Diagnostic value of birch recombinant allergens (rBet v 1, profilin rBet v 2) in children with pollen-related food allergy

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Abstract

Purpose: Pollen-related food allergy to fresh fruits and vegetables is a well-known clinical phenomenon. Bet v 1, the major birch pollen allergen, has been cloned and shows homologies to various food allergens (e.g. hazelnut, apple, celery, tomato). Allergy to profilin Bet v 2 was also described in 10-15% of patients sensitized to birch pollen. Objective of our work was to assess the diagnostic value of recombinant allergens (rBet v 1, rBet v 2) for diagnosis of children sensitized to birch pollen with associated food allergy.

Material and methods: The investigations were carried out on the group of 14 children aged 4-17 years, with a history of allergic reactions and sensitized to birch pollen with associated food allergy. Skin prick tests were performed with natural foods and commercial aeroallergens (Bencard). Sera-specific IgE antibodies to recombinant and other allergens (Pharmacia Upjohn) were measured with a fluoroimmunoenzymatic essay (UniCAP). Oral food challenge tests were performed to confirm adverse food reactions.

Results: 64% were sensitized to rBet v 1, 14% to rBet v 2, 7% to both of them. 50% of children with allergy to Bet v 1 had also concomitant allergy to other pollens and food allergy to fruits from family Rosaceae. Patients with positive reaction to Bet v 2 represented allergy to vegetables from family Umbelliferae. The most common form of allergic reactions were: allergic rhinitis in 64%, atopic dermatitis in 36%, oral allergy syndrom in 21% of investigated children.

Conclusions: Use of two recombinant allergens permits the diagnosis of birch pollen sensitization in children with food-pollen related allergy and gives the pattern of possible cross-reactivity between pollen and food allergens in children with allergic diseases.

Key words: birch recombinant allergens, cross allergy, children.

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Introduction

Pollen-related food allergy to fresh fruits and vegetables is a well-known clinical phenomenon. It concerns patients sensitized to pollens and suffering from allergic rhinitis and bronchial asthma and developed allergic reactions after ingesting some of plant-origin foods containing allergens from the same family [1,2]. The results of first clinical investigations were published by Eriksson, Onorato, Boccafolgi in the midle of last century [3]. They described association of hypersensitivity to birch pollen with allergy to apple, hazelnuts or coincidence between allergy to grass pollen and celery, carrot and tomato [1-3]. Many immunological data has been obtained using sera provided by birch pollen-sensitive patients with simultaneously occuring allergies to various fruits and vegetables. They explained immunological background and pathogenesis of cross reactivity. The scientifc experiments with new immunological methods (immunoblotting, RAST inhibition) suggested structural similarities in the allergic components responsible for these cross-reactivities [4-6].

Techniques of genetic engineering applied to allergens have enabled the production of highly pure proteins with homogenic structures, identified sequency of peptides and B cell and T cell epitopes (recombinant allergens) and allowed to better understanding of pathogenetic mechanism of cross allergy. In most cases, it concerns the presence of cross-reactive IgE epitopes in pollen and plant-derived food. By using specific

antibody probes, IgE immunoblott inhibition experiments and molecular biology techniques, it could be demonstrated that plant-derived food contains allergens that share epitopes with the major birch pollen allergen, Bet v 1, and profilin Bet v 2, a highly cross-reactive plant panallergen [7-9].

Materials obtained from natural sources often vary in composition and contain many allergens (major and minor), so a reliable separation of the different allergens contained in that material is not always easy to achieve. The epitopes of major allergens (Bet v 1, Mal d 1) show the highest IgE-affinity, but the different patterns of IgE-binding were also observed in individuals. In general, allergen extracts from plant materials contain many glycoproteins with N-linked glycans that bind IgE in a subgroup of patients sensitized to pollen and food [4,5,10].

Last years and new diagnostic methodes gave a better knowledge of allergen structure and the pathomechanism of association between pollen and food allergy. The investigations performed with recombinant and native allergens have confirmed the role of them in diagnosis of cross-reactivity, based on the similarity and homogenicity of protein structure. Most common are also skin tests with recombinant allergens and detection of specific IgE to recombinant polypeptides in the sera of patients. The differences between immune response to recombinant polypeptides can hence be used to decrease or even modulate specific IgE responses in vivo [4,9,11].

