

Influence of allergen avoidance at high altitude on serum markers of eosinophil activation in children with allergic asthma

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Summary

A cohort of 12 asthmatic children was followed over several months, during which they moved back and forth from an allergen-free to an allergen-rich environment at high and low altitude, respectively. The children were treated with non-steroidal anti-asthmatic drugs as clinically needed. Histamine PC20-FEV₁ was unaltered during the study period, whereas serum levels of eosinophil cationic protein (ECP) and eosinophil protein X (EPX) showed significant changes when the children were exposed to the offending allergens. The total IgE significantly increased during exposure. The serum levels of myeloperoxidase (MPO) as well as of chemotactic factors for both neutrophils and eosinophils were unaltered during allergen exposure. We conclude that the serum markers of eosinophil activity ECP and EPX are sensitive indices of allergen exposure in asthmatic atopic children.

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Introduction

A variety of circumstantial evidence suggests that eosinophil leucocytes are intimately involved in the inflammatory process in the bronchial mucosa of asthmatic patients [1,2]. During an asthmatic attack eosinophils are found both in bronchial walls and in airway lumen and they may exert tissue damage by oxygen-dependent as well as oxygen-independent mechanisms [3]. The former is based on the toxic oxygen metabolites and eosinophil peroxidase (EPO) and the latter on granule proteins such as eosinophil cationic protein (ECP), eosinophil peroxidase (EPO), eosinophil protein X/eosinophil derived neurotoxin (EPX/EDN) and major basic protein (MBP). Recent studies have suggested that the measurement of the levels in serum of some of these proteins, notably ECP, may be useful in reflecting the activity of circulating eosinophil granulocytes [4–11]. The ECP levels were related to bronchial hyperreactivity in asthmatic patients and to the development of late asthmatic reactions after bronchial allergen challenge [6]. Venge *et al.* have also indicated that there is a correlation between the degree of

activity of the eosinophils in the lungs and the serum levels of these proteins [11].

The role of eosinophils and neutrophils in bronchial asthma has been also examined by studying the serum levels of such chemotactic activity factors as heat-stable neutrophil chemotactic activity (HS-NCA), heat-labile neutrophil chemotactic activity (HL-NCA) and heat-labile eosinophil chemotactic activity (HL-ECA) [12]. All these factors are produced during natural exposure to allergen and are generally normal out of allergen season [12].

The monitoring of the levels of myeloperoxidase (MPO), as an indication of neutrophil activity, may be useful in defining the role of the neutrophils in allergen exposure but also in the distinction between other causes of elevated levels of the eosinophil markers ECP and EPX/EDN, such as acute infections [13].

As altitude increases the mite content of house dust decreases [14] and in an Alpine environment (above 1750 m) the pollen season is short, being usually limited to July and August. When asthmatic subjects who live at high altitude (low levels of allergens) move to sea level (high levels of allergens) at the end of Spring (June) and then return to a high altitude at the end of Summer (Sep-

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tember), they are exposed to a natural allergen exposure/avoidance challenge [15,16] for mites, grass pollen and *parietaria* (a weed in the Urticaceae family).

The aim of this study was to evaluate the effects of such a natural challenge on non-specific bronchial hyperreactivity (BHR), blood eosinophil counts, serum levels of ECP, EPX, MPO, eosinophil and neutrophil chemotactic factors as well as on total and *Dermatophagoides pteronyssinus* and *D. farinae* specific IgE.

Materials and methods

Subjects

A group of 12 asthmatic children (seven boys, five girls), with asthma as defined by the American Thoracic Society [17], participated in the study. All the children had skin-prick test and RAST positivity for *D. pteronyssinus* and *D. farinae* and nine of them were also allergic to grass pollen and/or *parietaria*. The characteristics of the study population are summarized in Table 1.

The children were treated at home between June and September with sodium cromoglycate (SCG), 20 mg dry powder four times a day on a regular basis and with inhaled salbutamol when needed. No treatment was required during the periods of stay at high altitude except occasional β -2 agonists.

Study design

The children were, in the different periods of the study, either guests of the residential house for asthmatic children Istituto Pio XII located at Misurina, by the

Italian Alps at 1756 m above sea level, an environment practically allergen-free, or they lived with their own families at sea level. The children stayed at high altitude from January 1988 to June 1988 and from September 1988 to December 1988.

