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Hypersensitivity to aeroallergens in adult patients with atopic dermatitis develops due to the different immunological mechanisms

Atopic dermatitis (AD) is a disease with a complex pathomechanism, it is very difficult to establish the exact factors which can either trigger or exacerbate the disease. Knowledge of the mechanisms involved in AD development can be increased by, among others, applying new diagnostic tests and careful assessment of the results obtained. The aim of this study was to determine the allergic mechanisms of hypersensitivity to selected aeroallergens in patients with AD. The study comprised 109 AD patients. In all the patients the total IgE level was measured and atopy patch tests and skin prick tests were performed. We also assessed the presence of specific IgE against house dust mite, birch-tree, mixed grass pollen and cat dander. The highest incidence of positive results was found for house dust mite allergens, irrespective of the test employed. Analysing hypersensitivity to all the examined allergens we revealed the presence of allergic mechanisms in 85.3% of the patients. In 30.2% of the examined individuals we proved a type I immunological response, in 45.9% – both types I and IV in 9.2% – only type IV in one patient. In 14.7% of the patients the results of all the tests performed were negative. Analysing hypersensitivity to particular aeroallergens, negative test results to house dust mite were observed in 25.8% of the patients. The percentage of positive results for birch pollen, grass pollen and cat dander were 45.0, 44.1 and 53.2, respectively. Analysis of the results showed that allergic reactions to the same aeroallergens may develop via different mechanisms. We also revealed that the coexistence of various mechanisms involved in the development of hypersensitivity to a particular aeroallergen may occur in individual patients.

Key words: Atopic dermatitis, atopy patch tests, skin prick tests, specific IgE

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The diagnostic criteria of atopic dermatitis (AD) introduced by Hanifin and Rajka [1] are universally accepted and widely used in clinical practice. However, the exact pathomechanisms of the disease are still not fully elucidated [2], and the classification of AD based on pathogenic factors is still questionable [3-5]. In 2001, the European Academy of Allergy and Clinical Immunology (EAACI) [6] introduced a new diagnostic classification which included mechanisms of the development of skin lesions. According to this approach, AD should be regarded as a syndrome composed of both allergic AD associated or not with IgE, and non-allergic AD. Positive results of atopy patch tests (APT) which were introduced into clinical practice some years ago, have confirmed very complex pathomechanisms of AD and have indicated contact hypersensitivity to aero- and food allergens as a possible trigger of skin lesion development [7-10]. The observations of many authors concerning the usefulness of APT in the diagnosis of AD indicate that this method may be regarded as a further diagnostic criterion of atopic dermatitis [11]. We can, however, suppose that in some cases of AD, multiple immuno-

logical mechanisms are involved in the disease pathomechanism. Their overlapping, sometimes observed in clinical practice, leads to the problems in classification of the individual patient to a certain subgroup of AD. Thus, the aim of our study was to determine types of immunological mechanisms involved in the development of hypersensitivity to selected aeroallergens in patients with AD.

Material and methods

The study comprised 109 AD patients (63 females and 46 males), aged 18 to 45 years old (mean 25.5). Diagnosis of AD was established when at least three major and three minor criteria of Hanifin and Rajka were met [1]. Additionally, in 47 of the examined patients (43%) allergic rhinitis, in 35 (32.1%) – allergic conjunctivitis, and in 19 (17.4%) – atopic asthma were diagnosed. Patients were diagnosed and treated at the Department of Dermatology, Central Clinical Hospital of Military Institute of Medicine in Warsaw. The prospective study was conducted on patients who were in

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complete remission of skin lesions. Patients did not take any medicines which could interfere with the obtained results. The patients gave their oral and written informed consent to participate in the study. The study protocol was approved by the Local Ethics Committee.

The assessment of specific serum IgE (sIgE) and skin prick tests (SPT) were performed to investigate type I hypersensitivity reactions, whereas atopy patch tests were conducted to determine type IV hypersensitivity responses. The following allergic extracts were employed: house dust mite mixture of *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, white birch pollen – *Betula alba*, composition of five types of grass (orchard grass *Dactylis glomerata*, meadow grass *Poa pratensis*, dandelion *Lolium perenne*, vernal grass *Anthoxanthum odoratum*, timothy grass *Phleum pratense*) and cat dander.

