

Differential patterns of circulating adhesion molecules in children with bronchial asthma and acute bronchiolitis

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The object of the study was to assess the levels of circulating forms of the cellular adhesion molecules ICAM-1, VCAM-1, E-selectin, L-selectin and P-selectin in young children with asthma and acute bronchiolitis. Thirty-nine children aged 12 to 84 months with mild or moderate asthma were studied at admission for acute asthma ($n = 15$) or in a stable phase ($n = 24$). Ten of the children with acute asthma were seen again after one month. Twenty-two children aged 1 to 17 months with acute bronchiolitis and nine non-atopic controls were also included in the study. In children with acute asthma, the mean concentration of circulating soluble ICAM-1 (sICAM-1) was increased compared to children with stable asthma (mean 442 $\mu\text{g/l}$ versus 363 $\mu\text{g/l}$; $p < 0.001$) and to controls (363 $\mu\text{g/l}$; $p < 0.05$). The levels of sICAM-1 remained high at follow up. In children with stable asthma, the mean serum concentration of soluble L-selectin (sL-selectin) (2080 $\mu\text{g/l}$) was significantly higher than in the controls (1664 $\mu\text{g/l}$; $p < 0.05$). The levels of circulating cellular adhesion molecules were similar in atopic and non-atopic asthmatics. Children with acute bronchiolitis had increased serum levels of soluble VCAM-1 (sVCAM-1) (1637 $\mu\text{g/l}$ versus 1019 $\mu\text{g/l}$ in the controls; $p < 0.01$) and sL-selectin (2041 $\mu\text{g/l}$ versus 1664 $\mu\text{g/l}$ in the controls; $p < 0.05$). There was no difference between the levels of circulating cellular adhesion molecules in children with respiratory syncytial virus (RSV) positive and RSV negative bronchiolitis. Soluble E-selectin (sE-selectin) and soluble P-selectin (sP-selectin) in serum were not significantly increased in any of the groups studied. In conclusion, our data suggest differential patterns of circulating cellular adhesion molecules in young children with acute asthma, stable asthma, and acute bronchiolitis, which may reflect differences in the underlying inflammatory processes in these obstructive pulmonary diseases.

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Bronchial asthma is an inflammatory disease, and leukocyte infiltration seems to be a key event in the pathogenesis of asthma (1, 2). The recruitment of leukocytes from the circulation into affected tissue is dependent on a series of cell adhesion molecule-mediated interactions between vascular endothelium and leukocytes (1, 3). The selectins, i.e. E-selectin, L-selectin and P-selectin, and their carbohydrate ligands mediate the initial capture and rolling phase of leukocyte adhesion (3), whereas intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), both members of the

immunoglobulin superfamily, and their integrin ligands contribute to the firm adhesion and transendothelial migration (3). The expression and function of the cellular adhesion molecules are modulated by a number of inflammatory mediators, e.g. histamine, platelet activating factor, leukotrienes and a variety of cytokines and growth factors (2). The demonstration of increased expression of adhesion molecules in the bronchial mucosa of patients with asthma (2, 4) has led to the postulation of an important role for these molecules in the inflammatory process of asthma (2, 4).

Recently, circulating forms of several adhesion molecules have been identified in peripheral blood and other body fluids, as well as in bronchoalveolar lavage aspirates, of healthy individuals and in disease (5). As these molecules seem to retain their functional activity, the circulating adhesion molecules may have physiological effects, e.g. by competing in cell-cell adhesion or by triggering a response in ligand-bearing cells (5–8). The serum concentration of soluble ICAM-1 (sICAM-1), soluble VCAM-1 (sVCAM-1) and soluble E-selectin (sE-selectin) have been reported to increase in a variable fraction of adults with acute asthma (9–12), but their pathophysiological role in this disease is still unclear. Moreover, it is not known whether the level or pattern of circulating adhesion molecules in asthma may reflect disease activity.

