

State of the Art and Perspectives in Food Allergy (Part I): Diagnosis

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Abstract: IgE-mediated food allergy is a major 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. This review focuses on novelties in the diagnosis of food allergy.

Correct diagnosis 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 are needed to confirm/exclude diagnosis. To a certain extent, novel *in vitro* diagnostics in the form of allergen component-based sIgE assays and flow-assisted quantification of *in vitro* activated basophils might help to discriminate between genuine allergy and merely sensitization. Furthermore they make it possible to establish individual risk profiles, to predict persistence of allergy, and facilitate therapeutic approach.

Keywords: Food allergy, basophil activation test, component resolved diagnosis, food allergy diagnosis.

IgE-MEDIATED FOOD ALLERGY: FROM BABYLONIAN SPEECH CONFUSION TO CORRECT DESCRIPTION

To harmonize the nomenclature about adverse food reactions, the European Academy of Allergy and Clinical Immunology has drawn up a mechanistic classification of these conditions [1]. Adverse food reactions are defined as any aberrant reaction due to consumption of a food or a natural or synthetic additive, and can be of non-toxic and toxic origin. Toxic reactions can occur in anyone, providing that the ingested amount is sufficient. In contrast, the occurrence of non-toxic reactions is dependent on the individual susceptibility and may result from immune (allergy) and non-immune mechanisms (intolerance), the latter mostly being idiopathic. Food allergy is subdivided into IgE-mediated, non-IgE-mediated but cellular, and finally mixed IgE-mediated and cellular disorders [2, 3]. Today about 65% of IgE-mediated food allergies are related to one of the 4 plant - or 3 animal food protein superfamilies [4]. Figure 1 shows an overview of the plant - and animal-food protein superfamilies an overview of protein superfamilies in food of plant or animal origin.

Exposure to food allergen predominantly occurs via the gut where various non-immunologic and immunologic mechanisms prevent food allergen to enter the body and/or to trigger the immune system. However, some food allergens “escape” from these surveillance mechanisms and encounter specialized dendritic cell populations in the gut, that leads to naive T-cell priming and polarization (generally to Th2). In IgE-mediated allergy, this process triggers immunoglobulin isotype switching and finally the synthesis of allergen-specific IgE by B cells. Tissue-resident effector cells, predominantly mast cells, are loaded with IgE. Upon re-exposure to the allergen, that cross-links adjacent specific IgE molecules bounded to the high affinity IgE Fe-receptor, these effector cells release various mediators responsible for the allergic reaction. Alternatively, IgE-mediated food allergy, particularly to plant food, can also be acquired indirectly via cross-reactivity towards structural homologous epitopes present in non related sources such as pollen and latex (Fig. 2). In these cases sensitization is not acquired

via the gastrointestinal system but via inhalation or cutaneous contact. Noticeably, the detection of food-specific IgE (sensitization) does not necessarily indicate genuine clinical allergy (see below).

IN VITRO DIAGNOSIS OF IgE-MEDIATED ALLERGY

Historical Perspective

Almost half a century ago, IgE was discovered as the “reagine” responsible for the so-called type I hypersensitivity reaction [5, 6], and about 5 years later, the first *in vitro* sIgE assay, named radio allergosorbent test (RAST) was born and commercialized. In the original RAST, native allergen extracts were covalently bound to a cyanogen-bromide activated paper disc, and sIgE that complexes with the coupled allergen, was quantified with radio(¹²⁵I)-labeled polyclonal antihuman IgE [7]. At present, traditional RAST is almost completely abandoned and substituted by automated and technically optimized enzyme immunoassays that generally display excellent analytic sensitivity, precision, reproducibility and linearity [8, 9]. Unfortunately, none of the currently available sIgE assays demonstrates an absolute predictive value. By consequence, a positive sIgE result should be interpreted with prudence as it does *de facto* not indicate the presence of overt allergic disease but can merely reflect (cross-)sensitization. For example, ubiquitous structures such as profilins and cross-reactive carbohydrate determinants (CCD) present on glycoproteins of plants and hymenoptera venom have repeatedly been shown to elicit clinically irrelevant positive sIgE results with an immense spectrum of cross-reactivity involving pollen, *Hevea* latex, plant food, and venoms [10, 11]. *Vice versa*, a negative sIgE result, by no means excludes allergy. Alternatively, it is imperative to realize that results and experience obtained with different sIgE assays are not readily interchangeable [8]. For example, data on levels of sIgE predictive for the outcome of food challenges [12, 13] or the presence of more severe disease [14] should be considered as strictly specific for the assay that was investigated. Thus, a lot of patients might still need double-blind placebo controlled food challenges (DBPCFC) for correct diagnosis. However, routine application of DBPCFC is hampered by several practical and mostly ethical limitations [15, 16] and might even fail to correctly predict the outcome [17].

