Basophil activation test in IgE-mediated food allergy : should we follow the flow?

Basophil activation test in IgE mediated food allergy: should we follow the flow?

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Introductory paragraph

IgE-mediated food allergy constitutes an important health issue affecting about 17.000.000 Europeans. Although double blind placebo controlled food challenges remain the gold standard for correct diagnosis, the technique has not entered mainstream use for obvious ethical, practical and economic reasons. Therefore, in clinical practise most physicians will rely upon quantification of sIgE antibodies and skin test to confirm their clinical suspicion. However, correct diagnosis of food allergy is not always straightforward, as these tests are not absolutely predictive for the clinical outcome. Therefore, basophil activation tests, that closely mirror the in vivo reaction, might constitute a significant diagnostic aid. This review focusses on the potential and limitations of basophil activation tests in IgE-mediated food allergy.

Abstract

IgE-mediated food allergy constitutes an important and increasing health issue with significant impairment of quality of life and significant morbidity and mortality. It affects children, as well as adolescents and adults. Correct diagnosis of food allergy relies upon history supplemented by quantification of specific IgE (sIgE) antibodies and/or skin tests. Unfortunately, as these tests do not demonstrate absolute predictive values, controlled oral provocation tests might be needed to confirm/exclude diagnosis. However, it is unlikely oral challenges to enter mainstream application, mainly because of obvious ethical reasons. Therefore, correct diagnosis of food allergy might benefit from novel *in vitro* diagnostics such as allergen component-based sIgE assays and flow cytometric quantification of *in vitro* activated basophils. As a matter of fact, these tests might prove to be particularly helpful in discriminating genuine allergy from merely sensitization. Furthermore, they might be useful in establishing individual risk profiles, predicting persistence of allergy, and facilitating therapeutic approach. This review focuses on the applications and limitations of the basophil activation test in IgE-mediated food allergy. Anno 2016 we believe that the utility and usefulness of basophil activation experiments need to be reevaluated thoroughly, in view of difficulties inherent to the correct preparation and storage of allergen extracts, optimizing and standardizing stimulation conditions and also the potential of alternative diagnostics such as component resolved diagnosis that are becoming more readily accessible.

Keywords: food allergy, basophil activation test, food allergy diagnosis

Basophil activation tests: a historical perspective

Basophils represent the most important effector cells for IgE-mediated allergy in the circulation. Functional *in vitro* basophil activation assays have initially focused on histamine and/or sulphidoleukotriene release (review: (1, 2)). However, the time- and cost-consuming two-step methods with cell incubation and quantification of mediators, have seriously impeded their application in general clinical practice. Actually, from these reviews it appears that with respect to IgE-mediated food allergy only a very limited number of papers on release of histamine and/or sulphidoleukotriene have been published. In these rather small and heterogeneous studies sensitivity and specificity of mediator release tests varied between 53%-85% and 78-100%, respectively. In the early 1990ies, the discovery of the tetraspanin CD63 (gp53) as a basophil activation marker has induced the development of a flow cytometric technique to analyze and quantify allergen-specific *in vitro* activation of basophils (3). Briefly, as shown in figure 1, upon activation with specific allergen, along with the release of histamine, basophils also up-regulate the expression of particular activation (CD203c) and degranulation (CD63) markers that can be analyzed by multi-color flow cytometry using specific monoclonal antibodies (4). During recent years, the technique, designated as basophil activation test (BAT), appeared to be an accessible, rapid and reliable diagnostic method allowing simultaneous testing of several putative allergens such as aeroallergens, latex, hymenoptera venom and particularly drugs (for review: (5-7)).

Basophil activation tests in IgE-mediated food allergy

In IgE-mediated food allergy the BAT was first adopted in the late 1990ies by Moneret-Vautrin *et al.* (8), who demonstrated the technique to be as sensitive and specific as the sulphidoleukotriene release assay and clearly more efficient than the traditional histamine release test.

Table 1-4, display the current applications of the BAT in IgE-mediated food allergy including studies regarding diagnosis with native foods and purified or recombinant food allergen

components, cross-reactivity, allergy outgrowing and risk stratification of patients, follow-up during immunotherapy and oral tolerance induction, allergen detection (traces), and effect of food processing (9).

