

Birch pollen-related foods trigger atopic dermatitis in patients with specific cutaneous T-cell responses to birch pollen antigens

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Background: Patients with inhalant allergy caused by birch pollen frequently demonstrate immediate symptoms to cross-reactive fruits, vegetables, or both. The nature of late eczematous reactions to pollen related food antigens has not been investigated in detail.

Objective: The purpose of this study was to find out whether isolated late eczematous reactions to birch pollen-related food antigens can be observed in patients with atopic dermatitis (AD) who are highly sensitized to birch pollen antigens. A possible linkage of such reactions with specific T-cell responses to birch pollen antigens in the blood and lesional skin was examined as well.

Methods: We examined 37 adult patients with AD and hypersensitivity to birch pollen but without any history of immediate responses to food challenges. These patients underwent an elimination diet, including all birch pollen-related food antigens, followed by a double-blind, placebo-controlled, oral provocation. Blood and skin biopsy specimens were taken to examine a birch pollen-specific lymphocyte response.

Results: Seventeen patients reacted with a deterioration of AD symptoms. Food- or birch pollen-specific IgE did not differentiate these patients from nonreactive patients. A significantly higher increase in the proportion of blood lymphocytes expressing the cutaneous lymphocyte antigen on incubation with birch pollen antigens was found in cells from reactive compared with nonreactive patients. The proliferative response of skin-derived T-cell lines from reactive patients to birch pollen extract or Bet v 1 was significantly higher than that of nonreactive patients. An enrichment of more than 25% of T-lymphocyte subpopulations defined by T-cell receptor-V β elements was detected in the majority of such antigen-stimulated T-cell lines from responsive patients. A higher frequency of birch pollen-reactive T cells was calculated from limiting-dilution assays, and a higher rate of birch pollen-specific T-cell clones was generated from cultures with skin-derived T cells from reactive patients.

Conclusion: Our results show, for the first time, that a subpop-

ulation of patients with hypersensitivity to birch pollen and AD reacts with worsening of eczema after oral challenge with birch pollen-related foods and that a birch pollen-specific T-cell response can be found in the lesional skin of these patients. (*J Allergy Clin Immunol* 1999;104:466-72.)

Key words: Atopic dermatitis, birch pollen-related foods, cross-reactivities, specific T cells

Atopic dermatitis (AD) is a chronic eczematous skin disease that often begins early in infancy and runs a course of remissions and exacerbations. At present, the pathogenesis of AD is not fully understood. Besides basophils, eosinophils, and cells of the mononuclear phagocyte lineage, skin lesions mainly contain CD4⁺ T lymphocytes.^{1,2} Elevated IgE is only found in 60% to 80% of all patients with AD and is therefore not a prerequisite for AD.³

Skin lesions might be provoked by food antigens in a subpopulation of patients with AD. Series of studies have been performed, including diet programs and oral provocations with various food antigens, in children with AD.⁴ Atherton et al⁵ observed a significant improvement of AD in children during a period of placebo-controlled diet. Sampson and McCaskill⁶ observed cutaneous symptoms in a significant percentage of children with AD some hours after oral provocation. Delayed eczematous reactions have been reported in other studies in which the suspected allergen was given daily for several days.⁷⁻⁹ Hill et al¹⁰ observed delayed eczematous reactions (ie, 24 hours or later after challenge) in 17 of 135 children with suspected cow's milk allergy. Interestingly, 10 of these 17 children had negative skin prick test responses to cow's milk antigens, suggesting a non-IgE-mediated pathogenesis.

If food allergens play a direct role in the pathogenesis of AD, food allergen-reactive T cells should be involved. Previously, we described a positive correlation between oral provocation tests with milk and specific proliferative reactions of blood T lymphocytes to casein in children with AD.¹¹ We also investigated the role of food antigens in adolescent and adult patients with AD⁹ and showed that individuals who experienced worsening of AD after oral provocation with milk had a higher incidence of casein-specific T-cell reactions compared with control subjects.

