



Circulating adhesion molecules in allergic and non-allergic asthma

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Received 22 January 2004; accepted 17 May 2004

KEYWORDS

Asthma;
Adhesion molecules;
Bronchial responsiveness;
Allergy

Summary Circulating forms of adhesion molecules (intercellular-adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin) are related to the turnover of these molecules on the cell surface. In contrast to the other molecules, the levels of E-selectin probably exclusively reflect the activity of endothelial cells.

The aim of this study was to compare levels of circulating adhesion molecules in patients with allergic (AA) and non-allergic asthma (NA) and to relate the levels of soluble adhesion molecules to methacholine responsiveness and lung function.

The study comprised 19 patients with AA, 15 patients with NA and 17 healthy subjects. Soluble adhesion molecules, spirometry, methacholine responsiveness and peak flow variability was measured.

The group of patients with AA had higher levels of sE-selectin than the reference group ($P = 0.046$). Serum levels of sE-selectin correlated significantly with bronchial responsiveness ($r = 0.76$) and peak flow variability ($r = 0.75$) ($P < 0.01$) in the NA but not in the AA group. All adhesion molecules in AA ($P < 0.05 - < 0.001$), but only sE-selectin in NA ($P < 0.05$), were correlated to airway conductance. sVCAM-1 was reduced by inhaled steroids ($P < 0.01$).

Our results indicate that endothelial cells are activated in asthma and that this activity has a bearing on airflow variability and bronchial responsiveness in NA.

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Introduction

Adhesion molecules such as the intercellular-adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin facilitate the recruitment and migration of inflammatory cells from the blood to the airway walls and therefore have an important role in the pathogenesis of asthma. Circulating forms of adhesion molecules can be measured in blood. ICAM-1 and VCAM-1 are shed from endothelial cells, epithelial cells and monocytes while E-selectin is only released from endothelial cells.¹

Increased levels of circulating ICAM-1 and E-selectin are found in exacerbations of asthma.^{2–5} Increased levels of soluble adhesion molecules are also found in acute infections,¹ in cystic fibrosis⁶ and in pulmonary tuberculosis.⁷ In asthma sICAM-1 levels increase after allergen provocation⁸ and decrease after introduction of inhaled steroids.⁹

We have previously found that patients with allergic (AA) and non-allergic asthma (NA) have comparable bronchial responsiveness to methacholine. Patients with AA and NA differ, however, in many other aspects such as responsiveness to cold air, adenosine monophosphate (AMP),¹⁰ levels of nitrogen oxide (NO) in expired air¹¹ and the level of eosinophilic inflammation in the airways.¹² This implies that the mechanism through which bronchial responsiveness is developed is different in these two sub-groups of asthma.

The aim of this study was to compare levels of circulating adhesion molecules in patients with AA and NA and to relate the levels of soluble adhesion molecules to methacholine responsiveness and lung function.

Population and methods

Patients

Nineteen patients with AA and 15 patients with NA were included in this study^{10,11} (Table 1). The patients were recruited from the Department of Respiratory Medicine and Allergology, at our hospital. Subjects were defined as having asthma; if they had a physician diagnosed asthma and had previously demonstrated an increased responsiveness to inhaled methacholine. The patients with asthma had current respiratory symptoms and a forced expiratory volume in 1 s (FEV₁) above 70% of the predicted value. Patients with asthma who had at least one positive skin prick test (mean diameter ≥ 3 mm) were defined as having AA. Skin prick

tests were carried out using Solu Prick (ALK, Denmark) standardised allergen extract against the following allergens: birch, timothy grass, mugwort, cat, dog, horse, *Dermatophagoides pteronyssinus*, *Cladosporium* and *Alternaria*.

Fourteen of the allergic patients with asthma and 12 of the non-allergic patients with asthma were on regular treatment with inhaled steroids (budesonide 200–1600 μ g ($n = 21$) or beclomethasone 50–400 μ g ($n = 5$)). Inhaled corticosteroid therapy was continued with no changes. All the patients but one with asthma were taking inhaled β_2 -agonists as required. None of the patients was being treated with theophylline compounds. One allergic subject with asthma and six non-allergic subjects with asthma were ex-smokers (stopped smoking 2–20 years previously).

