

Short communication

Circulating leukocyte adhesion molecules in stable asthma and nonobstructive chronic bronchitis

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Leukocyte adhesion molecules have been associated with airway inflammatory diseases such as asthma and obstructive chronic bronchitis. Lately, it has become possible to measure circulating forms of cell adhesion molecules (cCAMs) in body fluids. Elevated serum levels have been found in acute asthma and in obstructive chronic bronchitis. We investigated whether the patterns of cICAM-1, cVCAM-1, and cE-selectin could serve as markers for airway inflammation in stable asthma and stable nonobstructive chronic bronchitis. Small-volume bronchial lavage (BL) and serum from 15 controls, 13 asthmatics without steroid inhalation therapy, 11 asthmatics with regular steroid inhalation therapy, and 10 smokers with chronic bronchitis were analyzed. We found cICAM-1, cVCAM-1, and cE-selectin to be present in serum from patients with stable asthma and stable nonobstructive chronic bronchitis. Only cICAM-1 was found in BL fluid. No differences were seen between the subject groups for either cCAM, but levels of ECP were increased in the non-steroid-treated asthmatic group. Subject atopy or smoking did not increase the cCAM levels. In conclusion, the degree of airway inflammation in stable nonobstructive chronic bronchitis and stable asthma does not appear to be well associated with circulating ICAM-1, cVCAM-1, and cE-selectin.

G. C. Riise¹, S. Larsson¹, O. Löwhagen²,
B. A. Andersson³

Departments of ¹ Pulmonary Medicine, and
² Allergy, Sahlgrenska University Hospital,
Göteborg; ³ Department of Clinical Immunology,
University of Göteborg, Göteborg, Sweden

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Dr Gerdt C. Riise
Department of Pulmonary Medicine
Bruna Str. 11
Sahlgrenska University Hospital
S-413 45 Göteborg
Sweden

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Expression of leukocyte-endothelial adhesion molecules on endothelial cell surfaces is one of the factors behind the inflammation in airway disease (14, 24). Cell adhesion molecules (CAMs) serve as ligands for leukocyte cell receptors, mediating the adhesion of leukocytes to the endothelium prior to their extravasation into inflamed tissue (12). Inter-cellular adhesion molecule-1 (ICAM-1), a glycoprotein belonging to the immunoglobulin supergene family, is basally expressed on both endothelial and epithelial cell surfaces and upregulated by cytokines (23). ICAM-1 mediates the adhesion and trans-endothelial migration of leukocytes (14). Vascular adhesion molecule-1 (VCAM-1) is induced by interleukin-4 and has been shown to be involved in eosinophil adhesion (19).

E-selectin (formerly called endothelial-leukocyte adhesion molecule-1 (ELAM-1)) is, together with

other selectins, involved in the initial leukocyte rolling on the vessel wall in areas of inflammation prior to the manifest adhesion mediated by ICAM-1 and VCAM-1 (10). All three CAMs have been associated with the mucosal inflammation seen in asthma and allergic rhinitis (2, 5, 11, 24), and in obstructive chronic bronchitis (3).

Recently, circulating forms of CAMs (cCAMs) thought to originate from adhesion molecules expressed on activated cells have been determined (18, 21). Levels of circulating ICAM-1 (cICAM-1), cVCAM-1, and cE-selectin have been discovered in bronchoalveolar lavage fluid in interstitial lung disease (4) and serum in active tuberculosis (8). In patients with acute asthma, increased levels of cICAM-1 and cE-selectin have been found in serum (7, 13). The high levels remained unchanged in spite of high-dose corticosteroid treatment for 28 days,

indicating a possible role for cCAMs as indirect markers of acute airway inflammation in asthma (13). In patients with stable obstructive chronic bronchitis, increased levels of cICAM-1 in bronchial lavage (BL) fluid and eE-selectin in serum have recently been reported (17).

In the present study, we investigated the levels of cICAM-1, eVCAM-1, and eE-selectin in small volume BL fluid and serum in three groups of well-defined patients: nonsmoking asthmatics with and without inhaled steroid therapy, and smokers with nonobstructive chronic bronchitis. We asked whether the pattern of cCAMs could serve as a marker for airway inflammation in these patients with stable airway disease.

