

Original article

Frequency and intensity of responses to mite patch tests are lower in nonatopic subjects with respect to patients with atopic dermatitis

Background: So far the issue of patch tests with mite allergens in subjects not affected by atopic dermatitis (AD) has been poorly investigated. The aim of this study was to assess the frequency and intensity of responses to atopy patch tests with *Dermatophagoides* in non-AD subjects, and to compare them to the ones observed in AD patients.

Methods: Patch tests were performed, employing a mixture of *Dermatophagoides pteronyssinus* and *farinae* at two different concentrations (20 and 40% pet.), on 75 non-AD subjects, including 33 patients with allergic rhinitis and 42 healthy volunteers, and on 210 AD patients, as controls.

Results: Positive responses to 20% mite patch tests were observed in 17 non-AD subjects (23%) and in 102 AD ones (49%). The former showed a lower intensity of the responses, with a mean score of 1.4 compared with 2.1 in atopics.

Conclusions: Although positive responses to mite patch tests are also observable in subjects without AD, their frequency and intensity are significantly lower compared with AD patients.

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Atopic dermatitis (AD) is a multifactorial disease, mediated by both intrinsic abnormalities (immunological and pharmacological) and extrinsic factors, such as food, airborne and contact allergens, and irritants. Clinical data, pointing to a relationship between exposure to house dust mite (HDM) allergens and exacerbation of the dermatitis, together with the presence of mite-specific immunoglobulin E (IgE) in many AD patients, and the recent finding of IgE receptors on Langerhans cells (1–3), indicate that the role of mite allergens can be carried out through their transepidermal penetration, binding to Langerhans cells, and activation of a subset of lymphocytes, with the triggering of a delayed type hypersensitivity reaction.

Skin lesions characterized by erythema, papules and vesicles, showing microscopic and immunopathological similarities to eczematous lesions in AD, can be artificially induced by applying aeroallergens on the skin of AD patients for 24–72 h. These tests, evaluating delayed reactions, have been performed since 1982, by authors who, employing different substances and various techniques, reported variable frequencies of positive responses in patients with AD (4–18).

Most authors consider atopy patch tests (APT) as a diagnostic tool for identifying subjects presenting eczematous skin lesions upon exposure to allergens generally inducing IgE-mediated reactions, which may provide further diagnostic information in addition to the patient's

history and skin prick test results. Moreover, they are regarded as specific for patients with AD. However, in a few studies, also atopic subjects without dermatitis and nonatopics have been reported to show positive patch test responses (8, 13), and this would raise the question of the specificity and the clinical relevance of positive APT results.

The aim of this study was to assess the response to patch tests performed with HDM allergens in subjects not affected by AD, and to compare the data with those observed in AD patients. The influence of allergen concentration on the patch test response in the two different groups was also evaluated.

Patients, materials and methods

The study population included 42 healthy volunteers (HV), aged 22–35 years (mean $\pm$ SD = 28.2 ± 3.5), and 33 patients affected by allergic rhinitis (AR), aged 3–72 (mean $\pm$ SD = 43.8 ± 19.4).

The APTs were also performed on 210 subjects with AD, aged 2–60 years (mean $\pm$ SD = 12.9 ± 11.6), on whom AD was diagnosed according to the criteria of Hanifin and Rajka (19). Among the latter 53 patients also had allergic asthma and/or rhinitis.

None of the patients had been treated with systemic steroids, antihistamines with prolonged half-lives or cyclosporine for 4 month prior to the beginning of the study, and the administration of other drugs, including antihistamines, was stopped 7 days before patch

Table 1. Number (%) and intensity of positive responses to dermatophagoides (20% pet.)

	Non-AD subjects (HV + AR) [75]	HV [42]	AR [33]	AD patients [210]
Number (%) of positive responses	17 (23%)*	6 (14%)*	11 (33%)**	102 (49%)
Clinical score	1.4 ± 0.5*	1.3 ± 0.4*	1.5 ± 0.5*	2.1 ± 0.6

HV = healthy volunteers.

AR = subjects with respiratory atopy.

