

- ter intradermal allergen injection. *J ALLERGY CLIN IMMUNOL* 1982;70:236-42.
26. Britton JB, Hanley SP, Tattersfield AE. The effect of an oral leukotriene D₄ antagonist L-649,923 on the response to inhaled antigen in asthma. *J ALLERGY CLIN IMMUNOL* 1987;79:811-6.
 27. Shephard EJ, Malan L, McFarlane CM, Mouton W, Joubert JR. Lung function and plasma levels of thromboxane B₂, 6-ketoprostaglandin F_{1α}, and β-thromboglobulin in antigen-induced asthma before and after indomethacin pretreatment. *Br J Clin Pharmacol* 1985;19:459-70.
 28. Howarth PH, Durham SR, Kay AB, Holgate ST. The relationship between mast cell-mediator release and bronchial reactivity in allergic asthma. *J ALLERGY CLIN IMMUNOL* 1987;80:703-11.
 29. Westcott JY, Smith HR, Wenzel SE, et al. Urinary leukotriene E₄ excretion in patients with asthma: effect of airways reactivity and sodium cromoglycate [submitted].

Blood eosinophil number and activity in relation to lung function in patients with asthma and with eosinophilia

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*Blood eosinophil count, serum concentrations of eosinophil cationic protein (ECP), eosinophil protein X (EPX), myeloperoxidase (MPO), and peak expiratory flow (PEF) rate were studied in 23 patients with severe labile asthma characterized by eosinophilia at the start and end of a treatment period of 5 weeks. The mean blood eosinophil count was $808 \times 10^6/L$ at the start of the treatment period. Serum ECP and EPX were significantly raised compared with that of the references, whereas the mean serum MPO level was normal. The mean PEF was significantly and negatively correlated to both blood eosinophil count and serum ECP and EPX, but the predominant correlation was that between blood eosinophil count and PEF. At the end of the treatment period, PEF had increased and the blood eosinophil count and serum ECP and EPX were reduced when these values were compared with the values at the start of the treatment period. There was a significant and negative correlation of mean PEF to serum ECP but not to the blood eosinophil count. In individual subjects, the decreases in the blood eosinophil counts and serum EPX were significantly correlated to the individual increases of mean PEF. In conclusion, the present investigation indicates that in patients with asthma and pronounced eosinophilia, the lung function of the patients was principally related to the number of circulating eosinophils, whereas, when their eosinophilia was reduced to moderate levels, the patient's lung function was closer related to the activity of the eosinophils. (*J ALLERGY CLIN IMMUNOL* 1991;87:548-57.)*

Eosinophilia is an outstanding feature in patients with asthma.¹ A relationship has previously been demonstrated between the number of eosinophils in the blood and the lung function of the patients.²⁻⁴ Exten-

Abbreviations used

ECP: Eosinophil cationic protein
EDN: Eosinophil-derived neurotoxin
EPO: Eosinophil peroxidase
EPX: Eosinophil protein X
MBP: Major basic protein
MPO: Myeloperoxidase
PEF: Peak expiratory flow

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Supported by grants from Th Bergs stiftelse, the Swedish Medical Research Council, the Medical Faculty of the University of Uppsala, and the Swedish National Environment Protection Board.

Received for publication April 24, 1990.

Revised Sept. 24, 1990.

Accepted for publication Oct. 4, 1990.

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sive evidence has been presented to suggest that the eosinophil is the major cell responsible for the inflammatory events occurring in asthma. Secretion of granular components is probably of considerable importance for the inflammatory effects of the eosinophils. The major granule constituents are highly basic, cytotoxic proteins: ECP, EPO, EPX or EDN, and MBP.⁵

TABLE I. Clinical data of the patients at the start of the study

Pt	Age (yr)	Sex	Medical treatment*	Atopy	Duration of asthma (yr)	Mean PEF %	Min PEF %
L. B.	53	M	1, 2, 3, 4,	Yes	12	58	39
L. E.	42	M	1, 2, 3	No	11	55	27
C. B.	42	M	1, 2, 3, 4	Yes	2	65	50
T. P.	62	M	1, 2, 4	Yes	18	70	59
L. W.	55	F	1	Yes	8	72	51
E. Ö.	30	M	1	Yes	12	104	96
A. M. P.	43	F	1, 2, 3, 4	Yes	10	102	79
L. E.	41	M	1	No	7	80	58
B. R.	49	M	1, 2	Yes	9	76	67
P. S.	57	M	1, 2, 3, 4	No	5	58	51
L. B.	47	F	1, 2, 3, 4	No	10	85	70
S. N.	59	M	1	Yes	13	107	97
M. G.	44	F	1, 2, 3	Yes	14	72	32
A. S. C.	18	F	1, 2, 3	Yes	2	107	102
R. F.	47	F	1, 2, 3, 4	Yes	24	76	49
I. A.	20	F	1	Yes	2	85	62
I. R.	53	F	1, 2, 3, 4	No	2	73	54
E. L.	65	F	1, 3	No	60	92	66
B. S.	50	F	1	Yes	2	105	79
T. S.	60	M	1, 3	Yes	1	82	72
C. H. K.	22	M	1, 2, 3	No	1	96	86
Y. P.	39	F	1, 2, 3, 4	Yes	38	75	48
R. V.	45	M	1, 2, 3	Yes	7	103	83

Pt, Patient; min, minimum.

