

Expression of Fc γ RIII (CD16) on human peripheral blood eosinophils increases in allergic conditions

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Background: Blood eosinophils have mRNA for Fc γ RIIIB (CD16) but no or minimal spontaneous CD16 expression. Because IFN- γ and chemotactic factors induce eosinophil CD16 expression in vitro, we postulated that blood eosinophils could express CD16.

Objective: Blood of nonallergic controls and subjects with allergic rhinitis, allergic and nonallergic asthma, or hypereosinophilia of various etiologies were analyzed for leukocyte CD16 surface expression.

Methods: CD16⁺ eosinophils were identified on the basis of physico-optic characteristics, major basic protein, CD49b expression, and sorting by flow cytometry and microscope examination.

Results: Subjects with allergic rhinitis and subjects with asthma had higher median percentages of CD16⁺ eosinophils (8.1% [1% to 48.6%] and 7.3% [1.4% to 31.1%], respectively) than nonallergic controls and nonallergic asthmatics (3% [0% to 11%] and 4.6% [2.9% to 5.1%], respectively). In subjects with hypereosinophilia, CD16⁺ eosinophils were increased only in a case of drug allergy. When subjects with mild allergic asthma were challenged with a relevant aeroallergen, blood CD16⁺ eosinophils further increased during or after the late-phase response (6 to 48 hours after challenge; mean $\pm$ SEM, 9.4% $\pm$ 2.5% to 20.0% $\pm$ 3.0%). CD16⁺ eosinophils expressed more IL-5 receptor but less CD11b and IL-12p35 than did CD16⁻ eosinophils.

Conclusion: Upregulation of blood CD16⁺ eosinophils in allergic conditions and its association with a modified phenotype suggest that CD16 receptor could play a role in eosinophil activation in allergy. (*J Allergy Clin Immunol* 2002;109:463-9.)

Key words: Eosinophils, CD16, allergic rhinitis, asthma, hypereosinophilia, allergen challenge, IL-5 receptor, CD11b, IL-12p35

CD16 is a member of the IgG receptor family that also includes Fc γ RI (CD64) and Fc γ RII (CD32).¹ These receptors belong to the Ig superfamily and are widely expressed in hematopoietic cells. The CD16 receptor is present in 2

Abbreviations used

dSS: Depolarized side light scattering
EAR: Early asthmatic response
FS: Forward scatter light
LAR: Late asthmatic response
MBP: Major basic protein
PE: Phycoerythrin

isotypes, A and B. Fc γ RIIA is found on macrophages, activated monocytes, natural killer cells, T cells, and progenitors of B and T lymphocytes.¹ Fc γ RIIIB is specific to neutrophils and mediates exocytosis of granule proteins.² The A isoform is a transmembrane molecule with a distinct cytoplasmic domain. The B isoform is linked to the outer layer of the plasma membrane by a glycosylphosphatidyl-inositol anchor and is unable to induce cell signaling by itself.^{3,4} The 2 isoforms have the same extracellular domain with a low affinity for IgG complexes.¹

Eosinophils transiently circulate in the blood and migrate into tissues, where they likely play an important role in tissue homeostasis and various inflammatory processes.⁵ However, because the accessibility of blood eosinophils is easier than that of their tissue counterparts, blood eosinophils are frequently used to study biological features of these cells. Purification of blood eosinophils requires their discrimination from other, more numerous leukocytes. One major step in this procedure is the isolation of eosinophils from neutrophils. All neutrophils express CD16B.^{1,6} In contrast, previous studies reported minimal or no expression of CD16B on blood eosinophils in normal subjects and in disease conditions such as allergic rhinitis, asthma, and hypereosinophilic syndrome.⁷⁻¹⁰ Consequently, the technique of eosinophil purification by CD16⁻ immunomagnetic selection of neutrophils is commonly used to obtain pure eosinophil preparations.