[] = number of subjects in which APTs were performed.

* = significant in respect of AD patients ($P < 0.05$).

** = significant in respect of HV subjects ($P < 0.05$).

Table 2. Number (%) and intensity of positive responses to dermatophagoides (40% pet.)

	HV [17]	AD patients [45]
Number (%) of positive responses	4 (24%)*	23 (51%)
Clinical score	1.3 ± 0.5*	2.2 ± 0.8

HV = healthy volunteers.

[] = number of subjects in which APTs were performed.

* = significant in respect of AD patients ($P < 0.05$).

testing. Only topical treatment with emollients and antibiotics was continued, avoiding the skin on the back. At testing, all the patients were in a stationary phase of partial remission, with mild to moderate dermatitis, and no patient presented skin lesions on the back.

The patch test material consisted of a mixture in equal parts of whole bodies of *Dermatophagoides pteronyssinus* (DPT) and *Dermatophagoides farinae* (DF); 1 g contained 15 000 mites equal to 300 µg of DerP1. The material was provided by Euromedical (Lecco, Italy) at two different concentrations (20 and 40% pet.). The APTs with a higher concentration were performed in 17 HV and in 45 AD patients only.

About 20 mg of the allergenic material was applied to the healthy skin of the back with large Finn Chambers on Scanpor tape (Epi-test, Tuusula, Finland). Reading was performed at 72 h, 30–120 min after removal of the test chambers. Responses were evaluated according to the following scoring scale: erythema, infiltration = 0.5; erythema, infiltration, few papules = 1; erythema, infiltration, many papules = 2; erythema, infiltration, many papules spreading outside the patch test area = 3; erythema, infiltration, papules and vesicles = 4.

Prick tests were performed on all AD and non-AD patients, employing a prick test solution containing the same allergenic material as the patch test preparation (Euromedical).

Statistics

The Mann–Whitney test and the chi-square test were employed to check differences between patients and controls. A P value < 0.05 was considered statistically significant.

Results

The frequency (number of subjects) and intensity (mean clinical score) of positive responses to mite patch tests proved to be significantly lower in non-AD compared with AD patients for both concentrations.

The APT results with *DPT/DF* 20% pet. are summarized in Table 1. Of 75 non-AD subjects, 17 (23%) showed positive responses to mite patch tests. The frequency of positive reactions was significantly higher in the subgroup of subjects with AR compared with the healthy ones. As regards AD patients, 102 (49%) showed clear-cut positive reactions (mean score of 2.1). Irritant reactions were observed in four cases. Compared with AD patients, the intensity of the response was lower both in HV and in AR patients, with a mean score of 1.3 and 1.5, respectively.

Table 2 shows the results of APTs performed with the 40% preparation. The number of positive responses was higher than with the 20% concentration both in HV (24%) and in AD patients (51%). The difference between the two groups was significant. The mean score was 1.3 in the former and significantly higher in the latter (2.2). Among AD patients, three subjects showed irritant reactions to mite material at 40% pet.

Mite-specific IgE by prick tests were detected in 24% of AD patients, whereas only 13% of the controls were IgE-positive. In 36 and 28%, respectively, of non-AD and AD subjects reacting to APTs with HDM, prick tests were positive too, whereas 57 and 60% of non-AD and AD subjects, respectively, with mite-specific IgE showed positive APT responses.

None of the patients or controls showed respiratory symptoms or other side-effects during or after testing.

Discussion

Both immunological and nonimmunological factors play a role in the pathophysiology of AD, a chronic inflammatory skin disorder, characterized by eczematous lesions. In infants affected by AD food allergy seems to play a major role. Later on, the role of external antigens such as aeroallergens, especially *DF* and *DPT*, prevails. After an immediate or a delayed contact hypersensitivity reaction to mite allergens, the damaged skin surface may further facilitate their penetration, thus producing a vicious circle of chronic, recurrent contact dermatitis caused by HDM (20–23). Their enhanced presence in the homes of AD patients seems to be relevant for the exacerbation of dermatitis, and possibly

associated to a greater nutritional value of shed scales from which the mites feed on the skin of AD patients, or the lack of fatty acids in AD skin impairing mite development (21). For AD patients, the identification and elimination of triggering factors from the patients' environment represents the basic approach to disease treatment. In fact, these patients may obtain symptomatic relief if the environmental levels of HDM antigens are reduced (21–23), as the severity of allergen contact-induced dermatitis depends largely on the amount of the causative antigen to which the patient is exposed.

