Neopterin and circulating adhesion molecules as prognostic markers in childhood asthma

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Abstract

Introduction: The purpose of this study was to determine the serum levels of neopterin and circulating adhesion molecules sICAM-1, sE-selectin and sL-selectin in children with stable asthma in order to investigate their possible role in the pathogenesis of chronic inflammation in asthma. We also compared atopic and non-atopic asthmatic children in order to detect differences between groups, possibly reflecting different mechanisms involved in chronic inflammation of the airways.

Material and methods: The study included three groups of children: A, B and C. Group A consisted of 30 children with atopic asthma, group B of 30 children with non-atopic asthma and group C (control group) of 20 healthy children. All asthmatic children had been diagnosed with mild to moderate persistent asthma according to the *International Paediatric Asthma Consensus Group Reports* and were studied during a stable phase of their disease. The asthmatic children were divided into atopic and non-atopic, as judged by the presence of a positive or negative skin prick test or a positive specific IgE test.

Results: There were no differences in serum sICAM-1, sE-selectin or sL-selectin levels between groups A, B and C. Atopic asthmatic children had significantly higher levels of neopterin compared to the non-atopic asthmatics or healthy children ($p<0.001$).

Conclusions: Our data support the hypothesis that neopterin is produced and secreted by activated macrophages, the latter playing a role in chronic asthmatic inflammation. This may allow a better understanding of the clinical implications and more insight into the inflammatory processes of bronchial asthma.

Key words: asthma, neopterin, sICAM-1, sE-selectin, sL-selectin.

Introduction

Asthma is not a single, well defined disease entity, but consists of various different subtypes [1]. The causes of the disease are complex and are made up of a variety of genetic and environmental factors [2, 3]. Airway inflammation is a key in the pathogenesis of asthma [4]. The mechanisms underlying airway epithelial damage observed in patients with asthma have attracted a great deal of interest. Several inflammatory products may induce epithelial damage in asthma [5, 6].

One of the characteristic features in asthma during pulmonary inflammation is the leukocytic infiltration of the airway [7]. In this infiltrate, eosinophils

predominate but significant numbers of lymphocytes and macrophages are also present [8-10].

Thus, in the inflammatory response of the lung, circulating inflammatory cells accumulate in the pulmonary capillaries and migrate through the vascular endothelium to the submucosa of the asthmatic airway [11, 12].

Firm adhesion of leukocytes to vascular endothelium is mediated by the interaction of adhesion molecules expressed on the cell surfaces of leukocytes and vascular endothelial cells. Cell adhesion molecules are involved in several of the immunological processes relevant to the inflamed airway [13].

The migration of neutrophils from capillaries to the alveoli involves transient adhesion and rolling, followed by firm adhesion, and then transendothelial migration [14]. Transient adhesion and rolling are mediated by intercellular adhesion molecule-1 (ICAM-1) and selectins such as E-selectin and L-selectin, which have somewhat overlapping roles in neutrophil recruitment [15, 16].

ICAM-1 is expressed on activated leukocytes as well as on endothelial cells, epithelial cells, fibroblasts, dendritic cells and macrophages. It participates in leukocyte adhesion to activated endothelial cells, T cell/antigen presenting cell, T cell/T cell and T cell/B cell interactions [17].

E-selectin and L-selectin are involved in the initial association of leukocytes with the vessel wall in areas of inflammation. These selectins with the adhesion molecules are responsible for the early step in the movement of immunocompetent cells from the peripheral blood to tissues [18, 19].

There are soluble forms of all the above molecules. The soluble form is the small proportion that sheds in the serum [soluble intercellular adhesion molecule-1 (sICAM-1), soluble E-selectin (sE-selectin) and soluble L-selectin (sL-selectin)]. The soluble molecule levels correlate with acute phase reactant levels. It was suggested that increased circulating sICAM-1, sE-selectin and sL-selectin levels reflect increased expression of these molecules on the cell surface, as well as adhesiveness and signal transmission across cells in vivo, perhaps as a result of shedding of the parent molecule locally [20, 21].

The increased level of circulating soluble adhesion molecules is a key to understanding the prognosis and pathology of certain diseases [14, 15, 21].

Neopterin is an aromatic pteridine released primarily by human macrophages and monocytes on stimulation by T lymphocytes. Serum neopterin concentrations are increased in numerous infectious malignant and inflammatory diseases in childhood and correlate with disease activity in rheumatic diseases [22-24].

