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Review

IgE-Mediated food allergy diagnosis: Current status and new perspectives

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In June 2005, the work of the EU Integrated Project EuroPrevall was started. EuroPrevall is the largest research project on food allergy ever performed in Europe. Major aims of the project are to generate for the first time reliable data on the prevalence of food allergies across Europe and on the natural course of food allergy development in infants. Improvement of *in vitro* diagnosis of food allergies is another important aim of the project. The present review summarizes current knowledge about the clinical presentation of food allergy and critically reviews available diagnostic tools at the beginning of the project period. A major problem in diagnosis is a relatively poor ‚clinical specificity', *i.e.* both positive skin tests and *in vitro* tests for specific IgE are frequent in sensitized subjects without food allergy symptoms. So far, no *in vitro* test reliably predicts clinical food allergy. EuroPrevall aims at improving the predictive value of such tests by proceeding from diagnosis based on allergen extracts to purified allergen molecules, taking into account the affinity of the IgE–allergen interaction, and evaluating the potential of biological *in vitro* tests such as histamine release tests or basophil activation tests including assays performed with permanently growing cell lines.

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1 Introduction

1.1 Classification and nomenclature

Some 10 years ago the European Academy of Allergy and Clinical Immunology (EAACI) published a position paper [1] that classified adverse reactions to food as:

1) Toxic (adverse reactions that occur in any exposed individual provided that the dose is high enough)
2) Nontoxic (adverse reactions that depend on individual susceptibility to a certain food)
   a) Immune-mediated (food allergy)
      (i) IgE-mediated
      (ii) Non-IgE-mediated
   b) non-immune-mediated (food intolerance)
      (i) enzymatic (*e.g.* lactase deficiency)
      (ii) pharmacological (abnormal reactivity to substances such as vasoactive amines normally present in some foods)
      (iii) undefined (*e.g.* food additives intolerance).

On the basis of the subsequent EAACI position paper on nomenclature for allergy, nonimmune-mediated reactions' should be now called ‚nonallergic food hypersensitivity' [2]. Non-IgE-mediated food hypersensitivity will not be discussed in this paper. Since some differences between nomenclature and classifications of adverse food reactions used in Europe [1] (Fig. 1a) and in the USA [3] (Fig. 1b) still exist, in order to get a common nomenclature for

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Abbreviations: AD, atopic dermatitis; **APT,** atopy patch test; **CCD,** crossreactive carbohydrate determinants; **DBPCFC,** double-blind placebo-controlled food challenge; **EAACI,** European Academy of Allergy and Clinical Immunology; **OAS,** oral allergy syndrome; **OFC,** open food challenges; **SPT,** skin prick test

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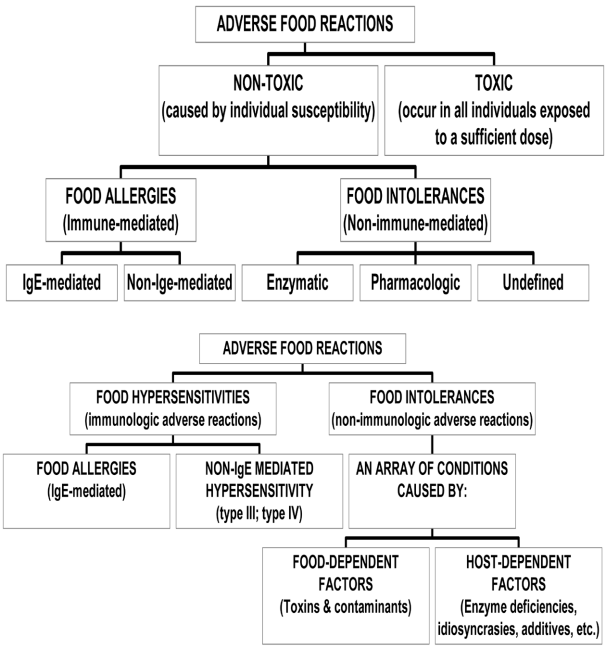

Figure 1. (a) Classification and terminology for food allergy most frequently used in Europe. (b) Classification and terminology for food allergy most frequently used in the USA.

allergy a specific committee of the World Allergy Organization (WAO) updated the EAACI 2001 position statement in 2003 [4]. This document states that the term ‚food allergy' is appropriate when immunological mechanisms have been demonstrated and that the term 'IgE-mediated food allergy' should be used if IgE is involved in the reaction. All other reactions should be referred as 'nonallergic food hypersensitivity' [4].

1.2 General remarks on food allergy

Most adverse food reactions are caused by intolerance [3], but it is food allergy that has become a major health concern in developed countries during the last few decades. In recent surveys, between 5 and 25% of adults believed that they or one of their relatives had a food allergy [5–7]. Although the prevalence of food allergies is certainly overestimated in the public perception, 6–8% of children experience food-allergic disorders during the first 3 years of life [8], in most cases from cows' milk and hen's egg. Fortunately, up to 85% of them outgrow their allergy in the first 5–10 years of life. In adults, food allergies are probably less prevalent than in young children; nonetheless, it is estimated that nearly 4% of the adult population in the USA have some kind of food allergy [9]. European reports suggest that 0.3–7.5% of children and about 2–4% of adults have food allergy [1, 10].

