Effects of inhaled steroid treatment on serum eosinophilic cationic protein (ECP) and low affinity receptor for IgE (FcεRII/sCD23) in childhood bronchial asthma

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Abstract

Serum eosinophilic cationic protein (ECP) and soluble low affinity receptor for IgE (FcεRII/sCD23) concentrations were measured in relation to symptom-medication scores, pulmonary function, and total IgE levels in 27 chronic allergic asthmatic children (17 boys, 10 girls), mean age 10.8 years, before and at the end of a three month inhaled corticosteroid (budesonide) treatment period. Serum ECP and sCD23 concentrations were also measured in age matched non-asthmatic controls with allergic rhinitis. All asthma patients had significantly higher serum ECP and sCD23 than the controls, whereas the mean serum IgE was not different. No correlation between total IgE concentrations and serum sCD23 could be detected in either group. At the end of the treatment period, symptom-medication scores and pulmonary function improved. Serum ECP and sCD23 concentrations were reduced; however, total IgE values did not change significantly. A significant relation was found between the improvement of symptom-medication scores and fall in both sCD23 and ECP concentrations. Although there was a significant correlation of pulmonary function values with serum ECP, no such relation was observed for sCD23. It appears that serum sCD23 and ECP concentrations could be good disease markers, particularly in asthma. Monitoring of serum inflammation markers, especially ECP, may be useful in the follow up of asthmatic children on anti-inflammatory treatment.

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Keywords: inhaled corticosteroid, soluble low affinity receptor for IgE (FcεRII/sCD23), eosinophilic cationic protein (ECP).

In bronchial asthma, bronchial tissue is the site of acute and chronic inflammatory cell infiltration. It is important to evaluate and grade asthma inflammation because this is a major risk factor for short and long term outcome.¹ Since bronchial biopsy and bronchoalveolar

lavage are not practical procedures for investigating bronchial inflammation, indirect inflammation markers in the sputum or peripheral blood samples are used more frequently in the monitoring of asthma. Of these, eosinophil cationic protein (ECP) is a specific indicator of eosinophilic activation,² and soluble low affinity Fc receptor for IgE (FcεRII/sCD23), which can be detected in the serum, is important in the regulation of IgE synthesis and allergic inflammatory reactions by affecting the activation, proliferation, and differentiation of B lymphocytes.³

To evaluate the effect of asthma treatment on serum ECP and sCD23 concentrations, we obtained measurements before and at the end of a three month inhaled corticosteroid (budesonide) treatment period in children with chronic allergic bronchial asthma. The results were compared with symptom-medication scores, pulmonary function tests, and total IgE level obtained before and after termination of treatment. Serum ECP and sCD23 concentrations were also measured in age matched non-asthmatic controls with allergic rhinitis.

Methods

Twenty seven children with moderate chronic perennial allergic asthma⁴ who had not received previous inhaled corticosteroid treatment or immunotherapy were studied. They were referred from other hospitals or general practitioners to the outpatient clinic of the department of allergy. Sodium cromoglycate and ketotifen treatment were stopped two weeks prior to the entry. Children with strictly seasonal allergic asthma were excluded from the study. Before entering the study, the patients discontinued their usual maintenance treatment for at least two weeks (run-in period). After the run-in period, the children started treatment with inhaled budesonide (Pulmicort inhaler, Astra) 600 µg twice daily for the first month and 400 µg twice daily for the next two months with a valved spacer device (Nebuhaler). In all patients inhaled β₂ agonists were used as necessary to control the respiratory symptoms. Fifteen healthy children with perennial allergic rhinitis, but no previous history suggestive of asthma, served as controls. During the two weeks of run-in period

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Table 1 Effect of budesonide on asthma symptom-medication scores, pulmonary function, serum IgE, and sCD23 and ECP concentrations in chronic allergic asthmatic children. Data are mean (SD) (range)