Although traditional sIgE remain necessary for the diagnostic work-up, they do not provide information about the risk profile of the patient and cannot discriminate between cross-reactivity and genuine sensitization. Therefore it is anticipated that *in vitro* allergy

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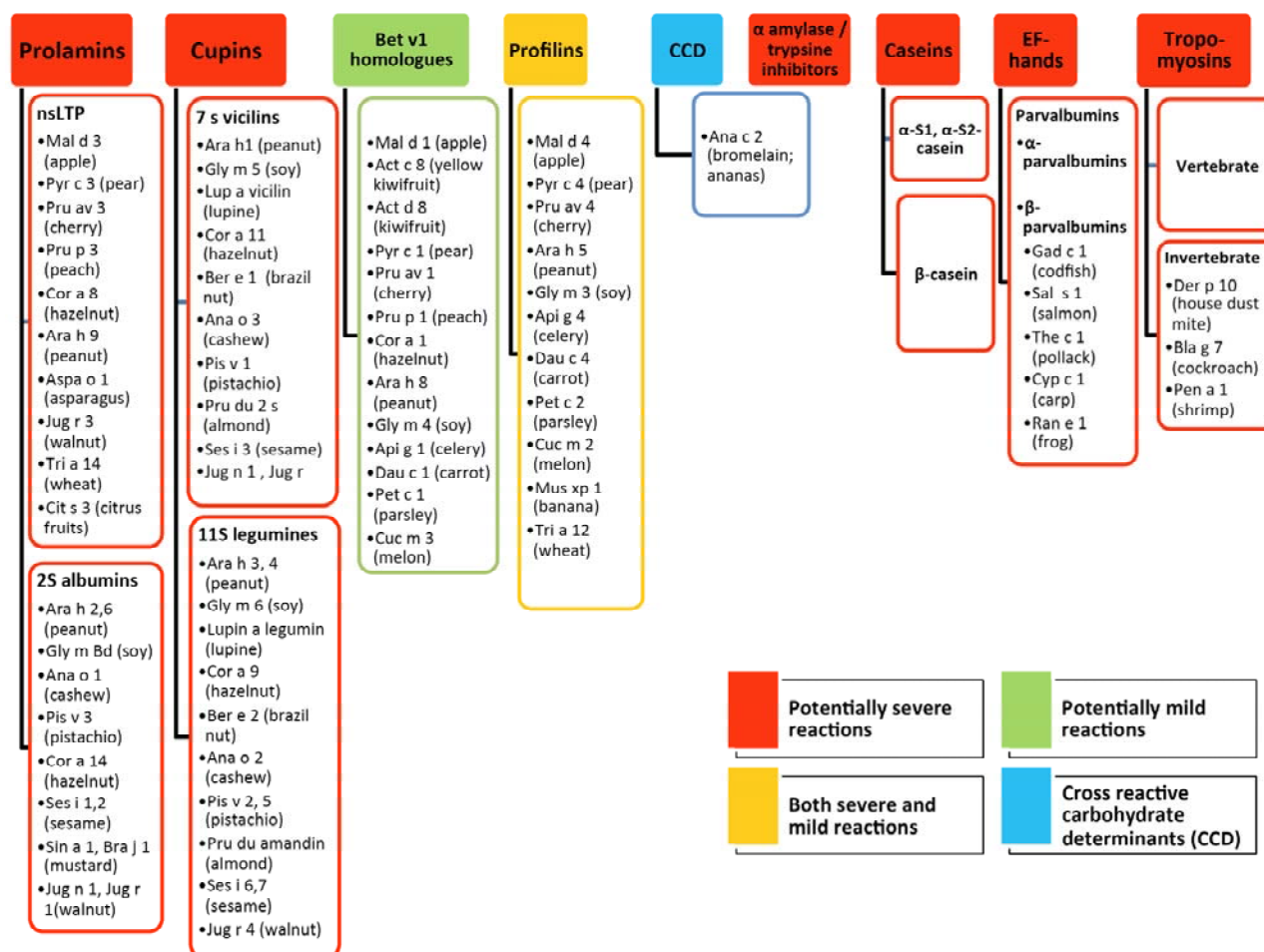


Fig. (1). Superfamilies and most representative allergen components of plant- and animal- derived food allergens (not exhaustive)

Allergen components are indexed according to the IUIS Allergen Nomenclature (www.allergen.org). The first three letters and the one after the space define the genus and the species respectively, whereas the cypher the number of allergens (generally in order of description). In brackets are specified the common name. For example Ara h 1 denotes *Arachis hypogaea*, the allergen number 1 of the peanut.

