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Mannose-binding lectin levels in children with asthma

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Mannose-binding lectin (mbl), one of the important components of innate immunity, can activate the lectin pathway of the complement system. After binding mannose containing carbohydrate structures of foreign antigen, mbl initiates and regulates the inflammatory responses. Asthma is a complex inflammatory disease of the lung involving many components of the immune system. Our objective was to investigate the serum mbl levels of asthmatic children in comparison with healthy controls. Serum mbl levels were determined by nephelometric assay in 72 asthmatic children (5–15 yr old) and 30 healthy age-matched controls. Mbl levels of asthmatic children were measured both during acute attack and after complete remission. There was no significant difference between the mbl levels during acute attack (median 4.1 mg/l) or quiescence of symptoms (median 3.6 mg/l). Serum mbl levels both during acute attack or quiescence of symptoms was significantly higher in asthmatic children than in the healthy controls (median 2.8 mg/l, $p < 0.0001$ for each). Furthermore, mbl levels of asthmatic children positively correlated with peripheral blood eosinophils ($r = 0.377$, $p < 0.001$), which is a systemic component of airway inflammation in asthma. Our findings indicate that mbl may be implicated in the pathogenesis of asthma by contributing to airway inflammation or by increasing the risk of developing asthma.

Aysen Uguz[1], Zafer Berber[1], Mesut Coskun[1], S. Halide Akbas[2] and Olcay Yegin[1]

[1]Division of Immunology and Allergy, Department of Pediatrics, Faculty of Medicine, [2]Central Laboratory, Clinical Biochemistry Unit, Faculty of Medicine, Akdeniz University, Antalya, Turkey

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Aysen Uguz MD, Division of Immunology and Allergy, Department of Pediatrics, Faculty of Medicine, Akdeniz University, Antalya, Turkey
Tel.: +90 242 227 43 43/33 566
Fax: +90 242 227 44 90
E-mail: aguz@akdeniz.edu.tr

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Asthma is a complex inflammatory disease of the lung caused by inappropriate immunological responses to many environmental antigens. One of the main characteristics of asthma is the persistent inflammatory reaction of the airways (1, 2). Among many cell types, eosinophils play a central role in both allergic and non-allergic asthma. Increased numbers of eosinophils have been documented in the peripheral blood, bronchoalveolar lavage fluid, airway epithelium, and sputum of patients with asthma. However, inflammation in asthma appears to be far more complex than a simple eosinophilic inflammation alone. Many components of the immune system including T cells, B cells, basophils, mast cells, neutrophils, macrophages and eosinophils, as cellular contributors and IgE, some mediators and complement system as humoral contributors have a role in asthma pathogenesis by contributing to airway obstruction as well as regulating Th2 responses after triggered by some stimuli such as allergens (3–7). Mannose-binding lectin (mbl), a C-type lectin, is a member of the collectin family. After binding mannose containing carbohydrate structures of foreign antigen such as microorganisms, mbl can activate the lectin pathway of the complement system by using two serine proteases (8–10). Recently, Varga et al. (11) showed the role of mbl in allergen-induced activation of the classical and lectin pathways of complement. Although the insufficiency of mbl makes some individuals susceptible to recurrent infections or autoimmune diseases (8, 10), high mbl levels are associated with some biological disadvantages by increasing inflammatory damage (12, 13). Considering that asthma is a chronic inflammatory disease in genetically susceptible patients

and mostly associated with allergen exposure in childhood, mbl may be a contributing factor to asthmatic inflammation in children. The aim of this study was to investigate the mbl levels of asthmatic children both during acute attack and quiescence of symptoms in comparison with healthy controls and whether there is a correlation between mbl levels and other asthmatic markers.

Materials and methods

Patients and controls

This case–control study comprised 72 asthmatic children (aged between 5 and 15 yr) and 30 healthy age-matched children as a control group. All patients were followed up in the Pediatric Immunology and Allergy Division of Akdeniz University Hospital in Antalya. The patients were subclassified according to clinical data and peak expiratory flow rate and spirometric measurements (14). Patients with any concomitant chronic disease were excluded from the study.

Control children were enrolled from those attended to the general pediatric outpatient department for non-allergic and non-immunologic diseases. Control subjects were admitted to the hospital because of minor medical problems (eating problems, vaccination, growth assessment, etc.) and were enrolled after exclusion of the infections and any immunologic disease.

An informed consent was obtained from both children and parents before enrollment. The Akdeniz University Ethic Committee approved the study.

