

The effect of immunotherapy on bronchial hyperresponsiveness and eosinophil cationic protein in pollen-allergic patients

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The effect of immunotherapy (IT) on bronchial response to histamine and on eosinophil cationic protein (ECP) in 40 birch pollen-allergic patients with a history of rhinoconjunctivitis and wheezing during the birch season was examined. Twenty patients started IT with birch extract (Pharmacia, Uppsala, Sweden) before the season. The other 20 patients were not treated with IT. Histamine-challenge tests were performed before, at the start, at pollen peak, at the end, and after the birch-pollen season. Blood samples for determination of ECP were collected at the same time. Skin prick tests and nasal provocation tests were also performed before and after the season. A significant increase in bronchial hyperresponsiveness was noted in the group treated with IT and in the untreated group during the season. The increase was greatest in the untreated group with the maximal difference between the two groups at the end of the pollen season ($p < 0.07$). IT treatment was followed by significantly less medication and higher peak expiratory flow values. The levels of ECP increased during the season in untreated patients ($p < 0.05$) but not in patients treated with IT. The ECP levels of patients from both groups correlated significantly with histamine sensitivity ($p < 0.001$). (J ALLERGY CLIN IMMUNOL 1988;82:470-80.)

Increased sensitivity to nonspecific stimuli is a cardinal feature of allergic and nonallergic asthma. It is also present in a number of patients with allergic rhinitis.¹ Specific airway responsiveness correlates to skin sensitivity, amount of allergen inhaled, and degree of nonspecific BHR.²⁻⁵

A number of cells and mediators have been identified in the course of the allergic reaction.^{6,7} Findings in some recent works focused attention on the role of the eosinophil cell. Increased eosinophil counts were found in fluid from BAL in allergic patients who developed LAR.⁸ In peripheral blood of patients who responded to allergen with dual reaction, the change in eosinophil count was found to correlate with methacholine PC₂₀.⁹ These findings indicate that eosinophils may play an important role in the inflammatory events of allergic asthma. IT is a well-established method for the treatment of allergic airway disease. However, the

Abbreviations used

IT:	Immunotherapy
BHR:	Bronchial hyperresponsiveness
ECP:	Eosinophil cationic protein
PC ₂₀ :	Provocation concentration corresponding to 20% decrease in FEV ₁
SPT:	Skin prick test
NPT:	Nasal provocation test
LAR:	Late asthmatic response
PEF:	Peak expiratory flow

mechanism of its action has not been clarified, and the effect on lower airways is still controversial.^{10,11}

The aim of this work was to study the effect of IT on the nonspecific BHR and on the levels of eosinophil degranulation product, ECT, in pollen-sensitive patients.

PATIENTS AND METHODS

Forty birch pollen-allergic patients, 26 female and 14 male patients, participated in the study. They were between 18 to 49 years of age, average age, 31 years. The diagnosis was based on a positive history of rhinitis and asthmatic symptoms during birch-pollen season, positive SPT, and positive NPT with birch-pollen antigen.

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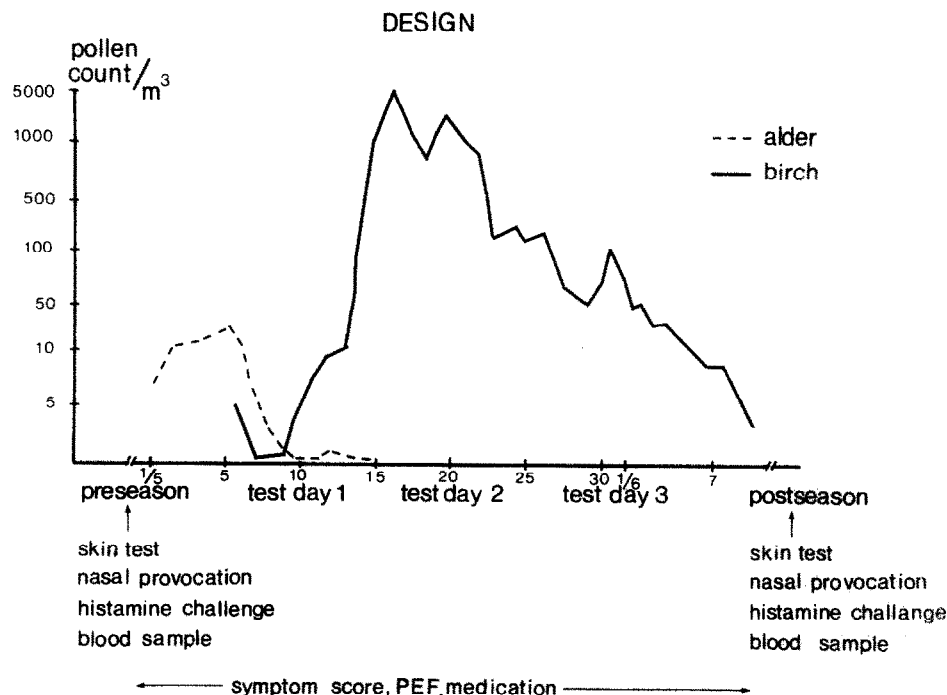


FIG. 1. Design of the study. Preseasonal testing was performed during winter months of 1985. In season 3 test days encompass pollinating of the alder and the birch tree. Postseasonal tests were performed in autumn of 1985.