Although CD16 expression is reported to be absent or minimal on eosinophils, blood eosinophils from subjects with mild eosinophilia express the mRNA for Fc γ RIIIB.¹¹ Western blot and immunofluorescence analyses demonstrated constitutive intracellular CD16B in eosinophils from mild atopic subjects.⁷ In vitro, IFN- γ induced de novo functional and sustained surface CD16B expression on eosinophils,¹¹ whereas the chemoattractants induced transient surface expression of CD16B.⁷ In our experience, poor recovery of eosinophils with the CD16⁻ immunomagnetic purification of blood from subjects with symptomatic allergic asthma suggested that at least in this

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TABLE I. CD16⁺ eosinophil counts in blood of patients with hypereosinophilia

Sex	Age (y)	Diagnosis	Blood eosinophil count: % (10 ⁹ /L)	CD16 ⁺ eosinophil count: %
F	68	Transient, drug-induced	18.0 (1.2)	74.0
F	50	Hypereosinophilic syndrome	42.4 (3.5)	7.3
F	73	Paraneoplastic	18.6 (2.5)	3.3
F	55	Transient, possibly drug allergy	13.4 (0.1)	11.5
M	89	Unknown	38.0 (0.7)	0.8
M	75	Eosinophilic cellulitis of unknown etiology	63.0 (9.2)	1.6
F	27	Churg-Strauss	69.7 (23.7)	4.8

condition, a large number of blood eosinophils expressed CD16 and were captured in the column.

The objectives of this study were to document the existence of blood CD16⁺ eosinophils, to evaluate their association with various clinical conditions associated with blood eosinophilia, and to compare their expression of various membrane receptors and intracellular cytokines with that of CD16⁻ eosinophils.

METHODS

Reagents

Monoclonal anti-CD16 (clone 3G8), CD69, CD44, CD11b, CD49d, IL-5 receptor, GM-CSF receptor, IL-12p35, IL-12p40, IL-2, IL-5, IFN- γ , GM-CSF antibodies, and isotype controls were purchased from BD Pharmingen (San Diego, Calif). Monoclonal anti-CD16 (clone LNK7) was from Serotec (Raleigh, NC). Anti-major basic protein (MBP) was kindly provided by Dr Redwan Moqbel (Edmonton, Alberta, Canada). Allergens for inhalation challenges were standardized house dust mite and cat fur extracts (Bencard, Mississauga, Ontario, Canada). FCS and RPMI 1640 were purchased from Canadian Life Technology (Burlington, Ontario, Canada).

Subjects

The Laval Hospital ethics committee approved the study, and all subjects signed informed consent forms. For the evaluation of CD16⁺ blood eosinophil expression and their phenotype, blood samples were obtained from nonallergic normal volunteers (9 women and 1 man; age [mean $\pm$ SEM], 30 $\pm$ 4 years; blood IgE, 45 $\pm$ 27 IU/mL; negative skin prick test results; blood eosinophils, 0.13 $\pm$ 0.02 $\times$ 10⁹/L; FEV₁, 104% $\pm$ 5% of predicted; PC₂₀ methacholine [geometric mean], 69.0 $\pm$ 1.3 mg/mL) and subjects with allergic (14 women and 9 men; age, 25 $\pm$ 1 years; IgE, 266 $\pm$ 39 IU/mL; positive skin prick test results; eosinophils, 0.30 $\pm$ 0.02 $\times$ 10⁹/L; FEV₁, 96% $\pm$ 2% of predicted; PC₂₀, 1.5 $\pm$ 1.3 mg/mL) and nonallergic (4 women and 1 man; age, 24 $\pm$ 1 years; IgE, <4 IU/mL; negative skin prick test results; eosinophils, 0.08 $\pm$ 0.02 $\times$ 10⁹/L; FEV₁, 88% $\pm$ 6% of predicted; PC₂₀, 2.3 $\pm$ 0.3 mg/mL) mild asthma and allergic (10 women and 10 men; age, 26 $\pm$ 1 years; IgE, 186 $\pm$ 39 IU/mL; positive skin prick test results; eosinophils, 0.22 $\pm$ 0.03 $\times$ 10⁹/L; FEV₁, 105% $\pm$ 2% of predicted; PC₂₀, 35 $\pm$ 1.3 mg/mL) rhinitis. Asthmatic subjects diagnosed according to the American Thoracic Society criteria¹² had mild symptoms and used only inhaled β -agonists as needed. They had stable asthma and had not used inhaled corticosteroids or any other anti-inflammatory drugs in the previous 3 months. The atopic rhinitic subjects were asymptomatic at the time of the study and were not taking any medication. Blood samples were also obtained from subjects with hypereosinophilia (>10%) of various etiologies for CD16 expression analysis on eosinophils (Table I). For the evaluation of blood eosinophil CD16 expression in response to an allergic inflammatory event, subjects

with mild atopic asthma (8 women and 6 men; age, 24 $\pm$ 1 years; IgE, 319 $\pm$ 46 IU/mL; positive skin prick test results; eosinophils, 0.32 $\pm$ 0.03 $\times$ 10⁹/L; FEV₁, 95% $\pm$ 3% of predicted; PC₂₀, 1.3 $\pm$ 1.4 mg/mL) underwent allergen challenge as described below.

