

Eosinophils in exercise-induced asthma

Per Venge, MD,* Jörn Henriksen, MD,** and Ronald Dahl, MD**

Uppsala, Sweden, and Århus, Denmark

The variations in serum levels of eosinophil cationic protein (ECP) have been measured after exercise challenge of 13 patients with asthma with exercise-induced asthma (EIA) and nine patients with asthma without EIA. The patients were treated before exercise in a randomized and blinded fashion with inhalation of one dose of either disodium chromoglycate, terbutaline, or budesonide and in an open study with 4 weeks of inhaled budesonide. In the group with EIA, there was, in some patients, an initial increase in serum levels of ECP after exercise, but 60 minutes after exercise, the levels were significantly reduced ($p < 0.001$). Disodium chromoglycate and 4 weeks of budesonide treatment inhibited this reduction. Histamine challenge of the group with EIA produced a similar fall in serum ECP levels ($p < 0.001$). The group without EIA had initially lower levels of ECP than the group with EIA ($p < 0.05$ to $p < 0.01$), and ECP stayed unaltered after exercise. The preexercise serum ECP levels correlated significantly to the maximal fall in peak expiratory flow in the untreated group ($r = 0.91$; $p < 0.001$) and in the group receiving one dose of budesonide ($r = 0.62$; $p < 0.05$). The blood eosinophil counts were unchanged after challenge and not related to lung function. The results suggest that the ECP content in serum reflects the degree of allergic inflammation in the lungs and thereby the degree of bronchial hyperreactivity. (J ALLERGY CLIN IMMUNOL 1991;88:699-704.)

Key words: Eosinophils, eosinophil cationic protein, exercise, asthma

The symptoms of chronic asthma are most likely a consequence of an inflammatory process in the lung. During recent years, it has become increasingly clear that the EOS plays an important role in the development of chronic asthma, possibly caused by its great cytotoxic potential.¹ We have previously investigated the relationship of the activity of the EOS to the asthmatic reaction after allergen challenge, both in the laboratory^{2,4} and during the pollen season.⁵ These studies demonstrated a relationship between the activity of the EOS, as measured by serum levels of ECP, and the asthmatic reaction. One of the conclusions from these studies was that the enhanced activity

Abbreviations used

EOS:	Eosinophil
EIA:	Exercise-induced asthma
LAR:	Late asthmatic reaction
PEF:	Peak expiratory flow
ECP:	Eosinophil cationic protein
DSCG:	Disodium chromoglycate

of the EOS is one prerequisite for the development of the LAR after allergen challenge.

In many subjects with asthma, exercise will induce an early asthmatic reaction. An LAR to exercise has been observed by some investigators, but the prevalence appears to be quite low.⁶⁻¹¹ The underlying causes of EIA are poorly understood. The reaction might reflect the inflammation in the lung of the individual with asthma, although no experimental data to confirm this hypothesis are available. The possible role, however, of the EOS in EIA has not been investigated. The aim of this study was to study the variations in activity of the EOSs, as measured by serum levels of ECP, after exercise challenge of patients with EIA with the hypothesis that the enhanced

From the *Laboratory for Inflammation Research, Department of Clinical Chemistry, University Hospital, Uppsala, Sweden, and **Department of Lung Medicine, University Hospital, Århus, Denmark.

Supported by grants from the Swedish Medical Research Council and AB Draco, Lund, Sweden.

Received for publication Dec. 23, 1989.

Revised Dec. 21, 1990.

Accepted for publication Feb. 8, 1991.

Reprint requests: Per Venge, MD, Laboratory for Inflammation Research, Department of Clinical Chemistry, University Hospital, S-751 85 Uppsala, Sweden.