In general, the technique proved useful when traditional diagnostic tests such as quantification of sIgE and skin tests are unavailable or yield equivocal or false negative results. In general, sensitivity and specificity of the BAT in IgE-mediated food allergy varies between 75 and 95%. In some studies it was also demonstrated that (to some extent) the BAT can discriminate between sensitisation and genuine allergy (10-13). Furthermore, in individual cases the BAT was useful to diagnose allergy to coriander, mandarin, pumpkin and the natural dye annatto (14-17).

Basophil activation tests have also been applied as biomarkers to study the natural evolution of food allergies and to predict the occurrence of tolerance to milk (18-21) or eggs (20), or to evaluate the therapeutic effect of Chinese herbal medicine (Food Allergy Herbal Formula-2, FAFH-2) (22).

Finally, other studies suggest that the BAT can be applied to study the (residual) allergenicity of food (by detecting traces of allergens) (23) and allows to study the effects of various means of processing (*e.g.,* heating (24), Maillard reactions (25)).

Particular considerations and perspectives

It is clear that the BAT creates new and unique opportunities in the diagnostic management and follow-up of IgE-mediated food allergies, mainly as it closely mirrors the in vivo situation. However, we believe that the utility and usefulness of basophil activation experiments need to be reevaluated thoroughly, mainly in view of difficulties inherent to the correct preparation and storage of allergen extracts, optimizing and standardizing stimulation conditions and also the venue of easier alternative diagnostics such as component resolved diagnosis (26). For example, we demonstrated that the outcome of the BAT in peanut allergy is significantly affected by the

applied cultivar and the processing of the source material (24). Actually, Sabato *et al* showed that various methods of (thermal) food processing can significantly alter the capacity of particular peanut cultivars to trigger basophils in patients with established severe peanut allergy. Moreover, effects appeared to be individually divergent and absolutely unpredictable by protein staining using SDS-Page nor IgE-immunoblotting. To a certain extent, similar observations were recently described by Cabanillas *et al* (27), demonstrating that heat and pressure of 3 varieties of commonly consumed peanuts significantly decreases IgE binding capacities, diminishes activation of passively sensitized basophils and reduces wheal sizes in skin prick tests. From these observations the conclusion is inevitable, like for other allergy diagnostics, reliability of the BAT is highly dependent on the stimulation conditions, particularly the (residual) composition, preparation and storage of the allergen extracts. Therefore, observations from different studies might not readily be interchangeable. In addition, the predictive value of basophil activation experiments might also exhibit significant geographical and age-dependent variations, mainly as a result of differences in sensitization profiles and nutritional habits. For example, in our regions, the absence of the labile Cor a 1.04 in a hazelnut (*Corylus avellana*) extract could significantly affect the outcome of BAT in adults with a birch-pollen related hazelnut allergy but have almost no effect on the reliability of BAT in hazelnut allergic children, as the latter are predominantly sensitized to Cor a 9 and/or Cor a 14 (28). Taken together, it is clear that further validation of the diagnostic utility of BAT in IgE-mediated food allergy is absolutely warranted to allow a more universal clinical use. As with any study of this nature, the major challenges will be to accurately identify the patients and control individuals and to apply well-defined and controllable stimulation conditions. In this context, it is our believe that the different applications of BAT in IgE-mediated food allergy will benefit from the increasing availability of purified and recombinant food components, as these will allow to compose better characterized and harmonized stimulation mixtures.

General conclusions

It is clear that the BAT is a valuable and safe diagnostic method in IgE-mediated food allergy, mainly as the technique more closely resembles the in vivo reaction than IgE quantification. However, utility of the BAT should be reassessed regarding difficulties with the preparation and proper storage of allergen extracts and the fact that results might not readily be interchangeable between different study populations. It seems theoretically justified to anticipate that the application of well-characterized single components, or mixtures thereof, might further improve reliability and utility of the technique in diagnosis and follow-up of IgE-mediated food allergy.