In addition to birch allergy, 15 patients were sensitive to grass pollen. However, the grass-pollen season in this area comes later and is well separated from the birch-pollen season; 17 of 40 patients were also sensitive to animal dander but were not knowingly exposed to this during the study.

None of the patients had previously been treated with IT, and no patient was receiving steroid treatment.

Clinical data

Diary cards were filled in daily from April 22, which was estimated to be about 1 week before the start of the birch-pollen season and continued during a period of 6 weeks.

Peak flow measured with a mini-Wright PEF meter was noted daily, morning and evening. Daily symptom scores for eyes and nose from 0 to 3 (0, no symptoms; 1, slight; 2, moderate; and 3, severe) were registered.

Topical medication for eyes and nose consisted of decongestants taken when they were needed (counted as number of drops), nasal steroids (in number of puffs), and antihistamines (in number of tablets). For asthmatic symptoms, β -agonists were used when they were needed by inhalation (number of puffs) and orally (amount of tablets). Number of puffs and tablets was noted each day.

Design (Fig. 1)

Tests were performed before, during, and after the pollen season 1985. These included SPT, NPT, histamine provocation, and blood sampling for ECP.

Tests were carried out during the birch-pollen season on

3 test days: test day 1, at the beginning of the season; test day 2, in the middle of the season close to the peak; and test day 3, at the end of the season. Histamine challenge and blood collection were performed on each test day (Fig. 1).

Symptom scores were counted for three 2-week periods, encompassing each test day, that is, period 1 comprised the first 2 weeks at the beginning of pollen season, followed by period 2 in the middle of the season, whereas period 3 comprised the last 2 weeks.

Skin test

The SPT was performed as described earlier.¹² Before season, all patients were tested with a standard panel of allergens consisting of birch, timothy, mugwort pollen, dog and cat dander, mite, and mold (*Cladosporium* and *Alternaria*). Further testing was done with birch, alder, and hazel because of known cross-reactivity among these deciduous trees. SPT was considered positive at >3 mm of diameter of the wheal at 10,000 BU/ml of allergen concentration.¹³ SPT with birch-pollen extract was repeated after season, 10 to 12 months later.

Change in skin sensitivity was estimated by comparing the wheal area (square millimeter) at given allergen concentration before and after the treatment.

Nasal provocation test

The nasal sensitivity to birch pollen was tested in a standardized NPT. The test was performed at the same time as SPT before and after season.

TABLE I. Symptoms score, medication, and PEF before and during birch-pollen season

	Period							
	Before season*		1†		2‡		3§	
	Non-IT	IT	Non-IT	IT	Non-IT	IT	Non-IT	IT
Symptoms								
Eye and nose	0.17	0.16	0.63	0.54	2.31	2.22	1.89	1.3
Airways	0.03	0.18	0.33	0.25	0.89	0.6	1.10	0.43
PEF	445	545	450	529	445	493	433	526
Medication								
Eye and nose	0.29	0.25	1.79	0.61	12.03	3.14	11.08	2.55
Airways	2.67	0.33	6.6	1.26	20.6	5.4	18.1	2.8

*One week before start of pollen.

†First 2 weeks of pollen season.

‡Two weeks after period 1, including the peak period.

§Two last weeks of the birch season.

Allergen extract was diluted with albumin diluent in step-wise increasing concentrations with 0.5 log step 3.2, 10, 32, etc., up to 10,000 BU/ml; 0.1 ml of each concentration was flushed with a syringe on the lower conchi in both nostrils under visual control.

Subjective symptoms graded 0 to 3 were scored after 8 and 15 minutes for the following symptoms: sneezing, rhinorrhea, and congestion (0, no symptoms; 1, slight; 2, moderate; and 3, severe). If the sum was ≥ 5 with any of the concentrations up to 10,000 BU/ml, the test was regarded positive.

The result of NPT was expressed as the concentration of allergen extract producing a positive response.

Histamine challenge

The histamine provocation test was performed as described earlier.¹⁴ FEV₁ of >75% of predicted value was required before each challenge. Twofold increasing concentrations of histamine solution were prepared starting from 0.03 mg/ml at preseasonal provocation and from 0.00047 mg/ml during pollen season. Two milliliters of histamine solution was nebulized in a Pari Inhaler Boy (Pari Ritzar Pari Werk, Starnberg, W. Germany) (output 0.75 ml/min) and inhaled by tidal breathing for 2 minutes. FEV₁ was then assessed after 2 and 4 minutes. The test was stopped when the fall in FEV₁ was $\geq 20\%$ of initial value. The results were expressed in PC₂₀ (histamine concentration) calculated from the dose-response curve.

