

- effect on bronchial responsiveness of inhaled thromboxane A₂ analogue (STA₂) in guinea pigs. *J Asthma* 1989;26:237-42.
14. Katsura M, Miyamoto T, Hamanaka N, et al. In vitro and in vivo effects of new powerful thromboxane antagonists (3-alkylamino pinane derivatives). *Adv Prostaglandin Thromboxane Leukotriene Res* 1983;11:351-7.
 15. Shulman ES, Newball HH, Demers LM, Fitzpatrick FA, Adkinson NF. Anaphylactic release of thromboxane A₂, prostaglandin D₂, and prostacyclin from human lung parenchyma. *Am Rev Respir Dis* 1981;124:402-6.
 16. Wenzel SE, Westcott JY, Smith HR, Larsen GL. Spectrum of prostanoid release after bronchoalveolar allergen challenge in atopic asthmatics and in control groups: an alteration in the ratio of bronchoconstrictive to bronchoprotective mediators. *Am Rev Respir Dis* 1989;139:450-7.
 17. Yukawa T, Yamai T, Watanabe S, et al. A selective inhibitor of thromboxane biosynthesis (OKY-046) reduces the airway response to inhaled leukotriene D₄ and acetylcholine in patients with asthma. *Jpn J Thoracic Dis* 1987;25:1309-14.
 18. Beasley RCW, Featherstone RL, Church MK, et al. Effect of a thromboxane receptor antagonist on PGD₂- and allergen-induced bronchoconstriction. *J Appl Physiol* 1989;66:1685-93.
 19. Lefort J, Rotilio D, Vargaftic BB. The platelet-independent release of thromboxane A₂ by PAF aceter for guinea pig lungs involves mechanisms distinct from those for leukotriene C₄ and bradykinin. *Br J Pharmacol* 1984;82:525-31.
 20. Fuller RW, Dixon CMS, Dollery CT, Barnes PJ. Prostaglandin D₂ potentiates airway responsiveness to histamine and methacholine. *Am Rev Respir Dis* 1986;133:252-4.
 21. Black PN, Fuller RW, Taylor GW, Barnes PJ, Dollery CT. Bronchial reactivity is not increased after inhalation of leukotriene B₄ and prostaglandin D₂. *Br J Clin Pharmacol* 1988;25:667.
 22. Hamberg M, Svensson J, Samuelsson B. Thromboxanes: a new group of biologically active compounds derived from prostaglandin endoperoxides. *Proc Natl Acad Sci USA* 1975;72:2994-8.

Secretion of granule proteins from eosinophils and neutrophils is increased in asthma

Marie Carlson, MB, Lena Håkansson, PhD, Christer Peterson, DMS,
Gunnemar Stålenheim, MD,* and Per Venge, MD Uppsala, Sweden

The activity of eosinophil and neutrophil granulocytes with respect to secretion of granule proteins was studied in 30 patients with asthma and with varying severity of their disease. Granulocytes were stimulated with serum-opsonized Sephadex particles, and the released amount of eosinophil cationic protein (ECP), eosinophil protein X (EPX), and myeloperoxidase was measured by means of specific radioimmunoassays. Eosinophils from patients with asthma released significantly more ($p < 0.001$) ECP and EPX after 20 minutes of incubation than cells from control subjects without asthma. The release of myeloperoxidase from neutrophils was also somewhat higher ($p < 0.03$). The serum concentrations of ECP and EPX were also significantly increased ($p < 0.001$) in the group with asthma. No significant relationships were found between clinical variables and the secretory activity of either eosinophils or neutrophils. We conclude that eosinophils and, to some extent, neutrophils from subjects with asthma have an increased propensity to release their granule proteins, which we suggest is a consequence of priming of these cells. (J ALLERGY CLIN IMMUNOL 1991;87:27-33.)

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

Supported by grants from the Swedish Medical Research Council, the Medical Faculty of Uppsala University, the Swedish National Environment Protection Board, the Swedish Association against Heart and Lung Diseases, and Bror Hjerpesteds stiftelse.

Received for publication Feb. 7, 1990.

Revised Aug. 3, 1990.

Accepted for publication Aug. 21, 1990.

Reprint requests: Marie Carlson, MB, Laboratory for Inflammation Research, Department of Clinical Chemistry, University Hospital, S-751 85 Uppsala, Sweden.