BAT in diagnosis of IgE-mediated food allergy

BAT with native food: diagnosis

Stimulus	*Reference test*	*Sensitivity (%)*	*Specificity (%)*	*N*	*Marker*	*Reference*
Carrot, celery, hazelnut	H	85-90	80-90	20	CD63	(29)
Apple	H	100[1] 88	100 75[2]	59	CD63	(10)
Macrobrachium rosenbergii	H + SPT + sIgE	100	100	45	CD63	(30)
Peanut	H + SPT + sIgE	81.3 89.5	95.4 97.2	75	CD63 CD203c	(31)
Egg	H + SPT + sIgE	77.4 57.4	100 96.4	67	CD63 CD203c	(31)
Anisakis simplex	H	97	100	88	CD63	(32)
Wheat	H + sIgE + PT	85	77	58	CD203c	(33)
Peanut	H or sIgE or PT	97.6 95.2	96 96	65	CD63 CD203c	(13)
Peanut, soy, birch pollen	H + sIgE or PT	79	86	91	CD63 CD203c	(34)
Hazelnut	H + sIgE or SPT + PT	100	97	40	CD63 CD203c	(35)

BAT with food components: diagnosis

Stimulus	*Reference test*	*Sensitivity (%)*	*Specificity (%)*	*N*	*Marker*	*Reference*
Apple (Mal d 1)	H	75	68	54	CD63	(36)
Celery (Api g 1)	H	75	77	54	CD63	(36)
Carrot (Dau c 1)	H	65	100	54	CD63	(36)
Peach (Pru p 3)	H + SPT or PT	77	97	30	CD63	(37)
Peach (Pru p 2)	H + SPT or PT	50-80	-	31	CD63	(38)

BAT for examination of cross-reactivity

Description	*Outcome*	*N*	*Marker*	*Reference*
Peanut and tree nut allergens	In vitro cross-reactivity of peanut-specific IgE antibodies with tree nuts causes effector cell activation, which is demonstrated by a stripped basophil activation assay.	2	CD63	(11)
Sesame (Ses i 6) and walnut	A modified BAT might help to distinguish between clinically	-	CD63	(12)

	relevant and irrelevant in vitro IgE cross-reactivity of seed storage proteins in nuts and seeds. However data not validated by challenge.		CD203c	
[1]Taken in to account the non-responders sensitivity is 90% [2] In an additional comparison between birch pollen allergic patients with and without apple-induced OAS				

Table 1. Basophil Activation Test (BAT) in diagnosis of IgE-mediated food allergy. **H;** History, **PT;** Provocation Test, **sIgE;** specific Immunoglobulin E measurement, **SPT;** Skin Prick Test

BAT for evaluation of allergy outgrowing and risk stratification				
Description	***Outcome***	***N***	***Marker***	***Reference***
Risk stratification in children with a cow's milk allergy	BAT can discriminate between children tolerating heat denatured milk, which have less severe reactions and commonly show an earlier outgrow, and children with allergic reactions upon heat denatured milk.	55	CD63 CD203c	(18)
Risk stratification in children with a cow's milk allergy	BAT is a valuable tool in helping to decide if an oral challenge can be safely undertaken in children with cow's milk allergy.	112	CD63	(19)
Risk stratification and evaluation of the outgrow of hen's egg and/ or cow's milk allergy	BAT is useful in decision making regarding whether or not to perform oral food challenges and to determine if children will outgrow hen's egg and/ or cow's milk allergy	71	CD203c	(20)
Risk stratification in children with allergy towards cow's milk.	Spontaneous basophil activation as well as milk specific basophil reactivity is greater in patients with more severe clinical milk reactivity.	132	CD63 CD203c	(21)
Basophil allergen threshold sensitivity (CD-sens) to peanut and Ara h 2 in relation to DBPCFC	CD-sens is an useful tool in the evaluation of peanut allergy. Negative CD-sens with peanut and Ara h 2 is predictive for negative DBPCFC to peanut	38	CD63	(39)
Prediction of the severity and threshold of reactivity to peanut during oral food challenges.	BAT is a marker for severity of allergic reactions and can be used to estimate the threshold of allergic reactions to peanut.	49	CD63 CD203c	(40)
Prediction of outcome and severity of reactions at DBPCFC to various foods: peanut, tree nut, shrimp, fish, sesame	BAT discriminates between allergic and non-allergic individuals and can act as a predictor of clinical severity of food allergy	67	CD63	(41)
Evaluation of the allergenic activity of 2 mutants of the profilin of Melon (Cuc m 2)	One of the mutants (Mut 2) displayed reduction of basophil activation	10	CD63	(42)
Evaluation of basophil allergen threshold sensitivity (CD-sens) to peanut and Ara h 8 in relation to an oral peanut challenge	CD-sens to Ara h 8 was positive in 85 % of children with peanut and Ara h 8 sensitization	20	CD63	(43)
Risk stratification of peanut allergy in children	Severe peanut allergy is significantly associated with higher basophil allergen sensitivity	27	CD63	(44)
Evaluation of basophil response to peanut allergens in Mediterranean patients.	BAT with Ara h 2 is able to discriminate peanut allergic subjects from peanut tolerating but peach allergic subjects (Pru p 3).	60	CD63	(45)