Treatment with oral β -agonists, β -agonist inhalants, theophyllines, and antihistamines was not allowed for 8, 4, 12, and 24 hours, respectively, before challenge.

Pollen count

Pollen count was registered daily with a Burkard (Burkard Manufacturing Co., Herts, U.K.) volumetric pollen trap placed on the top of the hospital building, approximately 15 m high. Besides birch pollen, alder and hazel were also counted, since these trees cross-react with birch.¹⁵ No other pollens were present at this time.

Immunotherapy

Twenty patients were allocated to treatment with IT, whereas the remaining 20 patients were not treated with IT. The groups were matched as to specific sensitivity to birch-pollen extract measured with SPT and NPT and to nonspecific sensitivity to histamine.

The patients in each group represented a similar range of BHR from pronounced (threshold value <0.5 mg/ml of histamine concentration), moderate (0.5 to 2 mg/ml), and mild (4 to 8 mg/ml). IT was performed with purified and standardized birch-pollen extract (Pharmacia, Uppsala, Sweden).

For SPT and NPT, a freeze-dried preparation was diluted with albumin diluent (Pharmacia), and for IT, with depot diluent (Pharmacia) containing 0.2% aluminum hydroxide.

The patients in the treated group received IT from November 1984 to February 1985. The initial concentration was 10 BU/ml of birch extract in all treated patients. The doses were then increased at weekly intervals until maximum tolerated dose was achieved (varying from 0.1 to 1 ml of concentration 100,000 BU/ml) and continued at 4- to 6-week intervals. During season, the maintenance dose was reduced by half.

Eosinophil cationic protein

Ten milliliters of blood was drawn on each test day. Serum samples were stored at -20°C and was analyzed according to the method of Venge et al.¹⁶

Statistics

The results of prechallenge FEV₁ at each test day were analyzed for the two treatment groups with two-sample Student's *t* test and analysis of variance.

To estimate PC₂₀, linear interpolation on a logarithmic scale was used. The analysis of PC₂₀ values was performed after logarithmic transformation to stabilize the variance. Results for each test day comparing values between the treatment groups and between preseason and each test

