

Increased adhesion to vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 of eosinophils from patients with asthma

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Adhesion of peripheral blood eosinophil and neutrophil granulocytes to the endothelial cell adherence receptors E-selectin, vascular cell adhesion molecule-1, and intercellular adhesion molecule-1 has been measured. The study included patients with allergic rhinitis, patients with mild allergic and nonallergic asthma, and healthy individuals; 10 persons were in each group. In addition, assay of eosinophil and neutrophil cell surface expression of the receptor complex CD11b/CD18 was performed. Increased eosinophil adhesion to vascular cell adhesion molecule-1 ($p < 0.05$) and intercellular adhesion molecule-1 ($p < 0.05$) was demonstrated in the patients with a more labile asthma, that is, a peak expiratory flow rate variability of more than 10%, suggesting a relationship to the degree of ongoing inflammation in the airways of the patients. The increased eosinophil adhesion was most probably due to a functional upregulation of the CD11b/CD18 and very late activation antigen-4 receptors, because the number of receptors measured as cell surface expression was unaltered. The increased eosinophil adhesion in the patients with high peak expiratory flow rate variability appeared independent of atopy. The increased adhesion was not entirely specific to the eosinophils, because neutrophils from patients with a peak expiratory flow rate variability of more than 10% also demonstrated increased adhesion to intercellular adhesion molecule-1 ($p < 0.05$) when compared with neutrophils from the patients with low peak expiratory flow rate variability. In conclusion, the demonstrated priming of eosinophil adhesion to vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 might be one contributing mechanism behind the selective accumulation of eosinophils in the lung tissue of patients with asthma. (J ALLERGY CLIN IMMUNOL 1995;96:941-50.)

Key words: Eosinophil, neutrophil, adhesion, E-selectin, VCAM-1, ICAM-1, CD11b/CD18, VLA-4, asthma

The eosinophil granulocyte is now considered to form an important part of the inflammatory process that is responsible for asthmatic disease. This hypothesis is based on the observation that eosinophils accumulate in the lung tissue of patients

Abbreviations used

BHK:	Baby hamster kidney
BHR:	Bronchial hyperresponsiveness
ECP:	Eosinophil cationic protein
FEV ₁ :	Forced expiratory volume in 1 second
ICAM:	Intercellular adhesion molecule
MPO:	Myeloperoxidase
PD ₂₀ :	Provocative concentration (of methacholine) causing a 20% fall in FEV ₁
PEF:	Peak expiratory flow
VCAM:	Vascular cell adhesion molecule
VLA:	Very late activation antigen

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with asthma, especially during an exacerbation of the disease.¹⁻³ Furthermore, increased amounts of eosinophil granule proteins indicated that the eosinophils were activated.^{4,5} Finally, eosinophil

granule proteins such as major basic protein, eosinophil peroxidase, and eosinophil cationic protein (ECP) have been shown in experiments to cause damage to epithelial cells similar to that seen in lung biopsy specimens from patients with asthma.⁶⁻⁹

The mechanism or mechanisms behind the selective accumulation of eosinophils in lung tissue appear to be an essential pathophysiologic cause of the inflammatory process in patients with asthma, because such mechanisms are the prerequisite for the presence of eosinophils in lung tissue. Previous investigations have demonstrated increased levels of chemotactic activity in bronchoalveolar lavage fluid obtained from patients with allergic asthma during pollen season.¹⁰ The chemotactic activity, however, attracted both eosinophils and neutrophils. Furthermore, priming of the eosinophil migratory responses, such as chemotaxis and chemokinesis, has been demonstrated by studies on blood eosinophils from patients with asthma.^{11, 12} These mechanisms could partly explain the selective accumulation of eosinophils but are not pronounced enough to be the only cause.

Adherence of eosinophils to the endothelial cells of the blood vessel wall is an absolute requirement for the migration of eosinophils into the tissue in response to chemotactic substances. Adhesion of granulocytes in general is mediated through the induction and activation of adherence receptors on granulocytes and endothelial cells.¹³⁻¹⁵ Eosinophils are able to adhere to the endothelial adherence receptors P-selectin, E-selectin, vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1.¹⁶⁻¹⁸ Adhesion to VCAM-1, which is exclusive for eosinophils compared with neutrophils, is mediated through the very late activation antigen (VLA)-4 (CD49d/CD29) molecule on the eosinophils, adhesion to ICAM-1 by the Mac-1 complex (CD11b/CD18), and adhesion to E-selectin by sialyl-dimeric Lewis^x¹⁹; but the counterreceptor for P-selectin on eosinophils has not been conclusively identified.