Food allergies cause a number of clinical conditions involving the gastrointestinal tract, the skin, the airways or the most dangerous of all allergic reactions, anaphylaxis. The clinical presentation of a food-induced allergic reaction depends on a number of variables including the physical/chemical characteristics of the allergen responsible for sensitization, dose (amount ingested), whether the food was ingested alone or in combination with other foods that may delay its absorption, the association with alcoholic beverages, aspirin or exercise and importantly, ill-defined 'host' factors. On the basis of the clinical presentation, physical/chemical features of allergens responsible for the allergic reaction, and underlying immunologic mechanisms, two forms of food allergy can be distinguished. In Class 1, food allergy sensitization occurs through the intestinal tract and is often caused by stable proteins. In contrast, Class 2 food allergy develops as a consequence of sensitization to airborne allergens [11]. The diagnostic approach always starts with a thorough medical and dietary history and physical examination, supported by a series of both *in vivo* and *in vitro* tests. Detecting the offending food is certainly essential, but in some instances the identification of the responsible protein(s) is even more important as distinct foods containing homologous, crossreacting allergens might pose a risk of further allergic reactions. In the future, the clinical approach might include the identification of the protein(s) responsible for the allergic reaction as a part of the risk assessment. Furthermore, one of the main objectives of the clinical allergist caring for a patient with food allergy should be to preserve the quality of life avoiding unnecessary dietary restrictions.

1.3 Sensitization *versus* allergy

A major obstacle in the field of food allergy diagnosis is that, probably with the exception of challenge tests with the offending food, no single *in vivo* or *in vitro* test is able to provide a reliable prediction of the clinical reactivity of a patient. To understand the basis of this shortcoming, it is necessary to distinguish between sensitization and (clinical) allergy. Sensitization is defined as the presence of a specific IgE response occurring upon exposure of the immune system to an allergen. *In vitro* assays detect such specific IgE in serum and *in vivo* tests like skin prick test (SPT) use mast-cell reactivity as a read-out for specific IgE. Although SPT additionally provides information on the potency of specific IgE antibodies to trigger a biological effect, *i.e.* the induction of mediator release from mast cells, both *in vitro* tests and SPT do not unequivocally predict clinical food allergy; they only assess sensitization. Neither the presence of specific IgE in serum nor the presence of biologically active IgE on mast cells in the skin will reliably predict clinical allergy upon exposure to food. Clinical allergy is defined as the development of symptoms upon consumption of food, and this cannot directly be predicted on the basis of sensitization. There is sensitization with and without clinical allergy. The chal-

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lenge is to develop novel diagnostics that better predict clinical reactivity.

1.4 EuroPrevall

The EuroPrevall consortium, a group of experts that was formed within the Sixth Framework Program of the EU, includes clinicians, scientists, epidemiologists, representatives of patients' associations, and representatives of the diagnostic industry and the food industry, and aims at investigating the prevalence and distribution of food allergies throughout Europe, determining the threshold doses for different allergenic foods, investigating the role of the environment in determining the different patterns of food allergy, measuring the socio-economic impact of food allergy and developing new diagnostic tools providing a better correlation of *in vitro* diagnostic results with the clinical situation. One important aspect in this context is the creation of a library of purified and well-characterized food allergens which will be used in a variety of diagnostic tests. This report summarizes current knowledge about the clinical presentation of food allergy and critically reviews available diagnostic tools at the beginning of the 4-year EuroPrevall project.

2 Clinical presentation of food allergy

Foods may induce a variety of symptoms in allergic persons and, notably, some symptoms may equally have an allergic or a nonallergic cause. There may be a clear temporal relationship between the intake of the food and the development of the allergic symptoms, but symptoms may also develop after hours making the relation with the ingestion of food less clear.

2.1 Oral allergy syndrome (OAS)

The most frequent symptom of food allergy, particularly in adults, is the so-called OAS, a particular type of IgE-mediated contact urticaria involving lips, oral mucosa, and pharynx [12, 13]. Symptoms develop within minutes and typically include local itching of lips, tongue, palate, throat, and/or ears and nose and/or swelling (angioedema) of the same areas. In most cases, the clinical course is mild with symptoms limited to the oropharynx and resolving within 1 h, but in some cases, the clinical course is more dramatic with potentially fatal pharyngeal swelling or progression towards a generalized anaphylactic reaction [9]. Although oral itching can be elicited by any food allergen, the classical OAS is associated with sensitization to heat-labile/pepsin-labile plant-derived proteins in patients with pollen-related food allergy. In this case, crossreactivity between homologous plant-derived proteins in pollen and vegetable foods is the basis of OAS. About 75% of birch pollen-allergic patients may experience oral allergy after the ingestion of raw fruits like apple and kiwi, nuts such as hazelnuts and walnuts and/or raw vegetables such as celery and carrot due to the presence of proteins homologous to Bet v 1, the major birch pollen allergen, in these foods (reviewed in [14]). Also, a proportion of patients sensitized to the plant pan-allergen, profilin, may experience OAS; in these subjects tomato, orange, melon are additional offending foods [15]. Most allergens involved in such crossreactivity reactions are easily destroyed by pepsin digestion and heat, explaining why symptoms of pollen-related food allergy are in most cases mild and why the majority of patients with OAS can ingest offending foods cooked or after other kind of thermal processing (*e.g.* pasteurization, canning, *etc.*) without difficulty. OAS is often induced by stable allergens as well; as a consequence, a subset of patients with this specific kind of food allergy may in some instances experience generalized and even life-threatening reactions.

2.2 Gastrointestinal disorders

IgE-mediated gastrointestinal problems may present with a variety of symptoms including nausea, vomiting, gastric retention, intestinal hyper-motility, abdominal pain due to colonic spasms and diarrhoea [3]. Symptoms usually develop within minutes to 2 h of the ingestion of the offending food. Food allergens causing gastrointestinal symptoms are generally pepsin-stable, and hence able to reach the gastrointestinal tract in an almost unmodified form or as (assembled) fragments with sufficient residual allergenicity. Immediate gastrointestinal hypersensitivity reactions are rarely isolated, and most often accompany allergic symptoms in other target organs (skin, nose, lungs and eyes).

