

The effect of immunotherapy on eosinophil accumulation and production of eosinophil chemotactic activity in the lung of subjects with asthma during natural pollen exposure

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Two groups of birch pollen-allergic patients with seasonal rhinoconjunctivitis and asthma were followed during two consecutive birch-pollen seasons, one group, N = 10, during a season with high pollen load, and one group, N = 15, during a season of low pollen load. Half the patients were treated with immunotherapy (IT) for 3 and 4 years, respectively. The other half of the patients served as control group (non-IT). Bronchoalveolar lavage (BAL) was performed once before each season and once during the pollen season. Eosinophil (EOS) numbers in BAL were increased ($p < 0.01$) during the season with high pollen load but not in the season with a low pollen load, and this increment was absent in the IT-treated group. Also, the EOS cationic protein levels were raised in the non-IT-treated group during the season with a high pollen load. The levels of EOS and neutrophil chemotactic activity were raised in BAL in both seasons in the non-IT-treated group compared with the IT-treated group ($p < 0.02$, $p < 0.003$, $p < 0.04$, and $p < 0.005$ in high- and low-load pollen season, respectively). Serum and BAL eosinophil chemotactic activity (ECA) were positively correlated ($p < 0.001$). We conclude that there is an influx of active EOSs into the lung of pollen-allergic patients with asthma during a pollen season, which may be abrogated by IT. Furthermore, the generation of ECA appears to be an extremely sensitive marker of antigenic exposure, and the potent inhibition of the generation of ECA by IT may provide a clue as to the mechanism of this treatment. (J ALLERGY CLIN IMMUNOL 1991;88:878-88.)

Key words: Asthma, bronchial hyperresponsiveness, inflammation, immunotherapy, bronchoalveolar lavage, eosinophil, birch allergy

Although IT treatment has been used in clinical practice since its introduction by Freeman and Noon¹ in 1911, little is known about the mechanism of its action.² A series of studies has been performed in which the effect of IT on clinical and biochemical parameters in birch pollen-allergic subjects with asthma was investigated. We³ and other investiga-

Abbreviations used

IT:	Immunotherapy
BAL:	Bronchoalveolar lavage
BALF:	Bronchoalveolar lavage fluid
BHR:	Bronchial hyperresponsiveness
ECP:	Eosinophil cationic protein
ECA:	Eosinophil chemotactic activity
NCA:	Neutrophil chemotactic activity
MPO:	Myeloperoxidase
PC ₂₀ :	Provocative concentration causing a 20% fall in FEV ₁
EOS:	Eosinophil

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tors⁴⁻⁶ have demonstrated that BHR increases during the pollen season in sensitive patients. The increase could be partly or entirely inhibited by sodium cromoglycate,^{3, 4, 6} steroid treatment,⁷ and IT preseasonally.⁸ In the latter study, the degree of histamine sen-

sitivity in the airways correlated to the levels of ECP in serum. The levels of heat-labile ECA and NCA increased in serum during natural birch-pollen exposure in birch pollen-allergic subjects.⁹ The IT treatment with birch-pollen extract preseasonally counteracted this response.¹⁰

Our previous investigations concentrated mainly on the number and the secretory activity of EOSs and ECA, since this cell is considered to be a marker of allergic and nonallergic asthma.¹¹ However, the changes described earlier⁸⁻¹⁰ occurred in the blood of the patients and may therefore not be applicable to the situation *in situ* in the lung. Therefore, we expect the BAL to provide more nearly accurate information about the role of the cell in seasonal inflammation in the bronchi. For this purpose, a group of pollen-allergic patients were lavaged before and during two consecutive pollen seasons; half of these patients were receiving continuing IT.

METHODS

Patients

Twelve birch pollen-allergic patients in high pollen season and 15 in the low season were recruited to the study. There were six men and six women in high pollen group, and the mean age of the patients was 29 years, ranging from 21 to 37 years. Two patients were occasional smokers. There were nine men and six women in the group participating in the study during low-load pollen season with the mean age of 28 years, ranging from 18 to 46 years. Two patients were occasional smokers. The diagnosis of allergy was based on history of rhinoconjunctivitis and wheezing during the birch-pollen season, positive skin prick test, and RAST. All patients demonstrated increased BHR, as measured by means of histamine sensitivity within the "asthmatic range" at time of admission to the IT study according to the method used.¹² The patients participated in the IT study for the past 3 and 4 years, respectively; six and seven patients (in high- and low-load pollen season, respectively) were IT treated with birch-pollen extract (Pharmacia Diagnostics, Uppsala, Sweden) and continued to receive a maintenance dose within the highest potency of the extract (100,000 BU/ml), and the other six and eight patients were control subjects (non-IT). The patients used β_2 -agonists occasionally, mainly during the pollen season, when nasal and ocular drops to control rhinoconjunctivitis symptoms were also allowed. None of the patients had a history of respiratory tract infection 4 weeks before examination. The clinical data and patients' characteristics are described in an earlier work.⁸