They returned home, at sea level, during the summer holidays (end of June–September), when they were exposed to a natural challenge to *D. pteronyssinus* and *D. farinae*, *parietaria*, and grass pollen. All the evaluations were performed within a few hours of departure from or on arrival at the residential home.

Assessment of bronchial hyperreactivity

The levels of bronchial hyperreactivity were evaluated by histamine challenge [18]. Briefly, histamine challenge was performed only if FEV₁ baseline values were $\geq 80\%$ of predicted values. Increasing concentrations of histamine acid phosphate (1, 2, 4, 8, 16 and 32 mg/ml) were delivered by a Wright nebulizer. The nebulizer output was 0.13 ml/min and the duration of inhalation was 2 min. FEV₁ was evaluated by a Vitalograph spirometer (Vitalograph Co, Ltd, Buckingham) 30 sec, 1 min 30 sec and 3 min after each inhalation and the best of three determinations was always considered. The histamine concentration which caused a 20% decrease of FEV₁ values (PC20-FEV₁), was considered in the analysis of the results. SCG and β -2 agonists respectively were not allowed 24 and 12 hr prior to the challenge. No child was receiving long-acting β -2 agonist.

Serum determinations

Blood was centrifuged at 1000 *g* for 10 min at room temperature and serum was immediately frozen and stored at -70°C until the assays were performed. Total IgE (PRIST) and specific IgE to *D. pteronyssinus* and *D. farinae* (RAST) levels were performed by radioimmunoassay (Pharmacia Diagnostics, Uppsala, Sweden). ECP, EPX and MPO levels were evaluated according to the method of Venge *et al.* [19,20,21]. The 95% ranges of ECP, EPX/EDN and MPO in normal serum are 5–55, 7–42 and 40–380 $\mu\text{g/l}$ respectively. The presence of chemotactic factors in serum was assayed by means of the leading front method in a modified Boyden chamber as previously described [22]. The incubation time was 1 hr and the migration distance was calculated from readings of six microscopic fields. HL-NCA and HL-ECA are expressed in per cent of a control serum and HS-NCA in $\mu\text{m/hr}$. All the assays were performed without knowledge of the study design.

Blood eosinophil determination

Total white blood cells were evaluated by an automatic haematology analyser Coulter S-7 (Coulter Electronics

Table 1. Patients' characteristics

Patient	Sex	Age	Skin test positivity
1	F	14	Df, Dp
2	M	10	Df, Dp, dog, grass, <i>parietaria</i>
3	M	14	Df, Dp, grass
4	F	13	Df, Dp, grass
5	F	13	Df, Dp
6	F	14	Df, Dp, grass
7	F	10	Df, Dp, grass
8	M	12	Df, Dp, grass
9	M	11	Df, Dp, cat, grass, <i>parietaria</i>
10	M	12	Df, Dp
11	M	14	Df, Dp, grass, <i>parietaria</i>
12	M	14	Df, Dp, grass

Df, *Dermatophagoides farinae*. Dp, *Dermatophagoides pteronyssinus*.

Table 2. Summary of the results of the investigated parameters during the study period (mean $\pm$ s.e.m.). The patients were exposed to allergens between June and September, then they lived at high altitude with no allergen exposure.

	June		September		December
PC20-FEV ₁	2.89 $\pm$ 0.91	—n.s.—	5.54 $\pm$ 2.51	—n.s.—	6.66 $\pm$ 2.34
Blood Eos	215 $\pm$ 56	—n.s.—	159 $\pm$ 51	—n.s.—	81 $\pm$ 13
ECP	23.95 $\pm$ 4.79	— <i>P</i> < 0.01—	64.95 $\pm$ 13.07	— <i>P</i> = 0.001—	9.77 $\pm$ 2.0
EPX/EDN	38.19 $\pm$ 5.11	— <i>P</i> = 0.02—	59.10 $\pm$ 10.69	— <i>P</i> < 0.01—	22.67 $\pm$ 1.99
MPO	196.70 $\pm$ 19.3	—n.s.—	208.33 $\pm$ 29.4	— <i>P</i> = 0.02—	156.50 $\pm$ 21.8
HL-NCA	118.35 $\pm$ 8.21	—n.s.—	108.14 $\pm$ 6.82	— <i>P</i> < 0.05—	121.78 $\pm$ 8.32
HL-ECA	109.78 $\pm$ 6.0	—n.s.—	109.92 $\pm$ 7.61	—n.s.—	117.71 $\pm$ 5.7
HS-NCA	11.52 $\pm$ 2.62	—n.s.—	12.05 $\pm$ 1.67	—n.s.—	8.40 $\pm$ 1.91
Total IgE	477.28 $\pm$ 1375	— <i>P</i> = 0.01—	680.65 $\pm$ 151.4	— <i>P</i> < 0.01—	472.56 $\pm$ 99.26
Dpt IgE	48.27 $\pm$ 8.97	—n.s.—	57.46 $\pm$ 11.64	— <i>P</i> < 0.05—	48.28 $\pm$ 10.59
Df IgE	21.79 $\pm$ 4.58	— <i>P</i> = 0.01—	33.35 $\pm$ 7.71	— <i>P</i> = 0.02—	25.2 $\pm$ 5.57