Specific serum IgE levels were determined by commercially available kits (Pharmacia CAP, FEIA System, Pharmacia-LKB company, Sweden) according to the manufacturer's instructions. The tests employed were based on fluoroenzymatic reaction. The results were automatically read and recalculated after standard serum specimen considerations. Results were expressed in kUA/L; results above 0.7 were regarded as positive.

Skin prick tests were performed in the generally accepted way, on clinically uninvolved skin of the left back, using sets of allergens from Allergopharma Company (Germany). Simultaneously negative (solution used to dissolve allergens) and positive controls (histamine, 10 mg/mL concentration) were carried out. Results were interpreted after 20 minutes using a standard ruler. Tests were evaluated by comparison of diameters between the wheal produced by histamine and the wheal produced by the tested allergens. A wheal diameter equal or bigger than the one caused by the histamine solution was regarded as a positive result.

Atopy patch tests (APT) were performed on clinically uninvolved skin of the right back using commercially available reagents of Stallergenes Company (France). Blotting paper squares of 1 × 1 cm, soaked in solutions of the allergens examined, as well as the control test composed of the vehicle (white petrolate), were applied on the skin. Subsequently, the blotting paper was covered with plastic foil (1.5 × 1.5 cm) for occlusion. Then hypoallergic adhesive tape was applied to fix the test dressings. Reagents composed of allergens in a concentration of 200 IR were used in the study [12, 13]. Test dressings were discarded after 48 h. The patches were removed after 48 h and the findings were assessed at 72 h after test application. Tests were classified as positive when reaction on the skin was estimated at least as one plus (+). The following scale was employed: (–) lack of any signs, (+/–) doubtful reaction, (+) erythema, induration, (++) erythema, a few papules (up to 3), (+++) erythema, papules (4 and more), (+++++) erythema, numerous, disseminated papules, (+++++) erythema, vesicles, exudates [12].

Total serum IgE was determined by commercially available kits (Pharmacia CAP, FEIA System, Pharmacia-LKB company, Sweden) according to the manufacturer's instruction. All measurements were performed using the same system as for sIgE level evaluation. Results were expressed in IU/mL (normal values ≤ 135 IU/mL).

In the statistical analyses, the chi-squared test with Yates's correction for small groups was applied. Statistical significance was set at p-value < 0.05.

Results

Eighty three patients (80.7%) presented at least one positive skin prick test and/or increased sIgE levels, whereas 60 patients (53.9%) had at least one positive atopy patch test. In the patients with AD in whom at least one positive SPT and/or increased level of sIgE were noted, atopic asthma was found in 21.7% (18/83) patients, rhinitis – in 50.6% (42/83) and conjunctivitis – in 37.3% (31/83). The percentage of the patients with negative test results was 3.8 (1/26), 23.1 (6/26), 15.3 (4/26) respectively. The differences were statistically significant ($p < 0.05$).

Among the patients with AD in whom at least one positive APT was noted, atopic asthma was found in 11.7% (7/60) patients, rhinitis – in 40 (24/60), and conjunctivitis – in 31.7 (19/60). The percentage of patients with negative tests results was 24.5 (12/59), 42.8 (21/59), 32.6 (16/59) respectively. The differences were not statistically significant.

In 50 patients APT to house dust mite 21 (42%) were assessed as +/ ++, 20 (40%), as +++ and in 9 (18%) as ++++/+++++. Percentage of positive APT to birch pollen were found in 73.6 (25/34), 14.7 (5/34), 11.7 (4/34), to grass pollen were found in 70.8 (17/24), 16.7 (4/24), 12.5 (3/24), and to cat dander were found in 68.75 (11/16), 18.75 (3/16), 12.5 (2/16) respectively.

Detailed frequency of hypersensitivity incidence to allergens obtained in different diagnostic tests is presented in figure 1.

Positive skin prick test results to a mixture of house dust mite allergens were observed in 57 patients (52.3%) whereas positive skin prick tests to birch pollen, mixed grass pollen and cat dander were noted in approximately 32.1% (35/109)-35.8% (39/109) of the patients. Increased sIgE levels for the allergens examined were found in different percentages of the patients, from 30.2% (birch pollen) to 43.1% (mixed grass pollen). Positive APT to house dust mite mixture were observed in 50 patients (45.8%) whereas for birch pollen, mixed grass pollen and cat dander they were observed in 31.2%, 22.0% and 14.7% of the individuals examined, respectively (figure 2).