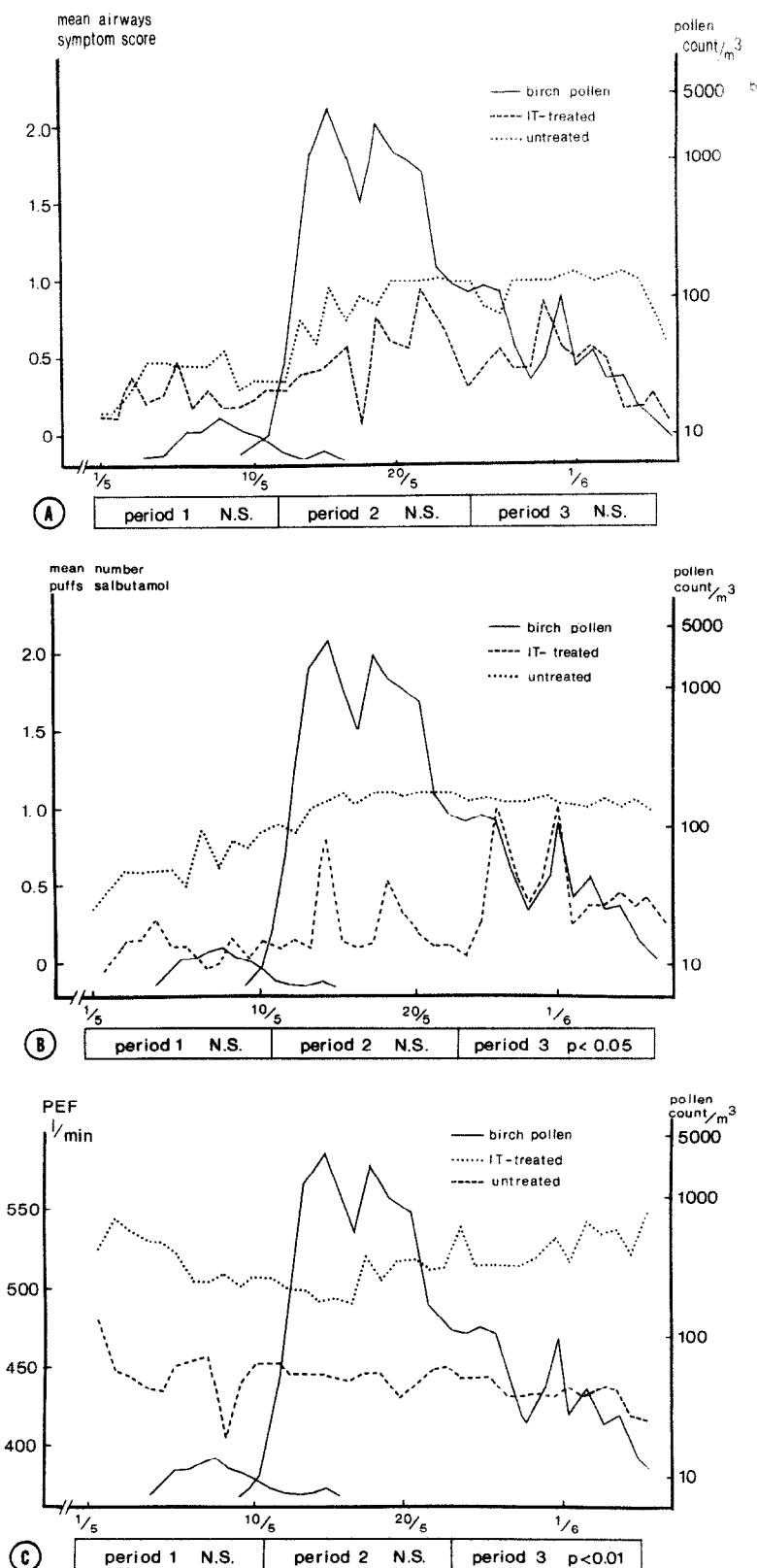


FIG. 2. Clinical data of the airway symptoms related to the pollen curve. **A**, Mean daily value of symptom score (0 to 3) in a group treated with IT and untreated group. **B**, Mean daily number of salbutamol puffs in each group during season. Significant difference between the groups at the third period, $p < 0.05$. **C**, Mean daily PEF value for each group. The difference between the groups was significant in the third period, $p < 0.01$.

TABLE II. Baseline FEV₁ and PC₂₀ histamine

	FEV ₁ (L)	Median PC ₂₀ (mg/ml)	Range PC ₂₀ (mg/ml)
IT-treated group			
Preseason	3.53	1.69	0.048-15.5
Test day			
1	3.58	0.51	0.018-10.0
2	3.59	0.12	0.001-4.0
3	3.66	0.075	0.0004-0.38
Postseason	3.65	1.51	0.05-15.0
Untreated group			
Preseason	3.13	1.25	0.021-10.27
Test day			
1	3.23	0.24	0.0019-5.0
2	3.28	0.078	0.0009-10.2
3	3.20	0.01	0.0004-1.8
Postseason	3.23	0.32	0.03-11.0

NS = not significant.

day within the group were analyzed with a Student's paired *t* test.

For symptom scores, peak flows and medication biweekly means were calculated and compared with two-sample Student's *t* test. The results of SPT and NPT were analyzed by use of two-sample Student's *t* test comparing preseasonal and postseasonal mean group values.

ECP values were evaluated by use of the paired Student's *t* test comparing mean ECP values between the groups and comparing preseasonal with seasonal test day results within the groups. The correlation coefficients were obtained by use of linear regression analysis. For statistical significance, *p* value was <0.05.

RESULTS

Birch-pollen season

The birch-pollen season of 1985 started late because of prolonged winter. The season was very poor in hazel and consisted mainly of birch and alder. It started with alder that peaked in the first week of May when only a few birch grains were noted. Birch pollen increased rapidly and peaked shortly after the middle of the month. Only small amounts of birch pollen were noted after the June 1 (Fig. 1). It was the most intensive birch-pollen season of the last 12 years with peak values up to 4000 grains/m³.

Clinical data (Table I)

Eye and nose symptom scores were very low before the start of the season, mean score 0.17 in untreated

and 0.16 in patients treated with IT. The symptoms increased during season, and the highest values were measured at the peak period, 2.31 and 2.22 for the two groups, respectively. Although decrease in symptom scores were noted at period 3 (1.89 and 1.3), the difference between the groups was significant *p* < 0.05. The mean medication score for eyes and nose rose from 0.29 (non-IT) and 0.25 (IT) before the onset of season to 12.03 and 3.14 during the peak pollen period 2, and 11.08 and 2.55 during period 3, respectively.

The difference between the groups was significant (*p* < 0.01) in all three periods.

Airway symptom scores were lowest before the start of the alder-pollen season, 0.03 in patients without IT and 0.18 in patients treated with IT. The highest mean values were recorded for untreated patients after the peak of pollen season, 1.1, and 0.43 for patients treated with IT. The difference was not significant (Fig. 2, A). Mean medication scores for airways were 2.67 (non-IT) and 0.33 (IT) before the pollen season. The difference was not significant. Highest consumption of medication for airways was observed in the second period, 20.6 and 5.4, respectively. The difference between the groups was significant at the third period, 18.1 and 2.8, respectively, and *p* < 0.05 (Fig. 2, B).

