

Recombinant Food Allergens in the Diagnosis of Pollen-Related Food Allergy

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Introduction

The most frequent form of food hypersensitivity in the adult population of Central and Northern Europe is the pollen – in particular birch pollen – related food allergy. Diagnostic procedures such as skin prick tests (SPT) using extracts prepared from natural sources and *in vitro* determination of specific IgE (RAST, CAP) currently used to assess the presence of a pollen-related food allergy, unfortunately, are unsatisfactory (1–5). SPT with commercially available food extracts are often affected by false negative reactions due to a lack of standardization in regard to total protein content, content of single allergens or biological activity (6). In our own studies within the framework of the EU-project “Adverse Reactions to Foods” (FAIR CT97-3224) in patients with confirmed pollen-related food allergy, i.e. with a positive double-blind placebo-controlled food challenge (DBPCFC), sensitivity of a SPT with a commercial carrot extract was just 26% (2) and in another study with a commercial cherry extract 25% (7). The allergenic potency of natural food extracts may vary between different batches of extracts and between extracts from different manufacturers (8). Thus, we observed, for instance in celery-allergic patients with a positive DBPCFC, a sensitivity of SPT with a celery extract from one manufacturer of just 48%, but of 86% when using the extract from another manufacturer in the same patients (1). The strain of food or even the degree of ripeness of fruits used for extraction may influence the quality of the extract (9). Furthermore, allergen extracts produced from natural source materials are heterogeneous products containing not only the allergenic proteins but also non-allergenic proteins or enzymes that may interact with allergens and causing their degradation. Moreover, even with well prepared extracts false positive SPT do occur due to clinically insignificant cross-reactions. Therefore, many commercially available extracts are not suitable for *in vivo* or *in vitro* diagnosis of pollen-related food allergy. Genetically engineered recombinant allergens, however, may offer a promising alternative to overcome the problems recognized by using natural allergenic products.

Recombinant allergens in large amounts, suitable for standardized diagnosis, are available from different sources (15), hazelnut (16) and extracts since containing peroxidase and proteinase.

***In vivo* use of recombinant allergens in pollen-related food allergy**

So far, there are no data on the use of pollen-related food allergens. Sommergruber et al. (17) performed a skin prick test with allergens derived from birch pollen. They resolved diagnosis of food allergy from the *Rosaceae* family with a panel of two cherry allergen rPru av 4 and a food allergen, i.e. rPru av 3, used as a reference. In an immunoblot result, 10 patients with a history of allergic Spanish patients included underwent skin prick tests (100 µg/ml, 100 µg/ml, 100 µg/ml, 100 µg/ml, 100 µg/ml, 100 µg/ml, 100 µg/ml, 100 µg/ml, 100 µg/ml, 100 µg/ml) reacted positively to rPru av 4, and one patient reacted to at least one of the two cherry allergens. Patients from Spain showed a reaction pattern to the birch pollen sensitized to the Bet v 1 and rPru av 3. More than 50% of the patients av 1 experienced oral cavity, sensory and respiratory symptoms.

To assess the value of the diagnosis of fruit allergy, we performed the specificity of birch pollen-allergy by skin prick test, provocation and challenge with three recombinant allergens in a control group including patients with conditions such as irritant contact dermatitis to the birch-pollen.

Recombinant allergens may be produced in heterologous expression systems in large amounts, suitable purity and batch consistency and, hence, may offer a perfectly standardized diagnostic material. Purified recombinant proteins are nowadays available from different foods of plant origin such as cherry (10-12), celery (13-14), apple (15), hazelnut (16) etc. These proteins are much more stable than antigens in food extracts since constituents of the plant matrix responsible for degradation, e.g. polyphenoloxidase and proteases (4), are absent.

