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Studies of Atopic Patch Tests

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35 patients were patch-tested for 72 h with house dust mite, timothy- and birch-pollen, Cladosporium herbarum and hen's egg white allergens, prepared in a cream in concentrations up to 1 000 times the concentration used for skin prick testing. 6 patients developed a total of 10 positive reactions. All the strong positive patch-test reactions occurred in patients with a strong positive skin prick test to the same allergen. Immunohistochemical studies of biopsied positive patch-test reactions demonstrated a mononuclear cell-infiltrate in the upper part of dermis, consisting mainly of T-cells, with a slight predominance of T-helper-cells as compared to T-suppressor cells, and about 10 % CD1 positive cells. No significant responses were obtained in peripheral blood mononuclear cell-cultures stimulated with the various allergens. A positive patch-test reaction to birch-pollen was successfully transferred passively to a non-allergic-recipient, suggesting that the positive reaction may depend upon sensitizing factor(s) in the serum. ***Key words: Atopic dermatitis; Patch tests; Allergy; Passive transfer.***

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During the last few years, several papers have been published, dealing with positive epicutaneous patch-test reactions (EPR) to atopic allergens, such as house dust mite and birch-pollen, in atopic patients (1, 2, 3). The possibility that allergens, brought in direct contact with the skin, for example airborne, may contribute in the pathogenesis of the dermatitis, is an interesting aspect of the relationship between atopic dermatitis and allergic reactions.

In the present work we ask the question if atopic allergens elicit EPR. Furthermore, we ask the question if the allergen-specific EPR is associated with the presence of high levels of allergen-specific IgE, and if the various allergens are able to stimulate peripheral blood mononuclear cells (PBMC). Finally, we demonstrate passive transfer of EPR to a non-allergic recipient.

MATERIALS AND METHODS

Patients

35 patients with atopic eczema, aged 9–72, mean 26 years, were included in the study. The diagnosis was based upon criteria outlined by Hanifin & Rajka (4). The severity-degree of atopic dermatitis, assessed by means of clinical criteria described elsewhere (5), was found to be mild in 11 patients, moderate in 12 and severe in another 12 patients. All patients had +++ or stronger reactions in skin prick test (SPT) to at least two of the allergens used in patch-testing. One of the patients (R.S.) had repeatedly noticed aggravation of the dermatitis during the birch-pollen season. Apart from this, none of the patients had a history of aggravation of the dermatitis related to contact with the allergens used for patch-testing. However, most of the patients with positive reactions in SPT to timothy- and birch-pollen reacted with rhinitis when exposed to these allergens.

Antisera

OKT6 and OKT8 monoclonal antibodies were obtained from Ortho Pharmaceutical Corp., Raritan, N.Y. Anti-Leu 4, anti-Leu 3a + 3b, and anti-HLA-DR antibodies were obtained from Becton-Dickinson, Sunnivale, CA.

Serum for passive transfer of EPR

Serum was drawn from one of the atopic patients (R.S., 9 years old) with positive reactions in SPT as well as in EPR to timothy-pollen, birch-pollen and hen's egg white. Three 1.0 ml aliquots of this serum (R.S.-serum) were incubated with: 78 PRIST discs (anti-IgE), 63 g_6-RAST discs (timothy) and 72 T_3-RAST discs (birch), respectively, at room temperature (23°C), on a "Rock and Roller" overnight. The discs were removed from the sera (650–700 µl), and the sera were then sterile filtered using a Milex 0.2 µm filter. Untreated and absorbed R.S.-serum were tested for total IgE levels and specific IgE to timothy, birch and egg white, using Phadebas g_6, t_3 and f_1 discs, respectively.