*Medical treatment: 1, inhaled β_2 -agonist and inhaled steroid; 2, oral β_2 -agonist; 3, oral theophylline; 4, oral steroid.

ECP and MBP have been demonstrated to cause damage to respiratory epithelium of the type observed in bronchial asthma.⁶⁻⁸

Recent studies suggest a relationship between the hyperreactivity of the asthmatic lung and eosinophil activity, measured in terms of serum levels of eosinophil granule proteins.^{9, 10} Raised levels of serum ECP and EPX were found before challenge in patients who developed a late asthmatic reaction on allergen challenge.⁹ In a study of patients with asthma with normal baseline levels of eosinophils and serum levels of eosinophil granule proteins by Durham et al.¹¹ a relative increase of serum EDN, EPO, and ECP was noted in parallel with the late asthmatic reaction after allergen challenge. A correlation between the prechallenge serum ECP levels and the extent of exercise-induced asthma has also been demonstrated.¹⁰ A study of bronchoalveolar lavage fluid from patients with asthma demonstrated high levels of MBP, which decreased when the patients were subjected to medical treatment.¹²

Another investigation, based on natural challenge of subjects with asthma, demonstrated that the serum levels of ECP were increased in birch-pollen atopic patients with asthma during pollen season. Furthermore, the increased ECP levels correlated to bronchial hyperreactivity, as measured by the provocative con-

centration of histamine causing a 20% decrease in FEV₁.¹³

The aim of the present investigation was to study the relationship between eosinophil number and activity and lung function in patients with asthma characterized by eosinophilia. A group of 23 patients with severe labile asthma and eosinophilia ($>400 \times 10^6/L$) were investigated at the start and end of a 5-week period of medical treatment and training. The lung function of the patients was followed and measured as PEF rate, and the activity of the eosinophils was assessed as the serum concentration of the eosinophil granule proteins, ECP and EPX.

Measurement of the serum concentration of MPO was included to evaluate the possible involvement of neutrophil activation in asthma.

MATERIAL AND METHODS

Patients (Table I)

Twenty-three patients with asthma, 11 women and 12 men, in the age range of 18 to 65 years (mean, 45 years), were selected for the study. One patient was a smoker, and of the remaining 22 patients, 14 were former smokers. Sixteen patients were considered atopic based on increased serum IgE (>10 IU/ml) or positive skin prick test to at least two of a panel of 10 common standardized test extracts. Seven patients were nonatopic. All patients had a blood eosinophil count of $>400 \times 10^6$ cells per liter. All patients

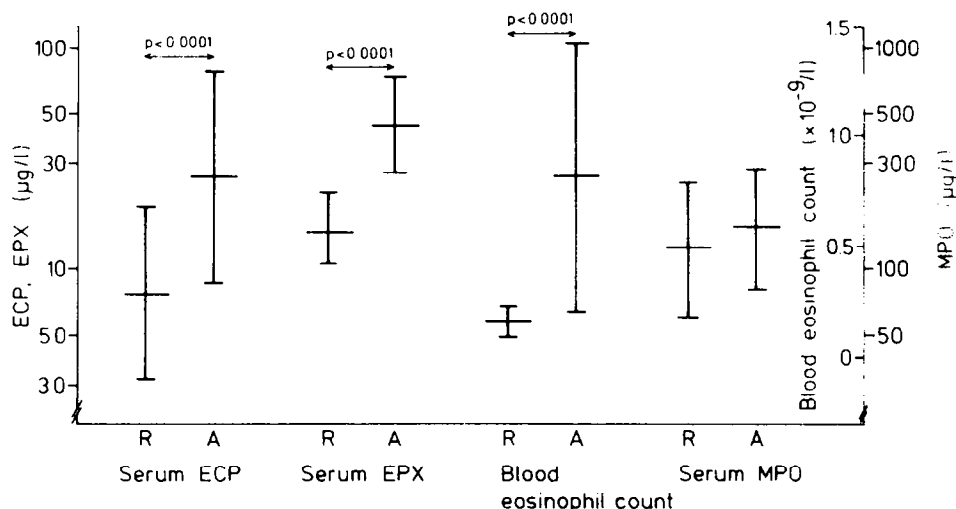


FIG. 1. Serum ECP, EPX, MPO, and blood eosinophil count in patients with asthma at the start of the study. The geometric means and ± 1 SD intervals and the significance of the differences between patients (A) and references (R) are indicated.