2.3 Skin disorders

The skin is frequently involved in IgE-mediated food allergy. Cutaneous symptoms may vary from pruritus, urticaria, and angioedema to morbilliform rashes. Acute urticaria, with or without angioedema, is the most common skin disorder in adult patients with food allergy [16]. As IgE-reactive components from foods are rapidly absorbed, urticaria may appear within minutes of ingesting the offending food and may last for some hours. Frequently patients with severe skin reactions are treated in the Emergency Department. The cause-and-effect relationship between a specific food and the skin reaction is often clear-cut.

Contact urticaria is a rather common disorder associated with the handling of foods; in some instances patients with contact urticaria have food allergy as well. Raw meats, fish, fruits and vegetables are the most commonly involved foods in these reactions [17].

Atopic dermatitis (AD) is a chronically relapsing inflammatory skin disease commonly associated with the presence of IgE specific for airborne and/or food allergens.

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AD is commonly associated with food allergy in children but rarely in adults. In a recent study, 43% of 336 children with AD scored positive on skin tests with milk and/or egg [18]. Because their disease is characterized by exacerbations and remissions, patients frequently think it is related to the ingestion of specific foods, but this is most often not the case; for these patients, the role of the allergist is to ascertain whether foods have a pathogenic role.

2.4 Respiratory disorders

Respiratory symptoms (rhinoconjunctivitis and bronchospasm) may occur in food-allergic patients following the ingestion of the offending foods in association with gastrointestinal and skin disorders but are rarely present as the only symptom [16]. In contrast, rhinoconjunctivitis and/or bronchospasm following inhalation of food dusts or vapours are not uncommon in food-allergic patients and have been associated with a number of foods, especially fish, crustaceans and legumes.

2.5 Anaphylaxis

According to the recent EAACI position paper on nomenclature [2], anaphylaxis is defined as a 'severe, life-threatening, generalized or systemic hypersensitivity reaction'. The anaphylactic reaction is the most dramatic allergic reaction and is always a medical emergency. Along with drugs, foods are one of the most common causes of anaphylaxis [19]. Anaphylaxis is caused by the abrupt, massive release of mediators from mast cells and/or basophils throughout the body, inducing gastrointestinal, skin, and respiratory symptoms, in some cases associated with cardiovascular symptoms including hypotension, collapse and dysrhythmia. Patients may react within minutes or even seconds after contact with (traces of) the food, with a generalized, life-threatening reaction characterized by a combination of the following symptoms: generalized urticaria, erythema, itching, nausea, vomiting, dyspnoea due to oedema of the glottis (throat tightness) and/or bronchospasm, dizziness, palpitations, fainting or even collapse. These reactions may be fatal or near-fatal; young adults with peanut or tree nut allergy and asthma are particularly at risk [20]. In less severe cases, symptoms are limited to some of those mentioned above. In a small proportion of cases, anaphylactic reactions can be biphasic [21] or prolonged, persisting for several days (typically 2–3 days) with multiple recurrences interrupted by asymptomatic periods lasting for hours [22, 23].

2.6 Management

There is presently no cure for food allergies. Therefore, the focus of food allergy management has to be on optimal avoidance of the offending food. To this end, patients have to be well taught how to recognize the early signs of a reaction, to read food labels and to recognize high risk food (particularly hidden food allergen sources). Patients at risk of severe reactions should be supplied with self-injectible epinephrine including a written emergency plan.

2.7 Natural history of food hypersensitivity

In the first few years of life (0–4 years), the prevalence of food hypersensitivity (but also the possibility to prevent it) is greatest. The most frequently involved foods are milk, egg, peanut, nuts, and fish, with regional differences possibly caused by local nutritional habits. Depending on the allergen, children may outgrow their allergy [24]. In a prospective study of milk hypersensitivity, 50% of the children had already lost their allergy by 1 year of age and 85% by 3 years of age [25]. Patients with a peanut, nut or fish hypersensitivity will mostly remain clinically reactive [15].

Oral allergy due to crossreactivity of pollen-specific IgE with food proteins usually develops in (young) adults.

About 75% of infants with food hypersensitivity and severe AD progress to allergic rhinitis and asthma at 4 years of age [26].

2.8 Limitations and problems

One important aspect in the clinical expression of food allergies is the discrepancy between objective and subjective symptoms. The OAS is a typical example of food-induced, IgE-mediated reaction that remains subjective unless angioedema of the lips and/or larynx or pharynx occurs. This has relevance to the choice of methods to be adopted for diagnostic purposes (see below).

3 *In vivo* tests in the diagnosis of food allergy

3.1 SPTs

SPTs are frequently used to screen for food-specific IgE [27]. They can be easily performed, are safe and cheap, and the results are available within 15 min. *In vitro* tests may be more sensitive in infants, and may be the method of choice in the case of extensive skin disease or dermographism, or if antihistamines cannot be discontinued.

