

Increased chemokinetic and chemotactic responses of eosinophils in asthmatic patients

E. GRIFFIN¹, L. HÅKANSSON², H. FORMGREN¹, K. JÖRGENSEN¹ & P. VENGE²

¹Asthma and Allergy Clinic, Åre Hospital, Åre and ²Laboratory for Inflammation Research, Department of Clinical Chemistry, University Hospital, Uppsala, Sweden

The aim of the present study was to investigate the migratory responses of eosinophil and neutrophil granulocytes from asthmatic patients compared with granulocytes from healthy individuals. Twenty-three patients with unstable and severe asthma and blood eosinophilia ($> 400 \times 10^6$ cells/l) were selected for the study. Eosinophil and neutrophil chemotactic and chemokinetic responses were tested twice, at the beginning and end of a 5 week treatment period. Lung function was followed by daily measurements of PEF. The eosinophils of the asthmatics demonstrated increased chemokinetic responses to albumin, autologous serum, and normal human serum (NHS), and an increased chemotactic response to NHS at the beginning of the treatment period compared with eosinophils from the references. At the end of the period, the eosinophil chemokinetic responses to albumin, autologous serum and NHS were still increased and so was the chemotactic response to zymosan-activated serum (ZAS). The neutrophil migratory responses were not increased compared with those of the references, except for the chemokinetic response to autologous serum, which was increased both at the beginning and end of the treatment period. Patients in whom the eosinophil migratory responses, to most of the agents used, decreased over the treatment period, demonstrated a significantly greater improvement of their lung function at the end of the period compared with patients in whom the eosinophil migratory responses increased. However, no direct relationship between eosinophil migratory responses and lung function of the patients was found. In conclusion, the present investigation demonstrated increased migratory responses of eosinophils from asthmatic patients. This enhanced responsiveness is proposed to be due to priming of the eosinophils *in vivo*, and might be one mechanism behind the selective recruitment of eosinophils to the lungs of asthmatics.

Key words: asthma; chemokinesis; chemotaxis; eosinophil.

Accepted for publication 7 October 1990

Asthma is often characterized by peripheral blood

eosinophilia. A positive correlation has been found between the number of eosinophils in the blood and the severity of the symptoms (14, 15, 28). Infiltration of the respiratory tissues is also a common feature of asthma. Large numbers of eosinophils are usually found in and around the airway tissues from patients who have died of an asthmatic attack (3, 5). Furthermore, bronchoalveolar lavage fluid (BAL) obtained from asthmatic patients during allergen challenge and from birch pollen-allergic asthmatics during the

* AS: autologous serum; BAL: bronchoalveolar lavage; CV: coefficient of variation; EAF: eosinophil-activating factor; ECP: eosinophil cationic protein; f-MLP: formyl-methionyl-leucylphenylalanine; GM-CSF: granulocyte-macrophage colony-stimulating factor; HL-ECA: heat-labile eosinophil chemotactic activity; HL-NCA: heat-labile neutrophil chemotactic activity; LTB₄: leukotriene B₄; NHS: pooled normal human serum; PAF: platelet-activating factor; PEF: peak expiratory flow; PMN: polymorphonuclear leu

Table 1

The patients' lung function and blood eosinophil count at the beginning (B) and end (E) of the study

	min. PEF (%) (mean $\pm$ SD)	max. PEF (%) (mean $\pm$ SD)	VPEF (l/min) (mean $\pm$ SD)	Blood eosinophil count ($\times 10^6/l$) (mean $\pm$ SD)
B	64 $\pm$ 22	94 $\pm$ 19	140 $\pm$ 64	818 $\pm$ 609
E	91 $\pm$ 23 ^a	107 $\pm$ 16 ^a	75 $\pm$ 51 ^a	475 $\pm$ 255 ^a

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

birch pollen season contained an increased number of eosinophils (21, 25).

The current hypothesis suggests that the eosinophils are recruited from the blood to the lung tissues by means of chemotactic factors produced by cells in the lung, such as mast cells, alveolar macrophages and T lymphocytes (6, 7, 16, 23). Raised eosinophil chemotactic activity has been demonstrated in BAL fluid as well as in serum from asthmatics during allergen challenge and from birch-allergic asthmatics during the pollen season (9, 22, 25, 26).

However, the production of chemotactic factors is probably not the sole mechanism behind the accumulation of eosinophils in the lungs of asthmatic patients. Thus, neutrophil chemotactic activity has also been demonstrated in BAL from asthmatics during allergen challenge and from birch pollen-allergic asthmatics during the pollen season, despite the fact that the number of neu-

trophils in BAL was not increased (21, 22, 25). Further, at least *in vitro*, essentially all chemotactic factors that attract eosinophils also attract neutrophils. This is the case with the well-known chemoattractants C5a, LTB₄, f-MLP and PAF, although PAF has been demonstrated to be a more potent chemoattractant for eosinophils than for neutrophils (13, 36). To explain the selective accumulation of eosinophils in the airways of asthmatics, we hypothesize that the eosinophils of these patients are primed, meaning that their responsiveness to chemotactic factors is increased.