1/1/31504

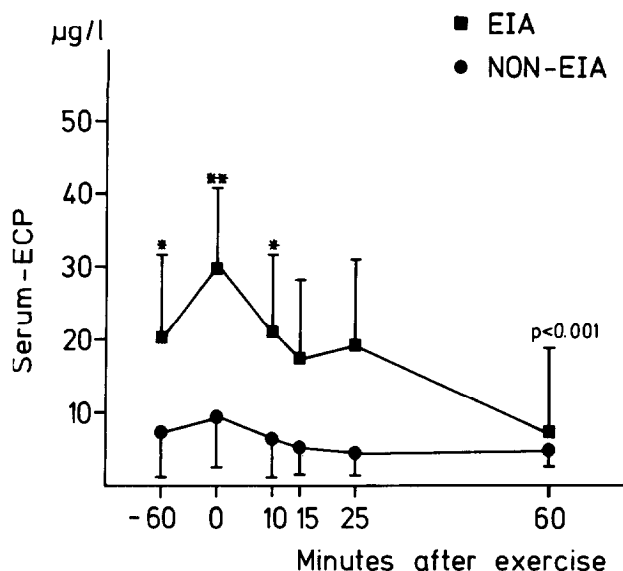


FIG. 1. Variations in serum ECP levels after submaximal exercise of patients with asthma; patients who reacted with an EIA, $N = 13$; patients who did not react with EIA, $N = 9$. Results are presented as means $\pm$ SD; * and ** $p < 0.05$ and $p < 0.01$ compared to the group without EIA; $p < 0.001$ at 60 minutes indicates the significant difference compared to the results before exercise.

activity of the EOS is one prerequisite for the development of EIA. To accomplish this aim, we pretreated a group of patients with asthma with various antiasthmatic drugs to modify the reaction to exercise, as has been described previously.

PATIENTS AND STUDY PROTOCOL

The patients consisted of two groups and were described in detail in a previous publication.¹² One group comprised 13 adult subjects with asthma (six women and seven men; average age, 29 years; range, 15 to 39 years) with EIA, defined as a $>15\%$ fall in PEF after submaximal exercise. The other group comprised nine adults with asthma without EIA (four women and five men; average age, 27 years; range, 22 to 34 years). The patients were not allowed to take any bronchodilators or DSCG within 8 hours before the exercise. Oral bronchodilators were withheld for 24 hours. Glucocorticosteroids were not allowed the last 3 months.

Pretreatments were administered in a randomized order in a double-blind fashion 15 minutes before exercise challenge and consisted of five puffs from pressurized aerosols of either budesonide, 200 μg per puff, terbutaline, 250 μg per puff, or DSCG, 1 mg per puff. In addition, 12 of the 13 patients were treated with budesonide aerosol, 400 μg in the morning and 600 μg in the evening, for 4 weeks, after which, patients performed a final exercise challenge. The protocol was approved by the Danish health authorities and the ethics committee of the Medical Faculty of Århus University.

METHODS

The exercise challenges consisted of 6 minutes of continuous exercise on a bicycle ergometer at a work load previously adjusted to 80% of the predicted maximal working capacity of the patients. Heart rate was measured by electrocardiogram monitor, and mean heart rate from the last 30 seconds of exercise was recorded as final heart rate. Room temperature and relative humidity were recorded at each visit.

PEF was measured by Wright's peak flow meter (best of three attempts) 15 minutes before exercise (before premedication), immediately before exercise (baseline), after 2, 4, and 6 minutes of exercise, and 1, 3, 5, 10, 15, 20, 25, 30, and 60 minutes after exercise. Thereafter, PEF was measured once every hour for the rest of the day to detect a possible LAR. The bronchial response to exercise was calculated as percentage fall in PEF:

$$\text{EIA \% fall} = \frac{\text{Baseline PEF} - \text{lowest PEF after exercise}}{\text{Baseline PEF}} \times 100$$

Histamine challenge test was performed according to a standardized method.¹³

Blood was obtained just before, just after, and 10, 15, 25, and 60 minutes after challenge with either exercise or histamine. Serum was freshly frozen in small aliquots and stored at -20°C until analysis. Serum levels of ECP were measured by a described method.¹⁴ Blood EOS counts were performed under the microscope after eosin staining of the cells with standard techniques.

Statistical methods

Wilcoxon's signed-rank test and linear regression analysis were used. All statistical calculations were performed on a personal computer by means of the statistical package, Statgraphics (STSC, Inc., Rockville, Md.).