1/1/25252

During recent years, a role for the eosinophil granulocyte in asthma has been suggested by a number of studies. Thus, the eosinophil contains several highly cytotoxic proteins, such as ECP, EPX (eosinophil-derived neurotoxin), eosinophil peroxidase (EPO), and major basic protein, which may cause damage to airway epithelium resembling the histopathologic findings in asthma.^{1,2} The eosinophils also produce the potent bronchoconstrictory agents, leukotriene C₄ and platelet-activating factor.^{3,4} Moreover, eosinophil accumulation is a common finding in the lung of patients

TABLE I. Degranulation of eosinophils and neutrophils expressed as percent of the total intracellular content (percent mean $\pm$ SEM)

Protein	Incubation time (min)	Reference group	Patients with asthma	p Value
ECP	0	0.2 $\pm$ 0.1 (N = 18)	0.1 $\pm$ 0.0 (N = 30)	NS
EPX	0	0.2 $\pm$ 0.1 (N = 17)	0.2 $\pm$ 0.1 (N = 30)	NS
MPO	0	0.1 $\pm$ 0.1 (N = 18)	0.2 $\pm$ 0.1 (N = 30)	NS
ECP	5	3.4 $\pm$ 0.4 (N = 18)	5.8 $\pm$ 0.7 (N = 29)	<0.004
EPX	5	6.1 $\pm$ 0.5 (N = 17)	9.4 $\pm$ 0.6 (N = 29)	<0.001
MPO	5	2.0 $\pm$ 0.3 (N = 18)	3.0 $\pm$ 0.3 (N = 29)	<0.02
ECP	20	7.9 $\pm$ 0.8 (N = 18)	13.0 $\pm$ 1.0 (N = 30)	<0.001
EPX	20	12.7 $\pm$ 1.3 (N = 17)	19.6 $\pm$ 1.2 (N = 30)	<0.001
MPO	20	4.0 $\pm$ 0.4 (N = 18)	6.1 $\pm$ 0.7 (N = 30)	<0.03

The statistic differences between the reference group and patients with asthma were calculated by means of the Mann-Whitney U test.

Abbreviations used

- ECP: Eosinophil cationic protein
- EPX: Eosinophil protein X
- EPO: Eosinophil peroxidase
- MPO: Myeloperoxidase
- PEF: Peak expiratory flow rate
- RIA: Radioimmunoassay

with asthma during day-to-day asthma or after allergen challenge in those patients who experience a late asthmatic reaction.⁵ In one study, patients with asthma were challenged with their allergen, and serum levels of ECP and the number of blood eosinophils were assayed as measures of activity and availability of the eosinophils. In those patients who developed a late asthmatic reaction, the eosinophil numbers and ECP levels were significantly raised before challenge in contrast to eosinophil numbers and ECP levels in those patients who only experienced an immediate reaction.^{6,7} Pretreatment with inhaled glucocorticosteroids inhibited the development of the late asthmatic reaction and concomitantly normalized the ECP levels but not the eosinophil numbers. This study thus suggested that the reduction of the activity of the eosinophil with respect to the secretion of its granule proteins is one prerequisite for the prevention of the late asthmatic reaction after allergen challenge. We therefore hy-

pothesized that the raised ECP levels might partly be explained by an increased propensity of the eosinophil to degranulate. The aim of the present study was to test this hypothesis. Thus, we have investigated the release of ECP and EPX in vitro from eosinophils obtained from patients with asthma and nonallergic individuals. For a comparison, the release of MPO from neutrophils was also measured.

MATERIAL AND METHODS

Patients

Thirty patients with asthma (according to the definition of the American Thoracic Society of 1962), 19 women and 11 men, with a mean age of 50 years (range, 22 to 79 years), were selected for the study. Six were smokers, nine were former smokers, 10 had never smoked, and the smoking history of five patients is not known. Two of the patients were considered atopic as a result of positive prick skin tests to more than one common allergen. The PEF rates at the time of examination had a mean value of 76% (34% to 123%) of expected. To evaluate further the severity of the disease, the amount of treatment needed to control their symptoms was registered. The patients were divided into four groups: (1) using only inhaled β_2 -agonists, four patients, (2) using inhaled steroids or oral β_2 -agonists in addition to inhaled β_2 -agonists, six patients, (3) using a combination of inhaled β_2 -agonists, inhaled steroids, and oral β_2 -agonists or theophylline, 17 patients, and (4) using oral corticosteroids, three patients. All patients had a well-controlled disease at the time of the study. The patients had had asthma for 2 to 50 years (median, 12 years). In the