Acute bronchiolitis is a viral disease of childhood with inflammatory obstruction of the small airways. A significant proportion of infants with bronchiolitis have hyperreactive airways during later childhood, and it has been speculated that bronchiolitis, especially if caused by respiratory syncytial virus (RSV), may trigger the development of asthma (13). RSV has been demonstrated to induce the expression of ICAM-1 by respiratory epithelial cells *in vitro* (14), and to enhance neutrophil and eosinophil adhesion to cultured respiratory epithelial cells by an ICAM-1 dependent mechanism (15). Recently, Smyth et al. demonstrated slightly elevated levels of circulating sICAM-1 in infants with RSV bronchiolitis (16). However, the role of adhesion molecules in the inflammatory response of bronchiolitis remains to be determined.

In this study our object was to assess the levels of several circulating adhesion molecules in different clinical states of childhood asthma and, whether in bronchiolitis, the pattern was similar to that demonstrated in asthma. We have determined sICAM-1, sVCAM-1, sE-selectin, soluble L-selectin (sL-selectin) and soluble P-selectin (sP-selectin) in serum samples from 39 young children with asthma, as well as in 25 children undergoing their first episode of acute bronchiolitis.

Material and methods

Study subjects

Patients with asthma: Patients aged 12 to 84 months were diagnosed as having mild or moderate asthma based on episodic cough and wheeze, with response to β_2 -agonists, persisting or recurring for at least 6 months without a history of another diagnosis (17). Thirty-nine patients (26 males and 13 females) were studied and divided into three separate groups.

The first group consisted of 15 children hospitalized for acute asthma (mean age 46 months; range 12 – 83 months). They all received inhalations with adrenalin or β_2 -agonist. In addition some were treated with intravenous theophylline. They were discharged without anti-inflammatory treatment, but some of them started prophylactic treatment at follow up, one month later. At this time four patients still had symptoms indicating mild bronchial obstruction. Five children refused to have blood samples taken at follow-up. The patients with acute asthma had no signs of bacterial infection at admission, as evaluated by clinical examination, serum C-reactive protein, total leukocyte counts and chest X-rays.

The other patients were classified to have stable mild asthma ($n = 11$; mean age 51 months; range 14 – 83 months) or stable moderate asthma ($n = 13$; mean age 54 months; range 22 – 82 months) according to internationally accepted criteria (17). All patients used bronchodilators on demand in symptomatic periods. No patient with asthma had been using prophylactic therapy (i.e. inhaled glucocorticoids or cromoglycate) before the study, but in accordance with therapeutic recommendations (17), most of the patients with moderate asthma received prophylactic treatment after the blood samples were taken.

None of the patients with stable asthma had experienced clinical symptoms of bronchopulmonary obstruction in the last week before inclusion, and none had clinical signs of infection. All patients with stable asthma and the controls had C-reactive protein and total leukocyte counts in the normal range.

Nineteen of the children with asthma were atopic and 20 were non-atopic. Atopy was defined as having at least one positive skin prick test to common allergens (egg, milk, pea, hazelnut, peanut, cod, shrimp, *Cladosporium herbarum*, *Dermatophagoides pteronyssinus*, cat, dog, grass pollen and tree pollen; Soluprick, ALK, Hørsholm, Denmark). The median ages of the atopic and non-atopic asthmatics were 62 and 42 months, respectively. The median total IgE for the atopic infants with asthma was 242 kU/l and for the non-atopic 16.9 kU/l.

Patients with acute bronchiolitis: This group consisted of 22 children aged 1–17 months (mean 6.4 months) needing hospitalization for their first episode of bronchiolitis. Bronchiolitis was defined as acute respiratory illness with tachypnoea, dyspnoea, prolonged expiration and wheezing on auscultation (18).