The aim of the present study was to investigate the migratory responses of eosinophils and neutrophils from asthmatic patients compared with those of cells from healthy individuals and in relation to their lung function, measured as peak expiratory flow. The chemotactic and chemokinetic responses of eosinophils and neutrophils

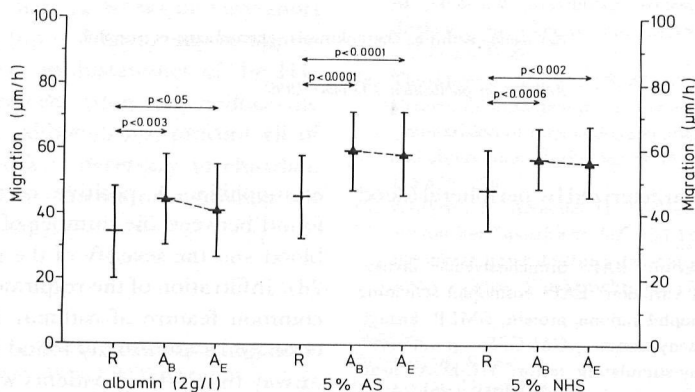


Fig. 1. Chemokinetic responses of eosinophils from asthmatics (A, $n = 23$) at the beginning (B) and end (E) of a treatment period of 5 weeks, compared with the responses of eosinophils from references ($n = 96$). Means and $\pm$ SD interval of the respective groups are indicated. Differences between the respective patient groups and the references were analysed by means of Student's *t*-test.

from 23 patients with unstable and severe asthma have been studied at the beginning and end of a 5 weeks stay in an asthma rehabilitation hospital.

MATERIAL AND METHODS

Twenty-three asthmatic patients (11 women and 12 men), in the age range 18–65 years (mean 45 years) were selected for the study. Sixteen patients were atopic, increased IgE (> 10 IU/ml) or positive skin prick test to at least two of a panel of 10 common standardized test extracts, and seven were non-atopic. All had a blood eosinophil count of more than 400×10^6 cells/l. They all suffered from unstable and severe asthma and were therefore referred from all parts of Sweden to the Asthma and Allergy Clinic, Åre Hospital, which specializes in the rehabilitation of asthmatics. After informed consent, the patients took part in the study during their stay, on average 5 weeks. The patients' lung function and blood eosinophil count are presented in Table 1.

During the first week of their stay at the clinic, corresponding to the first 2 study days, the patients continued the medical treatment they had received just prior to their arrival to the clinic. After the first week their medications were modified, such that the majority reached a stabilized clinical condition at an optimal lung function level at the end of the study.

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 when and if needed. Further, the patients inhaled high-dose (800–1600 μ g/day) topical steroids. Budesonide was administered as dose-aerosol via a spacer and beclometazone either as dry powder or as dose-aerosol via a spacer. In addition, most patients also needed sustained release theophylline tablets. The dose was titrated to a serum level of 60–90 μ mol theophylline/l. Nine patients received a dose of 5–20 mg 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 asthma

medication when used, was withheld for 24 h prior to blood tests.

Lung function was followed with peak expiratory flow measurements (PEF) recorded 5 times daily before and after inhaled β_2 -agonists over the whole study period. The highest and lowest PEF values were related to the patients' individual predicted normal PEF values and expressed in per cent, as max. PEF (%) and min. PEF (%) respectively. The PEF values recorded on the day of blood sampling were used in these calculations. The variation in PEF (VPEF) was expressed as the difference between the highest and lowest PEF values on each day (l/min).

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

Patients blood samples were drawn twice weekly, early in the morning. One healthy reference subject was selected for each test day. The sera obtained were frozen at -70°C , until later analysed for eosinophil and neutrophil chemokinetic and chemotactic activity and ECP concentration.

Migration assays for eosinophils and neutrophils were performed using a modification of the Boyden chamber assay described in detail elsewhere (13). Granulocytes were isolated from heparinized blood by dextran sedimentation (10). The granulocyte suspension obtained by this procedure had a purity of 66 ± 9 (SD) % (references), respectively 69 ± 9 (SD) % (asthmatics). The relative amount of eosinophils was on average 3 % (range 0–8), 10 % (range 0–25 %) respectively, in the granulocyte populations obtained from references and asthmatics. The final concentration of granulocytes was 1.5×10^9 /l in all assays. The filter pore size was 5 μ m (Millipore Corp. Bedford, MA, USA). Incubation was performed for 1 h at 37°C . The filters were stained with Mayer's Hämälunlösung and Chromotrope 2R, according to a procedure previously described, to enable detection of eosinophil and neutrophils (13). The migration of eosinophils and neutrophils was assessed in duplicated filters, according to the leading front method of Zigmond & Hirsch (37). A chemokinetic response is defined as the enhancement of random migration caused by the