Reference group

The control group comprised 17 subjects who responded to a request for volunteers or were recruited from the staff at our hospital; none had asthmatic symptoms or was on anti-asthmatic treatment and all were non-allergic^{10,11} (Table 1).

All the investigated subjects were non-smokers and free from respiratory infections for at least 6 weeks prior to the study and none had a history of cardiovascular disease. The investigation was carried out outside the birch and grass pollen season (April–August) in all subjects.

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee at the Medical Faculty at Uppsala University. Verbal informed consent was obtained prior to the study.

Measurement of circulation adhesion molecules

Whole blood was drawn in SST tubes and allowed to coagulate at room temperature for 60 min. The serum was then separated by centrifugation. After collection the serum was frozen at -70°C until analysed. Soluble adhesion molecules were measured using commercial kits obtained from R&D Systems (USA) according to the manufacturer's instructions. The sensitivity of the assays was 0.1 ng/l for sE-selectin, 0.35 ng/l for sICAM-1 and 2 ng/l for sVCAM-1.

Spirometry

The spirometry took place from 8.30 to 11 am. The subjects were instructed not to take any

Table 1 Demographics, lung function and medication in the reference and patient groups.

	Healthy (n = 17)	AA (n = 19)	NA (n = 15)
Age	37 ± 10	34 ± 11	39 ± 13
Women (n)	13	10	14
FEV ₁ (% pred)	95 ± 12	94 ± 15	90 ± 11
FVC ₁ (% pred)	94 ± 8	96 ± 14	92 ± 9
sGaw (% pred)	102 ± 52	81 ± 29	88 ± 60
Methacholine responsiveness (log transformed)	0.81 ± 0.42	1.41 ± 0.48***	1.16 ± 0.24**
PEF variability (%)	5 ± 2	7 ± 4*	8 ± 4**
Inhaled steroids (daily dose in µg)	—	382 ± 336	520 ± 426

(mean ± SD or N, *P < 0.05, **P < 0.01, ***P < 0.001 compared to the reference group).

short-acting β_2 -agonists 8 h before testing or any long-acting β_2 -agonists 24 h before testing. Specific airway conductance (sGaw), forced vital capacity (FVC) and FEV₁ were measured with a Masterlab Trans spirometer and a Masterlab body plethysmograph (Eric Jaeger AG, Würzburg, Germany). Lung function values are presented as a percentage of reference values.^{13,14}

Methacholine challenge

An automatic, inhalation dosimeter Spira Elektro 2 (Respiratory Care Centre, Hämeenlinna, Finland), was used in this study.¹⁵ The dosimeter was adjusted with a nebulisation onset of 50 ml and a nebulisation duration of 1 s. Inspiratory flow rate was 0.5 l/s. The dosimeter output was 16 ± 5 µl/breath. Each individual took five tidal breaths of each concentration and sGaw, FVC and FEV₁ were measured after 90 s. The next nebulising concentration started 3 min after the previous concentration had stopped. Baseline values were measured after an inhalation of saline. After the nebulisation of saline, methacholine chloride (National Corporation of Swedish Pharmacies) was delivered. The starting concentration of methacholine was 0.0625 mg/ml. The challenge was continued with inhalation of doubling concentrations of methacholine until the FEV₁ had decreased by 20% or the highest concentration (32 mg/ml of methacholine) was reached. The quantity of inhaled methacholine was calculated by weighing the amount of methacholine that was left in the dosimeter after each concentration and subtracting it from the starting dose.

The least-squares slope for methacholine was calculated from the regression equation for percentage decline in FEV₁ on the cumulative dose of methacholine using all the measured points.¹⁶

Peak flow variability

Peak flow diaries were kept during a 17-day period, starting on the day of inclusion in the study. The peak expiratory flow rate (best of three measurements) was recorded four times daily with a Mini-Wright peak flow meter (Clement Clarke, London, UK). Daily peak flow variability was calculated by the formula: ((highest PEF – lowest PEF) × 100)/mean PEF value of the day). Complete PEF-diaries were obtained from all but three patients with AA and one with NA.

Statistics

ANOVA and unpaired *t*-test was used to compare the levels of soluble adhesion molecules between the different patients groups and the reference population. Linear regression was used to study the relationship between bronchial responsiveness, lung function and the level of soluble adhesion molecules in the two groups of asthmatic patients. The levels of soluble adhesion molecules and the methacholine responsiveness slope was log transformed before performing the analyses. In order to avoid negative values the formula log(slope + 7) was used when transforming the methacholine responsiveness slope.

