

Patch test reactions to mite antigens: a GERDA* multicentre study

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We performed patch tests with *Dermatophagoides pteronyssinus* (Dp) antigens from 2 different sources in 355 non-randomly selected patients with atopic dermatitis (AD) and 398 subjects of a control group. The study demonstrated that contact sensitization to mites occurred in an appreciable % of AD cases (20.8%), using commonly available assay products. The differences recorded between the 2 materials tested were related to the concentration of P1 antigen. Non-atopic patients rarely showed positive reactions to Dp (0.75%), when strict criteria for readings were applied and if 2 readings were performed. Patients with positive patch tests did not necessarily show positive immediate skin tests. It would be useful to carry out tests systematically in atopic patients, even if it is not yet known what modern treatment would be best for the patient. Laboratories still do not provide standardized house dust mite preparations – measuring and codifying their biological activity – for use in patch tests. It is to be hoped that the extension of this type of test will lead to the production of better test materials, in syringes with homogeneous dispersion and concentration.

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Patients with atopic dermatitis (AD) usually show multiple positive immediate-type reactions to airborne allergens, especially the house dust mite, *Dermatophagoides pteronyssinus* (Dp). The rôle of such sensitization to inhalant allergens in cases of AD remained unclear until recently, particularly in the absence of respiratory symptoms. However, the eviction of mites appears to improve AD in cases of strong sensitization (1).

Several years ago, Barker et al. (2) and Bruynzeel-Koomen et al. (3) demonstrated that in patients with AD, Langerhans' cells carried receptors for the Fc fragment of IgE. This was a fundamental discovery, since IgE molecules were found bound

to Langerhans' cells in the epidermis and dermis of healthy or damaged skin in such patients. This abnormality was not encountered, either in cases of contact dermatitis (CD), or in the skin of asthma patients without skin lesions, nor in the skin of non-atopic subjects who were healthy or infected by a parasite (schistosomiasis), in spite of a high concentration of IgE. The presence of receptors of low affinity for FelgE (CD 23) on Langerhans' cells in the skin might thus provide a relatively reliable biological marker for AD.

However, it is already known that false-positive tests can be obtained, e.g., in inflammatory non-allergic dermatoses with a high IgE serum level (4),

such as in hyper-IgE associated symptoms (Buckley's syndrome).

Repeated open application (ROAT) or occluded patch tests, carried out at high concentration of mite antigens in solution, in patients with AD exhibiting positive prick tests to these antigens, can give positive reactions reproducing CD within 2 and 3 days, in both children and adults. These tests are not positive in asthma patients with positive prick tests to the same allergens but who do not have skin lesions (5).

Mitchell et al. (6) and Platts-Mills et al. (7) were the first to observe a positive patch test to Dp within 2 days, but they employed concentrations $100,000\times$ higher than those used in prick tests. Elliston et al. (8) subsequently showed in vitro lymphocyte proliferation in patients with AD in the presence of *Dermatophagoides farinae* (Df) antigen, suggesting that delayed hypersensitivity to mites might play a rôle in the pathogenesis of AD.

It has also been demonstrated that mite antigens are able to penetrate skin by coupling them with ferritin, but Gondo et al. (9) used scratch tests which may change the skin permeability. Tanaka et al. (10) confirmed these data by multiple cutaneous biopsies on 5 patients with AD, at different times after patch tests with inhalant allergens, without a previous scratch. Immuno-electronmicroscopy confirmed the penetration of inhalant allergens into the epidermis. Within the first 6 h, they were observed near IgE-bearing Langerhans' cells and after 48 h in the dermis, where they were taken up by macrophages and presented to lymphocytes.

Other authors (5, 11–13) obtained positive patch tests results with mites and other inhalant allergens. The concentrations tested seem increasingly comparable with those which exist in the environment of atopic patients.

In 1988, the GERDA undertook a multicentre study whose aim was to carry out systematic tests on AD using Dp in aqueous solution in a prick test (read after 20 min) and known concentrations of mite antigens in solution dispersed in white petrolatum in patch tests (read after 2, 3 or 4 days).

