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Elevation of serum soluble E-selectin and VCAM-1 in severe asthma

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Objective: **To investigate the significance of circulating adhesion molecules associated with leucocyte-endothelial cell interactions in asthma, serum levels of soluble E (sE)-selectin, soluble P (sP)-selectin, soluble L (sL)-selectin, and soluble vascular cell adhesion molecule-1 (sVCAM-1) were measured in mild, moderate and severe asthma.**
Method: **Serum levels of sE-selectin, sP-selectin, sL-selectin, and sVCAM-1 were measured in 32 women with asthma and 30 healthy donors using an enzyme-linked immunosorbent assay method. Twenty patients were suffering from severe asthma, and 12 from mild/moderate asthma.**
Results: **Serum sE-selectin and sVCAM-1 levels from patients with asthma were significantly higher than those observed in healthy donors ($p < 0.01$). The levels of sP-selectin were the same as those of controls. The level of sE-selectin exhibited an important increase in the severe asthmatic patients compared with mild/moderate asthma ($p < 0.01$). The sVCAM-1 level was increased in severe asthma when compared with healthy controls. There was no correlation between the levels of soluble selectins and the age of the patients. A significant correlation was found between sE-selectin and sVCAM-1 levels.**
Conclusion: **These data indicate that circulating soluble forms of the selectins may have different kinetics during the clinical course of asthma, suggesting that they may reflect different inflammatory pathways in severe asthma. Both sVCAM-1 and sE-selectin may be useful immunological markers for monitoring disease activity in asthma.**

Key words: Asthma, Inflammation, Selectins

Introduction

Adherence of leucocytes to endothelial cells (EC) is a primordial event in the sequence of inflammatory response. Specific cell adhesion molecules, expressed on the surface of leucocytes and/or EC, have been identified. As an initial event during inflammation, leucocytes in the blood stream roll along EC with loose contact, mediated by E-selectin, P-selectin and L-selectin.[1] In the inflammation phase, activation of leucocyte integrins occurs with expression of immunoglobulin-like adhesion proteins on EC, including intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 (VCAM-1). The leucocytes that are tightly bound to EC then migrate into the subendothelial tissue. Soluble forms of these adhesion molecules are released in circulation. Serum levels may be increased in several inflammatory diseases, and particularly in asthma. These circulating forms may be biologically active.[2]

Asthma is known as a bronchial chronic inflammatory disorder.[3] Immunological abnormalities during asthma are characterized by marked activation of the immune system, leading to increased cytokine production by activated effector cells and the induction of antigen expression on EC.[4] The factors modulating the activation and recruitment of circulating inflammatory cells to the lung remain partially unknown, but an early step in this process is the interaction of adhesion molecules on circulating cells with those on EC.[5] Severe asthma is supposed to involve different pathways as corticosteroids are much less efficient in reducing inflammation.

To investigate the significance of circulating adhesion molecules in the pathogenesis of asthma, we measured the serum levels of soluble E (sE)-selectin, soluble P (sP)-selectin, soluble L (sL)-selectin, and soluble VCAM-1 (sVCAM-1) in two asthma groups and compared them with healthy donors.

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Subjects and methods

Subjects

Patients were recruited from the Department of Respiratory Diseases (A. Mami Hospital, Ariana, Pavillon B). Thirty-two female asthma patients were classified depending on severity, according to National Heart Lung and Blood Institute (NHLBI) criteria.[6] All women were non-smokers. Twenty women suffered from severe asthma (mean age, 47.6 ± 1.3 years; range, 45–56 years), and 12 women had mild or moderate asthma (mean age, 36.6 ± 2.7 years; range, 22–38 years). Patients with severe asthma were treated by oral glucosteroids (10–20 mg/day) and theophylline. Patients were studied away from acute exacerbation. Controls were selected from the staff of our hospital, and showed no abnormalities on physical examination and laboratory tests (mean age, 40.2 ± 5.7 years; range, 28–44 years).

