

Seasonal intestinal inflammation in patients with birch pollen allergy

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Background: The pathophysiologic interactions of inflammatory reactions between the mucosa of the respiratory tract and that of the gastrointestinal tract in individuals with allergy are poorly studied, despite the fact that allergic symptoms in the airways and the gastrointestinal tract might arise in the same patient.

Objective: The objective of this study was to examine the inflammatory response histologically by enumerating eosinophils, IgE+ cells, and T cells in duodenal biopsy specimens in adult patients with IgE-mediated birch pollen allergy during the birch pollen season and off-season.

Methods: Nine patients with birch pollen allergy verified by skin prick test and serum IgE antibodies were investigated toward the end of the birch pollen season and again 6 months later (off-season). Duodenal biopsy specimens were obtained and studied by immunostaining for the eosinophil major basic protein (MBP), IgE, and CD3+ T cells.

Results: Oral allergy syndrome to birch-associated foods was present in all patients as indicated by questionnaire. Duodenal increases of MBP+ eosinophils and IgE-bearing cells were found in the patients during the birch pollen season as compared with off-season. No seasonal differences in the T-cell numbers in these patients were seen. Off-season, there was no significant difference between the patients and the control subjects regarding the intestinal frequencies of MBP+ eosinophils, mucosal IgE+ cells, and T cells.

Conclusion: Birch pollen exposure triggered a local inflammation with an increase in duodenal eosinophils and IgE-carrying mast cells in patients with allergy. Our study gives evidence for the interplay between immunologically active cells in the airways and the gut. (*J Allergy Clin Immunol* 2003;112:45-51.)

Key words: Birch pollen allergy, Bet v 1, duodenal mucosa, eosinophils, eosinophil major basic protein, immunoglobulin E, T lymphocytes

Abbreviations used

Bet v 1: Recombinant major birch pollen antigen
BLP: Basal lamina propria
IBS: Irritable bowel syndrome
kU_A/L: Kilounits of allergen-specific IgE per liter
MBP: Major basic protein
NS: Not significant
OAS: Oral allergy syndrome
SPT: Skin prick test

Inflammation in the nasal mucosa is associated with the presence of IgE, mast cells, activated eosinophils, and T lymphocytes in patients with rhinitis caused by birch or grass pollen allergies.¹⁻⁴ Birch pollen allergy is often accompanied by oral symptoms to fruits and vegetables, the so-called oral allergy syndrome (OAS). There is clinical documentation for a connection of symptoms and inflammation between the upper and lower respiratory mucosa in patients with allergy.⁵⁻⁷ Less is known about a similar link between allergy in the respiratory and gastrointestinal mucosa. Despite the presence of OAS in response to birch-associated foods, little is reported as to reactions in the intestinal mucosa in patients with an IgE-mediated allergy when exposed to birch pollen.

In patients with a proven food hypersensitivity, challenge with the relevant food induces a local gastrointestinal reaction that possesses similar features to reactions in the respiratory mucosa, with increased intestinal secretion of hyaluronan and eosinophils releasing eosinophil cationic protein (ECP) and major basic protein (MBP), as well as prostaglandins and albumin.⁸⁻¹¹ IgE antibody production is generally considered to be a systemic event in allergic diseases. However, a local production of IgE has been discussed in the nasal mucosa,^{6,7} the lungs,⁵ the lesional skin,¹² and the intestine¹³ during exposure to allergens. Features of local allergic reactions in the gastrointestinal mucosa have been demonstrated in atopic, nonatopic, and asthmatic patients, implying that much of the mucosal immune system is involved in these conditions.^{7,14-16} Gastrointestinal symptoms have also been noted in oral immunotherapy with birch pollen antigen, in which the patients experienced abdominal pain and discomfort after local exposure in the gastrointestinal tract.¹⁷⁻²⁰ The aim of this study was to assess whether there was an inflammatory response in the gastrointestinal tract of patients with birch pollen allergy during the birch pollen

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Asthma, rhinitis,
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season, compared to off-season and to healthy control subjects without allergy. This was assessed by immunohistochemical characterization of the local cellular infiltration in the duodenal mucosa during the birch pollen period and off-season.