Table 2. BAT for evaluation of allergy outgrowing and risk stratification

BAT for follow up during immunotherapy and oral tolerance induction				
Description	***Outcome***	***N***	***Marker***	***Reference***
Immunotherapy for milk allergy	BAT did show a significant decrease in constitutive expression of CD63 and CD203c with immunotherapy, potentially due to a reduced extrinsic basophil activation *in vivo*.	30	CD63 CD203c	(46)
Sublingual immunotherapy for peanut allergy	After 12 months of peanut sublingual immunotherapy, a significantly lower percent of activated basophils after stimulation with peanut extract was observed.	18	CD63	(47)
Sublingual immunotherapy for peanut allergy	The percentage CD63 positive values are significantly lower in subjects receiving peanut sublingual immunotherapy compared with placebo subjects.	40	CD63	(48)
Oral immunotherapy for peanut allergy	Basophil activation was significantly reduced within 4 months, in contrast to basophil reactivity in subjects in an observational study of peanut allergy.	39	CD63	(49)
Oral immunotherapy for peanut allergy	BAT shows evidence supporting the hypothesis that peanut oral immunotherapy induces pathway-specific basophil anergy.	28	CD63 CD203c	(50)
Milk oral immunotherapy	Basophil activation decreases over time with oral tolerance induction	142	CD63 CD203c	(51)
Evaluation of the inhibitory effect of IgG4	Peanut induced basophil activation was inhibited in the presence of plasma from non-allergic, peanut sensitized children and children submitted to peanut oral immunotherapy. Basophil activation was not inhibited	228	CD63 CD203c	(52)
Evaluation of the outcome of peanut sublingual immunotherapy	Patients responding to SLIT showed significant decrease in peanut induced basophil activation compared to non-responders (high rate of patients discontinued therapy)	40	CD63	(53)
Evaluation of sublingual immunotherapy with a Pru p 3 quantified peach extract	Basophil activation with peach peel and recombinant Pru p 3, in contradiction to other studies, increased during the first 6 months of treatment	31	CD63	(54)
Evaluation of BAT in patients with specific oral tolerance induction with egg	A significant decrease of antigen specific basophil responsiveness was observed in patients after oral tolerance induction with egg	10	CD63	(55)
Evaluation of BAT in patients with birch-pollen-associated apple allergy after an oral tolerance induction	Basophil reactivity and sensitivity to Bet v 1 and Mal d 1 did not significantly change after regular apple consumption, although clinical tolerance was obtained in the majority of patients.	40	CD63	(56)

