

Original article

T cell and eosinophil activation in mild and moderate atopic and nonatopic children's asthma in remission

Background: Inflammation in the pathogenesis of asthma is associated with products of activated T cells and eosinophils. The aim of this study was to determine whether ongoing inflammation persists in children with different phenotypes of asthma despite the disease in remission.

Methods: Serum samples were collected from 68 children with atopic or nonatopic asthma in remission and from 15 healthy children. Soluble interleukin-2 receptor (sIL-2R), IL-2 and IL-4 were examined by using an enzyme-linked immunosorbent assay. Total and specific immunoglobulin E, and eosinophil cationic protein (ECP) were analysed by fluoroimmunoassay (Pharmacia CAP System).

Results: In patients with moderate persistent atopic asthma, sIL-2R was increased significantly when compared with mild persistent atopic asthma ($P < 0.05$). No changes of sIL-2R were seen in nonatopic asthmatics compared with atopics and controls. The level of IL-2 was elevated in moderate persistent atopic and nonatopic asthmatic children compared with controls ($P < 0.05$ and $P < 0.05$ respectively) and compared with mild persistent atopic asthmatics and mild persistent nonatopic asthmatics ($P < 0.05$ in both cases). The levels of IL-4 in most patients and controls remained below the sensitivity of the assay. Eosinophil cationic protein levels in moderate persistent atopic and nonatopic asthmatics were significantly higher than in mild persistent asthma severity cases ($P < 0.001$ and $P < 0.01$ respectively) and in healthy children ($P < 0.01$ in both cases).

Conclusion: Changes in the concentration of sIL-2R, IL-2 and ECP reflect increased T cell and eosinophil activity in relation to the level of severity of asthma in atopic and nonatopic children, thereby proving the presence of persistent inflammation despite the absence of disease symptoms.

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Persistent inflammation of the respiratory mucosa, characterized by an eosinophilic infiltrate, and also involving other cell types (lymphocytes, mast cells, basophils and neutrophils) is thought to be important in the pathogenesis of asthma and is associated with airway hyper-responsiveness, a hallmark of the disease (1, 2). The initiation and regulation of pulmonary inflammatory responses are absolutely dependent on T-lymphocyte activities. For the most part, they orchestrate these responses through the synthesis and release of cytokines (3, 4). The severity of asthma has been correlated with many inflammatory indices including eosinophil number and activation, T-cell activation and cytokine expression (1, 5). Ongoing airway inflammation has been found in asthmatic children despite regular treatment (6). The inflammatory process is thought to be present even in symptom-free subjects during asthma remission (7, 8). However, studies regarding the presence of ongoing

airway inflammation during remission of asthma in children are lacking.

Most patients with asthma have atopy, determined by skin test positivity to common aeroallergens and elevated levels of serum immunoglobulin E (IgE). It is well established that CD4⁺ Th2 lymphocytes and Th2-associated cytokines [interleukin-4 (IL-4), IL-5, IL-13] play a crucial role in orchestrating the chronic inflammatory response in atopic asthma (AA) (1, 3, 9). Although the majority of asthmatics seem to be atopic, there remains about one-third of asthmatics, called nonatopic, in whom no sensitization to common aeroallergens and negative serum levels of allergen-specific IgE can be identified and they have normal or only slightly elevated serum IgE levels (10, 11). There is some evidence that nonatopic asthma runs in families and the increase in the prevalence of asthma is particularly because of an increase in nonatopic asthma (12, 13). So it is very important to

obtain valuable information on the natural course of different types of asthma and insight into optimal (and different?) therapeutic approaches to them. The immunological mechanisms occurring in patients with nonatopic asthma are less well characterized, particularly in children. There is conflicting evidence as to whether atopic and nonatopic asthma are similarly mediated (14, 15). Walker et al. (16) reported that IL-2 and IL-5, but not IL-4, are increased in bronchoalveolar lavage fluid and supernatants from purified peripheral blood T cells. They also stressed that nonatopic asthmatics have distinct patterns of T-cell activation and cytokine production, whereas Humbert et al. (17) and Bettiol et al. (18) tend to support the view that both types of asthma share a similar immunological disorder. A common feature of atopic and nonatopic asthma is eosinophilic inflammation of the airways (9, 17, 19). Serum levels of eosinophil cationic protein (ECP), a granular protein derived from activated eosinophils, are reflection of the degree of activation of the circulating pool of eosinophils and indirectly assume the intensity of ongoing eosinophilic inflammation in the airways (20).