In addition to food antigens, inhalant allergens may

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Abbreviations used

AD:	Atopic dermatitis
CLA:	Cutaneous lymphocyte antigen
ICAM-1:	Intercellular adhesion molecule-1
LPT:	Lymphocyte proliferation test
TCC:	T-cell clone
TCL:	T-cell line
TCR:	T-cell receptor

provoke flare-ups of the extrinsic variant of AD.¹² Because of extensive experimental work during the last 2 decades, it is now clear that skin-infiltrating inhalant allergen-specific T cells express a T_{H2}-like cytokine pattern in acute skin lesions^{13,14} but may also secrete IFN- γ in chronic lesions.^{15,16}

In this study we investigated adolescents and adults with AD who were highly sensitized to birch pollen antigens. In contrast to the majority of birch pollen-sensitized individuals,¹⁷⁻¹⁹ these patients belonged to the group of one third of all sensitized patients with AD who do not have immediate symptoms after ingestion of birch pollen-related foods. Therefore they did not maintain a birch pollen-related elimination diet before this study. We could show that (1) nearly half of the birch pollen-sensitized patients with AD react with a marked deterioration of their eczema on oral provocation with related foods and (2) this clinical reaction is associated with a specific T-cell mediated immune response to birch pollen antigens in lesional skin.

METHODS

Patients

Thirty-seven adult patients with AD, between 17 and 64 years of age, were recruited from the Department of Dermatology and Allergy of the Hannover Medical University between July 1996 and March 1998. A diagnosis of AD was made according to the criteria of Hanifin and Rajka.²⁰ All patients were sensitized to birch pollen (specific IgE, >17.5 kU/L; Pharmacia, Freiburg, Germany). Patients were selected from the general population of our outpatient clinic according to the following 3 criteria: (1) lack of a history of immediate symptoms to birch pollen-related food challenges; (2) sensitization to birch pollen; and (3) presence of AD. None of these patients had a history of food-responsive AD. The study protocol was approved by the ethics committee of the Hannover Medical University, and written informed consent was obtained from all patients.

Clinical study

All patients underwent an elimination diet for 4 weeks after instruction by a dietician, which excluded all birch pollen-related food. For oral provocation, 60 g of carrots, 60 g of celery, 20 g of hazelnuts, and 60 g of apple were mashed and masked with carob (Sinlac; Nestle, Munich, Germany) and orange flavor (Scientific Hospital Supplies, Liverpool, UK). Verum (containing 200 g of mixed fresh birch pollen-related foods) or placebo (carob, orange flavor, and rolled oats) were given to the patients on 2 consecutive days at the beginning of a week in random order according to a modified protocol, as previously described.⁹ The time period of

observation was 2 hours on each day of challenge, and no immediate symptoms were observed. The patients did not have baseline symptoms (ie, urticaria, angioedema, bronchospasm, or gastrointestinal symptoms) other than AD, which might have masked early reactions. The challenge was truly double blind; the patients could not guess correctly whether they received placebo or a true challenge. The body surface affected and severity of dermatitis were assessed daily with the SCORAD,²¹ which takes into account the intensity and extent of the eczema and subjective parameters, such as sleep loss and itching, by a physician unaware of the food given and of laboratory findings. An increase of at least 15 SCORAD points, which indicates a clinically marked deterioration of AD on the basis of our experience was considered to be positive.

Preparation of antigens for cell culture

Birch pollen extracts (Allergopharma, Hamburg, Germany) were found to be contaminated with LPS. LPS was removed from the protein fractions by polymyxin B coupled to a solid phase, as described.^{22,23} The protein fractions were incubated on a shaker for 24 hours at 4°C with polymyxin B agarose (Serva, Heidelberg, Germany) in the presence of octyl- β -D-glucopyranoside (Calbiochem-Novabiochem, Bad Soden, Germany). After centrifugation, the whole procedure was repeated, and the supernatants were dialyzed twice against pyrogen-free 0.9% NaCl. LPS concentrations were determined by the Limulus assay (Coatest Endotoxin, Pharmacia), according to the manufacturer's protocol. The detection limit was 50 pg LPS/mL.