The purpose of our study was to determine the serum levels of neopterin, sICAM-1, sE-selectin and sL-selectin in children with stable asthma in order to investigate their possible role in the pathogenesis of chronic inflammation in asthma. We also compared the above parameters in atopic and non-atopic asthmatic children in order to detect differences between groups, possibly reflecting different mechanisms involved in chronic inflammation of the airways.

Material and methods

The study included three groups of children: A, B and C.

Group A consisted of 30 children with atopic asthma, 18 boys and 12 girls, aged 8-14 years (mean age ±SE 10.33±0.44).

Group B consisted of 30 children with non-atopic asthma, 18 boys and 12 girls, aged 8-14 years (mean age ±SE 9.2±0.52).

Group C (control group) consisted of 20 healthy children, 10 boys and 10 girls, aged 8-14 years old (mean age ±SE 10.66±0.65). In this control group atopy was excluded on the basis of personal and family history for atopy and of total IgE value as well as of specific IgE tests measured by RAST tests.

All asthmatic children (groups A and B) were regularly attending the asthma outpatient clinic, had been diagnosed with mild to moderate persistent asthma according to the *International Paediatric Asthma Consensus Group Reports* [25] and were studied during a stable phase of their disease. Stable asthma was defined on the basis of lack of clinical symptoms such as wheezing, tachypnoea and prolonged expiration time, and lack of frequent use of reliever medicines.

Children receiving oral steroids and those with recent infection and/or asthma exacerbation were excluded from the study.

The asthmatic children were divided into atopic and non-atopic as judged by the presence of a positive or negative skin prick test, or a positive specific IgE test to the house dust mites *Dermatophagoides pteronyssinus* (Dp) and *D. farinae* (Df), and inhalation and food allergens as measured by RAST tests. All healthy children lacked specific IgE for house dust mites *D. pteronyssinus* (Dp) and *D. farinae* (Df), and inhalation and food allergens as measured by RAST tests.

None of the children had clinical signs of infection.

Informed consent was obtained from the parents of the children before participation in the study.

Peripheral venous blood samples were collected from all children. We observed the usual precautions for venepuncture. We preserved the chemical integrity of the blood specimens from the moment they were collected until they were assayed. We did not use grossly haemolytic, icteric or grossly lipaemic specimens. We did not use contaminated specimens. We allowed samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000xg. We removed serum and

stored samples in aliquots at –80°C. We avoided repeated freeze-thaw cycles.

The serum levels of sICAM-1, sE-selectin and sL-selectin were determined by enzyme-linked immnosorbent assay (ELISA) using commercially available ELISA Kits (R&D Systems, Mineapolis, MN). The respective kits were: BBE1B for sICAM-1, BBE2B for sE-selectin, and BBE4B for sL-selectin. Neopterin levels were determined by ELISA and we used a kit by IBL Hamburg (RE59321). We followed the method and procedures for the soluble adhesion molecules and neopterin assays as described in the literature [26, 27].

Statistical analysis

All data are expressed as median values and interquartile range (IQR). Kruskal-Wallis and Anova were employed to compare median values of sICAM-1, sE-selectin, sL-selectin and neopterin of children of groups A, B and C. For all tests a p value below 0.05 was considered significant.

Results

Levels of serum sICAM-1, sE-selectin, sL-selectin and neopterin are displayed in Figures 1, 2, 3 and 4

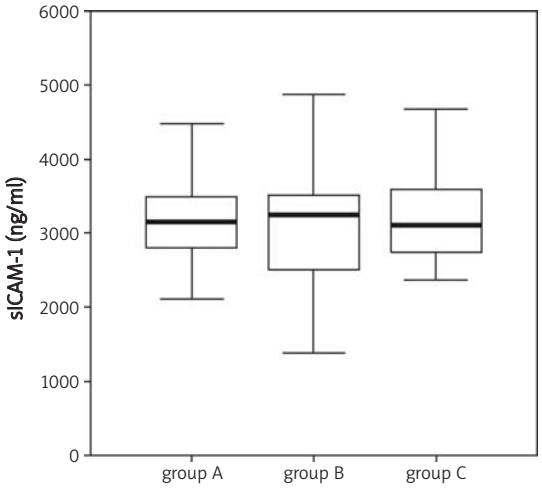

Figure 1. Serum sICAM-1 levels in groups A, B and C. The dark line within the box marks the median, the lower boundary of the box indicates the 25th percentile and the upper boundary of the box indicates the 75th percentile. Error bars above and below the box indicate the 90th and the 10th percentiles