To our knowledge, there are no studies that have examined the activation status of T cells and eosinophils in atopic and nonatopic asthma in children during remission and in relation to the level of severity of the disease. It is very important to measure peripheral blood markers (minimally invasive method) to assess airway inflammation in children with asthma. The aim of this study was to determine whether ongoing inflammation persists in children with different phenotypes of asthma despite the disease in remission. We report the results of analyses of IL-2 (Th1 cytokine), IL-4 (Th2 cytokine), soluble marker of T-cell activation *in vivo* – soluble IL-2 receptor (sIL-2R) and ECP level in the serum of mild persistent and moderate persistent asthmatics in remission.

Materials and methods

Patients

The study group comprised 48 children with AA, 20 children with nonatopic non-IgE-mediated asthma (NAA) and 15 normal control (C) subjects. The demographic characteristics of the patients are summarized in Table 1. The recruitment of asthmatics was in accordance with the Global Initiative for Asthma (GINA) (21) and a revised nomenclature for allergy (22). The atopic group comprised patients with a history of mild persistent atopic asthma (mlAA) and moderate persistent atopic asthma (moAA). The nonatopic group also comprised patients with mild persistent nonatopic asthma (mlNAA) and moderate persistent nonatopic asthma (moNAA). Atopic asthma was defined as a positive family and personal history of atopy, a positive skin prick test response to one or more of 25 common aeroallergens (including house dust mites; cat and dog dander; grass, tree and weed pollens; and moulds), at least one positive (≥ 0.35 kU/l) allergen-specific IgE and total serum IgE concentration > 150 IU/ml. Nonatopic non-IgE-mediated asthma subjects had negative skin test responses to common aeroallergens,

Table 1. Demographic characteristics of the study population

	Atopic asthmatic children	Nonatopic asthmatic children	Healthy controls
Number	48	20	15
Age in years (mean $\pm$ SEM)	9.2 $\pm$ 0.5	7.1 $\pm$ 0.9	9.9 $\pm$ 0.4
Gender (male/female)	22/26	10/10	7/8
Asthma severity (number of patients)			
Mild persistent	21	8	–
Moderate persistent	27	12	–

negative-specific IgE and serum IgE concentration within the normal range (0–150 IU/ml). All patients were recruited from the National Rehabilitation Hospital (sanatorium). They were referred from different outpatient clinics where they had been receiving the regular daily controller therapy (inhaled glucocorticoids or cromones, or leukotriene modifiers) in doses recommended for the level of severity by GINA. For our study, we selected only patients with remission of asthma who had not taken oral (2 months) or inhaled (2 weeks) glucocorticoids, leukotriene modifiers (2 months), cromones (2 weeks) and antibiotics (2 weeks) before the study. In our study, clinical remission of asthma was defined as the absence of asthmatic symptoms in subjects and near-normal peak expiratory flow rate and forced expiratory volume in 1 s ($\geq 80\%$ predicted), which were achieved prior to the study. A gradual reduction in maintenance therapy was achieved and the minimum therapy required to maintain control was temporarily interrupted prior to our study. A clinical history of at least 1 year prior to the study formed the basis for the documentation of the duration of asthma in patients. Patients were excluded from exposure to their usual home environmental trigger stimuli. Exclusion criteria included evidence of upper and lower respiratory tract infection within the previous 6 weeks, and other chronic diseases. The C group was comprised of healthy children with no family and personal history of asthma or other allergic diseases and negative skin prick test results to common aeroallergens.

The study was approved by the Lithuanian Ethics Committee and written informed consent was obtained from the parents of all participating children.