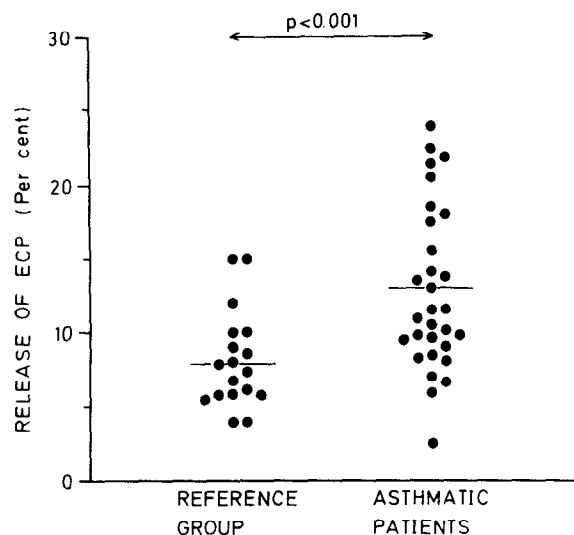


FIG. 1. Release of ECP from eosinophils of patients with asthma (N = 30) and from the reference group (N = 18) after 20 minutes of incubation with washed serum-opsonized particles. The respective means and the significant difference between the two groups, determined by Mann-Whitney U test, are indicated.

group with asthma, the mean blood eosinophil count was $484 \times 10^6/L$ (range, 30 to $1993 \times 10^6/L$).

References

The reference group consisted of 18 healthy nonallergic blood donors. Donors had no allergic symptoms and had serum concentrations of total IgE within the reference interval.

PEF

PEF rates were measured with a Wright peak flow meter. The highest value of three attempts was recorded.

Measurement of eosinophil and neutrophil degranulation

The release assay was performed according to the method of Winqvist et al.⁸ with some minor modifications. Granulocytes were isolated from heparinized blood by dextran sedimentation.⁹ The granulocyte mixture obtained by this procedure had a purity of $83\% \pm 6\%$ (SD) (references) and $82\% \pm 8\%$ (SD) (patients with asthma). The relative amount of eosinophils was, on average, 4% (range, 0% to 7%) and 9% (range, 1% to 28%), respectively, in the granulocyte populations obtained from references and patients with asthma. A volume of 300 μ l of washed serum-treated Sephadex G-15 (Pharmacia LKB Biotechnology, Uppsala, Sweden) particles (167 gm/L) was mixed with 150 μ l of granulocytes, $3 \times 10^9/L$, in plastic tubes, and incubated for 0, 5, and 20 minutes at 37°C. Hanks' buffer salt solution supplemented with Ca^{2+} , 0.74 mmol/L, and human serum albumin, 0.1%, were used as assay buffer. Degranulation

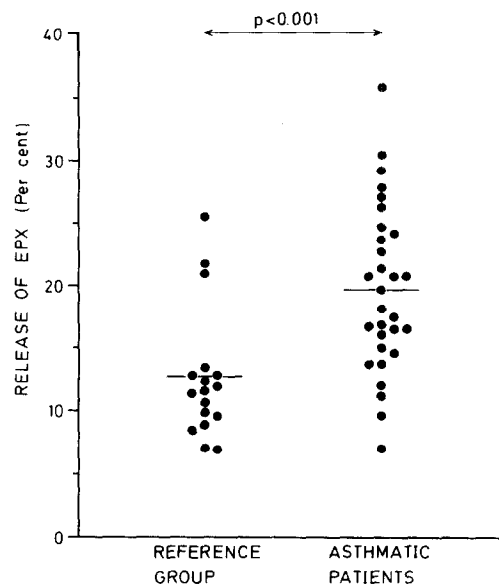


FIG. 2. Release of EPX from eosinophils of patients with asthma (N = 30) and from the reference group (N = 17) after 20 minutes of incubation with washed serum-opsonized particles. The respective means and the significant difference between the two groups, determined by Mann-Whitney U test, are indicated.