METHODS

Study population

Nine patients with birch pollen allergy and 9 control subjects were recruited by the Asthma and Allergy Research Group, Sahlgrenska University Hospital, Göteborg, Sweden. The mean age of the 3 men and 6 women was 40 years (range, 33 to 59 years). All had their diagnosis confirmed by a positive skin prick test (SPT) result ($n = 9$) (Soluprick; ALK, Hørsholm, Denmark) and serum IgE antibodies ($n = 9$) (Pharmacia CAP system) to the birch pollen antigen Bet v 1 (Pharmacia Diagnostics AB, Uppsala, Sweden). All patients reacted with seasonal allergic rhinitis. Additional symptoms in 5 patients were allergic conjunctivitis, asthma in 2 patients, and eczema in 1 patient during the birch pollen season. Seven of the patients had positive SPT results for other inhalant allergens (alder, hazel, timothy, mugwort, *Dermatophagoides pteronyssinus*, cat, dog, horse, guinea pig), and 3 patients had positive SPT results to foods (hazelnut, kiwi fruit, rye flour, soy protein). One of these 3 also had positive serum IgE antibodies against peanut and soy protein. Those 3 latter patients strictly avoided the foods they reacted to during the study. Six of the patients had an atopic family history, 4 had had eczema during early childhood, and 3 had had rhinitis/conjunctivitis since the early teens. Clinical and demographic characteristics of the subjects are shown in Table I.

The patients were requested to answer a questionnaire about their reactions of OAS to birch-associated foods (hazelnut, kiwi fruit, peanut, apple, pear) if and when they had gastrointestinal symptoms and if they fulfilled the irritable bowel syndrome (IBS) criteria (Rome II criteria, described elsewhere).²¹

Nine age- and sex-matched healthy volunteers with a mean age of 39 years (range, 30 to 58 years) and without a history of food or inhalant allergy or gastrointestinal symptoms and a negative serology for IgE antibodies as assessed by Phadiatop test (Pharmacia Diagnostics AB) were used as control subjects.

Experimental design

The patients were subjected to gastroscopy at the end of 2 different Swedish birch pollen seasons (late May to early June), when maximum exposure to the birch pollen was expected. The patients had to avoid food and drinks for at least 12 hours before the gastroscopy. A local anesthetic was applied as a spray in the throat about 5 minutes before the endoscopy started. The endoscope (Fujinon EG 200-S P; Fujinon Inc, Wayne, NY) was introduced orally and monitored on screen by the gastroenterologist, and biopsy specimens (4 to 6 biopsy specimens, 2 to 3 mm in size) from the proximal part of the duodenum were obtained. Duodenal biopsy and serum samples were obtained after a period of 7 days, when all birch-associated foods had been avoided that could give rise to OAS. The second biopsy specimens were taken after the birch pollen season in late autumn, when the patients were free of allergy symptoms. At least 6 months were allowed between the first and the second gastroscopies.

During both periods the patients were encouraged to fill in symptom protocols for 2 weeks, starting with the 7-day washout period when all foods that could cause symptoms were carefully avoided. During both periods antihistamines, corticosteroids, antileukotrienes, and sodium cromoglycates were withdrawn. Biopsies and sera were obtained in the same manner for the control subjects as for the patients. Informed consent was obtained from the

participants, and the Ethics Committee of the Medical Faculty, University of Gothenburg approved the study protocol.

Immunohistochemistry

The tissue specimens were immediately embedded in OCT compound (Tissue-Tek Miles, Inc, Elkhart, Ind), frozen in isopentane, pre-cooled by liquid nitrogen, and finally transferred into pure liquid nitrogen. Biopsy specimens and sera were kept at -70°C until analyzed.

The following mouse monoclonal antibodies were used: anti-human eosinophil MBP (15381A IgG1 κ ; PharMingen, San Diego, Calif), anti-IgE (CIA-E-7.12, IgG1 κ), anti-CD3 (UCHT1, IgG1 κ) (all from DAKO, Glostrup, Denmark).