PEF values were highest before the start of season, mean 445 L/min in untreated patients and 545 L/min in patients treated with IT.

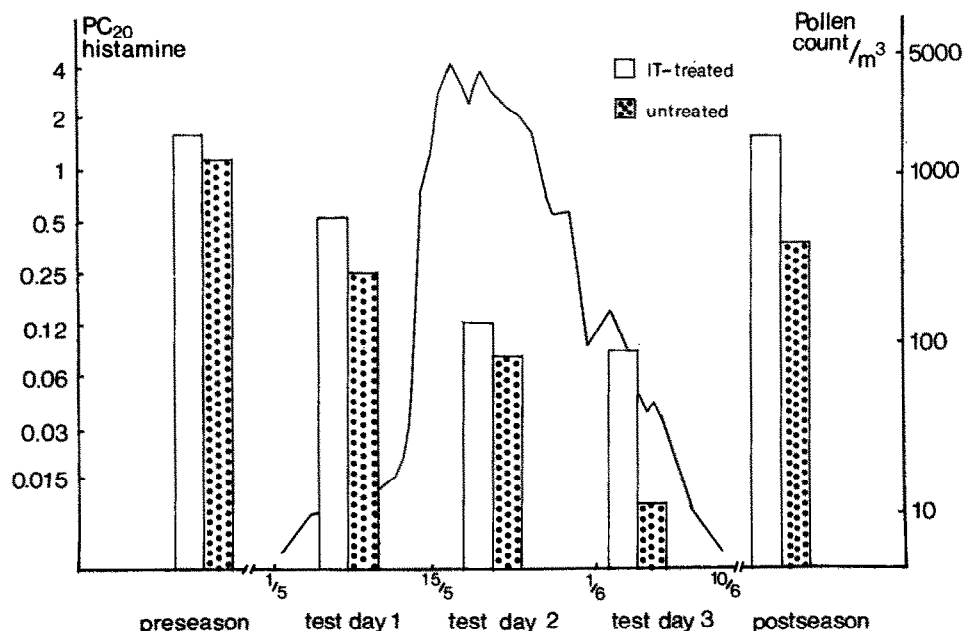


FIG. 3. Mean alder and birch-pollen count (curve) and median PC₂₀ histamine for patients treated with IT (open bars) and the untreated (dotted bars) group. At test day 3, the difference between median PC₂₀ of group treated with IT and the untreated group corresponded to $p < 0.07$.

The difference was significant ($p < 0.05$) in absolute values, but not as percentage of predicted values (different sex distribution within the groups). In both groups, PEF values dropped during the season. There was no significant difference between the two groups during period 2. The lowest mean value (433) was noted in the untreated group in period 3 in which there was significant difference between the groups (absolute values, $p < 0.01$) (Fig. 2, C).

Baseline FEV₁

Mean baseline FEV₁ value was 3.13 L (range 2.41 to 4.27) in the untreated group at the preseason test. In the group treated with IT, the mean preseason FEV₁ was 3.53 (range 2.35 to 5.17).

No significant difference in FEV₁ was observed within or between the groups on any of the test days (Table II).

Histamine challenge (Table II)

BHR was expressed in PC₂₀. In the untreated group, median PC₂₀ was 1.25 mg/ml at the preseason test and 0.24, 0.078, and 0.01 on the three test days during the season and 0.32 at the postseason test. The differences within the group between preseasonal PC₂₀ values and each test day (1 to 3) and postseasonal PC₂₀ values were statistically significant ($p < 0.05$, $p < 0.001$, $p < 0.001$, and $p < 0.05$, respectively). In the group treated with IT, median PC₂₀ value at preseasonal testing was 1.69, and 0.51, 0.12, and

0.075 during the season and 1.51 at the postseason test. Compared to the preseason value, the decrease in PC₂₀ on the three test days in season was statistically significant ($p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively). No significant difference was observed between preseason and postseason PC₂₀ in this group. The decrease in PC₂₀ was greatest in the untreated group at test days 2 and 3, but the difference between the groups was not statistically significant (at test day 3, $p < 0.07$). No significant differences were noted between the groups at any test day (Fig. 3).

Eosinophil cationic protein

The mean ECP values in micrograms per liter for each group and test occasion are presented in Fig. 4. In the untreated group, the increase from preseasonal value to test day 3 value was significant ($p < 0.05$). In the group treated with IT, there was no significant increase in ECP levels. There were no significant differences between the groups at any of the test days. The relationship between ECP levels and bronchial hyperreactivity was expressed as a correlation of ECP levels and PC₂₀ values (Fig. 5). A significant correlation was found for both patient groups, $r = 0.31$ and $p < 0.001$.