Assay methods

Serum levels of sE-selectin, sP-selectin, sL-selectin and sVCAM-I were measured using an enzyme-linked immunosorbent assay kit (sE-selectin, British Bio-Technology Products Ltd, Abingdon, UK; sL-selectin and sP-selectin, Bender MedSystems, Vienna, Austria; sVCAM-I, R&D Systems, Minneapolis, MN, USA).

Statistical analysis

Values are presented as mean ± standard deviation (SD). Differences between groups are analyzed using the Mann-Whitney *U*-test.

Results

The female asthma patients enrolled in this study were classified according to NHLBI criteria[6] as suffering from mild/moderate (group 1) and severe asthma (group 2). Values for sE-selectin, sP-selectin, sL-selectin and SVCAM-1 (Fig. 1A-D) were expressed as mean ± SD (ng/ml).

The sE-selectin levels (Fig. 1A) of the asthmatic patients (98.72 ± 22.5 ng/ml, $p < 0.001$) were significantly higher than those of healthy controls (42.6 ± 12.7 ng/ml). Patients with severe asthma exhibited a significant increase of sE-selectin level (128.53 ± 33.7 ng/ml, $p < 0.001$) compared with all asthmatic and mild/moderate asthma patients (63.82 ± 28.45 ng/ml).

Serum levels of sP-selectin (Fig. 1B) in asthma patients (150.6 ± 15.2 ng/ml, $p < 0.01$) were the same as healthy controls (168.7 ± 17 ng/ml). There was no difference in the levels of sP-selectin between mild/moderate asthma (148.78 ± 15.4 ng/ml) and severe asthma (154.2 ± 16.7 ng/ml, $p < 0.01$).

Serum sL-selectin levels (Fig. 1C) in the group of asthmatic patients (1589.7 ± 43.5 ng/ml) were similar to controls (1449.5 ± 47.9 ng/ml). A weak significant difference was observed between mild/moderate (1492 ± 53.0 ng/ml) and severe asthma (1662.7 ± 39.4 ng/ml, $p < 0.05$)

The sVCAM-1 levels (Fig. 1D) of asthmatic patients (753 ± 60.8 ng/ml) were higher than those of healthy controls (580 ± 70.7 ng/ml; $p < 0.01$). Serum sVCAM-1 levels showed no statistically significant differences between mild/moderate (761.5 ± 46.2 ng/ml) and severe asthma (727.3 ± 55.4 ng/ml).

Within the severe asthma group, there was a significant correlation between sE-selectin and sVCAM-1 ($r = 0.75$, $p < 0.001$). No significant correlation was found between serum sL-selectin or sP-selectin and sVCAM-1 (data not shown). No correlation was found between age and soluble selectins in our patients.

Discussion

The selectin family consists of three proteins designated by the prefixes E (endothelial), P (platelet), and L (leucocyte). These adhesion molecules are shed following proteolytic cleavage near the transmembrane domain or by expression of alternatively spliced mRNA lacking a transmembrane domain from the cell surface, following activation with T helper 2 (Th-2) cytokines.[2] It has been suggested that the soluble molecules can regulate cell adhesion by down-regulation as competitive inhibitors, or by upregulation as co-signaling factors.[7] More recently, soluble isoforms have been detected in the circulation. Increased levels of these molecules may reflect the inflammatory processes of certain diseases.[1,2]

In the present study, high levels of soluble selectins and VCAM-1 were found in asthmatic women. Our results suggest that the activation of leucocyte-endothelial interactions persists for mild, moderate and severe asthma even in the absence of acute exacerbation. High levels of soluble selectin during asthma reflect the upregulation of selectin expression, and that the soluble form of these molecules may be useful markers of persistant inflammation.[8]

An important finding in this work is the significant correlation found between soluble selectin levels and severe asthma. The expression of E-selectin is restricted to activated EC; increased levels of sE-selectin in the blood would be taken as conclusive evidence for the activation of EC[1,2] and to represent a marker for endothelial damage or activation in asthma. In patients with allergic asthma, E-selectin and VCAM-1 were overexpressed only on the endothelium.[9] It has also been reported that sE-selectin levels are increased in autoimmune diseases.[10] There is evidence that endothelin 1, a mediator released by

