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Immediate and Delayed Hypersensitivity Reactions to Birch Pollen in Patients with Atopic Dermatitis

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We investigated immediate and delayed hypersensitivity to birch pollen in 10 patients with atopic dermatitis (AD) who had experienced a worsening of their eczema during the birch pollen season. The patients were prick- and patch-tested and antigen-induced basophil histamine release and lymphocyte proliferation were measured. 9/10 birch pollen-allergic patients proved positive in the histamine release test and the results correlated with specific IgE levels measured by RAST. Birch pollen antigen induced lymphocyte proliferation in 6/10 patients, but a positive patch test result was obtained in only one case. Both peripheral blood monocytes and purified epidermal Langerhans' cells were able to present birch pollen antigen to T cells, although Langerhans' cells seemed to function less efficiently in this respect. ***Key words: Prick test; Patch test; Lymphocyte proliferation test; Histamine release test; Langerhans' cells.***

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Patients with atopic dermatitis (AD) exhibit diminished cell-mediated immunity and impaired regulation of T and B cell function, with undue reactivity to external antigenic stimuli. The patients exhibit reduced resistance to viral and fungal infections and diminished ability to respond to suboptimal (at least) concentrations of topical contact allergens (1). Relative deficiency of suppressor T cell function and increased production of interleukin 4 are crucial immunological abnormalities in AD. Interleukin 4 stimulates B cell IgE production and expression of low-affinity Fcε receptors on B cells, Langerhans' cells (LC), and macrophages (1,2). Other immunological abnormalities described in AD include enhanced synthesis or releasability of inflammatory mediators such as leukotrienes B_4 and C_4, prostaglandin E_2 and histamine (3–6).

Traditionally, allergic rhinitis and asthma – but not AD – have been regarded as manifestations of type I hypersensitivity. Recently both type I and type IV hypersensitivity reactions to environmental antigens have been connected with the pathogenesis of AD. House dust mite, animal dander and pollen antigens have been shown to induce positive patch test reactions or eczematous lesions in patients with AD (7–14).

Birch pollen is an important environmental antigen in Scandinavian countries, frequently causing respiratory allergy. AD patients may also exhibit flare-up of their eczema during the birch pollen season. We therefore measured birch pollen-induced type I and type IV hypersensitivity reactions both in vivo and in vitro in patients whose AD was exacerbated in the spring. In addition to the prick test and RAST, patch, basophil histamine release and lymphocyte proliferation tests were performed. We also studied whether epidermal LC are capable of presenting birch pollen antigen to T cells.

PATIENTS AND METHODS

Patients and controls

Twenty subjects took part in the study. Ten of them (mean age 23.8 years) were birch pollen allergic atopics who had experienced flare-ups of their eczema during the birch pollen season. These patients were regarded as allergic to birch pollen partly on the basis of their rhinitis symptoms during the birch pollen season, and also as a result of previous positive conjunctival challenge or prick test or RAST. The control group comprised 7 subjects with AD but without birch pollen allergy plus 3 healthy non-atopic persons. The study was performed after the birch pollen season had ended.

Skin tests and determinations of allergen-specific and total IgE

The patients and controls were prick-tested using the routine test series. Patch tests were performed on the upper arm with birch pollen allergen (10^6 SQ-E/ml, Aquagen, Allergologisk Laboratorium A/S, Copenhagen, Denmark) in Finn Chambers® on Scanpor tape® (Epitest Ltd, Hyrylä, Finland). A diluent was used as negative control. The test patches were removed on day 2 and read on day 3. Patch tests were performed on eczema-free skin which had not been treated with topical corticosteroids for at least one week. Commerical kits were used to measure specific IgE levels to birch pollen and total IgE (Pharmacia Diagnostics, Uppsala, Sweden).

Isolation of cells

Mononuclear cells were obtained by Ficoll-Isopaque centrifugation of venous blood. These cells were used in cultures or for isolation of T lymphocytes and monocytes. Monocytes were purified on the basis of their adherence to plastic surfaces and T lymphocytes by rosette formation with aminoethylisothiouronium bromide-treated sheep erythrocytes. The average purity of monocytes was 94% as assessed by non-specific esterase staining. T cell populations contained $< 0.2\%$ monocytes, $< 5\%$ OKB7-positive cells (B lymphocytes) and $> 90\%$ OKT3-positive cells (T lymphocytes).

Suction blisters were raised on the uninvolved abdominal skin of volunteer patients. Suction blister roofs were treated with 0.25% trypsin and 0.01% DNase I to obtain epidermal cells. Langerhans' cells were isolated by attaching them to IgG-coated erythrocyte monolayers in tissue culture dishes (15). The purity of LC-enriched cells was about 80% as assessed on the basis of OKT6-positive cells.