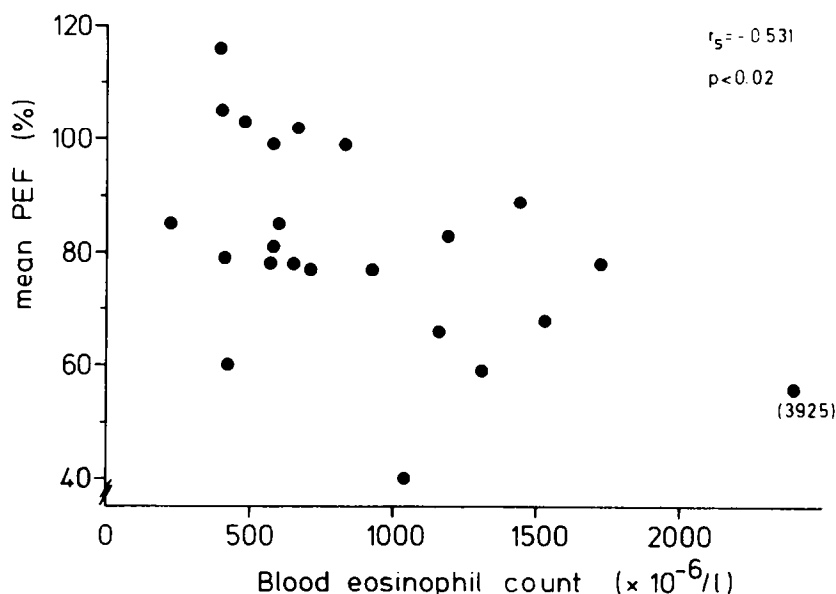


FIG. 2. Correlation between the blood eosinophil count and mean PEF at the start of the study. The coefficient of correlation and the significance of the correlation were calculated by Spearman's rank-correlation test.

suffered from unstable and severe asthma and stayed at the Asthma and Allergy Clinic, Åre Hospital, for a treatment period of, on average, 5 weeks. After informed consent, the patients participated in the study during their stay.

Medication criteria

During the first week of their stay at the clinic, corresponding to the first 2 study days, the patients received the

same medical treatment as the treatment they had received just before their arrival at the clinic. After the first week, their medications were modified to the extent that most patients reached a stabilized clinical condition at an optimal lung function level at the end of the treatment period.

Medication was fairly uniform, consisting of regular inhalation of β_2 -agonist dose, aerosols or dry powder (terbutaline or salbutamol), five times daily, and extra doses

TABLE II. Serum concentration of ECP and EPX and blood eosinophil count in atopic and nonatopic patients with asthma at the start of the treatment

	ECP (μg/L)	EPX (μg/L) (geometric mean ± SD range)	MPO (μg/L)	Blood eosinophil count (× 10 ⁶ /L) (mean ± SD range)
Patients				
Atopic (N = 16)	28 (10-78) <i>p</i> < 0.0001*	41 (27-60) <i>p</i> < 0.0001*	175 (108-286)	709 (381-1037) <i>p</i> < 0.0001*
Nonatopic (N = 7)	23 (6-82) <i>p</i> < 0.02*	54 (27-107) <i>p</i> < 0.0001*	119 (55-259)	1066 (102-2030) <i>p</i> < 0.0001*
References (N = 98)	7.7 (3-19)	15 (10-21)	127 (63-256)	160 (88-232)

*Significant differences between patients and references.

TABLE III. Lung function in patients at the start and end of the treatment period

	Min PEF (%) (mean ± SD)	Max PEF (%) (mean ± SD)	Mean PEF % (mean ± SD)
S	64 ± 22	94 ± 19	82 ± 18
E	91 ± 23*	107 ± 16*	98 ± 18*

Min, Minimum; *max*, maximum; *S*, start; *E*, end.

*Significant difference between the beginning and end of the study, *p* < 0.001 (Student's *t* test).

TABLE IV. Mean and minimum PEF of patients with blood eosinophil count and serum ECP or EPX above or within the reference intervals at the end of the treatment period

	Mean PEF (%) (mean ± SD)	Min PEF (%) (mean ± SD)	
Blood eosinophil count			
<400 × 10 ⁶ /L (N = 10)	102 ± 18	92 ± 23	
>400 × 10 ⁶ /L (N = 13)	96 ± 17	88 ± 22	
Serum ECP			
<46 μg/L (N = 21)	99 ± 18	91 ± 23	
>46 μg/L (N = 2)	99 ± 14	94 ± 17	
Serum EPX			
<30 μg/L (N = 14)	106 ± 18	97 ± 23	<i>p</i> < 0.006
>30 μg/L (N = 9)	91 ± 15	82 ± 17	
			<i>p</i> < 0.02

Min, Minimum.

The statistical significance of the differences between mean and minimum PEF, respectively, of the two groups was calculated by means of Mann-Whitney U test.

when and if doses were needed. Furthermore, the patients inhaled high-dose (800 to 1600 $\mu\text{g/day}$) topical steroids.

Budesonide was administered as aerosol via a spacer, and beclomethazone was administered either as dry powder or as aerosol via a spacer. In addition, most patients also received sustained-release theophylline tablets. The dose was titrated to a serum level of 60 to 90 μmol of theophylline per liter. Nine patients received 5 to 20 mg of prednisolone in the morning, in an alternate-day regimen from the beginning and throughout the whole study. Eight patients needed a short course of oral prednisolone during their stay to stabilize their condition. The starting dose was 30 mg in the morning, which was thereafter reduced every third day down to a 10 mg alternate-day regimen.