was stopped by adding 150 μ l of ice-cold 10 mmol/L of ethylenediaminetetraacetic acid in 0.15 mol/L of NaCl, followed by centrifugation at 600 g for 10 minutes at 4°C. A volume of 400 μ l of supernatant was removed and mixed with 200 μ l of 0.5% N-cetyl-N,N,N-trimethylammonium bromide in 0.15 mol/L of NaCl. In control experiments, granulocytes were incubated with buffer. All incubations were made in duplicate. For the measurement of the total cellular content of granule proteins, 200 μ l of granulocytes, $3 \times 10^9/L$, were mixed with 1 ml of 0.5% N-cetyl-N,N,N-trimethylammonium bromide.

RIA of ECP

The serum concentration and the released amount of ECP were assayed by means of a specific RIA.¹⁰

RIA of EPX

The serum concentration and the released amount of EPX were assayed by means of a specific RIA. EPX was purified according to the method of Peterson and Venge¹¹ and Peterson et al.,¹² and the concentration of purified EPX was determined with the extinction coefficient, E 1%, 1 cm = 15.5 at 280 nm.¹³ Antibodies against EPX were raised in rabbit with purified EPX. EPX was labeled with ¹²⁵I by means of the chloramin-T method.¹⁴ RIA of EPX was performed by means of a double-antibody assay. Antibodies to EPX (50 μ l), ¹²⁵I-labeled EPX (50 μ l), and the material to be assayed (50 μ l) were incubated for 3 hours at room temperature. Thereafter, 2 ml of Sepharose-antirabbit IgG (Pharmacia Diagnostics, Uppsala, Sweden) was added, and incubation continued for ½ hour at room temperature. Un-

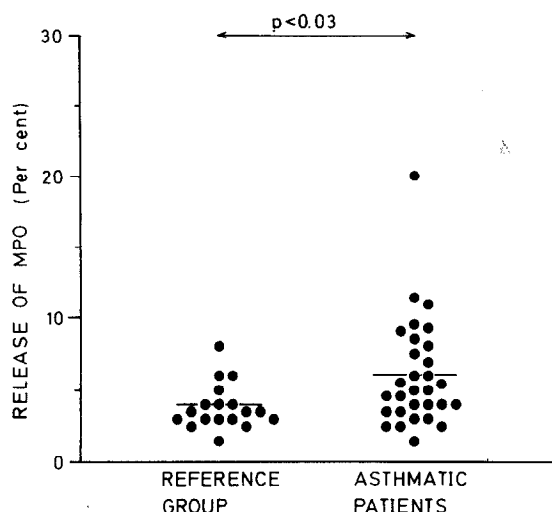


FIG. 3. Release of MPO from neutrophils of patients with asthma ($N = 30$) and from the reference group ($N = 18$) after 20 minutes of incubation with washed serum-opsonized particles. The respective means and the significant difference between the two groups, determined by Mann-Whitney U test, are indicated.

labeled and labeled EPX in complex was separated from unbound EPX by means of centrifugation at 1500 g for 10 minutes, followed by decantation of the supernatant. The sensitivity of the assay was approximately $0.7 \mu\text{g/L}$. The coefficient of variation of the assay was 7.9% (range, 5.5% to 10%) within the assay and totally, 9.6% (range, 6.6% to 13%). Cross-reactivity of the assay with ECP from eosinophils and lysozyme from neutrophils was $<0.1\%$.

RIA of MPO

The released amount of MPO was assayed by means of a specific RIA.¹⁵ There was no cross-reactivity of the assay with EPO.¹⁶

Calculation of released amount of ECP, EPX, and MPO

The amount of released ECP, EPX, and MPO was expressed as percent of the total cellular content that was calculated from a standard curve of serial dilutions of the respective cell extract. The amount of protein in control incubations was subtracted from the amounts obtained in incubations with particles. Results were calculated with regression analysis (ECP and MPO) or cubic spline program (EPX).

Blood eosinophil count

The number of eosinophil granulocytes in blood was counted according to the method of Forsham et al.¹⁷

Statistical evaluation

Wilcoxon's signed-rank test, Mann-Whitney U test, and Student's *t* test were used. All statistical calculations were

performed on a personal computer by means of the statistical package, Statgraphics (STSC, Inc., Rockville, Md.).