Nasopharyngeal mucus was examined for RSV by direct immunofluorescence in all patients (biomérieux, Marcy-l'Étoile, France). Eleven of the patients were RSV positive and 11 RSV negative. Seven of the patients had serum concentrations of total IgE above the normal range, and one of these

had atopic dermatitis. Infants with pre-existing lung disease (e.g. bronchopulmonary dysplasia) or signs of bacterial infection were not included.

Controls: Nine non-atopic infants without asthma or infection served as controls (mean age 62 months; range 13 - 86 months).

Ethics: The study was approved by the Regional Ethical Committee, and signed statements of informed consent were obtained from all parents.

Laboratory analysis

Venous blood was drawn at outpatient control or immediately after admission for acute asthma or bronchiolitis. Serum was aliquoted and stored at -70°C until analysis.

sICAM, sVCAM-1, sE-selectin, sL-selectin and sP-selectin were analysed by enzyme-linked immunosorbent assays (ELISA) (R&D Systems Europe Ltd, Abingdon, UK) according to the manufacturer's recommendations. All measurements were carried out in duplicate. The lower limit of detection was 0.35 $\mu\text{g/l}$ for sICAM-1, 2 $\mu\text{g/l}$ for sVCAM-1, 0.1 $\mu\text{g/l}$ for sE-selectin, 0.3 $\mu\text{g/l}$ for sL-selectin and 0.5 $\mu\text{g/l}$ for sP-selectin.

Serum total IgE was analyzed using the CAP system from Pharmacia (Uppsala, Sweden). Serum-IgE levels > 1 s.d. above the age related mean value was considered elevated. Total and differential leukocyte counts (Technicon H*2/H*3 or Coulter STKS) and C-reactive protein (immunoturbidimetric technique; Roche, Switzerland) were determined in all patients. All patients with bronchiolitis and most with acute asthma had a chest X-ray taken at admission.

Parameters of eosinophil inflammation, i.e. total eosinophil counts and serum concentrations of eosinophil cationic protein (ECP) and interleukin-5 were determined in all patients, the results of these measurements have been published earlier (19).

Statistical analysis

Significance of difference was determined by the Mann-Whitney U-test and Spearman's rank test was

used for correlation analysis. $P < 0.05$ was considered significant. The data are given as mean, median and quartiles.

Results

Acute asthma

The children with acute asthma had significantly higher mean serum concentration of sICAM-1 at admission than the children with stable asthma ($p < 0.001$) and the controls ($p < 0.05$) (Fig. 1a and Table 1). There was no significant relationship between the duration of symptoms before admission and the levels of sICAM-1. At follow up, the mean sICAM-1 concentration was only slightly lower than at admission (Table 1), and four patients had serum concentrations of sICAM-1 that were even higher than those at admission one month earlier. The mean serum concentration of sL-selectin was slightly higher in children with acute asthma both at admission and at follow up than in the healthy controls, but these differences did not reach statistical significance (Table 2). For sVCAM-1, sE-selectin and sP-selectin the serum concentrations in children with acute asthma were similar to those in the healthy controls both at admission and at follow up (Tables 1 and 2). In addition, there was no significant correlation between the serum levels of any of the adhesion molecules in acute asthma.

Stable asthma

In children with stable asthma, the mean concentration of sL-selectin was higher than in the controls ($p < 0.05$) (Fig. 1b and Table 2), and even in the subgroup of children with mild stable asthma the mean concentration of sL-selectin was elevated ($p < 0.01$). For sICAM-1, sVCAM-1, sE-selectin and sP-selectin, the levels were similar in children with stable asthma and in controls (Tables 1 and 2). There were no significant differences between the serum concentrations of any of the circulating adhesion molecules examined in mild and moderate stable asthma.

