

ORIGINAL ARTICLE

Atopy patch test with *Aleuroglyphus ovatus* antigen in patients with atopic dermatitis

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Abstract

Background Epicutaneous test made with dust mite antigens.

Objective Evaluation of the response of the epicutaneous test with *Aleuroglyphus ovatus* antigen in atopic patients.

Methods We patch tested 119 individuals, 48 with atopic dermatitis, 50 with respiratory allergy and 21 healthy controls. We compare the positive response frequency to a closed patch test using *Aleuroglyphus ovatus* antigen in different concentrations and 48 and 96h reading times among those individuals.

Results Six patients with atopic dermatitis (12.5%) and 4 with respiratory atopy (8.0%) had positive reactions. None of the non-atopic controls had a positive response. As the antigen concentration raised, the number of positive reactions to epicutaneous test raised as well.

Conclusion Our data suggest a positive relation between Atopy Patch Test positive responses and *Aleuroglyphus ovatus* antigen concentration, no matter the kind of the atopic clinical expression.

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Conflicts of interest

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Introduction

Atopic dermatitis (AD) is a chronic cutaneous disease frequently associated with asthma and allergic rhinitis and onset during the first 6 months of life.¹ The prevalence of AD is about 20% of the worldwide population.^{2,3} Some researchers believe that the increase in AD prevalence since World War II is due to changes in home environment, as wall-to-wall carpeting, poor insulation leading to increase in humidity and changes in household heating and air circulation, altogether contributing to house dust mites thriving.⁴

Atopic dermatitis develops in three phases: infantile, childhood and adult, each one with its own clinical findings. Hanifin and Rajka⁵ proposed a list of diagnostic features in view of major and minor features. Established scores may evaluate the disease's severity, as the SCORAD index⁶ and the rigorousness of skin signs are associated with indoor concentrations of mites, which act both as non-specific irritants and as allergens for AD skin lesions.⁷

Many of the aetiopathogenic aspects of AD remain controversial: 80–100% of AD skin lesions exhibit *Staphylococcus aureus*

colonization, what incites the synthesis of specific IgE⁸ and high levels of total IgE.^{9,10} Stored product mite extracts contain molecules that modulate the host microvascular endothelial cells.¹¹ The contact with aeroallergen can exacerbate AD¹⁰ and domiciliary fungi are implied in the disease aggravation.⁹

Among the aeroallergens, house dust mites, storage mites and arthropods are noteworthy as trigger factors of asthma and allergic rhinitis crisis.^{12–15} *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* are examples of house dust mites, extensively studied and evaluated in medical literature.^{16,17}

Evidences on the allergenic capacity of the storage mite beyond the sensitization to its allergens by individuals with occupational exposition are growing.^{18–21} However, they are also widely spread out of non-stored products, as domiciliary dust,²² being a true pest of storage products.^{23,24} *Aleuroglyphus ovatus* frequently pollutes grains particularly rich in proteins and fat, grain-storage facilities, rodent burrows, hen houses and barn dust^{23,24} and can cause cutaneous irritation or respiratory allergies in human beings.^{22,25,26}

Atopic dermatitis can be aggravated after contact with environment allergens, being the mechanism still uncertain. The initial event could be antigen inhalation or direct skin contact, as there is eczematous injury at the patch test site.^{27,28}

Patch tests with allergen proteins can induce a delayed sensitization in the tested place, particularly in patients with AD.^{26,29} There are some studies with aeroallergen patch tests using house dust mites, pollen and coat of animals, the atopy patch test (APT), with helpful results.^{29–33}

The main objective of this research is to evaluate the response to APT with *A. ovatus* antigen, a storage mite just a few times reported in medical literature in relation to AD patients and its usefulness.

Methods

Subjects

We studied 119 subjects. Out of them, 48 had AD diagnosed according to the criteria of Hanifin and Rajka,⁵ mean age 9.47 years (from 2 up to 31 years); 21 were men and 27, women. Out of the initial population, 50 had asthma and/or allergic rhinitis and no AD (28 men and 22 women, mean age 14.5 years; from 2 up to 40 years). The last 21 persons were healthy volunteers (eight men and 13 women, mean age 18.42 years; from 3 to 37 years). All patients were tested during clinical remission. Systemic steroids, oral antihistamines and topical treatment on the back, except for emollients, were discontinued for at least 7 days before testing. There was no ultraviolet radiation exposure for at least 4 weeks prior to the test. A detailed medical and allergic history was obtained. We used SCORAD index⁶ to evaluate the clinical severity of the skin lesions (mean, 25 points). Subjects with normal seric IgE and negative skin prick test (SPT) were included in the healthy control group. Both patients and controls came from the Dermatology outpatient facility of Clinic Hospital of University of Sao Paulo School of Medicine, SP, Brazil.

Informed consent was obtained from all subjects or their parents. The Ethics Committee of the University of São Paulo School of Medicine approved this study.

Atopy patch test

Patch testing and interpretation were performed by one investigator. The reactions were recorded as follows: +, erythema with slight induration; 2+, erythema with papules; and 3+, erythema, papules and vesicles.

Allergenic extract

The allergenic extract of *A. ovatus* was produced from total culture of mites, 85% of body of mites in accordance with the internal procedures of FDA Allergenic, Rio de Janeiro, Brazil. The extract purification was done by dialysis and a posterior sterilization in membrane 0.22 µm. The dosage of proteins for the Kjeldahl method was of 1 mg N₂/mL. Dialysed and not dialysed extracts were prepared for SPT. The allergens for patch test were prepared with the total culture of *A. ovatus* in the amount of 1.5×10^6 mites%, in the concentrations, 0.1%, 0.5%, 1.0%, 1.5% and 2.0%, in white petrolatum.

