EDITORIAL

The fascinating world of molecular diagnosis in the management of food allergy: nondum matura est

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In the last decade, the allergy literature has raised clinicians' expectations with the advent of a growing array of allergenic molecule [1], their purification, characterization and recombinant engineering (allergome: a platform for allergen knowledge, 2008; http://www.allergome.org, accessed 4 January 2011), touted as the harbinger of novel diagnostic applications [2]. By applying advanced microtechnology, qualified amounts of natural or recombinant allergens can now be spotted on activated biochip surfaces and minute quantities of serum are all that are needed to detect IgE antibody to almost any number of specific allergens in a single-step process [3]. This remarkable breakthrough has been hailed by some researchers as the allergy diagnostics of the future, in particular for infants with food allergy [4]. In 2006, *Current Opinion in Allergy and Clinical Immunology* (COACI) dedicated an article to the subject of component-resolved diagnostics (CRDs) in food allergy. It was then stated that 'this development is expected to bring about more informative ways of analyzing specific IgE responses in different food allergies which, successively, along with more sophisticated approaches to interpret such data, will enable higher diagnostic, predictive and prognostic power of the testing methods in clinical routine' [5].

Some years into the recombinant allergen diagnostic era however, the molecular diagnosis of allergy using multiplex technology such as ISAC microarray and component-resolved diagnosis approaches remain firmly circumscribed to research settings. The various methodologies and their applications to the field of allergy diagnosis and treatment are dealt with in the article by Sanz *et al.* [6] in this issue of COACI. In 2010, the World Allergy Organization Clinical Guidelines on cow's milk allergy (DRACMA) did not find diagnostic benefits from a GRADE-methodology review of studies of cow's milk allergen-specific IgE antibody measurement using microarrays [7–9]. The best performance characteristics found were a sensitivity of 60% and a specificity of 84%. In consequence, evidence-based medicine recommendations could not be formulated and it was suggested that allergen microarrays and CRDs should be used only in research [10]. Similarly, the recently issued NIAID diagnostic guidelines on food allergy list the performance characteristics of epitope or component protein-based assays among the 'knowledge gaps' of food allergy diagnosis and call for further studies to determine their relevance to clinicians outside the field of technology assessment [11,12].

Has something changed? Has any study substantially added about the benefits of molecular diagnosis in food allergy management? The current issue of COACI reviews the utility of these technologies in the fields of egg, milk and fruit allergies [13–15].

The diagnosis of food allergy

An alternative to replace the gold standard double-blinded placebo-controlled food challenge (DBPCFC) has been mooted in the allergy community for many years. Studies [16,17] claiming a very high accuracy using a combination of skin prick test, specific IgE and patch tests have failed to deliver on their promises. The diagnostic accuracy of a test must be checked against the existing diagnostic standard, in this case the DBPCFC. Most of the studies of molecular diagnostics have been in milk and egg allergies (see this issue [13,14]). Their performance characteristics have not been found clearly superior to those of existing methodologies and even their equivalence has not been demonstrated. Accuracy varies according to geography depending on the sensitization process that may involve different allergens, compounding the difficulty in generalizing the use of these novel technologies.

Immunoblotting studies [18–22] indicated that the major peanut allergens are Ara h 1, Ara h 2 and the highly homologous Ara h 6. However, in certain populations of peanut-allergic children, Ara h 3 is the prominent allergen [23]. In hazelnut allergy, sensitization to Cor a 1.04 is prevalent in the northern regions of Europe and is commonly associated with oral allergy syndrome. On the contrary, sensitization to hazelnut LTP (Cor a 8) is more common in patients from southern Europe [24].

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As the conventional ImmunoCAP literature remains largely experimental, we can expect that sensitization will not yield clinical conclusions *per se*. In allergic disease, sensitization is not linked to a single molecule, but several food allergens in the same food may contribute to the onset of clinical symptoms. Thus, it is not surprising that the sensitivity of a diagnostic test with a single molecule is often less clinically relevant, compared with that of a test in which an allergen extract contains several different allergens. Hence, skin tests with recombinant molecules need to include whole panels of recombinant allergens which cover immunodominant structures present in a given food [25]. For these reasons, the sensitivity of in-vivo and in-vitro microarray studies compares well with available extract-based assays but is still to be investigated by various studies in different areas of the world.

Assessment of severity

As specific IgE to allergenic proteins are the causative agents in the clinical manifestation of food allergy, one would expect a direct correlation between their titers and the probability/severity of the allergic symptoms. From experience with ImmunoCAP, we know that a direct relationship does not exist between IgE level and symptom severity [25]. The use of molecular diagnosis does not seem to add much in this respect. However, an association between symptom severity and a specific sensitization profile has been found in CMA [14]. Polysensitization to hazelnut allergen components is mostly observed in patients with severe symptoms [26]. In peanut allergy, studies [19,27,28] using skin prick tests indicate that polysensitization and sensitization to Ara h 2 are associated with severe reactions. In a population-based study [29] of 8-year-old children, peanut-sensitized patients underwent oral food challenges to peanut. Those allergic to peanut had higher levels of IgE antibodies to Ara h 1–3; in contrast, those tolerant to peanut had higher values to cross-reactive carbohydrate determinants (CCDs) and grass components (Phl p 1, 4, 5b). In hazelnut allergy, however, the detection of IgE to CCDs did not increase the ability to discriminate between patients with hazelnut allergy and hazelnut-tolerant patients [24].