The diagnostic accuracy of SPTs depends on the quality of the food allergen extracts used but, in contrast to extracts from aeroallergens, many commercially available food extracts are not standardized. In children with AD and food allergy to egg, milk, peanut and fish, SPTs with these foods have an excellent sensitivity and negative predictive accuracy (generally >90%), but poor specificity and positive predictive accuracy (50–85%) [28]. Thus, a negative skin test with these food extracts represents a good method to rule out an IgE-mediated food allergy, whereas a positive

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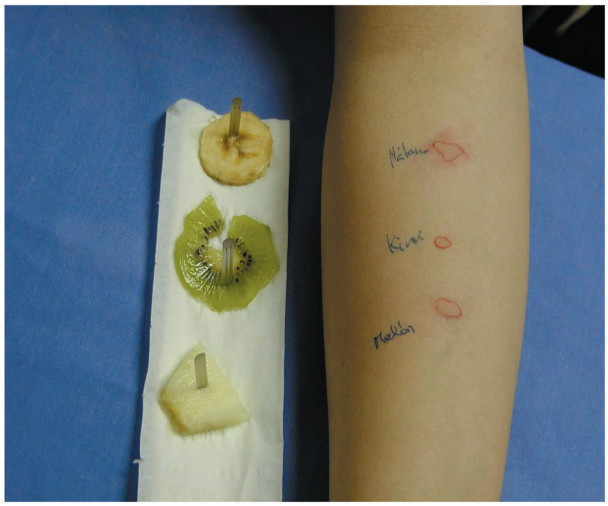

Figure 2. An SPT with fresh food carried out by the prick–prick technique.

test does not predict clinical reactivity accurately and the final diagnosis relies on the oral challenge test. To overcome these problems, diagnostic decision points predicting the outcome of oral food challenge tests have been recently developed for some foods and specific patient populations [18, 29].

For many foods (particularly plant-derived ones), commercial extracts for SPT show a low sensitivity resulting in a high rate of false-negative results. This phenomenon is related to the low abundance or the lack of stability of several allergens to endogenous enzymatic processes taking place in plant food extracts. In these cases, skin testing with native foods by the prick-prick technique shows a clearly superior performance [30]. In this test, the lancet is plunged several times into the food immediately before pricking patient's skin. Nowadays, this is the most reliable and common *in vivo* test with fruits and vegetables (Fig. 2). Prick-prick tests are also useful when there are discrepancies between a suggestive case history and a negative SPT with a commercial extract, or when a specific food extract is not available. The main drawbacks of prick–prick tests are the low specificity resulting in a high rate of false positive results, the impossibility to standardize the allergen source, and its dependence on the availability of the fresh food in question. The reduced specificity is, among other reasons, an expression of IgE crossreactivity with pollen or other related foods, and the only way to control clinical relevance of positive SPTs is by means of controlled oral challenges. Since the concentration of labile allergens is reduced drastically while stable allergens remain present in commercial extracts of plant-derived foods, it has been suggested to utilize this observation as a means to carry out a differential diagnosis between patients sensitized to stable (*e.g.* LTP, seed storage proteins, *etc.*) or labile (*e.g.* Bet v 1-like, profilin) allergens [14].

In the last years, purified natural and recombinant major plant food allergens have been applied in diagnosis, with the aim to improve the diagnostic accuracy of IgE tests. They have been mainly applied in *in vitro* tests, but when used in SPTs the sensitivity was higher than 70%, and the combination of several individual allergens from the same food increased the sensitivity [31, 32]. Because skin reactivity shows a certain degree of interpatient variability negative and positive controls are included in each session. The diluents used to preserve the allergen extracts or saline are generally employed as negative controls. Patients with dermographism will frequently show a wheal-and-flare reaction to the negative control which will make the interpretation of skin reactions at the allergen sites much more difficult [33]. Histamine 10 mg/mL is used as a positive control in most settings.

3.1.1 Limitations and problems

The term 'poor specificity' referring to SPT deserves some discussion as in healthy subjects SPT always scores negative. The positive skin tests that frequently characterize atopic patients who tolerate the corresponding foods reflect in all the cases, the presence of IgE-specific for that particular food, as a result of direct sensitization or on the basis of crossreactivity. The absence of clinical allergy in the presence of specific IgE may be caused by a number of variables including the absence of cofactors, extremely low levels of specific IgE, low affinity/avidity of specific IgE or a high threshold.

3.1.2 Possible future developments

Diagnosing food allergy at a molecular level will potentially have significant clinical (both prognostic and preventive) relevance. In fact, single food sources contain a number of allergens that may differ in their physical/chemical characteristics (pepsin stability, heat stability, *etc.*) and hence in their ability to induce mild or severe symptoms. Moreover, several allergens are highly conserved proteins and may crossreact with homologous proteins in unrelated sources. The possibility of using a large number of single allergenic proteins for the diagnosis of food allergies either *in vivo* or *in vitro* (see below), will have a considerable impact on the clinical management of food allergies [34].

3.2 Atopy patch test (APT)

For APT, allergens are applied epicutaneously and the induced eczematous skin lesions are evaluated. The usefulness of the APT is controversial. It appears that the APT has a high specificity [35] and, particularly in combination with the measurement of specific serum IgE, some investigators rely on the APT a useful tool in the diagnostic work-up of food allergy in infants and children with AD [36]. In contrast, other investigators reported that the APT has a poor reliability and does not increase the diagnostic accuracy in

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food allergy [37–39]. These controversial views might be explained by the fact that the APT is difficult to interpret, particularly by nondermatologists, as nonspecific reactions frequently occur.

3.3 Food challenges

3.3.1 General

The oral challenge is a diagnostic test which provides strong evidence of a food allergy, and allows the clinician to recommend a correct elimination diet. However, it is agreed that, in those patients with a history of a severe immediate systemic reaction (anaphylaxis) after the ingestion of an isolated food to which specific IgE is demonstrated, food challenges are not essential for confirming the diagnosis. Since oral tolerance may develop with time, particularly in children allergic to cow's milk and hen's egg, longitudinal provocations should be performed to reassess clinical reactivity.