Lymphocyte proliferation assays

Lymphocyte proliferation assays (LPTs) were performed before the elimination diets. None of the patients had been receiving steroids systemically for 3 weeks before the tests were carried out. PBMC were isolated from heparinized blood samples by density centrifugation on Ficoll-Hypaque (Nycomed Pharma AS, Oslo, Norway; density = 1.077 g/mL). PBMCs (2×10^5) were cultured in Iscove's medium (Biochrom KG, Berlin, FRG) supplemented with 4% heat-inactivated AB serum, glutamine, gentamycin, penicillin, streptomycin, and nonessential amino acids in the presence or absence of antigen. After determination of the optimal concentration in proliferation assays, PBMCs were stimulated with 10 μ g/mL PHA (as positive control), LPS-depleted birch pollen extract with a protein concentration of 5 μ g/mL, or recombinant birch pollen antigens Bet v 1 and Bet v 2 (Biomay, Linz, Austria) at a concentration of 1 μ g/mL. After 5 days, the cells were pulsed with tritiated thymidine (0.5 μ Ci/well for 4 hours), and the β -decay was counted in a liquid scintillation counter. The stimulation index was defined by the ratio of mean counts per minute of stimulated to unstimulated cultures.

Preparation of single cells from skin biopsy specimens

Punch biopsy specimens (5 mm in diameter) were taken from lesional skin. The epidermis and dermis were separated by overnight incubation in dispase (2.4 U/mL, Boehringer Mannheim) at 4°C, as previously described.¹⁶ The epidermis was incubated with 0.25% trypsin (Sigma Chemie, Munich, Germany) for 20 minutes at 37°C and washed in modified HBSS. Dermal tissue was incubated in HBSS (5 hours at 37°C) containing collagenase (0.1%), DNase (20 μ g/mL), dispase (1.2 U/mL, Boehringer-Mannheim), hyaluronidase (0.1%, Sigma), and 10% FCS. Dermal cell suspensions were washed and filtered through nylon gauze. Epidermal and dermal cell suspensions were mixed in subsequent cell cultures. The percentage of T cells was assessed by flow cytometry with an antibody to CD3 (Becton Dickinson GmbH, Heidel-

TABLE I. Serum IgE in patients sensitive to birch pollen antigens

	n	Specific IgE, Bet v 1 (kU/L)	Specific IgE, Bet v 2 (kU/L)	Specific IgE, carrot (kU/L)	Specific IgE, celery (kU/L)	Specific IgE, hazelnut (kU/L)	Specific IgE, apple (kU/L)	Total IgE (kU/L)
Responding patients	17	108.4*	1.5	9.6	8.4	17.4	6.1	3085
Nonresponding patients	20	231.3	1.3	4.4	3.1	8.6	4.0	3150

*Mean values are shown.

berg, Germany). T cells represented less than 15% of all cells in cutaneous cell suspensions. The ethical committee of the Hannover Medical University approved taking skin biopsy specimens from lesional skin.