Cryostat sections (7 μm) were stained as previously described.^{14,22} The slides were coded and evaluated in a random order. Positively stained cells were counted by using a computer-supported image analysis system Leica Q500MC (Leica, Cambridge, United Kingdom). All positive cells in at least 4 fields of the mucosa were counted, separating the villi from the basal lamina propria (BLP) regions. The positive cells were recorded as the number of stained cells/ mm^2 of tissue.

SPT

The SPT was performed and the reactions were graded according to the recommendations of the Standardization Committee of the Northern Society of Allergology.²³ Wheal reactions corresponding to the 10 mg/mL histamine reference were recorded as +++ and double this size as ++++; half the histamine reference size was recorded as ++ size and half of that as +.

Determination of IgE antibodies in serum

Serum IgE antibodies to inhalants and foods were measured with the Pharmacia CAP system, and sera of healthy control subjects were analyzed for the presence of atopy with a screening test, Phadiatop, according to the manufacturer's instructions. The Phadiatop is a common qualitative test for the presence of serum IgE antibodies against the aeroallergens most commonly occurring in the environment.

Statistical analysis

The values were reported as individual data points and median values. The statistical analyses were carried out by using Statview (SAS Institute, Inc, Cary, NC) on a Macintosh (Apple Computer, Cupertino, Calif) computer. The Mann-Whitney U test for non-paired data and Wilcoxon signed rank test for paired data were applied. The level for significance was set at P less than .05.

RESULTS

All the patients reacted with oral symptoms of itching and hoarse throat, sensation of swollen throat, or pain and redness in the throat after intake of birch-associated foods. Six patients experienced increased symptoms of OAS during the birch pollen season, and 3 did not associate such symptoms with the birch pollen season. Seven of the patients also complained of gastrointestinal symptoms such as abdominal distention, nausea, diarrhea, flatulence, abdominal pain, and constipation. The symptoms from the gastrointestinal tract affected 3 patients all year round, and 4 patients had severe abdominal symptoms during the birch pollen season. Five patients fulfilled the IBS criteria.²¹ Three of the patients experienced that the gastrointestinal symptoms depended on the severity of the birch pollen season. These results are shown in Table II.

TABLE I. Clinical features of the 9 patients with birch pollen allergy

Case no.	Age/ Sex	SPT/Serum IgE antibodies to birch pollen	SPT/Serum IgE antibodies to other inhalants	SPT/Serum IgE antibodies to foods	Birch pollen related symptoms
1	60/F	+++/4	+++/4	++/-	A, AR, AC, E
2	47/F	++/2	++++/-	-/-	AR
3	45/F	+++/3	+++/-	-/-	AR
4	38/M	++++/4	+++/-	-/-	AR, AC
5	37/M	+++/3	+++/-	-/-	AR
6	37/F	+++/3	-/-	++/-	AR, AC
7	36/M	+++/6	+++/3	+++/-	AR, AC
8	35/F	++++/4	-/-	-/-	AR
9	34/F	++/4	+++/-	-/-	A, AR, AC

Classes 1-6 depending on results of serum IgE antibodies or negative results (-). Other inhalant allergens are alder, hazel, timothy, mugwort, *D pteronyssinus*, cat, dog, horse, and guinea pig.

A, Asthma; AR, allergic rhinitis; AC, allergic conjunctivitis; E, eczema; +, positive results of the test, according to criteria for SPT; -, negative results of the test, according to criteria for SPT.