Oral food challenges may be performed openly, single-blind, or double-blind, and blinded challenges are controlled by placebo. The double-blind placebo-controlled food challenge (DBPCFC) is considered the gold standard for the diagnosis of food allergy and is preferred over open challenges. The current knowledge on DBPCFC is reviewed in a recent position paper by the EAACI [40]. DBPCFCs are especially required for research studies, in chronic disorders such as AD, for patients appearing to have multiple food allergies, and when the patient's subjective complaints may act to skew accurate assessment of symptoms.

Open food challenges (OFC) are useful to reintroduce a food in the diet after a lack of response to the elimination diet, and when no specific IgE to the food is detectable, the history is not suggestive, and the patient has been avoiding the food. OFCs may be also useful as a first step of the provocation procedures. If the OFC is negative, the food may be reintroduced into the diet.

Food challenges should be performed in a hospital setting where emergency care is immediately available. The food is given to the patient in fasting conditions, starting with a low dose unlikely to provoke symptoms, according to the eliciting dose reported in the medical history or in the last positive challenge test. Incremental amounts of food are given at time intervals (generally 15–60 min), until a positive reaction appears or the patient eats an amount of the food corresponding to a normal serving. To control for possible false-negative challenges, a negative DBPCFC is followed by an OFC with the culprit food or by reintroduction of the food into the diet.

As mentioned, the best available test for the diagnosis of food allergy is the DBPCFC [1, 41]. Placebo and active test food challenges are administered in a random order. The code is blinded to both the patient and the health care professionals involved in the test. Active and placebo tests foods are preferably administered on separate days. After evaluation of the test, the code is broken, followed by dietary advice to the patient. In case of a positive DBPCFC, the patient is advised to continue the elimination of the culprit food, whereas in case of questionable results (less frequent) the test has to be repeated.

For the active test food challenge, the suspected allergenic food is disguised in a food matrix (recipe) consisting of food components normally tolerated by the patient. Placebo and active test recipes should be as sensory equivalent as possible in terms of taste, smell, consistency, texture and colour so that the active meal cannot be distinguished from placebo. Validation of adequate blinding can be achieved by sensory testing in a professional food laboratory [42]. When challenging children, special care must be taken to acceptable taste and volume of the test food, so that even choosy eaters will actually eat the test food and the challenge test can be completed. For cow's milk challenges in young infants, cow's milk hydrolysate which the child usually drinks can be used as a food matrix for disguising the milk. In older children, solid recipes, such as pancakes, meat recipes or cookies are suitable. Recipes should be controlled for altered or lost allergenicity or by matrix effects during preparation and processing of the test meals. Loss of allergenicity is a well-known problem when incorporating some fresh foods, such as apple, in a food matrix [43].

Although the DBPCFC has been considered the gold standard in diagnosing food allergy since 1988 [41], to date, this test is still not performed in a uniform fashion, which makes comparison of results from different studies difficult. Because the test is time consuming and since there is a lack of uniform challenge protocols, DBPCFC are only performed in specialized centres. Recently, some proposals have been put forward aiming at the harmonization of the test procedures and validation of challenge materials [40]. However, standardization of many aspects of DBPCFC is still lacking; for instance, the use of uniform food matrices in all centres involved, incremental scales, acceptable test volumes, the state in which the allergenic food is administered (fresh, heated, freeze dried), minimum dose, maximum dose, assessment of symptoms, *etc*. Within the Euro-Prevall consortium, protocols have been developed in order to perform for the first time DBPCFCs in a uniform way in a large number of clinical centres.

3.3.2 Limitations and problems

One potential problem of oral food challenges, both open and blinded, is that the procedures do not reproduce what occurred to the patient when he/she experienced the adverse reaction(s). As a result, the challenge test may produce a false-negative result. The food-dependent, exercise-induced anaphylaxis (FDEIA) is a typical example in this sense. Patients with this condition have positive skin tests and detectable IgE specific for the offending food, but the reaction occurs only if the ingestion of the responsible food

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is followed by exercise within a time range of some minutes up to 3 h. Other cofactors are able to increase the intestinal absorption rate of allergen, including nonsteroidal anti-inflammatory drugs (aspirin, and other COX-inhibitors) and viral gastrointestinal infections.

Finally, matrices used to mask foods during DBPCFC procedures may influence the release and absorption of allergens [44].

4 Assays for specific IgE antibodies

Allergen-specific IgE antibodies (Fig. 3) are principal components in food-allergic reactions. Circulating IgE antibodies of defined allergen specificity can be measured in blood samples of subjects with suspected food allergy using commercially available assays and assessment of specific IgE is a frequently used and important element in the clinical investigation and diagnosis of food allergy.

4.1 Assays for clinical use

Several different assay systems for measurement of specific IgE antibodies are used in clinical routine [45, 46]. While all these assays have in common an allergen reagent for antibody specificity and an IgE-binding reagent for antibody isotype specificity, different types of assay design exist. In the most frequently used, allergosorbent-type of assay, a serum or plasma sample is incubated with a solid phase carrying an immobilized allergen preparation which captures specifically binding antibodies. Bound IgE can then be detected and quantified with a labelled IgE-specific antibody detection reagent, such as an enzyme-conjugated monoclonal anti-IgE antibody. In a different type of assay, serum IgE antibodies are first captured by an immobilized anti-IgE antibody and subsequent binding of a labelled allergen reagent is detected. In yet another assay type, serum antibodies are allowed to bind tagged allergen in fluid phase, followed by capture of the allergen–antibody complex *via* the allergen tag and subsequent detection of bound IgE with a labelled anti-IgE reagent. In all commonly used commercial assay systems which are based on immobilized antigens, a standard curve based on purified IgE calibrators is established and used to convert assay signals to mass units of allergen-specific IgE, given in International Unit (IU) *per* mL of serum or plasma.