The objectives were to define:

(i) whether contact allergy to Dp can be detected using mite antigen concentrations similar to those found in the environment (so as to avoid irritant or collective events);

(ii) whether the positive reaction is specific to AD and not encountered in cases of non-atopic CD, asthma patients who have never had skin reactions, or healthy subjects;

(iii) what the best vehicles and test concentrations are and the most reliable products for developing such contact allergens.

Materials and Methods

Products

Commercially available mite antigens are not marketed with a comparable unit measurement system (14), e.g., the AURs employed in the USA are based on RAST inhibition, while the IRs used in France are based on comparisons of clinical tests and thus do not have the same reaction-producing power. Therefore, we have evaluated the P1 antigen concentration of our extracts.

We began our study using products supplied by Miles-Dome-Hollister-Stier Laboratories (DHS) (Tour Bayer, 13 Rue Jean Jaurès, 92807 Puteaux Cedex). We patch tested a reference glycerine solution, containing 400 AUR/ml, as the reference prick test, and for patch tests, 2 series of 3 concentrations of 100, 400 and 1600 AUR/ml respectively, in glycerine solution, and in white petrolatum.

We then used other products obtained from Laboratoires Stallergènes (7 Allée des Platanes, 94264 Fresnes Cedex) for comparison. Thus, for patch tests, we used a glycerine-saline solution containing 100 IR, as the prick test reference, and solutions dispersed in white petrolatum containing 100, 200 and 500 IR/g.

Subjects

AD patients were included according to the criteria of Hanifin & Rajka (15).

Using DHS products, we tested a total of 86 AD patients (51 female and 35 male) aged between 1 and 79 years (mean 27 years). We tested 59 non-atopic patients (33 female and 26 male) aged between 5 and 78 years (mean 39 years).

With Stallergènes products, 269 patients with AD were tested (162 female and 107 male), aged between 2 and 80 years (mean 22.1 years). 340 non-atopic patients were tested (203 female and 137 male), their age ranging from 4 to 78 years (mean 36.6 years).

Testing

In each patient, a reference prick test (read after 20 min) was performed on the forearm. Patch tests were applied on normal skin of the back and read after 20 min, 2 and 4 days, using the procedure of the ICDRG. We did not perform any scratch or stripping tests.

Dosage of P1 antigen

The determination of house dust mite antigenic content in the samples was performed by ELISA using monoclonal antibodies. Microtiter plates

coated with Dp1 and Df1 specific monoclonal antibodies were incubated with antigen extracts, and bound antigen was detected with a second biotinylated monoclonal antibody recognizing another epitope common to Dp1 and Df1. Production and characterization of the monoclonal antibodies supplied by Platts-Mills and Chapman have previously been described (16, 17). After addition of an enzyme substrate system (streptavidine peroxidase with orthophenyldiamine), the concentration of Dp1 and Df1 antigen was obtained by comparing the optical density of the sample at 492 nm with a standard curve. Results were expressed in μg of Group I antigen levels, defined as the sum of Dp1 and Df1 per g of house dust.

Results

We obtained 74 positive patch tests (20.8%) among 355 AD patients and 3 positive patch tests (0.75%) among 399 subjects of the control group. However, it seems to be of more interest to give the results separately for each material used.

DHS products

In 86 patients with AD, 27 prick tests were positive and patch tests were positive in 6 patients (7%); all positive patch tests were seen with the highest concentration (1600 AUR), only 2 also being positive with 400 AUR. Moreover, 2 positive patients already showed an itchy erythematous reaction within the first 20 min.

9 patients (10.5%) had doubtful patch test reactions (?+) according to the ICDRG classification: they were not included in the positive cases. Doubtful results were obtained mainly at high concentrations (1600 AUR) and were sometimes pustular. Most positive results were with the glycerine solution and not the petrolatum suspension, and the results were much more obvious when the solution was used.

Among the 59 non-atopic patients, 6 prick tests were positive; none of the patch tests was positive, and there were 6 isolated erythematous reactions (10%).

Stallergènes products

68 of the 269 patients with AD showed at least 1 positive patch test for mite antigens (25.3%), including 30 whose tests were vesicular (11.2%). 4 had doubtful test results. We also observed 124 positive prick tests, but, in 20% of these there was no concordance between positive patch and prick tests.