PC20-FEV₁ = μ g/ml. ECP = μ g/ml. EPX/EDN = μ g/ml. MPO = μ g/ml. HL-NCA = % of control serum. HL-ECA = % of control serum. HS-NCA = μ m/hr. IgE = IU/ml.

Ltd, Luton, U.K.). Eosinophil percentage of total white cells was determined on May Grünwald-Giemsa stained smears obtained from the same blood samples.

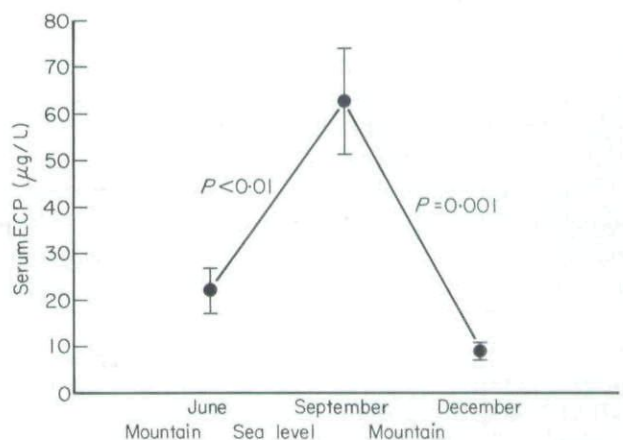
Data analysis

Data were evaluated by a repeated measurements analysis of variance (ANOVA). Where significant differences were shown, data were compared by two-tailed paired Student's *t*-test. Correlations between different parameters were performed by Pearson's correlation analysis. *P* values of < 0.05 were considered significant.

The study was approved by the local Hospital Ethics Committee.

Results

The results of the investigated parameters (BHR, number of eosinophils in the blood, ECP, EPX/EDN, MPO, HL-

**Fig. 1.** ECP serum levels (μ g/L, mean $\pm$ s.e.m.) during the study period.

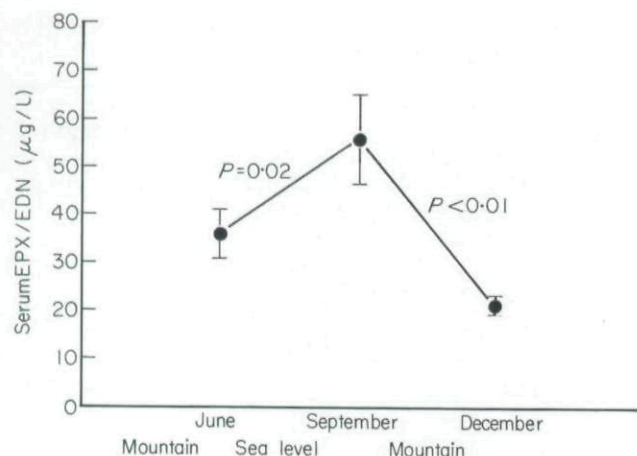


Fig. 2. EPX serum levels ($\mu\text{g/L}$, mean $\pm$ s.e.m.) during the study period.

NCA, HL-ECA, HS-NCA, total and specific serum IgE) are summarized in Table 2. No significant difference was observed at the different times of the study in the levels of bronchial reactivity and in the numbers of eosinophil granulocytes in peripheral blood. A significant increase in ECP levels was observed between June and September ($P < 0.01$) and a decrease was noted between September and December ($P = 0.001$) (Fig. 1). Highly significant differences in the serum levels of EPX/EDN were observed between June and September ($P = 0.02$) and between September and December ($P < 0.01$) (Fig. 2). The only significant change in MPO serum level is represented by the decrease shown between September and December ($P = 0.02$).

