

EDITORIAL

Clinical & Experimental Allergy

Component-resolved diagnosis of allergy: more is better?

This editorial discusses the findings of the paper in this issue by Scala et al. [8] pp. 911–921.

G. Salcedo and A. Diaz-Perales

Unidad de Bioquímica, Departamento de Biotecnología, E.T.S. Ingenieros Agrónomos, Universidad Politécnica, Madrid, Spain

The diagnosis of type I allergy by *in vitro* (e.g. specific IgE determination), *ex vivo* (e.g. basophil activation test) or *in vivo* [e.g. skin prick test (SPT); nasal, oral or bronchial provocation] methods has been mainly performed by testing crude extracts from different allergenic sources (e.g. mites, pollen, foods). These extracts are mixtures of allergenic and non-allergenic components, usually non-standardized for their major allergens. When extracts are poorly extracted, degraded or inactivated during the preparation, the major allergen may be present in low amounts (or even absent) or not – in the native conformation of the allergenic source used [1]. Likewise, immunotherapy protocols have also included such crude and complex extracts to treat all patients with allergy to a particular allergenic source regardless of the potential differences in their individual sensitization profiles. The isolation and characterization of an increasing number of natural allergens during the last decades, together with the production of their recombinant forms, have resulted in an enhanced capacity for diagnosis and treatment of allergic disease [1, 2].

Panels of purified allergens, and/or more recently peptides deduced from their amino acid sequences, which represent the relevant allergenic components of inhalant or ingested sources, have been used to carry out what has become known as component-resolved diagnosis, which leads to a better definition of the molecular sensitization profile for each patient [3–5]. SPTs, but much more frequently allergen-specific IgE determination by commercial (e.g. Immuno CAP) or home-developed ELISA assays have been used. Attempts (e.g. ADVIA Centaur platform) to refine these *in vitro* methods, avoiding the

recognition of low-affinity IgE responses and testing lower allergen amounts, have been recently developed [6].

The introduction of microarrays with a much larger number of purified allergens constitutes a further development in the diagnosis of allergic diseases [7]. Simultaneous measurement of specific IgE to multiple molecules using minimum amounts of allergen and sera can be achieved, thus allowing the screening of many samples in molecular epidemiological studies. The work published by Scala et al. [8] in this issue of the journal is a good example of the potential and limitations of using this technology, in particular a version of the ISAC microarray system. Specific IgE to a panel of 75 allergens from different sources was determined by a semi-quantitative assay in sera from 23 077 random and consecutive Italian subjects referred for suspected allergic disease. This survey revealed IgE to at least one allergen in 71% of the sera, and allowed the ranking of sensitization to the molecules tested, with Cup a 1 from *Cupressus arizonica* pollen, Der f 2 from the mite *Dermatophagoides farinae* and Phl p 1 from *Phleum pratense* (grass) pollen being the top-ranked allergens. Further analysis of specific IgE prevalences uncovered divergent sensitization to defined molecular markers according to gender (e.g. male subjects showed higher sensitization rates to those from grass, olive or plane tree pollens), as well as differential age profiles of several allergen sensitizations (e.g. the highest IgE prevalence below the age of 5 years for cow's milk and hen's egg allergens, while 15–25 years of age for the major grass pollen marker Phl p 1). Thus, the study by Scala et al. [8] highlights the potentiality and advantages of the ISAC microarray as a current tool for diagnosis of allergy. Up to now, ISAC has been used as a research tool and this large epidemiological survey is a good example of the method's potential. However, before its use can be generalized as a feasible alternative for allergy diagnosis, there are several constraints that need to be addressed. The first issue regards the lack of clinical information to help to interpret

Correspondence:

Gabriel Salcedo, Unidad de Bioquímica, Departamento de Biotecnología, E.T.S. Ingenieros Agrónomos, Universidad Politécnica, 28040 Madrid, Spain. E-mail: gabriel.salcedo@upm.es

Cite this as: G. Salcedo and A. Diaz-Perales, *Clinical & Experimental Allergy*, 2010 (40) 836–838.

the relevance of sensitization. A careful clinical history of each recruited patient, confirmed by the appropriate *in vitro* (i.e. IgE determinations) or *in vivo* (i.e. SPTs) tests and provocation challenges when necessary [9], is the ideal background to raise questions on the actual relationship between molecular markers and the type of clinical symptoms (e.g. local and mild vs. systemic and severe), sensitization route (e.g. inhalation vs. ingestion), cross-reactivity (co-sensitization) with other allergenic sources, etc. The EU-funded EuroPrevall project is a good example of how a multidisciplinary approach is needed and can be managed to develop clinically relevant and validated diagnosis tools.

A second and more important point concerns the criteria to include an allergen in the microarray [10, 11]. The relevance (prevalence of sensitization and clinical symptoms) of different allergenic sources and their individual allergens so far identified [12], the presence of allergen families (e.g. pan-allergens) comprising cross-reactive members from diverse biological species [13], the potential lack of equivalence between natural and recombinant forms of a determined molecule [14] and the differential sensitization profiles found in different geographical areas [15], should be taken into account. In this context, the version of the ISAC microarray used by Scala et al. [8] appears to present some limitations. Thus, regarding allergens of plant origin: (1) the panel appears to have a bias towards Central and Northern European sensitization profiles, at the expense of those shown by patients from the Mediterranean area (e.g. olive allergens) [3,15]; (2) some relevant allergenic sources are not represented in the microarray, both for Central (peanut and soybean) or Southern (melon and *Salsola kali* pollen) Europe. The same is true considering the poor selection of allergens representing certain cereal (e.g. wheat) or legume (e.g. lentils, peas) seeds [16]; (3) there is too much redundancy within some allergen families, such as profilins or Bet v 1-homologous group; (4) it is not clear how far recombinant forms included in the panel have been compared with their natural counterparts to ascertain the equivalence of their structural and immunological properties [14]; (5) the role of cross-reactive carbohydrate determinants (CCDs) in the IgE-binding to some allergens (e.g. nCup a 1, nOle e 1) appears to need further evaluation, although Scala et al. [8] rule out a significant interference of CCDs on IgE reactivity based on indirect experimental evidence.