Material and methods

Design

Flexible fiberoptic bronchoscopy, together with standardized small-volume (20 ml) lavage of the large airways, was performed to obtain samples predominantly reflecting the status of the bronchial epithelium (9, 16). Levels of cICAM-1, eVCAM-1, eE-selectin, and eosinophil cationic protein (ECP) were measured in BL fluid as well as in serum.

Atopy was defined as a history of upper or lower airway symptoms caused by common aeroallergens, as verified by positive skin prick test or by the radioallergen sorbent test for specific IgE (Phadiatop[®], Pharmacia, Uppsala, Sweden). All bronchoscopies were performed outside the pollen allergy season, and by the same investigator (G.R.).

Subjects

Group 1: 15 healthy controls (14 lifelong nonsmoking volunteers and one ex-smoker for 20 years). All had normal spirometry, negative methacholine test, provocative concentration causing $>20\%$ fall from baseline FEV_1 ($PC_{20} > 16$ mg/ml), and no signs of respiratory infection during the past 4 weeks.

Group 2: 24 patients with bronchial asthma. They were defined according to the criteria of the American Thoracic Society (22), including positive metha-

choline challenge test ($PC_{20} < 4$ mg/ml) or an increase in FEV_1 of $>20\%$ on β_2 agonist inhalation. All were lifelong nonsmokers (except for one ex-smoker) and with no signs of respiratory infection or obstructive attacks during the past 4 weeks. These patients were divided into two subgroups:

- Thirteen patients with mild asthma requiring symptomatic therapy with inhaled β_2 agonists only occasionally. None used either inhaled or oral steroid medication, or oral antiasthmatic medication.
- Eleven patients with bronchial asthma and receiving regular medication with inhaled steroids administered by powder inhaler devices for more than 1 year (mean 836 μ g beclomethasone or budesonide per day), together with inhaled β_2 agonists medication as required.

Group 3: 10 patients with nonobstructive chronic bronchitis. All were current smokers (mean 33.7 pack years) with productive daily cough for at least 3 consecutive months each year during the past 2 years. They had normal spirometry, negative methacholine tests ($PC_{20} > 16$ mg/ml), and no increase in FEV_1 on β_2 agonist inhalation. None had a history of recurrent infectious exacerbations. For further patient data, see Table 1.

Of the 24 asthmatic patients, four used oral β_2 agonists regularly, and one used oral theophylline preparations (all in group 2b). Of the 10 patients with chronic bronchitis, one used ipratropium bromide for inhalation at irregular intervals, and the others used no airway medication.

The duration of the respiratory symptoms of the asthmatics ranged between 3 and 30 years (mean 9 years), and of the bronchitics between 2 and 20 years (mean 8.5 years).

Ventilatory lung function (FEV_1) was measured with a Bernstein spirometer or a Vitalograph[®] in a standardized manner, according to the directions of the European Community for Coal and Steel (15). All methacholine provocation tests were performed within 4 weeks before the bronchoscopy.

Criteria for exclusion were abnormal chest x-ray, bronchial hypersecretion caused by factors other

Table 1. Clinical details of subjects. Sex, number of subjects with atopy, age, FEV_1 (in % of predicted normal), and level of positive methacholine provocation test (PC_{20})

	n	Atopy n	Age (years)		FEV_1 (% pred)		PC_{20} (mg/ml)	
			Mean	(range)	Mean	(SD)	Mean	(range)
Group 1: healthy controls (11 F 4 M)	15	4	39	(20–62)	106	(12)	>16.0	
Group 2a: asthma without inhalation steroids (10 F 3 M)	13	9	40	(22–62)	92	(8)	0.75	(0.5–2.0)
Group 2b: asthma with inhalation steroids (4 F 7 M)	11	7	47	(22–63)	93	(6)	4.00	(2.0–8.0)
Group 3: chronic bronchitis (6 F 4 M)	10	3	45	(31–62)	100	(11)	>16.0	

than cigarette smoking, age >65 years, and, for safety reasons, a methacholine test of <0.5 mg/ml in the steroid-untreated asthmatic patients (group 2a). The study design was approved by the ethics committee of the University of Göteborg, Sweden, and all subjects gave their consent after both written and oral information.