4.2 Assay quality and performance requirements

For the proper and reliable function of an assay for specific IgE, certain quality and performance criteria need to be considered, regardless of the particular design of the assay. Such criteria include: antibody isotype specificity, absence of nonspecific antibody binding to the solid phase, level of sample-independent background signals, controlled interference by total IgE and specific IgG antibodies present in the sample, sample dilution linearity, minimal intra- and interassay variation, lot-to-lot consistency and adequate detection limit and measuring range.

Given a well designed and robust assay platform, the performance of a test for allergen-specific IgE is to a large extent determined by the composition, quality and stability of the allergen preparation used. Provided a highly potent allergen preparation is applied, specific IgE antibodies will be detectable in the vast majority of patients with clinically proven allergy and a negative test result can therefore be used to reliably exclude sensitization to the particular allergen tested.

Similarly, a negative test result will be obtained in most nonallergic subjects and in subjects lacking relevant sensitization to the allergen being tested for. However, as discussed below, the presence of structurally similar allergens in different foods and pollens may in some cases cause antibody binding and test positivity to foods against which the subject does not react or has never even consumed. The positive predictive value of IgE-based diagnostic methods is therefore generally lower than their negative predictive value.

4.3 Quantitation of specific IgE antibodies

For an analyte which is causally involved in the allergic disease mechanism, such as specific IgE antibody, a correlation between analyte concentration and probability or severity of allergy symptoms would be expected. Indeed, such a relationship between the concentration of specific IgE and probability of a reaction upon controlled challenge has been demonstrated for several allergenic foods, including peanut, milk, egg and fish [9, 47–52]. Although reported studies on this aspect of specific IgE assessment have mostly been performed in children and adolescents, a recent study carried out on both adults and children demonstrated a relationship between peanut-specific IgE antibody concentration and severity of reaction in DBPCFC to peanut in adult subjects [53].

Studies reporting a positive correlation between specific IgE concentration and clinical expression of reactivity have all concerned foods with no known specific association to pollen sensitization. Systematic studies of this issue in pollen-related allergy are lacking. Further, interpretation of quantitative IgE-binding data obtained with commercial fruit and vegetable extracts may be complicated by the fact that no information on allergen quality is available in the majority of studies. However, in pollen-related food allergies, specific IgE concentration may not be as strongly related to manifestation of symptoms or reactivity upon food challenge.

In order to take optimal advantage of their reported utility in the diagnostic workup of certain food allergies, specific IgE quantitation results must be interpreted with

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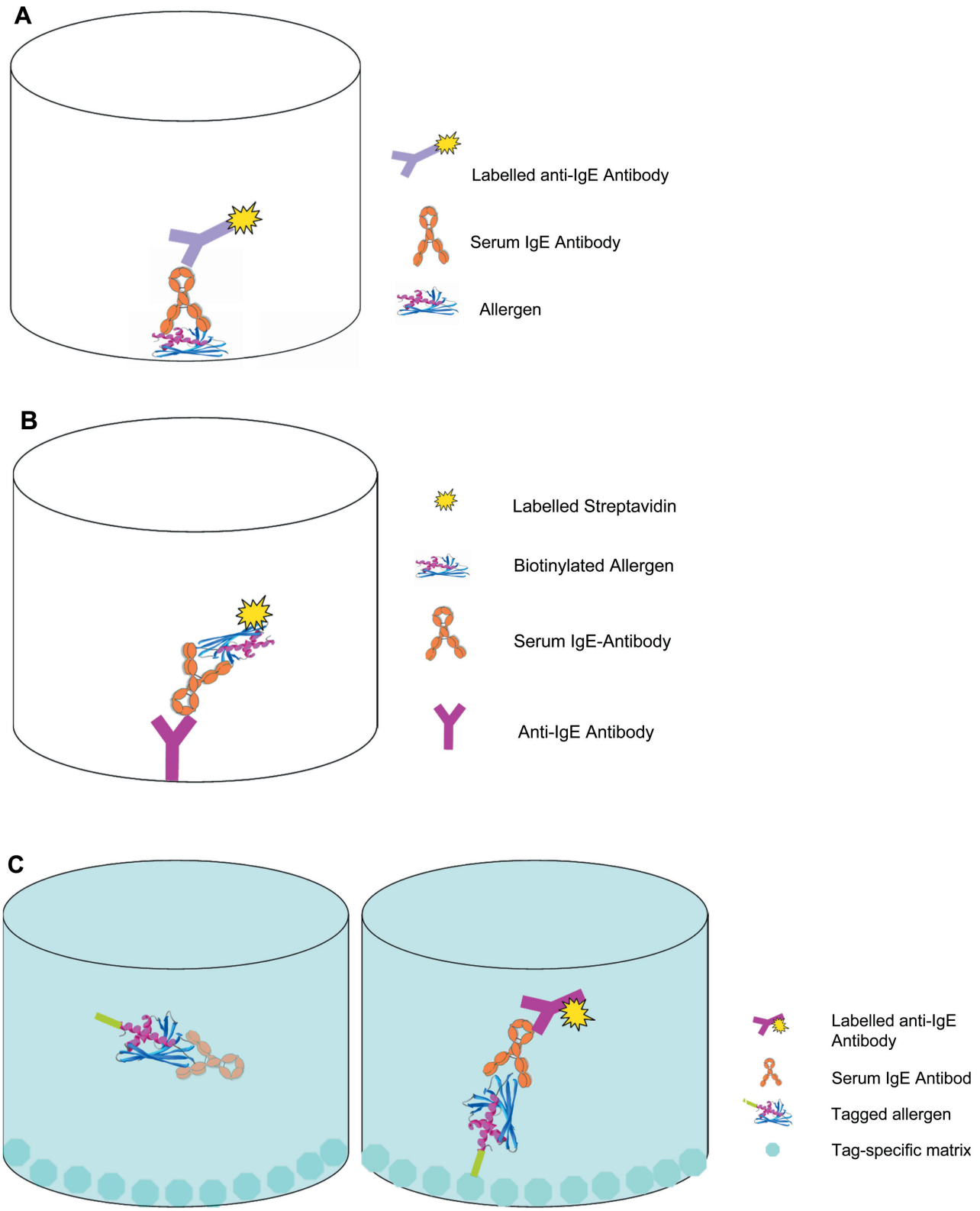