The aim of this investigation was to study the adherence function of eosinophils from patients with asthma and to compare it with that of neutrophils and of eosinophils from healthy individuals. By means of a newly developed method that uses fibroblasts transfected with E-selectin, VCAM-1, and ICAM-1, the adhesion of eosinophil and neutrophil granulocytes to E-selectin, VCAM-1, and ICAM-1 has been selectively measured.²⁰ In addition, assays of the cell surface expression of

CD11b, CD18, and CD49d on eosinophils and neutrophils were performed by means of immunoflow cytometry.

METHODS

Study population

The individuals participating in this study were recruited from the European Community Respiratory Health Survey^{21, 22} in Uppsala. A previous study, performed 2 years earlier, initially included 3600 persons, ranging in age from 20 to 44 years. Approximately 700 of these patients, including all patients with asthma and patients with possible asthma, were examined with skin prick tests, a methacholine provocation test, and symptom analysis.^{21, 23, 24} From this latter population 40 persons were recruited: 10 with allergic rhinitis, 10 with allergic asthma, 10 with nonallergic asthma, and 10 healthy control subjects. The control subjects had no symptoms of asthma or allergic rhinitis, no bronchial hyperresponsiveness (BHR), and a negative skin prick test result. (One of the 10 individuals was omitted because of eosinophilia $>400 \times 10^6/L$.) All individuals were nonsmokers. Subjects with allergic rhinitis had atopy and reported symptoms of hay fever but not of asthma. Subjects with allergic asthma had symptoms of asthma (see following), BHR, and atopy. Subjects with nonallergic asthma had symptoms of asthma and BHR, but they did not have atopy.

For this classification the following definitions were used: BHR was defined as a reduction in forced expiratory volume in 1 second (FEV₁) by at least 20% after inhalation of 2 mg or less of methacholine with a Mefar dosimeter (Mefar, Brescia, Italy).²⁵ Atopy was defined as a skin prick test reaction to at least one of 10 common allergens with a mean diameter of ≥ 3 mm.²⁶ Symptoms of asthma were defined as a positive answer to one or more parts of the following question. Have you at any time during the last 12 months had wheezing or whistling in the chest, a daytime attack of breathlessness during exercise, a daytime attack of breathlessness while resting, or a nighttime awakening because of an attack of breathlessness?

The investigation was performed outside pollen season and in all cases was done in the morning within a fixed time interval of 1 hour. The patients included were taking only intermittent short-acting β -agonists, had not been treated with corticosteroids of any kind during the 4 weeks preceding the examination, and had not had any infection during the 3 preceding weeks.

All persons gave their informed consent. The protocol of the study was approved by the Ethics Committee of the Medical Faculty of Uppsala University.

Lung function

FEV₁ was measured with the Spiro Medics computed dry-rolling seal spirometer system 2130 (Sensor Medics,

Anaheim, Calif.). The predicted values were calculated for each patient.²⁷

Peak flow diary

Each subject was asked to register his or her peak expiratory flow (PEF) rate (best of three measurements) with a Mini-Wright peak flow meter (Clement Clarke, London, U.K.) four times daily during 2 weeks. PEF variability was calculated during the second week by dividing the difference between the highest and lowest daily PEF reading by daily mean PEF value.²⁸

Symptom analysis

An assessment of current asthma symptoms was done with the question mentioned previously. A positive answer to any part of that question was given a score of 1 point, and thus the range of possible scores was 0 to 4.