RESULTS

The average fall in PEF without pretreatment was in the group with EIA, 36% (range, 15% to 62%), and in the group without EIA, 6% (range, 3% to 11%). None of the patients experienced an LAR after exercise. In a previous publication, the effect of the different medications on EIA was documented.¹² Briefly, terbutaline, DSCG, and 4 weeks of budesonide treatment significantly reduced the fall in PEF to, on average, 8%, 17%, and 17%, respectively ($p < 0.001$, $p = 0.002$, and $p = 0.01$, respectively), whereas, one dose of budesonide was without significant effect (average 26% fall in PEF).

The variations of ECP levels in serum after exercise of patients with asthma are illustrated in Fig. 1. In patients with EIA, the ECP levels revealed a slight tendency to increase immediately after exercise. After this slight increase, however, the levels were reduced

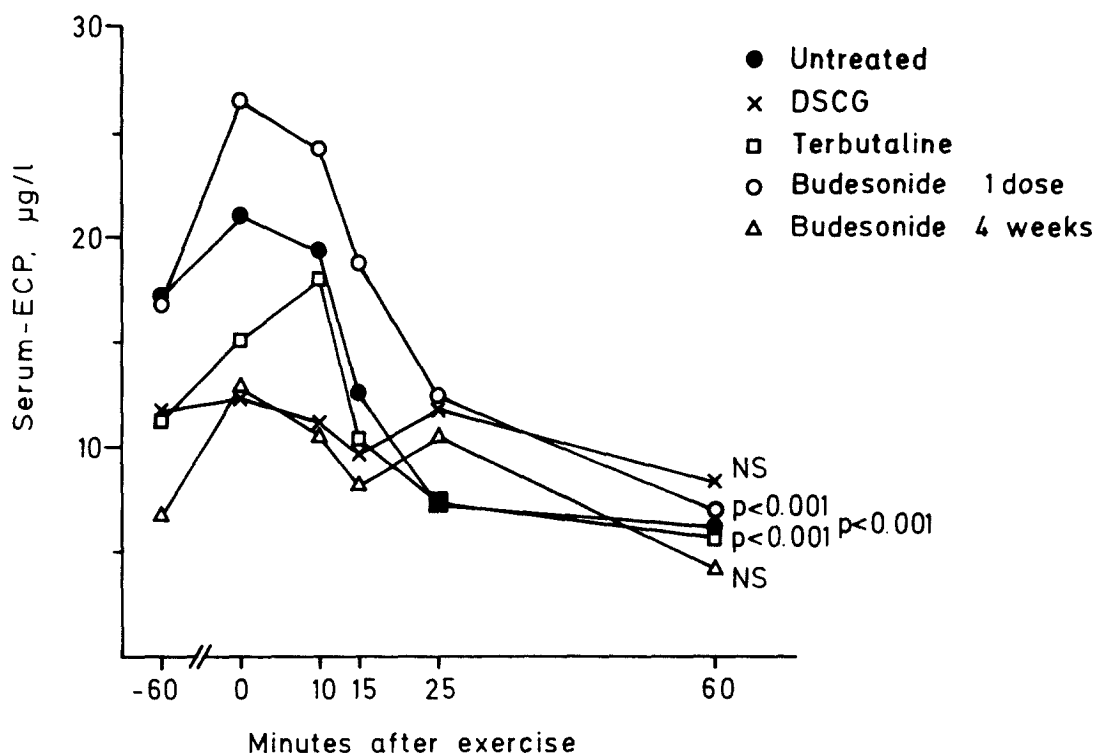


FIG. 2. Variations in serum ECP levels after submaximal exercise of patients with asthma and the effects of antiasthmatic treatment. Results are presented as means. The p values at 60 minutes after exercise indicate differences compared to the results before exercise.

and, significantly so, 60 minutes after exercise ($p < 0.001$). In patients without EIA, the ECP levels were unaltered during the whole observation period and significantly lower than levels observed in the group with EIA before, 0, and 10 minutes after exercise ($p < 0.05$ to $p < 0.01$).

