizinga *et al.* (1989) demonstrated that FcγRIII on neutrophils promotes the binding of immune complexes, particularly small complexes, whereas FcγRII is essential for triggering of the respiratory burst and phagocytosis. Therefore, the role of FcγRIII may be to co-operate with FcγRII to facilitate cell activation. The absence of FcγRIII on eosinophils may be responsible for their poor phagocytic ability compared with the neutrophil. The major role of the eosinophil is the extracellular killing of parasites and these large, highly opsonized targets are probably sufficient to cross-link FcγRII on the eosinophil and cause cell activation without the involvement of FcγRIII (Huizinga *et al.*, 1989).

There is discrepancy in the literature regarding Fcγ receptor expression on eosinophils, but, except for obvious differences in cell preparation methods and assay systems, the explanation for differing results is not clear. Fleit, Wright & Unkless (1982) reported the presence of considerable numbers of FcγRIII on the eosinophil (43,200 sites/cell) using two anti-CD16 mAb (3G8 and 4F7), and this was supported by Looney *et al.* (1986) and most recently by Graziano *et al.* (1989), both using mAb 3G8. The latter two studies also found similar expression of FcγRII on eosinophils and neutrophils using mAb IV3. Whilst Looney *et al.* (1986) concluded that CD16 was too sparsely present on eosinophils to contribute to binding of antibody-coated erythrocytes, Graziano *et al.* (1989) (who estimated 5000 CD16 sites/eosinophil) demonstrated killing of opsonized erythrocytes (but not hybridoma cells) by eosinophils via CD16. However, Kulczycki (1984), who investigated IgG Fc receptor expression by electrophoresis of material isolated from radioiodinated neutrophils and eosinophils, found two bands from neutrophils (FcγRII and FcγRIII) but only a single band from

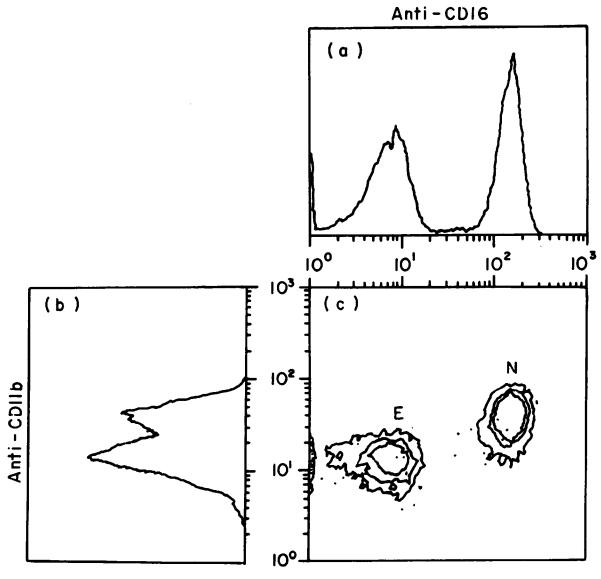

Figure 5. Two-colour immunofluorescence of a mixture (1:1) of eosinophils and neutrophils. (a) Histogram of logarithmic fluorescence intensity of CD16 staining of 10,000 cells. The neutrophils had very bright fluorescence whereas the eosinophils were negative. (b) Histogram of logarithmic fluorescence intensity of CD11b staining of 10,000 cells. The fluorescence of the two cell types overlapped. (c) Double staining of CD16 (horizontal axis) and CD11b (vertical axis). The eosinophils were CD16 negative and expressed slightly less CD11b than the neutrophils, which were CD16 positive.

eosinophils (FcγRII). In agreement with this, Yazdanbakhsh *et al.* (1985) showed that neutrophils but not eosinophils stained with anti-CD16 mAb 3G8 and CLB-FcR-1. In our hands, using anti-Leu 11b, no CD16 expression was observed on eosinophils, leading us to investigate the use of this marker to distinguish eosinophils from neutrophils in flow cytometry. Having sorted a mixture of eosinophils and neutrophils stained for CD16 with anti-Leu 11b (data not shown), we were confident that all the CD16-positive cells were neutrophils and all the CD16-negative cells were eosinophils. Differentiation of eosinophils and neutrophils is facilitated by the exceptionally high density of CD16 expression on neutrophils (Figs 2 and 5).

The other receptors investigated using specific mAb, i.e. LFA-1α, CR3α, p150,95-α, common β-chain, ICAM-1 and CR1, were expressed over a similar range on eosinophils and neutrophils, but both differed from the monocyte (Figs 3 and 4). The surface area of eosinophils and neutrophils has been shown to be similar (Yazdanbaksh *et al.*, 1985), so the density of these receptors on the cell surface must be comparable.

A number of studies have found that low density eosinophils are 'activated' compared with normal density cells in terms of metabolic activity and IgG Fc and complement receptor expression measured by rosetting (Bass *et al.*, 1980; Pincus *et al.*, 1981; Winqvist *et al.*, 1982). In this study we found that low density eosinophils were less granular than normal density eosinophils but that receptor expression was, in general, very similar (Table 2), except in the case of the common β-chain which was less well expressed by low-density cells (Fig. 4). The significance of reduced β-chain expression is unclear since there was no parallel reduction in expression of the alpha chains. It may be an indication that low-density eosinophils in the circulation have undergone down-regulation of their capacity to adhere to the vascular endothelium. The discrepancy between our findings and the results of others, in terms of Fcγ and complement receptor expression, may be due to the different methods employed to assess receptor expression and differences in the source of the low density eosinophils used.

Although the density of expression of a number of functionally relevant receptors appears to be very similar on eosinophils and neutrophils, there may be differences in intracellular signalling mechanisms of these two cell types which give rise to a difference in the quality of the response to a given stimulus. In addition, while the use of specific mAb and flow cytometry gives an accurate measure of the number of receptors on the surface of the cell, it gives no indication of the affinity of the receptor. Therefore, there are a number of ways in which an apparently similar level of receptor expression could give rise to a very different level of response to a stimulus in different cell types.

Investigation of these well characterized cell surface receptors showed only one major difference between eosinophils and neutrophils, that is the lack of CD16 expression on eosinophils (Fig. 2 and Table 2). The functional consequences of this difference are unclear. Eosinophils have very distinctive cytotoxic proteins within their granules, which are crucial to their effector function and which clearly distinguish them from other inflammatory cells. It is possible that they also possess specialized cell-surface receptors which require further characterization.

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