TABLE II. Birch-related and gastrointestinal features in the 9 patients with birch pollen allergy

Case no.	Birch-associated symptoms	Increased OAS during birch pollen season	Gastrointestinal symptoms	Increased gastro-intestinal symptoms during birch pollen season	Fulfills IBS criteria	Gastrointestinal disorder depending on worse/mild pollen season
1	OAS	-	AD, D, F, N	-	+	-
2	OAS	+	AP, D, E, V from soy, peanuts, nuts, seafood	+	+	-
3	OAS	+	AD, AP, F, from birch-associated foods and biogenic amines in foods	+	+	+
4	OAS	+	---	-	-	-
5	OAS	+	---	-	-	-
6	OAS	+	AD, D from birch-associated foods	+	+	+
7	OAS	-	AD, AP, D	-	-	-
8	OAS	-	AD, AP, C, D, F	-	+	-
9	OAS	+	D, F from birch-associated foods	+	-	+

AD, Abdominal distention; AP, abdominal pain; C, constipation; D, diarrhea; F, flatulence; N, nausea; V, vomiting; E, eczema; +, positive results of the test, according to criteria for SPT; -, negative results of the test, according to criteria for SPT.

Immune parameters in the serum

There were significantly elevated levels of serum IgE against Bet v 1 in the patients during the pollen season, 35.7 kilounits of allergen-specific IgE (kU_A/L), which were decreased off-season to 29.7 kU_A/L ($P = .02$). The control subjects showed low levels of IgE anti-Bet v 1 regardless of season, 0.5 kU_A/L and 0.4 kU_A/L , respectively ($P = .0003$).

Immune parameters in the duodenal mucosa

Frequency of eosinophils in the duodenal mucosa. During the birch pollen season the biopsy specimens from the patients showed an increased number of MBP+ eosinophils and pronounced mucosal infiltration of MBP+ cells throughout the villi and BLP (Fig 1, A). The frequency of MBP+ eosinophils was significantly higher in the patients during the birch pollen season (Fig 2, A) as compared with off-season (Fig 2, B) ($P = .008$), in the villi and in the BLP or compared to the control subjects

(Fig 2, C) in season ($P = .009$ in the villi and $P = .001$ in the BLP). Off-season, there were no significant differences in the eosinophil numbers comparing the patients and the control subjects.

Frequency of IgE-positive cells in the duodenal mucosa. During the birch pollen season (Fig 2, D) more IgE+ cells were present in the duodenal mucosa of the patients compared with the patient samples taken off-season (Fig 2, E) ($P = .008$ in the villi and $P = .01$ in the BLP). The patients showed significantly higher numbers of IgE+ cells as compared with the control subjects during the birch pollen season ($P = .002$ in the villi and $P = .009$ in the BLP) (Fig 1, B). Off-season, the cell counts in the villi did not differ between the patients and the control subjects (Fig 2, F). However, there were still slightly higher numbers of IgE+ cells in the BLP of the patients as compared with the control subjects ($P = .05$). Morphologic analysis showed that the IgE+ cells were mainly mast cells, and only a few were IgE+ eosinophils.