Fiberoptic bronchoscopy

Premedication was given with diazepam, 5 mg orally, followed by 0.5–1 ml morphine-scopolamine i.m. For topical anesthesia, 1% preservative-free tetracaine was sprayed with a deVilbiss nebulizer in the larynx, with additional anesthesia applied through the bronchoscope for the lower respiratory tract. Bronchoscopy was performed via the transoral route with Olympus flexible fiberoptic bronchoscopes of various models. Throughout the procedure, all subjects in the study, including the healthy controls, received intravenous infusion of terbutaline at a rate of 2.5 mg/min. Supplemental 100% oxygen was delivered nasally at a rate of 2–3 l/min, and the blood oxygen saturation was monitored continuously with an Ohmeda pulse oximeter (Ohmeda, Louisville, KY, USA).

Collection of samples

All samples were collected between 8.30 and 10 a.m. Serum was separated from the 10-ml venous blood sample according to Ahlstedt et al. (1), and frozen at -20°C for later analysis of cCAMs. BL fluid was obtained by installing 20-ml sterile phosphate-buffered saline (PBS) with the bronchoscope in an unwedged position in the anterior segment in the right upper lobe. The fluid was immediately aspirated, collected in a siliconized, endotoxin-free container, and transported on ice to the laboratory. Cells were sedimented by centrifugation, and the supernatant was stored at -20°C for later analysis of cCAMs, ECP, and albumin.

Analysis of cCAMs, ECP, and albumin

Soluble ICAM-1, soluble VCAM-1, and soluble E-selectin were analyzed as single determinations by commercial assays (R&D Systems Europe Ltd, Abingdon, UK). The serum samples were diluted 1:20 and the small-volume BL samples 1:4 in sample diluent. The lower limits of detection were for ICAM-1 9 $\mu\text{g/l}$, for VCAM-1 22 $\mu\text{g/l}$, and for E-selectin 2 $\mu\text{g/l}$. The assay is an enzyme-linked immunosorbent assay (ELISA) using one unconjugated and one conjugated monoclonal antibody directed to different determinants on the antigen (i.e., the compound to be analyzed). The unconjugated

antibody is coated onto the walls of microtiter wells. Any antigen present forms a bridge between the unconjugated and the conjugated antibody. The assay is standardized against a purified soluble form of recombinant ICAM-1 or E-selectin. By analyzing standards of known concentration coincident with samples, a curve of signal versus concentration can be constructed, and the concentration of unknowns determined. The inter- and intracoefficients of variation of the ELISAs used in the study have been investigated and accounted for in the assay instructions issued by the manufacturers.

Radioimmunoassay kits for analysis of ECP and albumin (Pharmacia Diagnostics AB, Uppsala, Sweden) were used according to the instructions of the manufacturers.

Statistical evaluation

Results were expressed as arithmetic mean and standard deviation (SD). The Kruskal-Wallis nonparametric test was used to analyze the significance of differences in data among the four groups of subjects. The Mann-Whitney U-test was used for comparison of the distribution of cCAMs, ECP, and albumin between atopic and nonatopic subjects. Spearman's rank correlation test was used to detect possible associations among cCAMs, albumin, and clinical parameters. *P* values <0.05 were considered to be statistically significant.

Results

High levels of all three cCAMs were found in the serum samples. Measurable levels of cICAM-1 could be detected in BL fluid (Fig. 1), but the BL levels of cVCAM-1 and cE-selectin were below the limits of detection. No statistically significant differences in serum or BL levels of either cICAM-1, cVCAM-1, or cE-selectin could be found among the four groups of subjects (Figs. 1 and 2).

In the 23 atopic subjects, the mean serum levels of cICAM-1 (401 vs 339), cVCAM-1 (612 vs 512), and cE-selectin (74 vs 62) were higher than in the 26 nonatopic subjects. These differences, however, were not statistically significant.