	Before treatment	After treatment	Mean changes (SD)*	p	Control (allergic rhinitis)	p†
Asthma symptom score (max. 6)	4.26 (1.44) (1.24-6.0)	1.58 (0.37) (0.04-2.90)	-2.45 (0.12)	< 0.01	0	
Relief medication (number of puffs/day)	3.48 (1.27) (1.86-5.34)	0.24 (0.6) (0.02-1.12)	-3.19 (0.17)	< 0.001	0	
PEF (am) (l/min)‡	275 (67) (120-430)	302 (78) (144-460)	24 (11)	< 0.01	318 (44) (158-465)	< 0.01
PEF (pm)	292 (60) (140-458)	310 (66) (158-470)	21 (10)	< 0.01	322 (40) (164-477)	< 0.01
FEV ₁ (l)§	1.98 (0.62) (1.35-2.88)	2.12 (0.54) (1.44-3.08)	0.17 (0.23)	< 0.05	2.30 (0.39) (1.80-3.25)	< 0.05
FEF ₂₅₋₇₅ (l/min)¶	1.80 (0.72) (1.26-2.70)	1.96 (0.84) (1.38-3.0)	0.18 (0.28)	< 0.05	2.21 (0.36) (1.40-3.48)	< 0.01
Total IgE (IU/ml)	48.2 (11) (6.5-90.0)	36.5 (18) (2.0-82.5)	-9.4 (0.6)	> 0.05	52.4 (18) (12-100)	> 0.05
sCD23 (U/ml)**	84.26 (31.7) (48-156)	66.72 (28.4) (34-130)	-15.0 (8.7)	< 0.05	42.5 (12.3) (17-90)	< 0.02
ECP (µg/l)††	29.6 (2.4) (15.2-42.7)	13.8 (2.9) (8.3-19.0)	-16.5 (1.4)	< 0.01	9.5 (3.2) (6.0-18.4)	< 0.001

Data presented as mean (SD), (range).

* Mean compared from baseline at end point for efficacy variables.

† Compared with controls cases before therapy.

‡ Peak expiratory flow rate.

§ Forced expiratory volume in one second.

¶ Forced expiratory flow mid-expiratory phase.

** Soluble low affinity receptor for IgE.

†† Eosinophilic cationic protein.

and three months of follow up, the day and night symptoms of the patients were determined separately with a scoring method (0, no symptoms; 1, mild; 2, moderate; 3, severe; total daily score = 6). Peak expiratory flows (PEF) were measured two times daily at home with a peak flow metre (Asses peak flowmeter, Health Scan Products Inc, Cedar Grove, NJ, USA). Forced expiratory volume in one second (FEV₁) and forced expiratory flow, mid-expiratory phase (FEF₂₅₋₇₅) were measured by means of flow-volume curves with an autospiro device (Minato, AS-600, Osaka, Japan) before and after the inhaled corticosteroid treatment by the same blinded technician. Patients were seen monthly to monitor their progress. Patients were diagnosed as having asthma according to criteria of the American Thoracic Society.⁵ Allergic rhinitis patients were defined as those having rhinitis symptoms but showing no evidence of bronchial hyper-responsiveness. Patients were tested by the skin prick test with the 25 most common allergens (ALK, Copenhagen, Denmark). Atopy was defined as a radioallergosorbent test score (RAST) (Pharmacia Ltd, Uppsala, Sweden) more than 2 and a positive skin prick test to at least three inhalant allergens with a mean of horizontal and vertical diameters more than 3 mm.

Blood samples were obtained before and at the end of third months of inhaled corticosteroid treatment at 8.00-8.30 am and stored at -70°C until later analysis. Patients did not receive antihistaminic drugs, theophylline, β_2 agonist, and systemic steroid therapy for at least two weeks before the assay. No asthmatic attack or infectious disease were noted at least for three weeks preceding the test. Total IgE levels were studied with a microparticle enzyme assay (Abbott Diagnostic Division, USA), sCD23 was measured by sandwich enzyme linked immunoassay (ELISA) (Cell-free CD23 Test Kit, T Cell Diagnostics Inc,

Woburn, MA, USA). Serum ECP concentrations were studied using radioimmunoassay (Pharmacia, Uppsala, Sweden) as previously described⁶ after the run-in period and at the end of the study. The detection limit of the ELISA for sCD23 was 15 U/ml and the intra-assay coefficient of variation of the radioimmunoassay for ECP was 6.1%. All assays were done blind.

