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Patch Test Reactions to Inhalant Allergens in Atopic Dermatitis

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To study whether inhalant allergens could induce eczematous reactions on normal skin of atopic patients we applied birch pollen and house dust mite antigens at 500 times the concentration used for prick testing as epicutaneous tests. Six out of 17 patients with atopic dermatitis in remission had positive delayed type reactions to birch pollen and three to house dust mite. Only one out of 13 atopic patients without history of atopic dermatitis but with seasonal allergic rhinitis had a positive patch test reaction to birch pollen and no patient had positive test reactions to house dust mite. No positive patch test reactions to birch pollen or house dust mite were seen in the ten healthy control subjects. In patients with positive test reactions biopsies from the test sites revealed epidermal spongiosis and vesiculation. Immunostaining of the epidermis revealed keratinocytes displaying both CD1 and HLA-DR. The present study suggests that inhalant allergens can exacerbate atopic dermatitis. *Key words: Inhalant allergens; Birch pollen; House dust mite; Patch test reactions.*

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Many patients with atopic dermatitis show skin reactions of the immediate type, i.e. positive prick test reactions, when tested with various inhalant allergens. There is generally a good correlation between positive prick test reactions and immediate atopic symptoms such as allergic rhinitis. However, it has proved difficult to correlate these positive prick test reactions to the patient's eczema, which is a delayed type reaction. So far delayed type skin reactions have been mainly ascribed to house dust mite (1, 2).

We recently described a group of patients with a birch pollen allergy of the immediate type but who also showed seasonal exacerbation of the atopic dermatitis during the spring birch pollen season (3). The present study investigates patch test reactions to birch pollen and house dust mite in these patients and in other patients with atopic dermatitis. In addition to findings reported earlier (3) some new results on HLA-DR and CD1 expression of keratinocytes will be presented.

PATIENTS AND METHODS

Patients and control subjects

We studied 17 patients with atopic dermatitis. Thirteen of these patients had positive prick test reactions to birch pollen, and nine had positive prick test reactions to house dust mite. As controls we studied 13 atopic patients without dermatitis, 11 of whom had positive prick test reactions to birch pollen. As further controls we studied nine healthy subjects.

Allergens

We used lyophilized preparations of Aquagen SQ 108 birch pollen (*Betula verrucosa*) and Aquagen SQ 503 house dust mite (*Dermatophagoides pteronyssinus*). The allergens were purchased from Allergologisk Laboratorium A/S, Copenhagen, Denmark. As vehicles we used distilled water and white petrolatum. The allergens were used at 500-fold concentration as compared to the standardized prick test to 1 HEP (histamine equivalent prick). White petrolatum was used as control.

Patch testing and skin biopsies

The patch tests were performed with Finn Chambers (Epitest Ltd., Helsinki, Finland) applied to clinically normal skin on the back. The chambers were removed after 48 h. The patch test reactions were read at 48 h and optionally after 72 and 96 h.

Skin biopsies were taken from both positive epicutaneous test reactions and control sites.

Processing of skin biopsies

The biopsies were divided into two parts. One was processed for immunohistochemistry for quantitation of inflammatory cell subtypes; the other was used for quantitation of mast cells and basophils according to the methods described by Dvorak et al. (4) and Hénocq and Gaillard (5).

Immunostaining

The following primary antibodies were used: OKT3, OKT4, OKT6, OKT8, OKT9, OKM1, OKIa1 (all from Ortho Diagnostic Systems Inc., Raritan, NJ, USA), Leu7 (Becton-Dickinson, CA, USA) and anti B cell antibody (Dakopatts A/S, Copenhagen, Denmark). The immunoperoxidase staining was performed with the avidin-biotin-complex (ABC) method (Vectastain ABC kit mouse IgG PK-4002, Vector Laboratories Inc.; Burlingame, CA, USA) as described by Hsu, Raine and Fanger (6). The sections were incubated in the

Table I. *Positive test results from skin testing with birch pollen*

Clinical diagnosis	Number of patients	Positive prick tests	Positive patch tests
Atopic dermatitis	17	13	6
Allergic rhinitis (no eczema)	13	11	1
Healthy	10	0	0

dark with 0.5% 3,3'-diaminobenzidine tetrahydrochloride (DAB, Sigma Chemical Co., MO, USA) and 0.01% H_2O_2 in phosphate-buffered saline (PBS), counterstained with haematoxylin, dehydrated in a graded alcohol series, cleared in xylene and mounted in Histoclad (Clay Adams, NJ, USA).