Parenteral and oral medication was withheld for 24 hours before blood tests.

Lung-function measurements

Lung function was followed with PEF rate measurements five times daily. The highest, the lowest, and the mean of the five PEF values each day were related to the patients' individual predicted normal PEF values and expressed as percent of the predicted normal value, as maximum PEF (percent), minimum PEF (percent), and mean PEF (percent), respectively.

Reference population

Ninety-eight apparently healthy individuals, with no history of asthma or allergy and who were free from infections for at least 2 weeks before the tests, were recruited as normal references. All subjects participated in the study after informed consent.

Blood samples

Blood samples for determination of serum ECP, EPX, and MPO, and blood eosinophil of the patients were drawn twice weekly, early in the morning. Blood samples from the references were obtained at the same time of day. The sera obtained were frozen at -70°C until later analyzed for concentration of ECP, EPX, and MPO.

Measurements of serum ECP, EPX, and MPO

The levels of ECP, EPX, and MPO in serum were measured by means of radioimmunoassays.¹⁴⁻¹⁶ The coefficients of variation of the radioimmunoassays for ECP, EPX, and MPO were 7%, 8%, and 7%, respectively. All assays were performed without knowledge of the protocol.

Eosinophil blood count

Quantitation of eosinophils in whole blood was done by use of a modified eosin stain. Cell counts were done in a Fuchs Rosenthal (Firma Walter Schrenck GmbH, Hoffheim am Taunus, Germany) chamber.

Statistical methods and calculations

Student's *t* test, Mann-Whitney U test, Spearman's rank-correlation test (r_s), and linear regression analysis (r) were used in the statistical evaluation of the results.

RESULTS

Serum concentration of ECP, EPX, and MPO in patients with asthma and with eosinophilia

At the start of the treatment period, that is, the first 2 days of the study, the patients had a mean blood eosinophil count of 808×10^6 cells per liter. The patients had significantly raised serum levels of ECP and EPX (Fig. 1) compared with that of the references. In contrast, the average levels of MPO in serum of the patients and references were similar.

Both the atopic and nonatopic patients demonstrated raised serum levels of ECP and EPX compared with that of the references (Table II).

Relationship of the serum concentrations of ECP, EPX, and MPO and the blood eosinophil count to the lung function (PEF)

At the start of the treatment period, blood eosinophil count (Fig. 2) and serum ECP ($r_s = -0.433$; $p < 0.05$) and EPX ($r_s = -0.447$; $p < 0.04$) demonstrated significant negative correlations to mean PEF. Furthermore, blood eosinophil count and serum EPX also demonstrated significant negative correlations to minimum PEF ($r_s = -0.464$ and $p < 0.03$ and $r_s = -0.421$ and $p < 0.05$, respectively) and blood eosinophil count to maximum PEF ($r_s = -0.472$; $p < 0.03$). In contrast, serum MPO was not related to the lung function of the patients ($p > 0.05$).

Blood eosinophil count, serum ECP and EPX, and lung function at the end of the treatment period

At the end of the treatment period, most patients demonstrated an improvement of their lung function, as demonstrated by the significant increase of their average minimum, maximum, and mean PEF (Table III). In addition, a significant decrease of the mean blood eosinophil count, as well as the serum concentration of ECP and EPX (Fig. 3), was found. Serum ECP (Fig. 4) was significantly and negatively correlated to mean PEF, and serum EPX was correlated to maximum PEF ($r_s = -0.504$; $p < 0.02$), whereas blood eosinophil count was not related to any of the PEF values ($p > 0.05$).

The level of blood eosinophil count and serum concentration of ECP and EPX in relation to PEF

At the end of the treatment period, nine patients had a serum level of EPX above the reference interval ($>30 \mu\text{g/L}$), and these patients also demonstrated a significantly lower mean and minimum PEF when patients were compared with patients who had an EPX