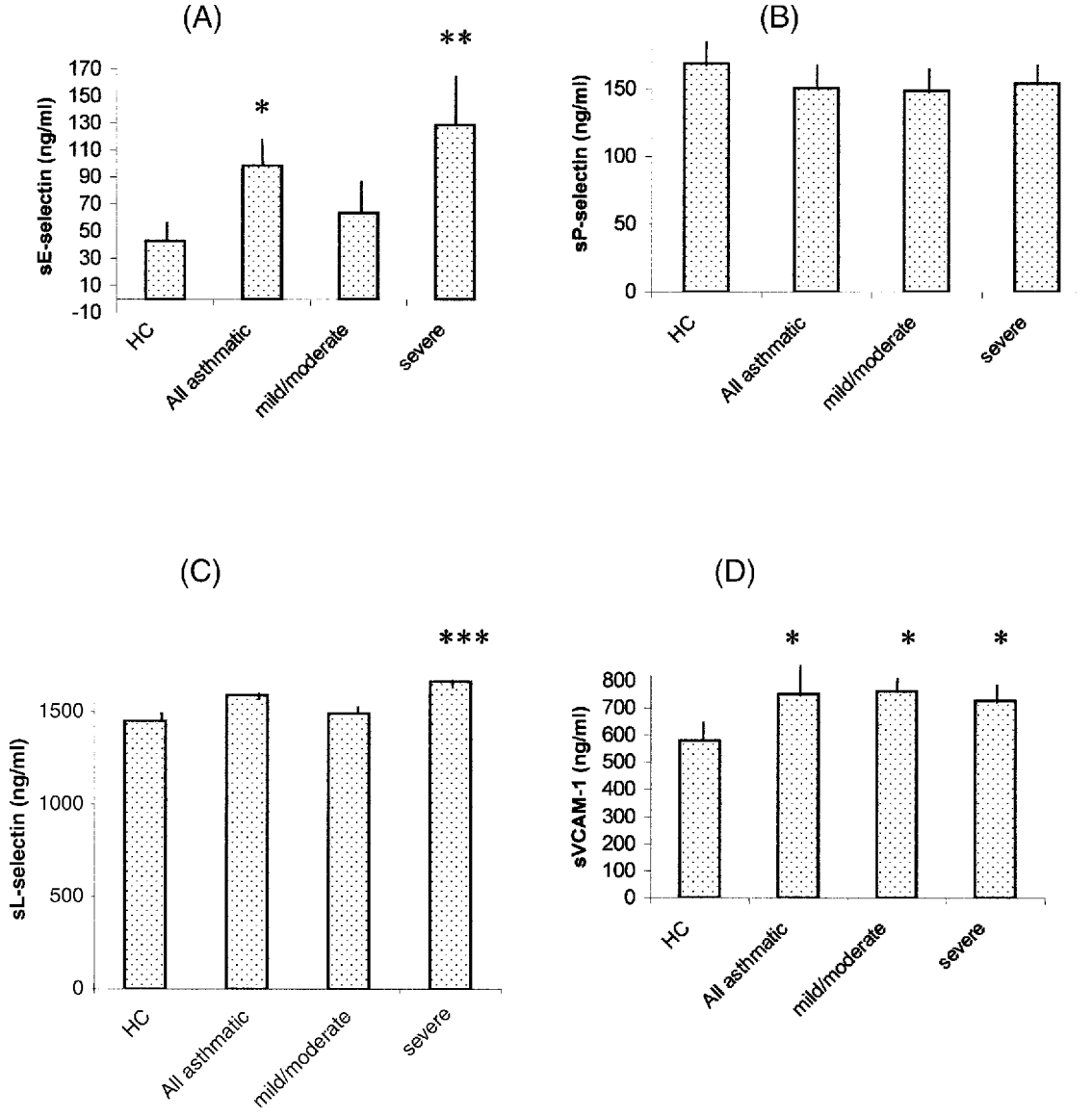

FIG. 1. Expression of (A) sE-selectin, (B) sP-selectin, (C) sL-selectin and (D) sVCAM-1 in female asthma patients and in healthy controls (HC). Patients were selected in the mild/moderate asthma group and the severe asthma group. * Significantly different when compared with healthy controls ($p < 0.01$), ** significantly different when compared with all asmathic patients and with patients with mild/moderate asthma ($p < 0.001$), and *** significantly different when compared with patients with mild/moderate asthma ($p < 0.05$).