Isolation of granulocytes

Granulocytes were isolated according to a procedure earlier described.²⁰ Briefly, heparinized blood was mixed with an equal volume of phosphate-buffered saline solution in a 50 ml tube, and one volume of Percoll (Pharmacia, Uppsala, Sweden) (67%) was placed in the bottom of the tube. After centrifugation at 1000 g was performed for 30 minutes at room temperature, the mononuclear leukocytes on top of the Percoll gradient and the gradient were removed. The erythrocytes were lysed by the addition of NH_4Cl (155 mmol/L) and incubation on ice for 15 minutes. The remaining granulocytes were washed twice with the assay buffer containing NaCl (140 mmol/L), KCl (5 mmol/L), MgCl_2 (1 mmol/L), CaCl_2 (1 mmol/L), glucose (5.6 mmol/L), human serum albumin (30 mg/L), and N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (10 mmol/L) with a pH of 7.4; and the granulocyte concentration was adjusted to $1 \times 10^9/\text{L}$. The purity of the granulocytes was $\geq 99\%$. In granulocyte suspensions from healthy individuals, $6.6\% \pm 3.0\%$ SD of the cells were eosinophils, and in granulocyte suspensions from patients with allergic rhinitis, allergic asthma, and nonallergic asthma, respectively, $6.3\% \pm 3.1\%$, $12.0\% \pm 7.1\%$, and $8.4\% \pm 3.9\%$ of the cells were eosinophils.

Adherence assay

The adherence of eosinophil and neutrophil granulocytes to E-selectin, VCAM-1, and ICAM-1 was measured according to a recently described method²⁰ with cell lines of hamster fibroblasts that selectively express E-selectin, VCAM-1, and ICAM-1. Baby hamster kidney (BHK) fibroblasts, normal fibroblasts, and transfected cell lines were maintained in Dulbecco's modified Eagle medium (Gibco, Paisley, U.K.) supplemented with 10% fetal calf serum, 100 U penicillin per milliliter, 100 μg streptomycin per milliliter, and 4.5 gm glucose per liter. Methotrexate (Lederle Laboratories, Pearl River, N.Y.) was added to the medium of transfected cell lines to obtain a final concentration of 1 $\mu\text{mol/L}$. The fibroblasts

were cultured at 37° C in a 5% CO_2 atmosphere and were routinely passaged every third or fourth day. From the continuously cultured fibroblast cell lines, 96-well microtiter plate cultures were prepared for adhesion measurement. The fibroblasts and transfected cell lines were seeded at 5×10^3 per well and cultured overnight before the adhesion assay was performed.

To measure granulocyte adhesion the fibroblast monolayers in 96-well microtiter plates were washed three times with assay buffer, and 1×10^5 granulocytes in assay buffer with 0.5 mmol/L of MnCl_2 were added to the wells and incubated for 1 hour at 37° C. Granulocytes in assay buffer without MnCl_2 were also added to the native BHK fibroblasts. After incubation was performed, nonadherent granulocytes were removed from the wells, and the plates were washed twice with buffer. The amount of adherent neutrophils and eosinophils was selectively analyzed by the use of radioimmunoassays to measure myeloperoxidase (MPO) (Kabi Pharmacia Diagnostics AB, Uppsala, Sweden) and eosinophil cationic protein (ECP) (Kabi Pharmacia Diagnostics), respectively, as follows. The adherent cells were treated with a detergent solution containing N,N,N'-cetyl-triammonium bromide (5 gm/L) and NaCl (8.8 gm/L) for 1 hour at room temperature. The supernatants were collected by centrifugation at 600 g for 10 minutes. Assays of the amounts of ECP and MPO, respectively, were performed by radioimmunoassay (Pharmacia Diagnostics AB, Uppsala, Sweden), and the percentage of adherent neutrophils and eosinophils of the total amount that had been added to the wells was calculated from a standard curve consisting of serial dilutions of an extract of the initial amount of added granulocytes by means of regression analysis.

Flow cytometry

Granulocytes at a concentration of $5 \times 10^9/\text{L}$, isolated according to the procedure described previously, were incubated with monoclonal antibodies against CD11b, CD18, and CD49d (Immunotech) for 30 minutes at +4° C. After two washings were performed with phosphate-buffered saline solution, the granulocytes were further incubated with phycoerythrin-conjugated goat anti-mouse immunoglobulins (Dakopatts, Glostrup, Denmark) for 30 minutes at +4° C, washed twice, and incubated with fluorescein-isothiocyanate-conjugated anti-CD9 (Dakopatts). After two final washes were performed, the cells were resuspended to a concentration of $1 \times 10^9/\text{L}$ in phosphate-buffered saline solution with human serum albumin (1 gm/L) and analyzed in an EPICS II flow cytometer (Coulter Company Inc., Hi-aleah, Fla.). Based on the pattern of forward and side scatter, a granulocyte gate was set. Eosinophils were identified as CD9-positive granulocytes^{29, 30} and neutrophils as CD9-negative granulocytes. The mean specific fluorescence of CD11b, CD18, and CD49d was calculated by subtracting the mean background fluorescence of eosinophils/neutrophils labeled with an irrelevant