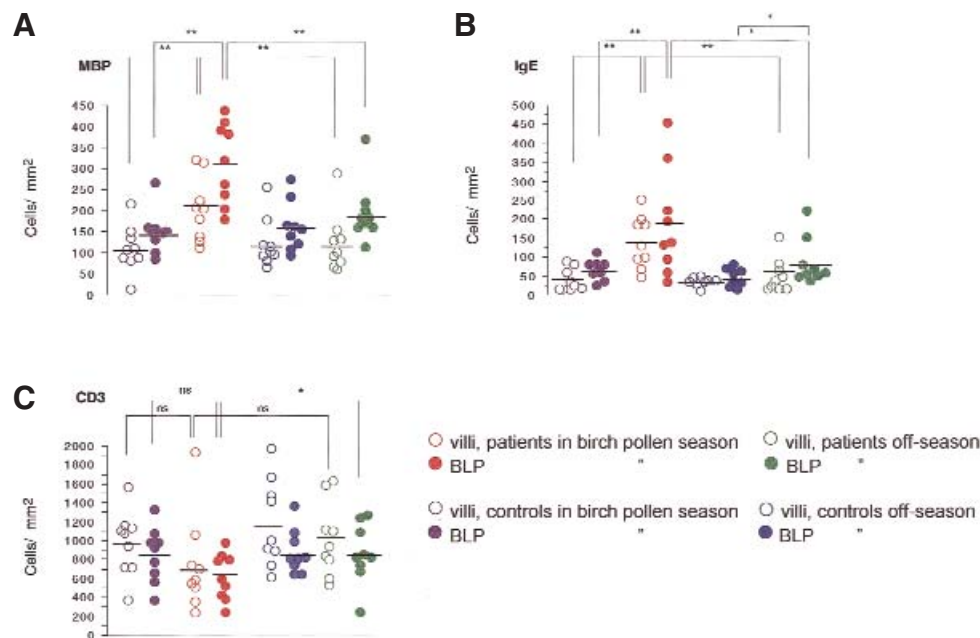


FIG 1. Numbers of MBP+ eosinophils (**A**), IgE stained cells (**B**), CD3+ T cells (**C**) in duodenal biopsy specimens taken from patients with birch pollen allergy and healthy control subjects in season and off-season ($n = 9$). Results are expressed as individual data points and the median is indicated. * $P < .05$. ** $P < .005$. *** $P < .001$.

Frequency of CD3+ T cells in the duodenal mucosa. The numbers of CD3+ T lymphocytes were determined in the villi and in the BLP of the duodenal biopsy specimens. In the patients during the birch pollen season the number of CD3+ T cells was comparable to that seen off-season (Fig 1, C) in the villi ($P = .09$) and in the BLP ($P = .05$). However, during the pollen season the patients did not show any significant differences in the overall numbers of T cells ($P = .07$ in the villi and $P = .1$ in the BLP), as compared with the healthy control subjects. Off-season, no differences in the T-cell number were found in any of the comparisons. The CD4+ and CD8+ T-cell subsets behaved very similarly in the groups studied (data not shown) and followed the general trend of the common T-cell marker CD3.

Despite the infiltration of inflammatory cells in the patients during the birch pollen season, there were no signs of gross morphologic changes like crypt hyperplasia, epithelial damage, edema, or villus atrophy.

No positive correlations were found between any of the inflammatory markers in patients with additional positive SPT/serum IgE to foods with single or inhalation allergens. The inflammatory patterns in patients with and without gastrointestinal symptoms were similar. Furthermore, there were no significant differences between patient biopsy specimens taken during a season rich in birch pollen compared to seasons with moderate pollen levels.

DISCUSSION

In this study we have demonstrated the presence of allergic inflammation in the duodenal mucosa of subjects

with birch pollen allergy during the birch pollen season. Eosinophil infiltration and increased numbers of IgE+ cells primarily characterized the inflammation. In an earlier study we have shown that food-intolerant patients with gastrointestinal symptoms and a diagnosis confirmed by double-blinded, placebo-controlled food challenge, but negative SPT results and serum IgE antibody to tested foods, had a similar gastrointestinal inflammatory pattern as the patients with birch pollen allergy with a classic IgE-mediated reaction.¹⁴ Reactions in the gastrointestinal tract might be explained by the deposition of the dissolved inhalants in the oropharynx and a successive passage into the gastrointestinal tract. Furthermore, aerolized administration of radiolabeled pollen grains of *Poa pratensis* to adults showed that most of these airborne particles affected the oropharyngeal mucosal surfaces and then were ingested with only a minor part ending up in the bronchi.²⁴ However, the allergenicity of swallowed inhalant allergens has been questioned. In vitro experiments have demonstrated that after 30-minute incubation in duodenal juices most birch and timothy pollen proteins had been hydrolyzed, leaving only 10% of the original birch and timothy allergenic activity.²⁵

During the birch pollen season, the patients in this study showed increased numbers of MBP+ eosinophils compared to off-season and to control subjects. Eosinophilic inflammation has been observed previously in the duodenal mucosa of atopic, nonatopic, and asthmatic subjects. This would imply that much of the mucosal immune system might be involved in such conditions.^{7,14,16,26} Such a view is supported by studies showing an increased proportion of eosinophils, neu-