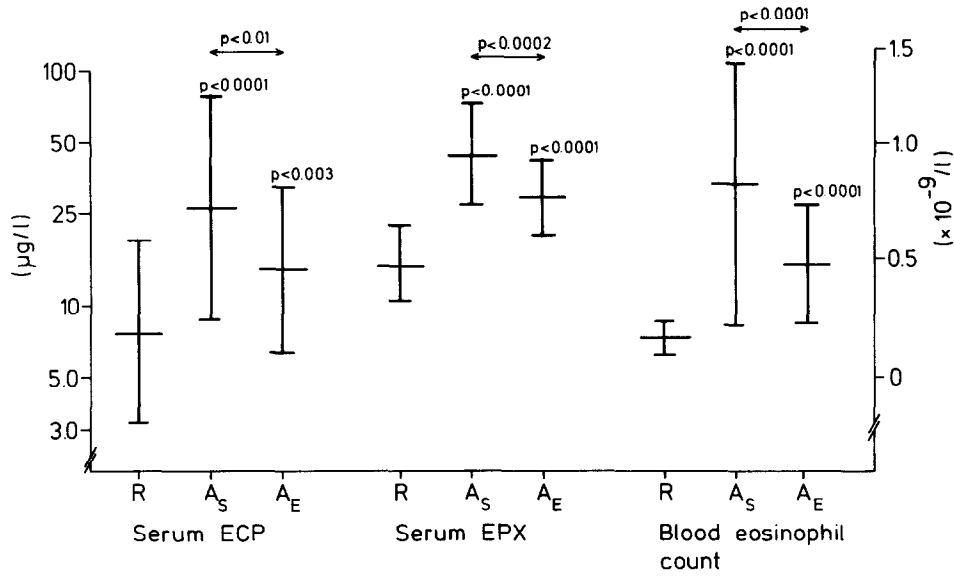


FIG. 3. Serum ECP and EPX and blood eosinophil count at the start and end of the treatment period. The geometric means and ± 1 SD intervals and the significance of the differences between references (R) and patients at the start (A_S) and end (A_E) of the treatment period are indicated. The concentration of EPX was significantly higher than that of ECP in both patient groups ($p < 0.008$ and $p < 0.0001$) and in the reference group ($p < 0.0001$).

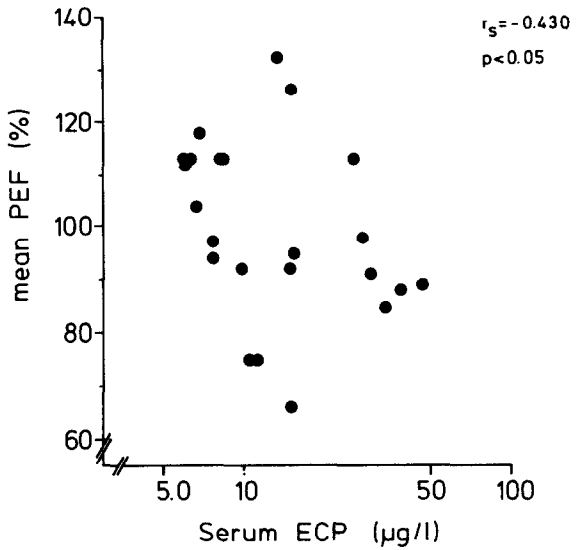
level within the reference interval (Table IV). The patients with a blood eosinophil count or a serum ECP level above the respective reference intervals demonstrated mean and minimum PEF values that were similar to values of the patients with a blood eosinophil count or a serum ECP level within the respective reference intervals.

Relationship between changes in lung function and eosinophil number and activity during the treatment period

The individual changes in blood eosinophil count demonstrated a significant and negative correlation to the changes in mean PEF (Fig. 5) and minimum PEF ($r_s = -0.679$; $p < 0.002$). Furthermore, the individual changes of serum EPX demonstrated a negative correlation to the changes in minimum PEF ($r_s = -0.500$; $p < 0.02$).

Influence of oral steroid treatment on eosinophil number and activity and PEF

Nine patients received oral steroid medication from the start and throughout the study (group S); six patients did not receive any oral steroids at all (group NS), whereas eight patients received oral steroids later during the treatment period (group ST) (Table V). At the start of the treatment period, the patients in groups S and ST had significantly lower mean and minimum PEF values than the patients in group NS. The patients



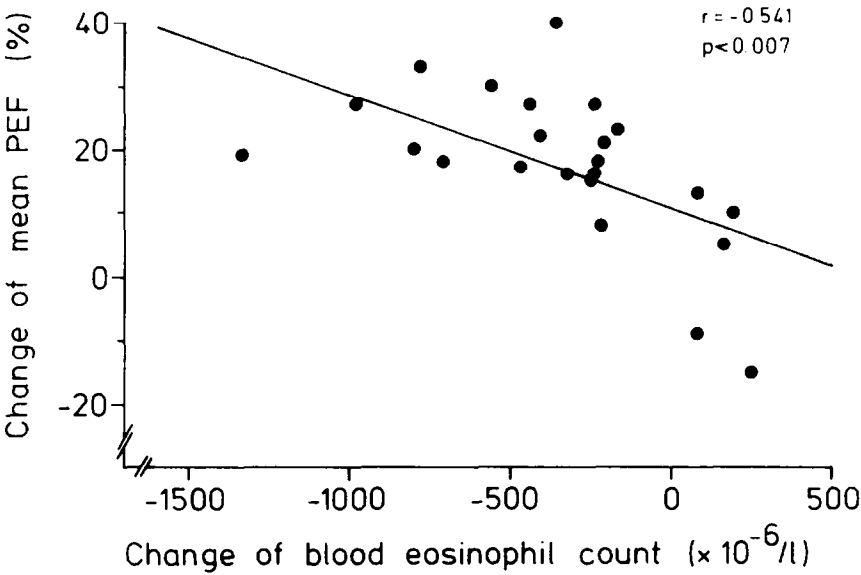


FIG. 5. Relation between the increase in mean PEF and decrease of the blood eosinophil count during the treatment period. The coefficient of correlation and the significance of the correlation were calculated by linear regression analysis.