BL levels of ECP were significantly higher in the asthmatic patients without inhaled steroid therapy (group 2a) than in controls and asthmatic patients with regular inhaled steroid therapy (group 2b) ($P<0.05$) (Table 2). Serum levels of ECP were also higher in the asthmatic patients without inhaled steroid therapy ($P<0.05$) (Table 2).

The subjects with atopy had significantly higher mean BL levels of ECP (47.5 $\mu\text{g/l}$) than those without atopy (15.5 $\mu\text{g/l}$) ($P<0.05$), but their serum ECP levels did not differ significantly. The BL ECP levels

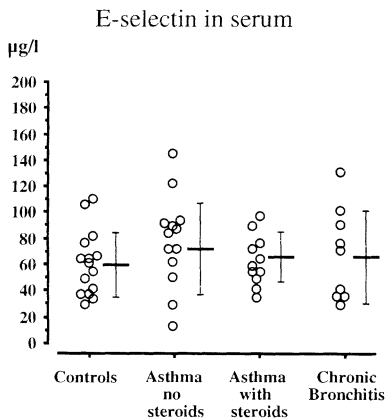
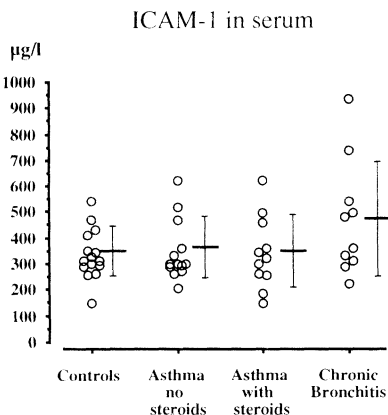
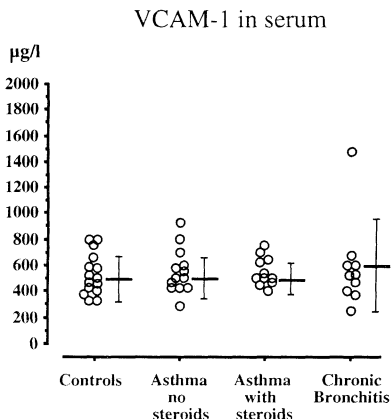
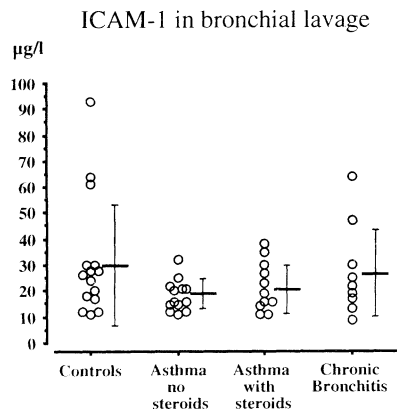


Fig. 1. Circulating intercellular adhesion molecule-1 (cICAM-1, $\mu\text{g/l}$) in BL and serum fluid in healthy controls, asthmatics without steroid inhalation therapy, asthmatics with regular steroid inhalation therapy, and patients with chronic bronchitis. Vertical bar represents mean values ± 1 SD.

Fig. 2. Circulating vascular adhesion molecule-1 (cVCAM-1, $\mu\text{g/l}$) and circulating E-selectin ($\mu\text{g/l}$) in serum in healthy controls, asthmatics without steroid inhalation therapy, asthmatics with regular steroid inhalation therapy, and patients with chronic bronchitis. Vertical bar represents mean values ± 1 SD.

correlated significantly with the ECP serum levels ($\rho = 0.46$, $P < 0.05$) in the atopic patients (data not shown).

The levels of albumin in BL fluid were comparable among the groups (Table 2). The BL levels of albu-

Table 2. Bronchial lavage (BL) and serum (s) levels of eosinophil cationic protein (ECP), and BL levels of albumin for each subject group. Mean percentage and one standard deviation (SD) are presented

	BL-ECP µg/l	s-ECP µg/l	BL-albumin mg/l
Group 1: healthy controls	7.8 (10.1)	6.7 (4.7)	98.2 (327.1)
Group 2a: asthma without inhalation steroids	53.0* (102.7)	15.5* (12.2)	17.2 (13.4)
Group 2b: asthma with inhalation steroids	30.1 (32.0)	8.5 (3.4)	21.2 (24.9)
Group 3: chronic bronchitis	35.7 (42.7)	11.7 (7.5)	23.0 (23.9)

* $P < 0.05$ compared to controls (group 1), and asthmatic patients taking inhalation steroids (group 2b).

min were found to correlate significantly with BL levels of cICAM-1 ($P < 0.001$, $\rho = 0.68$).