Table 1. The concentrations of circulating adhesion molecules of the immunoglobulin superfamily in sera from young children with asthma or acute bronchiolitis¹⁾

	n	sICAM-1	sVCAM-1
Acute asthma (admission)	15	442 (430; 411-492) $\mu\text{g/l}$ ²⁾	1054 (990; 845-1240) $\mu\text{g/l}$
Acute asthma (follow-up)	10	415 (430; 339-473) $\mu\text{g/l}$	1124 (952; 790-1367) $\mu\text{g/l}$
Stable asthma	24	363 (364; 328-394) $\mu\text{g/l}$	1171 (1120; 965-1360) $\mu\text{g/l}$
Acute bronchiolitis	25	409 (398; 336-470) $\mu\text{g/l}$	1637 (1432; 1310-1785) $\mu\text{g/l}$ ³⁾
Controls	9	363 (386; 291-431) $\mu\text{g/l}$	1019 (935; 797-1197) $\mu\text{g/l}$

¹⁾ The data are given as mean (median; quartiles).

²⁾ $p < 0.05$ as compared to healthy controls, and $p < 0.001$ as compared to children with stable asthma.

³⁾ $p < 0.01$ as compared to healthy controls.

Table 2. The concentrations of circulating selectins in sera from young children with asthma or acute bronchiolitis¹⁾

	n	sE-selectin	sL-selectin	sP-selectin
Acute asthma (admission)	15	111 (97; 85-149) µg/l	1971 (1989; 1653-2290) µg/l	113 (108; 88-142) µg/l
Acute asthma (follow-up)	10	91 (96; 75-112) µg/l	1957 (1768; 1509-2225) µg/l	119 (117; 96-136) µg/l
Stable asthma	24	97 (97; 73-113) µg/l	2080 (2152; 1724-2401) µg/l ²⁾	103 (106; 77-129) µg/l
Acute bronchiolitis	25	138 (106; 90-173) µg/l	2041 (2192; 1695-2339) µg/l ²⁾	128 (112; 81-152) µg/l
Controls	9	103 (76; 66-160) µg/l	1664 (1767; 1509-1802) µg/l	103 (101; 68-138) µg/l

¹⁾ The data are given as mean (median; quartiles).

²⁾ $p < 0.05$ as compared to healthy controls.

In addition, there were no significant correlations between the concentrations of any of the circulating adhesion molecules in stable asthma.

Acute bronchiolitis

In acute bronchiolitis, the mean serum concentration of sVCAM-1 was significantly higher than in the controls ($p < 0.01$) (Fig. 2a and Table 1). In addition, the mean level of sL-selectin was significantly higher in the patients than in the controls ($p < 0.05$) (Fig 2b and Table 2). Both the serum concentrations of sE-selectin and sP-selectin were slightly higher in acute bronchiolitis than in the controls (Table 2), but these differences did not reach significance. The levels of sICAM-1 in acute bronchiolitis were similar to those of the controls (Table 2). There were no significant correlations between the levels of any of the circulating adhesion molecules in bronchiolitis. In addition, there were no differences in the serum

concentrations of any of the circulating adhesion molecules between children with RSV-positive and RSV-negative bronchiolitis.

Atopy and parameters of eosinophil inflammation

The levels of circulating adhesion molecules were similar in atopic and non-atopic children. There were no significant correlations between total eosinophil counts, serum ECP concentrations or serum interleukin-5 concentrations and the concentrations of any of the circulating adhesion molecules measured in this study.

Discussion

Recently, circulating isoforms of several cellular adhesion molecules retaining their functional capacities have been demonstrated and increased levels of sICAM-1, sVCAM-1 or sE-selectin have been reported

