



Recombinant allergens in the diagnosis of food allergy: Which are the advantages?

Les allergènes recombinants dans le diagnostic de l'allergène alimentaire : quels sont leurs avantages ?

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Abstract

To date, neither in vitro nor in vivo test, showing full correlation with clinical food allergy, exists, and there is a pressing need for improved diagnostic approaches. A multitude of recently reported findings indicate that the use of recombinant allergens might particularly improve clinical sensitivity in the diagnosis of food allergy if compared to conventional allergen extracts. Furthermore, use of panels of recombinant allergen molecules may allow refined clinical information to be obtained, regarding the likelihood or severity of an allergic food reaction.

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Résumé

À ce jour, aucun test in vitro ni in vivo n'est parfaitement corrélé à l'allergie alimentaire. Les démarches diagnostiques doivent impérativement être améliorées. De nombreuses et récentes études montrent que l'usage des allergènes recombinants pourrait améliorer considérablement la sensibilité clinique du diagnostic d'allergie alimentaire par rapport aux extraits allergéniques conventionnels. De plus, la mise en oeuvre de panels de molécules d'allergènes recombinants peut affiner l'information clinique en ce qui concerne la possibilité ou la sévérité d'une réaction allergique alimentaire.

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Skin prick testing (SPT) and in vitro determination of food specific IgE are still the primary tools for the diagnosis of food allergy in the daily clinical practice [1]. Both diagnostic approaches are mainly based on the use of food extracts. Many commercial food extracts for use in the diagnosis of food hypersensitivity, however, lack appropriate biological standardization or a precise adjustment of the content of individual allergens. Allergenic extract preparations for in vivo and in vitro diagnosis contain a mixture of allergenic and non-allergenic components that are usually obtained from allergenic

source materials by relatively simple buffer extraction procedures. Degradation of allergenic proteins during the extraction procedure, caused for instance by inherent enzyme activities, and variable composition of the extracts, are unavoidable problems [2]. Inadequate quality of food-derived allergen extracts often results in a poor correlation between the clinical history or the outcome of a controlled food challenge and the skin test or in vitro results.

Most importantly, in pollen related food allergy, the sensitivity of extract based skin testing or in vitro determination of food specific IgE is often low due to the fact that the Bet v 1 homologous food proteins are particularly prone to degradation during extraction procedures. In studies using DBPCFC as a reference for positivity, the sensitivity of SPT or in vitro

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determination of specific IgE using commercially produced food extracts of celery, carrot, cherry or hazelnut, ranged from 20 to 80% [3–6]. Moreover, even with well-prepared food extracts, positive SPT or specific IgE results are frequently observed in the absence of clinical symptoms of allergy due to the phenomenon of subclinical sensitisation or allergen cross-reactivity [7,8].

1. Diagnosing food allergy by recombinant food allergens: general remarks

To date, neither in vitro nor in vivo test, showing full correlation with clinical food allergy, exists and there is a pressing need for new and improved diagnostic approaches. It cannot be expected that the use of novel allergen reagents, such as recombinant allergen components, alone will resolve the dilemma of sensitization not accompanied by clinical allergy, but improved clinical sensitivity is a realistic goal to be achieved.

Recombinant allergens in the diagnosis of food allergy might be used in different ways:

- (1) using the traditional extract-based diagnostic approach but to determine additionally specific IgE to recombinant food allergens that are underrepresented in the commercial food extract;
- (2) enhancing the sensitivity of the extract-based diagnostic approach by supplementing the extract with the recombinant allergens, that are underrepresented in the extract (spiking of extracts);
- (3) determining specific IgE to a whole panel of allergens derived from one source, which enables assessment of the sensitization profile in individual patients, a concept that has been defined as “component resolved diagnostics” (CRD) [9];
- (4) determining specific IgE to mixes of recombinant allergens derived from one source, that is, to a highly standardized “artificial” extract.