The different colours indicate the potential severity of clinical reaction associated with sensitization with a particular component.

diagnosis could benefit from the introduction of standardized and well-characterized allergen components as well as functional assays such as flow-assisted basophil activation that enables assessment of the ability of an extract to trigger the effector cells. In this review we will further focus on the state of the art and new horizons of these techniques.

Component Resolved (Allergy) Diagnosis (CRD)

When in the late 1980s the major allergen Der p 1 (*Dermatophagoides pteronyssinus*) from house dust mite was cloned [18], the birth of another instrument in the diagnosis of IgE-mediated allergy, i.e. component-resolved diagnosis (CRD) was witnessed. In contrast to traditional sIgE assays, CRD assays do not rely upon whole extract preparations from native allergens but on quantification of sIgE antibodies to single protein or polypeptide components, purified from natural sources or obtained by recombinant techniques. There are various databases on allergen components and protein families, such as the official "International Union of Immunological Societies (IUIS)" allergen nomenclature database that is available from <http://www.allergen.org> and the "Allfam" allergen database grouping allergens into protein (sub) families (<http://www.meduniwien.ac.at/allergens/allfam>) [19].

The principles, applications and pitfalls of CRD that can be performed by traditional single-plexed assays as well as by microar-

ray technology are extensively reviewed elsewhere [20-26]. Briefly, CRD involves specific (unique) marker components and/or substructures (epitopes) to study the genuine sensitization of patients towards a particular allergen and the presence of sIgE antibodies to cross-reactive components or epitopes, such as ubiquitous panallergens or cross-reactive carbohydrate determinants (CCD) that point to cross-sensitization to several even unrelated allergen sources. These so-called "gatekeeper" tests create an opportunity to establish individual primary and cross-reactive sensitization profiles, in other words which parts of allergen(s) are recognized by an individual patient, as demonstrated for inhalant allergy [27-31], food [32-41], *Hevea latex* [11, 42], and venom allergy [43-46].

Furthermore CRD can help discriminating between sensitization and genuine allergy [11, 32, 36, 42, 47, 48]. For example, by using sIgE to the 2S albumin Ara h 2 from peanut (*Arachis hypogaea*), with a decision threshold of 0.35 kUA/L, almost 98% of the population was correctly classified, with a correct diagnosis of peanut allergy in all patients. The misclassification rate using a traditional peanut sIgE cut-off of 15 kUA/L was about 18% [47, 49].

In addition, CRD allows an individual risk assessment of severity [38, 39, 47, 50-52]. For example, IgE analysis to peanut allergen components has repeatedly been demonstrated to distinguish between peanut-sensitized individuals at risk of severe symptoms with (co-)sensitization to Ara h 1, 2, 3 or 9 and those likely to present