The immunostaining was controlled as follows: 1) the first antibody was replaced with PBS or a nonrelevant monoclonal antibody and 2) tissue sections were stained for endogenous peroxidase activity without antibody treatment.

Mast cells and basophilic granulocytes

Mast cells and basophils were stained in paraffin sections with toluidine blue and Giemsa's stain.

RESULTS

Patch test reactions

Positive patch test reactions to birch pollen were seen in six out of 17 patients with atopic dermatitis and in one out of 13 patients with allergic rhinitis without dermatitis (Table I). All six patients with positive patch test reactions had positive prick test reactions to birch pollen. The only patient with a positive patch test reaction to birch pollen without atopic dermatitis had a positive prick test reaction to birch pollen. No positive patch test reactions to birch pollen or house dust mite were seen in the healthy subjects. Three out of nine patients with positive prick test reactions to house dust mite had positive epicutaneous test reactions to the mite. All three patients had positive epicutaneous test reactions to birch pollen as well.

Histopathology

In all patients with positive patch test reactions the biopsy from the test site revealed an eczematous skin reaction with epidermal spongiosis and vesiculation.

Keratinocyte staining for HLA-DR and CD1

We saw positive immunostaining of keratinocytes for HLA-DR and CD1 in all five patients with positive patch test reactions (Table II).

Immunostaining of the dermal infiltrate

Immunostaining of frozen sections with monoclonal antibodies showed dermal cell infiltrates consisting mainly of T cells with a CD4 to CD8 ratio of 2–6/1. 50–90% of the infiltrating cells were HLA-DR positive. There was a smaller proportion (0–30%) of CD1-positive Langerhans cells or indeterminate cells in the dermal infiltrate.

Mast cells and basophilic granulocytes

The proportion of mast cells and basophils in tissue sections was usually 5–10% and never exceeded 15%.

DISCUSSION

Platts-Mills and co-workers (1, 2, 7) have earlier shown that by using the house dust mite antigen P1 it is possible to produce a delayed type of response both in vivo as a patch test reaction and in vitro as blast transformation in atopic patients with positive prick test reactions to the same antigen. It has also been shown in a double-blind, controlled study that repeated exposure to house dust mite antigen produces a mild eczematous reaction of both eczematous and clinically uninvolved skin (8). Other workers have also confirmed that house dust mite or other inhalant allergens exacerbate atopic dermatitis (9–11). These findings provide further clinical evidence for a role of exposure to inhalant allergens in the pathogenesis of atopic dermatitis. In the present study we chose the birch pollen antigens in addition to house dust mite for the patch tests. Unlike house dust mite, the birch

Table II. *Positive keratinocyte staining for HLA-DR and CD1 antigens in biopsies from positive epicutaneous test reactions to inhalant allergens*

Patient	Allergen	Time (h)	Antigen	
			HLA-DR	CD1
1	Birch pollen	72	+	+
	House dust mite	72	+	+
2	Birch pollen	72	–	+
	House dust mite	72	+	+
3	Birch pollen	48	+	+
4	Birch pollen	48	+	+
	Birch pollen	72	–	+
5	Birch pollen	72	+	+

pollen antigens provide the possibility to study patients in an allergen-free environment for most of the year. Our observation of a group of patients with birch pollen allergy of the immediate type who also show a seasonal exacerbation of the atopic dermatitis during the birch pollen season in spring, suggests that inhalant allergens may play a role in the pathogenesis of atopic dermatitis. The present study included four such patients, three of whom showed a positive delayed-type reaction to birch pollen.

The HLA-DR expression of keratinocytes is induced by gamma interferon present in the lymphocytes underlying the epidermis and possibly in the epidermis itself (12). Barker and co-workers have shown that allergic contact dermatitis is followed by HLA-DR expression of keratinocytes (13, 14). These authors did not find similar HLA-DR expression in the keratinocytes in natural lesions of atopic dermatitis. This was taken as evidence that atopic dermatitis differs from allergic contact dermatitis and hence is not a delayed type of hypersensitivity response. In contrast, we found HLA-DR positive keratinocytes in most of the positive patch test reactions. Recently similar results have been reported for natural lesions of atopic dermatitis (15). Keratinocytes displaying HLA-DR can, however, be seen in many other inflammatory conditions with underlying T cell infiltrates (16, 17). Similarly keratinocytes displaying CD1 have been observed in various dermatoses (18).

In conclusion, some patients with atopic dermatitis have delayed type hypersensitivity reactions to inhalant allergens and these reactions may be clinically relevant (3, 15). However, the precise mechanism of these reactions is still unclear.

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