Antigens

The hen's egg white antigen solution was prepared by diluting egg white from fresh eggs (less than 24 h old) with an equal volume of 0.15 mol/l NaCl (6). Freeze-dried allergens from timothy-pollen, house dust mite (D. farinae), cladosporium herbarum and birch pollen, were intermediate products for Spectralgen® (Pharmacia, Uppsala, Sweden), supplied by Nycomed (Oslo, Norway). The allergens were dissolved in 0.15 mol/l NaCl and then diluted in a fatty cream to the following concentrations (w/v): 0.3 g/l (house dust mite), 10 g/l (cladosporium herbarum), 2 g/l (birch-pollen), 1.6 g/l (timothy-pollen). These concentrations are up to 1 000 times the con-

centrations used in skin prick testing. Phazet® (Nycomed, Oslo, Norway) was used in SPT for all allergens, except for egg white, which was prepared as described.

Skin prick test (SPT)

SPT was performed using Phazet-histamine® as the positive reference (1 HEP), defined as +++; and Phazet-negative® as the negative reference. Reactions that were half the histamine-reference (defined as ++) or stronger, were recorded as positive (7).

Patch tests

Patch tests were performed using Finn Chambers® (Epitest Ltd., Helsinki, Finland), applying the various allergens at clinically normal skin on the back for 72 h. Two chambers served as controls, both containing the cream without any allergens added. In the passive transfer experiments the chambers were removed after 2, 6, 12, 72 and 120 h for recording of the reactions. Following the first four recordings, i.e. 72 h, the allergens were reapplied at the same test sites.

The patch-test reactions were recorded as +: erythema, ++: erythema and papules/oedema and +++: erythema, papules/oedema and vesicles.

Urticarial reactions observed in the passive transfer experiments were expressed as the products of the two widest perpendicular diameters of the wheals measured in mm.

Passive transfer test

The recipient was a non-allergic volunteer (one of the authors) being negative in SPT to timothy, birch and egg white and with total IgE in serum less than 10 U/ml. Each of the four R. S.-sera were used for intradermal infiltration of three different sites on the volar aspect of the forearm, each site being infiltrated with 0.1 ml serum. Infiltration with the untreated R.S.-serum in a skin area that was not patch-tested, served as a control. 24 h later, allergen preparations containing timothy-pollen, birch-pollen and egg white were applied so that each of the three allergens were tested against each of the four sera. As control, the allergens were also applied in skin areas that had not been infiltrated with R. S.-serum. All tests were read blindly.

Separation of PBMC

PBMC were separated by means of flotation on Lymphoprep® (Nycomed, Oslo, Norway) as described by Bøyum (8).

Cell culture techniques

Lymphoprep-isolated PBMC, in medium RPMI-1640 with L-glutamine (Gibco Bio-Cult, Glasgow, Scotland) supplemented with penicillin, streptomycin and 20% pooled human serum, from 8 patients with atopic dermatitis and positive SPT as well as patch tests to some of the various allergens, and 3 controls, were incubated with various dilutions (range 1 mg/ml – 1^{-10} mg/ml) of timothy-pollen, birch-pollen, house dust mite (D. farinae), cladosporium herbarum and hen's egg white allergens in round-bottomed microtitre plates ($5 \cdot 10^4$ and 10^5 cells per well) for 6 days in a humid 5% CO_2 atmosphere. ^{3}H-thymidine was added 18 h before harvesting with a semi-automatic multiple cell culture harvester (Skatron, Lierbyen, Norway). Incorporation was measured by means of a liquid scintillation counter and expressed as mean ± SD of triplicates. PBMC cultures without antigen served as negative controls, and cultured with PPD as positive controls. All the patients were tuberculin sensitized through vaccination. PBMC from tuberculin sensitive individuals cultures with purified protein derivative of tuberculin (PPD) served as additional positive controls.

Immunohistochemical studies

Biopsy specimens were taken from a positive EPR to timothy-pollen in one of the patients (S. R.) and the passively transferred EPR to birch-pollen. The samples were embedded in OCT (Tissue Tek), snap-frozen in liquid nitrogen and stored at −20°C.