Among the clinical parameters, age, duration of disease, smoking habits, inhaled steroid medication, level of PC_{20} , and level of FEV_1 (% of predicted), no significant correlations with levels of cCAMs were found.

Discussion

Earlier studies using immunohistochemical analysis of CAMs have reported increased endothelial cell expression of ICAM-1, VCAM-1, and E-selectin in bronchial biopsies from patients with allergic asthma (2, 5), and in nasal biopsies from patients with allergic rhinitis (11). We investigated whether levels of circulating CAMs in serum and BL fluid from stable asthmatics could reflect these findings. Our results indicate that they do not. Circulating ICAM-1, cVCAM-1, and cE-selectin molecules in serum and BL fluid did not differ among the non-steroid-treated asthmatic patients, the asthmatic patients with regular steroid inhalation therapy, and the healthy controls. No significant differences were found between atopic and nonatopic individuals. The levels of ECP, however, were increased both in the atopic group, and in the non-steroid-treated asthmatics, indicating activation of eosinophils in these patients. Our results agree with the findings of Montefort et al., who reported increased serum levels of cICAM-1 and cE-selectin only in acute asthmatic subjects, and not in asthmatic or atopic individuals in a stable condition (13). Kobayashi et al. recently reported increased serum levels of cICAM-1 and cE-selectin in acute asthma, regardless of atopic status (7). In contrast, Hashimoto et al. found intermediate serum levels of cICAM-1 in atopic stable asthmatics compared to nonatopic controls and acute asthmatics,

but the time of sampling in relation to debut of asthma symptoms was not documented (6). Schmitt et al. did not find increased levels of serum cICAM-1 in stable atopic asthmatics compared to controls during allergen challenge, but the subjects were children and they were observed for only 24 h (20).

Only cICAM-1 was detected in BL fluid, and the levels were comparable among the groups. This reflects bronchial biopsy findings that airway epithelial cells express ICAM-1, in contrast to endothelial cells which express ICAM-1, VCAM-1, and E-selectin (2, 5).

In the smokers with nonobstructive chronic bronchitis, we found levels of cCAMs comparable to those of the nonsmoking asthmatic patients and controls. It therefore seems unlikely that exposure to cigarette smoke in itself affects cCAMs. We earlier reported that smokers with stable obstructive chronic bronchitis had a marked increase in serum cICAM-1 and cE-selectin compared to nonsmoking controls, and that serum levels of cE-selectin correlated inversely with FEV_1 for the obstructive patients (17). The smokers in the present study differ clinically from the obstructive patients in our earlier study not only by having normal lung function; they were also younger and without a history of infectious exacerbations. However, their smoking habits were similar. It is therefore possible that the elevated levels of cCAMs observed in the previous study could be linked to airways obstruction, repeated airways infections, or old age, rather than smoking as such or presence of chronic bronchitis.

In accordance with the findings of Gosset et al., we found no correlations between cCAMs and clinical markers of disease severity such as FEV_1 or airway responsiveness (5). cICAM-1 in BL fluid correlated significantly with albumin, possibly indicating some passive leakage of this molecule from serum to the bronchial surface.

In conclusion, the degree of underlying airway inflammation in stable nonobstructive chronic bronchitis and asthma is not well described by serum and BL fluid levels of cICAM-1, cVCAM-1, and cE-selectin. Smoking and atopy did not appear to affect the levels either. The role of circulating CAMs in inflammation has not yet been established, and their theoretic potential as modifiers of leukocyte adhesion remains to be verified. We agree with Montefort et al. (13) that the potential diagnostic use of cCAMs in stable asthma and nonobstructive chronic bronchitis appears to be limited.

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