Investigation of T-cell lines and T-cell clones

Polyclonal T-cell lines (TCLs) were generated in the presence of the birch pollen antigens Bet v 1 and Bet v 2. Cloning was performed by limiting dilution in the presence of antigen and IL-2, as previously described.^{9,14,16} Responder cells from patients and a mixture of Bet v 1 and Bet v 2 antigens (each at a concentration of 1 µg/mL) were added to limiting-dilution wells (96-well, round-bottom trays; Nunc GmbH, Roskilde, Denmark) together with 2×10^4 autologous, antigen-presenting cells (PBMCs irradiated with 55 Gy, ³⁷Cs). The cultures were kept in Iscove's medium supplemented with 4% heat-inactivated AB serum, glutamine, gentamycin, penicillin, streptomycin, nonessential amino acids, and 10 U/mL recombinant (r)IL-2. After 12 to 14 days, wells demonstrating growth of greater than 50 lymphoblasts were scored as positive. Frequencies of responding CD3⁺ T cells were determined by the minimum χ^2 method, according to the method of Taswell,²⁴ and calculated as described.²⁵ Because cultures were expanded from only dilutions with 0.3 proliferating cells or less, there was a high probability of clonal cell growth.

Phenotyping of PBMCs, TCCs, and T-cell clones

Birch pollen-stimulated PBMCs were investigated with antibodies directed to the cutaneous lymphocyte antigen (CLA; HECA 452, Pharmingen, Hamburg, Germany) or to the intercellular adhesion molecule-1 (ICAM-1; Immunotech, Hamburg, Germany). Expression of T-cell surface antigens on TCLs or T-cell clones (TCCs) was investigated with FITC- or PE-labeled antibodies recognizing CD3, CD4, CD8, T-cell receptor (TCR)2 (α/β), V β 1, V β 2, V β 3, V β 6.7, V β 9, V β 13.1/13.3, V β 14, V β 16, V β 17, V β 18, V β 20, V β 21, or V β 22 (Immunotech). Antibodies against V β 5.1-3, V β 8, or V β 12.1 were obtained from DPC (Bad Nauheim, Germany). After the staining procedure, 1×10^4 cells per sample were analyzed with a FAC-Scan flow cytometer (Becton-Dickinson). Viable cells were identified by staining with propidium iodide.

Statistical analysis

The comparison of the medians of nonparametrical distributed results of different patient groups was performed with the Mann-Whitney rank-sum test. Mean values of parametrical distributed results were compared by means of *t* tests. The numbers of reactive TCCs from responders versus nonresponders were compared by means of the χ^2 test.

RESULTS

Outcome of the clinical study

Double-blind, placebo-controlled food challenges were carried out in 37 adult patients by using birch

pollen-related foods. Seventeen patients reacted with worsening of eczema and experienced additional itching and sleeplessness within 48 hours after oral provocation, with a median increase of 21 SCORAD points (Fig 1). There were neither significant differences of the skin conditions before provocation nor of associated respiratory atopic diseases nor of total serum IgE values between the patient groups. Specific IgE to at least one birch pollen-associated food (>1 kU/L) was found in all but one patient. Food-specific, but not Bet v 1-specific, IgE levels were higher in the group of reactive patients (Table I).

Evaluation of lymphocyte responses to birch pollen antigens of blood lymphocytes

Birch pollen extract or recombinant Bet v 1 or Bet v 2 antigens served as antigens in LPTs. No proliferative responses were observable with lymphocytes from nonatopic control individuals when birch pollen allergens were tested (with a mean of 450 ± 23 background counts, data not shown). Proliferative responses with stimulation indices between 2 and 3 were observable to birch pollen extract and to Bet v 1 both in responsive and in nonresponsive patients. Recombinant Bet v 2 did not lead to significant proliferative responses in either patient group (data not shown). Flow cytometric evaluation of birch pollen-stimulated PBMCs revealed that the rate of ICAM-1⁺ lymphocytes was higher on stimulation with birch pollen antigens in both patient groups. In contrast, significant increases of CLA⁺ lymphocytes were observable in responding patients ($P < .01$, *t* test), but not in nonresponding patients. Fig 2 compares the increases of CLA⁺ and ICAM-1⁺ lymphocytes from both groups.