After premedication with terbutaline and one dose of budesonide, the results in treated patients were very similar to the results in untreated patients with significant reductions of ECP 60 minutes after exercise ($p < 0.01$ to $p < 0.001$). In contrast, after premedication with DSCG and after 4 weeks of budesonide treatment, ECP levels stayed unaltered during the observation period (Fig. 2).

The alteration in ECP levels after exercise demonstrated no relation to the severity of EIA. However, in the untreated group, the ECP levels before exercise were significantly correlated to the maximal fall in PEF after exercise ($p < 0.001$) (Fig. 3), as well as in the one-dose budesonide-treated group ($r = 0.63$; $p < 0.05$). This relationship was not observed after DSCG, terbutaline, or 4 weeks of budesonide treatment.

Blood EOS numbers before exercise were similar in all groups (Table I) and demonstrated no relation

to the fall in PEF after exercise. Blood EOS numbers after exercise are illustrated in Fig. 4 and demonstrated no difference between the two exercise groups. However, the EOS counts were significantly elevated at time 0 after exercise in both groups, as compared to the counts before exercise ($p < 0.05$).

The serum levels of ECP after histamine-challenge of 12 patients with EIA are illustrated in Fig. 5. The ECP levels were significantly ($p < 0.001$) reduced 25 and 60 minutes after challenge.

DISCUSSION

In previous studies we have demonstrated that ECP will rise significantly in serum during the early asthmatic reaction after allergen inhalation challenge in about one third of the patients but that this rise is subsequently replaced by a quick reduction in ECP levels.^{2,3} In the remaining two thirds of the patients, only the fast reduction in serum ECP levels after challenge is observed. The reasons for these two patterns are not clear, although it is likely that the initial rise is a reflection of the activation of the EOSs as a consequence of the challenge. The reduction has been postulated to be dependent on the exclusive and accelerated elimination of ECP by mechanisms induced

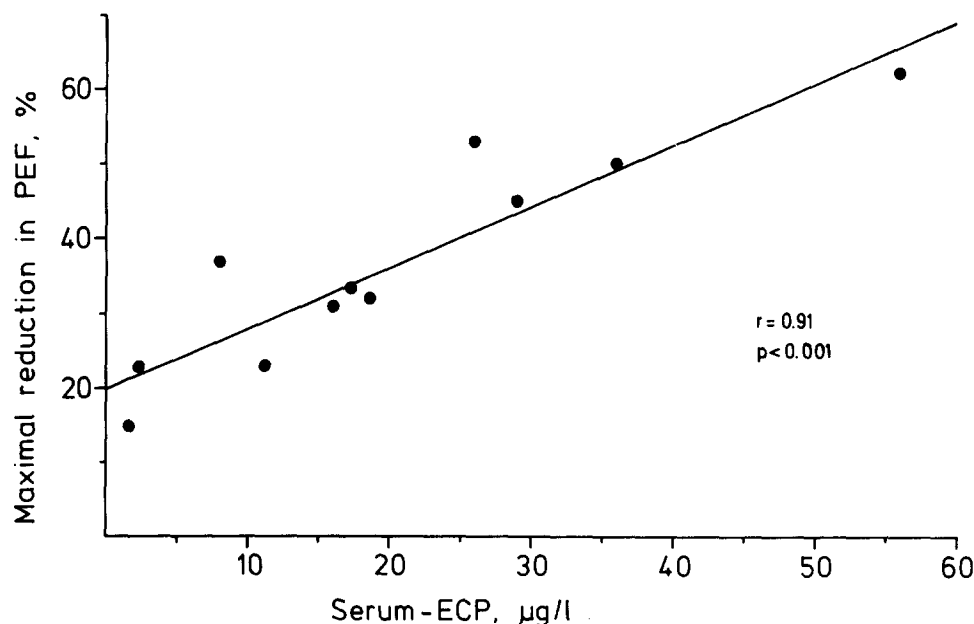


FIG. 3. Correlation between the serum ECP levels before exercise in the untreated group and the maximal fall in PEF after exercise.

