

Do we have suitable in-vitro diagnostic tests for the diagnosis of food allergy?

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Purpose of review

Positive standardized food challenges represent the gold standard of diagnostic procedures in food-related reactions suspected to be of allergic nature. Skin prick testing and in-vitro diagnosis is helpful in most cases and can help to avoid cumbersome food challenges. This review considers recent progress in the use of in-vitro tests in the diagnosis of food allergy.

Recent findings

Recent studies have addressed the characterization of 'new' food allergens which might now be used more accurately in the in-vitro diagnosis of food allergy. Additionally, while in-vitro tests must always be interpreted in line with the allergen tested and the clinical history, levels of food-specific immunoglobulin E can be correlated with the outcome of challenges to foods such as tree nuts as well as egg, according to two recent studies.

Finally, epitope binding patterns of specific food allergens might help to predict which patients will most likely outgrow their food allergy, or which patients are clinically tolerant. This might help to avoid food challenges, which carry a risk for a potentially severe outcome.

Summary

Recent studies of in-vitro diagnosis of food allergy have helped to provide safer and more accurate tests in the diagnosis and prognosis of food allergy.

Keywords

food allergy, food hypersensitivity, CD63, IgE, CAP RAST

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Abbreviations

IgE immunoglobulin E
LTP lipid transfer protein
SPT skin prick test

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Introduction

The gold standard of any diagnostic procedure in food allergy remains the positive outcome of a standardized food challenge. Of great help to an accurate diagnosis are skin prick tests (SPTs) as well as in-vitro diagnostic tests. In both these procedures, standardization of the method is most important to collect reliable results. In the case of in-vitro diagnostic tests, only a few commercially available serum immunoglobulin E (IgE) testing methods are currently available resulting in the scientific advantage of having most studies performed with one or the other method and allowing having larger patient samples to evaluate results. Further possible improvements in in-vitro diagnosis include correlation with clinical diagnosis, standardization of food extracts and characterization of new allergens, defining clinically relevant and irrelevant cross-reactivities and, finally, the possibility to use in-vitro diagnosis to predict tolerance or clinical sensitivity. This review will consider recent progress made in each of these subjects.

In-vitro diagnosis and correlation with clinical features

In a retrospective review and later in a prospective study, Sampson defined cut off points for the clinical diagnosis of allergy [1,2]. These were defined for cow's milk, hen's egg, fish, soy, wheat and peanut. However, other important foods such as tree nuts had not yet been studied. Clark and Ewan [3•] recently reviewed, in a cross-sectional study, 1000 patients allergic to peanuts or tree nuts. All patients had SPTs performed, 223 patients had CAP assays (by the CAP FEIA system; Pharmacia, Uppsala, Sweden) performed and these results were correlated with the clinical history in absence of a standardized food challenge. First, the authors found that there was no correlation between the CAP levels and the severity of the reaction. Looking at correlations between in-vivo and in-vitro testing, they found that 22% of patients with a clinical reaction to nuts had a positive SPT while they had a negative CAP result. Furthermore only 30% of these patients had a CAP assay to nut higher than or equal to 15 KU/l, which is the cut off level for peanut, as defined by Sampson. When they looked at subjects tolerant to specific nuts, they found that 5% of those had a CAP results to nut of 15 KU/l or higher while 60% had negative CAP results and 35% a positive CAP result in the range of 0.35–14.99 KU/l. When they looked at the correlation between SPT and CAP, they found that by taking a cut-off point of 3 mm

or more for SPT and 0.35 KU/l or higher for CAP, there was a concordance between SPT and CAP assay of 64%. As already reported, a positive SPT and a negative CAP result largely accounted for the discordance between the results with both methods. This pattern was found for all nuts (peanut, Brazil nut, almonds, hazelnut) when individually examined. The authors concluded that while (at least in the UK) most testing done by non-allergists is by in-vitro methods, one should strongly also consider SPTs, especially in patients with a strongly suggestive history of nut allergy. Furthermore, the cut-off point of 15 KU/l strongly suggests clinical reactivity to the different tree nuts, similar to that defined for peanut by Sampson [2].

Defining levels of food-specific IgE to predict clinical reactivity is definitely an important task to pursue for accurate diagnosis of food allergy. Osterballe and Bindselev-Jensen [4**] recently published a report in which they looked at threshold levels for clinical reactivity to egg allergy as well as to specific IgE levels with regards to the clinical outcome of food challenges. They found a significantly higher titer of egg-specific IgE by two different methods – CAP FEIA system and Magic Lite system – in patients with positive food challenge when compared with patients with negative food challenges. With the CAP FEIA system, the values were 4.6 versus 0.6 KU/l and for the Magic Lite system, 35 versus 3.6 SU/ml. They found that above 1.5 KU/l by the CAP system and 10.8 SU/ml by the Magic Lite system, there was a greater than 95% likelihood of the patients having egg allergy. It is most interesting to see, as already shown by other studies, that one cannot consider a unified value to predict clinical reactivity in all patient populations. Again, it must be stressed that each population should be looked at individually in order to gather accurate cut-off levels.