RESULTS

Eosinophil and neutrophil degranulation

Eosinophil degranulation was measured by means of the release of ECP and EPX. Eosinophils from the patients with asthma, after 20 minutes of incubation, released significantly higher ($p < 0.001$ and $p < 0.001$, respectively) amounts of both ECP and EPX than eosinophils from the reference group (Figs. 1 and 2).

Neutrophil degranulation was measured by means of MPO release. Neutrophils from the patients with asthma, after 20 minutes of incubation, released significantly higher amounts ($p < 0.03$) of MPO than neutrophils from the reference group (Fig. 3).

As presented in Table I, a significantly increased release of ECP ($p < 0.004$), EPX ($p < 0.001$), and MPO ($p < 0.02$) was demonstrated already after 5 minutes of incubation of cells from the patients with asthma compared with that from the reference group.

The intracellular content of ECP and EPX

The amount of ECP and EPX per eosinophil was estimated. There was no difference between the amount of ECP in eosinophils from the patients with asthma ($34 \mu\text{g}/10^6$ eosinophils) compared with that of eosinophils from the reference group ($33 \mu\text{g}/10^6$ eosinophils). The intracellular concentrations of EPX was $17 \mu\text{g}/10^6$ eosinophils from both the group of patients with asthma and the reference group.

Kinetics of eosinophil degranulation

Degranulation of ECP and EPX was studied. After 5 minutes of incubation with the washed serum-opsonized Sephadex G-15 particles, the relative release of EPX was significantly higher than that of ECP in both the group with asthma and in the reference group ($p < 0.001$ and $p < 0.001$, respectively) (Fig. 4, A and B). After 20 minutes of incubation, the mean level of EPX degranulation was even more pronounced and significantly higher than that of ECP in the group with asthma as well as in the reference group ($p < 0.001$ and $p < 0.001$, respectively) (Fig. 4, A and B). The level of the ECP and EPX in medium at time zero was negligible, both in the group with asthma and the reference group, which indicate that the cells were viable and not unspecifically activated before the experiments.

Serum concentration of ECP and EPX

In the group with asthma, the mean serum concentration of ECP was $45.4 \mu\text{g/L}$ (range, 3.1 to 155