Clinical evaluation

Atopy was defined as the presence of at least 1 positive response (wheal diameter $\geq$ 3 mm at 10 minutes) to skin prick tests with a battery of 26 common airborne allergens.¹³ Expiratory flows were measured according to the American Thoracic Society's recommendations.¹⁴ Nonspecific airway hyperresponsiveness was determined by methacholine challenge¹⁵ and expressed as the concentration that caused a 20% decrease in FEV₁ (PC₂₀; in mg/mL). Serum IgE was measured by immunoenzymofluorometry (normal value, <100 IU/mL), and blood eosinophils were counted on a Coulter Gen-S system 2 (Beckman Coulter, Hialeah, Fla).

Allergen challenge

The dose of allergen extract for inhalation was determined through use of a modification of the Cockcroft equation, which takes into account the results from the skin titration and the PC₂₀ to methacholine.¹⁶ The starting dose of allergen was 4 doubling doses below the calculated dose of allergen predicted to give a 20% fall in FEV₁. Allergen was inhaled for 2 minutes, and FEV₁ was recorded 10 minutes later. Doubling dilutions of the starting dose were administered until the 10-minute FEV₁ had fallen by 15% or more. The early asthmatic response (EAR) was reached if FEV₁ fell by 15% during the first hour; the late asthmatic response (LAR) was reached if FEV₁ fell by 10% between 3 and 7 hours after allergen inhalation. Each subject had to develop an LAR to be included in the study. Blood samples were taken before challenge (baseline), at the LAR, and 24 and 48 hours after challenge. The mean EAR and LAR were 27% $\pm$ 3% and 22% $\pm$ 3%, respectively. To verify any effect of the circadian cycle on eosinophil CD16 expression, we collected blood at the same time of day in 3 of these subjects at another visit without allergen challenge.

Blood process and flow cytometry analysis of leukocyte preparations

Blood samples (10 mL) were collected in vials with EDTA (Vacutainer, Becton Dickinson, Franklin Lakes, NJ). Erythrocytes were lysed with ammonium chloride solution (150 mmol/L NH₄Cl, 10 mmol/L KHCO₃, 0.1 mmol/L Na₂EDTA, pH 7.4), and the resulting white blood cell pellet was washed with PBS. Cells (1 $\times$ 10⁶) were incubated with the anti-CD16-phycoerythrin (PE) (clone 3G8), anti-MBP, anti-CD49d-PE, or matched isotype control for a 30-minute incubation at 4°C. Cells were washed with ice-cold PBS, fixed for 10 minutes on ice with paraformaldehyde 4%/PBS, and permeabilized with saponin 0.1%/PBS, as previously described.¹⁷ Cells were then analyzed by flow cytometry on an Epics Elite ESP (Beckman Coulter). Eosinophils were discriminated from other

leukocytes by autofluorescence and physico-optic cell properties through use of forward scatter light (FS) and depolarized side light scattering (dSS), measured after fixation and permeabilization.¹⁷ Monoclonal anti-MBP antibody was used in some experiments to further confirm eosinophil identity. Monoclonal anti-CD49d antibody staining was used as a control to further discriminate eosinophils from neutrophils in all experiments. A total of 5000 eosinophils were depicted in FS-dSS cytograms for CD49 and CD16 expressions in each blood sample.

Effect of recombinant human IFN- γ on eosinophil CD16 expression

Blood CD16⁺ eosinophils were purified by immunomagnetic cell selection through use of anti-CD16 antibody-conjugated beads (Miltenyi Biotec, Hamilton, Ontario, Canada), as previously described.¹⁸ Purified eosinophils were incubated with increasing concentrations of recombinant human IFN- γ (10^{-4} to 10^3 μ g/mL) in RPMI 1640 medium complemented with 10% FBS for 24 hours. The CD16 level was determined by flow cytometry.

Measurement of IFN- γ level in plasma of subjects who underwent an allergen challenge

Blood samples were taken at baseline and at 24 and 48 hours after challenge; plasma was kept at -70°C . The dosage of IFN- γ was done in the same plate and at the same time for all samples through use of ELISA for human IFN- γ (OptEIA Human IFN- γ Set, BD Pharmingen), performed according to the manufacturer's instructions (detection limit, 4 pg/mL).