Design

The study consisted of two lavages; the first, BAL 1, was performed during February and March, before the start of the pollen season, and the second lavage, BAL 2, in May during the birch-pollen season. The seasonal lavages started shortly after the peak count of birch pollen and during the following week, thus ensuring the same period of priming

and maximum exposure during peak count period for all patients. According to our earlier experience,⁸ this is also the period of highest intensity of inflammation as judged by PC₂₀ values. It would also be practically impossible to perform all lavages during 1 day, considering requirement of the time of the day for both BAL 1 and 2, and for the same investigator to perform all bronchoscopies. Patients from both groups were lavaged randomly. Histamine provocation tests were performed the day before BAL according to the procedure described earlier,¹² as were chest x-ray and electrocardiogram. Blood samples were collected on the BAL day. All subjects gave their consent, and the study was approved by the local ethical committee.

Pollen sampling

Registrations were performed with Burkard's 7-day-recording volumetric trap (Burkard Manufacturing Co., Ltd., Rickmansworth, Hertfordshire, England). The trap was placed on the roof of the hospital and reflected pollen distribution in the region. The tapes were removed from the trap, and the number of pollen grains was counted and expressed as total number in cubic meters during 24 hours. The recording in the study area started at the beginning of March, when the first pollens of hazel were expected, followed by alder. The birch-pollen season usually starts at the beginning of May (Fig. 1).

BAL procedure

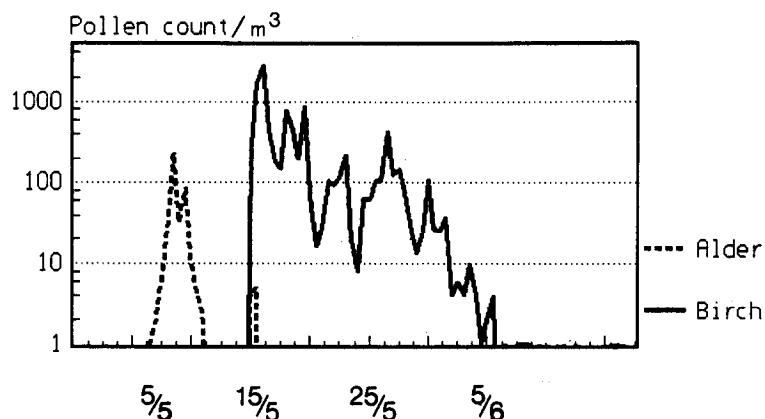
Bronchoscopy was performed through the mouth with a flexible fiberoptic bronchoscope (Olympus BF1 T, 4B 2, and P10, Olympus Corp., Lake Success, N.Y.) under local anesthesia with 2% Xylocaine (Astra Pharmaceuticals, Södertälje, Sweden). The patients were premedicated with morphine-scopolamine and two puffs of salbutamol, 0.1 mg per puff, 15 minutes before the bronchoscopy procedure. The bronchoscope was wedged in the middle or lower lobe bronchus. Two hundred milliliters of sterile saline solution at 37° C was instilled in 50 ml portions and gently aspirated into plastic test tubes and put on ice. The same investigator performed bronchoscopy, at the same time of the day, using the same bronchoscope before and during the season. The patients remained at the clinic 4 hours after BAL was performed.

Laboratory techniques

The BAL volume was measured. To avoid cell loss, no filtration was performed.¹³ The cells were pelleted at 400 g for 10 minutes at +4° C and then resuspended in 8 to 10 ml of RPMI 1640 (medium), supplemented with gentamycin and 15% fetal calf serum. The cell suspension was kept cold during transport to the laboratory and was again centrifuged and resuspended, this time in Earl's balanced salt solution without phenol red, supplemented with gentamycin (50 μ g/ml) and 15% fetal calf serum. Total cell counts were performed with a Bürker chamber. Cytochrome preparations for differential cell counts were made with Cytospin 2 (Shandon Southern Products, Cheshire, England).

Approximately 5×10^4 cells suspended in 100 μ l balanced salt solution were cytochrome centrifuged at 500 rpm for 3

POLLEN SEASON 1987



POLLEN SEASON 1988

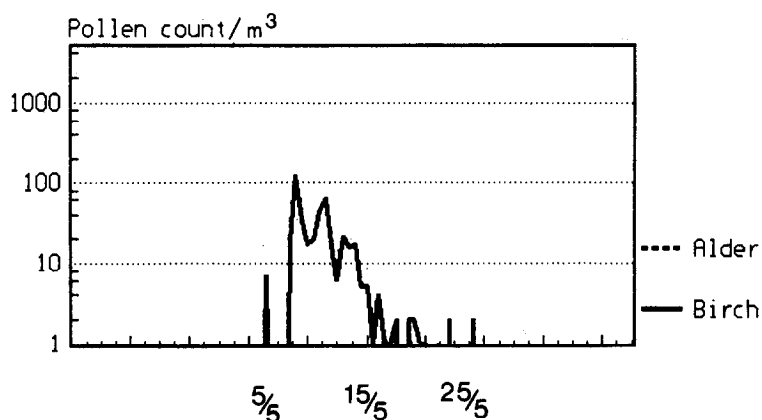


FIG. 1. The birch-pollen season, 1987, high-pollen load; 1988, low-pollen load. BALs were performed during the week after pollen peak, starting May 17, 1987, and May 10, 1988.