Mite cultures

The Immunochemistry Laboratory of the Ponce School of Medicine, Puerto Rico, USA, provided mite cultures for the allergenic *A. ovatus* extracts. The mite extracts were made from 28-day-old, live *in vitro* cultures at the maximum growth phase of *A. ovatus*. Prior to processing, live cultures were verified for culture purity and mite identification.³⁴ *Aleuroglyphus ovatus* cultures were free of any contamination. Food-free mite bodies were obtained.³⁵ The food-free mite bodies (99.5% bodies with faecal particles and 0.5% food particles) were placed on a sterile 50-mL conical tube and stored until use at 5°C.

Statistical analysis

To analyse the relationship between two variables, we used linear regression with 95% confidence interval.

Table 2 Frequency of combined responses of atopy patch test (APT) and skin prick test (SPT) in subjects with atopic dermatitis (AD) using *Aleuroglyphus ovatus* dialysed antigen

	APT+ (%)	APT– (%)	Total (%)
SPT+	0 (0)	7 (16.2)	7 (16.2)
SPT–	6 (14.0)	30 (69.8)	36 (83.8)
Total	6 (14.0)	37 (86.0)	43 (100)

Table 1 Positive atopy patch test in atopic dermatitis (AD) and respiratory atopy groups using different concentrations of *Aleuroglyphus ovatus* antigen

Concentration (%)	AD (N = 43) (%)	Respiratory atopy (N = 47) (%)	Healthy controls (N = 21)
0.1	0	2 (4.25)	0
0.5	2 (4.65)	2 (4.25)	0
1.0	2 (4.65)	3 (6.38)	0
1.5	2 (4.65)	4 (8.51)	0
2.0	5 (11.62)	4 (8.51)	0
Negative controls (petrolatum and fish food)	0	0	0

Results

There were no positive responses in the healthy control group. Six patients in the AD group and four in the respiratory allergies (RA) group showed positive responses to APT with *A. ovatus* antigen. All six patients, APT positive and SPT positive, had moderate SCORAD index (mean, 22.3 points) and high IgE levels (mean, 411.0 IU/mL).

Table 1 shows the positivity rate for each patient group at different concentrations of *A. ovatus* antigen. A comparison between dialysed *A. ovatus* extract SPT and *A. ovatus* antigen APT is shown in Table 2.

Discussion

The immunopathological mechanism involved in APT includes T-cell intervention via IgE-bearing Langerhans cells, as observed in the T-helper Th2-cytokine pattern in the T-cell infiltrate in the skin during the initial phases of APT reactions and confirmed by demonstration of the switch from an initial Th2 response to a late Th1 response *in situ* in APT.^{36–38}

The enzymes secreted by mite digestive tract are capable of eliciting strong IgE responses in allergic patients³⁹ and are found in high concentrations in mite faecal pellets small enough to be transferred by air as protein allergens.⁴⁰ The hypothesis that APT positivity could be related to hyper-reactivity of atopic skin has been denied by the reproducibility of the test.^{31,41} In dogs with AD, there is cross-reactivity among house dust and storage mites, at least *in vitro*.⁴²

In literature, the percentage of APT-positive subjects wide-ranges from 15% to 100%, probably because of the lack of standardized techniques and the dissimilarities in allergen purification. As for the APT technique, it was standardized both by the European Task Force on Atopic Dermatitis⁴³ and the Brazilian Group in Studies of Contact Dermatitis.³⁶ Since there is no agreement on the optimal occlusion time for the APT, we choose to read the results at 48 and 96 h.

As some APT studies point to a dilution of 20% in white petrolatum,⁴⁴ we decided to maintain these concentrations and vehicle to study the possible sensitization to the storage mite *A. ovatus*, which is becoming domestic in the State of São Paulo.⁴⁵

Since there were no previous parameters, we decide to use concentrations that varied from 0.1% to 2.0%, obtaining some positive results. Despite the low positivity, the outcomes show that *A. ovatus* allergens are able to elicit eczematous skin lesions on unchanged skin in a subgroup of patients with AD and we believe that patch test with *A. ovatus* has a good potential and should be observed in future studies.

All patients with negative SPT against the dialysed extract of *A. ovatus* showed clear-cut positive APT reactions, indicating there is no correlation between these two tests.

Data implied a strong relation between the concentration of *A. ovatus* and the number of subjects with positive test, which

could be due to irritation. APT, as any other epicutaneous test, should be interpreted according to the patient's history, signs and symptoms.

As AD is the most common inflammatory skin disease in children and its diagnose is based mainly on clinical findings and the contact with aeroallergens can cause flares of AD in some patients, mite antigen APT can be used as an additional diagnostic tool for some provocateurs. Relevant new allergens either for diagnosis or for immunotherapy are needed.⁴⁶

In short, our data suggest that there is no correlation between APT and SPT, and that there is a strong correlation between *A. ovatus* antigen concentration and APT positivity. Therefore, APT with *A. ovatus* antigen has a good potential and should be observed in future research studies. Besides, this test could be used as a diagnostic tool when flares of AD in some patients are suspected to be provoked by aeroallergens.

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