Evaluation of CD16⁺ and CD16⁺ eosinophil phenotype

CD16⁺ eosinophils were isolated from subjects with allergic asthma as described above.¹⁸ The column was removed from the magnetic field and washed out with PBS to recover the CD16⁺ cell fraction. Both cell populations were fixed for 10 minutes on ice with paraformaldehyde 4%/PBS. Direct surface staining was done for CD69 and CD44, 2 activation markers for human eosinophils,¹⁹ and CD11b, an integrin involved in adherence and FcR-mediated degranulation in neutrophils,²⁰ with appropriate mAbs. Intracellular staining was done to evaluate expression of IL-5, GM-CSF, IL-2, IL-12p35, IL-12p40, and IFN- γ by eosinophils. Indirect surface staining was done for IL-5 and GM-CSF receptors with anti-mouse-IgG-antibody-biotin/streptavidin-PE complexes. Eosinophils were discriminated from neutrophils through use of the techniques described above. For the analysis of surface antigens, permeabilization with saponin for the FS-dSS eosinophil discrimination was done after incubation with antibodies. Staining and analysis were done simultaneously for CD16⁺ and CD16⁺ cell populations for each subject.

RESULTS

Cytometry analysis of blood eosinophils

A clear differentiation of eosinophils from neutrophils was obtained through use of the FS-dSS method during cytometry analysis of blood cell preparations. Cells with high dSS were sorted in 3 experiments and examined on Diff-Quik-stained (Dade Diagnostics, Aguada, Puerto Rico) cytocentrifuged preparations: they were mostly eosinophils (>99%), as previously described.¹⁷ Moreover, almost all cells with high dSS were MBP⁺ (data not shown; measures repeated 3 times). Fig 1 shows exam-

ples of blood leukocyte CD49 and CD16 expression measurements. CD49 immunostaining confirmed that the cells depicted as eosinophils by the FS-dSS discrimination method were CD49d⁺ (Fig 1, B).²¹ Fig 1, C shows that a portion of the eosinophils (19.8%) was CD16⁺. Interestingly, the fluorescence intensity of CD16 expression on CD16⁺ eosinophils was similar to that observed on CD16⁺ neutrophils. These cytometry analyses confirmed that blood eosinophils can express CD16 at their surface and showed that the CD16 expression level of CD16⁺ eosinophils is similar to that of neutrophils.

CD16 expression on blood eosinophils

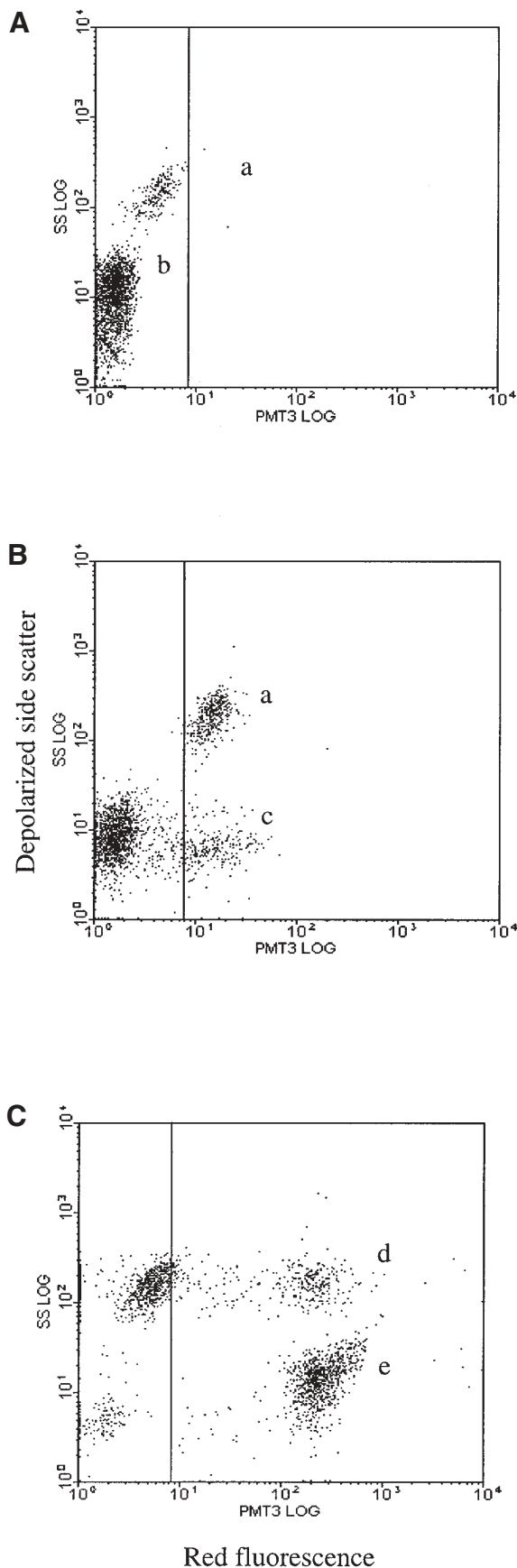
The expression of CD16 was very low or absent on eosinophils of control subjects (median [range], 3.0% [0% to 11.0%]; Fig 2) and nonallergic asthmatic subjects (4.6% [2.9% to 5.1%]). In contrast, there was a wide range of CD16 expression on eosinophils from subjects with allergic asthma (7.3% [1.4% to 31.1%]) or allergic rhinitis (8.1% [1.0% to 48.6%]). The CD16⁺ eosinophil counts were higher in allergic asthmatic and rhinitic subjects than in normal subjects and nonallergic asthmatic subjects ($P < .05$ by Kruskal-Wallis test). In some individuals, the CD16⁺ eosinophil count reached >20% of the total eosinophil number. For all subjects tested ($n = 58$), no correlation was found between CD16⁺ eosinophil counts and total blood eosinophil counts. Analysis of CD16 expression on blood samples obtained from subjects with hypereosinophilia of various etiologies showed variable CD16⁺ eosinophil counts (Table I). With the exception of drug allergy, CD16⁺ eosinophil counts of other hypereosinophilia conditions were similar to those of normal subjects despite very high total blood eosinophil counts.