Table 3. BAT for follow up during immunotherapy and oral tolerance induction

BAT for food allergen risk management				
BAT for detection of hidden allergens				
Description	***Outcome***	***N***	***Marker***	***Reference***
Detection of trace amounts of peanut in biscuits and chocolates	BAT is a highly sensitive and specific tool to detect traces of functionally active food allergens.	10	CD63	(23)
Detection of biologically active peanut protein in household dust	Peanut proteins in household dust are able to activate basophils from children with peanut allergy	6	CD63	(57)
BAT for measuring variety and potential allergenicity of processed food				
Description	***Outcome***	***N***	***Marker***	***Reference***
Allergenicity of thermally processed peanut	BAT, rather than SDS-PAGE or IgE binding, can predict the impact of thermal processing on the allergenicity of peanuts.	10	CD63	(24)
Allergenicity of thermally processed or Maillard reacted hazelnuts	BAT can help to assess the allergenicity of hazelnut extracts after modification of constituent proteins/allergens through thermal processing and the Maillard reaction	11	CD63	(25)
Evaluation of the allergic potential of two different tomato cultivars	BAT underpinned the clinical differences evoked by the different tomato cultivars	9	CD203c	(58)
Diagnosis of different subtypes of Wheat-dependent exercise induced anaphylaxis (WDEIA)	BAT with various preparations of wheat proteins is highly useful in predicting causative allergens in patients with WDEIA.	10	CD203c	(59)
Allergenicity of thermally processed peanut	Basophils, passively sensitized with IgE from peanut allergic patients, showed lower activation upon exposure to heat/pressure-treated peanut	8	CD203c	(27)

Table 4. BAT for food allergen risk management

Legends

<u>Figure 1.</u> Principle of flow cytometric analysis of basophils: immunophenotyping and histamine release at a single cell level

Top: Upon cross-linking of membrane-bound IgE, basophils up-regulate the expression of specific activation and degranulation markers such as CD203c, CD63 and expression of the inhibitory receptor CD300a.

Bottom: In the left quadrant co-expression analysis of CD63 and CD203c up-regulation in activated basophils is shown and 3 different subpopulations are demonstrated: $CD203c^{bright+}CD63^{-}$ (R1, green), $CD203c^{bright+}CD63^{dim+}$ (R2, blue) and $CD203c^{bright+}CD63^{bright+}$ (R3, red). In the central and right quadrant the histamine intracellular staining by meaning of DAO coupled fluorochrome is displayed. Histamine release is revealed by reduction of DAO positive cells. Of note the complete release of histamine can be observed only in $CD203c^{bright+}CD63^{bright+}$ basophils (red).

References

1. Crockard AD, Ennis M. Basophil histamine release tests in the diagnosis of allergy and asthma. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 2001;31(3):345-50.
2. Demoly P, Lebel B, Arnoux B. Allergen-induced mediator release tests. Allergy. 2003;58(7):553-8.
3. Knol EF, Mul FP, Jansen H, Calafat J, Roos D. Monitoring human basophil activation via CD63 monoclonal antibody 435. The Journal of allergy and clinical immunology. 1991;88(3 Pt 1):328-38.
4. Bridts CH, Sabato V, Mertens C, Hagendorens MM, De Clerck LS, Ebo DG. Flow cytometric allergy diagnosis: basophil activation techniques. Methods in molecular biology. 2014;1192:147-59.
5. 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 Part B, Clinical cytometry. 2008;74(4):201-10.
6. Hoffmann HJ, Santos AF, Mayorga C, Nopp A, Eberlein B, Ferrer M, et al. The clinical utility of basophil activation testing in diagnosis and monitoring of allergic disease. Allergy. 2015;70(11):1393-405.
7. Mangodt EA, Van Gasse AL, Decuyper I, Uyttebroek A, Faber MA, Sabato V, et al. In vitro Diagnosis of Immediate Drug Hypersensitivity: Should We Go with the Flow? International archives of allergy and immunology. 2015;168(1):3-12.
8. 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. Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology. 1999;82(1):33-40.
9. Faber M, Sabato V, De Witte L, Van Gasse A, Hagendorens MM, Leysen J, et al. State of the art and perspectives in food allergy (part I): diagnosis. Current pharmaceutical design. 2014;20(6):954-63.
10. 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 Part B, Clinical cytometry. 2005;64(1):28-33.
11. 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. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 2005;35(8):1056-64.
12. 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. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 2007;37(6):929-38.
13. Santos AF, Douiri A, Becares N, Wu SY, Stephens A, Radulovic S, et al. Basophil activation test discriminates between allergy and tolerance in peanut-sensitized children. The Journal of allergy and clinical immunology. 2014;134(3):645-52.
14. Ebo DG, Bridts CH, Mertens MH, Stevens WJ. Coriander anaphylaxis in a spice grinder with undetected occupational allergy. Acta clinica Belgica. 2006;61(3):152-6.
15. Hagendorens MM, Carrette M, Bridts CH, Stevens WJ, Ebo DG. Allergy from giant pumpkin (Cucurbita maxima) is not a fairy tale. Allergy. 2009;64(11):1694-6.
16. Ebo DG, Ingelbrecht S, Bridts CH, Stevens WJ. Allergy for cheese: evidence for an IgE-mediated reaction from the natural dye annatto. Allergy. 2009;64(10):1558-60.
17. Ebo DG, Ahrazem O, Lopez-Torrejon G, Bridts CH, Salcedo G, Stevens WJ. Anaphylaxis from mandarin (Citrus reticulata): identification of potential responsible allergens. International archives of allergy and immunology. 2007;144(1):39-43.
18. Wanich N, Nowak-Wegrzyn A, Sampson HA, Shreffler WG. Allergen-specific basophil suppression associated with clinical tolerance in patients with milk allergy. The Journal of allergy and clinical immunology. 2009;123(4):789-94 e20.