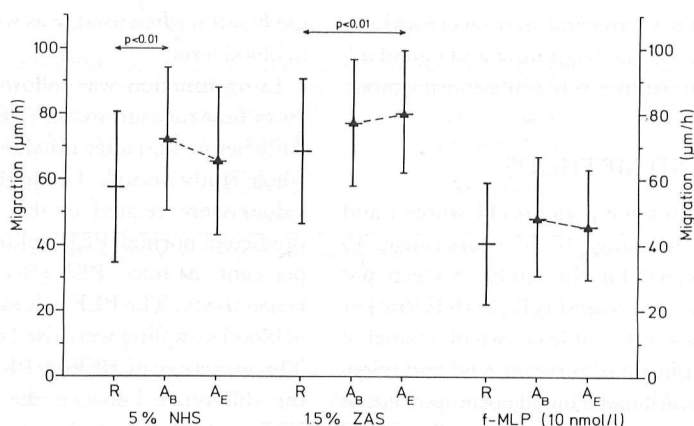


Fig. 2. Chemokinetic responses of eosinophils from asthmatics (A, $n = 23$) at the beginning (B) and end (E) of a treatment period of 5 weeks, compared with the responses of eosinophils from references ($n = 96$). Means and $\pm$ SD interval of the respective groups are indicated. Differences between the respective patient groups and the references were analysed by means of Student's t -test.

presence of a chemokinetic stimulus in the cell suspension (11). A chemotactic response is defined as the increased migration caused by the attraction of the cells by a chemotactic agent (12, 17).

The chemokinetic responses of eosinophils and neutrophils were measured with albumin (2 g/l) (AB Kabi, Stockholm, Sweden), 5% autologous serum (AS) and 5% normal fresh frozen human serum (NHS) as chemokinetic agents.

The chemotactic responses of eosinophils and neutrophils were measured using 5% NHS,

1.5% ZAS (12) and f-MLP, 10 nmol/l (Sigma Chemical Company, St. Louis, MO, USA) as chemoattractants.

The eosinophil and neutrophil chemokinetic and chemotactic activities of fresh frozen serum were assayed using granulocytes from healthy blood donors and with NHS as reference (= 100%) (11, 12, 26, 33). The inter-day variation of the method was 12.8% (CV), and 10.5% (CV), respectively, of the assay for the chemotactic and chemokinetic activity of serum. The pre-

Table 2

Neutrophil migratory responses of healthy persons and asthmatics at the beginning (B) and end (E) of the study

Test	References (<i>n</i> = 97)	Patients	Patients
		B (<i>n</i> = 23)	E (<i>n</i> = 23)
Migration (μm/h)			
Chemokinetic response			
Albumin (2 g/l)	60 ± 16	59 ± 17	62 ± 15
5 % autologous serum	57 ± 15	66 ± 16 ^a	69 ± 19 ^b
5 % NHS	60 ± 16	63 ± 13	63 ± 15
Chemotactic response			
5 % NHS	79 ± 27	80 ± 27	79 ± 23
1.5 % ZAS	96 ± 23	96 ± 23	98 ± 21
f-MLP (10 nmol/l)	73 ± 28	72 ± 28	74 ± 29

Results are given as means $\pm$ SD.

^a Significant difference between patients and references, $P < 0.01$ (Student's t -test).

^b Significant difference between patients and references, $P < 0.001$ (Student's t -test).

sent method is designed to measure the heat-labile (56°C , 30 min) eosinophil and neutrophil chemotactic activity (HL-ECA, HL-NCA) of serum (9).

The level of ECP in serum was measured by means of a radioimmunoassay (35). In the present study, the geometric mean of the concentration of ECP in serum from healthy individuals was $8\text{ }\mu\text{g/l}$, (range $1\text{--}50\text{ }\mu\text{g/l}$).

Statistical methods and calculations.

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

RESULTS

Chemotactic and chemokinetic responses of eosinophils and neutrophils

The results from the 2 study days of the first week, when the medical treatment was unaltered, were used for evaluation of the migratory responses of patient cells at the beginning of the treatment period. As shown in Figs. 1 and 2, eosinophils from the asthmatics demonstrated increased chemokinetic responses to albumin, autologous serum and NHS and a significantly increased chemotactic response towards NHS at the beginning of the treatment period, compared with that of eosinophils from the references.

The results of the last 2 study days were used to evaluate the migratory responses of the granulocytes when the clinical condition of the patients had improved (Table 1). Figs. 1 and 2 show that eosinophils from the asthmatics still demonstrated increased chemokinetic responses to albumin, autologous serum and NHS, and an increased chemotactic response towards ZAS at the end of the treatment period.

Neutrophils from asthmatics did not, in general, demonstrate increased chemokinetic or chemotactic responses compared with those from the references. The only exception was the significantly ($P < 0.01$) increased chemokinetic re-

sponse to autologous serum of patient neutrophils, both at the beginning and end of the treatment period, compared with that of the reference neutrophils (Table 2).

There were no differences in the eosinophil or neutrophil migratory responses between atopics and non-atopics ($P > 0.05$).

Eosinophil and neutrophil chemokinetic and chemotactic activities of serum.