Results

The characteristics of the two patient groups and the reference group are presented in Table 1. There were no differences between the groups with AA and NA. Both asthmatic groups had higher methacholine responsiveness and peak flow variability than the reference group.

The group of patients with AA had higher levels of sE-selectin than the reference group ($P = 0.046$). A similar trend was also found between the NA group and the reference group ($P = 0.091$) (Fig. 1). No significant differences were found in the levels of sICAM-1 or sVCAM-1 between any of the groups (Fig. 2).

A significant correlation was found between sE-selectin and bronchial responsiveness in the group of patients with NA while no corresponding association was seen in the AA group (Fig. 3). There was also a significant correlation between sE-selectin and peak flow variability in the NA but not in the AA group (Fig. 4).

A significant correlation was found between sE-selectin and sGaw in both asthmatic groups (Table 2). Significant correlations between sGaw and sICAM-1 and sVCAM-1 were found in the AA but not in the NA group. There were no significant correlations between any of the soluble adhesion molecules and FEV₁ or FVC.

There was no correlation between age of the patients or asthma duration and soluble adhesion molecules in any of the asthmatic groups.

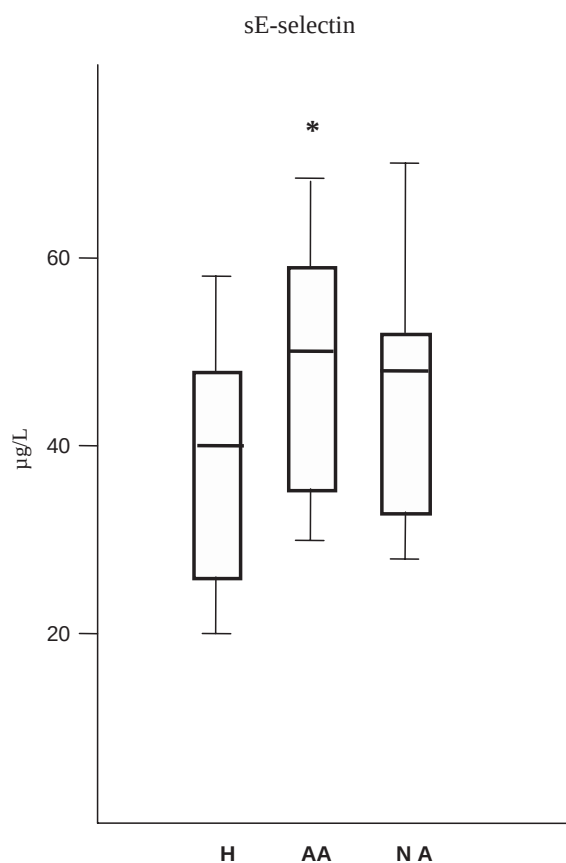


Figure 1 Levels of sE-selectin in healthy controls (H), patients with AA and patients with NA (10th, 25th, 50th, 75th and 90th percentile, * $P < 0.05$).

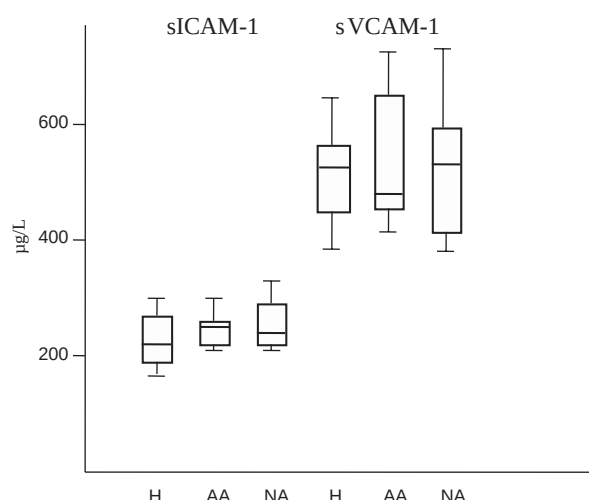


Figure 2 Levels of sICAM-1 and sVCAM-1 in healthy controls (H), patients with AA and patients with NA (10th, 25th, 50th, 75th and 90th percentile).