TABLE I. Clinical data

Group	No.	Age (yr)	Male/ female	Lung function		PD ₂₀ (mg)	Symptom score
				FEV ₁ (%)	PEF (%)		
Healthy individuals	9	31 (22-43)	0/9	104 (70-115)	82 (74-122)	>2.0	0
Patients							
Allergic rhinitis	10	35 (23-42)	3/7	100 (85-114)	84 (68-102)	>2.0 (0.3->2.0)	0 (0-1)
Allergic asthma	10	33 (24-46)	5/5	88* (65-104)	83 (65-116)	0.2† (0.01-2.0)	1.5‡ (0-3)
Nonallergic asthma	10	36 (23-47)	2/8	88* (63-108)	81 (57-96)	0.6† (0.05-2.0)	1.0 (0-3)

The data presented are median and range. Statistically significant differences among the healthy individuals and patients calculated by the Mann-Whitney U test are indicated.

**p* = 0.03.

†*p* < 0.01. Measurements of BHR (PD₂₀) were performed on an earlier occasion.

‡*p* = 0.001. Statistically significant differences among patients with allergic rhinitis and asthma, allergic and nonallergic, are also shown.

TABLE II. Hematologic data

Group	Blood counts				Serum concentration	
	Neutrophils (10 ⁹ /L)	Eosinophil (10 ⁶ /L)	Lymphocytes (10 ⁹ /L)	Monocytes (10 ⁶ /L)	ECP (μg/L)	MPO (μg/L)
H	3.3	110	2.1	300	9.9	373
	2.8-3.6	90-170	1.6-2.4	280-370	7.7-16.6	321-595
AR	3.3	135	1.8	345	9.8	374
	3.0-3.4	100-185	1.3-2.0	290-380	6.4-19.1	279-411
AA	3.0	270*	1.7	360	10.4	431
	2.4-3.2	120-395	1.1-2.0	260-445	8.2-26.3	287-547
NAA	2.9	130	2.1	460	9.3	350
	2.3-3.7	80-200	1.5-2.5	360-490	5.1-17.3	309-475

The presented data are median and lower and upper quartiles. Statistically significant differences among patients with allergic asthma and healthy individuals are represented. H, Healthy individuals; AR, patients with allergic rhinitis; AA, patients with allergic asthma; NAA, patients with nonallergic asthma.

**p* = 0.025.

primary antibody and the secondary phycoerythrin-labeled antibodies from the value obtained with anti-CD11b, anti-CD18, and anti-CD49d, respectively.

Measurement of concentrations of ECP and MPO

Serum samples for measurement of ECP and MPO were handled according to the instructions of the manufacturer. Assays of the serum concentrations of ECP and MPO were performed by means of double-antibody radioimmunoassays (Pharmacia Diagnostics AB). The intraassay and interassay coefficients of variation are less than 11% in ECP and less than 13% in MPO. The detection limit is less than 2 μg/L in ECP and less than 8 μg/L in MPO.

Statistics

Results are expressed as the median and lower and upper quartiles, or median and range, respectively. Statistical evaluations were made with Mann-Whitney U-test, and Spearman's rank order correlation was used

to analyze relationships between different variables, both by the use of CSS Statistica (StatSoft Inc., Tulsa, Okla.).

RESULTS

Lung function and hematologic data for patients and healthy individuals

The patients who were classified as having asthma, allergic and nonallergic, had significantly lower values of FEV₁ than the healthy control subjects (Table I). The patients with allergic asthma also had significantly higher symptom scores and blood eosinophil counts as compared with healthy individuals. No significant differences were seen in the serum levels of ECP or MPO among the four groups of subjects (Table II).