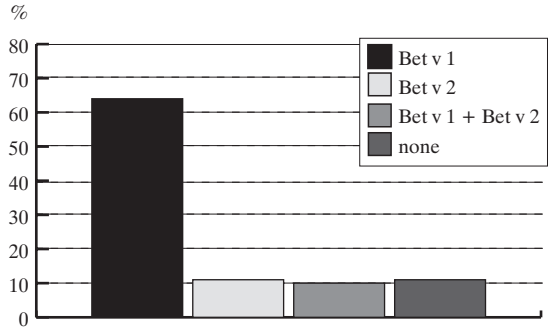
These recent studies concern also allergens of Betula verrucosa. The presence of rBet v 1 (major allergen) and rBet v 2 homologues allergen (profilin) in tree extracts was investigated by IgE immunoblott experiments. Bet v 1 is one of the most important environmental allergens and it shares B cell and T cell epitopes with related allergens present in the pollens of many trees and plant-derived food. Furthermore, more than 95% of birch pollen allergic patients react with Bet v 1, and more than 60% are sensitized exclusively against Bet v 1. Proteins that share common epitopes with Bet v 1, the major birch pollen allergen, occur in pollens of several tree species: apples, stone fruits, celery etc. Molecular analysis has proved the higher amount of IgE-binding epitopes in rBet v 1 allergens versus rBet v 2. Allergy to profilin Bet v 1 is also specific to patients with food allergy to fruits form family Rosaceae [6,9,12].

The first results of investigations with recombinant proteins indicated the necessity of further experiments with plant food and pollen allergens. It is important not only to comprehension of cross allergy, but, first of all, the use of modified recombinant allergens with reduced allergic activity lead to new modalities of specific immunotherapy.

Material and methods

The investigations were carried out on the group of 14 children (10 boys, 4 girls) aged 4-17 years, with a history of clinical allergic reactions to food and pollen allergens. The diagnosis of cross allergy was confirmed on the basis of clinical symptoms and skin prick tests performed as with birch pollen as with fruits and vegetables. In all patients, food allergens were tested in native form by means of a modified skin prick technique (prick by prick tests). To perform tests with aeroallergens (trees and birch pollen, grass, weed pollen), commercial extracts from Bencard Company were used. Negative (saline serum) and positive (9% Codeine) controls were also included. The criterion for positivity of the test was a wheal diameter of 3 mm to tested allergens with a negative glycerosaline control result.

Figure 1. **Positive sIgE to birch pollen recombinant allergens (N=14)**



Sera-specific IgE antibodies to recombinant and other allergens (Pharmacia Upjohn) were measured with a fluoroimmunoenzymatic essay (UniCAP) as detailed by the manufacturer and considered positive if >0.7 kU/l. To confirm adverse food reactions, labial and oral food challenge tests (DBPCFC) were performed, following standards.

Results

All 14 patients who had been included in this study showed positive prick test reactions to birch pollen; sensitization to other pollens was found in the majority of cases. Allergy to birch pollen was also confirmed on the basis of IgE specific results. They were positive in all of investigated patients. 64% of children were sensitized to rBet v 1 (5 of them has IgE antibodies in 3rd class), 14% to rBet v 2, 7% to both of them (major allergen rBet v 1 and profilin rBet v 2) (*Fig. 1*). These, who had reaction neither to Bet v 1 nor to Bet v 2 (14%), presented lower levels of birch IgE in the sera than the others, but had elevated sIgE to artemisia (Artemisia vulgaris) and timothy (Phleum pratense) and suffered from pollen-related food allergy to fruits from family Rosaceae and Rutaceae (citrus). 50% of children with allergy to Bet v 1 had also concomitant allergy to other pollens and food allergy to fruits from family Rosaceae (apple, peach, cherry). But all children with positive reactions to rBet v 2 demonstrated allergy to some fruits and vegetables from family Umbelliferae (celery, carrot, parsley). The most common clinical form of allergic reactions were: allergic rhinitis (64%), atopic dermatitis 36%, bronchial asthma (7%) and multiorgans symptoms as (bronchial asthma, allergic rhinits, atopic dermatitis) observed in 50% of children. 21% of patients suffered from oral allergy syndrom after ingesting of some fruits (apple, peach, citrus, cherry). Analysis of clinical symptoms is presented in *Tab.1*.