TABLE I. Blood EOS numbers before exercise in patients with EIA

Treatment	Median $\times 10^6/L$	Actual range $\times 10^6/L$
Untreated	265	102-709
DSCG	479	90-715
Terbutaline	321	68-828
Budesonide 1 dose	298	129-676
Budesonide 4 weeks	293	124-1013

None of the differences in EOS numbers were statistically significant. Wilcoxon's signed-rank test was used.

by the asthmatic reaction, since another EOS-specific protein, eosinophilic protein X, did not elicit any such reduction after challenge. One speculation as to this specific elimination of ECP is related to the demonstration of the binding of ECP to the plasma protein α -2-macroglobulin,¹⁵ since this requires the brief attack of α -2-macroglobulin by proteases, such as thrombin and cathepsin G, both of which may be produced during the acute allergic reaction. It is therefore of interest that exercise of subjects with asthma who react with an asthmatic reaction induces almost identical patterns. As was suggested in the earlier studies,³ these patterns in the variation of the levels of ECP are not merely the consequence of the smooth

muscle reaction, since the bronchoconstrictor, metacholine, does not affect the ECP levels and since, as is also demonstrated in this study, the most effective drug in inhibiting the bronchoconstriction, terbutaline, was without effect in this respect. Also similar to the allergen challenge studies, prolonged treatment with budesonide prevented the reduction in ECP after challenge. However, in contrast to previous findings with metacholine, histamine challenge, in fact, produced a similar reduction in serum ECP levels. The underlying mechanism is obscure but may indicate that histamine has some other effects on lung cells besides the contraction of bronchial smooth muscles. Indeed, a number of studies have described the interaction between histamine and various EOS activities, such as secretion^{16, 17} and migration.¹⁸⁻²¹ Also histamine induces changes in lung permeability, seemingly independent of smooth muscle contraction.²² We therefore conclude, as to this part of the study, that the variations in serum ECP levels after EIA are similar to variations observed after allergen-induced asthma and that both procedures induce the formation of active principles, which enhance the elimination of ECP. This reduction in ECP is mimicked by histamine challenge, suggesting a relation to endogenously released histamine.^{23, 24}

The other major question which is addressed in this study is why some subjects with asthma react with

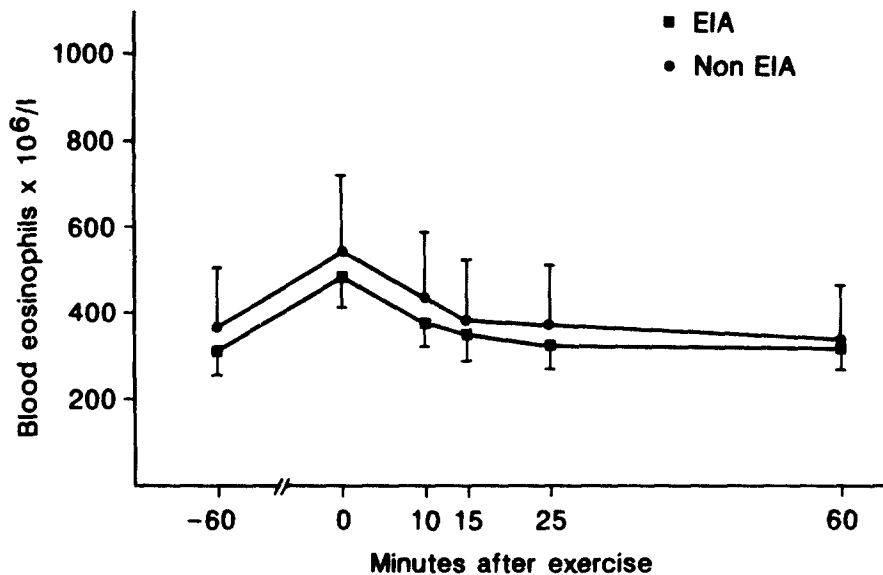


FIG. 4. Blood EOS counts after exercise challenge of patients with asthma; patients who reacted with EIA, N = 13; patients who did not react with EIA, N = 9. The blood EOS counts are significantly elevated ($p < 0.05$) in both groups at time 0 after exercise compared to before exercise.