19. Rubio A, Vivinus-Nebot M, Bourrier T, Saggio B, Albertini M, Bernard A. Benefit of the basophil activation test in deciding when to reintroduce cow's milk in allergic children. Allergy. 2011;66(1):92-100.
20. Sato S, Tachimoto H, Shukuya A, Kurosaka N, Yanagida N, Utsunomiya T, et al. Basophil activation marker CD203c is useful in the diagnosis of hen's egg and cow's milk allergies in children. International archives of allergy and immunology. 2010;152 Suppl 1:54-61.
21. Ford LS, Bloom KA, Nowak-Wegrzyn AH, Shreffler WG, Masilamani M, Sampson HA. Basophil reactivity, wheal size, and immunoglobulin levels distinguish degrees of cow's milk tolerance. The Journal of allergy and clinical immunology. 2012.
22. Wang J, Jones SM, Pongracic JA, Song Y, Yang N, Sicherer SH, et al. Safety, clinical, and immunologic efficacy of a Chinese herbal medicine (Food Allergy Herbal Formula-2) for food allergy. The Journal of allergy and clinical immunology. 2015;136(4):962-70.e1.
23. Sabato V, van Hengel AJ, De Knop KJ, Verweij MM, Hagendorens MM, Bridts CH, et al. Human basophils: a unique biological instrument to detect the allergenicity of food. Journal of investigational allergology & clinical immunology. 2011;21(3):179-84.
24. Sabato V, van Hengel AJ, De Knop KJ, Verweij MM, Hagendorens MM, Bridts CH, et al. Basophil activation reveals divergent patient-specific responses to thermally processed peanuts. Journal of investigational allergology & clinical immunology. 2011;21(7):527-31.
25. Cucu T, De Meulenaer B, Bridts C, Devreese B, Ebo D. Impact of thermal processing and the Maillard reaction on the basophil activation of hazelnut allergic patients. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association. 2012;50(5):1722-8.
26. Van Gasse AL, Mangodt EA, Faber M, Sabato V, Bridts CH, Ebo DG. Molecular allergy diagnosis: status anno 2015. Clinica chimica acta; international journal of clinical chemistry. 2015;444:54-61.
27. Cabanillas B, Cuadrado C, Rodriguez J, Hart J, Burbano C, Crespo JF, et al. Potential changes in the allergenicity of three forms of peanut after thermal processing. Food chemistry. 2015;183:18-25.
28. Faber MA, De Graag M, Van Der Heijden C, Sabato V, Hagendorens MM, Bridts CH, et al. Cor a 14: missing link in the molecular diagnosis of hazelnut allergy? International archives of allergy and immunology. 2014;164(3):200-6.
29. 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. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 2003;33(5):607-14.
30. Ebo DG, Bridts CH, Hagendorens MM, De Clerck LS, Stevens WJ. Scampi allergy: from fancy name-giving to correct diagnosis. Journal of investigational allergology & clinical immunology. 2008;18(3):228-30.
31. Ocmant A, Mulier S, Hanssens L, Goldman M, Casimir G, Mascart F, et al. Basophil activation tests for the diagnosis of food allergy in children. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 2009;39(8):1234-45.
32. Gonzalez-Munoz M, Luque R, Nauwelaers F, Moneo I. Detection of Anisakis simplex-induced basophil activation by flow cytometry. Cytometry Part B, Clinical cytometry. 2005;68(1):31-6.
33. Tokuda R, Nagao M, Hiraguchi Y, Hosoki K, Matsuda T, Kouno K, et al. Antigen-induced expression of CD203c on basophils predicts IgE-mediated wheat allergy. Allergol Int. 2009;58(2):193-9.
34. Rentzos G, Lundberg V, Lundqvist C, Rodrigues R, van Odijk J, Lundell AC, et al. Use of a basophil activation test as a complementary diagnostic tool in the diagnosis of severe peanut allergy in adults. Clinical and translational allergy. 2015;5:22.
35. Brandstrom J, Nopp A, Johansson SG, Lilja G, Sundqvist AC, Borres MP, et al. Basophil allergen threshold sensitivity and component-resolved diagnostics improve hazelnut allergy diagnosis. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 2015;45(9):1412-8.
36. Erdmann SM, Sachs B, Schmidt A, Merk HF, Scheiner O, Moll-Slodowy S, et al. In vitro analysis of birch-pollen-associated food allergy by use of recombinant allergens in the basophil activation test. International archives of allergy and immunology. 2005;136(3):230-8.