minutes. The cytocentrifuge preparations were stained according to May-Grünwald Giemsa, and 400 cells were counted. The results were expressed as percent of the total cell number. The BALF and the serum were kept frozen at -70°C , and the ECP and MPO concentrations and the ECAs and NCAs were measured in serum as recently described.^{9, 10, 14, 15} For activity in BALF, the samples were diluted 1:4, 1:8, and 1:16 in Gey's solution. The migration was assayed as described earlier.⁹ The chemotactic activity (units) of the BALF was calculated as the maximal migration (micrometers per hour) multiplied by the actual dilution of the sample. Blood EOSs were stained and counted in a Fuchs-Rosenthal chamber.

Statistics

Data are expressed as medians and actual ranges. For comparison of data within the groups, Wilcoxon's signed-rank test was used. For between groups, Mann-Whitney U test was used. Correlations were estimated with Spearman's

rank-correlation test. The statistical calculations were performed on a personal computer by means of the statistical package, Statgraphics (STSC, Rockville, Md.).

RESULTS

All patients tolerated the BAL procedure well. One patient had a limited bronchospasm during BAL 1, which was resolved easily with salbutamol administered intraluminally. This patient was not accepted for BAL during the season. Another patient did not participate in the second lavage because of a surgical emergency. Both patients were in the high pollen-season group.

Pollen counts

The birch- and alder-pollen counts of the two pollen seasons are illustrated in Fig. 1. The pollen season of 1987 was a season of high pollen load compared with

TABLE I. Clinical data

	FEV ₁ (L/sec)		PC ₂₀ (mg/ml) of histamine	
	Preseason	Season	Preseason	Season
Pollen season				
High-load				
IT	4.42 (3.17-5.19)	4.98 (3.17-5.51)	4.3 (0.05-32)	2.9 (0.16-32)
Non-IT	3.4 (3.05-4.55)	3.58 (3.1-4.87)	2.7 (0.65-4.87)	0.93* (0.07-1.65)
Low-load				
IT	3.85 (2.52-5.48)	4.09 (2.46-5.08)	3.9 (0.69-32)	1.06 (0.03-32)
Non-IT	3.86 (2.64-4.58)	4.12 (2.8-4.72)	1.5 (0.48-5.19)	0.35 (0.026-16)

FEV₁ and PC₂₀ median values and ranges.

*Significant decrease in PC₂₀ during high-load pollen season in the non-IT-treated group compared to preseasonal values.

that of 1988. During the season of 1987, the peak pollen counts of birch and alder were tenfold higher than counts of 1988.

Baseline FEV₁ and PC₂₀

The clinical data of baseline FEV₁ and PC₂₀ before BAL 1 and BAL 2 are presented in Table I. FEV₁ was similar in the two groups in the high pollen season and on the two occasions when FEV₁ was adjusted to percent of predicted. Although median histamine PC₂₀s tend to be higher in the IT-treated group before the season, no statistical differences were observed. In the control group, histamine PC₂₀ values decreased in all patients during the season, and the difference was statistically significant ($p < 0.05$), compared with preseasonal values, in contrast to an unaltered sensitivity in the IT-treated group. During low pollen season, similar FEV₁ values were observed before and during the season in both IT-treated and untreated patients. There was no significant difference between PC₂₀ values of the groups before season. During the season, PC₂₀ values decreased in six but increased in two patients. The difference was not significant ($p < 0.2$). The changes in the season were not significantly different between the groups.

Fluid recovery and albumin concentration in BAL

Fluid recovery and albumin concentration in BAL are presented in Table II. During both high and low pollen seasons, fluid recovery was similar in the two groups before the season and stayed unaltered during the season. In the high pollen season, albumin concentrations were the same during pre-season, but increased during the season in untreated patients, and

the difference in albumin concentrations between IT-treated and untreated patients was significant ($p = 0.036$). In contrast, in the low pollen season, albumin concentration values were decreased significantly in untreated patients ($p < 0.05$).

Cell findings in BAL

In the birch-pollen season with high pollen load, the total number of recovered cells in the BALF was similar in the two groups before and during the season (Table III). The number of recovered specific cells was also similar with two exceptions. Thus, the number of lymphocytes decreased significantly during the season in the IT-treated group ($p < 0.01$), and number of EOSs increased significantly in BAL in the control group during the season ($p = 0.008$). A significantly increased number of EOSs in the control group was also evident compared with the number in the IT-treated group ($p = 0.01$) (Fig. 2). In low pollen season, total number of recovered cells was similar in both groups and remained unaltered during the pollen season with the exception for neutrophils. The number of neutrophils decreased significantly in season compared with pre-season ($p = 0.02$) but only in the IT-treated group. No increase in EOS number was noted in low pollen season in the untreated group. When the number of EOSs in the BALF of untreated patients was compared between high and low pollen seasons, difference was significant ($p = 0.0043$).

