Clinical & Experimental Allergy, 1–2 doi: 10.1111/j.1365-2222.2009.03287.x

© 2009 Blackwell Publishing Ltd

EDITORIAL

Clinical & Experimental Allergy

Basophil activation tests in food allergy

This editorial discusses the findings of the paper in this issue by Ocmant et al. [15]

D. G. Ebo

Department of Immunology, Allergology and Rheumatology, Antwerp University, Antwerpen, Belgium

The 'gold standard' for diagnosis of IgE-mediated food allergy remains a properly performed double-blind, placebo-controlled food challenge (DBPCFC) [1, 2]. However, there are different practical (long duration, blinding) and ethical (e.g. severe anaphylaxis) limitations that hamper entrance of DBPCFC in daily clinical practice. As a result, physicians predominantly use history as a 'copper standard' and try to document their clinical suspicion by commercially available specific IgE (sIgE) assays and skin test extracts. However, it has repetitively been demonstrated that none of these tests possesses an absolute predictive value. Furthermore, IgE testing does not predict features such as reaction severity; nor can it discriminate cross-reactivity from multiple sensitizations [3]. Consequently, there is a consensus that there is room for additional more reliable *in vitro* tests that could eventually replace or at least reduce the need for DBPCFC.

Although basophils are the least abundant circulating leucocyte, they represent the most important effector cell for IgE-mediated allergy in the circulation. Functional *in vitro* basophil activation assays have generally focused on histamine and/or sulphidoleukotriene release (for a review, see [4, 5]). However, the time- and cost-consuming two-step approach, i.e. (a) cell incubation and (b) quantification of mediators, has limited their application. From these reviews it appears that with respect to food allergy only a limited number of papers on histamine and/or sulphidoleukotriene release have been published. In these rather small and heterogeneous studies sensitivity and specificity varied between 53–85% and 78–100%, respectively.

In the early 1990s, the discovery of the basophil activation marker CD63 has induced the development of a flow cytometric technique to analyse allergen-specific *in vitro* activation of basophils [6]. Briefly, as shown in Fig. 1, upon activation with specific allergen basophils not only secrete quantifiable mediators but also up-regulate the expression of particular activation markers such as CD63 and CD203c that can be analysed flow cytometrically using specific monoclonal antibodies. During recent years, the technique has proven to be an easily accessible, rapid and reliable diagnostic method that allows simultaneous testing of several putative allergens such as aeroallergens (e.g. house dust mite, pollen, epithelia), natural rubber latex, hymenoptera venom and drugs (e.g. curarising neuromuscular blocking agents, β-lactam antibiotics) with a minimal amount of blood (for a review, see [7, 8]). In food allergy, the BAT was first applied in the late 1990s by Moneret-Vautrin et al. [9], who demonstrated the technique to be as sensitive and as specific as the sulphidoleukotriene release assay and more efficient than the traditional histamine release test. Since then, the BAT has been validated in different primary food allergies (e.g. *Anisakis simplex*, *Macrobrachium rosenbergii* and wheat). From these studies it appears that the BAT can constitute a sensitive and specific instrument that can complement conventional tests such as quantification of sIgE and skin tests. The technique seems particularly useful when traditional tests are unavailable or yield equivocal or false-negative results. In general sensitivity and specificity of the BAT varies between 75% and 95%.

The BAT has also been evaluated in different pollen-related food allergies such as the oral allergy syndrome for apple, carrot and celery [10–12]. In one of these studies it was also demonstrated that to some extent the BAT can discriminate between sensitization and true allergy [12], an observation that was confirmed recently [13, 14]. In individual patients the BAT confirmed the diagnosis of an allergy to coriander, lychee, mandarin and the natural dye annatto.

The study published by Ocmant et al. [15] in this issue of *Clinical and Experimental Allergy* investigates the usefulness of the BAT in diagnosis of peanut and egg allergy. For this purpose, data from 63 children with suspected peanut or egg allergy were compared with results obtained in 51 healthy control children and 28 children sensitized but tolerant for peanut or egg. For the BAT, two different

Correspondence:
D.G. Ebo, Department of Immunology, Allergology and Rheumatology, University Antwerp, Campus Drie Eiken T401, Universiteitsplein 1, B 2610 Antwerpen, Belgium.
E-mail: immuno@ua.ac.be

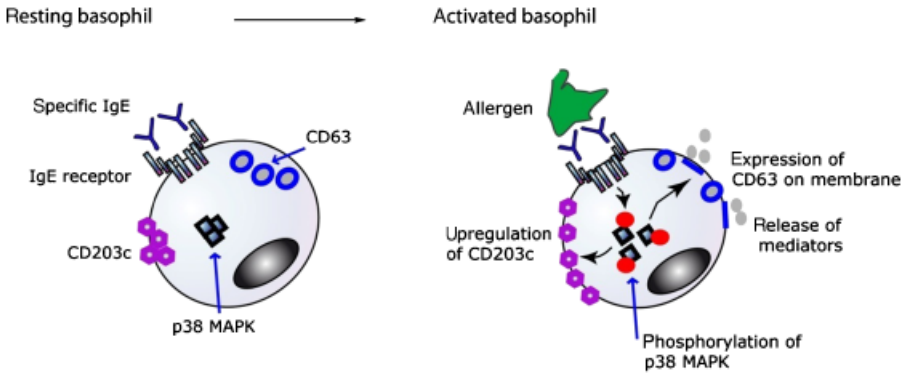

Fig. 1. Upon cross-linking of membrane-bound IgE, basophils not only secrete particular mediators, but will also up-regulate the expression of specific activation markers such as CD63 and CD203c. The exact mechanism(s) that govern basophil degranulation remain elusive but it has been demonstrated that phosphorylation of p38 mitogen-activated protein kinase (MAPK) exerts a pivotal role in cell activation. Both, the surface markers and p38 MAPK can be detected flow cytometrically [16] (with permission Ebo DG et al., *Acta Clin Belg* 2009).