Positive SPT and/or increased sIgE with simultaneously negative APT for the tested aeroallergens proved involvement of type I allergic mechanisms in hypersensitivity

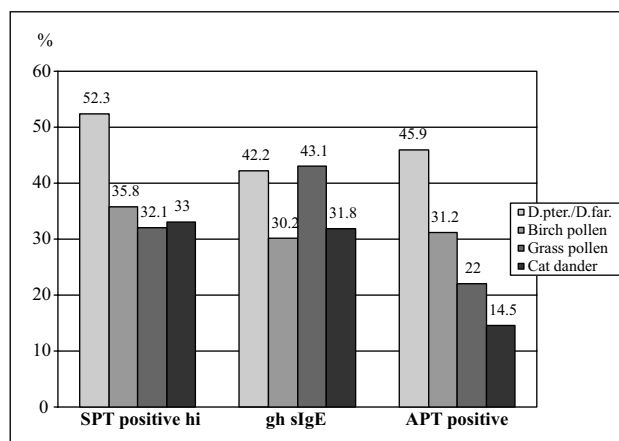


Figure 1. Incidence of positive skin prick tests, specific IgE levels and atopy patch tests with the aeroallergens examined in 109 patients with AD.

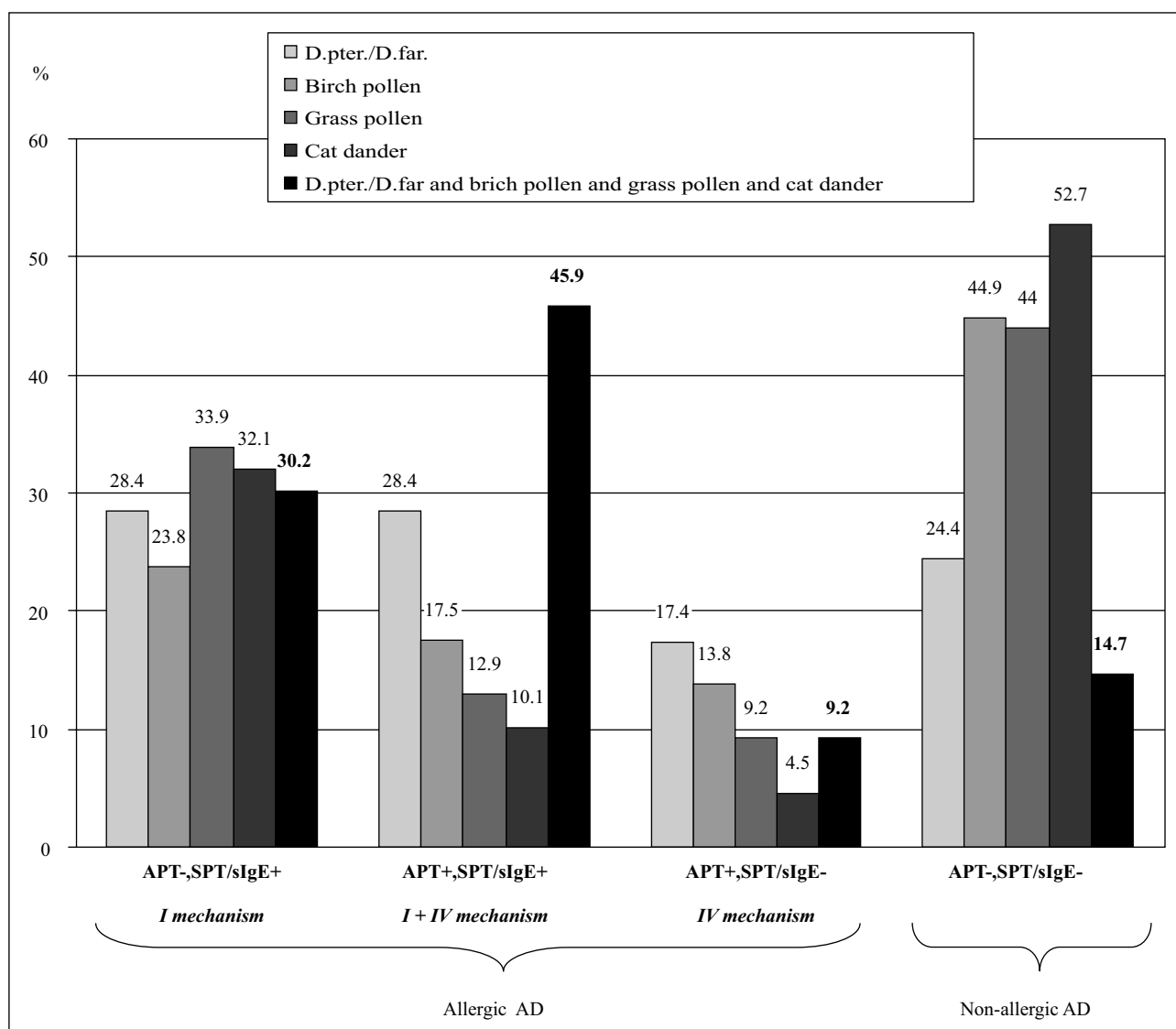


Figure 2. Incidence of the different immunological mechanisms observed to individual aeroallergens and to the groups of aeroallergens examined in the group of 109 AD patients.

development. The percentage of the patients with those results was quite similar and ranged between 23.8% and 33.9%. In 10.1%-28.4% of the patients we observed the presence of both type I and IV mechanisms. In this group of AD patients both positive APT as well as SPT and/or increased sIgE levels were noted. Isolated type IV allergic mechanism (positive APT only) was most frequently observed to house dust mite allergens (17.4%) and most rarely to cat dander allergens (4.6%). Negative results for house dust mite allergens, obtained in all the tests applied, were found in 25.8% of the patients examined. The percentage of negative tests for birch pollen, mixed grass pollen and cat dander were 44.0, 44.1 and 53.2 respectively.

Figure 2 also demonstrates the percentages of patients according to the immunological reaction caused jointly by four groups of aeroallergens.

We showed a type I allergic mechanism, based on at least one positive test (SPT and/or sIgE) in 33/109 patients (30.2%). Simultaneous occurrence of type I and IV mechanism was observed in 50 patients (45.9%). Type IV allergic

mechanism was demonstrated in 10 patients (9.2%), whereas all tests were found to be negative in 16 patients (14.7%).

Increased levels of total IgE were found in 20/33 patients (60.6%) in whom involvement of type I mechanism was proven. In patients with the involvement of two immunological mechanisms (I and IV), total IgE was increased in 48.0% (24/50 patients) whereas in those in whom only type IV mechanism was found, total IgE was raised in only 2 out of 10 patients. In the group of patients in whom no test result was positive, IgE was raised in only 3 individuals.