Comparison of polyclonal lymphocyte responses to birch pollen antigens from blood and lesional skin

Frequencies of proliferating T cells were determined in limiting-dilution cultures with birch pollen extract or rBet v 1 and rBet v 2. There was no significant difference in the rate of blood lymphocytes reacting to birch pollen antigens in either group when limiting-dilution cultures from 3 patients in each group (ie, responding vs nonresponding patients) were compared. In contrast, the rate of skin-derived lymphocytes growing in the presence of birch pollen antigens was markedly higher when cells from responding patients were compared with cells from nonresponders (Fig 3).

Skin biopsy specimens were taken from 14 randomly selected patients to generate polyclonal TCLs in the pres-

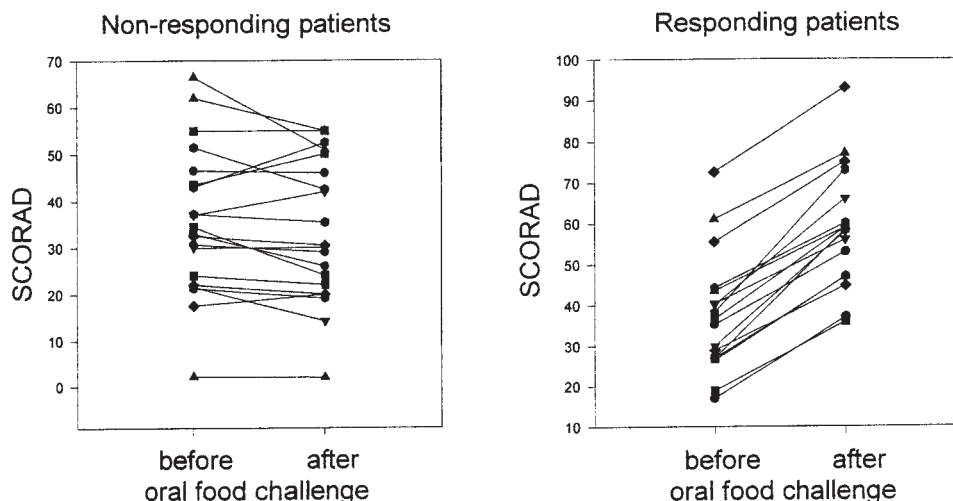


FIG 1. Clinical results of oral provocation with birch pollen-associated foods. The results for 37 individual patients with AD before and 48 hours after oral provocation are shown. The *left panel* summarizes the results of patients who did not respond to oral provocation; the *right panel* shows the results of positive oral provocations. The median SCORAD score was 38 versus 36 before and 59 versus 33 after oral provocation in responsive versus nonresponsive patients.

ence of rBet v 1 and rBet v 2. There were significant differences between the stimulation indices when skin-derived TCLs from responders were compared with TCLs from nonresponders on restimulation with birch pollen extract or with rBet v 1 ($P < .05$, Fig 4). In contrast, the proliferative responses of TCLs derived from peripheral blood showed no significant differences when cells from patients responding to oral provocation and nonresponders were compared (not shown). There was, however, a marked increase of CLA⁺ lymphocytes in blood-derived TCLs from responders on stimulation with birch pollen antigens. As expected, the rate of CLA⁺ lymphocytes was high (>70%) in TCLs from the skin, even without in vitro stimulation, and did not further increase during incubation with birch pollen antigens (not shown).

Fourteen reactive polyclonal TCLs from the blood or the skin of responding patients were stained with a panel of 16 different TCR-V β -specific antibodies. An enrichment of T-lymphocyte subpopulations expressing certain V β elements was detected in 9 antigen-stimulated TCLs (Table II).

Comparison of clonal lymphocyte responses to birch pollen antigens from blood and skin

The rate of TCCs showing reactivity to birch pollen antigens in restimulation tests was not significantly different when 214 blood-derived TCCs from responders were compared with 77 cultures from nonresponders (27% vs 35%). In contrast, a significantly higher proportion of skin-derived TCCs from responding patients proliferated on restimulation with birch pollen antigens compared with TCCs from nonresponders (40% vs 9%; $P < .05$, χ^2 test). Of allergen-specific TCCs, 92% were found to be CD4⁺.