Figure 3. Specific IgE detection assays used in clinical routine. Allergen-specific IgE antibodies present in human serum samples are detected using commercially available assays of different designs. Following the formation of antibody–allergen complex, a labelled detection reagent is added to quantify the amount of allergen-bound IgE (A, C) or IgE-bound allergen (B).

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appropriate caution. As high levels of specific IgE sometimes are present in subjects with mild or no clinical reactivity and strong reactions occasionally occur in subjects with low levels of specific IgE, concentration of specific IgE cannot be used as an absolute diagnostic parameter but rather as tool to assess risk of allergic reaction. Despite this limitation and because risk assessment is considered by many a relevant element in the management of a complex disease such as food allergy, quantitative measurement of specific IgE is likely to become an increasingly important parameter in the clinical investigation of food allergy.

4.4 Strengths and limitations of specific IgE as an analyte in allergy diagnosis

There is evidence from numerous publications and from its widespread and increasing use that specific IgE is the best objective marker so far available in routine clinical diagnosis of allergic disease. It should be noted, however, that allergy is a progressive disorder in which sensitization represents an early stage and the onset of disease symptoms represents a later stage. From this course of development of the disease, it follows that frequently detectable sensitization will at some point be present prior to the occurrence of manifest symptoms of allergy. Conversely, at late stages of the disease, specific IgE antibodies may be generated against structures which are only rarely involved in the elicitation of symptoms (see below). Further, as indicated above, even though the probability of allergic reactivity increases with increasing magnitude of sensitization (*i.e.* concentration of specific IgE), there is no absolute cut-off level above which symptoms necessarily occur upon allergen contact. Therefore, in a population of individuals tested for specific IgE antibodies, certain individuals may exhibit sensitization in the absence of allergic symptoms. Such observations highlight the fact that specific IgE is a marker of allergic sensitization, not of allergic disease.

Another biological factor that needs to be taken into consideration when interpreting specific IgE results is the complex nature of allergens, particularly the relationships between different allergen sources, taxonomically close or distant. Due to similarity between homologous proteins in different allergen sources, IgE antibodies formed against one particular allergen source may crossreact to proteins of another source to which the subject was never sensitized or even exposed. Such crossreactions may be weak or strong and may or may not generate clinical symptoms upon exposure or challenge. Examples of crossreactive structures which sometimes give rise to seropositivity, but on their own rarely are associated to allergic symptoms, are profilins from certain food sources and crossreactive carbohydrate determinants (CCD) [54–56]. There is, however, evidence that profilin sensitization may be clinically relevant in some patients with pollen allergy, inducing OAS to foods such as melon, tomato, citrus fruits, banana and *Rosaceae* fruits [15, 57]. An antibody assay will generally not be able to discriminate between genuine sensitization and serological crossreaction to a given allergenic structure.

It should be clear from the limitations discussed here that specific IgE test results need to be evaluated in the context of the patient's case history and relevant clinical observations.

Information in the test result is lost when it is interpreted as positive or negative: strongly positive results are more strongly associated with clinical sensitivity than results just above the threshold between negative and positive, and completely negative results are more strongly associated with clinical tolerance than results just below the threshold. The best use of allergy test results can be made by consideration of likelihood ratios [58].

4.5 Difficulties and problems with current specific IgE assays

Besides the basic assay design issues discussed above and the biological strengths and limitations of specific IgE as a diagnostic tool, assays for specific IgE are critically dependent on allergen reagents of sufficient potency and adequate allergen representation. In the area of food allergy diagnostics, this aspect is often regarded as problematic. Preparation of food allergen extracts is generally difficult and both scarcity of particular allergen components in the raw material and susceptibility to inactivating damage occurring during extraction are known sometimes to compromise the quality of extracts of several important foods. Clearly, if allergens are lost in preparation of extracts, the sensitivity of tests will be compromised. Instructive examples of allergen deficiency in natural food extracts have been reported for certain fruits of the *Rosaceae* family (*e.g.* apple and cherry), soybean and peanut. In these foods, a particular class of allergen component, belonging to the PR-10 protein family, is severely underrepresented or even missing, causing false-negative test results in patients exclusively sensitized to this group of allergens [31, 59–62]. Recombinant PR-10 allergens from several of these foods are now available and will become useful complements to natural extracts in diagnostic tests for specific IgE.

4.6 Future developments

Specific IgE antibody testing is open to two major lines of development: use of pure allergens and allergen arrays. Dramatic progress has been made in both over the past few years.