Among the 340 non-atopic patients, 73 prick tests and 3 patch tests, were positive (0.88%), but not vesicular (+), and 17 patch tests were doubtful (5%) and not vesicular, being irritant in appearance and not persisting after 3 and/or 4 days.

Irritant tests were particularly found with the 500 IR concentration but were infrequent with the 2 other concentrations. Tests which were clearly positive were generally positive at both the 2 highest concentrations, i.e., 200 and 500 IR. This led us to think that the 200 IR concentration was more reliable, since it provided positive results with little irritation.

Tests with 100 IR were rarely positive and therefore of little value.

While using products from 2 suppliers, we observed 2 cases of 'angry back' and in 4 cases, an exacerbation of lesions in patients who still had activity at the time of the tests, but in whom it was difficult to obtain a remission prior to testing.

On the other hand, some patients who had incompletely recovered but who were expected to show a positive result (strong clinical indication) were negative, as if the immune system was too heavily involved elsewhere at that particular time (18).

Dosage of P1 antigen

Several assays were carried out with the reference prick test solutions (it is impossible to perform this assay with products dispersed in white petrolatum) and we found the following values:

DHS 400 AUR/ml 6.3 to 7 $\mu\text{g}/\text{ml}$
Stallergènes 100 IR/ml 5.5 to 6 $\mu\text{g}/\text{ml}$

Table 1. Summary of multicentre study results

	DHS atop.	DHS non-atop.	Stall. atop.	Stall. non-atop.
no. of cases	n=86	n=58	n=269	n=340
mean age	27	39	22.1	36.6
prick test +	27 (31%)	6 (10%)	124 (46%)	73 (21%)
patch test +	6 (7%)	0 (0%)	68 (25.3%)	3 (0.88%)
patch test ?+	9 (10.5%)	6 (10%)	4 (1.5%)	17 (5.0%)

Discussion

The number of positive results (Table 1) observed with the 2 products differed, with $3.5 \times$ as many tests being positive with 1 product as with the other. This led us to determine the respective P1 antigen concentrations in the 2 solutions adequate for prick tests.

Similar values were found for concentrations of 100 IR (Stallergènes) (minimum concentration used) and 400 AUR (Dome-Hollister-Stier) (medium concentration). The 2 products for prick tests thus contained satisfactory levels of mite antigen. But there were differences in the method of preparation of patch test material and it was impossible at that time to determine the P1 antigen concentration in petrolatum preparations.

In addition, several investigators involved in this study have noticed marked differences in series carried out using the same Stallergènes syringes, as if the concentration varied substantially within the syringe itself, indicating that dispersion in the petrolatum was heterogeneous. Some of us have had numerous positive tests which could be photographed, whereas others have had none at all. This also tends to imply that the various syringes supplied to participants in the study were not homogeneous.

The production of individual units of this unusual dispersion of mite antigen in white petrolatum certainly does not give the precision and homogeneity of normal patch test products which are prepared on a large scale using standardized, tried and tested semi-industrial methods.

The interest of our study is first the number of tested patients: 355 AD and 399 controls. The other studies of the literature appear more restricted. Moreover, the different studies in the literature show considerable differences in the method used, in the times of reading and in the test method. We think that the best way is for the dermatologist to use the method that he is trained in. Therefore, we performed patch tests without scratch or stripping. The 2nd reading at 4 days is essential to eliminate false-positive responses which are generally weak and disappear within 3 days.

Comparing the 2 materials employed, the 2nd seems to be the more efficient and the concentration of 200 IR appears to be the most relevant, because of false-negative tests obtained with 100 IR and false-positive irritant tests obtained with 500 IR.

With this material, if we take into account the vesicular responses only, we found a % of 11.2% real positive eczematous reactions, which is comparable with the incidence of positive nickel patch

tests in contact dermatitis. We only tested one inhalant allergen (Dp), but other studies indicate that positive patch tests can be observed with other inhalant allergens such as pollen extracts, animal extracts, moulds, etc. (6, 11, 13).

It therefore seems to be of great interest to perform patch tests with inhalant allergens in AD. Multicentric assays must determine which allergens should be tested in the future in a new AD standard series and establish optimal concentrations and vehicles.

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