activated EC, is involved in asthmatic bronchial inflammation and remodeling pathways.[11]

The higher levels of sE-selectin could be influenced by the estrogen receptor that accumulates in the cytoplasm of smooth muscle cells.[12] Soluble selectins were found increased in lactating women.[13] Our asthma patient group was composed of women. Recent evidence suggest a role for hormonal factors in the etiology of asthma. However, we found no correlation between soluble selectins and female sex steroid hormones, neither with estrogen nor progesterone (data not shown). In the same way, a large study reported no association between hormone replacement therapy in postmenauposal women and asthma severity.[14]

sE-selectin is reported to be only a weak inhibitor of E-selectin-mediated adhesion,[15] while it may have cytokine-like functions that activate the polymorphonuclear cell CD11b integrin receptor, serving as a chemoattractant.[16] Furthermore, Th-1 lymphocytes, but not Th-2, are able to bind to E-selectin.[17] The particular increase of this selectin in severe asthma could suggest an involvement of Th-1 cells in addition to Th-2 in this group of patients.

P-selectin is expressed on EC and platelets.[1] sP-selectin was shown to prevent activated neutrophil adhesion to EC *in vitro*.[18] In the present report, no differences were observed in sP-selectin levels between normal controls and asthmatic patients.

L-selectin is constitutively expressed on most leucocytes but with varying density.[1] The sL-selectin levels in our asthmatic patients were increased, particularly in severe asthma. It has been reported that patients with sepsis or HIV infection have increased levels of sL-selectin and that these soluble forms can inhibit L-selectin-mediated adhesion at physiological concentrations.[19]

VCAM-1 is expressed on EC, epithelia, macrophages, and dendritic cells.[15] Soluble forms of VCAM-1 are shed from cytokine-activated EC.[1] Serum sVCAM-1 levels in our patients were increased compared with healthy controls, but were similar in mild/moderate and severe asthmatic patients. Our results indicate that there is no specific change of sVCAM-1 levels depending on disease severity.

As several cytokines (tumor necrosis factor-α, interleukin-1, interleukin-4) stimulate the expression of adhesion molecules and allergen exposure induces the expression of endothelial adhesion molecules in passively sensitized human bronchus,[1,2,20] increased levels of soluble adhesion molecules were expected to be found in asthmatics. Leucocyte adhesion mechanisms play an important role in the development of allergic inflammation in bronchial asthma. Increased serum levels of sE-selectin and soluble intercellular adhesion molecule-1 were observed during asthma exacerbation.[21] The role of soluble selectins in asthma is still unknown. The elevated levels of sE-selectin, and sVCAM-1 appear to correlate with disease severity of asthma. It is probable that these molecules play a significant role in localization of leucocytes to the site of lung vascular activation in asthma, as has been shown in other immunologically mediated diseases.[1,2]

In the presented patients, the increase in soluble selectins could not be induced by treatment as theophylline *in vitro* inhibited the L-selectin shedding induced by PAF on both eosinophils and neutrophils.[22] In the same way, increases in sE-selectin, kinins, and albumin after segmental allergen challenge of asthmatic subjects were inhibited by glucocorticoids.[23]

It has been reported that increased soluble selectins could be a promising serological marker of the severity of inflammation in bronchial asthmatic children.[24] The present study suggests that elevated levels of these molecules may reflect the inflammatory process in asthmatic adults. Because attachment of circulating cells to endothelium is a key event in their recruitment to the inflammatory sites, the association of higher concentrations of soluble selectins with severe asthma suggests that E-selectin and VCAM-1 are involved in the inflammatory pathways of this kind of asthma.

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