For HL-NCA values, we observed a significant ($P < 0.05$) increase between September and December results; no other significant difference was seen. No significant changes were observed in HL-ECA and HS-NCA serum values. The increase in total IgE levels between June and September ($P = 0.01$) and the decrease between September and December ($P < 0.01$) were statistically significant. There was a significant decrease for *D. pteronyssinus* specific IgE ($P < 0.05$) between September and December. The *D. farinae* specific IgE values show a significant difference between June and September ($P = 0.01$) and between September and December ($P = 0.02$).

Correlation analysis between different parameters gave positive correlations only between serum levels of ECP and EPX at the June ($r = 0.709$, $P < 0.01$) and September ($r = 0.515$, $P = 0.5$) observations. No relationships were observed between histamine PC20-FEV₁ and either of the blood measurements.

Discussion

The bronchial non-specific responsiveness of our study population did not significantly increase during the allergen exposure despite the activation of the circulating pool of eosinophil granulocytes, as indicated by the significant increase of ECP and EPX serum levels. These findings are unexpected in comparison with the results of previous studies, which showed close temporal correlations between rise in serum ECP levels during seasonal birch pollen exposure and increase in lung reactivity to histamine [23]. However, our patients received regular treatment with SCG during the period of allergen exposure at sea level. SCG has been shown to be able to inhibit the increase in BHR which is observed following both allergen challenge [24–26] and natural allergen exposure [27]. *In vitro* observations have shown that SCG is able to inhibit the release of ECP from eosinophils stimulated by incubation with sepharose C3b, but only at high concentrations (up to 10^{-3} M), when it becomes toxic to cultured cells [28]. The lack of effect of this drug on eosinophil activity *in vitro* confirms the *in vivo* observations. The increase in serum eosinophilic proteins (ECP and EPX/EDN) appears to be a more sensitive index of allergen exposure than increase in non-specific BHR, particularly in asthmatics who are treated with drugs which prevent the increase in BHR. The results of this study suggest that the levels of ECP and EPX in serum may be useful in monitoring the effectiveness of environmental control manoeuvres in the homes of allergic patients. Similar considerations can be made for specific IgE serum levels, which increased after prolonged exposure to aero-allergens and decreased after complete allergen avoidance at high altitude. It has been observed that the mite content of house dust decreases as altitude increases [14,29] and indeed, the routine around the year analysis of samples from mattresses in the residential house Istituto Pio XII always showed negative results (class 0 guanine test) (Boner, unpublished data). In contrast, class 2 positivity for guanine test for mites was shown in almost all the homes of 40 different asthmatic children living in the area served by the residential house, regardless of the season, due to the high level of humidity of that region (L. Sette unpublished data). However, we cannot speculate on the level of antigen exposure necessary to induce an increase in ECP-EPX/EDN serum levels because no quantification of the antigenic content of the mattresses in the homes of the children was performed specifically for the present study. In this study no change in chemotactic activities was observed in serum. In contrast, previous studies performed on adults showed a dramatic increase in eosinophil chemotactic activities both in serum and in BAL fluid [12,22]. This finding, as

well as the lack of modification in bronchial reactivity to histamine, might be due to treatment with SCG during the period of allergen exposure. Previous studies on the development of late asthmatic reactions showed that SCG inhibited the generation of chemotactic activities in serum in parallel with the abrogation of the late asthmatic reaction [30,31].

The development of lung hyperreactivity most likely requires the attraction of activated eosinophils to the lung. Allergen exposure in our children led to activation of the circulating pool of eosinophils, but, probably due to the action of sodium cromoglycate, not to the generation of the necessary signals in the lung to attract these cells. Our data support a previous hypothesis about the importance of chemotactic signals for the development of BHR and asthma and suggest that one important mechanism of action of the anti-allergic effects of the cromoglycates could be the inhibition of the generation of such signals.

We conclude that allergen exposure activates the eosinophil granulocytes, as indicated by raised serum levels of ECP and EPX/EDN, and that this activation is not affected by non-steroidal anti-asthmatic medications. The absence of a rise in eosinophil and neutrophil chemotactic activities, however, is most likely due to the therapeutic effects of SCG. These results support the relevance of serum measurements of eosinophil markers, such as ECP and EPX/EDN, as useful clinical tools in the monitoring of allergen exposure.

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