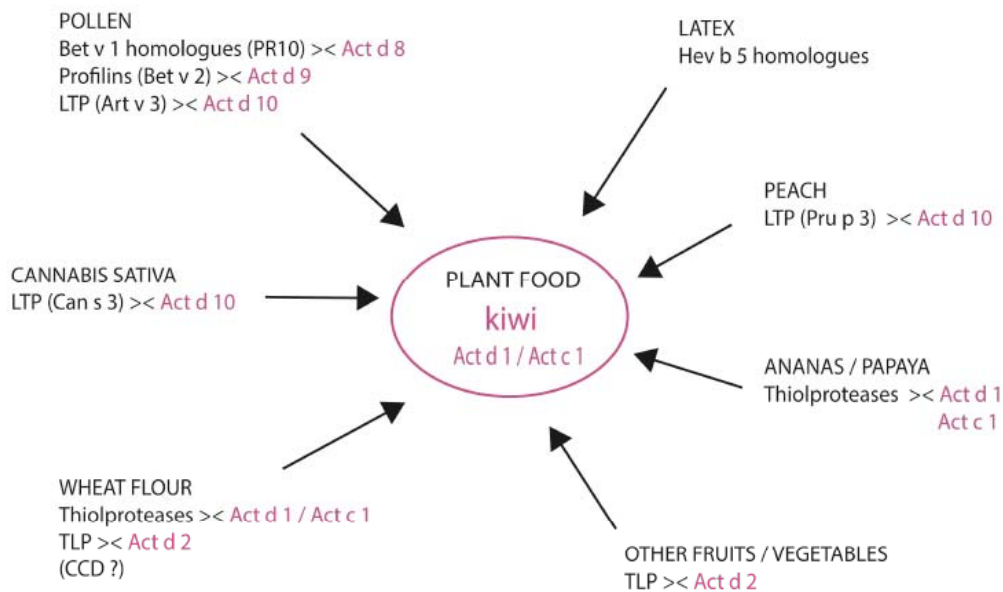


Fig. (2). Different allergen sensitization patterns for plant food allergy: the kiwifruit (*Actinidia deliciosa* and *Actinidia chinensis*) model

Kiwi fruit sensitization and allergy can result from various routes that demonstrate geographic distributions. In western/central and eastern Europe kiwi fruit allergy is dominated by a sensitization to Act d 8, the homologue of the major allergen Bet v 1 from birch (*Betula verrucosa*) that belongs to the PR10 group. In southern Europe sensitization to the non-specific lipid transfer protein (LTP) Act d 10 and might relate to a sensitization to the ns-LTP from peach (*Prunus persica*). Likely alternative sensitization routes for ns-LTP are Art v 3 from *Artemisia vulgaris* (mugwort) and Can s 3 from *Cannabis sativa* (weed/marijuana). Furthermore kiwi allergy can result from sensitization to other plant food allergens such as profilins (Act d 9), the acidic protein Hev b 5 from *Hevea brasiliensis* (natural rubber latex), thiolproteases (Act d 1, Act c 1), thaumatin-like proteins (Act d 2).

milder or even no symptoms to peanut if sensitized to pollen allergens and their peanut homologue allergens such as Ara h 5 and 8 [53-57].

In our region we observed children predominantly exhibiting sensitization to hazelnut (*Corylus avellana*) storage proteins Cor a 9 and Cor a 11. These allergens are unrelated to birch pollen allergy and are predominantly associated with more severe symptoms on consumption of raw or processed nuts. In contrast, adults generally present with a mild oral allergy syndrome due to an extensive cross-reactivity between the themolabile Cor 1.04 and Bet v 1. Alternatively, adults from a Mediterranean non-birch endemic region are rarely sensitized to Cor a 1.04 but predominantly react against the lipid transfer protein Cor a 8 and frequently display severe symptoms (for review: [58]). Geographic and/or age-related sensitization profiles with distinct clinical characteristics have also been disclosed for apple [59], peanut [60], cherry [61], peach [62] and carrot [41] and most recently kiwifruit [63]. As also reviewed elsewhere [64], in most North European regions, plant food allergies in adults are generally characterized by an oral allergy syndrome (OAS) due to sensitization to the labile homologs of Bet v 1. In contrast, children below the age of 6 years frequently demonstrate more severe and generalized reactions that are often not related to pollen allergy but are frequently due to sensitization to different seed-storage proteins (e.g., vicillins and legumines from the cupin superfamily) and 2S albumins belonging to the superfamily of prolamins. In contrast, in the Mediterranean non-birch endemic region plant food allergy originates frequently from sensitization to non-specific lipid transfer proteins (ns-LTPs). LTPs are members of the prolamin superfamily and have been identified in various fruits, vegetables, nuts, cereals, pollen and latex [for review: [64] [65] but also in *Cannabis sativa* [66], [Ebo DG under revision] (Fig. 1). Figure 2 shows the currently established or suspected sensitization profiles and routes for kiwifruit as a model (adapted from [67, 68]).

Component resolved diagnosis can also provide important information about the tolerability of baked food. For example higher titers of sIgE to ovomucoid (Gal d 1) were observed in egg-allergic children reacting to both raw and heated egg compared to those only reacting to heated egg [69]. Similar findings were recently observed by using a microarray technique [70]. Caudet *et al.* showed that baked egg-reactive children had higher ovalbumin and ovomucoid ratios of IgE/IgG₄ (and mediator release in the rat basophil leukaemia-based assay) than did tolerant children [71]. In another study, they found that casein-specific IgE demonstrated superior accuracy to predict reactivity to baked milk compared with cow's milk-specific IgE [72]. Likewise high titers of casein-sIgE are associated with milk allergy in children and might be associated with prolonged allergy [73-76].