All investigated children were underwent labial and oral food challenge with potential harmful food allergens. Positive results were obtained in 50% of children in labial challenge tests

Table 1. **Clinical symptoms in the investigated group of children (N=14)**

Clinical symptoms / Specific IgE to birch	Allergic rhinitis N (%)	Atopic dermatitis N (%)	Bronchial asthma N (%)	Oral allergy syndrome N (%)
Bet v 1 (N=9)	6 (43%)	2 (14%)	-	2 (14%)
Bet v 2 (N=2)	4 (14%)	-	1 (7%)	1 (7%)
Bet v 1 + Bet v 2 (N=1)	1 (7%)	1 (7%)	-	-
None (N=2)	-	2 (14%)	-	-

Table 2. **Positive results of food challenge tests in children with birch-pollen allergy (N=5)**

Patient (initials)	sIgE to birch recombinant allergens			Food allergens (food challenge tests)			
	Bet	Bet v1	Bet v2	Apple	Orange	Peach	Cherry
K.K.	3rd class	3rd class	3rd class	(+)	(-)	(+)	(-)
M.W.	3rd class	3rd class	0 class	(+)	(+)	(+)	(+)
Ł.M.	3rd class	3rd class	0 class	(+)	(+)	(+)	(-)
M.S.	2nd class	0 class	0 class	(+)	(+)	(-)	(-)
M.R.	2nd class	0 class	0 class	(+)	(+)	(-)	(-)

and 36% in oral provocation tests, most often to apple. The results of food challenge tests with comparison to sensitization to recombinat birch allergens are presented in *Tab. 2*.

Discussion

Increasing numbers of clinically relevant cross-reactivities between different pollen and food allergens have been recognized during the last years, due to the progress of molecular biology and immunological studies. Patients with pollen allergy, also to birch, often experience allergic reactions to various fruits, vegetables and nuts [1-3,12]. The major birch pollen allergen is Bet v 1 and the results of investigations showed that more than 95% of birch allergic patients react with Bet v 1 and more than 60% are sensitized exclusively against Bet v 1. This recombinant protein has also homology to other trees of the order Fagales (oak, nut). Although allergic reactions after the ingestion of apple, peach observed in patients with birch-pollen allergy are well described, there are few reports on the homology of epitopes between some fruits, vegetables and second, highly cross-reactive birch pollen allergen – profilin rBet v 2, cloned and expressed in Escherichia coli [9,11].

Patients with food-birch pollen related allergy are more often sensitized to Bet v 1 than to Bet v 2. It has been proved in Rossi et al. investigations carried out on the group of 65 patients presenting rhinoconjunctivitis or asthma and sensitized to tree pollens of trees of the order Fagales. All subjects reacted to at least one of the recombinant birch allergens: 43% to Bet v 1, 30.7% to Bet v 2 and 26% to both of them. Patients with a history of oral allergy syndrome after eating apples were monosensitized and reacted to Bet v 1 in 57%, but subjects allergic to Bet v 2 were polysensitized to other pollens and fruits [13]. Our clinical results indicate also that 64% of children had specific IgE against Bet v 1 and only 14% against Bet v 2. Bet v 1 allergic subjects demonstrated clinical symptoms after ingesting of apple, peach, orange and they with Bet v 2 allergy didn't tolerate celery, parsley.

The clinical statement of pollen-related food allergy is well known, but the clinical suspicion of this coincidence should be confirmed by food challenge tests [14,15]. It is very important, because of the lack of positive correlation between results of skin and food provocation tests. In many cases, skin prick tests with food extracts are not standarized and affected by false negative reactions in patients with clinical symptoms, after eating of some products [14]. The significantly greater importance for evidence of sensitization and correlation with clinical symptoms came from detection of specific antibodies in the serum of patients [16-18]. Serious systemic reactions after eating apples, peach, nuts in birch pollen sensitizied patients, were noticed only in cases with high level of specific IgE antibodies. In contrast, patients with positive prick tests and negative specific antibodies demonstrated only isolated local symptoms (oral allergy syndrom) [13]. Our clinical studies confirm the neccessity of following positive skin prick tests by food challenge tests. Only 5/14 investigated children reacted in provocation tests to apple, orange, peach, cherry. Positive SPT and specific IgE in 2nd and 3rd class were noticed in all subjects.