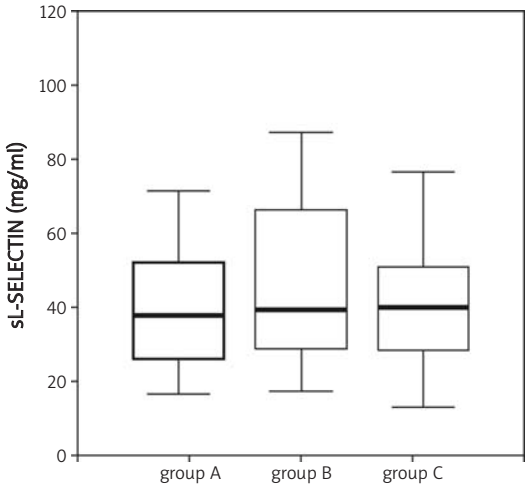

Figure 2. Serum sL-selectin levels in groups A, B and C. The dark line within the box marks the median, the lower boundary of the box indicates the 25th percentile and the upper boundary of the box indicates the 75th percentile. Error bars above and below the box indicate the 90th and the 10th percentiles

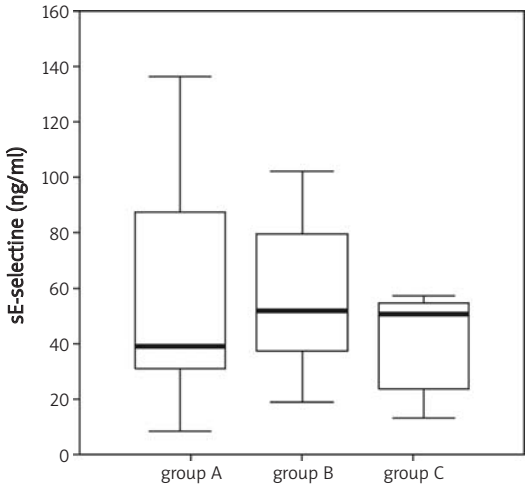

Figure 3. Serum sE-selectin levels in groups A, B and C. The dark line within the box marks the median, the lower boundary of the box indicates the 25th percentile and the upper boundary of the box indicates the 75th percentile. Error bars above and below the box indicate the 90th and the 10th percentiles

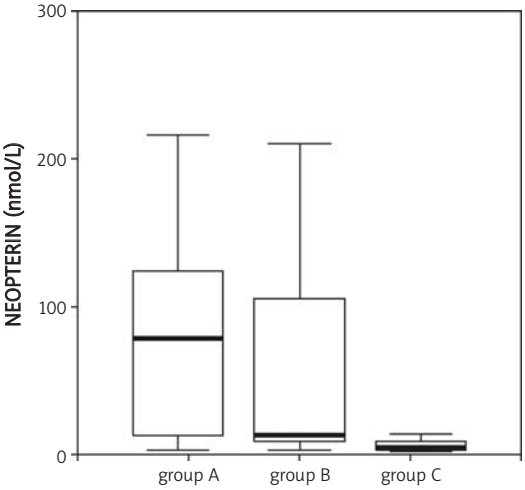

Figure 4. Serum neopterin levels in groups A, B and C. The dark line within the box marks the median, the lower boundary of the box indicates the 25th percentile and the upper boundary of the box indicates the 75th percentile. Error bars above and below the box indicate the 90th and the 10th percentiles

Table I. Levels (median and interquartile range, IQR) of serum sICAM-1, sE-selectin, sL-selectin and neopterin in groups A, B and C

	sICAM-1 (ng/mL) Median (IQR)	sL-selectin (ng/mL) Median (IQR)	sE-selectin (ng/mL) Median (IQR)	Neopterin (nmol/L) Median (IQR)
Group A	3143.5 (719.75)	37.79 (27.77)	39.46 (62.88)	78.91 (112.22)
Group B	3247 (1006)	39,28 (38.26)	51,77 (43.84)	13,53 (101.57)
Group C	3114.5 (891)	40.14 (23.635)	50.9 (31.43)	4.19 (6.42)
	p=0.18	p=0.13	p=0.14	**p<0.001**

and summarized in Table I as median values and interquartile range (IQR) for each patient group.