ECP and MPO in BAL

The levels of ECP and MPO are presented in Table IV. In the high pollen season, MPO levels were unaltered before and during the season and were similar in the two groups. The ECP levels were significantly

TABLE II. Recovery of lavage fluid and albumin concentration

	Recovery (ml)		Albumin concentration (mg/L)	
	Preseason	Season	Preseason	Season
Pollen season				
High-load				
IT	151 (108-164)	145 (114-161)	35 (25-70)	32.5 (17.5-62.5)
Non-IT	135 (125-154)	121 (117-134)	47.5 (15-207.5)	67.5 (37.5-100)
Low-load	NS	NS	NS	<i>p</i> = 0.036
IT	153 (119-158)	151 (117-158)	56 (43-110.2)	62 (33.3-101.8)
Non-IT	149 (112-165)	151 (90-158)	75.9 (31.1-107.7)	36.4* (25.3-94.9)

NS, Not significant.

*Significantly lower concentration of albumin was noted in BAL of the non-IT-treated group during low-load pollen season compared to preseason values (*p* < 0.05).**TABLE III.** Number of cells in lavage fluid of IT-treated and non-IT-treated patients during high-load and low-load antigen seasons

	IT-treated		Non-IT treated	
	Preseason	Season	Preseason	Season
High pollen season				
Total No. cells ($\times 10^6$)	1770 (1140-2439)	1400 (480-2450)	1860 (520-3200)	1640 (720-2700)
Macrophages	1637 (1037-2588)	1350 (418-2235)	1762 (472-2670)	1345 (666-2380)
Neutrophils	3.7 (0-26.5)	3.5 (0.18-22.6)	6.2 (5.0-14)	8.6 (2.6-18)
EOSs	7.8 (5.2-43.9)	5.84 (0.67-13.4)	13.6 (2.8-23.8)	45.5* (33.4-56)
Lymphocytes	66.4 (32.3-85.4)	36.28 (17.06-50.3)	53.07 (13.3-459.6)	43.45 (11.8-251.1)
Low pollen season				
Total No. cells ($\times 10^6$)	940 (780-2800)	1560 (200-3800)	1240 (340-2800)	1320 (200-2280)
Macrophages	836 (618-2730)	1282 (18-3600)	1008 (230-2738)	1060 (174-2174)
Neutrophils	6.6 (0.8-20)	0.28† (0-3.8)	0.9 (0-23)	1.84 (0-12.2)
EOSs	6.8 (0-20.8)	6.8 (0-48)	5.8 (1.5-16.6)	7.7 (0.8-18)
Lymphocytes	96 (29.6-698)	148 (1-808)	84 (25.6-234)	110 (23.8-362)

*Significantly lower EOS number in seasonal BALF of non-IT-treated patients during low-load pollen season compared to number of EOS in high-load pollen season.

†Significantly lower number of neutrophils during nonpollen season in BALF of IT-treated patients compared to preseasonal values.

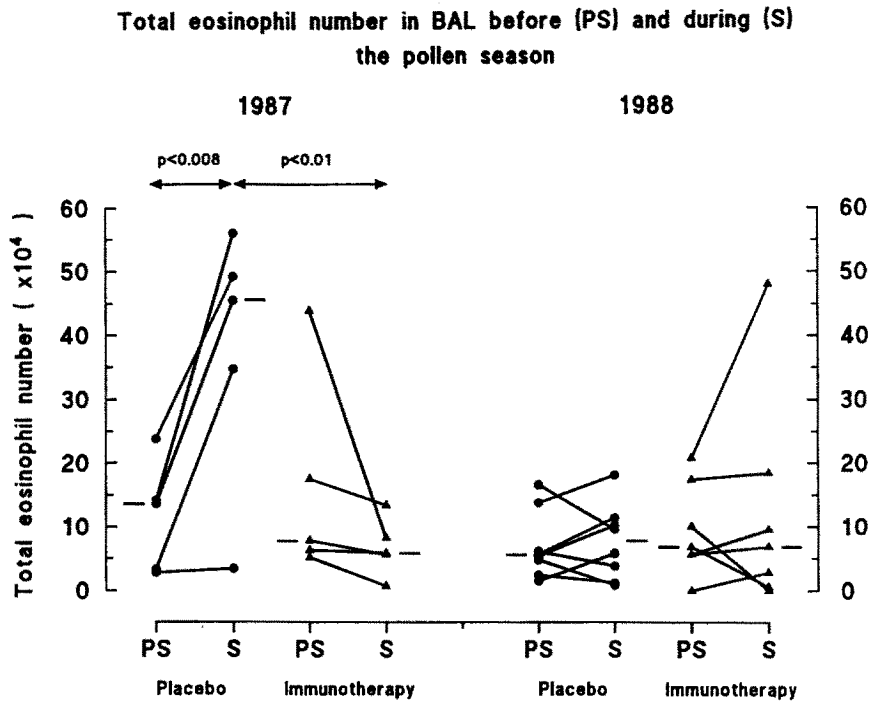


FIG. 2. Total EOS number ($\times 10^4$) (individual values and median) in lavage fluid before (BAL 1) and during (BAL 2) the pollen season. During the high-load pollen season, the number of EOSs in the untreated patients increased significantly compared with preseason increase ($p = 0.008$). Difference in EOS number between the groups during season was significant ($p = 0.01$). During low-load pollen season, no significant differences were observed within or between the groups.