Cell cultures

Mononuclear cells and T lymphocytes supplemented with 5% LC or monocytes were stimulated in cultures with birch pollen antigen (Aquagen-SQ, Allergologisk Laboratorium A/S). The cells were suspended in 20% autologous plasma-RPMI 1640 at a density of $0.25 \cdot 10^6$ cells/ml and 0.1-ml volumes of this suspension were pipetted per well of V-bottomed microplates. Thereafter, RPMI 1640 (controls) or various concentrations of birch pollen antigen in RPMI 1640 were added to the plates. The cultures were terminated after 6 days of incubation. Sixteen hours before harvesting, 0.125 μCi of iododeoxyuridine was added per well. The uptake of the isotope was measured with a gamma counter. The results were expressed as a stimulation index (SI). SI = uptake of isotope in stimulated culture/uptake of isotope in non-stimulated control culture.

Table I. *Prick test and laboratory test determinations to birch pollen in AD patients and controls.*

Subjects[a] Number/age	Severity of eczema	Total IgE (kU1)	Prick test[b]	RAST (PRU/ml)	Histamine release (%)[c]	Lymphocyte proliferation (SI)[d]
1/24	mild	320	+++	28.5	61	17.3
2/15	mild	202	+++	7.7	19	8.1
3/28	moderate	917	+++	0.9	59	2.1
4/29	mild	528	+++	39.5	45	0.8
5/29	severe	2445	++	12.4	70	2.6
6/19	severe	1610	++	24.7	44	3.8
7/29	mild	71	+++	2.5	86	1.5
8/17	moderate	7200	+++	84.0	43	3.5
9/23	moderate	3990	+++	290.0	56	8.9
10/25	severe	1970	+++	9.1	38	3.5
11/34	moderate	395	–	<0.4	n.d.[e]	1.0
12/60	mild	139	–	<0.4	10	1.9
13/41	mild	<5	–	<0.4	3	0.9
14/27	mild	<5	–	<0.4	12	1.0
15/21	mild	217	–	<0.4	6	2.6
16/10	moderate	347	–	<0.4	10	2.9
17/30	mild	10	–	<0.4	8	2.6
18/36	–	<5	–	<0.4	18	1.2
19/28	–	14	–	<0.4	15	2.7
20/35	–	6	–	<0.4	17	2.5

[a] Numbers 1–10 were AD patients with birch pollen-induced rhinitis (patients 3, 6, 8 and 9 also had asthma); 11–17 were atopics without birch pollen allergy and numbers 18–20 were healthy controls.
[b] Grading of reaction: ++ denotes equal to half the reaction to histamine, +++ = same as histamine.
[c] The results are expressed as stimulation indices = proliferation in antigen-stimulated culture/proliferation in control culture. The results obtained with optimal antigen concentration (10–100 μg/ml; 1200–12000 SQ-E/ml) are shown. Stimulation indices ≥ 3.0 are regarded as positive.
[d] The results are expressed as percentages of antigen-induced histamine release of total blood histamine. Results obtained with optimal antigen concentration (0.01–1 μg/ml) are shown.
[e] n.d. = not done.

Basophil histamine release test

The birch pollen allergen (Aquagen-SQ, Allergologisk Laboratorium A/S) was diluted in PIPES-calcium buffer, pH 7.4, over a wide concentration range. Equal volumes of the allergen and heparinized peripheral blood were incubated at 37°C for 60 min. Tubes for spontaneous histamine release were incubated in the absence of allergen. In addition, tubes for total blood histamine measurement (cells disrupted by ultrasonic treatment) were included for each test. After incubation, the tubes were centrifuged at 700 *g* for 10 min, whereafter the histamine content of the supernatants was measured by radio-immunoassay (Pharmacia Diagnostics). As a rule, spontaneous histamine release accounted for less than 20% of the total histamine level; values exceeding this percentage were thus considered positive.

Table II. *Birch pollen-induced T lymphocyte proliferation with purified monocytes or Langerhans cells as antigen-presenting cells.*

Subjects[a]	Proliferation responses[b] with	
	Monocytes	Langerhans cells
1	10.6	3.6
2	9.9	2.7
3	1.5	2.0
4	2.4	1.9
5	0.8	0.5
11	0.7	0.9

[a] Patients and control numbered as in Table I.
[b] Results expressed as stimulation indices.

RESULTS

Delayed hypersensitivity to birch pollen

Six out of 10 AD patients exhibited a positive lymphocyte proliferative response (SI > 3.0) to birch pollen; all the controls were negative (Table I). Birch pollen appeared to be a weak stimulant even in positive cases. Peripheral blood monocytes and epidermal LC were also compared for their capacity to present birch pollen antigen to T lymphocytes, and in the 2 positive cases, monocyte sseemed to function more efficiently than LC (Table II). Only one subject (number 1, Table I) with the highest proliferative response in the cultures was also positive in the patch test.