Methods

A detailed history was obtained from the parents for the demographic information, drug therapy and severity of the disease. The details on emergency attendance, hospital admissions, outpatient visits, and school attendance were also recorded. Following a general physical examination and lung function measurements, venous blood samples were taken and collected under standardized conditions to minimize sources of pre-analytic variation. For measurement of serum mbl level and total and specific IgE, blood samples were centrifuged at 3000 ***g*** for 10 min. Sera were separated and stored at −70°C until analysis. At the end of the study, all frozen samples were thawed and analyzed in a single run in duplicate. Serum mbl concentrations were determined by a nephelometric immunoassay (N Latex MbP; Dade Behring Diagnostic, Marburg, Germany) on a Dade Behring Nephelometer II (BN II; Dade Behring Diagnostic) according to the recommendations of the manufacturer. Sensitivity of the test is 0.09 mg/l. It was performed twice for each asthmatic child (during the attack and 2 wk after complete remission) Serum mbl was measured once in the control group at enrollment. Control groups and asthmatic children who were in quiescence period required to be free from infection were verified by detailed history, physical examination and complete blood count for 2 wk prior to obtaining blood samples.

Serum total and specific IgE measurements were performed by commercially available fluoroenzymeimmunoassay kits (UniCAP Total IgE FEIA, UniCAP Specific IgE FEIA; Pharmacia Diagnostics AB, Upsala, Sweden) on a UniCAP 100 instrument. Complete blood and absolute eosinophil counts were performed on an automated hematology analyzer (Gen-S System; Coulter Corporation, Miami, FL, USA).

All patients and controls had skin tests (Allergopharma, Reinbek, Hamburg, Germany) using the percutaneous method for airborne allergens including house dust mites, molds, animal danders, feathers, cockroaches, and seasonal pollens (trees, grasses, and weeds). A mean wheal diameter of more than 3 mm was considered positive. Atopy was defined as an increased specific IgE or a positive skin prick test result for the allergens tested.

Statistical analysis

Statistical analysis was performed in SPSS 11.0. Differences between patients and controls were analyzed using Mann–Whitney *U*-test. For paired comparison, Wilcoxon signed ranks test was used. Pearson *r* was used to relate the numeric variables. A p-value < 0.05 was considered statistically significant.

Results

The characteristics of the patients and controls are presented in Table 1. Seventy-two asthmatic children and 30 healthy controls were included in the study. Their respective mean ages were 9.3 ± 2.7 and 8.5 ± 3.0 yr ($p > 0.05$). Male and female distribution was similar in the patient and control group. All asthmatic patients and three controls were atopic. None of the control subjects had any allergic diseases. The number of asthmatic subjects with coexisting allergic rhinitis and coexisting eczema were 44 (65.7%)

Table 1. Characteristics of the patients and controls

	Patients (n = 72)	Controls (n = 30)
Age, yr (mean ± s.d.)	9.3 ± 2.7	8.5 ± 3.0
Gender, M/F	43/29	17/13
Atopic subjects, n (%)	72 (100)	3 (10)
Reactivity to aeroallergens, n		
House dust mite	49	2
Grasses	17	1
Weeds	13	0
Trees	3	0
Molds	7	0
Cockroaches	2	0
Dog dander	1	0
Cat dander	1	0
Feathers	1	0
Asthmatic subjects, n (%)	72 (100)	0 (0)
Concomitant allergic rhinitis, n (%)	44 (65.7)	0 (0)
Concomitant eczema, n (%)	5 (7.5)	0 (0)

Table 2. Comparison of the laboratory features of patients and controls

	Patients (n = 72)	Controls (n = 30)	p
Mbl levels during quiescence (mg/l)	3.6 (1.9–7.8)	2.8 (0.3–4.6)	<0.0001
Mbl levels during acute attack (mg/l)	4.1 (2.1–8.5)	2.8 (0.3–4.6)	<0.0001
IgE levels (kIU/l)	421 (46–2264)	26 (4–94)	<0.0001
Absolute eosinophil count (cells/μl)	530 (50–2720)	109 (12–321)	<0.0001
Total leukocyte count (cells/μl)	7475 (4980–13100)	7900 (5050–12500)	>0.05

Data are expressed as median (range). Absolute eosinophil and total leukocyte counts of asthmatic patients are from children in quiescence period.

and 5 (7.5%) respectively. Mean FEV_1 value of asthmatic children was 88.2 ± 13.0% for quiescence period and 60.1 ± 9.0% for acute attack (compared with predicted). Asthmatic patients were classified as: 23, intermittent; 44, mild persistent; and 5, moderate persistent. None of the asthmatic patients had severe asthma.

Serum mbl levels were significantly higher in the asthmatic children than in the healthy controls ($p < 0.0001$). The median serum mbl concentration during acute asthma (4.1 mg/l) and that of the same children after remission of the attack (3.6 mg/l) was statistically higher than the control value, being 2.8 mg/l (Table 2). Furthermore, serum mbl levels during attacks and after quiescence were not different and showed significant positive correlation. Comparison of the laboratory features of the patients and controls is presented in Table 2. Serum total IgE levels and absolute eosinophil count were significantly higher in the asthmatic than in the control subjects ($p < 0.0001$). There was no significant difference in the blood total leukocyte counts between the two groups ($p > 0.05$). Serum mbl concentration of the asthmatic children was not associated with some variables such as gender, FEV_1 values, family history of atopy, concomitant allergic rhinitis or eczema (data not shown). Serum mbl concentrations were not different between genders in the control group. The failure to demonstrate a relation between disease severity and serum mbl levels could be attributable to the factor that most of our patients had mild asthma.