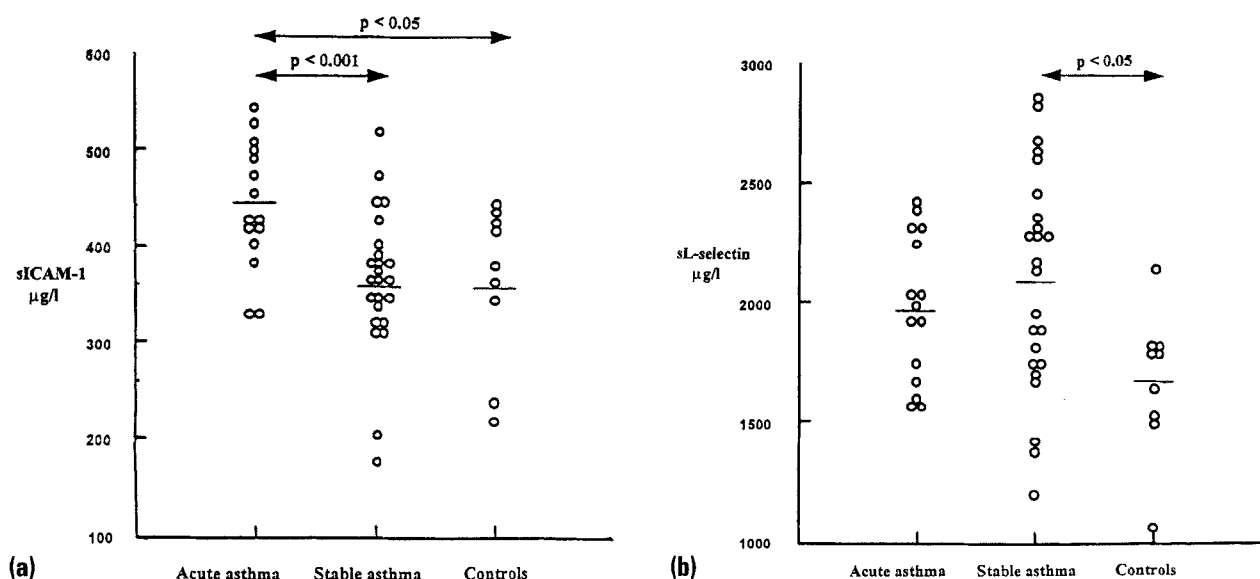


Fig. 1. sICAM-1 (a) and sL-selectin (b) in children with acute asthma, stable asthma and controls. Lines indicate mean values.

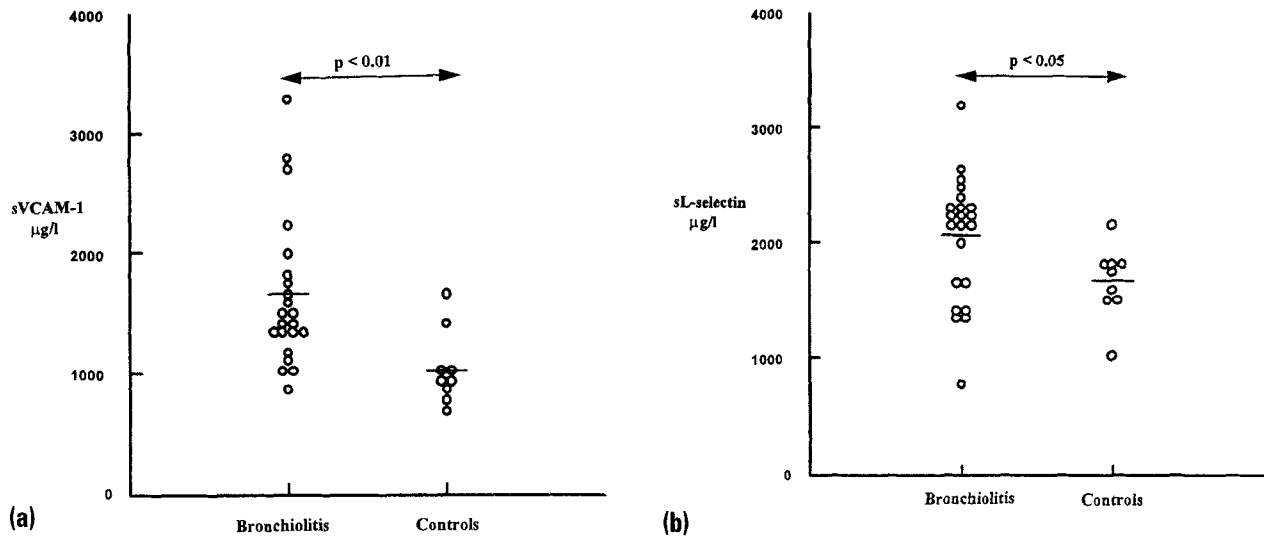


Fig. 2. sVCAM-1 (a) and sL-selectin (b) in children with bronchiolitis. Lines indicate mean values.