The influence of inhaled steroid dosage on the level of soluble adhesion molecules was studied with the two groups of asthmatics combined. A dose-dependent decrease in the levels of sVCAM-1 with increasing steroid doses was found (Fig. 5). Despite this no significant difference in the level of sVCAM-1 was found between the diagnostic groups after adjustment for inhaled steroid treatment. No significant relation between steroid doses and sE-selectin or sICAM-1 was found.

Discussion

The main findings in this study were that patients with AA and NA in a stable phase tend to have higher serum levels of sE-selectin than healthy subjects whereas no differences in the other two measured soluble adhesion molecules sICAM-1 and sVCAM-1 was found. Higher level of sE-selectin was related to decreased airway conductance in both asthmatic groups whereas a relation between sE-selectin and airflow variability and methacholine responsiveness was only found in patients with NA.

The role of adhesion molecules in asthma is supported by several studies showing an increased expression of adhesion molecules in bronchial epithelial and endothelial cells from asthmatics.^{17,18} Increased eosinophil adhesion to VCAM-1 and ICAM-1 has also been demonstrated in patients with labile asthma¹⁹ and during pollen season in birch allergic subjects.²⁰ The level of soluble adhesion molecules in the circulation is related to the turnover of these molecules on the cell surface.²¹ Measuring soluble adhesion

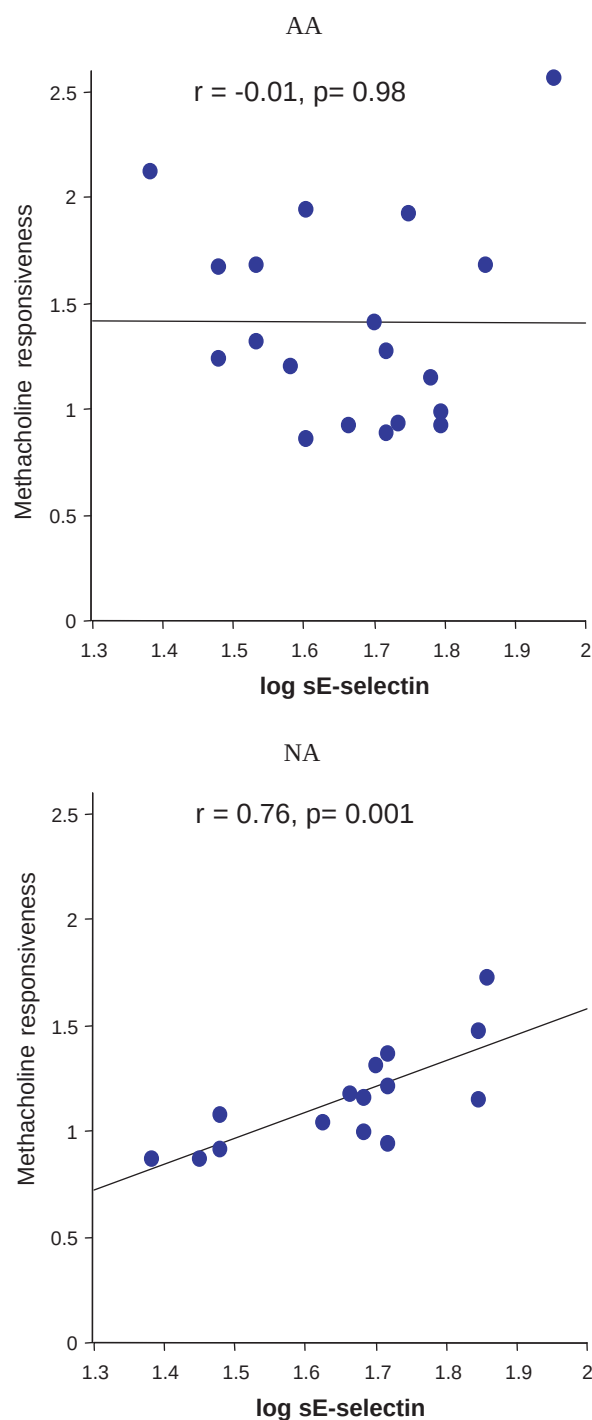


Figure 3 Correlation between sE-selectin and methacholine responsiveness (both variables are log transformed).

molecules in serum has therefore been proposed as a marker of inflammation in asthma and allergy.²²