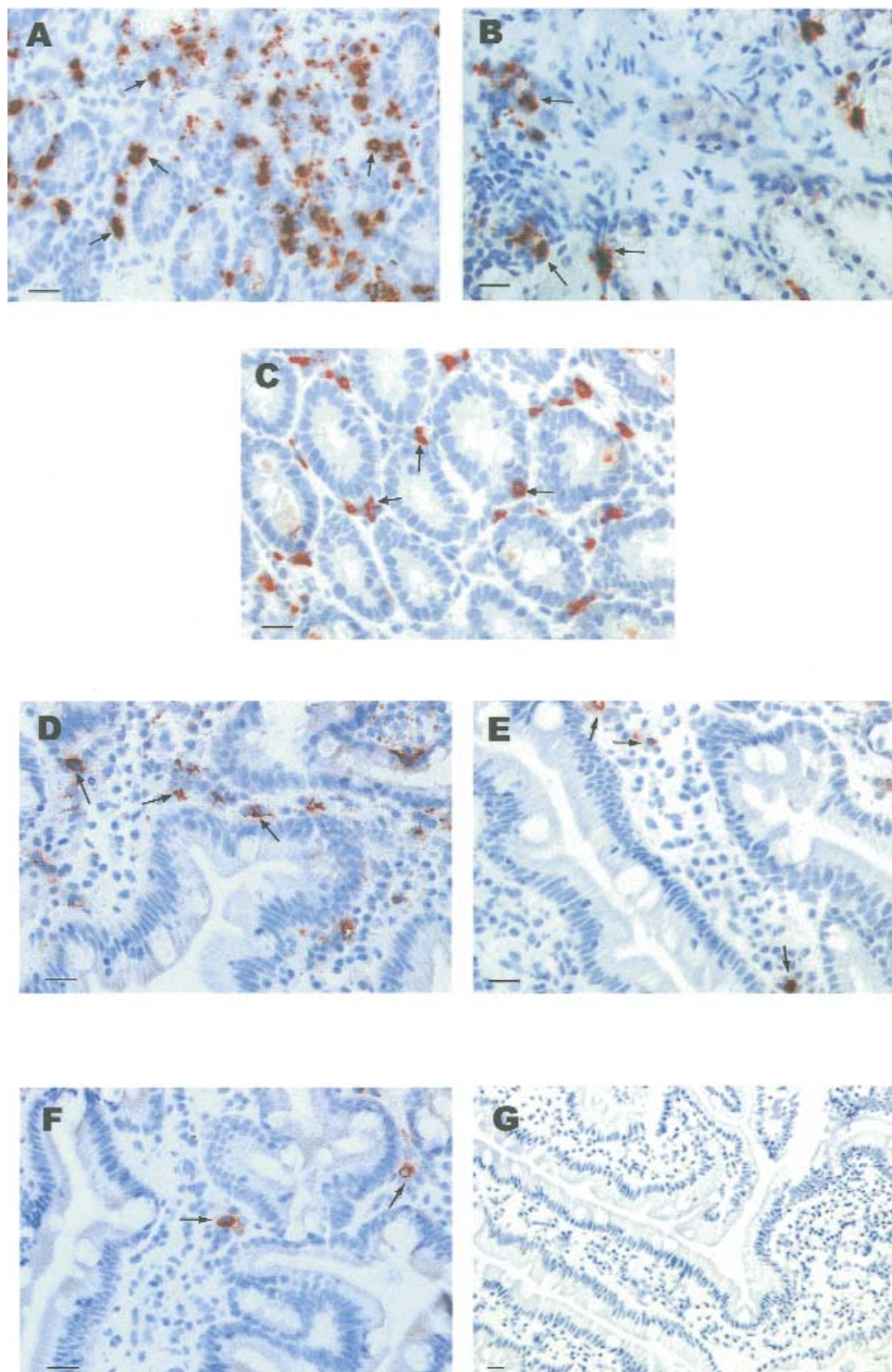


FIG 2. Immunostaining of duodenal biopsy specimens from patients with birch pollen allergy and control subjects. *Red-brown color* indicates positive staining; *blue color* is counterstaining of nuclei. *Black bars* indicate 30 μm. (A) Marked increase of MBP-stained eosinophils in a patient during the birch pollen season; (B) MBP+ cells in a patient off-season; (C) MBP-stained eosinophils in a healthy control subject; (D) enhanced presence of IgE-stained cells in a patient during birch pollen season; (E) presence of IgE-stained cells in a patient off-season; (F) IgE-stained cells in a healthy control subject; (G) isotype control staining.