Serum samples

Blood samples were drawn in serum tubes, clotted at room temperature for 60 min and centrifuged for 10 min at 1300 g. Serum samples were immediately frozen at -70°C until assay.

ELISA for IL-2, IL-4 and sIL-2R

Interleukin (IL)-2, IL-4 and sIL-2R were measured in serum by using an enzyme-linked immunosorbent assay (ELISA). The kits were obtained from Immunotech (Marseille, France) and were used according to the manufacturer's instructions. The detection limits were 5–1000 pg/ml of IL-2, 5–1000 pg/ml of IL-4 and 10–640 IU/ml of sIL-2R. The absorbance was measured with ELISA autoreader (Multiscan MS; Labsystem, Helsinki, Finland).

Measurement of IgE and ECP concentration

Total and specific IgE and ECP concentration tests were performed according to the instructions of the manufacturer (Pharmacia CAP system fluoroimmunoassay; Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden).

Statistical analysis

Values are presented as the mean $\pm$ SEM. Means were compared with two-tailed Student's *t*-test. Spearman's rank correlation test was used for the analysis of correlation between serum IL-2 level and ECP concentration in serum. $P < 0.05$ was considered statistically significant.

Results

Serum level of sIL-2R

Figure 1 shows that in patients with moAA, the level of sIL-2R in serum is increased significantly (128.93 ± 26.98 IU/ml) when compared with C children (63.04 ± 10.05 IU/ml, $P < 0.05$) and children with mlAA (60.62 ± 16.42 IU/ml, $P < 0.05$) and moNAA (56.58 ± 6.75 IU/ml, $P < 0.05$). There were no statistically significant differences between the serum level of sIL-2R results for mlNAA (40.37 ± 5.25 IU/ml), moNAA and C children (Fig. 1).

Serum level of IL-2 and IL-4

There were no statistically significant differences between IL-2 levels in serum of mlAA (89.81 ± 32.51 pg/ml), mlNAA (36.13 ± 12.00 pg/ml) and C subjects (57.89 ± 18.49 pg/ml) (Fig. 2). Serum levels of IL-2 were significantly elevated in both moAA and moNAA when compared with normal children (229.58 ± 58.55 pg/ml, $P < 0.01$; 214.25 ± 65.99 pg/ml, $P < 0.05$ respectively) and with mlAA and mlNAA ($P < 0.05$) in both cases.

We did not obtain statistically significant differences between IL-4 level in serum of patients with mlAA, mlNAA, moAA and moNAA in remission compared

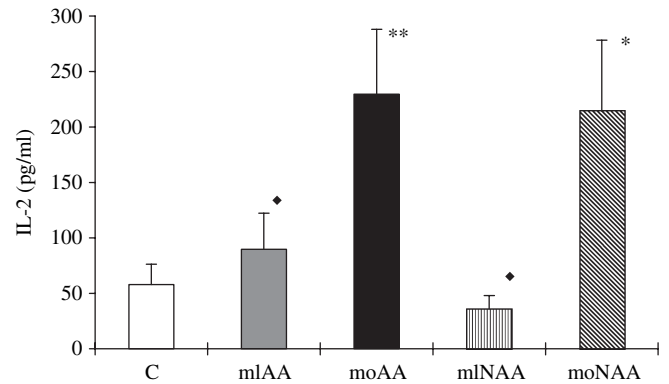


Figure 2. Serum interleukin-2 (IL-2) (pg/ml) in controls (C), mild atopic asthmatics (mlAA) and moderate atopic asthmatics (moAA), mild nonatopic asthmatics (mlNAA) and moderate nonatopic asthmatics (moNAA) during remission. Increase is significant in moAA patients vs C (** $P < 0.01$) and vs mlAA patients (* $P < 0.05$); in moNAA patients vs C (* $P < 0.05$) and vs mlNAA (* $P < 0.05$).

with C, because IL-4 values were low and near to the detection limits.