read-outs were applied (CD63 and CD203c: see Fig. 1) and data were compared with the production of leukotrienes. The key message of the study is that the BAT constitutes an additional test that can improve diagnosis from peanut and egg allergy, as it generally discriminates between real allergy and asymptomatic sensitization.

In conclusion, the BAT definitely creates new opportunities in the diagnosis of IgE-mediated food allergy. As the technique closely resembles the *in vivo* pathway leading to symptoms, it is anticipated that the technique might mostly be adopted for objectives such as differentiation between clinically relevant and irrelevant IgE serology, to predict spontaneous outgrow and to perform a functional risk analyses of trace amounts of (hidden) food allergens.

References

1 Bruijnzeel-Koomen C, Ortolani C, Aas K *et al.* Adverse reactions to food. European Academy of Allergology and Clinical Immunology Subcommittee. *Allergy* 1995; **50**:623–35.

2 Asero R, Ballmer-Weber BK, Beyer K *et al.* IgE-mediated food allergy diagnosis: current status and new perspectives. *Mol Nutr Food Res* 2007; **51**:135–47.

3 Cox L, Williams B, Sicherer S *et al.* Pearls and pitfalls of allergy diagnostic testing: report from the American College of Allergy, Asthma and Immunology/American Academy of Allergy, Asthma and Immunology Specific IgE Test Task Force. *Ann Allergy Asthma Immunol* 2008; **101**:580–92.

4 Crockard AD, Ennis M. Basophil histamine release tests in the diagnosis of allergy and asthma. *Clin Exp Allergy* 2001; **31**:345–50.

5 Demoly P, Lebel B, Arnoux B. Allergen-induced mediator release tests. *Allergy* 2003; **58**:553–8.

6 Knol EF, Mul FP, Jansen H, Calafat J, Roos D. Monitoring human basophil activation via CD63 monoclonal antibody 435. *J Allergy Clin Immunol* 1991; **88** (Part 1):328–38.

7 Ebo DG, Hagendorens MM, Bridts CH, Schuerwegh AJ, De Clerck LS, Stevens WJ. In vitro allergy diagnosi: should we follow the flow? *Clin Exp Allergy* 2004; **34**:332–9.

8 Ebo DG, Bridts CH, Hagendorens MM, Aerts NE, De Clerck LS, Stevens WJ. Basophil activation test by flow cytometry: present and future applications in allergology. *Cytometry B Clin Cytom* 2008; **74**:201–10.

9 Moneret-Vautrin DA, Sainte-Laudy J, Kanny G, Fremont S. Human basophil activation measured by CD63 expression and LTC4 release in IgE-mediated food allergy. *Ann Allergy Asthma Immunol* 1999; **82**:33–40.

10 Erdmann SM, Heussen N, Moll-Slodowy S, Merk HF, Sachs B. CD63 expression on basophils as a tool for the diagnosis of pollen-associated food allergy: sensitivity and specificity. *Clin Exp Allergy* 2003; **33**:607–14.

11 Erdmann SM, Sachs B, Schmidt A *et al.* In vitro analysis of birch-pollen-associated food allergy by use of recombinant allergens in the basophil activation test. *Int Arch Allergy Immunol* 2005; **136**:230–8.

12 Ebo DG, Hagendorens MM, Bridts CH, Schuerwegh AJ, De Clerck LS, Stevens WJ. Flow cytometric analysis of in vitro activated basophils, specific IgE and skin tests in the diagnosis of pollen-associated food allergy. *Cytometry B Clin Cytom* 2005; **64**:28–33.

13 de Leon MP, Drew AC, Glaspole IN, Suphioglu C, Rolland JM, O'Hehir RE. Functional analysis of cross-reactive immunoglobulin E antibodies: peanut-specific immunoglobulin E sensitizes basophils to tree nut allergens. *Clin Exp Allergy* 2005; **35**:1056–64.

14 Wallowitz ML, Chen RJ, Tzen JT, Teuber SS. Ses i 6, the sesame 11S globulin, can activate basophils and shows cross-reactivity with walnut in vitro. *Clin Exp Allergy* 2007; **37**:929–38.

15 Ocmant A, Mulier S, Hanssens L *et al.* Basophil activation tests for the diagnosis of food allergy in children. *Clin Exp Allergy* 2009, doi: 10.1111/j.1365-2222.2009.03292.x.

16 Ebo DG, Dombrecht EJ, Bridts CH, Aerts NE, De Clerck LS, Stevens WJ. Combined analysis of intracellular signalling and immunophenotype of human peripheral blood basophils by flow cytometry: a proof of concept. *Clin Exp Allergy* 2007; **37**:1668–75.