Serum mbl levels of the asthmatic children did not correlate with serum total IgE levels ($p > 0.05$). However, absolute eosinophil counts of the asthmatic children also showed statistically significant correlation with serum mbl levels measured during quiescence ($r = 0{,}377$, $p < 0.001$).

Discussion

In this study, serum mbl levels were significantly higher in asthmatic children compared with healthy controls. Mbl levels of asthmatic children were not different between acute attack and after quiescence of symptoms. Furthermore, a significantly positive correlation between serum mbl levels and peripheral blood eosinophils was found. These data could support the hypothesis that the mbl may have a role in the pathogenesis of allergic asthma.

Asthma is a chronic inflammatory disease of the airways associated with atopy and acquired immunity (15). Previous studies showed that in addition to acquired immune responses, the innate immune system such as the complement system is involved in the pathogenesis of asthma (16–20).

Mannose-binding lectin is a component of innate immunity. After binding carbohydrate structures of foreign antigen mbl can activate the complement system. Recently Currie et al. (21) suggested a role for both C-type lectins and β2 integrins in the binding and phagocytosis of carbohydrate-containing allergenic particulates by alveolar macrophages. They hypothesized that this interaction between alveolar macrophages and allergenic particulates likely involved the carbohydrate-binding receptors. These receptors have specificities for terminal sugar groups rarely found in mammalian systems but which are common constituents of pollen, bacteria, and fungi. In another study, involvement of the lectin pathway in allergen-induced activation of complement was demonstrated. The findings of this study indicated that allergen extracts can bind purified C1q and mbl, but they require IgG for efficient complement activation. Depending on

the allergens, this activation may be initiated through C1, mbl or both (11). Considering the carbohydrate content of some allergens, such as pollens and house dust (22), they could be recognized by the mbl.

Most studies have focused on the consequences of mbl deficiency rather than on the effects of increased mbl levels (23, 24). However, high levels of mbl are harmful in some settings such as increasing inflammation or vulnerability to intracellular infections such as tuberculosis (12, 13, 25) In line with this, recent studies indicate that mbl may initiate complement activation following oxidative stress and therefore potentially aggravate the resulting inflammation (26, 27). In normal condition mbl does not interact with the body's own tissues, but cellular hypoxia was recently shown to alter cell surface glycosilation, leading to increased mbl deposition (26). It is well known that increased oxidative stress is implicated in the pathogenesis of asthma (28–30). Therefore, increased oxidative stress in asthmatic airways may stimulate mbl synthesis and resulting complement activation. This activation may contribute to airway inflammation. Because most of our patients are mild asthmatics, we could not find any correlation between mbl levels and disease severity.

Different methods such as nephelometric method, ELISA, and genotyping have been suggested to give different results for evaluation of the patients' mbl status (31, 32). In our study, we found significantly increased mbl levels in asthmatics in comparison with controls using nephelometric method under the same assay conditions.

There are some studies that have investigated mbl levels in asthmatic children. Nagy et al. (33) proposed the association between variant mbl alleles and recurrent or chronic *Chlamydia pneumoniae* infection in asthmatic children. More recently, Cedzynski et al. (34) investigated the mbl levels in children with recurrent respiratory tract infection and found an association between low mbl levels and the occurrence of recurrent respiratory tract infection. In their patient group, a subgroup of the children had allergies (allergic rhinitis, atopic dermatitis, and asthma) or high IgE levels and had lower mbl levels than controls. In both studies, mbl levels were investigated in a selected group of asthmatics. Therefore, it is difficult to generalize this for all asthmatic children.

Asthmatic children in our study group was different from those used in other studies because none of the children had extraordinary or recurrent infection. Therefore, significantly increased mbl levels in asthmatics and correlation of mbl with eosinophils are the important findings and require further investigations such as possible relation between mbl and other markers of allergic inflammation like ECP and EPX.

Mannose-binding lectin is a weak acute phase reactant. Blood mbl levels are influenced by responses to the environment such as infection and surgery causing an increase in the acute phase response of up to threefold (8). However, it seems unlikely that our findings of increased mbl levels in asthmatics are due to non-specific stimulation of hepatic protein synthesis. First, there is no difference between mbl levels in acute attack and after quiescence of symptoms and both values were higher than controls. Secondly, leukocyte numbers of the asthmatics during quiescence were not significantly different from healthy controls. Thirdly, mbl levels of asthmatics significantly correlated with blood eosinophils, which are systemic components of airway inflammation in asthma.

In conclusion, our findings on increased mbl levels in asthmatics suggest that being a component of innate immunity, mbl may have a role in the pathophysiology of asthma by contributing to airway inflammation or increasing the risk of developing asthma. The mbl utility in relation to other parameters of asthmatic inflammation should be investigated.

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