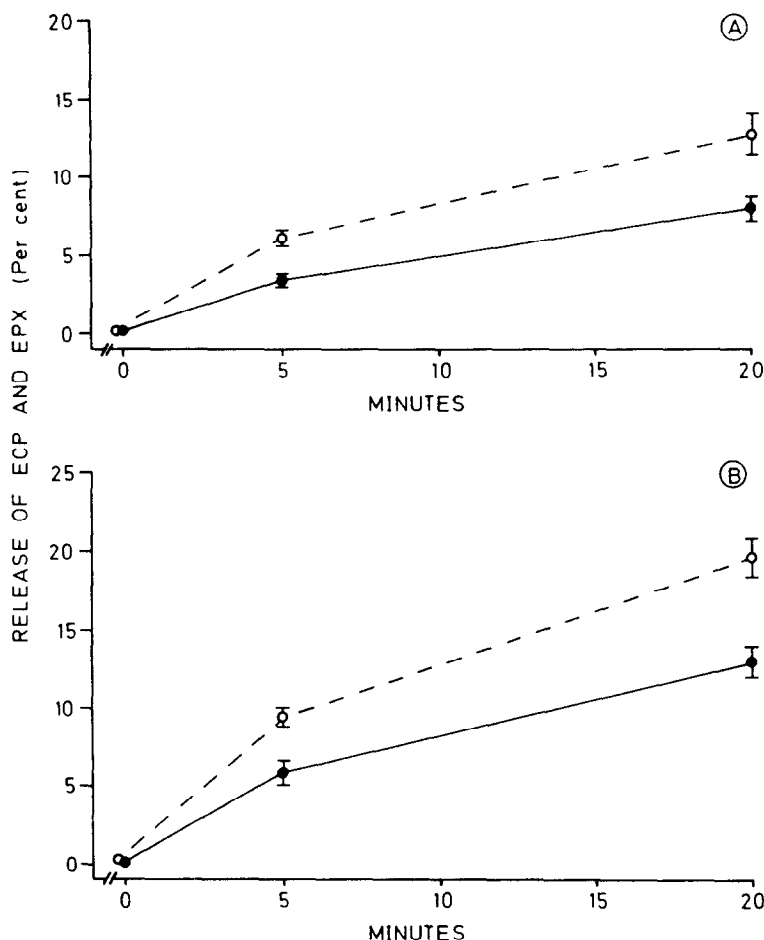


FIG. 4. A, Kinetics of ECP (N = 18) (●-●-●) and EPX (N = 17) (○-○-○) release from eosinophils of the reference group are listed together with the mean $\pm$ SEM for each incubation time. The relative release of EPX was significantly higher than the ECP release after 5 as well as 20 minutes of incubation ($p < 0.001$ and $p < 0.001$, respectively) (Wilcoxon's signed-rank test). B, Kinetics of ECP (N = 30) (●-●-●) and EPX (N = 30) (○-○-○) release from eosinophils of the patients with asthma are listed together with the mean $\pm$ SEM for each incubation time. The relative release of EPX was significantly higher than the ECP release after 5 as well as 20 minutes of incubation ($p < 0.001$ and $p < 0.001$, respectively) (Wilcoxon's signed-rank test).

$\mu\text{g/L}$) and of EPX, $41.7 \mu\text{g/L}$ (range, 14.4 to $121 \mu\text{g/L}$). Both values were significantly higher ($p < 0.001$ and $p < 0.001$, respectively) than the mean concentrations of ECP and EPX in the reference group (Table II). There were no correlations between serum levels and degranulation of ECP and EPX, respectively, in vitro.

Relationships between the clinical conditions of the patients and the degranulation of eosinophils and neutrophils

The released amount of ECP, EPX, and MPO and the serum concentrations of ECP and EPX were evaluated in relation to the patients' lung function, mea-

sured as PEF, the duration of their disease, and the amount of medical treatment needed to control their disease. No discernible relationships were, however, found.

DISCUSSION

The present study has demonstrated that eosinophils and, to some extent, neutrophils obtained from the peripheral blood of patients with asthma have an increased propensity to release their granule proteins to the extracellular environment. Thus, our hypothesis presented in this study has gained some support. The stimulus used to induce degranulation was the washed serum-opsonized Sephadex G-15 particle, which is a noningestible stimulus and which probably mediates

TABLE II. Serum concentration of ECP and EPX in the patients with asthma and in a reference group

Variables	Reference group	Patients with asthma	<i>p</i> Value
S-ECP	11.6 µg/L (N = 27) (range, 0.3-43.3)	45.4 µg/L (N = 28) (range, 3.1-155)	<0.001
S-EPX	18.4 µg/L (N = 28) (range, 10.1-33.8)	41.7 µg/L (N = 28) (range, 14.4-121)	<0.001

The statistic differences between the reference group consisting of apparently healthy blood donors and laboratory employees and the patients with asthma were calculated by means of Student's *t*-test.

its action via the C₃b-receptor.⁸ This stimulus was chosen since it is more physiologic than most other candidate stimuli, such as phorbol esters or calcium ionophores.

The reason for the enhanced degranulation is not well understood. It cannot, however, be explained by differences in content of the granule proteins. It might involve the enhancement of the C₃b-receptors or the general activation of the cells to respond more readily to any stimulus. Thus, C₃b-receptor enhancement of neutrophils and on blood monocytes has been demonstrated in subjects with asthma after allergen challenge.¹⁸ In other studies, we demonstrated that the responsiveness of the eosinophils to chemotactic and chemokinetic stimuli was enhanced in subjects with asthma, which indicates that the enhanced responsiveness is not restricted to a single function of the eosinophil but a more general phenomenon.¹⁹

The mechanism behind the enhancement of eosinophil activities in asthma is not known, but tentatively, it involves the activation of the mature eosinophil by cytokines, such as interleukin-5 or other eosinophil-activating factors described to be produced by the monocyte and the T-lymphocyte.^{20, 21} In the present study, however, there were no obvious relationships to number of eosinophils or to the serum levels of either ECP or EPX, although these variables were all very much raised. This finding was in contrast to the enhanced migratory responsiveness of the eosinophils that was higher in patients with eosinophilia and even in some variables linearly correlated to the serum ECP levels.¹⁹ These differences may therefore indicate the possibility that the enhancement of degranulation is governed by mechanisms different from mechanisms that cause the enhanced migratory response. Furthermore, the enhanced migratory responsiveness was more clearly restricted to the enhancement of the eosinophil response only, whereas the results in this study suggest that the neutrophils also were activated, which would support this notion.