In 4 out of 50 patients, in whom simultaneously type I and IV hypersensitivity reactions were diagnosed, coexistence of distinct immunological reactions to the groups of allergens examined was observed (table 1). In patient No. 1 we observed a type IV allergic reaction (APT +) to grass pollen and a type I to birch pollen. Patient No. 2 was characterized by positive APT results (type IV mechanism) for house dust mite and birch pollen allergens and positive SPT (type I mechanism) for grass pollen and cat dander allergens. In

Table 1. Atopy patch tests, skin prick tests results and specific IgE levels with individual groups of aeroallergens observed in four patients who presented distinct immunological reactions

Patient	Test		
	APT +	SPT +	high sIgE
No 1	Grass pollen	birch pollen	-
No 2	House dust mite, birch pollen	grass pollen, cat dander	-
No 3	House dust mite	birch pollen	-
No 4	House dust mite	-	cat dander

the third patient contact hypersensitivity (APT +) to house dust mite and immediate hypersensitivity (SPT +) to birch pollen were observed. Contact hypersensitivity to house dust mite and immediate hypersensitivity to cat dander were found in patient No. 4.

Discussion

The studies performed revealed that the coexistence of atopic diseases of the respiratory tract did not influence the results of APT. These observations are comparable to other authors [11]. Like the other authors [14] we too found that positive SPT and increased levels of specific IgE were statistically more often present in the AD patients in whom other atopic diseases occurred.

It is worth mentioning that, in accordance with other authors' observations [15, 16], the increased APT reaction to house dust was the strongest.

The results obtained confirm the complex pathomechanism of AD [17-19]. Positive skin prick tests and/or increased sIgE levels directed against aeroallergens are well known, characteristic features of the disease [20-23]. In our study this was confirmed as these parameters were observed in 30.1-52.3% of the patients, and the percentage depended on the aeroallergen studied (*figure 1*).