Eosinophil and neutrophil adhesion

Eosinophil and neutrophil adhesion to E-selectin, VCAM-1, and ICAM-1 showed no statistically

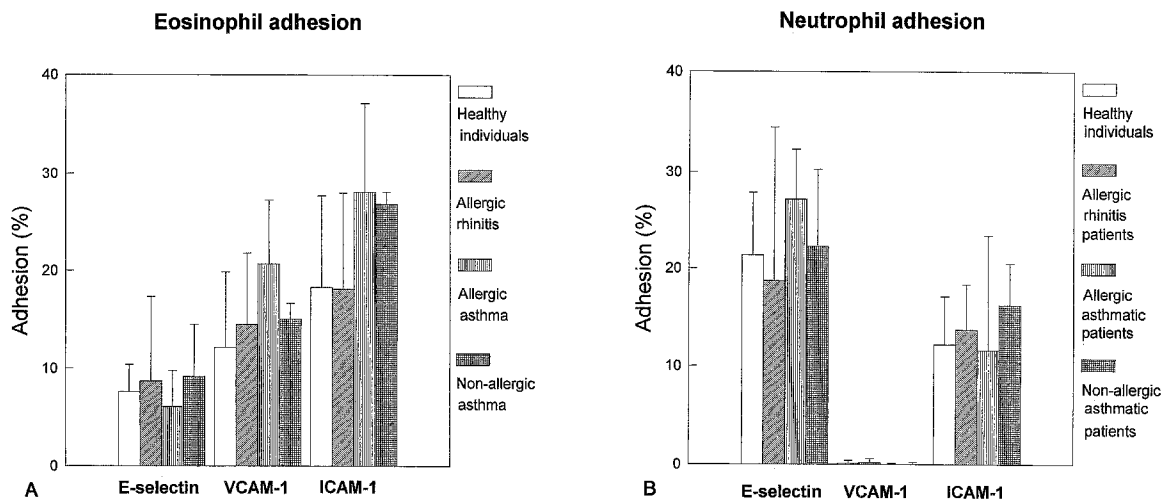


FIG. 1. Adhesion of eosinophil (A) and neutrophil (B) granulocytes from healthy individuals ($n = 9$), patients with allergic rhinitis ($n = 10$), patients with allergic asthma ($n = 10$), and patients with nonallergic asthma ($n = 10$) to E-selectin, VCAM-1, and ICAM-1. Results shown are median values and upper quartiles. Zero level represents background adhesion to native BHK fibroblasts.

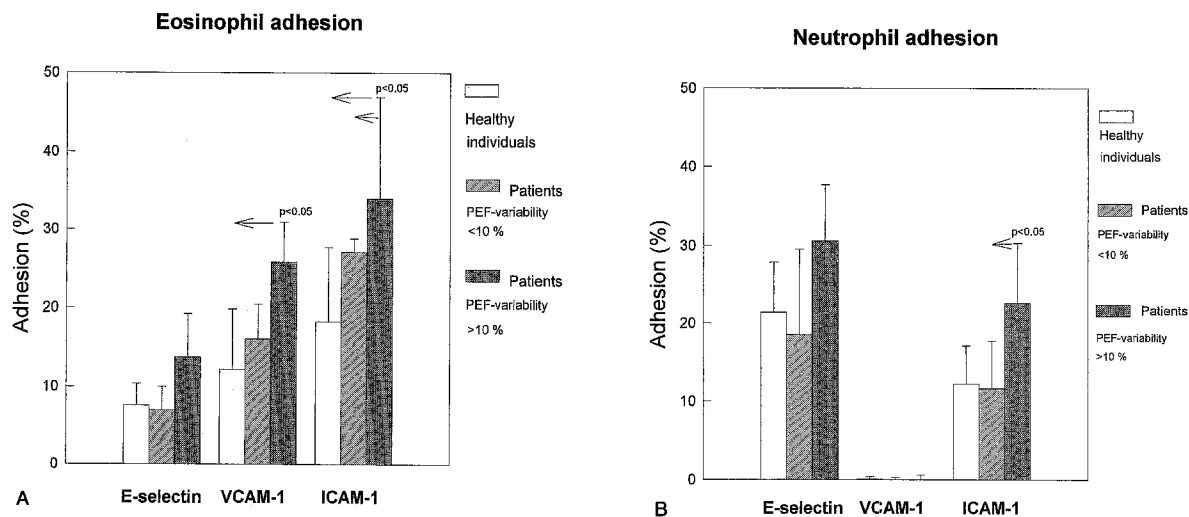


FIG. 2. Adhesion of eosinophil (A) and neutrophil (B) granulocytes from healthy individuals ($n = 9$), patients with PEF variability of less than 10% ($n = 12$), and patients with PEF variability of more than 10% ($n = 9$) to E-selectin, VCAM-1, and ICAM-1. Results shown are median values and upper quartiles. Significances of difference among results of patients with >10% PEF variability, healthy individuals, and patients with <10% PEF variability, calculated by means of the Mann-Whitney U test, are indicated.