While in-vitro diagnosis mostly relies on the titers of specific IgE antibodies, other parameters have raised interest, one of those being the measurement of CD63 expression on basophils in whole blood after stimulation with the antigen *in vitro*. Erdmann *et al.* [5] investigated patients with allergy to carrot, celery and hazelnut associated with pollen allergies. They found a slightly higher sensitivity for the induction of CD63 expression on basophils than for allergen-specific IgE measurement, as well as a good correlation between IgE reactivity and the number of CD63 positive basophils for all food allergens tested. As flow cytometers are currently available to most laboratories, it might become in some cases a useful additional tool for in-vitro diagnosis. However, these findings will need to be validated by other clinical studies before routine use can be recommended.

Defining new allergens and characterizing cross-reactivity by in-vitro diagnosis

Most allergy testing is currently done with whole protein food extracts. However, it is of importance to better characterize the specific proteins involved in allergic reactions. Wang *et al.* [6] recently cloned the major cashew allergen Ana o 2. This vicilin was found to be reactive by IgE immunoblotting to 13 of 21 sera from cashew allergic patients. Fruit allergy represents one of the major problems, especially in the Mediterranean countries. A group from Spain studied Pru p 3, a major peach allergen, in its recombinant and its natural form [7•]. They showed by circular dichroism analysis that natural and recombinant Pru p 3 forms were similarly folded. Furthermore, there was no difference in IgE binding capacity of the two molecular versions. As such, both proteins, the natural and the recombinant one, could be used for in-vitro or in-vivo diagnosis. Indeed it is most important that such tests are done before synthetic proteins or peptides are used for routine diagnostic testing.

Proteins found in multiple different foods or vegetables are most often responsible for clinical allergies in taxonomically related foods. Lipid transfer protein (LTP) is one such protein. Pastorello *et al.* [8] studied LTP using sera from a population of patients anaphylactic to maize. They found that denaturated maize had a decreased ability to bind IgE when compared with the raw sample. However, the IgE binding capacity of the LTP remained unchanged after thermal treatment although its secondary structure was altered. Again, better characterization of allergens with regard to their resistance to heat denaturation might improve in-vitro diagnosis.

Grape and wine allergy is quite uncommon. Pastorello *et al.* [9] describe several severe allergic reactions to grape or wine. They identified a 24 kDa protein homologous to the cherry thaumatin-like allergen as a minor allergen, and an LTP homologous to peach LTP as a major allergen. Patients with grape or wine allergy may therefore benefit from in-vitro diagnostic tests involving both these protein families.

Finally, some new insights into cross-reactivity among foods were brought by the study of Fernandes *et al.* [10•]. They looked at Orthodox Jews, unexposed to shrimp by Kosher dietary laws, who reacted positively to skin tests for shrimp. All had symptoms of perennial allergic rhinitis, asthma, atopic dermatitis or sinusitis with positive skin test to house dust mites. It was shown that the positivity to the major shrimp allergen Pen a 1 was due to cross-reactivity to tropomyosin of cockroaches or dust mites.

Predicting tolerance acquisition in food allergic patients

One of the major tasks in providing care for patients with food allergy is to predict the natural acquisition of tolerance as seen in most food allergies. To date, the measurement of specific IgE levels is of some help, but standardized food challenges are necessary to definitively assess the clinical status of the patient. Recent work has been done by looking at conformational and linear IgE binding epitopes in cow's milk allergy [11–13]. It is now well defined that a minority of patients can also lose their peanut allergy. Specific IgE levels can be of some help to identify these patients and Beyer *et al.* [14••] looked at peptide-specific IgE as an additional tool to do so. They looked at a group of 15 patients with symptomatic peanut allergy, 10 subjects who were peanut allergic but who had outgrown their allergy and six patients who never had clinical reactions to peanut but a positive test. Immuno-dominant IgE binding epitopes had been previously identified on Ara h 1, Ara h 2 and Ara h 3. These epitopes were synthesized on SPOT membranes and the sera of the patients were assayed for IgE binding. While all three immuno-dominant epitopes on Ara h 2 were recognized by most patients with symptomatic peanut allergy, only 10% of tolerant subjects had antibody to those epitopes. Again, most patients with peanut allergy recognized immuno-dominant epitopes on Ara h 3 while one third of tolerant participants also had positive tests. The four immuno-dominant epitopes previously defined on Ara h 1 were only recognized by 20–40% of those with symptomatic peanut allergy but less than 10% of tolerant patients. Differences in epitope recognition between patients with symptomatic peanut allergy and tolerant subjects were not dependent on the peanut-specific IgE levels. The results in this study show that similar to milk allergy, testing immuno-dominant epitopes in peanut allergy, especially to Ara h 1 and Ara h 2, can be of great help to predict tolerance development in peanut allergy. If these results are further clinically validated, they will definitely provide great help to a better care of food allergic patients.

Conclusion

In conclusion, to answer the question 'do we have suitable in-vitro diagnostic tests?', recent work addressing in-vitro diagnosis of food allergy has considered specific IgE testing and the correlation with either the clinical diagnosis or the follow-up of clinical sensitivity to food, improving the overall clinical accuracy of IgE testing. Cross-reactivity will need further studies to be better understood and to define clinically relevant tests. Food challenges should be done safely and will probably need to be used also in the future to assess clinical reactivity. Cut-off levels provide some tools to better predict which patients do not need a standardized food challenge for diagnosis or follow-up. However, these data need careful

interpretation with regard to the type of population. Finally, specific epitope binding tests might be a promising tool to define which patient will become tolerant or might already be tolerant to a specific food.

Acknowledgements

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- of special interest
- of outstanding interest

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