Cryostat sections of the skin biopsy samples were incubated with the various monoclonal antibodies in the alkaline phosphatase anti-alkaline phosphatase (APAAP) staining technique as described by Cordell et al. (9). The numbers of positively stained cells were estimated in a light microscope.

Radio-allergosorbent test (RAST) and Paper immunosorbent test (PRIST)

Specific and total IgE in serum were determined by means of RAST and PRIST, respectively, according to the recommendations of the manufacturer (Pharmacia, Uppsala, Sweden).

RESULTS

EPR

Positive EPR were recorded in 6 patients (17%) (Table I). Except for a weak EPR to house dust mite in one patient, all other reactions were accompanied by positive reaction to the same allergen in SPT.

Total and allergen-specific IgE in R. S.-serum

The results from determination of total and allergen-specific IgE in the 4 different R. S.-sera, are presented in Table II. The results show that the adsorption of IgE using PRIST discs, significantly reduced both total- and specific-IgE levels. Adsorption of specific IgE using RAST discs did, however, only reduce the concentration of IgE specific for the allergen on the absorbing discs.

Stimulation of PMBC with the various allergens

No stimulation was obtained with the PBMC from the patients with a range of concentrations of the various allergens, while stimulation was obtained after stimulation with PPD.

Immunohistochemical studies of positive EPR

The immunohistochemical studies of the EPR demonstrated a dermal infiltrate consisting of approximately 90% T-cells, with a slight predominance of CD4 positive cells as compared to CD8 positive cells. Approximately 90% of the cells in the dermal infiltrate were HLA-DR positive, and about 10% carried

CD1 antigens. There were also increased numbers of CD1 and HLA-DR positive cells in the epidermis.

Passive transfer experiment

2–12 h after the application of the allergens in the passive transfer experiment, a number of urticarial reactions appeared at the various test sites, as shown in Table III. At 72 h a positive EPR against birch-pollen had developed at the test site that had been infiltrated with the R. S.-serum containing the lowest amount of antibodies against birch. This test site was the only one being tested with birch, that did not develop an urticarial reaction during the first day (Table III). The positive EPR, appearing as itchy erythema with small papules, increased until day 5, when a biopsy specimen was taken. Light microscopical investigation demonstrated a mononuclear, perivascular infiltrate in the upper part of dermis and

Table I. *Patients with positive epicutaneous patch reactions*

ERP = epicutaneous patch reaction. SPT = skin prick test

Patient/age	Allergen	EPR	SPT
R. S./9[a]	Birch	+++	>++++
	Timothy	+++	>++++
	Egg white	+++	+++
S. R./33[a]	Timothy	+++	++++
O. R./25	Birch	++	+++
E. K./20	Cladospore	+	+++
	House dust mite	+	–
P. B./10[a]	Birch	+++	+++
H. G./18	Birch	++	+++
	Egg white	++	++

[a] These patients were retested. All reactions were reproduced.

Table II. *Concentrations of total IgE and specific IgE to timothy, birch and egg white in untreated R. S.-serum and R. S. serum pre-incubated with PRIST discs and g_6-, t_3- and f_1-RAST discs*

		R. S.-serum incubated with		
Test	Untreated R. S.-serum	PRIST IgE discs	RAST g_6 timothy discs	RAST t_3 birch discs
PRIST IgE (total)	1 668 U/ml	164 U/ml	1 376 U/ml	1 772 U/ml
RAST g_6 timothy	23.1 PRU/ml	7.7 PRU/ml	3.5 PRU/ml	28.7 PRU/ml
RAST t_3 birch	21.7 PRU/ml	3.5 PRU/ml	27.3 PRU/ml	14.7 PRU/ml
RAST f_1 egg white	13.3 PRU/ml	1.3 PRU/ml	13.3 PRU/ml	13.3 PRU/ml

Table III. *Urticarial lesions[a] during 2–12 h in passive transfer experiment*

	R. S.-serum used from infiltration of test site				
Allergen	Untreated	Low tot-IgE	Low anti-birch	Low anti-timothy	No serum (controls)
Egg white	25	0	100	64	0
Birch	100	100	4	60	0
Timothy	16	4	0	0	0

[a] Urticarial lesions are expressed as the products (mm^2) of the two widest perpendicular diameters of the wheal.

slight spongiosis in epidermis. Immunohistochemical studies of the dermal infiltrated showed a pattern essentially similar to that observed in the positive EPR in the patient.