In the present study, we found no differences in the serum levels of sICAM-1 and sVCAM-1 in any of the asthma groups compared to the control group. As the patients were in a clinically stable phase this is in accordance with some previous studies,^{3,23,24}

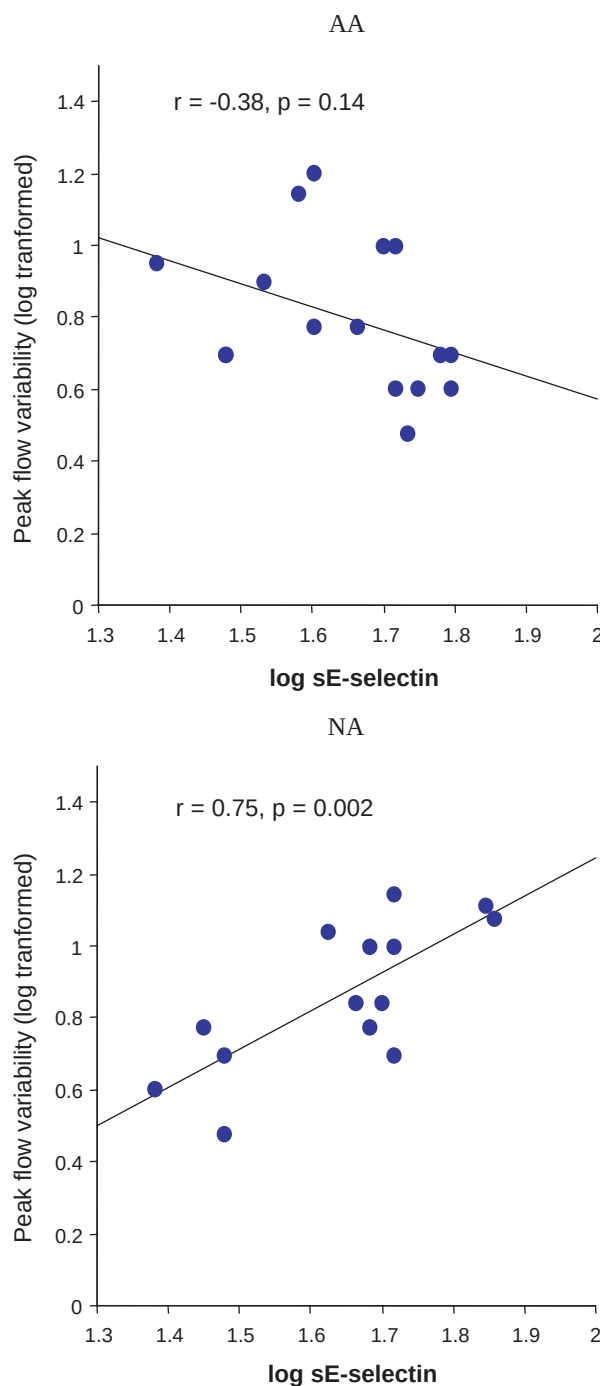


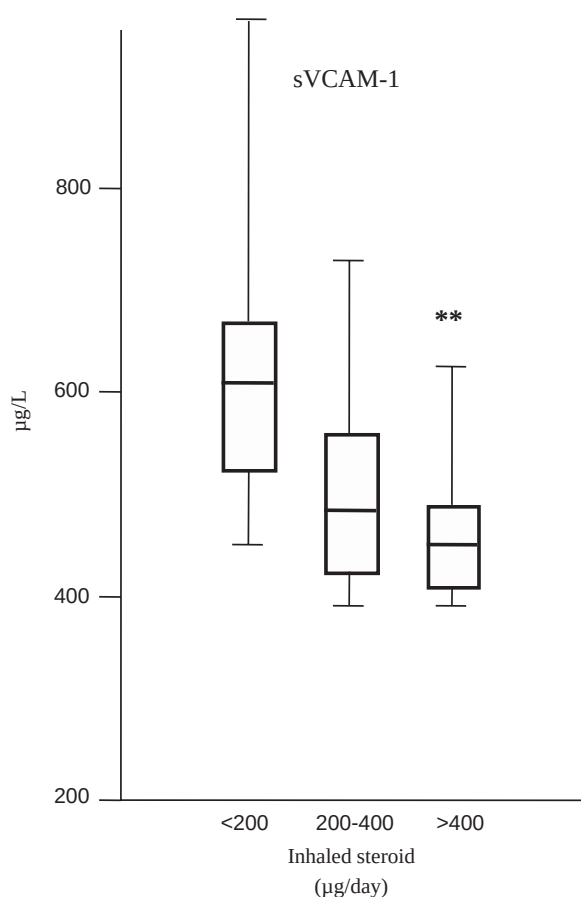
Figure 4 Correlation between sE-selectin and peak flow variability (both variables are log transformed).