TABLE IV. Total ECP and MPO in lavage fluid (median and range)

	ECP (ng)		MPO (ng)	
	Preseason	Season	Preseason	Season
High-load pollen season				
IT	353 (203-716)	376 (267-751)	285 (126-472)	278 (76-888)
Non-IT	472 (303-1258)	768 (506-2032)	381 (276-3040)	410 (203-1106)
	NS	$p = 0.036$	NS	NS
Low-load pollen season				
IT	310 (123-537)	465 (186-1311)	276 (170-607)	332 (152-964)
Non-IT	282 (201-455)	437 (189-3046)	443 (216-560)	458 (47-4663)
	NS	NS	NS	NS

Statistical differences between IT and non-IT are presented.

higher at BAL 2 in the control group ($p = 0.03$). The ECP levels of all patients during the season correlated positively with the total numbers of EOSs in BAL ($r = 0.74$; $p = 0.02$). There were also significant correlations between ECP in BAL and albumin and

MPO, in BAL, respectively ($r = 0.54$; $p = 0.01$ and $r = 0.70$; $p = 0.001$) and between MPO and albumin ($r = 0.45$; $p = 0.04$). In the low pollen season, the MPO and ECP levels were similar, preseasonally, and unaltered during the pollen season.

Eosinophil chemotactic activity in BAL fluid from birch allergic asthmatics, treated with placebo and immunotherapy, before (PS) and during pollen season (S)

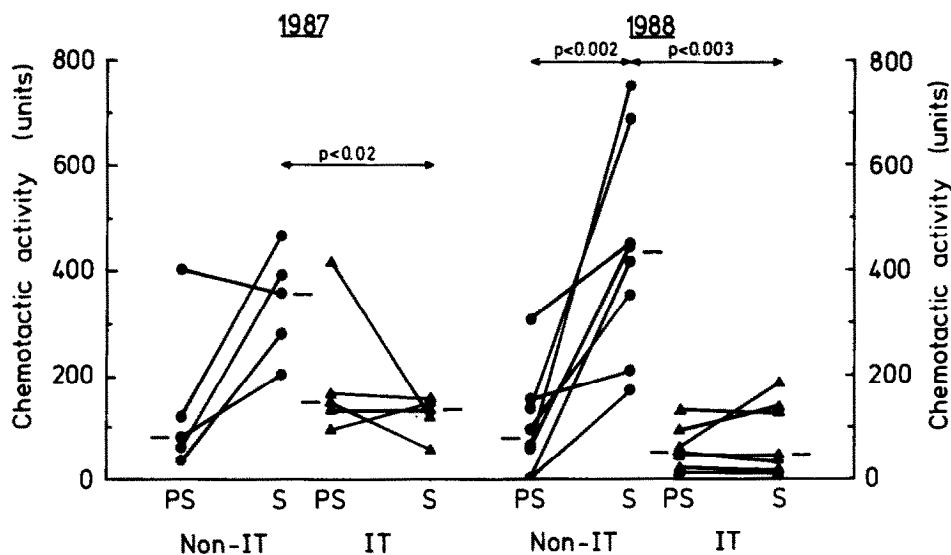


FIG. 3. Levels of the ECA of the lavage fluid expressed in units (maximal migration times dilution) (individual values). Significantly higher ECA levels ($p < 0.02$) were observed in the untreated group compared with levels in the IT-treated group during the high-load pollen season. During the low-load pollen season, ECA levels of the control group rose significantly compared with preseason levels ($p < 0.02$) and with in-season levels of the IT-treated group ($p < 0.003$).

ECA and NCA in BAL

In the high pollen season, the ECA in BAL was similar in the groups before the season (Fig. 3). During the season, ECA increased in all but one patient in the control group, compared with preseasonal values. There was a significant difference ($p < 0.02$) in seasonal ECA values between the groups.

In the low pollen season, the ECA in preseasonal BAL was similar in the groups. During season, a significant increase was noted in the untreated but not IT-treated group ($p < 0.002$). In the IT-treated group, no increment was recorded, and the difference between seasonal values of the two patients groups was significant ($p < 0.003$).

The NCA in BAL during the high pollen season was also similar in the two groups before the season. During the season, NCA was higher in three patients in the control group, and the activity was significantly elevated compared with the findings in the IT-treated group ($p < 0.04$) (Fig. 4).