Studies on patch tests with mites, beginning with the one by Mitchell et al. in 1982 (4), have varied widely in the allergenic materials and methods used, obviously yielding percentages of positive responses related to variables such as skin integrity, vehicle, and type and concentration of allergenic material (14, 15). In spite of the identification of the main antigens of DPT and farinae in mite feces or bodies (Der p1 and Der f1, and Der p2 and Der f2, respectively), most patch test studies have been performed employing whole mite cultures or culture extracts, because of the extremely high cost of the purified mite allergens. The concentration of most materials was specified indicating the number of mite bodies per gram of the test substance or the protein nitrogen units (PNU), i.e. the protein content. However, these proteins do not necessarily correspond to the allergenic ones, thus making the concentration of the material indefinable. The material in the present study, which contained 15 000 mites per gram, was titrated with regard to its content in Der p1. This makes our results highly reproducible and the dilutions meaningful and valid.

The specific nature of the response to APTs in AD patients was demonstrated by means of the characterization of allergen-specific T cell clones derived from skin specimens taken from aeroallergen patch test sites (24, 25), and the identification of a typical time-dependent cytokine pattern expression *in situ* was identified (26). Therefore, APTs are considered a diagnostic procedure enabling the identification of subjects reacting with an eczema after exposure to mites. However, so far, the issue of APTs in non-AD subjects has been poorly investigated and with contrasting results. In the GERDA multicentre study, less than 1% of 399 nonatopics reacted to DPT (11). In 85 HV no reactions to HDM and grass pollen APTs were detected by Langeveld-Wildschut et al. (15). Darsow et al. did not find any positive APT responses to

aeroallergens in patients with respiratory atopy or control subjects (14). Recently, reactions to HDM patch tests were reported in 10–27% of HV (27, 28). In this study, positive reactions, even if less frequent and intense compared with those of AD patients, were observed also in HV and in patients affected by AR, confirming our previous findings (9, 13).

Darsow et al. compared patch tests performed with two allergen concentrations (1000 and 10 000 PNU) in AD patients and observed a clear dose-response relationship between allergen concentration and positive reactions (16). However, the correlation between allergen concentration and the frequency of positive responses probably reaches a plateau at a certain point when all reactive subjects are identified. In fact, based on our data, the increase in allergen concentration does not improve the results: both the frequency and the intensity of positive responses to mite allergens do not rise when employing the 40% concentration in AD patients. On the contrary, the positivity rate increases (although not significantly) in HV indicating an unspecific effect probably based on the irritant properties and the proteolytic activity of the mite material (18). In fact, by employing a concentration much higher than ours (300 mg/g of Der p1) in 18 HV, Jamora et al. reported 83% of positive responses showing a decrescendo or persistent pattern, interpreted as false positive reactions (29).

The present data show that skin reactivity to mite allergens can be observed also in subjects without atopic eczema, although the frequency of these responses is lower and the intensity of the reactions reduced compared with AD patients. Thus, the eczematous reaction upon exposure to aeroallergens does not only apply to patients affected by AD. We could imagine a spectrum of reactivity characterizing different skin types modulated by percutaneous penetration capability, sensitivity threshold, and efficiency of specific and unspecific effector mechanisms promoting and enhancing the inflammatory response.

If atopy may be defined as a condition characterized by an enhanced Th2 reactivity, a positive response in healthy subjects may be equivalent to the finding of specific IgE by skin prick tests or RAST, indicating an atopic state. However, for the occurrence of clinical manifestations of AD, other skin factors should be concomitant and these could be of importance both in the induction and the elicitation phase of dermatitis.

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