In this study, we measured the release of two eosinophil-specific proteins, ECP and EPX. The kinetics of the release demonstrated a much higher relative release of EPX in both the group with asthma and the reference group. This finding might indicate that the eosinophils selectively release their granule proteins, depending on type of stimulus to which the cells are exposed. Selective release has been demonstrated previously in vitro with eosinophils exposed to IgE or IgG-mediated stimuli.²² Thus, IgE-mediated stimuli preferentially caused the release of EPO, whereas ECP was preferentially released with IgG stimuli. The preferential release of EPX from eosinophils has been demonstrated after exposure of the cells to the triglyceride IpOCOC₉,²³ which is an activator of protein kinase C in the plasma membrane.²⁴ The mechanism behind the possible selective release of granule proteins from eosinophils may involve differences in the compartmentalization of the proteins, since both peroxidase-positive and peroxidase-negative granules have been identified and which both contain ECP and EPX.^{23, 25}

There was no simple relationship between the increased degranulation of eosinophils and neutrophils and the clinical activity of the asthma disease of the patients. The reason might be explained by the fact that all patients had a well-controlled disease with respect to their pulmonary functions. The asthma medication may also have modified and reduced the release of the granule proteins from both the eosinophils and the neutrophils, since both β₂-agonists and glucocorticosteroids are known to reduce the release of the granule proteins.^{6, 26} Therefore, the results presented in this study may not correctly reflect the real activity of the cells in a patient with asthma. Rather, the activity may have been underestimated. However, the mere fact that we were able to demonstrate an enhanced propensity of granulocytes from patients with asthma to degranulate their proteins in spite of these medications is indeed intriguing and may explain

some of our data on serum-ECP measurements in asthma,^{6, 7, 27-29} as well as data reported from other investigators.³⁰

We conclude that eosinophils, and to a somewhat lesser extent, neutrophils, obtained from a group of patients with well-controlled asthma have an increased propensity to release their granule proteins. This phenomenon may reflect the fact that these cells have been primed by unknown factors, tentatively, some cytokine, as part of the asthmatic inflammatory process. The increased propensity to release cytotoxic proteins, such as ECP and EPX, may be an important pathophysiologic mechanism in asthma that may enhance the cytotoxic potential of the eosinophil.

We appreciate the skillful technical assistance of Kerstin Lindblad and Lena Moberg.