There was a significant correlation between ECA and NCA in BAL from all patients ($r = 0.76$; $p = 0.001$). ECA correlated positively to the total number of EOSs in BAL ($r = 0.46$; $p = 0.03$). No correlation of NCA to the number of neutrophils was discernible, nor were there any correlations be-

tween either ECA or NCA to ECP or MPO levels in BAL.

During the low pollen season, NCA levels in preseasonal BAL were similar for both groups of patients. During the season, a significant increase was observed in untreated patients ($p < 0.02$), whereas the NCA levels in the IT-treated group remained unaltered. There was a significant difference between the seasonal NCA values of the groups ($p < 0.005$).

Findings in blood

In Table V, blood EOS counts, serum ECP and MPO levels, and the activities of heat-labile ECA and NCA in serum are presented. In the high pollen season, a significant seasonal increase was found in the blood EOS number ($p = 0.046$) and the ECA in the control group ($p = 0.05$) compared with preseasonal values. No other differences were discernible between the groups. In the low pollen season, significant increases were observed between preseasonal and seasonal values of ECA and NCA ($p < 0.02$ and $p < 0.02$, respectively) of untreated patients. The seasonal levels of both were unaltered in IT-treated patients. When seasonal values between the groups were compared, ECA and NCA levels were significantly higher in untreated patients ($p < 0.007$ and

Neutrophil chemotactic activity in BAL fluid from birch allergic asthmatics, treated with placebo and immunotherapy, before (PS) and during pollen season (S)

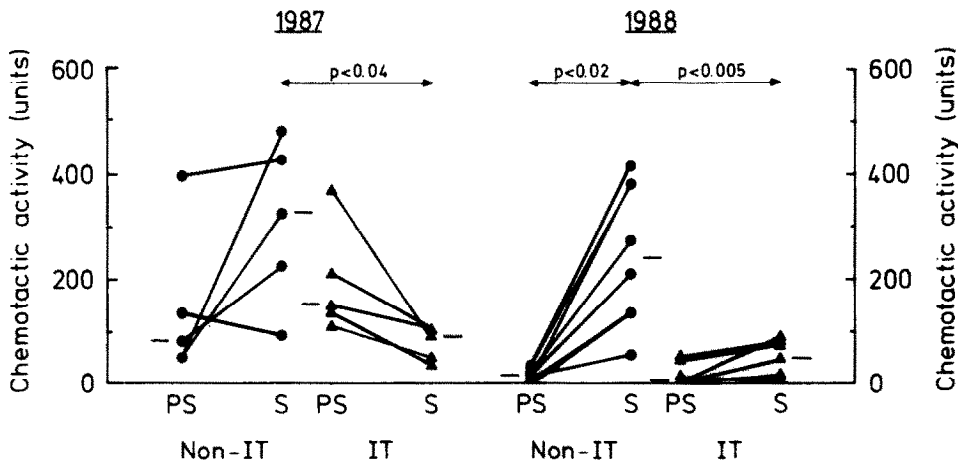


FIG. 4. Levels of the NCA of the lavage fluid expressed in units (maximal migration times dilution) (individual values). A significantly higher activity was observed in untreated patients compared to activity in IT-treated patients during the high-load pollen season ($p < 0.04$). During the low-load pollen season, NCA levels rose significantly in control patients compared with preseasonal values ($p < 0.02$) and with seasonal values of IT-treated patients ($p < 0.005$).

TABLE V. Blood EOS count and serum levels of ECP, MPO, ECA, and NCA

	Blood EOS count ($\times 10^6/L$)		ECP ($\mu g/L$)		MPO ($\mu g/L$)		ECA (%)		NCA (%)	
	S ₁	S ₂	S ₁	S ₂	S ₁	S ₂	S ₁	S ₂	S ₁	S ₂
Pollen season										
High-load										
IT	20 (20-480)	140 (40-240)	30 (7.7-68)	10.6 (3.2-46)	196 (126-417)	162 (57-235)	100 (77-113)	100 (92-118)	91 (84-114)	109 (84-122)
Non-IT	100 (40-180)	360* (100-480)	17.9 (7.9-82)	9.0 (7.1-155)	150 (48-666)	113 (50-266)	102 (92-139)	141† (94-167)	96 (83-109)	107 (95-138)
Low-load										
IT	100 (0-380)	180 (20-600)	29 (18.8-45)	32 (16.5-116)	190 (48-251)	165 (95-139)	112 (93-127)	98 (89-140)	100 (89-140)	92 (75-142)
Non-IT	70 (60-200)	40 (0-140)	28.9 (9.7-300)	45.5 (9.3-155)	142 (49-192)	165 (80-240)	101 (74-112)	141‡ (119-149)	92 (76-108)	121§ (102-144)

The samples was collected at the same time as BAL and named S₁ before season and S₂ during season.
*Significantly higher EOS number in non-IT-treated patients during high-load pollen season compared to preseasonal values ($p < 0.008$).
†Significant increase of ECA in non-IT-treated patients compared to preseasonal values during high-load pollen season ($p < 0.05$).
‡Significant increase of ECA in non-IT-treated group compared to preseasonal values ($p < 0.02$) and to seasonal values of IT-treated group ($p < 0.005$) during low-load pollen season.
§Significant increase of NCA in non-IT-treated patients compared to preseasonal values ($p < 0.05$) and seasonal values of the IT-treated group ($p < 0.02$) during low-load pollen season.