The eosinophil and neutrophil chemokinetic activities of sera from the asthmatics were significantly raised compared with those of reference sera, both at the beginning and end of the treatment period (Table 3). However, the heat-labile eosinophil and neutrophil chemotactic activities of sera from the asthmatic patients were not altered compared with those of sera from the references (Table 3).

Eosinophil migratory responses in relation to number of eosinophils in the blood.

Originally, the patients were selected for the study based on the criteria of a blood eosinophil count of $> 400 \times 10^6/\text{l}$. As shown in Figs. 3 and 4, patients who still had a blood eosinophil count of $> 400 \times 10^6/\text{l}$ at the end of the study also had significantly increased eosinophil migratory responses to all of the chemokinetic factors and to all of the chemoattractants tested, except f-MLP, compared with that of the references. Patients with a blood eosinophil count of $< 400 \times 10^6/\text{l}$ at the end of the study showed increased eosinophil chemokinetic migration only in autologous serum, compared with that of the references ($P < 0.01$) (Table 4).

A significant correlation between the number of eosinophils in the blood and the eosinophil chemokinetic response to autologous serum, $P < 0.04$ ($r_s = 0.265$) was demonstrated on the first 2 days of the study. However, the eosinophil migratory responses to the other chemokinetic and chemotactic agents did not correlate with the number of eosinophils in the blood ($P > 0.05$). On the last 2 days of the study, there was a

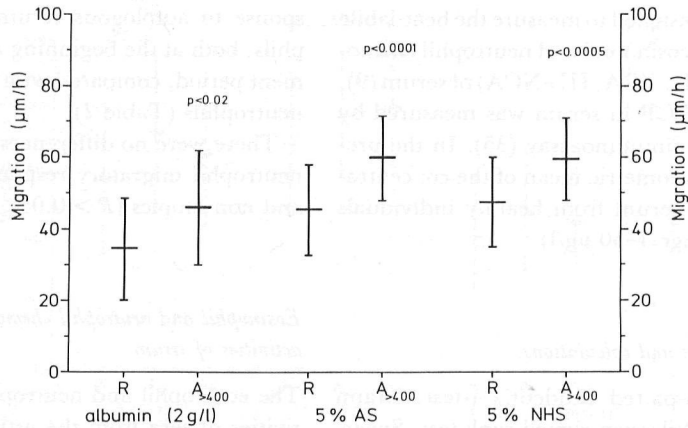


Fig. 3. Chemokinetic responses of eosinophils from asthmatics with $>400 \times 10^6$ eosinophils/l on the last 2 days of the treatment period ($n = 15$). Means and $\pm$ SD interval of the respective groups are indicated. The significance of the differences between patient group and references was calculated by means of Mann-Whitney test.

correlation between the blood eosinophil count and both the chemokinetic and chemotactic responses to NHS of eosinophils from the patients, $P < 0.01$ ($r_s = 0.340$) and $P < 0.02$ ($r_s = 0.311$), respectively.

Data from the reference group demonstrated correlations between the blood eosinophil count and the chemokinetic and chemotactic responses of eosinophils to NHS, $P < 0.02$ ($r_s = 0.203$) and $P < 0.01$ ($r_s = 0.232$), respectively.

Eosinophil migratory responses in relation to serum ECP

A significant correlation between the concentration of ECP in serum of the asthmatics and the chemokinetic response of their eosinophils to autologous serum was found at the beginning of the treatment period (Fig. 5), whereas no correlation between these variables was found with data from the references.

Table 3

Chemokinetic and chemotactic activities of serum from references and asthmatics at the beginning (B) and end (E) of the treatment period

Test	References (<i>n</i> = 73)	Patients	Patients
		B (<i>n</i> = 23)	E (<i>n</i> = 23)
Migration (%)			
Chemokinetic activity of serum			
Eosinophil	93 ± 19	103 ± 23 ^a	106 ± 16 ^b
Neutrophil	92 ± 14	102 ± 18 ^b	108 ± 17 ^b
Chemotactic activity of serum			
Eosinophil (HL-ECA)	94 ± 19	98 ± 16	90 ± 28
Neutrophil (HL-NCA)	94 ± 21	99 ± 20	96 ± 23

Results are given as means $\pm$ SD.

^a Significant difference between patients and references, $P < 0.02$ (Student's *t*-test).

^b Significant difference between patients and references, $P < 0.001$ (Student's *t*-test).

Table 4

Migratory responses of eosinophils from asthmatics with $< 400 \times 10^6$ eosinophils/l on the last 2 days of the treatment period

Test	References (<i>n</i> = 97)	Patients (<i>n</i> = 12)*
Migration ($\mu\text{m/h}$)		
Chemokinetic response		
Albumin (2 g/l)	34 ± 14	36 ± 17
5% autologous serum	45 ± 13	56 ± 16^a
5% NHS	47 ± 12	51 ± 9
Chemotactic response		
5% NHS	57 ± 23	53 ± 26
1.5% ZAS	68 ± 22	79 ± 18
f-MLP (10 nmol/l)	41 ± 18	42 ± 16

Results are given as means $\pm$ SD.^a Significant difference between patients and references, $P < 0.01$ (Mann-Whitney test).* Eight patients had a blood eosinophil count of $< 400 \times 10^6$ on both of the last 2 days, four patients on one of the last 2 days.*Alteration of eosinophil migratory responses in relation to lung function (PEF), blood eosinophil count and serum ECP.*

There were no significant differences in granulocyte migratory responses related to the patients' lung function, measured as PEF ($P > 0.05$).