Blood eosinophil CD16 expression after allergen challenge

Within 48 hours after allergen challenge, 13 of 14 asthmatic individuals presented an increase in CD16⁺ eosinophil: $9.4\% \pm 2.5\%$ to $20.0\% \pm 3.0\%$ (expressed as absolute numbers: 19.2 ± 3.2 to $40.8 \pm 8.3 \times 10^6/\text{L}$; Table II; $P < .05$ by repeated-measures ANOVA through use of baseline values as covariate). A baseline expression of CD16 >10% was predictive of an early increase of this expression at the LAR after the allergen challenge ($P < .01$ by Fisher test). There was no circadian variation of the CD16 expression ($n = 3$; data not shown). The increase in CD16 was not related to any other clinical parameter, such as eosinophil blood count, severity of the EAR or LAR, serum IgE level, or PC₂₀.

Role of IFN- γ in increased CD16 expression after allergen challenge

The in vitro dose-response curve showed a close correlation between the dose of IFN- γ and the percentage of CD16 expression on eosinophils ($r^2 = 0.91$; $P < .0001$). No increase in CD16 expression was observed below an IFN- γ concentration of 0.01 μ g/mL. The blood level of IFN- γ measured before the allergen challenge (15 ± 3



pg/mL) did not reach the concentration that is required to induce eosinophil CD16 expression *in vitro*. No correlation was found between IFN- γ levels and blood CD16⁺ eosinophil counts. A significant decrease in blood IFN- γ level was observed at the LAR (2 ± 4 pg/mL) and 24 hours after the challenge (2 ± 4 pg/mL), and a partial recovery to the baseline level occurred at 48 hours (9 ± 4 pg/mL; $P < .0001$ by ANOVA).

CD16⁺ and CD16⁻ eosinophil phenotypes

CD16⁺ and CD16⁻ eosinophils were compared for expression of (a) the surface markers CD44, CD69, and CD11b, (b) the cytokines IL-5, GM-CSF, IL-2, IL-12, and IFN- γ , and (c) GM-CSF and IL-5 receptors. No significant differences were found between CD16⁺ and CD16⁻ eosinophils for CD44 ($74\% \pm 19\%$ and $80\% \pm 7\%$, respectively) or CD69 ($11\% \pm 7\%$ and $30\% \pm 13\%$, respectively). The CD11b⁺ cell count was slightly lower in CD16⁺ eosinophils than in CD16⁻ ones ($73\% \pm 7\%$ and $91\% \pm 3\%$, respectively; Fig 3, A; $P = .03$ by Student paired *t* test). The number of IL-12p35⁺ cells was lower in CD16⁺ eosinophils ($27\% \pm 10\%$) than in CD16⁻ eosinophils ($56\% \pm 9\%$; Fig 3, B; $P = .04$ by Student paired *t* test). No significant differences were observed between the 2 eosinophil populations for IL-5 ($27\% \pm 16\%$ and $18\% \pm 6\%$ for CD16⁺ and CD16⁻ cells, respectively), GM-CSF ($19\% \pm 8\%$ and $32\% \pm 8\%$), IL-2 ($29\% \pm 7\%$ and $26\% \pm 11\%$), IFN- γ ($20\% \pm 7\%$ and $12\% \pm 6\%$), or IL-12p40 ($34\% \pm 12\%$ and $39\% \pm 6\%$). Expression of GM-CSF receptors was similar on both CD16⁺ and CD16⁻ eosinophils ($20\% \pm 8\%$ and $23\% \pm 10\%$, respectively). In contrast, 9 of 12 subjects showed an increase in cells positive for IL-5 receptor within the CD16⁺ eosinophil population ($21\% \pm 8\%$ to $39\% \pm 8\%$; Fig 3, C; $P = .07$ by Student paired *t* test).