TABLE V. Influence of oral steroid treatment on the blood eosinophil count, serum concentration of ECP and EPX, and PEF of the patients

Patient group	S (N = 9)	NS (N = 6)	ST (N = 8)
Mean age (yr)	49	44	47
Men/women	4/5	4/1	4/4
Blood eosinophil count (x 10 ⁶ /L)			
(mean ± 1 SD)			
Start*	927 ± 862	531 ± 130	972 ± 390
End*	505 ± 268	434 ± 271	469 ± 239
		***	***
Serum ECP (μg/L)			
(geometric mean [± 1 SD])			
Start	21 (7-59)	26 (7-98)	34 (13-96)
End	16 (7-33)	13 (4-41)	14 (8-25)
			*
Serum EPX (μg/L)			
(geometric mean [± 1 SD])			
Start	41 (23-73)	42 (23-75)	51 (35-76)
End	30 (20-44)	27 (18-41)	30 (21-43)
		*	**
Mean PEF (%)			
(mean ± 1 SD)			
Start	73 ± 17	97 ± 14	78 ± 14
End	92 ± 15	111 ± 14	99 ± 21
	***	**	**
Min PEF (%)			
(mean ± 1 SD)			
Start	55 ± 17	84 ± 17	54 ± 21
End	82 ± 22	107 ± 16	90 ± 22
	***	**	***

S, Oral steroid treatment at the start and throughout the treatment period; NS, no oral steroid treatment; ST = oral steroid treatment after the first two days of the study; min, minimum.

Start and end of the treatment period.

All statistical calculations were done by means of Mann-Whitney U test: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

Group NS demonstrated a significant decrease of serum EPX.

Group ST demonstrated significant correlations between the individual changes in the blood eosinophil count and mean, minimum, and maximum PEF ($r_s = -0.755$ and $p < 0.05$; $r_s = -0.762$ and $p < 0.05$; and $r_s = -0.833$ and $p < 0.03$, respectively) and between the individual changes in serum ECP and minimum PEF ($r_s = -0.786$; $p < 0.04$), whereas the two other groups did not demonstrate any significant relationships in this respect ($p > 0.05$).

Relationship between blood eosinophil count and serum concentration of ECP and EPX

The concentrations of ECP and EPX in serum from the patients were correlated to the blood eosinophil count of the patients both at the beginning ($r = 0.488$ and $p < 0.006$ and $r = 0.666$ and $p < 0.0001$, respectively) and end ($r = 0.372$ and $p < 0.02$ and $r = 0.626$ and $p < 0.0001$) of the treatment period. The concentration of EPX in serum from the references demonstrated a significant correlation to their blood eosinophil count ($r = 0.506$; $p < 0.0001$).

DISCUSSION

The aim of the present study was to investigate the relationship between eosinophil number and activity in a group of patients with severe labile asthma characterized by eosinophilia. The patients were subjected to a treatment period of 5 weeks, during which their lung function improved and their mean blood eosinophil count decreased. At the start of the treatment period, the lung function of the patients, measured as PEF, was demonstrated to be related both to number and activity of the eosinophils, measured as serum ECP and EPX. However, the most pronounced relationship was that between the number of eosinophils in blood and PEF. On the contrary, at the end of the treatment period, a relationship was found between the activity of eosinophils, as measured by serum ECP and EPX and PEF, rather than with the number of eosinophils. Serum MPO, which was included to reveal neutrophil activation, did not demonstrate any relationship to PEF, and the average MPO level of the patients was similar to that of the references.

Previous investigations have demonstrated inverse relationships between blood eosinophil count and FEV₁ and provocative concentration of histamine causing a 20% decrease in FEV₁^{2,4} in patients with asthma. The relationships between serum ECP and EPX and lung function were investigated in studies on the late asthmatic reaction induced by allergen inhalation.^{9, 17} The studies indicated that serum ECP

and EPX were more closely related to the late asthmatic reaction than to the number of circulating eosinophils. These findings suggested that the activity of the eosinophils might be more important for the development of the late asthmatic reaction than the number. In the present study of patients with asthma with pronounced eosinophilia, the PEF correlated much better with the number of eosinophils than with the serum ECP and EPX. However, when the eosinophilia of the patients was reduced by medical treatment, the relationship between the serum ECP and EPX and PEF remained, but there was no detectable relationship between the number of cells and PEF. One interpretation of the present data would be that at high eosinophil counts, the activity of the eosinophils recruited to the lung is of minor importance; instead, the mere presence of a very high number of cells that release putative precipitating agents, such as the eosinophil granule proteins, ECP and MBP, and the lipid mediators, Leukotriene C₄ and PAF, is sufficient to induce the inflammatory process involved in asthma. However, in cases with normal or moderately high eosinophil numbers, the reduced availability may be compensated for by the increased activity of the cells, which is enough to achieve sufficient concentrations of the inducers of the inflammatory process.