significant differences among patients with allergic rhinitis or allergic or nonallergic asthma and healthy individuals (Fig. 1). Eosinophils from the patients with allergic asthma, however, demonstrated a tendency toward higher adhesion to VCAM-1 and ICAM-1. PEF variability exceeded 10% in nine of the 22 patients who had recorded their PEF variability. These patients also had significantly decreased FEV₁ and increased symp-

tom scores compared with healthy individuals, and they had a higher degree of BHR (provocative dose of methacholine causing a 20% fall in FEV₁ [PD₂₀]) than the patients with low PEF variability (Table III). This patient group consisted of one patient with allergic rhinitis, five patients with allergic asthma, and three patients with nonallergic asthma. Eosinophils from the patients with high PEF variability demonstrated signifi-

TABLE III. Clinical data on patients classified according to PEF variability

Group	No.	Diagnosis	FEV ₁ (%)	PEF (%)	PD ₂₀ (mg)	Symptom score
Healthy individuals	9		104 (70-115)	82 (74-122)	>2.0	0
Patients	13	AR: 6	97 (63-114)	85 (71-100)	1.4 (0.05->2)	1* (0-2)
PEF variability:		AA: 3				
<10%		NAA: 4				
Patients	9	AR: 1	89* (64-108)	76 (57-116)	0.37† (0.04-2.0)	1* (0-3)
PEF variability:		AA: 5				
>10%		NAA: 3				

The data presented are median and range. Statistically significant differences between healthy individuals and patients and between the two patient groups were calculated by the Mann-Whitney U test. Measurements of BHR (PD₂₀) were performed on an earlier occasion.

Abbreviations as in Table II.

**p* < 0.05 between healthy individuals and patients.

†*p* < 0.05 between the two patient groups.

TABLE IV. Background adhesion of eosinophils and neutrophils in absence and presence of Mn²⁺

Mn ²⁺ (0.5 mmol/L)	Adhesion (%)			
	Eosinophils		Neutrophils	
	–	+	–	+
Healthy individuals	3.2 (1.1-4.4)	7.2* (3.3-10.3)	4.3 (0.9-4.7)	9.6† (2.5-11.9)
Patients				
PEF variability <10%	3.4 (2.6-5.4)	8.0† (3.8-9.8)	4.0 (2.6-5.4)	12.3† (5.6-15.8)
PEF variability >10%	2.7 (2.1-3.6)	4.8* (4.0-7.4)	2.3 (1.8-2.9)	7.9* (3.9-10.1)

Background adhesion of eosinophils and neutrophils was measured as the adhesion to native BHK fibroblasts. The presented data are median and lower and upper quartiles. The significance of the differences between adhesion in absence and presence of Mn²⁺ are indicated.

**p* < 0.05.

†*p* < 0.01.

TABLE V. Blood eosinophil count and serum concentration of ECP in patient groups classified according to PEF variability

Group	Blood eosinophil count (×10 ⁶ /L)	Serum concentration of ECP (μg/L)
Healthy individuals	110 (90-170)	9.9 (7.7-16.6)
Patients		
PEF variability <10%	180 (100-310)	10.4 (6.8-24)
PEF variability >10%	170 (120-220)	10.2 (6.9-17.3)

The presented data are median and lower and upper quartiles.

cantly increased adhesion to VCAM-1 and ICAM-1 when compared with those from healthy individuals (Fig. 2, *A*) and increased adhesion to ICAM-1 compared with those from the patients who had

low PEF variability. In the same group neutrophil adhesion to ICAM-1 was significantly increased when compared with that of the patients who had low PEF variability (Fig. 2, *B*). Eosinophils from both patient groups demonstrated increased (*p* < 0.01) adhesion to ICAM-1 when compared with neutrophils from the same individuals. Adhesion to ICAM-1 of eosinophils and neutrophils showed a significant correlation in both patient groups (*r* = 0.78, *p* < 0.01 and *r* = 0.93, *p* < 0.01) and in the healthy control group (*r* = 0.81, *p* < 0.05). Background adhesion of eosinophil and neutrophil granulocytes, in absence and presence of Mn²⁺, demonstrated comparable levels in all three groups, whereas the presence of Mn²⁺ induced a slight but significant general increase in adhesion (Table IV). Blood eosinophil count and serum concentration of ECP demonstrated comparable mean levels in both patient groups and in the healthy individuals (Table V).