DISCUSSION

Previous studies reported minimal or no expression of CD16 receptor on freshly isolated blood eosinophils.⁷⁻¹⁰ Hartnell et al⁸ observed no trace of CD16 on purified eosinophils of subjects with hypereosinophilic syndrome. Zhu et al⁷ found less than 7% of CD16⁺ eosinophils in purified blood granulocyte preparations of donors with mild atopy. Using flow cytometry analysis of unpurified blood leukocyte preparations, we demonstrat-

FIG 1. Eosinophils and neutrophils express CD16 at similar fluorescence levels. This is a typical example of flow cytometry analysis of blood leukocytes obtained from an asthmatic subject. **A**, Iso-type staining of leukocytes. Eosinophils (a) were discriminated from other leukocytes (b) through use of autofluorescence and dSS characteristics of eosinophils after fixation and permeabilization and CD49 positivity. **B**, CD49 immunostaining. All eosinophils (a) were positive for CD49. Some cells with low dSS (c), probably lymphocytes, were also positive for CD49. **C**, CD16 immunostaining. Some eosinophils expressed CD16 (d). The intensity (fluorescence level) of CD16 expression of CD16⁺ eosinophils was similar to that of neutrophils (e). Other leukocytes with low dSS did not express CD16. Note that because of their smaller size and weaker autofluorescence in comparison with granulocytes, the lymphocyte population was not entirely captured in this dot-plot histogram.

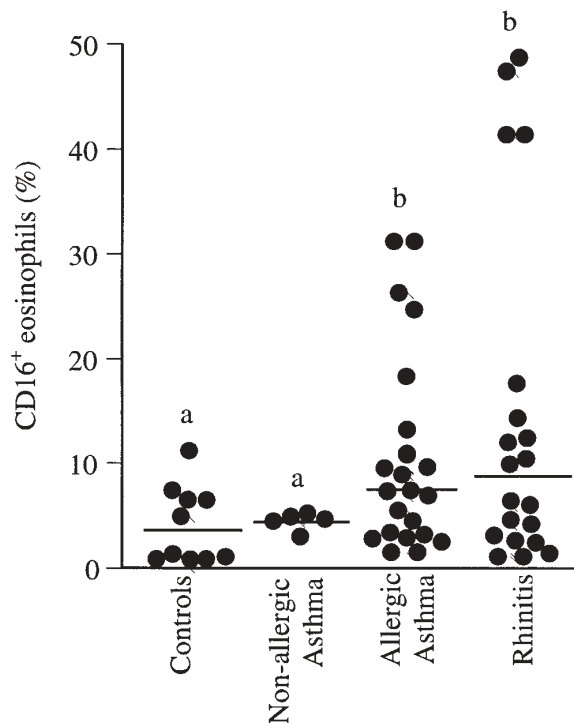


FIG 2. Eosinophils from subjects with allergic rhinitis and allergic asthma express more CD16 than those from normal controls and nonallergic asthmatic individuals. For each individual, the dots represent the percentage of CD16⁺ eosinophils and the lines represent the median value. Columns with different letters are statistically different ($a \neq b$; $P < .05$ by Kruskal-Wallis test).

ed that a subpopulation of blood eosinophils expresses surface CD16 *in vivo* and that CD16⁺ eosinophil counts increase in allergic diseases. Moreover, we showed that the phenotype of CD16⁺ eosinophils differs from that of CD16⁻ eosinophils.