Skin test (Table III)

Skin sensitivity to birch pollen before initiation of the treatment was 70 mm² (geometric mean), range 33 to 122 in untreated patients, and 68 mm², range

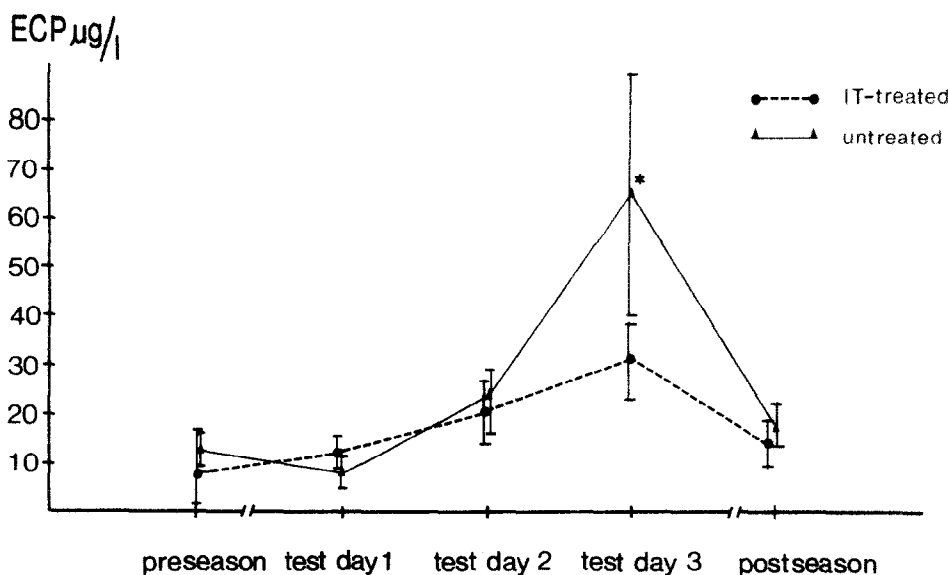


FIG. 4. ECP mean values and SEM in the group treated with IT and untreated patient group. There was a significant difference in the untreated group at test day 3 compared with preseasonal value ($p < 0.05$).

19 to 144, in the group treated with IT. The difference between the groups was not significant. After 10 to 12 months of treatment with IT, the values were 30 mm² (range 17 to 50) and 59 mm² (range 22 to 109), respectively. This difference was significant ($p < 0.001$).

Nasal provocation test (Table III)

Nasal sensitivity diminished significantly in the group treated with IT after 1 year of treatment ($p < 0.001$) but was unchanged in the untreated group.

Mean nasal sensitivity to birch pollen measured before IT was 1900 BU/ml (range 100 to 10,000) in the untreated group and 2100 BU/ml (range 320 to 10,000) in the patients treated with IT. The difference was not significant. After season, the values were 1900 BU/ml (range 320 to 10,000) and 10,000 (range 3200 to 100,000), respectively ($p < 0.001$).

DISCUSSION

In this study, nonspecific BHR increased significantly during the birch-pollen season, which is in agreement with an earlier study.¹⁷ In patients treated with IT, PC₂₀ values demonstrated a lesser decrease, the difference approaching significance ($p < 0.07$), compared with untreated patients.

Eosinophil degranulation product, ECP, increased significantly at the end of the birch-pollen season in untreated patients but not in the treated group. ECP

levels correlated significantly to PC₂₀ values and were especially high in the most sensitive patients of both groups.

The birch-pollen season in Sweden is an excellent natural provocation model with a clear-cut time period and no interference from other pollens. The birch-pollen season of 1985 was extremely intensive with unusually high pollen counts.

Alder pollinated in higher amounts than usual. The alder season precedes the birch-pollen season and causes allergic symptoms in most birch-pollen sensitive patients. Before the birch season there is usually a hazel season, but 1985 was very poor in hazel with no pollen in the area because of extremely low temperatures in the spring. Partial immunochemical identity of major antigen in alder, hazel, and birch explains the high degree of clinical cross-reactivity.¹⁸

Our patients were free from perennial allergies, such as mite and mold, and those patients sensitive to animal dander were not exposed to it. Thus, allergic symptoms during the birch-pollen season were an effect of pollen exposition alone and were only influenced by prescribed medication.