There were no differences observed in serum sICAM-1, sE-selectin or sL-selectin levels between groups A, B and C. Atopic asthmatic children (group A) had significantly higher levels of neopterin than the non-atopic asthmatics or healthy children ($p<0.001$).

Discussion

Asthma-associated airway inflammation ultimately results in the accumulation of inflammatory cells from circulation participating in upregulation of the endothelial adhesion molecules [6, 28].

Neopterin is an activation marker of macrophages and these cells become activated in bronchial asthma. As far as we are aware, though there are plenty of references in the literature investigating the role of neopterin as a marker for various inflammatory disorders in childhood, there are only a few investigating its relationship with asthmatic inflammation, and most of them have been performed in adults [29-31].

In our study, though all asthmatic children were in stable condition, levels of serum neopterin were found to be significantly higher in atopic children. The results of the study seem to indicate that in patients with mild to moderate stable bronchial asthma alveolar macrophages are activated especially in those with coexisting atopy. As neopterin is produced and secreted by activated macrophages, these data support a role for macrophages in the pathogenesis of asthma inflammation [31-33].

Neopterin is elevated in conditions associated with stimulation of cellular immunity and enhanced macrophage activity, for example in viral infections [23, 32]. Acute exacerbations of bronchial asthma are frequently precipitated by viral infections of the upper airways.

A recently published study investigated the levels of neopterin in both serum and bronchoalveolar lavage fluid (BAL) in stable asthmatics and reported higher levels of neopterin in BAL, although serum neopterin levels were normal [24].

Adhesion molecules may play a pivotal role in the inflammatory processes associated with asthma. 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. However, their roles in the development of airway inflammation and airway hyper-responsiveness are not clear [14, 15].

According to our results, there were no differences observed in serum sICAM-1, sE-selectin or sL-selectin levels between asthmatic and non-asthmatic children. Previous studies conducted in adult asthma patients have shown that sICAM-1 and sE-selectin levels increased in sera obtained during asthmatic exacerbation [21, 34, 35].

Studies performed in paediatric populations have also reported increased levels of sICAM-1, sVCAM-1 and sE-selectin in children with acute asthma [15, 21, 36, 37]. In our study, asthmatic children were enrolled in the study only if they were stable. Children receiving oral steroids were also excluded from our study, since they were categorized as suffering from acute asthma. We also found no differences in serum sICAM-1, sE-selectin or sL-selectin between atopic and non-atopic asthmatic patients. Laan et al. [34] studied paediatric patients and found no differences in sICAM-1 and sE-selectin levels among children with stable atopic asthma, stable non-atopic asthma, atopic dermatitis and healthy children.

Peribronchial inflammation contributes to the pathophysiology of allergic asthma. In many vascular beds, adhesive interactions between leukocytes and the endothelial surface initiate the recruitment of circulating cells.

L-selectin is an adhesion molecule involved in leucocyte attachment to the endothelium at sites of inflammation. Recently, it has been reported that when neutrophils from allergic patients were challenged with specific allergens that produce clinical allergy symptoms, L-selectin was down-regulated from the surface of those cells, accom-

panied by a concomitant up-regulation of soluble L-selectin in the supernatant [18]. Children in our study were studied during a stable phase of their asthma, possibly due to absence of exposure to specific allergens.

Visser et al. [31] reported lower concentrations of sICAM-1 in a group of asthmatic hyper-responsive children compared to non-hyper-responsive ones. Those children also had more atopic features (positive RAST, high IgE, eczema) and lower levels of FEV1.

A recent study by Janson et al. [16] compared levels of circulating adhesion molecules in patients with atopic and non-atopic asthma and related the levels of soluble adhesion molecules to methacholine responsiveness and lung function. They found that levels of all adhesion molecules in atopic asthmatics, but only sE-selectin in non-atopic asthmatics, were correlated with airway conductance. It was suggested that endothelial cells were being activated in asthma and that this had a bearing on airflow variability and bronchial responsiveness in non-atopic asthma.

Pulmonary function test criteria were not utilized in this study as not all of the children were able to cooperate. Thus, further studies will be necessary to see if there is a correlation between pulmonary function testing results and soluble adhesion molecule concentrations in serum of children with asthma.

Conclusions

Our data support the hypothesis that neopterin is produced and secreted by activated macrophages, and that these macrophages play a role in the inflammation of chronic asthma. This may allow a better understanding of the clinical implications and more insight into the inflammatory processes of bronchial asthma.

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