In the present study, blood samples were not processed (except for red cell lysis), the intent being to minimize potential alteration of eosinophil membrane expression; total blood leukocyte populations were analyzed. Physico-optic characteristics were used to discriminate eosinophils from the other leukocytes by flow cytometry analysis, as previously described.¹⁷ The identity of the eosinophil population was further confirmed by sorting and microscope examination and by evaluating the expression of MBP and CD49b expressions, a mediator and a receptor largely expressed by eosinophils but not by neutrophils.²¹ These analyses clearly demonstrated the expression of CD16 on a subpopulation of eosinophils in normal controls and its increase in subjects with allergic rhinitis and asthma but not in nonallergic asthmatic individuals. In these allergic subjects, the observed increase in CD16 expression was not related to blood eosinophil count. Subjects with hypereosinophilia did not present increased CD16⁺ eosinophil counts (except for the subject with drug allergy). Moreover, blood CD16⁺ eosinophil counts augmented significantly after allergen challenge in subjects with allergic asthma. These observations suggest

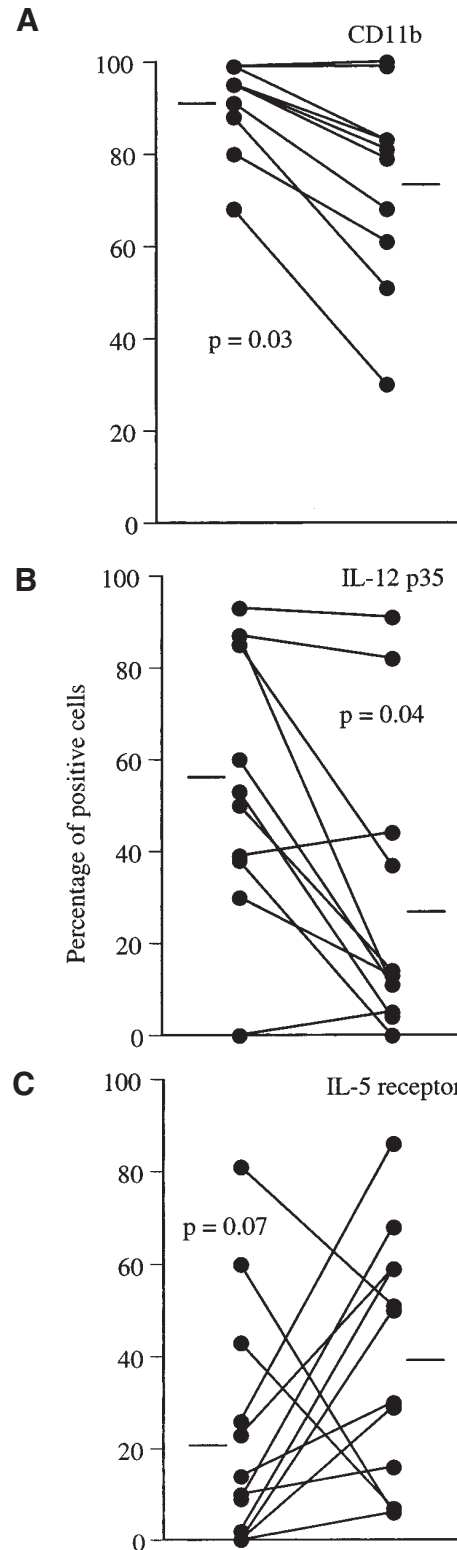


FIG 3. CD16⁺ eosinophils express less CD11b (A) and IL-12p35 (B) but more IL-5 receptor (C) than do CD16⁻ eosinophils. The horizontal lines represent the means. Values for each individual are linked by a line.

TABLE II. Percentage of CD16⁺ eosinophils in blood of asthmatics before and after allergen challenge

Subject no.	Baseline	LAR	24 h	48 h
1	31.1	42.0	3.3	5.2
2	2.8	6.3	9.9	15.2
3	26.2	34.3	15.2	2.2
4	7.3	2.8	20.1	3.0
5	2.7	0.7	0.0	5.0
6	4.4	6.4	15.9	5.0
7	18.2	4.2	15.6	8.8
8	10.7	22.4	17.4	0.0
9	1.4	5.0	22.1	3.0
10	8.8	4.0	10.8	3.6
11	1.4	1.6	12.6	0.0
12	3.3	1.9	4.3	13.5
13	2.4	1.8	8.1	17.7
14	1.8	28.1	2.4	2.4

A significant increase in the eosinophil CD16⁺ population was observed during the 48-hour period following the challenge ($P < .05$ by repeated-measures ANOVA). For each subject, the number in bold represents the maximum percentage of CD16⁺ eosinophils observed.

that the allergic inflammatory process upregulates eosinophil CD16 expression.