Introduction of APT for AD diagnosis demonstrated that aeroallergens may trigger not only type I hypersensitivity reactions but also type IV [24, 25]. Although at present there is no agreement regarding the aeroallergen concentrations used in APT, the availability of commercial tests gives the opportunity to perform comparable studies. In our study, type IV hypersensitivity reactions were found in 14.7-45.8% of the patients examined (*figure 1*). Positive results for both mechanisms were most often demonstrated to house dust mite allergens (*figure 2*). The above results are consistent with the literature data [3] and confirm the very important role of the house dust mite in AD pathogenesis [26, 27].

The patients enrolled to this study were divided into 4 groups based on the results obtained from the tests applied which, according to Fabrizi *et al.* [3] testify which immunological mechanisms are responsible for hypersensitivity development (*figure 2*). The classification proposed by Fabrizi *et al.* [3] corresponds to AD classification according to the EAACI consensus [6]. The first group comprised patients in whom skin lesions were associated with IgE dependent allergic mechanism (type I mechanism). This group was characterized by negative APT and positive skin prick tests and/or increased sIgE levels. The second group

included the patients with positive APT and skin prick tests and/or increased sIgE levels (types I and IV mechanism). The third group consisted of the patients in whom only positive APT were noted (type IV mechanism). The patients with negative results in all the tests performed were classified into group 4 (non-allergic mechanism).

The number of patients in the groups varied and it was related to the groups of allergens investigated (*figure 2*). The most significant differences could be observed especially when hypersensitivity to the allergens examined was taken into account. In the group of patients presenting both type I and IV allergic responses, the incidence of hypersensitivity to separate groups of allergens ranged from 10.1% to 28.4%, whereas the incidence of hypersensitivity to all the groups of allergens examined increased to 45.9%. However, analysis of the "non-allergic" patient group showed that this proportion significantly decreases (14.7%) in case of the evaluation of all the groups of allergens examined when compared to the evaluation of the hypersensitivity incidence to the separate allergens (range from 25.8 to 53.2%) (*figure 2*). Such observations might be the result of totally distinct reactions to the separate allergens observed in the same patient. Although the majority of patients in this group presented hypersensitivity to the allergen examined (mainly house dust mite allergens) in both mechanisms at the same time, the possibility of the development of reactions to different allergens via different mechanisms should be highlighted. It could be illustrated for example by the patient number 1 (*table 1*). Analyzing reactions to mixed grass pollen, house dust mite and cat dander, this patient would be placed in the group characterized by type IV allergic reactions. When the results with birch pollen allergens are taken into account, this patient would be moved to the group characterized by mixed mechanisms i.e. both types I and IV. If we do not know the composition of the grass pollen allergens, this patient would belong to the group characterized by type I allergic reaction. On the other hand, when hypersensitivity to cat dander and house dust mite allergens is considered, this patient should be placed in the group characterized by negative tests results (non-allergic mechanism). It should be expected that these proportions would significantly change if more aeroallergens and/or food allergens were included in the study.

The relationship between increased total IgE and AD development is not yet fully explained [4, 13, 28, 29]. Fabrizi *et al.* [3], in a group of 72 patients, showed the lowest levels of total IgE in the patients with skin lesions resulting from type IV allergic reactions and in the patients in whom the development of skin lesions did not demonstrate any relationship with allergic mechanisms. Similar results are presented in our study. Increased levels of IgE were present in all the groups of patients examined, irrespective of the immunological mechanism involved in the skin lesion development. However, the levels were three times lower in the group of patients in whom no type I allergic reaction was discovered. Concluding, assessment of total IgE concentration proved to be the least useful parameter in the evaluation of type of allergic reactions.

The results obtained have clinical significance as they prove the usefulness of APT in detecting agents triggering AD development.

APT indicate the presence of IgE bound on Langerhans cells (LC) surfaces. LC, the main antigen presenting cells in the epidermis, present the antigen to T cells and in this way

lead to IgE-dependent contact dermatitis [30]. APT are valuable additional diagnostic tests as SPT can only detect the presence of IgE bound on the surface of skin mast cells [31].

Conclusion

The study performed on patients with AD showed the possibility of allergic reaction development to the same aeroallergen through different mechanisms and the coexistence of the various mechanisms of allergic reactions to individual aeroallergens in the same patient. Therefore classification of AD as the allergic or non-allergic type should be linked to the single, specific allergen. ■

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