Determination of allergen sensitization profiles might also be useful in diagnosing particular food allergies such as exercise-induced wheat allergy froms ω -5-gliadin (Tri a 19 from *Triticum aestivum*) and $\alpha/\beta/\gamma$ gliadin sensitization [77-79] and plant-food anaphylaxis from lipid transfer proteins (Tri a 14 and Pru p 3 from *Prunus persica*) related to intake of non-steroidal drugs and exercise [80].

Epitope Mapping

To be immunogenic, food allergens must (at least partially) resist digestion and absorption from the gastrointestinal tract. This has led to the assumption that individuals producing IgE antibodies recognizing a diverse number or specific profiles of sequential (or linear non-conformational) epitopes are more likely to present overt clinical allergy rather than asymptomatic sensitization [for review: [25, 81]. Today, exploration of the IgE response beyond the level of allergen components has focused on peptide epitopes from allergens from milk [82-87] egg [88, 89], peanut [90, 91], wheat [92], shrimp [93] and a glycan epitope from mammalian meat. From

Table 1. Basophil Activation Test (BAT) in food allergy: diagnosis (native allergens and components), evaluation of cross-reactivity, follow-up and risk-stratification, follow-up during immunotherapy. detection of hidden allergens and effect of food processing.

BAT with native food: diagnosis						
<i>Stimulus</i>	<i>Reference test</i>	<i>Sensitivity</i>	<i>Specificity</i>	<i>N</i>	<i>Marker</i>	<i>Reference</i>
Carrot, celery, hazelnut	H	85-90	80-90	20	CD63	[123]
Apple	H	100 ¹	100	59	CD63	[124]
Apple	H	88	75 ²	59	CD63	[124]
Macrobrachium rosenbergii	H + ST + sIgE	100	100	45	CD63	[125]
Peanut	H + ST + sIgE	81,3 89,5	95,4 97,2	75	CD63 CD203c	[126]
Egg	H + ST + sIgE	77,4 57,4	100 96,4	67	CD63 CD203c	[126]
Anisakis simplex	H	97	100	88	CD63	[127]
Wheat	H + sIgE + FCT	85	77	58	CD203c	[128]
BAT with food components: diagnosis						
Apple (Mal d 1)	H	75	68	54	CD63	[129]
Celery (Api g 1)	H	75	77	54	CD63	[129]
Carrot (Dau c 1)	H	65	100	54	CD63	[129]
BAT for examination of cross reactivity						
<i>Description</i>	<i>Outcome</i>			<i>N</i>	<i>Marker</i>	<i>Reference</i>
Peanut and tree nut allergens	BAT showed that cross-reactivity of peanut-specific IgE antibodies with tree nuts causes effector cell activation.			2	CD63	[110]
Sesame (Ses i 6) and walnut	BAT might help to distinguish between clinically relevant and irrelevant <i>in vitro</i> IgE cross-reactivity of seed storage proteins in nuts and seeds. However data not validated by challenge.			-	CD63 CD203c	[111]
BAT for evaluation of allergy outgrowing and risk–stratification						
Risk–stratification of children with milk allergy	BAT can help to determine children with a favorable diagnosis by discriminating heatdenatured milk tolerating and non-tolerating individuals.			55	CD63 CD203c	[116]
Reintroduction of cow’s milk	The BAT could be a valuable tool in the management of children with cow’s milk allergy , by contributing in determining whether an oral challenge can safely be undertaken			112	CD63	[117]
Outgrow of hen’s egg and/ or cow’s milk allergy	BAT is useful to determine if children will outgrow hen’s egg and/ or cow’s milk allergy as well as in decision making regarding whether or not to perform oral food challenges			71	CD203c	[118]
Clinical tolerance of cow’s milk among children with allergy towards cow’s milk.	BAT confirms the finding that the majority of patients with milk allergy are able to tolerate some forms of baked milk in their diets			132	CD63 CD203c	[119]

(Table 1) Contd....