In vivo use of recombinant allergens for component-resolved diagnosis in pollen-related food allergy

So far, there are few experiences using recombinant allergens for skin testing in pollen-related food allergy. First results have been presented by Hoffmann-Sommergruber et al. using rApi g 1 in celery-allergic patients (17). Recently, we have performed a skin prick test study evaluating the diagnostic use of different recombinant allergens derived from one pollen-related allergenic food for a so-called component-resolved diagnosis (7). For that study we have chosen cherry as a model food derived from the *Rosaceae* family. For the first time *in vivo* and *in vitro* testing was performed with a panel of two pollen-related recombinant food allergens, i.e. the Bet v 1-related cherry allergen rPru av 1 and the cherry profilin rPru av 4 and one non pollen-related food allergen, i.e. the nonspecific lipid transfer protein (LTP) rPru av 3. DBPCFC was used as a reference method for positivity, and SPT results were compared with immunoblot results. In that study, we have included 33 cherry-allergic subjects: 24 Swiss patients with a history of allergic reactions to cherry and a positive DBPCFC and 9 Spanish patients from Barcelona with a clear-cut history of a cherry allergy. All subjects included underwent skin prick testing with rPru av 1, rPru av 3, and rPru av 4 in dilutions (100 µg/ml, 50 µg/ml, 10 µg/ml). 92% of Swiss cherry-allergic patients (DBPCFC-positive) reacted to rPru av 1, 17% of patients were sensitized to cherry profilin rPru av 4, and one patient (4%) to cherry LTP. Thus, the sensitivity of a positive SPT to at least one of the three recombinant allergens was 96%. By including 9 cherry-allergic patients from Spain, we observed an impressive geographic difference of the sensitization pattern to the three recombinant allergens. None of the Spanish patients was sensitized to the Bet v 1-related cherry allergen rPru av 1 but 8/9 patients to the cherry LTP rPru av 3. More important, whereas more than 50% of patients monosensitized to Pru av 1 experienced allergic symptoms after cherry consumption strictly localized to the oral cavity, sensitization to cherry LTP was generally associated with more severe symptoms.

To assess the impact of clinically insignificant cross-reactions on *in vitro* and *in vivo* diagnosis of fruit allergy we have included two different control populations for assessing the specificity of SPT with recombinant allergens: control group 1 consisted of 23 birch pollen-allergic patients without a cherry allergy as determined by a negative open provocation and control group 2 of 23 nonatopic subjects. The specificity using the three recombinant allergens was 100% when calculated in relation to the nonatopic control group indicating that recombinant cherry allergens do not elicit unspecific reactions such as irritative skin reactions. If specificity was calculated, however, in relation to the birch-pollen allergic control group, it was poor (17%) as a result of clinically

insignificant cross-reactions with Bet v 1 and Bet v 2-related epitopes on rPru av 1 and rPru av 4. Since no side effects were observed in the 79 subjects included, we concluded from that study that skin prick testing with recombinant allergens is highly sensitive and safe. However, similar to extracts, skin prick testing with recombinants does not distinguish between clinically relevant and irrelevant sensitizations.

***In vitro* use of recombinant allergens for component-resolved diagnosis in pollen-related food allergy**

Seven birch pollen allergens have been published (18-24), and six have been included in the official allergen list of the WHO/IUIS allergen nomenclature subcommittee (<http://www.allergen.org/List.htm>). Three of these allergens, Bet v 1, Bet v 2, and Bet v 6 are known to cross-react with food allergens. For two allergens Bet v 7, a cyclophilin, and Bet v 8, a pectin esterase, IgE cross-reactions to homologous food proteins are likely, but has not yet been published in peer-reviewed papers. IgE to certain N-glycans of plant glycoproteins is known to occur in 10-20% of pollen-allergic subjects (reviewed in 25), and these antibodies are highly cross-reactive to almost all kinds of plant foods. In particular, Bet v 1 and Bet v 2-related allergens derived from many different plant foods have been characterized, sequenced and produced in recombinant form. The prevalence of IgE reactivity against these individual recombinant allergens has been determined in food-allergic subjects. There are, however, hardly any studies using a whole panel of different recombinant allergens derived from one allergenic food for component-resolved *in vitro* diagnosis. To date, just one study addressing this issue has been published by Scheurer et al. They used three different cherry allergens (Pru av 1, Pru av 3, and Pru av 4) for component-resolved *in vitro* diagnosis in 101 German cherry-allergic subjects and found a prevalence of sensitization to Pru av 1 in 96%, to Pru av 3 in 3%, and to Pru av 4 in 16% of the patients (11).