asthma to exercise and other subjects do not and what determines the extent of the reaction. Two findings in this study may provide some answer to this question. The ECP levels in those patients who did not have EIA were significantly lower before challenge than levels found in the group with EIA, which would indicate that the activity of the EOSs in the group without EIA was lower. This finding is reminiscent of the previous observation that patients with asthma who reacted with an LAR to inhalation allergen challenge had much higher serum ECP levels before challenge than patients who did not have an LAR.^{3, 4} We interpreted this to mean that one prerequisite for the development of an LAR was the presence in the blood of activated or primed EOSs ready to be attracted to the lung. Another possibility might be that the serum ECP levels reflect the extent of EOS inflammation in the lung, since we have recently found a good correlation between the ECP levels in bronchoalveolar lavage fluid and serum in a group of subjects with mild asthma.²⁵ Therefore, EIA may reflect the ongoing inflammatory process in the lung. The very good correlations between the fall in PEF and the ECP levels before exercise found in the untreated group and in the one-dose budesonide-treated group support this hypothesis.

We conclude from this study that the variations of levels of ECP in EIA are similar to variations observed after allergen inhalation challenge and histamine challenge of patients with asthma but that the variations

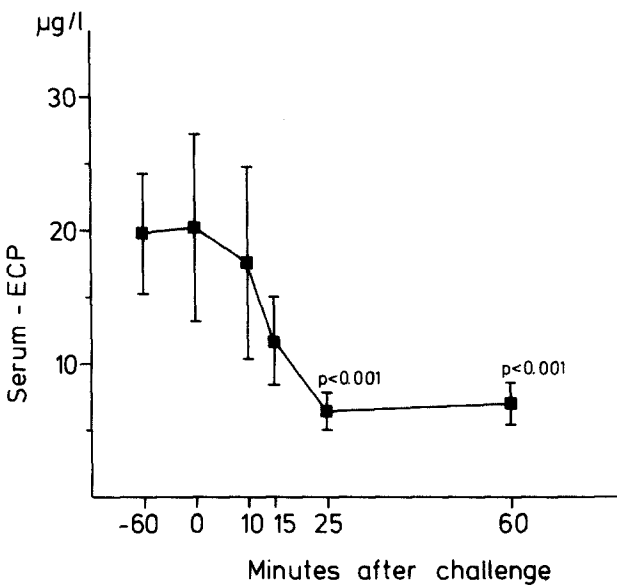


FIG. 5. Serum ECP levels after histamine challenge of 12 patients with asthma with EIA. Levels after 25 and 60 minutes were significantly reduced compared to levels before challenge.

are independent of the asthmatic reaction, as measured by PEF. We also conclude that EIA is dependent on the extent of the involvement of EOS in the inflammatory process of the lungs of patients with asthma, as reflected by serum ECP levels. Serum ECP level measurements may therefore be clinically useful as

objective signs of the inflammatory process of the lung in patients with asthma.