in subgroups of adults with asthma (5–12). The circulating forms can bind to their complementary receptors and inhibit leukocyte-endothelial cell or leukocyte-epithelial cell adhesion (5, 6), as well as modulate the functional status of key cells in the allergic inflammation, e.g. macrophages, T cells and eosinophils (8). This indicates that the role of adhesion molecules in inflammatory lung diseases goes beyond leukocyte recruitment, and it has been postulated that circulating adhesion molecules might serve as markers for the inflammatory activity in asthma, as well as targets for new therapeutic approaches (5, 7).

Asthma

In this study, we have demonstrated that the serum concentrations of sICAM-1 were significantly higher in young children with acute asthma than in healthy controls, whereas the levels of sICAM-1 in children with mild or moderate stable asthma were similar to those measured in the controls. In our patients, as in adults (9, 10, 12), there was significant overlap between the concentrations of sICAM-1 in the asthmatics and the healthy children. Moreover, the concentrations of sICAM-1 one month after admission remained high, and in four of nine patients the levels were even higher at follow up than at admission. Hence, the increased levels of sICAM-1 in young children with acute asthma suggest a pathophysiological process that does not significantly level off in the first month after the acute attack (10), but which may be reduced at least with oral glucocorticosteroid therapy (12).

In adults, the serum concentrations of sE-selectin has been reported to increase in acute asthma (9,

10), whereas in a study of stable asthma, sE-selectin was similar to controls (20). In this study, the levels of sE-selectin did not increase in acute asthma. It is possible that the difference between our results on acute childhood asthma and those found in adults may reflect differences in disease activity. However, other explanations, like different cytokine patterns in acute childhood and adult asthma cannot be ruled out.

The serum concentrations of sL-selectin were significantly increased in the group of children with mild and moderate stable asthma. L-selectin is shed from activated leukocytes after endoproteolytic cleavage, and serum sL-selectin is probably derived both from leukocytes activated in the circulation and in adjacent tissues (6). It has been postulated that the shedding of L-selectin from large numbers of leukocytes is required to induce noticeable elevations of sL-selectin in serum (21). Hence, increased amounts of sL-selectin even in children with mild asthma may support the current opinion that there is a significant inflammation in the airways of even mild asthmatics, implicating therapeutic considerations concerning prophylactic treatment (22).

There is a need for objective tools to document inflammation in small children with obstructive pulmonary disease, as measurements of airway flow and bronchial responsiveness are usually not possible in children less than 5 years old. Thus, it is of interest that sL-selectin, in contrast to total eosinophil counts, serum ECP, serum interleukin-5, sICAM-1, sVCAM-1, sE-selectin and sP-selectin, was the only parameter of inflammation that was significantly increased in children with stable asthma, and it is possible that sL-selectin could be of

value for the evaluation of disease activity in these children. However, sL-selectin in inflammatory lung disease has to our knowledge not been reported earlier, and further studies, including longitudinal studies of larger patient cohorts and of effects of anti-asthmatic therapy, are needed to determine the possible usefulness of sL-selectin measurements in the management and follow-up of children with asthma.

The role of sL-selectin in asthma is not known, but the increased serum levels in stable asthma may suggest effects on the asthmatic inflammation, possibly through inhibition of leukocyte recruitment (6). During acute asthmatic attacks, the levels of sL-selectin were slightly lower than in stable asthma. sL-selectin binds to activated venular endothelium in regions of pulmonary inflammation (21). Thus, during acute asthmatic attacks, inflammatory stimuli may induce strong upregulation of endothelial L-selectin ligands that would bind circulating L-selectin and tend to reduce the concentration in serum. A similar mechanism seems to be of significance in surgical patients at risk of developing the adult respiratory distress syndrome (23).