BAT for follow up during immunotherapy				
The safety and efficacy of immunotherapy for milk allergy	BAT did show a significant decrease in expression of CD63 and CD203c with therapy, potentially suggesting a state of reduced extrinsic basophil activation <i>in vivo</i> .	30	CD63 CD203c	[130]
Sublingual immunotherapy for peanut allergy	Basophil reactivity was diminished after 12 months of peanut sublingual immunotherapy, as indicated by the significantly lower percent of CD63+ activated basophils after stimulation with peanut extract.	18	CD63	[131]
The safety, efficacy, and immunologic effects of peanut sublingual immunotherapy.	BAT showed the percentage CD63+ values to be significantly lower for peanut sublingual immunotherapy subjects compared with placebo subjects, indicating an effect on basophil activation.	40	CD63	[132]
Peanut oral immunotherapy	Basophil activation was significantly reduced within 4 months. In an observational study of peanut allergy, no reduced basophil activity was seen.	39	CD63	[133]
Basophil and peanut oral immunotherapy	BAT shows evidence supporting the hypothesis that peanut oral immunotherapy induces pathway-specific basophil anergy. However data are not clinical controlled.	-	CD 63 CD203c	[134]
BAT for detection of hidden allergens				
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	[120]
BAT for measuring variety and potential allergenicity of processed food				
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	[135]
Allergenicity of thermally processed or Maillard reacted hazelnuts	BAT can make an assessment of the activatory potential of hazelnut extracts after modification of constituent proteins/allergens through thermal processing and the Maillard reaction	11	CD63	[121]
Investigation of the allergic potential of two different tomato cultivars	BAT underpinned the clinical differences evoked by the different tomato cultivars	25	CD203c	[136]
Diagnosing the subtypes of WDEIA (Hydrolyzed wheat protein -WDEIA and conventional -WDEIA)	Activation of basophils induced by various preparations of wheat proteins is highly useful in predicting causative allergens in patients with WDEIA.	10	CD203c	[137]

¹Taken 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.

most of these studies it appears that analysis of epitope binding patterns (also called epitope mapping) might further improve diagnosis of food allergy and enable better estimation of severity, phenotypic expression and prediction of natural history (persistence versus outgrow). For example, Chatchatee *et al* [94] identified 6 major and 3 minor IgE-binding regions on alpha(s1)-casein. Two IgE-binding regions (AA 69-78 and AA 173-194) were recognized by the majority of patients over 9 years of age with persistent cow's milk allergy (67% and 100%, respectively) but by none of the children less than 3 years of age who are likely to outgrow cow's milk allergy. Flinterman *et al* [90] demonstrated a positive correlation between IgE epitope diversity and clinical sensitivity, such that patients demonstrating the greatest epitope diversity were more sensitive than those with a lower diversity. However, no specific epitopes were associated with severe reactions to peanut. Furthermore linear epitope mapping can also be applied to study and pre-

dict the absence or presence of cross-reactivity [95-97] For example, Rosenfeld *et al* [97] showed that although they share a quite high degree of homology with the corresponding regions of walnut allergens, the sequence stretches previously identified as sequential IgE binding epitopes of Ara h 1, Ara h 2 and Ara h 3 from peanut (*Arachis hypogaea*) have no IgE binding equivalents in walnut allergens. Recently, epitope analysis also revealed to be useful to identify allergy to deaminated gluten in wheat tolerant individuals [98].

It has been recognized that the constituent proteins of plant (e.g. pollen, vegetables, fruit) and invertebrate allergens (e.g., hymenoptera venom) frequently bear immunogenic $\alpha(1,3)$ -fucose and/or $\beta(1,2)$ xylose residues that can elicit IgE responses [10, 99]. As these glycan residues are ubiquitously present in non-mammalian glycoproteins they are generally referred as cross-reactive carbohydrate determinants (CCD). The role of CCDs as a cause of allergic