2. Use of single recombinant allergens to improve the diagnosis of food allergy

Problems in diagnosing food allergy to soy and peanut in particular subgroups of patients, is one example demonstrating the potential of using recombinant allergens for improved diagnostic procedures. Allergy to soy and peanut is generally thought to be acquired by a primary sensitization via the gastrointestinal tract. In primary food allergy to peanut and soy, extract-based diagnostic approaches do have an adequate diagnostic sensitivity [7]. In recent studies, however, we have demonstrated that, in central Europe, soy and peanut are also clinically relevant as birch pollen-related allergenic foods [10,11]. Gly m 4 and Ara h 8 were identified as the Bet v 1-related molecules in soybean and peanut, respectively, and produced in recombinant form [10,11]. In addition to complete the panel of soybean and peanut allergens, Gly m 4 in particular

facilitated a relevant improvement of diagnostic sensitivity when used as an ImmunoCAP reagent. While only 10 out of 22 (45%) subjects with pollen-related allergy to soybean tested positive with the soybean extract-based test, all but one (96%) showed IgE binding to rGly m 4 coupled to streptavidin-coated ImmunoCAP tests [10]. Nowadays, rGly m 4 and rAra h 8 are commercially available as ImmunoCAP tests (Phadia, Uppsala, S). A sequential approach in the diagnosis of birch pollen related soy and peanut allergy is recommended, that is, an initial determination of specific IgE to soy or peanut extract and, if negative, to recombinant Gly m 4 or Ara h 8, respectively.

Furthermore, different food extracts have already been spiked by recombinant food allergens for in vitro determination of specific IgE to increase the diagnostic sensitivity such as hazelnut extract ImmunoCAP by rCor a 1 (Phadia, Uppsala, S). It is important to be aware, that by this procedure, the IgE binding capacity of the spiked extract has drastically increased and that an actual increase in concentration of specific IgE to hazelnut in individual patients just mirrors the improved sensitivity of the spiked hazelnut ImmunoCAP and not an effective increase in sensitization.

3. The CRD concept in the diagnosis of food allergy

A recently published review provides a comprehensive summary on studies regarding CRD in food allergy [12].

The purpose of CRD is to establish associations between subpopulations of specific IgE antibodies, measured by the use of individual allergen components or parts thereof, and clinically relevant aspects of the allergic disease.

For instance, allergy to a particular food may induce differently severe symptoms depending on which allergen component the individual is sensitized to. For example, sensitization to LTPs in Rosaceae fruit allergic patients in the mediterranean area is often accompanied with a history of systemic reactions while this is rarely the case in north european subjects where the sensitization to Rosaceae fruits is mainly directed to Bet v 1-related food allergens [13–15]. Thus, we have recently applied the CRD concept in cherry allergy investigating the suitability of the recombinant cherry allergens rPru av 1 (Bet v 1 homologous allergen), rPru av 3 (lipid transfer protein) and rPru av 4 (profilin), and in addition a mix of the three recombinant allergens, as in vitro diagnostic tools [16]. Characteristic geographic differences in the sensitisation pattern and clinical food reactions to cherry between Swiss/German and Spanish patients have been detected. In the central European population, sensitisation to Pru av 1 was dominant and little sensitisation to Pru av 3 was present, whereas the opposite was true for the Spanish population. Sensitisation to Pru av 4 was of minor importance in both populations. Furthermore, as expected, systemic reactions were more common in patients from the Mediterranean region, predominantly sensitised to the lipid transfer protein Pru av 3. LTP sensitisation was detected in 11% of cherry allergic subjects from central Europe but this group was found to be clinically different from Spanish LTP sensitised subjects, as no clear

correlation with symptom severity was found. Consequently, our data suggest that even if rPru av 3 may carry diagnostic significance over rPru av 1 in relation to severe food reactions, the association exists mostly in southern Europe.

In the same study, an ImmunoCAP containing a combination of rPru av 1, rPru av 3 and rPru av 4 (rMIX) proved to be highly sensitive when used to detect specific IgE in the study population, which comprised more than 140 cherry allergic subjects. The sensitivity was greater than or equal to 95% in both the central European and Spanish populations investigated, which is remarkably high for food allergy. The natural extract ImmunoCAP displayed significantly lower sensitivity than the rMIX ImmunoCAP in the central European population, in which it failed to detect specific IgE in 35% of the subjects. The findings reported in that study demonstrated that the rMIX ImmunoCAP was an excellent diagnostic reagent, displaying a diagnostic sensitivity and quantitative capacity for specific IgE measurement clearly exceeding the performance of the natural food extract.

4. In conclusion

Recombinant allergens are becoming increasingly available and can be used to map, characterize and exploit the clinical significance of individual IgE reactivity profiles. This development is expected to bring about more information on specific IgE responses in different food allergies which will enable higher diagnostic and prognostic power of the testing methods in the clinical routine.

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