However, when the individual alterations of the eosinophil migratory responses over the treatment period were evaluated in comparison with the lung function of the patients a relationship emerged. Those whose eosinophil responses had decreased (by more than $10 \mu\text{m/h}$) at the end compared with the beginning of the treatment period, in at least four of the six tests of chemokinetic and chemotactic responses (Group 1),

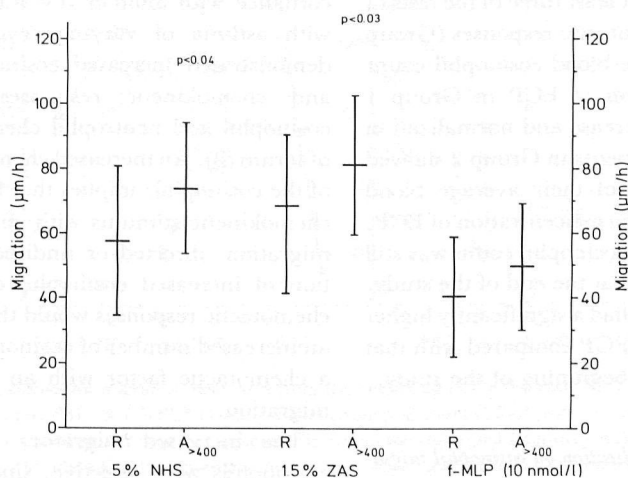


Fig. 4. Chemotactic responses of eosinophils from asthmatics with $> 400 \times 10^6$ eosinophils/l on the last 2 days of the treatment period ($n = 15$). Means and $\pm$ SD interval of the respective groups are indicated. The significance of the differences between patient group and references was calculated by means of Mann-Whitney test.

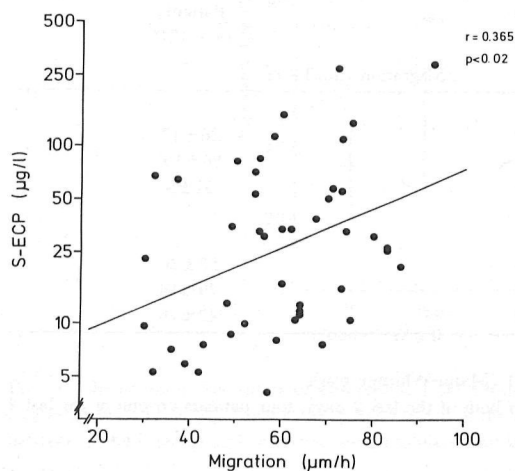


Fig. 5. Relationship between eosinophil chemokinetic response to autologous serum and log serum concentration of ECP. Calculation was based on values from the first 2 days of the study period. Coefficient of correlation calculated by linear regression analysis is given. The geometric mean of serum ECP concentration was 31 $\mu\text{g/l}$ on the first day of the study period and 23 $\mu\text{g/l}$ on the second day ($P > 0.05$).

demonstrated a significantly greater improvement in lung function (min. PEF and max. PEF) at the end of the treatment period than those whose eosinophil responses had increased (by more than 10 $\mu\text{m/h}$) in at least three of the tests of chemokinetic and chemotactic responses (Group 2) (Fig. 6). The average blood eosinophil count and serum concentration of ECP in Group 1 showed a significant decrease and normalization over the period. The patients in Group 2 showed no significant decrease of their average blood eosinophil count or serum concentration of ECP, and their average blood eosinophil count was still above the reference level at the end of the study. However, only Group 1 had a significantly higher serum concentration of ECP compared with that of the references at the beginning of the study.

Influence of oral steroid medication on eosinophil migratory responses

Comparison of the migratory responses to eosinophils from patients who received oral steroid med-

ication and those who did not, revealed no significant differences between the respective groups ($P > 0.05$). Further, the eight patients who had not taken prednisolone before, but who were treated with prednisolone during their stay at the hospital did not, in general, show any significant changes of their eosinophil migratory responses during the treatment period.