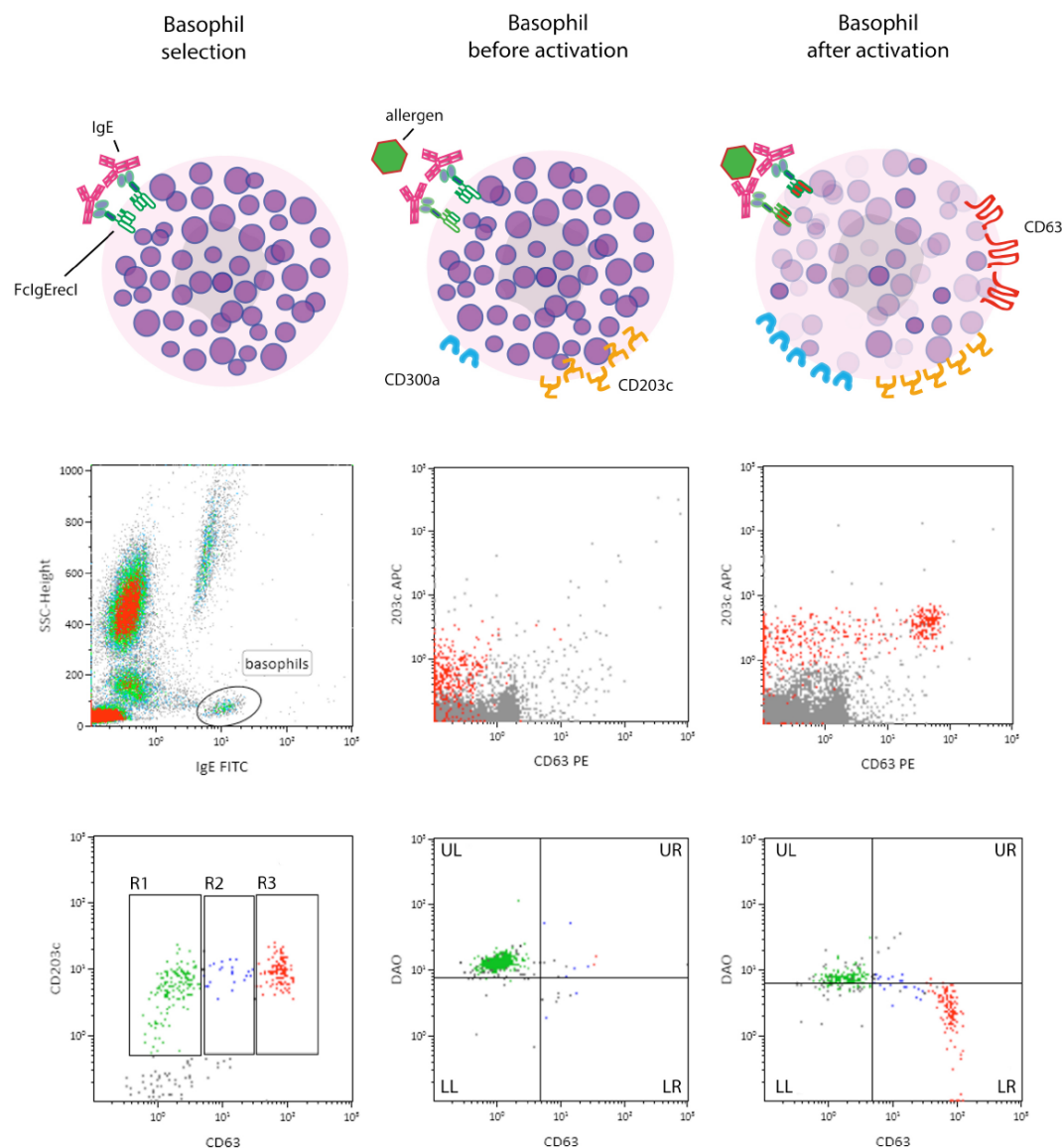


Fig. (3). Principle of flow assisted analysis of basophils: immunophenotyping and histamine release at a single cell level

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

Mid: histamine release after 20 min by hazelnut-activated basophils from a hazelnut allergic patient. Basophils are characterized using side scatter (SSC)/anti-IgE (ellipse gate in panel A). Panel B shows the non-stimulated basophils (red) expressing almost no CD63 or CD203c. Panel C shows the allergen-activated basophils with appearance of CD63 and up-regulation of CD203c.

Bottom: The co-expression analysis of CD63 and CD203c up-regulation in activated basophils reveals 3 different subpopulation: CD203c^{bright}CD63^{dim} (R1, green), CD203c^{bright}CD63^{dim} (R2, blue) and CD203c^{bright}CD63^{bright} (R3, red) (panel D). Panel E and F show the histamine intracellular staining by meaning of DAO coupled fluorochrome. 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.

symptoms is still controversial. Although sensitization to CCD is prevalent in our regions and affects about 25% of patients with grass pollen allergy [10], the general view is that these anti-glycan IgE antibodies have minimal, if any, clinical relevance. The exact reason(s) for the poor biological function of these anti-CCD antibodies remain(s) elusive. Although, recent studies disclosed that sIgE antibodies to galactose- α (1,3)-galactose (α -gal) epitopes present on asparagine at position 88, in the murine heavy chain Fab portion of the chimeric mouse-human IgG1 mAb cetuximab, can trigger life-threatening and even fatal reactions[100]. Because α -gal is present on proteins of most non-primates, Chung *et al.* [100] also performed IgE quantification for cat, dog, beef and pork proteins

and identified several patients who reported having had episodes of delayed anaphylaxis or serious angioedema 1 to 3 hours after eating beef or pork. Today, sensitization to the α -gal epitope of mammalian meat has been described by several authors (for review: [101]). For a review on epitope mapping in allergy the reader is referred elsewhere [102].