REFERENCES

- 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.
- Slifman NR, Peterson CGB, Gleich GJ, Dunette SL, Venge P. Human eosinophil-derived neurotoxin and eosinophil protein X are likely the same protein. *J Immunol* 1989;143:2317-22.
- Shaw RJ, Cromwell O, Kay AB. Preferential generation of leukotriene C₄ by human eosinophils. *Clin Exp Immunol* 1984;56:716-22.
- Lee T-c, Lenihan DJ, Malone B, Roddy LL, Wasserman SI. Increased biosynthesis of platelet-activating factor in activated human eosinophils. *J Biol Chem* 1984;259:5526-30.
- De Monchy JGR, Kauffman HF, Venge P, et al. Bronchoalveolar eosinophilia during allergen-induced late asthmatic reactions. *Am Rev Respir Dis* 1985;131:373-6.
- 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.
- 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.
- Winqvist I, Olofsson T, Olsson I. Mechanisms for eosinophil degranulation; release of the eosinophil cationic protein. *Immunology* 1984;51:1-8.
- Håkansson L, Venge P. The influence of serum on random migration and chemotaxis of polymorphonuclear leukocytes: methodological evaluation using sera from infection-prone patients and normals. *Scand J Immunol* 1980;11:271-82.
- Venge P, Roxin L-E, Olsson I. Radioimmunoassay of human eosinophil cationic protein. *Br J Haematol* 1977;37:331-5.
- Peterson CGB, Venge P. Purification and characterization of a new cationic protein—eosinophil protein-x(EPX)—from granules of human eosinophils. *Immunology* 1983;50:19-26.
- Peterson CGB, Jörnvall H, Venge P. Purification and characterization of eosinophil cationic protein from normal human eosinophils. *Eur J Haematol* 1988;40:415-23.
- Gleich GJ, Loegering DA, Bell MP, Checkel JL, Ackerman SJ, Kean DJ. Biochemical and functional similarities between human eosinophil-derived neurotoxin and eosinophil cationic protein: homology with ribonuclease. *Proc Natl Acad Sci USA* 1986;83:3146-50.
- Hunter WM, Greenwood FC. Preparation of iodine 131 labelled human growth hormone of high specific activity. *Nature* 1962;194:495-6.
- Olofsson T, Olsson I, Venge P, Elgefors B. Serum myeloperoxidase and lactoferrin in neutropenia. *Scand J Haematol* 1977;18:73-88.
- Öberg G, Lindmark G, Moberg L, Venge P. The peroxidase activity and cellular content of granule proteins in PMN during pregnancy. *Br J Haematol* 1983;55:701-8.
- Forsham PH, Thorn GW, Prunty FTG, Hills AG. Clinical studies with pituitary adrenocorticotropin. *J Endocrinol* 1948;8:15-66.
- Durham SR, Carroll M, Walsh GM, Kay AB. Leukocyte activation in allergen-induced late-phase asthmatic reactions. *N Engl J Med* 1984;311:1398-1402.
- Håkansson L, Carlson M, Stålenheim G, Venge P. Migratory responses of eosinophil and neutrophil granulocytes from asthmatic patients. *J ALLERGY CLIN IMMUNOL* 1989;85:743-50.
- Silberstein DS, Austen KF, Owen WF Jr. Hemopoietins for eosinophils: glycoprotein hormones that regulate the development of inflammation in eosinophilia-associated disease. *Hematol Oncol Clin North Am* 1989;3:511-34.
- Silberstein DS, Ali MH, Baker SL, David JR. Human eosinophil cytotoxicity-enhancing factor: purification, physical characteristics, and partial amino acid sequence of an active polypeptide. *J Immunol* 1989;143:979-83.
- Capron M, Leprevost C, Prin L, et al. Immunoglobulin-mediated activation of eosinophils. In: Morley J, Colditz I, eds. *Eosinophils in asthma*. London, New York, Tokyo: Academic Press, 1989:49-60.
- Peterson CGB, Garcia RC, Carlson MGCh, Venge P. Eosinophil cationic protein (ECP), eosinophil protein x (EPX) and eosinophil peroxidase (EPO): Granule distribution, degranulation and characterization of released proteins. In: Peterson C, ed. *Eosinophil granule proteins. Biochemical and functional studies [Thesis]*. Uppsala, Sweden; Uppsala University, 1987: 1-19.
- Olsson H, Bergstrand H. Activation of human neutrophil protein kinase C in vitro by 1,2-isopropylidene-3-decanoyl-sn-glycerol (IpOCOC₃). *Cell Sig* 1989;1:405-10.
- Egesten A, Alumets J, Von Mecklenburg C, Palmegren M, Olsson I. Localization of eosinophil cationic protein, major basic protein and eosinophil peroxidase in human eosinophils by immunoelectron microscopic technique. *J Histochem Cytochem* 1986;34:1399-1403.
- Dahl R, Venge P. Blood eosinophil leucocyte and eosinophil cationic protein. In vivo study of the influence of beta-2-adrenergic drugs and steroid medication. *Scand J Respir Dis* 1978;59:319-32.
- Dahl R, Venge P, Olsson I. Variations of blood eosinophils and eosinophil cationic protein in serum of patients with bronchial asthma. *Allergy* 1978;33:211-215.
- Venge P, Zetterström O, Dahl R, Roxin L-E, Olsson I. Low levels of eosinophil cationic proteins in patients with asthma. *Lancet* 1977;20:373-5.
- Dahl R, Venge P, Fredens K. The eosinophil. In: Barnes PJ, Rodger I, Thomson N, eds. *Asthma: basic mechanisms and clinical management*. London: Academic Press, 1988:115-30.
- 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.