DISCUSSION

Increased eosinophil chemokinetic and chemotactic responses and raised eosinophil and neutrophil chemokinetic activities of serum were found in the group of asthmatic patients studied. At the end of the treatment period when the patients' lung function had improved, their eosinophils still demonstrated raised chemokinetic and chemotactic responses and the chemokinetic activity of their sera remained increased, irrespective of what medical treatment they had been subjected to. These findings support our hypothesis that the eosinophils of asthmatics are primed, meaning that their eosinophils have an increased responsiveness to chemokinetic and chemotactic agents. The results indicate that the priming of eosinophils from asthmatic patients is inherent or that the period of 5 weeks rehabilitation and treatment is not sufficient to change the activity of these cells. Furthermore, the present results are in accordance with another investigation of patients with asthma of varying severity, which also demonstrated increased eosinophil chemotactic and chemokinetic responses and increased eosinophil and neutrophil chemokinetic activity of serum (8). An increased chemokinetic response of the eosinophils implies that the cells react to a chemokinetic stimulus with an enhanced rate of migration, directed or undirected. A combination of increased eosinophil chemokinetic and chemotactic responses would theoretically enable an increased number of eosinophils to respond to a chemotactic factor with an enhanced rate of migration.

The increased migratory responses of the eosinophils were selective, since, generally, the neutrophil migratory responses were not raised. That the neutrophil chemokinetic response to autologous serum was increased probably relates

to an increased activity of serum and not of the neutrophils.

The mean eosinophil chemotactic activity of sera from the patients did not differ from that of the reference sera, which is in contrast to the other investigation of asthmatic patients (8), where an increased HL-ECA was found. The difference may possibly be explained by the unstable asthmatic condition of the patients in the present investigation and also by their use of β_2 -agonists and steroids. Beta₂-agonists and steroids have previously been shown to inhibit the production of heat-labile chemotactic activity during allergen inhalation challenge (33).

Several findings in the present investigation indicate a connection between the stimulation of the various functional activities and the increased blood eosinophil count in asthmatics. The eosinophil chemokinetic response to autologous serum was correlated to the serum level of ECP and the blood eosinophil count. Furthermore, the patients who still had a blood eosinophil count of more than $400 \times 10^6/l$ at the end of the treatment period also demonstrated increased eosinophil chemokinetic and chemotactic responses. This

provides grounds for suggesting that the priming component may influence both eosinophil number and activity. The component may affect the production of eosinophils in the bone marrow and thereby also influence eosinophil numbers in the blood, whilst stimulation of the eosinophils in the blood may thereby lead to an increased eosinophil migratory and secretory activity. Although the increased chemokinetic activity of serum was not related to any other variable it may also reflect the activity of a priming component. The cell and tissue source of this priming agent remains speculative. However *in vitro* studies of granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin 3 and interleukin 5 have shown that these cytokines are able to stimulate eosinopoiesis as well as the activity of mature eosinophils (18, 19, 20, 27, 31). Further, a protein called eosinophil-activating factor (EAF), distinct from GM-CSF, produced by monocytes from individuals with moderate eosinophilia has also been described (29, 30).

No direct relationship was found between the eosinophil migratory responses and the patients' PEF values. However, when the changes in in-

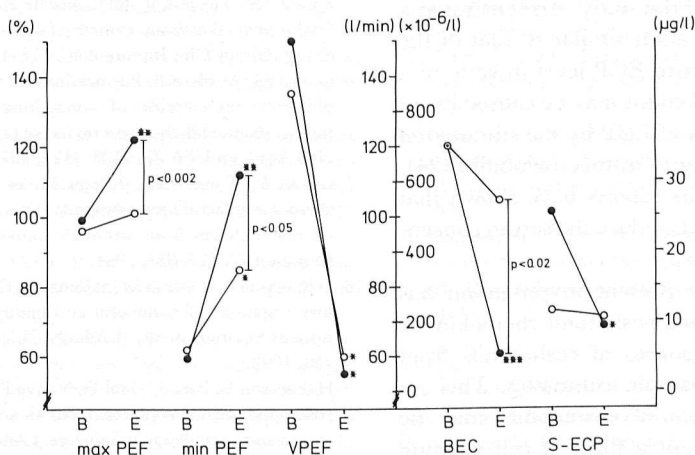


Fig. 6. Alterations in eosinophil migratory responses from the beginning (B) to the end (E) of the treatment period compared with max. PEF (%), min. PEF (%), VPEF (l/min), blood eosinophil count (BEC) ($\times 10^6/l$) and serum concentration of ECP ($\mu g/l$). Group 1 ($n = 5$), closed circles, represents patients in whom the eosinophil migratory responses decreased in at least four variables. Group 2 ($n = 6$), open circles, represents the group in whom the eosinophil migratory responses increased in at least three variables. Respective medians of the two groups are given in the figure. The significance of the differences between max. PEF, min. PEF and blood eosinophil count of the two groups at the end of the period were calculated by Mann-Whitney test. The significance of the differences between variables at the beginning and end of the period are indicated, ** $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ (Wilcoxon signed rank test).

dividuals were examined, some patients demonstrated a decrease in eosinophil migratory responses over the treatment period, in conjunction with an increase in PEF values. The results suggest that the decrease in eosinophil migratory responses may relate to the improvement of the lung function, possibly due to the treatment, in this group of patients. In speculation, the reduced migratory activity of eosinophils may result in diminished recruitment of these cells to the lungs and thereby a reduction in the damage to respiratory tissues that these cells can cause (1, 4, 32) and an improved lung function. However, there was no general relationship between the changes in the patients' PEF values and eosinophil migratory responses, which may be expected, since the average min. and max. PEF values increased over the treatment period, while the average eosinophil migratory responses remained increased.