Basophil Activation Tests

Basophils represent the most important effector cells for IgE-mediated allergy in the circulation. Functional *in vitro* basophil activation assays have generally focused on histamine and/or sulphidoleukotriene release (review: [103, 104]). However, the time-

and cost-consuming two-step methods with cell incubation and quantification of mediators, has seriously hampered their application. From both reviews it appears that with respect to food allergy only a limited number of papers on release of histamine and/or sulphidoleukotriene 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 tetraspanin CD63 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 [105]. Briefly, as shown in (Fig. 3), upon activation with specific allergen, basophils up-regulate the expression of particular activation (CD203c) and degranulation (CD63) markers that can be analyzed flow cytometrically using specific monoclonal antibodies. During recent years, the technique proved to be an easily accessible, rapid and reliable diagnostic method allowing simultaneous testing of several putative allergens such as aeroallergens, latex, hymenoptera venom and particularly drugs (for review: [106, 107]. In food allergy the BAT was first adopted in the late 1990s by Moneret-Vautrin *et al.* [108], who demonstrated the technique is as sensitive and specific as the sulphidoleukotriene release assay and clearly more efficient than the traditional histamine release test.

Table 1 summarizes the application of BAT in the food allergy including studies regarding diagnosis with native foods and allergen components, cross-reactivity, allergy outgrowing and risk stratification of patients, follow-up during immunotherapy, allergen detection (traces), and effect of food processing.

Generally regarding diagnosis, the technique seems 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%. In some studies it was also demonstrated that (to some extent) the BAT can discriminate between sensitisation and genuine allergy [109-111]. Furthermore, in individual patients the BAT was useful to diagnose allergy to coriander, mandarin, pumpkin and the natural dye annatto [112-115].

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 [116-119] or eggs [118], or to evaluate the effect of Food Allergy Herbal Formula-2 (FAFH-2) (see Part 2).

Finally, other studies suggest that the BAT can be applied to study the allergenicity of food (by detecting traces of allergens) [120] and allows to study the effects of processing (*e.g.*, heating, Maillard reactions [121]).

In conclusion, the BAT creates new opportunities in the diagnostic management of IgE-mediated food allergy. As the technique closely mirrors the *in vivo* pathway leading to symptoms, it is anticipated that the technique might mostly be applied for objectives such as differentiation between clinically relevant and irrelevant IgE serology, helping to assess the clinical outcome of particular sensitization patterns (by performing BAT with specific components), to predict spontaneous outgrow and to perform a functional risk analyses of trace amounts of (hidden) food allergens.

Finally we recently described a novel flowcytometric technique, namely Histaflow [122], useful to determine the intracellular histamine content and release. Traditionally, histamine release is quantified using fluorimetric and colorimetric techniques that measure its concentration in the supernatant of activated cells. The Histaflow technique is based on an enzyme-affinity method using the histaminase diamine oxidase (DAO) conjugated to a laser-excitable fluorochrome and allows to combine immunophenotyping and quantification of mediators at a single-cell level. Normally this data are not captured by the traditional techniques, since their results represent an average of isolated cells analyzed.

The principles of BAT and Histaflow are summarized in (Fig. 3).

GENERAL CONCLUSIONS

From this review it is clear that the cornerstone for an optimal management for food allergy is a correct diagnosis. The component resolved allergy diagnosis, and flow-assisted analysis of *in vitro* activated basophils constitute reliable instruments that can add to correct diagnosis in our allergic patients.

In particular they seem helpful in predicting the severity of reaction, the probability of spontaneous outgrow and the tolerance for extensively heated egg and milk.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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Declared none.

ABBREVIATIONS

α -gal	=	Galactose- α (1,3)-galactose
Ara h	=	Arachis hypogaeae
BAT	=	Basophil Activation Test
Bet v	=	Betula verrucosa
CCD	=	Cross-reactive Carbohydrate Ceterminants
Cor a	=	Corylus Avellana
CRD	=	Component Resolved Diagnosis
DAO	=	Diamine oxidase
DBPCFC	=	Double Blind Placebo Controlled Food Challenge
Der p	=	Dermatophagoides pteronyssinus
FAFH-2	=	Food Allergy Herbal Formula-2
Gal d	=	Ovomucoid
IUIS	=	International Union of Immunological Societies
Ns- LTP	=	Non specific- Lipid Transfer Protein
Pru p	=	Prunus persica
RAST	=	Radio Allergosorbent Test
WDEIA	=	Wheat-Dependent Exercise Induced Anaphylaxis
Tri a	=	Triticum aestivum

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