$p < 0.05$, respectively) than in IT-treated patients. The blood EOS number was significantly higher during the season with high pollen load compared with the season with low pollen load ($p = 0.008$). When

calculations were made on all patients before and during the season of high pollen load, significant correlations were found between the total number of EOSs in BAL and blood ($r = 0.43$; $p = 0.05$), as was a

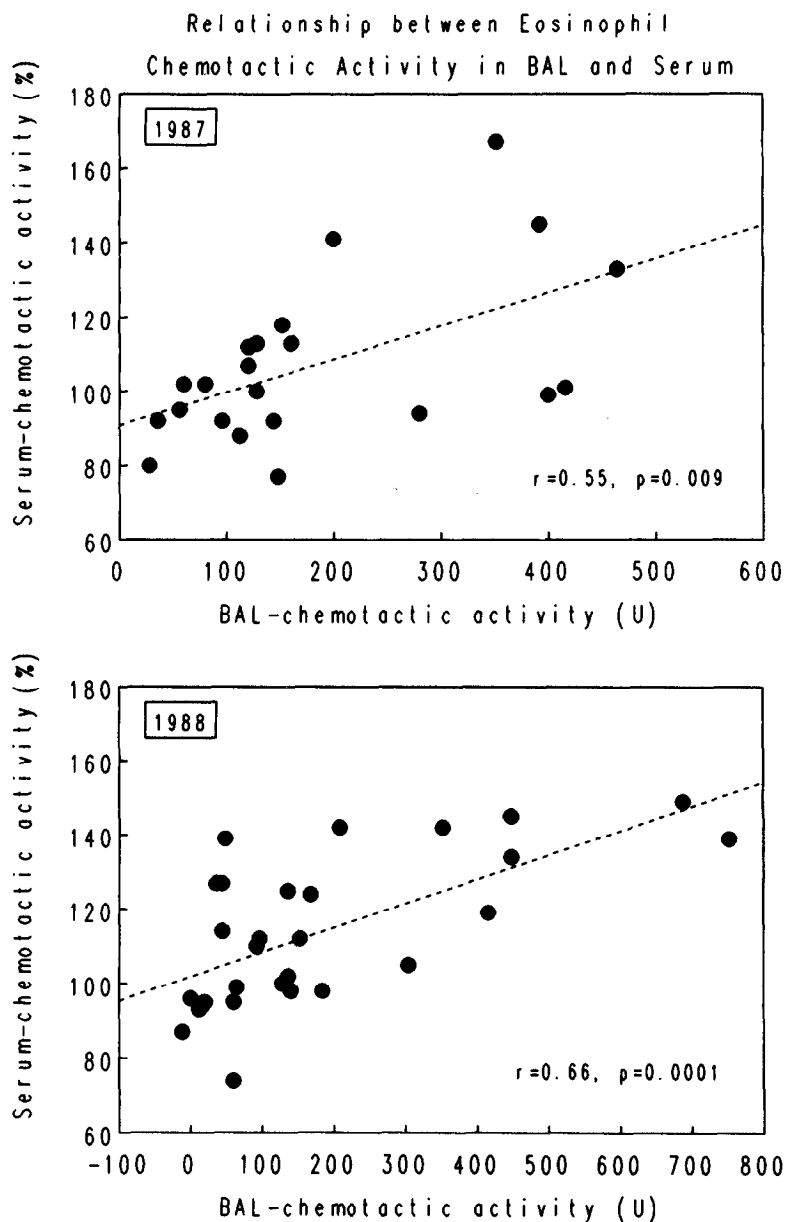


FIG. 5. A significant correlation was observed between serum and BAL ECA during the high-load ($r = 0.55$; $p = 0.009$) and the low-load ($r = 0.66$; $p = 0.0001$) pollen seasons.

positive correlation between the ECA in BAL and serum ($r = 0.55$; $p < 0.01$) (Fig. 5).