Proteomics techniques have been applied for the identification and characterization of novel and/or low abundance protein allergens from a wide range of different sources [63, 64]; further, cloning and biotechnological production of a growing number of allergens will overcome the limitations associated with the use of natural extracts [65]. It is

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expected that, by using such purified allergen preparations in combination with biological *in vitro* allergy tests, an improved correlation of test results with the clinical situation may be achieved. Further, reagent compositions optimized with respect to both clinical sensitivity and specificity can be established, and geographical differences in sensitization pattern between patient populations can be accommodated in a better way than would otherwise be possible [66]. Furthermore, the clinical consequences of component resolved diagnosis of food allergy are potentially enormous. The identification of diagnostic marker allergens responsible for sensitization or particularly severe clinical reactivity will profoundly influence the management of allergic patients. Moreover, the molecular approach will open new ways towards the introduction of specific immunotherapy as a therapeutic strategy for food allergy, using new allergy vaccines on the basis of recombinant allergens, for example genetically engineered molecules with a reduced allergenicity but retained immunogenicity.

Secondly, whereas specific IgE assays have so far been designed predominantly as single allergen tests, recent developments in protein array technologies are opening possibilities for simultaneous measurement of IgE antibodies of many specificities, using complex arrays comprising large numbers of allergens [67].

While clinical applications drawn from these areas of technological development are still in their infancy, it is likely that they will have significant impact on the design and performance of specific IgE testing in the future.

5 Other *in vitro* tests in the diagnosis of food allergy: Biological allergy tests

Apart from specific IgE assays, several biological tests have been used for the diagnosis of food allergy. These are based on the *in vitro* activation of basophils sensitized with IgE. The general concept for introducing cellular tests has been that activation of the basophil more closely resembles the *in vivo* situation. Several test systems have been developed: (i) allergen-induced histamine release, (ii) sulfidoleukotriene release from the patients own basophils, (iii) determination of *in vitro* activated patient basophils by FACS analysis of surface markers such as CD 63 and/or CD 203c and (iv) allergen-induced release of histamine or sulfidoleukotriene from donor basophils passively sensitized with serum from allergic patient. The advantage of using sensitized basophils is that a wide range of foodstuff can be used, including raw material and highly purified natural or recombinant allergens. The disadvantage of using basophils as a diagnostic tool is that basophils from certain patients (nonresponders) with a verified allergy do not react to the relevant allergens, Further, the basophil reactivity to relevant allergens can be reduced within 24 h after blood sampling from some patients (storage of blood samples before testing is therefore short). At present, basophil test systems are generally in accordance with SPT results but they cannot replace oral challenge tests, in particular, DBPCFC. The diagnostic sensitivity and specificity varies between different foods and different studies.

EuroPrevall will approach the cellular *in vitro* tests by:

(i) Performing larger prospective diagnostic studies with basophil tests taking the prevalence of specific food allergies in different regions into account (the prevalence to be determined by the epidemiological studies).

(ii) Extended validation of tests by investigating samples from clinically well-defined patient panels.

(iii) Examining the influence of time from blood sampling to testing on the diagnostic outcome and by introducing simplifications which can be applied to many clinics with a minimum of technical expertise/equipment.

(iv) Identifying serum factors that cause experimental error, *i. e.* failure of cell lysis in tests utilizing passive sensitization protocols.

(v) Examining the diagnostic value of using cell lines to replace the basophil as a diagnostic tool.

6 Unproven diagnostic procedures

Alternative approaches are used widely in the diagnosis of food allergy. Although they are posted on websites advertising their effectiveness, the usefulness of the measurement of food-specific IgG or IgG4 antibodies, cytotoxic food tests, kinesiology, provocation testing using sublingual or intradermal provocation tests or electrodermal testing has not been proven by properly performed studies [68]. Therefore, these approaches cannot be recommended as a meaningful element in the diagnostic workup of food allergy.

7 Novel diagnostic approaches within EuroPrevall

In many ways, EuroPrevall represents a unique approach: It is an EU-funded multidisciplinary integrated project (IP) involving 16 European member-states, Bulgaria (a candidate country), Switzerland, Iceland, and Ghana. Of the 54 partners, there are 15 clinical organizations as well as the leading allergy research organizations in Europe. Epidemiological studies will be performed to establish the true occurrence, prevalence and distribution of food allergy and hypersensitivity in children and adults as well as influences during pregnancy and early years, involving a birth cohort of 10 000 babies, yet to be born across Spain, Germany, Iceland, Greece, Poland and the UK. Defined DBPCFC materials will be developed that, together with a library of well-defined food allergens, will be used in the development of new diagnostic tools. The EuroPrevall diagnosis studies will include a variety of different approaches:

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(i) Allergen molecules derived from one food will be used in a highly sensitive commercial IgE assay system to increase predictive and prognostic potential of IgE-binding tests. Positive subjects will be confirmed by DBPCFC, while several groups of IgE-positive controls without food allergy symptoms (for example pollen-allergic subjects without food allergy) will be included. The presence of IgE against structures rarely causing symptoms will be assayed separately.

(ii) The affinity/avidity of specific IgE antibodies will be taken into consideration.

(iii) Multiallergen protein biochips will be developed and used to study well-defined patient populations including small children for which microchips represent the only possible tool for multiantigen testing. Microchips containing arrays of epitopes derived from major allergen molecules will also be included to increase the prognostic value of serological diagnosis.

(iv) Biological allergen assays (Section 5) utilizing the mechanism of the type I-allergic reaction will be studied to improve the correlation between the test results and the clinical situation.

It is not expected that EuroPrevall will generate the ultimate diagnostic tool for food allergy. However, since the projects will include one of the most systematic and comprehensive approach to the existing problems in the field, at least relevant progress can be expected.

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9 Addendum

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