The mechanisms modulating blood eosinophil CD16 expression are not known. The expression of CD16 on eosinophils in allergic subjects could be induced by cytokines or chemoattractants present in blood. Because expression of CD16 was induced in vitro by IFN- γ ¹¹ and because IFN- γ level increases in serum during acute severe asthma attacks²² and in bronchoalveolar lavage fluid after antigen challenge in allergic subjects,^{23,24} we measured IFN- γ concentration in the blood of asthmatic individuals after allergen challenge. The serum IFN- γ level decreased after allergen challenge; consequently, no correlation was found between CD16⁺ eosinophil number and serum IFN- γ concentration. These observations suggest that IFN- γ is probably not responsible for the increased blood CD16⁺ eosinophil counts observed after allergen challenge.

The role of CD16 with respect to eosinophils is not known. In neutrophils, the IgG complex reaction is mainly mediated by CD32 receptors, whereas CD16 receptors act as traps for immune complexes to enhance CD32 signaling. Because the level of CD16 expression on positive eosinophils is similar to that of neutrophils, this receptor might play a similar role with respect to eosinophils. This hypothesis is supported by the report of Valerius et al,⁹ who showed that blocking antibodies for CD16 (3G8) only partially abrogated FcR-mediated cytotoxicity in comparison with blocking antibodies for CD32 in freshly purified and IFN- γ -stimulated eosinophils.⁹ After allergen challenge, some aeroallergens are detected in serum and form complexes with specific IgG.²⁵⁻³¹ Allergen-specific IgG is also found in the lung; its role in asthmatic allergic reaction has not yet been defined. The possible interactions of allergen-IgG complexes and CD16⁺ eosinophils might be implicated, at least in part, in the chronicity of inflammation in allergy.

CD11b (MAC-1) plays a role in FcR-immune complex interactions in neutrophils by facilitating adherence and actin reorganization that precede phagocytosis and oxygen radical generation.²⁰ CD11b expression was decreased on CD16⁺ eosinophils in comparison with CD16⁻ cells, but its level remained relatively high. Further investigations are needed to determine whether CD11b is an important integrin involved in immune complex-mediated eosinophil activation. The decrease of intracellular IL-12 expression in CD16⁺ eosinophils is particularly interesting. In animal models, rIL-12 inhibits blood and bronchoalveolar lavage eosinophilia and bronchial hyperreactivity induced by allergen challenge. IL-12 inhibits T cell production of IL-5, a cytokine promoting eosinophil priming and survival.³²⁻³⁴ Eosinophil-derived IL-12 might have an autocrine or paracrine inhibitory effect on eosinophils.³⁵ In asthmatic individuals, the numbers of cells expressing IL-12 mRNA are reduced in bronchial tissue and blood leukocytes in comparison with normal controls.³⁶ Moreover, asthmatic subjects' blood leukocytes in vitro produced less IL-12 after stimulation.³⁷

Herein we have shown that CD16⁺ eosinophil counts are increased in allergic inflammatory processes and that these cells express less IL-12. Expression of IL-5 receptors is upregulated in eosinophils freshly released from bone marrow.³⁸ In this study, the CD16⁺ blood eosinophil population presented an increase of IL-5 receptor expression that tended to be significant ($P = .07$) in comparison with CD16⁻ cells. This observation suggests that CD16⁺ eosinophils probably result from active recruitment of eosinophils from bone marrow induced by an allergic reaction. Moreover, increased IL-5 receptor expression suggests that these eosinophils could be more efficiently activated by IL-5. Decreased IL-12 expression and increased IL-5 receptor expression could make CD16⁺ eosinophils more active proinflammatory cells in T_H2-like inflammatory processes.

The high counts of CD16⁺ blood eosinophils observed in allergic conditions should be taken into account in studies of purified blood neutrophils and eosinophils. The presence of eosinophils in neutrophil preparations should be ruled out when CD16 is used as a unique marker to sort out these 2 types of cells. The presence of a variable population of CD16⁺ eosinophils should also be considered when eosinophils from atopic subjects and eosinophils from nonatopic subjects are being compared after purification. A significant number of eosinophils, the CD16⁺, could be discarded by the purification process in allergic conditions.

In conclusion, we have shown that an important subpopulation (up to 50%) of blood eosinophils are CD16⁺ in allergic asthmatic and allergic rhinitic subjects and that allergen exposure can transiently increase this subpopulation in asthmatic individuals. This observation suggests that CD16 could be a marker of eosinophil activation during the allergic response. The role of CD16 expression and of the modified phenotype that presents CD16⁺ eosinophils needs to be better defined.

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