STATISTICAL ANALYSIS

For within group comparisons, the Wilcoxon matched pairs signed ranks test was used. For comparisons between asthma and control groups the Mann-Whitney U test was applied. Spearman's rank correlation analysis was used for correlations.

Results

Twenty seven asthmatic children (17 boys, 10 girls), mean age 10.8, SD 2.4 years, range 7.5 to 15.0 years, mean asthma duration 5.6 (2.0) years, completed the study. Two children were excluded from the study group because of non-compliance and failure to complete the symptom diaries. Fifteen healthy non-asthmatic children (12 boys, three girls), mean age 12.0 (1.8) years, range 9.0 to 14.5 years, with perennial allergic rhinitis were recruited as a control group. All children were allergic to three or more inhalant allergens as determined by skin prick testing and RAST. The most common allergens were house dust mite and grass pollens. At the end of the treatment, the mean symptom and medication scores were significantly reduced ($p < 0.01$, $p < 0.001$). Mean morning and evening PEF, FEV₁, and FEF₂₅₋₇₅ increased significantly ($p < 0.01$, $p < 0.01$, $p < 0.05$, and $p < 0.05$ respectively) from the run-in period in all patients within the three months of treatment (table 1).

There was no significant difference ($p > 0.05$) in total serum IgE concentration between the asthmatic and control groups, and serum IgE

Figure 1 Relation between the decrease in (A) mean symptom score ($r = 0.62$, $p < 0.01$) and (B) medication score ($r = 0.57$, $p < 0.02$) and the decrease in serum eosinophilic cationic protein (ECP) during the treatment period.

did not change after budesonide treatment ($p > 0.05$). No correlation between total IgE and serum sCD23 could be detected in either group. The mean serum ECP and sCD23 values obtained before treatment were higher than the values in the control group ($p < 0.001$, $p < 0.02$ respectively). They decreased significantly ($p < 0.01$, $p < 0.05$ respectively) to control levels after treatment (table 1).

While there was a weak correlation between the decrease in sCD23 levels and the reduction in symptom scores ($r = 0.42$, $p < 0.05$), a significant correlation was observed with the decrease in ECP concentrations ($r = 0.62$, $p < 0.01$) (fig 1). A similar correlation was present between the reduction in β_2 agonist requirement and decrease in sCD23 and ECP concentrations ($r = 0.36$, $p < 0.05$; $r = 0.57$, $p < 0.02$ respectively) (fig 1). Likewise, the improvement in pulmonary function tests showed a significant relation with the decrease in ECP concentrations (mean $FEV_{1.2}$, $r = -0.46$, $p < 0.04$; FEF_{25-75} , $r = -0.38$, $p < 0.05$; morning PEFR, $r = -0.41$, $p < 0.05$; evening PEFR, $r = -0.43$, $p < 0.05$) (fig 2), whereas no significant association was observed between the decrease in sCD23 concentrations and the improvement in pulmonary function tests (mean $FEV_{1.2}$, $r = -0.21$; FEF_{25-75} , $r = -0.22$; morning PEFR, $r = -0.16$; evening PEFR, $r = -0.18$).

Discussion

Decision to start regular anti-inflammatory prophylactic treatment is made based on the frequency and severity of asthmatic symptoms, along with the results of pulmonary function tests, and the follow up is made according to

Figure 2 Relation between the increase in (A) $FEV_{1.2}$ ($r = -0.46$, $p < 0.04$) and (B) FEF_{25-75} ($r = -0.38$, $p < 0.05$) and the decrease in serum eosinophilic cationic protein (ECP) during the treatment period.

the same indices.⁴ However, it is known that these variables are not always objective indicators. In a recent study that was performed on adult patients with asthma, worsening of symptoms occurred without significant deterioration in pulmonary function tests in 45% of patients. In 10% of the patients there was a fall in pulmonary function, although they were not aware of any change in their symptoms. A relation between the asthma symptoms and pulmonary function tests was found in only 45% of the patients.⁷ Therefore, in clinical practice it was suggested that in addition to pulmonary function testing, asthma symptoms, bronchodilator requirement, and inflammatory markers must also be evaluated.^{2,8,9}