When the relationship between the effect of the treatment on the lung function and the number and activity of eosinophils is considered, the degree of individual improvement of mean and minimum PEF of the whole patient group was revealed to be related to the degree of decrease of the blood eosinophil count and serum EPX, respectively. When the patients were divided into three groups, depending on when and if they had received oral steroid medication, the group treated with oral steroids (ST) demonstrated a significant relationship between the individual changes in PEF and blood eosinophil count and serum ECP, respectively. The patients who were not treated with oral steroids demonstrated a significant decrease of serum EPX but not of the blood eosinophil count. Their average blood eosinophil count was, however, significantly lower than that of group ST already at the beginning of the study. It should also be kept in mind that the patients were not untreated at the start of the study, although they did not receive the optimal medical treatment. Previous studies have demonstrated that steroid medication reduces the serum levels of ECP and EPX.⁹ This finding may explain the low levels of ECP and EPX, in spite of the high blood eosinophil count, in the patient group that received oral steroid medication at the start of the study.

Previous investigations on the subcellular distribution of ECP and EPX in eosinophils demonstrated

a similar pattern of the two proteins, although EPX was present at a relatively higher content in the membrane fraction.¹⁸ The total intracellular content of ECP and EPX in normal eosinophils was estimated at 25 and 10 $\mu\text{g}/10^6$ eosinophils, respectively, whereas eosinophils from patients with hypereosinophilia demonstrated reduced contents of both proteins.¹⁹⁻²¹ The present investigation, however, demonstrated a significantly higher serum concentration of EPX than of ECP in the reference group. Furthermore, the difference between the serum levels of ECP and EPX was even more pronounced in sera from the patients with asthma in which there was hardly any overlapping between the EPX levels of patients and references in contrast to the levels of ECP. These results indicate a selective regulation of the release and/or elimination of EPX and ECP. An investigation of the secretion by eosinophils from normal individuals and patients with asthma revealed a relatively higher secretion of EPX compared with that of ECP.¹⁵ Previous investigations of the serum level of ECP and EPX in patients with asthma have also suggested an increased elimination of ECP, which was not evident for EPX.^{22, 23} Another fact that might indicate a differentiated secretion and/or elimination of ECP and EPX was the comparatively closer correlation between the blood eosinophil count and EPX than between the blood eosinophil count and ECP.

In conclusion, the present investigation of patients with asthma, characterized by eosinophilia, demonstrated a significant decrease of the eosinophil number and serum ECP and EPX during the treatment period in conjunction with an improvement of the lung function that was in contrast to the lack of effect of the treatment on the enhanced eosinophil migratory activity previously described.²⁴ The evaluation of the relationship between eosinophil number and activity and PEF demonstrated that, when the patients had pronounced eosinophilia ($\approx 800 \times 10^6/\text{L}$), PEF demonstrated the closest relationship to the blood eosinophil count. At the end of the treatment period, when the patients had moderate eosinophilia ($\approx 500 \times 10^6/\text{L}$), PEF was related to the activity of the cells measured as serum ECP and EPX. From a clinical point of view, the interpretation of the results of the present study would be that, in patients with asthma and with pronounced eosinophilia, the measurement of serum ECP and EPX offers little additional information, as compared with the blood eosinophil count. However, in patients with a normal or moderately high blood eosinophil count, the serum level of ECP and EPX offers extended information compared with the number of cells.^{9, 10, 13, 17}

The technical assistance of Mr. Peter Vornfeld is greatly appreciated. We also thank Eva Holmström, Marianne Lemmelä, Kerstin Lindblad, Birgitta Månsson, Anna-Greta Sjödin, and Viveka Widlund for laboratory assistance.

REFERENCES

1. Schatz M, Wasserman S, Patterson R. The eosinophil and the lung. *Arch Intern Med* 1982;142:1515-9.
2. Horn BR, Robin ED, Theodore J, van Kessel A. Total eosinophil counts in the management of bronchial asthma. *N Engl J Med* 1975;292:1152-5.
3. Taylor KJ, Luksha AR. Peripheral blood eosinophil counts and bronchial responsiveness. *Thorax* 1987;42:452-6.
4. Durham SR, Kay AB. Eosinophils, bronchial hyperreactivity, and late-phase asthmatic reactions. *Clin Allergy* 1985;15:411-8.
5. Spry CJF. Eosinophils: a comprehensive review and guide to the scientific and medical literature. Oxford: Oxford Medical, 1988:40-73.
6. Venge P, Dahl R, Fredens K, Peterson C. Epithelial injury of human eosinophils. *Am Rev Respir Dis* 1988;138:S54-7.
7. Ayars GH, Altman LC, Gleich GJ, Loegering DA, Baker CB. Eosinophil- and eosinophil granule-mediated pneumocyte injury. *J ALLERGY CLIN IMMUNOL* 1985;76:595-604.
8. Gleich GJ, Flavahan NA, Fujisawa T, Vanhoutte PM. The eosinophil as a mediator of damage to respiratory epithelium: a model for bronchial hypersensitivity [Aspen Allergy Conference]. *J ALLERGY CLIN IMMUNOL* 1988;81:776-81.
9. 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.
10. Venge P, Dahl R, Henriksen J. ECP in serum in exercise-induced asthma (EIA) [submitted].
11. Durham SR, Loegering DA, Dunnette S, Gleich GJ, Kay AB. Blood eosinophils and eosinophil-derived proteins in allergic asthma. *J ALLERGY CLIN IMMUNOL* 1989;84:931-6.
12. Frigas E, Loegering DA, Solley GO, Farrow GM, Gleich GJ. Elevated levels of eosinophil granule major basic protein in the sputum of patients with bronchial asthma. *Mayo Clin Proc* 1981;56:345-53.
13. 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.
14. Venge P, Roxin L-E, Olsson I. Radioimmunoassay of human eosinophil cationic protein. *Br J Haematol* 1977;37:331-5.
15. Carlson M, Håkansson L, Peterson C, Stålenheim G, Venge P. Secretion of granule proteins from eosinophils and neutrophils is increased in asthma. *J ALLERGY CLIN IMMUNOL* 1991;87:27-33.
16. Olofsson T, Olsson I, Venge P, Elgefors B. Serum myeloperoxidase and lactoferrin in neutropenia. *Scand J Haematol* 1977;18:73-80.
17. 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-34s.
18. Peterson CGB, Garcia RC, Carlson MGCh, Venge P. Eosinophil cationic protein (ECP), eosinophil protein X (EPX), and eosinophil peroxidase (EPO): granule distribution, degranu-