All individuals had histories of rhinoconjunctivitis and mild asthma. Asthmatic symptoms were satisfactorily controlled with β_2 -agonists, although a few patients used oral theophyllines occasionally. Tested pre-seasonally, 33 patients demonstrated histamine sensitivity within asthmatic range PC₂₀ <4 mg/ml.¹⁹ The other seven patients had PC₂₀ values >4 but <16. When median PC₂₀ and range of histamine sensitivity

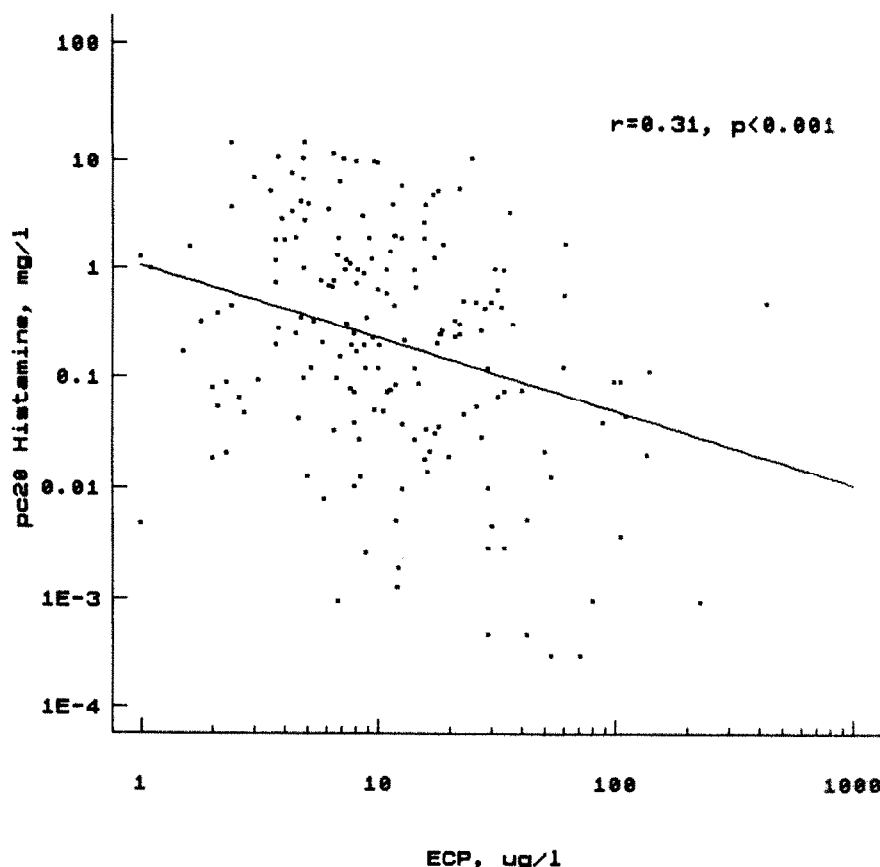


FIG. 5. Correlation between individual ECP levels and PC₂₀ values in all patients, $r = 0.31$ and $p < 0.001$.

and atopic state (allergen sensitivity in SPT and NPT) were taken into consideration, the treated and untreated groups were clinically similar at the start of the study.

The higher lung-function values in the group treated with IT are explained by different sex distribution within the groups (more women in groups treated with IT). When percent of predicted values were calculated, mean values of FEV₁ and PEF for groups before the season were similar. The effect of treatment with IT on sensitivity to birch pollen was demonstrated by a significant decrease in skin and nasal response to birch-pollen extract after 1 year.

Symptoms of rhinoconjunctivitis followed the pollen curve closely up to the pollen peak. Thereafter, symptoms were significantly less pronounced in the group treated with IT. Need of medication for eye and nose symptoms was also significantly less in the treated group during the whole pollen season. Also, in the airways, lesser use of β -agonist and higher PEF measurements in the treated patients indicated a significant improvement after IT.

It is reasonable to assume that the striking increase in BHR during the season is caused by high pollen

exposure producing a high degree of allergic inflammation in the airways. In a previous study with lower pollen count, we did not observe such great changes in BHR.¹⁷

The increase in histamine responsiveness during grass-pollen season was described earlier by Altounyan.²⁰ Boulet et al.²¹ examined methacholine sensitivity during ragweed season and found a significant increase during pollen exposition. The magnitude of change in their study was smaller than in the present study.

The intensive birch-pollen season in 1985 may also explain the relatively limited IT effect on nonspecific sensitivity in the treated patients. The short course of preseasonal therapy could be an additional reason.

In spite of low levels of alder and birch grains at the beginning of the season and very few allergic symptoms, BHR increased significantly in both groups during this period. Thus, the increase in nonspecific sensitivity appears to be the earliest indicator of allergen-induced bronchial inflammation. The inflammatory changes at this time do not reach the point where they cause perceptible asthmatic symptoms.

TABLE III. Mean values of SPT and NPT before and after season and difference between treated group with IT and untreated group

	SPT geometric mean (mm ²)		NPT mean allergen concentration (BU/ml)	
	Preseason	Postseason	Preseason	Postseason
IT-treated (n = 20)	68	$p < 0.001$	30	2100
	NS		NS	$p < 0.001$
Untreated (n = 20)	70	NS	59	1900
				NS

NS = not significant.