ECP concentration in serum

The values of serum ECP detected in patient's groups and C group are presented in Fig. 3. There were no statistically significant differences between ECP levels in serum of mlAA (6.78 ± 0.50 μ g/l), mlNAA (6.57 ± 1.17 μ g/l) and C subjects (6.34 ± 1.49 μ g/l). However, serum ECP levels in moAA (14.57 ± 1.81 μ g/l) were significantly higher than in mlAA ($P < 0.001$) and in C ($P < 0.01$). Eosinophil cationic protein concentration in the moNAA

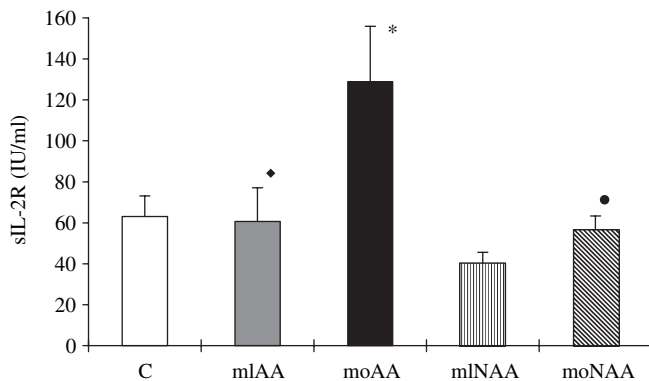


Figure 1. Serum soluble interleukin-2 receptor (sIL-2R) (IU/ml) in controls (C), mild atopic asthmatics (mlAA) and moderate atopic asthmatics (moAA), mild nonatopic asthmatics (mlNAA) and moderate nonatopic asthmatics (moNAA) during remission. Increase is significant in moAA patients vs C (* $P < 0.05$), vs mlAA patients (* $P < 0.05$) and vs moNAA patients (* $P < 0.05$).

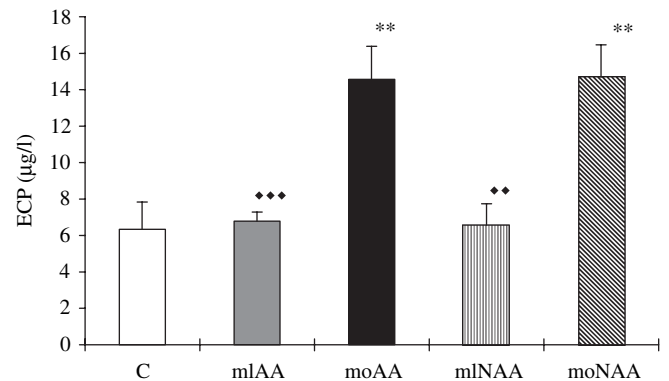


Figure 3. Eosinophil cationic protein (ECP) concentration (μ g/l) in serum from controls (C), mild atopic asthmatics (mlAA) and moderate atopic asthmatics (moAA), mild nonatopic asthmatics (mlNAA) and moderate nonatopic asthmatics (moNAA) during remission. Increase is significant in moAA and moNAA vs C (** $P < 0.01$); in mlAA vs moAA (*** $P < 0.001$) and mlNAA vs moNAA (** $P < 0.01$).

group was $14.68 \pm 1.78 \mu\text{g/l}$, which was significantly higher compared with the mNAA ($P < 0.01$) and C groups ($P < 0.01$).

Association of IL-2 levels with serum ECP

There was no significant correlation between serum IL-2 and ECP or eosinophil number in all asthmatic children.

Discussion

In this study, we examined T-cell activation *in vivo* by measuring sIL-2R concentration in serum of AA and NAA subjects and compared these findings with those of C. Although all asthmatic subjects were in remission, we observed, that in patients with moAA, that the level of sIL-2R was increased significantly compared with mNAA, moNAA and C children. However, we did not detect any differences in sIL-2R concentration in serum between mNAA and moNAA or in comparison with C subjects. These results suggest that T cells in moAA patients represent an acute activation pattern even in remission, most likely because of previous allergen exposure. Elevated plasma sIL-2R levels in asthmatics have with asthma attacks been reported (23, 24). Whether the serum sIL-2R level can reflect the severity of asthma is a subject of considerable debate, and for pediatric asthma, there is a scarcity of data. The reports by Kim et al. (25) and Tang and Chen (26) suggest that the serum level of sIL-2R in asthmatic children correlated positively with the severity of exacerbation, significantly higher than at clinical remission and could be a potential index of asthma severity. Our data are in agreement with Sahid El-Radhi et al. (27) who stressed that the serum concentration of sIL-2R in asthmatic children remained significantly higher than in controls despite apparent clinical remission. The results suggest that in cases of more severe asthma T-cell activation could be increased, as characterized by the presence of persistent inflammation despite clinical remission. The fact remains that insufficient medication in cases of more severe AA fails to suppress the elevation of sIL-2R levels.