Immediate hypersensitivity to birch pollen

All 10 AD patients proved positive in the birch pollen prick test and also gave positive RAST results (Table I). 9/10 patients were positive in the basophil histamine release test, i.e. the amount of antigen-released histamine in the supernatant exceeded 20% of the total blood histamine, whereas none of the controls was positive (Table I). The concentration of birch pollen allergen causing maximal histamine release varied individually between 0.01 and 1 μg/ml (1.2–120 SQ-E/ml). How-

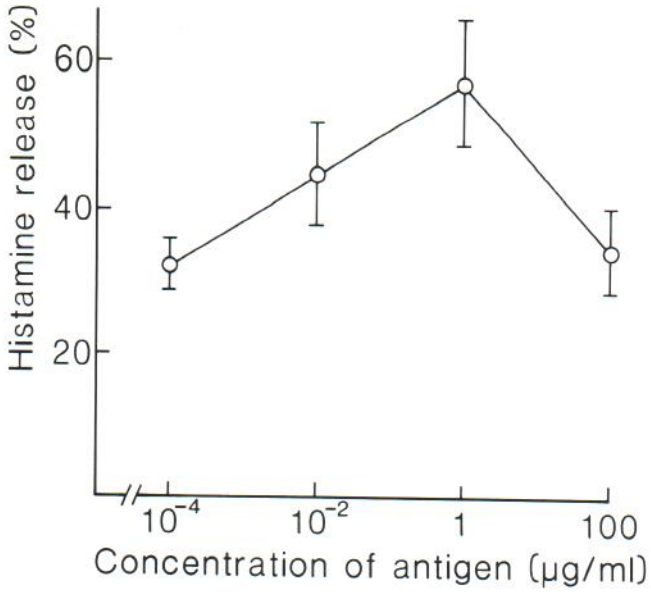

Fig. 1. Birch pollen allergen-induced basophil histamine release as percentage of total blood histamine (mean ± SE from ten experiments).

ever, a clear dose response was evident when the histamine release results from all patients were taken into account (Fig. 1).

The proportion of histamine released at optimal antigen concentration correlated positively with RAST ($p < 0.001$) and total IgE level ($p < 0.01$; Spearman rank correlation test). In addition, there was a significant relationship between total IgE and RAST ($p < 0.001$). No significant correlations were found between lymphocyte proliferative responses and type I hypersensitivity tests.

DISCUSSION

The present results show that birch pollen can elicit delayed hypersensitivity reactions in at least some AD patients whose condition is exacerbated during the birch pollen season. However, the observed in vitro reactivity of the patients to birch pollen was of low magnitude, and only one of the 10 patients proved positive in the patch test.

The allergen-induced histamine liberation test seems to be a useful in vitro test with which to measure type I hypersensitivity. This assay has been used with inhalant, food, animal epithelium and latex rubber allergens and, as a rule, has given results consistent with those of other tests of immediate hypersensitivity (16–18). Compared with RAST, the histamine release test is more time-consuming and expensive, but on certain occasions it can give valuable additional information (18).

In infancy or childhood, food antigens commonly exacerbate atopic eczema. Later in life the role of external antigens in the pathogenesis of AD may be greater than generally anticipated. Large antigenic molecules such as those derived from house dust mite have been shown capable of penetrating the epidermis (9). Pre-existing eczema or abrading or scratching the skin obviously facilitates penetration by antigens. After penetration of the epidermis, external antigens may be captured and processed by LC, which then present them to T lymphocytes. In addition to LC, other antigen-presenting cells may participate in T cell activation, especially in eczematous skin. Antigenic exposure may also lead to eczematous lesions via release of inflammatory mediators. Receptors for IgE are expressed on a substantial proportion of LC and monocytes/macrophages in AD (19–21). It has been suggested that IgE immune complexes, after binding to IgE receptors, activate monocytes to produce increased amounts of prostaglandins (22). Macrophages or dendritic cells bearing cytophilic IgE may also be activated to release inflammatory mediators upon exposure to specific antigens.

The percentages of positive patch test reactions to birch pollen have varied between 11% (4/35 patients positive; 23) and 35% (6/17 patients positive; 7) when patch-testing has been performed on clinically normal non-eczematous skin. Heterogeneity in patient materials and differences in patch test antigen preparations may underlie the disparity between the results. The patients, even if allergic to birch pollen, may differ in their patterns of reaction to this antigen. Some may be more prone to delayed and others to immediate-type hypersensitivity reactions. In our experience the lymphocyte proliferation test to birch pollen antigen proved positive in 6/10 cases, whereas Langeland et al. (23) observed no positive responses. Again, the discrepancy may be due to differences in antigen preparations, or methodology.

We and others have previously observed that epidermal LC are more potent accessory cells than peripheral blood adherent cells when T lymphocytes are stimulated with low-molecular-weight haptens such as nickel (24–26), chromium (27) or corticosteroids (28). In contrast to these findings, LC seemed to function less efficiently than monocytes in birch pollen-induced T cell proliferation (present data) or latex antigen-induced T cell activation (18). It is possible that the weak binding or defective processing by LC underlies these results. In these studies, LC were obtained from uninvolved abdominal skin. The situation may be different in eczematous skin, which may contain functionally activated LC or other cells with antigen-presenting capacity.

Many cell types obviously participate in the development of an eczematous skin reaction in AD. Accumulation of eosinophils, T lymphocytes of predominantly helper cell phenotype, basophils, mast cells, LC with surface-associated IgE and other dendritic cells have been described as occurring at the sites of positive patch test reactions or eczematous lesions (7, 8, 11). The histology of the lesions may represent an IgE-mediated late-phase response or type IV delayed reaction, or bear features from both (29). However, detailed data on the role of the different cell types and their interactions in the development of AD must await further investigations.

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