The group of patients whose eosinophil migratory responses decreased also demonstrated a significant decrease of their blood eosinophil count and serum ECP level, while the group whose eosinophil migratory responses increased showed no significant decrease of their blood eosinophil count or serum ECP. However, the latter group, already at the start of the study, demonstrated a serum ECP concentration similar to that of the references. A low serum ECP level in spite of a high blood eosinophil count may be caused by an increased elimination of ECP by the stimulation of the specific binding to α_2 -macroglobulin (24). Furthermore, previous reports have shown that β_2 -agonists and steroids reduce the serum concentration of ECP (2, 34).

In conclusion, the present investigation has demonstrated increased eosinophil chemokinetic and chemotactic responses of eosinophils from both atopic and non-atopic asthmatics. This enhanced responsiveness of eosinophils may be inherent, and represent a form of cell priming which could in part account for the selective recruitment of these cells to the respiratory tissues in asthma. Our results further suggest that the priming mechanism which is involved in the stimulation of the migratory responses also affects the secretory activity of the eosinophils as well as the blood eosinophil count. The cell

source of this component remains a matter for determination.

ACKNOWLEDGEMENTS

The present work was 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. The technical assistance of Mr Peter Vornefeld is greatly appreciated. We also thank Marianne Lemmelä, Kerstin Lindblad, Birgitta Månsson, Anna-Greta Sjödin, Agneta Trulsson and Viveka Widlund for laboratory assistance.

REFERENCES

- 1 Ayars GH, Altman LC, Gleich GJ, Loegering DA, Baker CB. Eosinophil- and eosinophil granule-mediated pneumocyte injury. *J Allergy Clin Immunol* 76, 595-604, 1985.
- 2 Dahl R, Venge P. Blood eosinophil count and eosinophil cationic protein. In vivo study of the influence of beta-2-adrenergic drugs and steroid medication. *Scand J Respir Dis* 59, 319-332, 1978.
- 3 Dunhill MS. The pathology of asthma. In Middleton E, Reed CE, Ellis EE (eds.): *Allergy: principles and practices*, pp. 678. Mosby, St. Louis, Missouri, 1978.
- 4 Gleich GJ, Flavahan NA, Fujisawa T, Vanhoutte PM. The eosinophil as a mediator of damage to respiratory epithelium: A model for bronchial hypersensitivity. *J Allergy Clin Immunol* 81, 776-781, 1988.
- 5 Gleich GJ, Motojima S, Frigas E, Kepart GM, Fujisawa T, Kravis LP. The eosinophil leukocyte and the pathology of fatal bronchial asthma: evidence for pathologic heterogeneity. *J Allergy Clin Immunol* 80, 412-415, 1987.
- 6 Goetzl EJ, Austin KF. Purification and synthesis of eosinophilotactic tetrapeptides of human lung tissues: identification as eosinophil chemotactic factor of anaphylaxis. *Proc Natl Acad Sci USA* 72, 4123-4127, 1975.
- 7 Gosset P, Tonnel AB, Joseph M, et al. Secretion of a chemotactic factor for neutrophils and eosinophils by alveolar macrophages from asthmatic patients. *J Allergy Clin Immunol* 74, 827-834, 1984.
- 8 Håkansson L, Carlson M, Stålenheim G, Venge P. Migratory responses of eosinophil and neutrophil granulocytes from asthmatic patients. *J Allergy Clin Immunol* 85, 743-750, 1990.
- 9 Håkansson L, Rak S, Dahl R, Venge P. The formation of eosinophil and neutrophil chemotactic activity during pollen season and after allergen challenge. *J Allergy Clin Immunol* 83, 933-939, 1989.
- 10 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* 11, 271-282, 1980.
- 11 Håkansson L, Venge P. Partial characterization and identification of chemokinetic factors in serum. *Scand J Immunol* 18, 531-537, 1983.