Interleukin-2 concentration was increased in moAA and moNAA patients. Interleukin-2 is mainly produced by T helper cells of the Th1-phenotype. It is considered to be the autocrine growth factor for Th1 and promotes Th2 clone proliferation (28). Increased IL-2 concentration indicates Th1 cell activity. Commonly, immunopathogenesis of AA and other atopic diseases is related to Th2 cells and their cytokines. Although some authors report that in the chronic course of atopic diseases (29, 30) and in children with difficult asthma (31), the response of Th cells is far more complicated, involving the interplay of both Th1 and Th2 activities. In different experimental systems, Th1 cells provide signals (i.e. in addition to allergens) necessary for Th2 cells to be effectively recruited

to the airways (30). Walker et al. (16) observed that nonallergic asthmatics produce increased levels of IL-2. In agreement with our results, these observations suggest that the pathogenesis of asthma may include activation of Th1 cellular pathways. In our study, the serum levels of IL-4 did not differ among asthmatic patients of different clinical categories or when compared with healthy children and were close to the sensitivity of the assay. These results may be due to the physiologically small concentration of IL-4 in serum.

In our study, we also examined eosinophil activation status during AA and NAA remission by measuring ECP levels in serum of asthmatic and healthy children. Our data demonstrate that the serum levels of ECP in patients with AA and NAA were significantly higher compared with C children. In agreement with Ludviksdottir et al. (32) and Nja et al. (33), data of ECP concentration in serum of AA patients were not statistically different from those of NAA subjects. Also, we evaluated the relationship between ECP level and disease severity. Several studies have shown that ECP is higher in symptomatic than in asymptomatic asthmatics or healthy controls, and is related to the activity and severity of asthma (34, 35). However, some reports have failed to reveal such relationships (36, 37). We found a significant increase of ECP in serum of moAA and moNAA patients compared with C children and mild forms of asthma. Although moAA and moNAA patients showed low ECP levels ($< 16 \mu\text{g/l}$), suggesting disease remission, ECP values of these asthmatics were significantly higher than those of healthy children. We interpret this elevation of serum ECP as representing the presence of persistent eosinophilic inflammation in asthma even during remission.

Eosinophilic inflammation is a feature of asthma; however, the mechanisms of eosinophil activation, proliferation and migration are not fully understood and are regulated by cytokines released by different cell types. Rand et al. (38) reported that IL-2 was a potent chemoattractant for eosinophils. Other investigators showed that IL-2 acted by stimulating T cells to produce increased amounts of IL-5 (39). Lampinen et al. (40) concluded that the direct effect of IL-2 on eosinophil migration is inhibitory, but did not exclude that IL-2 may contribute to eosinophilia by stimulating the production of IL-5 by T cells. We analysed the relationship between serum IL-2 level and serum ECP or eosinophil number in blood in AA and NAA. However, no correlation was found between serum IL-2 and ECP or eosinophil number values in our asthmatic subjects (data not shown). Whether the principal effect of IL-2 on the function of eosinophils is direct, or indirect through other cells and mediators, needs to be established.

In conclusion, our data suggest that eosinophilic and T-cell mediated airway inflammation may persist in childhood atopic and nonatopic asthma despite apparent

clinical remission. In agreement with other investigators, the results of this study indicate that the therapeutic strategy for asthma should be targeted at inflammatory phenomena rather than at symptoms alone.

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