trophils, and IL-8 in sputum in nonasthmatic patients with food allergy, in comparison with control subjects, which might suggest a subclinical airway inflammation in these patients.¹⁵

Increased numbers of IgE+ cells were noted in the duodenal mucosa in the patients compared with off-season and control subjects. This is in concordance with other findings demonstrating elevated specific IgE to inhalant allergens in intestinal washings from nonatopic children.¹³

In the present study we did not find any significant increase of T lymphocytes in patients during the birch pollen season, compared with the patients off-season and the control subjects. In asthma, T-cell-mediated mechanisms have been demonstrated, although the role of the T cells in IgE-mediated inflammation is quite unknown.²⁷⁻²⁹

Increased numbers of eosinophils and IgE-bearing cells in the duodenal mucosa off-season further support the idea that a more extended systematic inflammatory reaction takes place in these patients with birch pollen allergy. Allergic inflammation has been reported in a remote and "upstream" organ (nasal) after a local allergen challenge with grass pollen in a segmental bronchus of the right middle lobe.³⁰ Furthermore, recent evidence suggested that allergic rhinitis might be linked to development of systemic allergic manifestations such as allergic asthma.³¹ These findings suggest that the allergic inflammation is systemic and that lymphocytes of nasal, bronchial, and enteric mucosa might belong to the same recirculating pool.

The inflammatory response of IgE+ cells in the intestine occurred primarily during the birch pollen season but still remained in the BLP, albeit to a lesser extent, 6 months after the birch pollen season. A prolonged inflammatory response still present even when the antigen is not present has previously been described in the nasal mucosa of patients with allergic rhinitis.^{32,33} One might speculate that the subclinical inflammation off-season seen in our study was aggravated by allergies both to seasonal and perennial inhalant allergens as seen in the majority of the patients (7 of 9). However, this view was contradicted by the fact that the 2 patients not allergic to any additional inhalants did not differ from the other patients in regard to their numbers of inflammatory cells in season or off-season.

In our study the majority of the patients with birch pollen allergy complained about gastrointestinal symptoms, and 4 patients had severe abdominal symptoms during the birch pollen season. A few patients also reported severe symptoms related to the birch pollen counts during the season. Although a connection between the respiratory system and the gut has been discussed, there has not been any clear-cut clinical experience of a connection between birch pollen allergy and gastrointestinal symptoms according to some studies.^{7,34,35} On the other hand, a relation between allergies in the respiratory tract and the gut has been suggested in other studies.³⁶ A recent investigation showed an allergic reaction to Bet v 1 after colonoscopic allergen provocation in patients with allergy to birch pollen.^{37,38}

Gastrointestinal symptoms are difficult to diagnose, and 5 patients in our study fulfilled the IBS criteria. This might imply that patients with pollen allergy and gastrointestinal symptoms can be misdiagnosed with IBS and thus not be adequately treated. Furthermore, a subset of IBS might be seasonally determined, and this is explained in part by somatization.³⁹ There might be pathophysiologic links between IBS and allergy. Thus mast cells have been discussed to be included in the pathogenesis of IBS, and increased numbers of mast cells in biopsy specimens in cecum of patients with IBS have been found. In those studies, however, numbers of plasma cells, lymphocytes, eosinophils, neutrophils, and macrophages were not elevated.⁴⁰ Furthermore, similar to our findings, the prevalence of asthma has been noted more commonly among patients with IBS than in control subjects.⁴¹

In conclusion, this study showed that there was a significant increase during the pollen season of the numbers of eosinophils and IgE-positive mast cells in the duodenal mucosa of adults with birch pollen allergy and a history of rhinitis and OAS. In a majority of these patients this was accompanied by subjective gastrointestinal symptoms.

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