37. Gamboa PM, Caceres O, Antepara I, Sanchez-Monge R, Ahrazem O, Salcedo G, et al. Two different profiles of peach allergy in the north of Spain. Allergy. 2007;62(4):408-14.
38. Palacin A, Tordesillas L, Gamboa P, Sanchez-Monge R, Cuesta-Herranz J, Sanz ML, et al. Characterization of peach thaumatin-like proteins and their identification as major peach allergens. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 2010;40(9):1422-30.
39. Glaumann S, Nopp A, Johansson SG, Rudengren M, Borres MP, Nilsson C. Basophil allergen threshold sensitivity, CD-sens, IgE-sensitization and DBPCFC in peanut-sensitized children. Allergy. 2012;67(2):242-7.
40. Santos AF, Du Toit G, Douiri A, Radulovic S, Stephens A, Turcanu V, et al. Distinct parameters of the basophil activation test reflect the severity and threshold of allergic reactions to peanut. The Journal of allergy and clinical immunology. 2015;135(1):179-86.
41. Song Y, Wang J, Leung N, Wang LX, Lisann L, Sicherer SH, et al. Correlations between basophil activation, allergen-specific IgE with outcome and severity of oral food challenges. Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology. 2015;114(4):319-26.
42. Tordesillas L, Gamboa P, Sanz ML, Palacin A, Gomez-Casado C, Cuesta-Herranz J, et al. A mutant of the major melon allergen, Cuc m 2, with reduced IgE binding capacity is a good candidate for specific immunotherapy. Mol Immunol. 2011;49(3):504-11.
43. Glaumann S, Nilsson C, Johansson SG, Asarnoj A, Wickman M, Borres MP, et al. Evaluation of basophil allergen threshold sensitivity (CD-sens) to peanut and Ara h 8 in children IgE-sensitized to Ara h 8. Clin Mol Allergy. 2015;13(1):5.
44. Homsak M, Silar M, Berce V, Tomazin M, Skerbinjek-Kavalar M, Celesnik N, et al. The relevance of basophil allergen sensitivity testing to distinguish between severe and mild peanut-allergic children. International archives of allergy and immunology. 2013;162(4):310-7.
45. Mayorga C, Gomez F, Aranda A, Koppelman SJ, Diaz-Perales A, Blanca-Lopez N, et al. Basophil response to peanut allergens in Mediterranean peanut-allergic patients. Allergy. 2014;69(7):964-8.
46. Keet CA, Frischmeyer-Guerrerio PA, Thyagarajan A, Schroeder JT, Hamilton RG, Boden S, et al. The safety and efficacy of sublingual and oral immunotherapy for milk allergy. The Journal of allergy and clinical immunology. 2012;129(2):448-55, 55 e1-5.
47. Kim EH, Bird JA, Kulis M, Laubach S, Pons L, Shreffler W, et al. Sublingual immunotherapy for peanut allergy: clinical and immunologic evidence of desensitization. The Journal of allergy and clinical immunology. 2011;127(3):640-6 e1.
48. Fleischer DM, Burks AW, Vickery BP, Scurlock AM, Wood RA, Jones SM, et al. Sublingual immunotherapy for peanut allergy: a randomized, double-blind, placebo-controlled multicenter trial. The Journal of allergy and clinical immunology. 2013;131(1):119-27 e1-7.
49. Jones SM, Pons L, Roberts JL, Scurlock AM, Perry TT, Kulis M, et al. Clinical efficacy and immune regulation with peanut oral immunotherapy. The Journal of allergy and clinical immunology. 2009;124(2):292-300, e1-97.
50. Thyagarajan A, Jones SM, Calatroni A, Pons L, Kulis M, Woo CS, et al. Evidence of pathway-specific basophil anergy induced by peanut oral immunotherapy in peanut-allergic children. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 2012;42(8):1197-205.
51. Elizur A, Appel MY, Goldberg MR, Yichie T, Levy MB, Nachshon L, et al. Clinical and laboratory 2-year outcome of oral immunotherapy in patients with cow's milk allergy. Allergy. 2015.
52. Santos AF, James LK, Bahnson HT, Shamji MH, Couto-Francisco NC, Islam S, et al. IgG4 inhibits peanut-induced basophil and mast cell activation in peanut-tolerant children sensitized to peanut major allergens. The Journal of allergy and clinical immunology. 2015;135(5):1249-56.
53. Burks AW, Wood RA, Jones SM, Sicherer SH, Fleischer DM, Scurlock AM, et al. Sublingual immunotherapy for peanut allergy: Long-term follow-up of a randomized multicenter trial. The Journal of allergy and clinical immunology. 2015;135(5):1240-8 e1-3.
54. Garrido-Fernandez S, Garcia BE, Sanz ML, Echechipia S, Lizaso MT, Tabar AI. Are basophil activation and sulphidoleukotriene determination useful tests for monitoring patients with peach allergy receiving sublingual immunotherapy with a Pru p 3-enriched peach extract? Journal of investigational allergology & clinical immunology. 2014;24(2):106-13.