REFERENCES

1. Venge P, Peterson CGB. Eosinophil biochemistry and killing mechanisms. In: Morley J, Colditz I, eds. *Eosinophils in asthma*. New York, London: Academic Press, 1989:163-77.
2. Dahl R, Venge P, Olsson I. Variations of blood eosinophils and eosinophil cationic protein in serum of patients with bronchial asthma. *Allergy* 1978;33:211-5.
3. Venge P, Dahl R, Peterson CGB. Eosinophil granule proteins in serum after allergen challenge of asthmatic patients and the effects of anti-asthmatic medication. *Int Arch Allergy Appl Immunol* 1988;87:306-12.
4. Venge P, Dahl R. Are blood eosinophil number and activity important for the development of the late asthmatic reaction after allergen challenge? *Eur Respir J* 1989;2(suppl 6):430s-4s.
5. Rak S, Löwhagen O, Venge P. The effect of immunotherapy on bronchial hyperresponsiveness and eosinophil cationic protein in pollen-allergic patients. *J ALLERGY CLIN IMMUNOL* 1988;82:470-80.
6. Iikura Y, Inui H, Nagakura T, Lee TH. Factors predisposing to exercise-induced late asthmatic responses. *J ALLERGY CLIN IMMUNOL* 1985;75:285-9.
7. Lee TH, Nagakura T, Cromwell O, Brown MJ, Causon R, Kay AB. Neutrophil chemotactic activity and histamine in atopic and nonatopic subjects after exercise-induced asthma. *Am Rev Respir Dis* 1984;129:409-2.
8. Lee TH, Nagy L, Nagakura T, Walport MJ, Kay AB. Identification and partial characterization of an exercise-induced neutrophil chemotactic factor in bronchial asthma. *J Clin Invest* 1988;69:889-99.
9. Lee TH, Anderson SD. Heterogeneity of mechanisms in exercise-induced asthma. *Thorax* 1985;40:481-7.
10. Godfrey S. Controversies in the pathogenesis of exercise-induced asthma. *Eur J Respir Dis* 1986;68:81-8.
11. Nagakura T, Lee TH, Assoufi BK, Newman Taylor AJ, Denison DM, Kay AB. Neutrophil chemotactic factor in exercise- and hyperventilation-induced asthma. *Am Rev Respir Dis* 1983;128:294-6.
12. Venge P, Henriksen J, Dahl R, Håkansson L. Exercise-induced asthma and the generation of neutrophil chemotactic activity. *J ALLERGY CLIN IMMUNOL* 1990;85:498-504.
13. SEPCR working group. Guidelines for standardization of bronchial challenge with (non-specific) bronchoconstrictor agents. *Bull Eur Physiopathol Respir* 1983;19:495-514.
14. Venge P, Roxin L-E, Olsson I. Radioimmunoassay of human eosinophil cationic protein. *Br J Haematol* 1977;37:331-5.
15. Peterson CGB, Venge P. Interaction and complex formation between the eosinophil cationic protein (ECP) and alpha-2-macroglobulin. *Biochem J* 1987;245:781-7.
16. Kownatski E, Till G, Gagelmann M, Terwort G, Gerns D. Histamine induces release of an eosinophil immobilising factor from mononuclear cells. *Nature* 1977;270:67-9.
17. Pincus SH, DiNapoli A-M, Schooley WR. Superoxide production by eosinophils: activation by histamine. *J Clin Invest* 1982;79:53-7.
18. Clark RAF, Gallin JI, Kaplan AP. The selective eosinophil chemotactic activity of histamine. *J Exp Med* 1975;142:1462-76.
19. Clark RAF, Sandler JA, Gallin JI, Kaplan AP. Histamine modulation of eosinophil migration. *J Immunol* 1977;118:137-45.
20. Turnbull LW, Kay AB. Eosinophils and mediators of anaphylaxis histamine and imidazole acetic acid as chemoattractants for human eosinophil leucocytes. *Immunology* 1976;31:797-802.
21. Turnbull LW, Evans DP, Kay AB. Human eosinophils, acidic tetrapeptides (ECF-A), and histamine interactions in vitro and in vivo. *Immunology* 1977;32:57-63.
22. Rees PJ, Shelton D, Chan TB, Eiser N, Clark TJH, Maisey MN. Effects of histamine on lung permeability in normal and asthmatic subjects. *Thorax* 1985;40:603-6.
23. Belcher NG, Murdoch R, Dalton N, Clark TJH, Rees PJ, Lee TH. Circulating concentrations of histamine, neutrophil chemotactic activity, and catecholamines during the refractory period in exercise-induced asthma. *J ALLERGY CLIN IMMUNOL* 1988;81:100-10.
24. Broide DH, Eisman S, Ramsdell JW, Ferguson P, Schwartz LB, Wasserman SI. Airway levels of mast cell-derived mediators in exercise-induced asthma. *Am Rev Respir Dis* 1990;141:563-8.
25. Ådelroth E, Rosenhall L, Johansson S-Å, Linden M, Venge P. Inflammatory cells and eosinophilic activity in asthmatics investigated by bronchoalveolar lavage: the effects of antiasthmatic treatment with budesonide or terbutaline. *Am Rev Respir Dis* 1990;142:91-9.