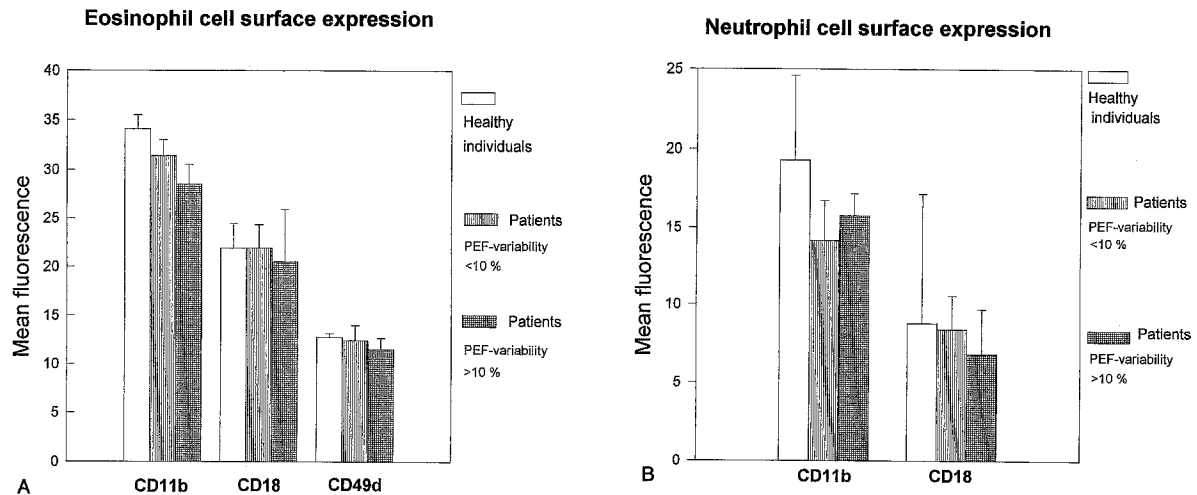


FIG. 3. Specific eosinophil (A) and neutrophil (B) cell surface expression of CD11b, CD18, and CD49d in healthy individuals ($n = 9$), patients with PEF variability of less than 10% ($n = 12$), and patients with PEF variability of more than 10% ($n = 9$). Results shown are median values and upper quartiles. Zero level represents background fluorescence (mean, 1.1 U; range, 1.0 to 1.66 U). Neutrophils do not express CD49d (mean, 0.0; range, 0.0 to 0.005).

Eosinophil and neutrophil cell surface expression of adherence molecules

The increased adhesion of granulocytes may be caused by either a qualitative or a quantitative upregulation of the adherence receptors on the cells, or both. Measurement of the expression of CD11b, CD18, and CD49d on the cell surface of the eosinophils showed the same levels on cells from patients with a PEF variability of more than 10%, patients with a PEF variability of less than 10%, and the healthy individuals (Fig. 3, A). Neutrophil expression of CD11b and CD18 also demonstrated the same levels on cells from patients and healthy individuals (Fig. 3, B).

Relationship between eosinophil adhesion and lung function

Regression analysis demonstrated no significant relationships between eosinophil or neutrophil adhesion and the registered measures of lung function (FEV_1 and PD_{20}) or symptom score.

DISCUSSION

This study revealed a priming of the adhesion of peripheral blood eosinophils obtained from patients with mild asthma. The measured increased eosinophil adherence responses could be defined as priming because only the specific adhesion to VCAM-1 and ICAM-1 was increased, whereas background adhesion was unaffected. The priming of eosinophil adhesion was related to the severity of the patient's asthmatic disease estimated as

variability of PEF. Previous investigations have concluded that a variation of PEF during the day of more than 10% to 12% is greater than normal.^{31, 28} Furthermore the variability of PEF was related to BHR to histamine. In this study patients with high PEF variability and increased eosinophil adhesion showed decreased FEV_1 compared with healthy individuals and increased BHR compared with patients with low PEF variability, although no direct correlation was found between eosinophil adhesion and FEV_1 or PD_{20} . Airway hyperresponsiveness of asthma is considered to be related to airway inflammation,³² which suggests an association between the ongoing inflammatory process in the lungs of the actual patients and priming of eosinophil adhesion. Finally, eosinophil priming was not related to allergy, because the group of patients with the higher degree of PEF variability and increased eosinophil adherence responses consisted of persons with and without allergy.