DISCUSSION

We succeeded in provoking positive EPR to all the allergens used in patch-testing. There is no simple method to decide whether the positive EPR were allergic reactions or simply due to irritation by the test preparations. The following observations indicate that the positive reactions depended upon allergy and not irritation: Firstly, there were only a few positive reactions, 10 out of 175 tests. Secondly, it was not the same allergen preparation that elicited all or most of the reactions. Except for a weak ERP to house dust mite allergens, all the positive reactions appeared in patients being sensitized to the allergen, presenting positive reaction to the allergen in SPT. Thirdly, a positive EPR to birch was transferred passively to a non-allergic recipient.

The immunological basis for the positive EPR is not clear. The presence of specific IgE against the allergen in patients with positive EPR, which is in accordance with other reports (1, 3), may suggest that IgE-antibodies are involved. Since, however, a number of patients with strong IgE-mediated reactions to the allergens did not develop positive EPR, the possibility exists that the positive reaction might not be dependant on allergen-specific IgE to occur, but mainly on another or additional factor. The successful passive transfer of a positive EPR might suggest that an additional factor is present in serum, and that this factor is capable of sensitizing the skin of the recipient.

The finding that the only test site reacting with a positive EPR to birch allergens was the one that had been injected with the serum containing least antibodies against birch-pollen, supports such a hypothesis.

The various allergens did not stimulate lymphocyte proliferation of the PBMC cultures obtained from the patients with positive EPR to the same allergens. Antigen stimulation of T-cells requires that the antigen is presented by an accessory antigen-presenting cell. PBMC are easily in-vitro stimulated by antigens like PPD (11) and in most cases also with other antigens, for example herpes simplex virus (12). Stimulation of T-cells with nickel sulphate in nickel sensitive subjects however, is often weak or negative when peripheral blood monocytes/macrophages are used as the antigen-presenting cells. Using epidermal Langerhans cells as antigen-presenting cells, in comparison, induce strong nickel-specific T-cell responses in the same subjects (13), indicating that Langerhans cells may be highly specialized in presenting certain antigens to T-cells. Since positive EPR may involve and need antigen presentation by Langerhans cells to occur, the lack of Langerhans cells in the PBMC-cultures may explain the negative reactions, similar to the findings with nickel sulphate. If an unknown serum factor present in the patients sera is necessary for the reaction to occur, another explanation might be that we used 20% pooled human serum (not from the patients) in the culture medium. Further studies are under way to elucidate this.

In allergic as well as irritant contact dermatitis the predominant cell type present is the T lymphocyte with variations in the helper/suppressor T-cell ratios between patients and within time periods (10). In the positive EPR to timothy we found a predominance of T-lymphocytes, but a considerable number of CD1 positive cells, in all probability Langerhans cells, were present in the dermal infiltrates, and increased in epidermis. The Langerhans cells are antigen presenting cells for T-cells (11), and the presence and increased number of Langerhans cells may reflect an antigen presenting function in the positive timothy reaction. Our results are in agreement with those of Reitamo et al. (3) who biopsied positive reactions to birch-pollen and house dust mite.

The allergens, being applied directly on clinically unaffected skin, are apparently capable of penetrating the epidermis, although most of them are molecules greater than 10000 daltons. This is also indicated by the development of urticarial reactions in the passive transfer experiment. The observation that some patients also react to these allergens with positive EPR suggests that such allergens may be of significance in the pathogenesis of atopic dermatitis in some patients.

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