In our study of young children with asthma, the levels of circulating cellular adhesion molecules were similar in atopic and non-atopic patients. In the youngest children with asthma, infection is often the main trigger of symptoms even in atopic individuals, and this may explain why the results were similar in the two groups. In a study of adult asthmatics, increased expression of ICAM-1 was demonstrated in bronchial biopsies from atopic asthmatics compared to non-atopic asthmatics (4). However, only one (24) of five (9, 10, 20, 25) earlier studies has indicated association between atopic asthma and elevated serum levels of sICAM-1 in adults.

Acute bronchiolitis

Several studies indicate a role for adhesion molecules in the immune response in viral infections (14–16, 26). This study demonstrated increased serum concentrations of sVCAM-1 in children with acute bronchiolitis as compared to healthy controls. VCAM-1 seems to contribute to the recruitment of T cells and eosinophils in tissue inflammation (1, 27). Hence, the high levels of sVCAM-1 may have influenced the mobilization of these leukocytes into the inflamed lungs of children with acute bronchiolitis.

Recently, it has been reported that children with wheezing continuing 5 months after the first episode of bronchiolitis, demonstrate increased production of interleukin-4 after stimulation, compared to lymphocytes from subjects who did not wheeze (28). As

interleukin-4 is associated with a TH-2 response, the results suggests a link between a TH-2 response and wheezing at this age group (28, 29). Interleukin-4 is the main cytokine that stimulates the upregulation of VCAM-1 on endothelial cells (1). Thus, elevated levels of sVCAM-1 in our children with bronchiolitis are consistent with a role for this interleukin and a TH2-dependent cellular response even at the first episode of bronchiolitis.

The children with acute bronchiolitis had significantly higher levels of sL-selectin than the control children. As eosinophils seem to be involved in the inflammation in bronchiolitis (30), increased levels of sL-selectin may suggest a role for this molecule in the regulation of eosinophil recruitment even in bronchiolitis.

RSV infections of respiratory epithelial cell monolayers enhance the cellular expression of ICAM-1 *in vitro*, promoting adhesion of neutrophils and eosinophils to infected epithelium (14, 15). Recently, slightly elevated sICAM-1 levels have also been demonstrated in serum from infants with RSV bronchiolitis (16). On the basis of these findings it has been postulated that ICAM-1 could play a role in the inflammation of the lower airways during RSV infection (15, 16). In our study, neither RSV positive or RSV negative bronchiolitis was associated with increased levels of sICAM-1.

The results of measurements of circulating cellular adhesion molecules in asthma and other inflammatory lung diseases should be interpreted with caution for several reasons (5). First, the measured levels do not determine the amount of bioactive adhesion molecules, and the fraction of active material may vary between disease states. Second, the concentrations of circulating adhesion molecules do not necessarily reflect the levels in the affected pulmonary tissue. Third, a significant proportion of shed adhesion molecules may also be captured on complementary receptor-bearing cells. Fourth, little is known on the rate of clearance of circulating adhesion molecules. Thus, further studies should be carefully designed to elucidate both the mechanisms controlling the release of adhesion molecules and their clearance, and their possible relevance for patient management.

Children with obstructive pulmonary disease during the first years of life represent a heterogeneous group, and distinct subgroups may be difficult to define (31). Moreover, the pathogenesis of early wheezing and its relation to subsequent development of asthma is poorly understood. However, our data indicating differential elevation of circulating molecules in children with acute bronchiolitis, stable and acute asthma suggest that there are differences in the underlying inflammatory responses in

these obstructive pulmonary disorders. The possible clinical use of monitoring serum levels of circulating adhesion molecules remains to be determined.

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