- lation, and characterization of released proteins [Dissertation]. In: Peterson C, ed. Eosinophil granule proteins: biochemical and functional studies. Uppsala, Sweden: Medical Faculty, University of Uppsala, 1987:74-84.
19. Peterson C, Venge P. Purification and characterization of a new cationic protein-eosinophil protein-X (EPX)-from granules of human eosinophils. *Immunology* 1983;50:19-26.
 20. Venge P, Dahl R, Fredens K, Hällgren R, Peterson CGB. Eosinophil cationic proteins (ECP and EPX) in health and disease. In: Yoshida T, Torisu M, eds. *Immunobiology of the eosinophils*. New York, Amsterdam, Oxford: Elsevier Publishing, 1983:164.
 21. Dahl R, Venge P, Olsson I. The content of eosinophil cationic protein in eosinophil leukocytes: study on normal controls and patients with bronchial asthma. *Allergy* 1978;33:152-4.
 22. Dahl R, Venge P, Olsson I. Variations of blood eosinophils and eosinophil cationic protein in serum in patients with bronchial asthma: studies during inhalation challenge test. *Allergy* 1978;33:211-5.
 23. Peterson CGB, Venge P. Interaction and complex formation between the eosinophil cationic protein and α_2 -macroglobulin. *Biochem J* 1987;245:781-7.
 24. Griffin E, Håkansson L, Formgren H, Jörgensen K, Venge P. Increased chemokinetic and chemotactic responses of eosinophils in asthmatic patients [in press]. *Allergy*.

Airway effects of inhaled bradykinin, substance P, and neurokinin A in sheep

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*The effects of inhaled bradykinin (BK), substance P (SP), and neurokinin A (NKA) on pulmonary resistance and airway responsiveness to carbachol were studied in conscious allergic sheep. Inhaled BK (20 breaths, 0.1 to 5.0 mg · ml⁻¹) caused dose-dependent increases in pulmonary resistance. Neither inhaled SP nor NKA (20 breaths, 0.1 to 1.0 mg · ml⁻¹) produced significant bronchoconstriction in allergic sheep. However, the response to SP could be enhanced (p < 0.05) by pretreatment with the neutral endopeptidase inhibitor, thiorphan (40 breaths, 1 mg · ml⁻¹). Sheep that were allergic to *Ascaris suum* antigen were 5.9 times (p < 0.05) more sensitive to the constrictor effects of BK than nonallergic sheep. BK-induced bronchoconstriction was blocked in a dose-dependent fashion by the BK β_2 -receptor antagonist, NPC 567 (D-arginine{hydroxyproline³, D-phenylalanine⁷}BK). Atropine (0.2 mg · kg⁻¹, intravenously) and nedocromil sodium (1 mg · kg⁻¹ in 3 ml of saline, aerosolized) significantly inhibited the BK-induced bronchoconstriction by 97% and 43%, respectively. Chlorpheniramine (2 mg · kg⁻¹, intravenously) had no effect. NKA caused a transient increase in airway responsiveness in allergic sheep, producing a mean 1.9-fold leftward shift in dose-response curves to aerosolized carbachol (p < 0.05). This hyperresponsiveness was not evident 24 hours after NKA challenge. Neither SP nor BK changed airway responsiveness. Thus, in allergic sheep, inhaled BK caused a more pronounced bronchoconstriction than that observed in nonallergic sheep. The bronchoconstriction was blocked by a BK-receptor antagonist and appeared to be partially mediated via cholinergic reflexes. The sensory neuropeptides, unlike BK, were not potent bronchoconstrictors when they were administered by inhalation, but NKA may play a role in modulating airway responsiveness. (J ALLERGY CLIN IMMUNOL 1991;87:557-64.)*

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Supported by National Institutes of Health Grant HLBI-33897 and the American Lung Association of Florida.

Presented at the Annual Meeting of the American Thoracic Society, Cincinnati, Ohio, May 1989.

Received for publication July 18, 1990.

Revised Oct. 1, 1990.

Accepted for publication Oct. 4, 1990.

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1/1/25932