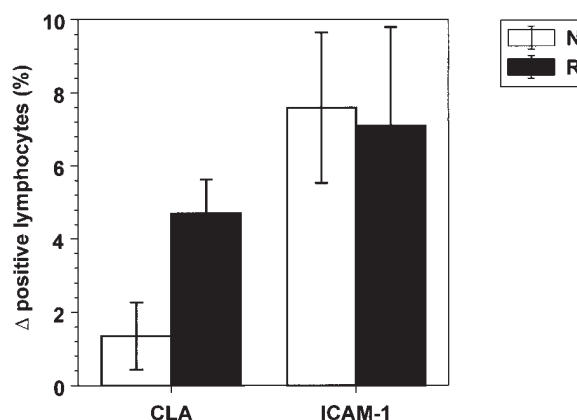


FIG 2. Increases of CLA⁺ or ICAM-1⁺ PBMCs on stimulation with birch pollen antigens. PBMCs of nonresponding patients (N) or patients who responded to oral provocation (R) with birch pollen-related food were cultured in the absence or presence of 1 μ g/mL rBet v 1 and 1 μ g/mL rBet v 2 for 6 days. Immunofluorescence was performed, and the results were analyzed by flow cytometry. The mean percentage of increases ($\pm$ SEM) of CLA⁺ or ICAM-1⁺ lymphocytes are shown. With the anti-CLA antibody HECA-452, significantly higher increases of positive cells were found in responding patients compared with nonresponders ($P < .05$, t test). With the anti-ICAM-1 antibody, increases did not differ significantly.

DISCUSSION

In this study the clinical relevance of pollen-related food challenges for patients with AD was evaluated. All patients enrolled in this study were sensitized to birch pollen antigens but did not have immediate reactions on ingestion of birch pollen-related food antigens. A subpopulation of these patients reacted to double-blind, placebo-controlled oral provocation with a deterioration

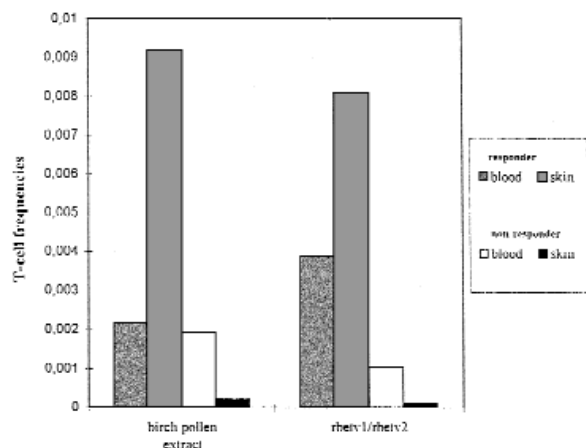


FIG 3. Investigation of the rate of proliferating T cells in the presence of birch pollen antigens. Frequencies of proliferating T cells derived from the skin and the blood of patients with AD after oral provocation with birch pollen-related foods are compared. Limiting-dilution cultures were started with PBMCs or skin cell suspensions in the presence of birch pollen extract plus IL-2 or in the presence of rBet v 1 plus rBet v 2 plus IL-2. Frequencies of responding CD3⁺ T cells were determined by the χ^2 method, according to the method of Taswell.²⁴ A frequency of 1.0 would mean that 100% of all tested T lymphocytes react in this system. Results from 3 randomly selected patients from each group are shown.