Prognosis

Another foreseeable application of CRDs is the individualized management plan through the patient profiling. Immunoblotting studies suggest that persistent milk allergies are associated with increased epitope recognition [30]. Recent studies have suggested a potential role for sequential IgE-binding epitopes as biomarkers for characterizing various phenotypes of food allergy. Studies of allergens in milk, peanut, egg and wheat have shown a correlation between sequential IgE epitope diversity and patients' allergy severity or persistence [31]. Peanut, tree nuts, fish and shellfish allergies are usually persistent and patients typically avoid all the responsible foods for life. A recent study [32] utilizing CRDs indicated that at least in a subset of patients allergic to shrimp, clinical reactivity might decrease in parallel with a shift of epitope recognition. In this study, sensitization to shrimp proteins in general, and to specific epitopes in particular, was greater in children and appeared to decrease with age. With the progress of applications designed for specific allergies (lab on a chip) we can foresee that one day we will be able to apply the right treatment through accurate patient profiling with respect to sensitizing antigens, sequences more likely to induce symptoms and individual keys to treatment. Despite limitations, current methods of IgE-binding epitope identification need to be addressed before they can be applied in the prognosis of food allergy.

Cross-sensitization in patient profiling: applications for more precise uses in immunotherapy

In most food allergic children, the oral food challenge is able to confirm food allergy to no more than one or two foods, and a few foods account for the majority of food allergies in paediatrics [33]. Avoiding unnecessary food eliminations based on a presumptive cross-reactivity is a classical problem in food allergy, and the solution is based on serial food challenges. For instance in the leguminosae family cross-sensitization without allergic reactions is well documented [34,35]. In the attempt to understand more deeply the relationship between sensitization to peanut components and cross-reactivity, it has been shown that Ara h 1 sensitization is associated with allergy lentil, pea and soybean [36]; Ara h 2 with lupine and tree nuts allergy [37]; Ara h 3 to soybean, pea and tree nuts [38]. However, in no case is the association unequivocal and therefore CRDs cannot substitute challenge tests in the evaluation of cross-reactivity. On the contrary, the finding that sensitization to different legumes and nuts is linked to similar molecules indicates that for severely allergic patients avoidance of one member of a family should translate into avoidance of all members, such as for fish and tree nuts. Sensitization to a fish parvalbumin (e.g. Gad c 1 from cod [39] and Cyp c 1 from carp [40]) suggests caution in administration of all fish species to reactive patients.

A lesson we learned from molecular studies is that it is possible to distinguish between primary sensitization through pollens and original sensitization to vegetable foods. In patients allergic to birch pollen, the occurrence of peanut allergy is mediated by cross-reactivity of Bet v 1 with the homologous peanut allergen Ara h 8 [41]. In a population-based study [42], 95% of patients sensitized to

Ara h 1, 2, 3, and 9 reported symptoms after peanut intake, whereas in Ara h 8 monosensitized patients only 18% reported symptoms. However, in no case, the molecular profile *per se* was able to exclude reactions to the incriminated food. Thus, CRD cannot be used as a rule-out test in the diagnosis of food allergy. Conversely, although Ara h 2-specific and Ara h 9-specific IgE antibodies are often associated with systemic and more severe reactions in addition to oral allergy syndrome, especially in southern Europe [43], the specificity of this finding to sensitization is not absolute. For us seasoned allergists who remember the saga of the RAST controversy, a lesson to be drawn from this slew of molecular information is a risk of overdiagnosis, over-referral and overtreatment of food allergy due to the higher precision and the sheer amount of information available in non-initiated hands.

Conclusion

Although there is no doubt regarding the immense potential of these platforms and technological applications, the future still belongs to the allergist. Clinically speaking, the questions to be answered remain overwhelming but only initial answers have begun to appear in the literature. In particular, the performance characteristics of the new diagnostics remain unknown in the context of individual food allergies, and whether their predictive values hold over the long term of the natural history of food allergy. This would be of tremendous impact for children who have received oral induction of tolerance, for instance. Cost-effectiveness should also be compared with standard procedures. The illusion that CRD technology will substitute food challenges in diagnosis is likely to remain such.

For the present, we are bound to reflect that, despite its availability, the use of CRDs in routine practice is largely premature. Our attitude as allergists therefore should mirror that of Phaedrus in the fable of the fox and the grapes: 'Nondum matura est. Nolo acerbam sumere' (It is not yet ripe. Do not eat them sour) [44].

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