Changes in BHR do not completely parallel symptoms or pollen counts. The increase in BHR was most pronounced at the end of the pollen season when only a few birch grains were still in the air. Some authors also reported delayed symptoms in hay fever²² and asthma late in the pollen season when pollen count is low. The greatest increase in BHR at this point probably reflects a maximum of the bronchial inflammation.

We have previously studied the seasonal increase in BHR during the birch-pollen season and demonstrated that the antiallergic drug, disodium cromoglycate, inhibits the increase during pollen season,¹⁷ but not otherwise.²³

Seasonal BHR changes have also been confirmed during ragweed and grass-pollen seasons.^{20, 21, 24}

In seasonal asthma, the early and late asthmatic responses could not be separated because of continuous allergen exposition. In a laboratory bronchial provocation model out of season, appearance of late asthmatic reaction was followed by an increase in BHR.²⁵ We do not know if there were late responders among our patients because no preseasonal or post-seasonal bronchial provocation with allergen was performed. In one study,²¹ seasonal increase in BHR was mainly limited to patients with the ability to develop late reaction.

Our experience from birch pollen-allergic patients does not fully agree with this finding. In a recent study, birch pollen-allergic patients were tested after the season. Methacholine test was performed 2 days after provocation with birch pollen. Preliminary results demonstrated increased response to methacholine in patients both with and without clear late reaction.²⁶

The clinical effect of IT on rhinoconjunctivitis has been well documented,²⁷ especially in pollen allergy caused by ragweed,²² grass,²⁸ birch,²⁹ and mountain cedar,³⁰ but this was not the main objective of this study. The results of earlier studies designed to evaluate the efficacy of immunotherapy in asthma are often contradictory.³¹ Frankland and Augustin³² treated

grass-allergic patients with hay fever and asthma with good clinical results. Aas³³ studied 93 children with severe asthma caused by dust allergy and demonstrated a significant decrease in specific bronchial sensitivity in children treated with IT. New studies with better standardized and partially purified extracts demonstrated good clinical effects of IT on subjects with asthma sensitive to cat,³⁴⁻³⁶ dog,³⁷ and mite.³⁸ In contrast, Hill et al.³⁹ found no clinical effect of limited hyposensitization with grass-pollen extract on seasonal asthma in children.

Only a few studies have been carried out to investigate the effect of IT on nonspecific BHR. Thus, in cat-allergic subjects with asthma, prolonged IT did not reveal any improvement in BHR in some studies,^{34, 35} whereas Sundin et al.³⁶ demonstrated a decrease in histamine sensitivity after 9 months of treatment with partially purified cat extract. In mite-sensitive subjects with asthma, increase in BHR was observed in children⁴⁰ and adults⁴¹ after IT with mite extract. It appears more difficult to influence BHR with IT when allergic patients are continuously exposed to antigen in an uncontrolled manner. Three recent studies reported the effect of IT on frequency of late allergic reaction.^{38, 42, 43} Metzger⁴² demonstrated improvement in PD₂₀ methacholine after IT with *Alternaria* extract in allergic subjects with asthma. The effect of treatment on the appearance of late reaction may be important because of a documented relationship between the two phenomena.²⁵

The pollen season is also a suitable period for studies on mediator release. Eosinophil-derived protein ECP levels increased in untreated patients during the birch-pollen season. The increase coincided with a pronounced decrease in PC₂₀ values in all patients. At the same time the PEF values were lowest and the symptom score from airways highest in untreated patients, which can be interpreted as being an expression of the intensity of inflammatory changes.

A correlation was found between ECP levels and PC₂₀ values in all patients. The strongest correlation

was between ECP and PC₂₀ in the most hyperreactive patients from both groups. The mechanism of action of IT treatment on eosinophil accumulation and activation is not known.

Bronchial hyperreactivity appears to reflect an inflammatory state in the asthmatic airway. The role of eosinophils in the pathology of asthma is well recognized. The amount of peripheral blood eosinophils was demonstrated to correlate inversely to FEV₁ in subjects with intrinsic asthma.⁴⁴ Durham and Kay⁹ found that eosinophils also correlated to BHR expressed in methacholine PC₂₀. In the same study the number of eosinophils increased 24 hours after antigen challenge in patients who developed LAR. This increase also correlated inversely to PC₂₀ values.

In BAL performed on allergic subjects with asthma after antigen challenge, the number of eosinophils and ECP levels increased in individuals who developed LAR, but not in the other lavaged patients.⁸ The eosinophil-derived proteins with highly cationic charge are strongly cytotoxic to airway epithelium.^{45, 46} The increase in eosinophil numbers and ECP levels during LAR and correlation to degree of BHR strengthen the link between cellular factors and clinical features of asthma.

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