while others have found increased levels of sICAM-1 even in stable or mild asthma compared to controls.^{4,5,25} A constant finding in previous studies is that sICAM-1 levels are increased in acute asthma^{2-5,25} and thus to some extent related to clinical asthma severity. In the present study, higher sICAM-1 and sVCAM-1 levels were related to decrease in airway conductance in the AA group, while no other correlation was found between

Table 2 Correlation (*r*-values) between specific airway conductance (sGAW) and soluble adhesion molecules.

	Allergic asthma	Non-allergic asthma
sE-selectin	−0.77***	−0.60*
sICAM-1	−0.50*	−0.26
sVCAM-1	−0.51*	−0.19

(**P* < 0.05, ****P* < 0.001).

**Figure 5** Levels of sVCAM-1 in relation to the daily dose-inhaled steroids (budesonide or beclomethasone (10th, 25th, 50th, 75th and 90th percentile, ***P* < 0.01).

these two soluble adhesion molecules and asthma severity.

The levels of sE-selectin were higher in the asthmatic population than in the reference group. This is in contrast to previous studies where no differences in sE-selectin levels were found between stable asthmatics and controls.^{3,5,23,24} The differences were, however, small with a large overlap between the asthmatic groups and the reference group. Of greater interest is the finding

of fairly close correlations between sE-selectin levels and airway lability (methacholine responsiveness and peak flow variability) in the non-allergic asthmatic but not the allergic asthmatic group. Soluble E-selectin is of special interest since it probably is a specific marker of endothelial cell activity,²² whereas sICAM-1 and sVCAM-1 are released from both endothelial cells and monocytes.^{1,26} Our results therefore indicate that endothelial cell activity may play a greater role in airflow variability and bronchial responsiveness in NA than AA. The mechanism behind this is uncertain, but could reflect the direct involvement of endothelial cells in the development of airway lability in NA. However, we have previously shown that neutrophils are abundant in the airways of patients with NA¹² and it is well established that the expression of E-selectin on endothelial cells is of major importance for the accumulation neutrophils in tissues. Our findings could therefore also reflect a role of neutrophils in the development of airway lability. A previous study showed correlations between E-selectin and lung functions in patients with COPD, another disease in which neutrophils are abundant.²³

The notion that adhesion molecules may have a different role in AA than NA is not new. Several studies have investigated this but the results have been conflicting. Bentley et al. reported that there was an increase in the staining of ICAM-1 and E-selectin in the submucosa of intrinsic asthmatics but not in extrinsic asthma.²⁷ Gosset et al. found the opposite with increased expression of adhesion molecules in epithelial and endothelial cells in AA but not NA.¹⁷ In accordance with the present study, previous studies have showed similar serum levels of sICAM-1 and sE-selectin in AA and NA.^{2,25}

In our study, there was a significant correlation between sVCAM-1 and the daily dosage of inhaled steroids. In contrast with the results of Cengizlier et al. we found no correlation between inhaled steroid treatment and sICAM-1.⁹ There was also no correlation between sE-selectin and the daily dose of inhaled steroids. VCAM-1 is selectively upregulated by IL-4.²⁸ The lower sVCAM-1 levels in patients with higher doses of inhaled steroids may therefore be caused by a steroid-induced inhibition of IL-4 production.²⁹

In conclusion NA patients with higher levels of sE-selectin have increased airflow variability and bronchial responsiveness, while this relationship was not found in patients with AA. As sE-selectin is a marker of endothelial activity, our results indicate that endothelial cell activity may play a greater role in airflow variability and bronchial responsiveness in NA than AA.

Acknowledgements

We thank our research nurse Ulrike Spetz-Nyström and research technician Monica Hall for their assistance with collecting data and technical assistance with the bronchial challenges. We acknowledge the support of the Swedish Heart–Lung Foundation, the King Oscar II Jubilee Foundation, and the Bror Hjerpstedt Foundation.

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