55. Vila L, Moreno A, Gamboa PM, Martinez-Aranguren R, Sanz ML. Decrease in antigen-specific CD63 basophil expression is associated with the development of tolerance to egg by SOTI in children. Pediatric allergy and immunology : official publication of the European Society of Pediatric Allergy and Immunology. 2013;24(5):463-8.
56. Kopac P, Rudin M, Gentinetta T, Gerber R, Pichler C, Hausmann O, et al. Continuous apple consumption induces oral tolerance in birch-pollen-associated apple allergy. Allergy. 2012;67(2):280-5.
57. Brough HA, Santos AF, Makinson K, Penagos M, Stephens AC, Douiri A, et al. Peanut protein in household dust is related to household peanut consumption and is biologically active. The Journal of allergy and clinical immunology. 2013;132(3):630-8.
58. Dolle S, Lehmann K, Schwarz D, Weckwert W, Scheler C, George E, et al. Allergenic activity of different tomato cultivars in tomato allergic subjects. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 2011;41(11):1643-52.
59. Chinuki Y, Kaneko S, Dekio I, Takahashi H, Tokuda R, Nagao M, et al. CD203c expression-based basophil activation test for diagnosis of wheat-dependent exercise-induced anaphylaxis. The Journal of allergy and clinical immunology. 2012;129(5):1404-6.