Patients with birch pollinosis and oral allergy syndrom frequently develop adverse reactions to hazelnuts, what is well known and very common [6,9,12,18]. But in medical history they often include walnuts among causative foods, however, skin prick tests with commercial walnut extracts are almost invariably negative in these subjects, what has been confirmed by recent study performed by Asero et al. The study were carried out on the group of 36 birch-pollen-hypersensitive adult patients reporting OAS after ingestion of nuts. No patients was positive on SPT with fresh walnut and only two with commercial extract, but all patients were positive on SPT with fresh hazelnut. On the basis of these findings, the possible explanation is that walnut does not express any Bet v 1-like allergen and the only way left definitely to diagnose walnut hypersensitivity in these patients

would be the food challenge. In our study in 5/14 children allergy to hazelnut was confirmed; none of them had specific antibodies to walnut [19].

Our and other investigations showed that cross allergy between different fruits (cherry, peach, pear) in patients with allergy to Bet v 1, concerns only those, who have allergy to Bet v 1 (*Tab. 2*) [9,20,21].

The comparative study of Pauli et al., with two recombinant birch pollen allergens, confirmed that sensitization to rBet v 1 is specific for birch and Rosaceae allergies, whereas sensitization to birch profilin, Bet v 2, is encountered in multisensitized subjects and is not always related to Umbelliferae allergy [11]. Immunochemical and molecular biology studies indicate that rBet v 1 contain more IgE binding epitopes than rBet v 2, what explained clinical importance of major Bet v 1 allergen. Rosaceae fruit allergy associated with birch pollinosis is typical to inhabitants in Central and Northern Europe. Rosaceae fruit allergy can occasionally be observed in patients without pollinosis. In those patients, profilin and Bet v 1-related structures are not involved in pathogenesis of food allergy symptoms [21]. This thesis can be counted as controversial but is the first step to further clinical analysis.

Type I allergic symptoms in the oropharyngeal mucosa upon contact with plant-derived food in patients with pollen allergies have been termed oral allergy syndrome (OAS). IgE cross-reactivity between pollen and food allergens represents the molecular basis for this phenomenon. Patients with OAS exhibit a broad variety of symptoms on direct contact of the oral mucosa with plant food as apple, peach, nuts etc., especially during and after the pollen season. This type of food sensitization often concerns people with birch-pollen allergy [2,3,6,12]. IgE immunoblot inhibition experiments demonstrated that plant-derived foods share epitopes with the major birch pollen allergen, rBet v 1 [6]. In the investigated group of children OAS was noticed in 3 of them. Because of the fact that proteins appeared to be strongly degraded in commercial extracts, diagnostic procedures should be based on the native allergens comming from natural sources. In addition, recombinant allergens can be beneficial in diagnostic of OAS [11,13,22].

The actual question remains the value and correlation of skin prick tests, concentration of specific IgE and clinical symptoms in patients with allergy to birch recombinant allergens. In the study of Oster, Pauli, assessing the value of diagnostic tests, total correlation between them was noticed in patients reacted to Bet v 1. In skin prick tests was noticed higher sensitivity with recombinant allergen rBet v 2 I.b., contrary to serum concentration of specific IgE [16].

The recent studies indicate also that only 75% of patients with confirmed IgE cross allergy to Bet v 1 – Mal d 1, demonstrated clinical symptoms after ingesting of apple. Some experiences with recombinant allergens show that these IgE binding with Bet v 1 and its homology in apple Mal d 1 can be clinically irrelevant because of cross-reactive carbohydrate determinants (CCD) [8,23,24]. They are frequently present in patients with adverse reactions to certain foods and responsible for false positive laboratory tests. These observations can explain the absence of specific IgE to birch recombinant allergens (Bet v 1, Bet v 2) obsreved in our two patients with presence of specific IgE to mixture of birch allergens (Bet). Clinically, they demonstrated the severe atopic dermatitis with allergic rhinonjunctivitis and showed positive skin tests to some of inhalant and food allergens (orange, apple, nuts). Food allergy was confirmed on the basis of food challenge tests. Fernandez--Rivas et al. investigations indicate that allergy to Rosaceae fruits (apple, peach) can be observed in patients without a related pollen allergy and profilin and Bet v 1-related structures are not involved in this kind of fruits allergy [21].

Recombinant allergens are not only very important in diagnostic procedures. Some recent progress has led to the production of modified recombinant allergens: the synthesis of recombinant polypeptides corresponding to T epitopes, the production of isoform recombinant allergens with reduced allergenic activity [25,26]. These isoforms can be used as a new tool in specific immunotherapy and reduce the risk of systemic reactions [26].

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