- 12 Håkansson L, Venge P. The chemotactic activity of normal and complement-activated serum as measured by the leading-front method using a Boyden chamber. *Scand J Immunol* 19, 63-73, 1984.
- 13 Håkansson L, Westerlund D, Venge P. New method for the measurement of eosinophil migration. *J Leukocyte Biol* 42, 689-696, 1987.
- 14 Honsinger RW, Silverstein D, Van Arsdel PP. The eosinophil and allergy. Why? *J Allergy Clin Immunol* 49, 142-155, 1972.
- 15 Horn BR, Robin ED, Theodore J, van Kessel A. Total eosinophil counts in the management of bronchial asthma. *N Engl J Med* 92, 1152-1155, 1975.
- 16 Kay AB, Stechschulte DJ, Austin KF. An eosinophil leukocyte chemotactic factor of anaphylaxis. *J Exp Med* 133, 602-619, 1971.
- 17 Keller HU, Wilkinson PC, Abercrombie M, et al. A proposal for the definition of terms related to locomotion of leukocytes and others cells. *Clin Exp Immunol* 27, 377-380, 1977.
- 18 Lopez AF, Sanderson CJ, Gamble HD, Young IG, Vadas MA. Recombinant human interleukin 5 is a selective activator of human eosinophil function. *J Exp Med* 167, 219-224, 1988.
- 19 Lopez AF, To LB, Yang YC, et al. Stimulation of proliferation, differentiation, and function of human cells by primate interleukin 3. *Proc Natl Acad Sci USA* 84, 2761-2765, 1987.
- 20 Metcalf D, Begley CG, Hohnson GR, et al. Biologic properties in vitro of recombinant human granulocytemacrophage colony-stimulating factor. *Blood* 67, 37-45, 1986.
- 21 Monchy de JGR, Kauffman HF, Venge P, et al. Bronchoalveolar eosinophilia during allergen challenge induced late asthmatic reaction. *Am Rev Respir Dis* 131, 373-376, 1985.
- 22 Monchy de JGR, Postma DS, Kauffman HF, Venge P, Weeke FW, de Vries K. Pretreatment with inhaled corticosteroids reduces eosinophil chemotaxis of BAL fluid following house dust mite challenge. *ICACI, Montreux* 1988. Abstract. p 249.
- 23 Parish WE, Luckhurst E. Eosinophilia VI. Spontaneous synthesis of chemokinetic, chemotactic, complement receptor-inducing activities for eosinophils by bronchial T-lymphocytes of asthmatic-bronchitic patients. *Clin Allergy* 12, 475-488, 1982.
- 24 Peterson CGB, Venge P. Interaction and complex formation between the eosinophil cationic protein (ECP) and alpha-2-macroglobulin. *Biochem J* 245, 781-787, 1987.
- 25 Rak S, Björnson A, Håkansson L, Sörenson S, Venge P. Immunotherapy prevents eosinophil accumulation and production of eosinophil chemotactic activity in the lung of asthmatics during natural allergen exposure. In: *Bronchial hyperresponsiveness and cellular and humoral factors involved in allergic inflammation during pollen season. Dissertation from the Medical Faculty, University of Gothenburg, Sweden.* pp. 161-180, 1988.
- 26 Rak S, Håkansson L, Venge P. Immunotherapy abrogates the generation of eosinophil and neutrophil chemotactic activity during pollen season. *J Allergy Clin Immunol* (to be published 1990).
- 27 Silberstein DS, Owen WF, Gasson JC, et al. Enhancement of human eosinophil cytotoxicity and leukotriene synthesis by biosynthetic (recombinant) granulocyte-macrophage colony-stimulating factor. *J Immunol* 137, 3290-3294, 1986.
- 28 Taylor KJ, Luksza AR. Peripheral blood eosinophil counts and bronchial responsiveness. *Thorax* 42, 452-456, 1987.
- 29 Thorne KJ, Richardson BA, Taverne J, Williamson DJ, Vadas MA, Butterworth AE. A comparison of eosinophil-activating factor (EAF) with other monokines and lymphokines. *Eur J Immunol* 16, 1143-1149, 1986.
- 30 Thorne KJ, Richardson BA, Veith MC, Tai P-C, Spry CJ, Butterworth AE. Partial purification and biological properties of an eosinophil-activating factor. *Eur J Immunol* 15, 1083-1091, 1985.
- 31 Tomonaga M, Golde DW, Gasson JC. Biosynthetic (recombinant) human granulocyte-macrophage colony-stimulating factor: effect on normal bone marrow and leukemia cell lines. *Blood* 67, 31-36, 1986.
- 32 Venge P, Dahl R, Fredens K, Peterson C. Epithelial injury of human eosinophils. *Am Rev Respir Dis* 138, S54-S57, 1988.
- 33 Venge P, Dahl R, Håkansson L. Heat-labile neutrophil chemotactic activity in subjects with asthma after allergen inhalation: Relation to the late asthmatic reaction and effects of asthma medication. *J Allergy Clin Immunol* 80, 679-688, 1987.
- 34 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* 87, 306-312, 1988.
- 35 Venge P, Roxin L-E, Olsson I. Radioimmunoassay of human eosinophil cationic protein. *Br J Haematol* 37, 331-335, 1977.
- 36 Wardlaw AJ, Moqbel R, Cromwell O, Kay AB. Platelet-activating factor. A potent chemotactic and chemokinetic factor for human eosinophils. *J Clin Invest* 78, 1701-1706, 1986.
- 37 Zigmond SH, Hirsch JG. Leukocyte locomotion and chemotaxis. New methods for evaluation, and demonstration of cell-derived chemotactic factors. *J Exp Med* 137, 387-410, 1973.

Address:

Lena Håkansson, Ph. D.
 Laboratory for Inflammation Research
 Department of Clinical Chemistry
 University Hospital
 S-751 85 Uppsala
 Sweden

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.