In a recent report it was shown that ECP concentration was increased in both allergic asthma and intrinsic asthma patients.¹⁰ Similarly, there was no difference in sCD23 levels between allergic and non-allergic children after 3 years of age.¹¹ Thus it is emphasised in published reports that sCD23 and ECP concentrations, particularly in bronchial asthma, are useful as markers of disease rather than of allergy as such.^{2,10-12} As in other studies, the concentrations of sCD23 and ECP in asthmatic children were found to be higher than in non-asthmatic children with allergic rhinitis in our study. In allergic rhinitis the target tissue is smaller than in asthma so the markers of inflammation may not be detected in the peripheral blood. At entry, although our asthmatic children were asymptomatic they had higher concentrations of sCD23 and ECP than non-asthmatic children. This finding suggests subclinical

inflammation in asthmatic children during symptom-free intervals. This was already reported by Van Bever *et al.*,¹³ who found that asymptomatic asthmatic children have higher levels of soluble interleukin-2 receptor and ECP than normal children.

Inhaled corticosteroids are effective in all patients irrespective of asthma severity. Generally, quite marked and rapid clinical improvements and changes in pulmonary function tests can be seen even with very low daily doses (around 100 µg) even in children with moderate and severe asthma,¹⁴ whereas higher doses (around 600 µg/day) and longer treatment are required to control hyper-reactivity.^{14,15} In our patients after three months' treatment both symptoms and pulmonary function tests improved significantly and bronchodilator requirement decreased in most of them. Recently a long term study has provided information on the beneficial clinical effects associated with long term continuous use of inhaled budesonide. It was realised that budesonide significantly increased the rate of growth in lung function with age in comparison with the children not receiving inhaled steroids.¹⁶ These results suggest that bronchial inflammation decreases with the inhaled corticosteroid therapy.

Total IgE concentrations did not decrease with inhaled steroid therapy in our patients and there was no correlation between IgE and sCD23 concentrations before and after the treatment. In patients undergoing bone marrow transplantation, sCD23 concentrations increase initially, followed several days later by a rise in total IgE. This indicates that these two variables do not increase concomitantly.¹⁷ Despite there being no change in IgE levels with inhaled steroid therapy, sCD23 concentrations decreased significantly. In an *in vitro* investigation, the pharmacological modulation of the CD23 expression on monocytes was investigated. Corticosteroids (dexamethasone or betamethasone) inhibited in a dose and time dependent manner the interleukin-4 induced CD23 expression on human monocytes. In contrast to corticosteroids, ketotifen did not alter the same reaction. β_2 Agonists potentiated the interleukin-4 induced CD23 expression.¹⁸ While a weak correlation was found between the improvement in the symptom-medication scores and the reduction in sCD23 levels, no correlation was found with pulmonary function tests in our study. Hoeger *et al.*¹⁹ showed a weak correlation between sCD23 levels and asthma severity in asthmatic children older than 10 years, while there was no significant relation in younger children.

Studies in a large number of patients have shown that the ECP concentrations are increased in chronic asthmatics and decreased during effective anti-inflammatory treatment.^{2,10,20,21} In our study, serum ECP decreased significantly at the end of the inhaled steroid treatment period. Studies by Djukanovic *et al.*²² in patients with asthma and by Lozewicz *et al.*²³ in patients with allergic rhinitis have shown that treatment for two to six weeks with topical corticosteroids decreased

the numbers of activated eosinophils in, respectively, the bronchial and nasal mucosa of these patients. In many studies, serum ECP has shown a definite correlation with a disease in activity of asthma.^{9,20} However, this has not been the case in all studies.^{21,24} A significant correlation was observed between the reduction of symptom-medication scores with improvement in pulmonary function tests and the decrease in ECP concentrations in our patients.

In conclusion, our results show that inhaled corticosteroids decrease the concentrations of both ECP and sCD23 in serum, presumably by inhibiting inflammation in the airways. Monitoring of serum inflammation markers, particularly ECP, may be useful in the follow up of asthmatic children on anti-inflammatory treatment.

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