Nevertheless, the real question in relation to the benefit of microarrays is whether or not 'more is better'. In other words, is it actually necessary to test a high number of purified allergens from very different allergenic sources to reach an accurate diagnosis of most patients? Besides the substantial cost for each sera analysed, is the information provided from a microarray with around 100 molecular markers useful and easy to interpret by most physicians

[17]? Large microarrays can be very helpful in epidemiological and basic research studies looking for correlations between specific IgE profiles and clinical manifestation of allergic diseases. In contrast, clinical practice probably requires a limited set of well-validated sensitization markers that can facilitate the diagnosis and predict potential cross-reactivities and clinical symptoms. In clinical practice, the use of diagnostic tools aims to solve a diagnostic problem. Thus, a careful selection of the allergens included based on their diagnostic value and not on their availability is needed. The validation of this new diagnostic tool will need the collaboration of basic researchers, clinical groups and industrial partners.

Acknowledgements

We thank Dr Domingo Barber for helpful discussions on component-resolved diagnosis.

References

- 1 Valenta R, Lidholm J, Niederberger V, Hayek B, Kraft D, Grönlund H. The recombinant allergen-based concept of component-resolved diagnostics and immunotherapy (CRD and CRIT). *Clin Exp Allergy* 1999; **29**:896–904.
- 2 Chapman MD, Smith AM, Vailes LD, Arruda LK, Dhanaraj V, Pomes A. Recombinant allergens for diagnosis and therapy of allergic disease. *J Allergy Clin Immunol* 2000; **106**:409–18.
- 3 Barber D, de la Torre F, Feo F *et al.* Understanding patient sensitization profiles in complex pollen areas: a molecular epidemiological study. *Allergy* 2008; **63**:1550–8.
- 4 Bauermeister K, Ballmer-Weber BK, Bublin M *et al.* Assessment of component-resolved in vitro diagnosis of celeriac allergy. *J Allergy Clin Immunol* 2009; **124**:1273–81.
- 5 Lin J, Bardina L, Shreffler WG *et al.* Development of a novel peptide microarray for large-scale epitope mapping of food allergens. *J Allergy Clin Immunol* 2009; **124**:315–22.
- 6 Fromberg J. IgE as a marker in allergy and the role of IgE affinity. *Allergy* 2006; **61**:1234.
- 7 Hiller R, Laffer S, Harwanegg C *et al.* Microarrayed allergen molecules: diagnostics gatekeepers for allergy treatment. *FASEB J* 2002; **16**:414–6.
- 8 Scala E, Alessandri C, Bernardi ML *et al.* Cross-sectional survey on IgE reactivity in 23,077 subjects using an allergen molecule-based microarray detection system. *Clin Exp Allergy* 2010; **40**: 911–921.
- 9 Asero R, Fernandez-Rivas M, Knulst AC, Bruijzeel-Koomen CAFM. Double-blind, placebo-controlled food challenge in adults in everyday clinical practice: a reappraisal of their limitations and real indications. *Curr Opin Allergy Clin Immunol* 2009; **9**:379–85.
- 10 Lucas JS, Atkinson RG. What is a food allergen? *Clin Exp Allergy* 2008; **38**:1095–9.
- 11 Mari A. When does a protein become an allergen? Searching for a dynamic definition based on most advanced technology tools. *Clin Exp Allergy* 2008; **38**:1089–94.
- 12 Kummeling I, Mills ENC, Clausen M *et al.* The EuroPrevall surveys on the prevalence of food allergies in children and

- adults: background and study methodology. *Allergy* 2009; **64**:1493–7.
- 13 Radauer C, Bublin M, Wagner S, Mari A, Breiteneder H. Allergens are distributed into few protein families and possess a restricted number of biochemical functions. *J Allergy Clin Immunol* 2008; **121**:847–52.
- 14 Diaz-Perales A, Sanz ML, Garcia-Casado G *et al.* Recombinant and natural Pru p 3, a major peach allergen, show equivalent immunological reactivity: a new tool for diagnosis of fruit allergy. *J Allergy Clin Immunol* 2003; **111**:628–33.
- 15 Fernandez-Rivas M, Bolhaar S, Gonzalez-Mancebo E *et al.* Apple allergy across Europe: how allergen sensitization profiles determine the clinical expression of allergies to plant foods. *J Allergy Clin Immunol* 2006; **118**:481–8.
- 16 Tatham AS, Shewry PR. Allergens in wheat and related cereals. *Clin Exp Allergy* 2008; **38**:1712–26.
- 17 Eigenmann PA. Component-resolved diagnosis in food allergy, are micro-array assays helpful to the clinician? *Allergy* 2008; **63**:1519–20.