Since allergy to *Apiaceae* is one of the most prevalent pollen-related food allergies in Central Europe, we have taken carrot as a model allergenic food to assess the use of component-resolved *in vitro* diagnosis by means of a standard ELISA procedure (unpublished data). We have cloned, sequenced, and characterized the carrot allergens identified in our former study (2) by immunoblot experiments to be relevant, i.e. the Bet v 1-related allergen Dau c 1, i.e. Dau c 1.0104 [accession No. Z81362, described by Hoffmann-Sommergruber as Dau c 1.3; (26)], and the carrot profilin, Dau c 4 (accession No. AF456482). To assess IgE reactivity to cross-reactive carbohydrate determinants we used N-linked glycopeptides purified from pineapple stem bromelain. IgE reactivity to that panel of allergens was assessed in 40 carrot-allergic patients. Dau c 1 was clearly identified to be the major allergen, since 98% of carrot-allergic patients were sensitized to that protein, 38% recognized Dau c 4, and 20% CCD.

The observation that the allergenicity of certain isoallergens is significantly reduced (27) has an important implication for the diagnostic use of recombinant allergens. The identification of high allergenic isoforms is crucial to achieve a good diagnostic sensitivity with recombinant allergens. But what about specificity? To get insight into the diagnostic use of different isoallergens, we cloned, in addition to Dau c 1.0104, a different isoform of Dau c 1, i.e. Dau c 1.0201 (accession No. AF456481), sharing only 50%

amino acid sequence. In a study with 40 carrot-allergic patients recognizing Dau c 1.0104 and Dau c 1.0201 with a sensitivity of 98% and 50%, respectively, we found that 10 patients recognized both isoforms, 10 patients recognized only Dau c 1.0104, and 20 patients recognized only Dau c 1.0201. Thus, sensitivity of the test was 98% (specificity of 95%). In a nonatopic control group of 40 patients, Dau c 1.0104 and Dau c 1.0201 were not recognized, respectively. Thus, the use of both isoforms can be more closely related to the clinical picture of such isoforms.

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amino acid sequence identity with Dau c 1.0104. IgE from just 65% of carrot-allergic patients recognized rDau c 1.0201 (unpublished data) resulting in a low diagnostic sensitivity. Again, to assess the impact of clinically insignificant cross-reactions we have included two different control populations for assessing the specificity of ELISA analysis with recombinant allergens: Control group 1 consisted of 21 birch-pollen allergic patients without a carrot allergy and control group 2 of 20 nonatopic subjects. Birch pollen-allergic patients with no carrot allergy recognized the same allergenic components as carrot-allergic patients but with a much lower incidence. Just one single birch pollen-allergic patient with no carrot allergy, however, showed IgE reactivity to rDau c 1.0201. Thus, sensitization to Dau c 1.0201 was much more specific for carrot allergy (specificity of 95%) than sensitization to Dau c 1.0104 (specificity of 62%). None of the nonatopic control subjects reacted with the recombinant carrot allergens Dau c 1.0104 and Dau c 4, one patient was positive for Dau c 1.0201 confirming for those allergens a specificity of the test procedure in relation to nonatopic patients of 100% and 95%, respectively. Thus, we conclude from our results that certain isoforms of allergens may be more closely related to a clinically relevant sensitization and that the diagnostic use of such isoforms may increase diagnostic specificity.

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