The increased eosinophil adhesion to VCAM-1 and ICAM-1 seemed to be a sensitive measure of eosinophil activation, because the serum concentration of ECP, otherwise used as a marker of eosinophil activation,^{33, 34} did not reveal any difference between the patients with PEF variability of more than 10% and the healthy individuals. Priming of eosinophil adhesion was, however, not entirely selective, because the neutrophils also demonstrated an enhanced adhesion to ICAM-1, although neutrophil adhesion to ICAM-1 of patients with the higher degree of PEF variability, as

in general,²⁰ showed a significantly lower level than eosinophil adhesion to ICAM-1.

The increased adhesion of eosinophils to VCAM-1 and ICAM-1 and of neutrophils to ICAM-1 in the patient group with a high degree of PEF variability was most probably caused by a functional upregulation of the receptors CD11b/CD18 and VLA-4, because the number of receptors per cell, measured as cell surface expression, was unchanged. The functional upregulation of neutrophil adhesion via CD11b/CD18 is a well-known phenomenon that has been shown to occur in vitro after stimulation with phorbol esters, lipopolysaccharide, and the cytokines granulocyte-macrophage colony-stimulating factor, interleukin-3, and interleukin-5.³⁵⁻³⁹ The mechanisms behind this functional upregulation have not been fully clarified but have been suggested to involve phosphorylation of the intracellular domain of CD18 and association of the CD11b/CD18 complex with cytoskeleton proteins. In this case the priming agent of eosinophil adhesion can only be speculated. Interleukin-5, which has been proposed by many investigations to be involved in the inflammatory process of asthma,^{40, 41} has in a previous investigation been demonstrated to increase eosinophil adhesion.⁴²

Manganese ions are known to regulate the adhesive functions of both β_1 (VLA-1, VLA-4, and VLA-5) and β_2 (CD11a-b/CD18) integrins.^{36, 43-46} In the method described in this study, addition of Mn^{2+} was shown to be a necessary and reliable way to selectively induce eosinophil adhesion to VCAM-1 and eosinophil and neutrophil adhesion to ICAM-1.²⁰ The need for Mn^{2+} ions is probably because the change in integrin avidity is the most important factor for their adhesion to the complementary ligands⁴⁷ and because the results obtained with this method are exclusively due to the functional alterations of the granulocytes. The priming of eosinophils demonstrated in this investigation resulted in an increased response to Mn^{2+} ions, which was still selective because background adhesion in the presence of Mn^{2+} was similar in patients and control subjects.

The consequences of the increased eosinophil adhesion to VCAM-1 and ICAM-1, found in the patients with a more labile asthmatic disease, would be an increased accumulation of eosinophils at sites where endothelial cells express VCAM-1 and ICAM-1 on their surface, and subsequently emigration of eosinophils into the adjacent tissue would be facilitated. Previous investigations have shown that ICAM-1 is upregulated on inflamed

bronchial epithelium and endothelium in vivo.^{48, 49} Furthermore in a primate model of asthma, treatment with anti-ICAM-1 prevented the influx of eosinophils from the bloodstream.⁵⁰ The expression of VCAM-1 on endothelium from lung tissue from patients with asthma has yet only been investigated in one study, which demonstrated a relationship between VCAM-1 expression and eosinophil activation.⁵¹

In conclusion, priming of eosinophil adhesion to endothelial cell adherence receptors was shown to be a sensitive measure of eosinophil activation, because it occurred in patients with mild asthma but was suggested to be related to the degree of ongoing inflammation in their airways. It was, however, not entirely selective, because neutrophil adhesion to ICAM-1 was also increased. Finally, priming of specific eosinophil adhesion might, in combination with upregulation of the expression of VCAM-1 and ICAM-1 on endothelial cells, be an essential mechanism for the selective accumulation of eosinophils in lung tissue of patients with asthma.

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