TABLE II. TCR-V β pattern of polyclonal T cells from patients with positive oral challenges

Origin of the T cell	Designation of T cell	TCR-V β element (proportion of positive T cells)
Blood	1-B2	V β 5.1 (28%)
Blood	2-B3	V β 16 (76%)
Blood	3-B2	V β 8 (82%)
Blood	4-B2	V β 9 (59%)
Blood	5-B1	V β 13 (56%)
Skin	1-H3	V β 2 (95%)
Skin	2-H4	V β 2 (96%)
Skin	3-H4	V β 13 (68%)
Skin	4-H1	V β 17 (98%)

A total of 14 T cells had been generated from the blood or lesional skin of food-responsive patients in the presence of rBet v 1 (0.1 μ g/mL), rBet v 2 (0.1 μ g/mL), and IL-2. After 2 weeks, all T cells were classified with anti-TCR-V β antibodies. The results of 9 T cells with a marked expansion of subpopulations expressing certain TCR-V β elements are shown.

of their eczematous skin lesions. Of note, none of these patients had been aware of a possible role of these foods in her or his skin condition before this study.

We included only patients with AD who had high serum levels of birch pollen-specific IgE. As expected, IgE to the major birch pollen allergen Bet v 1 did not differentiate responders from nonresponders. In addition, IgE to the corresponding food antigens was detectable in both groups, but birch pollen profilin (Bet v 2) caused few positive results.

Former studies on passively sensitized individuals demonstrated that immunologically active food proteins can enter the circulation and are distributed throughout

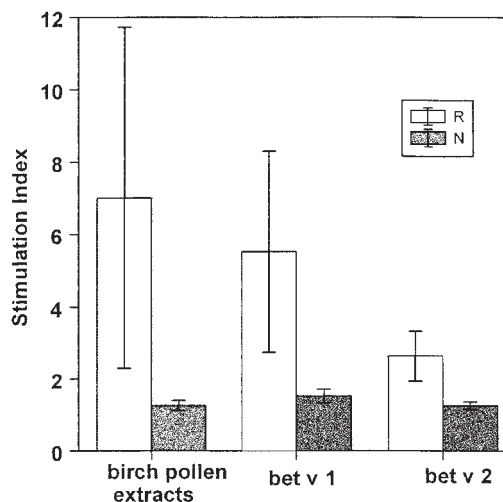


FIG 4. Proliferative response of T cells from the skin of responding versus nonresponding patients with AD. Fourteen T cells from the lesional skin of patients with AD that had been expanded with IL-2 and PHA for 2 weeks and that had worsened after oral provocation with birch pollen-associated foods (R) are compared with 14 T cells generated from the lesional skin of patients with AD not responding to the food challenge (N). All T cells were then stimulated with the given antigens for 5 days. The mean background count was 1853 ± 211 for all T cells. The mean values $\pm$ SD of the stimulation index to the investigated stimuli are shown. They were significantly higher in T cells from responding patients stimulated with birch pollen extracts or Bet v 1. ($P < .05$, Mann-Whitney U test.)

the body, including skin sites.^{26,27} Such food antigens may well cause a T cell-mediated immune response, which subsequently leads to worsening of AD. Because IgE-independent mechanisms may be involved in eczematous reactions to foods in some patients,^{5,9,10} we concentrated on the investigation of cellular reactions in this study.

Oral food challenges represent the gold standard for the diagnosis of food allergy.²⁸ Immediate reactions can easily be related to the suspected foods in most cases, but the causes of late eczematous reactions are difficult to identify. Repeated provocation of the same food antigens on at least 2 subsequent days has been proposed by some investigators as evidence of a causal effect.⁷⁻¹⁰

The clinical association between birch pollen and food allergy is known to be based on the presence of cross-reacting allergens. Bet v 1 is the major allergen in birch pollen and may cross-react with related proteins in apple,²⁹ celery,³⁰ or hazelnut.³¹ Bet v 2 belongs to the group of profilins, which share strong homologies between different plants.³² A third cross-reactive structure between birch pollen and food extracts was identified in the 60-kD range.³³

Here we studied lymphocyte responses to rBet v 1 and rBet v 2. The proliferation of PBMCs to birch pollen extract or Bet v 1 was higher than that found in control cultures in both patient groups but not significantly enhanced in responsive compared with nonresponsive patients with AD.