DISCUSSION

In the present study, the effect of IT on EOS accumulation and activation in the lungs of birch pollen-allergic patients with asthma during pollen season was investigated. The investigation was performed during two consecutive pollen seasons, one with a high pollen load and the next season with a low pollen load. During the high-load pollen season, as compared with

preseason, the number of EOSs, the concentration of ECP, as well as the ECA and NCA, increased in BAL and serum of the untreated patients, in contrast to increase in the IT-treated patients in which no such alterations were observed. These results support earlier findings of specific EOS accumulation and activation after allergen inhalation challenge.¹⁶ Furthermore, in the IT-treated patients, the BHR was not changed during pollen season, whereas it increased in the untreated patients. During the low-load pollen season, a raised ECA and NCA were demonstrated in BAL and serum of the untreated patients, whereas the

other variables were not significantly changed. Thus, our current results suggest that one of the mechanisms behind clinical effect of IT is the prevention of EOS accumulation and activation in the lungs of pollen-allergic patients with asthma. This finding is in agreement with a previous study in which a similar inhibitory effect of IT on the rise of EOS number and activation and ECA in serum during pollen season was noted.¹⁰

Our data from the two pollen seasons suggest a quantitative relationship between the allergen (pollen) load and EOS activation. During the low-load pollen season, only the ECA demonstrated a significant rise. The high-load pollen season, in contrast, resulted in increment of all measured aspects of EOS involvement, such as number of EOSs, ECP concentration, and ECA. The interpretation would be that the formation of ECA is the mechanism that is most sensitive to the pollen exposure. With an increasing pollen load, mechanisms that stimulate EOS secretion and eosinopoiesis are also activated. Although the formation of chemotactic activity was revealed to be very sensitive to pollen exposure, there was no linear relationship between pollen load and chemotactic activity, which indicates that a mechanism that modulates the production of chemotactic factors is also operative. The relationship between EOS number and ECA in BAL indicate that chemotactic factors are essential for the accumulation of EOSs in the lungs of patients with asthma. Furthermore, the relationship between ECA in BAL and serum suggests that the ECA in serum reflects the formation of chemotactic factors in the lungs. The relationship may either be due to release of the same chemoattractants into blood and BALF or to different factors produced by the same degree of activation of a cell or humoral system.

The exclusive accumulation of EOSs can, however, not be completely explained by the production of chemotactic factors. The NCA was also increased during pollen season and was significantly correlated to the eosinophil activity, but no increase in neutrophil number was observed. Our hypothesis is that EOSs from patients with asthma are primed to an enhanced responsiveness to chemotactic factors. Indeed, studies of the migration of EOSs from patients with asthma demonstrated increased chemotactic and chemokinetic responses in contrast to the neutrophils, thus supporting the hypothesis.^{17, 18}

In some studies with the BAL technique, the EOS accumulation has not been entirely specific but also involved a certain degree of neutrophil accumulation.¹⁹ In toluene diisocyanate-induced asthma, neutrophil accumulation was, in fact, the major finding, with only low numbers of EOSs present.²⁰ Thus, the

findings appear to vary, depending on the stimulus used to induce asthma and very likely on timing of the sampling, with more EOSs early after the challenge.

In years with medium- to high-pollen count, natural exposure to antigen will cause an increase in nonspecific BHR in all sensitive, untreated patients.^{3, 8} Also, a low-count pollen exposure results in increased sensitivity in the most sensitive patients but not in all patients. In the present study, a significant increase of BHR was found in all untreated patients (5/5) during high-load pollen season and in most patients (6/8) during low-load pollen season. In the IT-treated patients, the responses were more variable, and no significant changes were found. Half the recruited patients had been IT treated from 3 to 4 years and were clinically improved already during first season of IT treatment.⁸

The relationship between seasonal improvement and the out-of-season sensitivity is not known. Nevertheless, the differences in BHR to histamine preseasonally were not statistically different between the two groups neither before high- or low-load pollen season.

The seasonal increase BHR appeared to be associated with a specific accumulation of EOSs in the lungs of allergic patients during high-load pollen season. Exposure to a minor amount of antigen during low-load pollen season was neither sufficient to increase BHR nor to induce EOS influx to airway lumen. The association between clinical disease and presence and activation of EOS is, however, complex. In an earlier study in which the BHR increase was very marked, the PC₂₀ values correlated to ECP levels only in the most sensitive patients. It could be speculated that BHR is the net result of secreted cytotoxic proteins, sensitivity of the airways, the reparation mechanisms involved in neutralizing these agents, and finally other noninflammatory mechanisms. It is currently believed that, however dominating, the role of the EOS in allergic inflammation is not exclusive. All these factors are probably parts of the explanation of the lacking correlation between PC₂₀ histamine, EOS number, and ECP levels in some patients during low-load pollen season. Also, other investigators have made observations of discrepancies between recruitment of EOSs and BHR in atopic and asthmatic disease.²¹

Our current hypothesis is that blood EOSs of patients with asthma are primed to an enhanced response to chemotactic signals and that specific priming plays an important role in the exclusive accumulation of EOSs in the lung. The number and activity of primed cells could be directly linked to the amount of sensitizing antigen that is supported by the lower number

and secretory activity of EOSs during the low-load pollen season and vice versa during the high-load pollen season. The results also suggest that one important mechanism of action of IT is to prevent EOS accumulation and activation. The identification of the cell that produces the lung ECA may therefore not only be a major clue to the understanding of the effect of IT but also to the understanding of the allergic asthmatic reaction.

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