Because LPS may substantially influence the proliferation of PBMCs,^{9,23} we used LPS-depleted allergen preparations throughout our experiments. Our results indicate that circulating T cells reactive to birch pollen are present both in food-responsive patients with AD and in patients with AD who are sensitized but nonreactive to birch pollen antigens. The role of Bet v 2, which did not lead to significant proliferative responses in our study, has to be addressed in further investigations with larger patient groups. Perhaps more importantly, the diagnostic value of LPTs performed with well-characterized birch pollen-related food antigens, which are now available, has to be investigated in future studies.

Previously, Santamaria-Babi et al,³⁴ who studied proliferative responses in circulating T-lymphocyte subpopulations, observed a mite-specific, but not tetanus toxoid-specific, response in patients with AD sensitized to mite antigens in the subset expressing the skin-homing molecule CLA. In contrast, in sensitized patients with allergic bronchial asthma, mite-dependent proliferation was found in the CLA⁺ subset.³⁴ This observation reveals an immunologic mechanism that is possibly responsible for immigration of aeroallergen-specific T cells into the skin in patients with AD.

The subsequent finding that CLA⁺ mononuclear cells from children with milk-inducible AD can be expanded in vitro with casein suggested a possible significance of food-specific T-cell responses in the pathogenesis of AD in these patients.³⁵ Here we extended this finding to birch pollen-related foods in antigen-responsive patients with AD. The rate of CLA⁺ blood lymphocytes from these patients, but not from nonresponders, increased significantly on in vitro stimulation with birch pollen antigens. In contrast, the proportion of ICAM-1⁺ cells, which are easily inducible by IFN- γ on T cells, was equally high on cell suspensions from both patient groups. Our LPT results suggest that birch pollen leads to lymphocyte activation in both sensitized patient groups. Food-responsive patients with AD appear, however, to react to birch pollen in a way that enables lymphocytes to "home" into inflamed skin.

Our frequency analysis revealed similar growth rates of lymphocytes in limiting-dilution assays in the presence of birch pollen antigens when blood lymphocytes from responders were compared with cells from nonresponders. In contrast, a clear difference of proliferating cells in response to birch pollen extract and a mixture of rBet v 1 and rBet v 2 was detectable when cutaneous lymphocytes were compared. TCLs that had been generated from lesional skin in the presence of Bet v 1 and Bet v 2 responded to birch pollen extract and Bet v 1, but not Bet v 2, to a high degree. The majority of TCLs from reacting patients showed a clear overrepresentation of lymphocyte subpopulations expressing defined TCR-V β elements. This suggests that the presence of birch pollen antigens led to a strong selection of reactive T cells in these cultures. The role of allergen-specific skin-infiltrating T cells was further stressed by our finding that the proportion of specific TCCs generated from limiting-

dilution cultures of the skin was significantly higher when we compared cells from reactive with cells from nonreactive patients.

A current study addresses the clinical response to individual birch pollen-related foods to determine whether any of these foods are more of a problem than the others. Moreover, the role of T cells reacting with the recently identified Bet v 1 isotypes that do not elicit IgE responses³⁶ should be addressed in further studies. This study includes patients with the "intrinsic" variant as well, which is defined by the lack of environmental or food-specific IgE but may well be associated with circulating and skin-infiltrating T lymphocytes expressing a distinct functional phenotype.³⁷ The proposed investigation of these patients will elucidate whether allergen-specific T lymphocytes are sufficient to trigger eczematous reactions to ingested birch pollen-related food antigens, even in the absence of specific IgE.

A major conclusion from the present study is that a clinical relevance of food allergy should be suspected in adolescent and adult patients with AD who are sensitized to pollen antigens, even if no history of immediate reactions to related foods is presented.

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