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Research paper

Diagnostic oral food challenges: Procedures and biomarkers

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ABSTRACT

Oral food challenge (OFC) is the gold standard for the diagnosis of food allergy. They are conducted to confirm whether an allergy to food exists (initial challenge) or to monitor for resolution of a food allergy. The history of an immediate allergic reaction, when supported by positive tests for specific IgE antibodies to the suspect food, is often sufficient to establish a diagnosis without OFC. Additionally, higher concentrations of food-specific IgE or larger allergy prick skin test wheal sizes correlate with an increased likelihood of a reaction upon ingestion. Although these food-specific IgE tests are helpful biomarkers of allergy, their limited sensitivity and specificity often necessitates the use of OFC to establish reactivity. Furthermore, the pathogenesis of non-IgE-mediated food allergy, such as food protein-induced enterocolitis (FPIES) or proctocolitis and food allergy due to mixed IgE and non-IgE mediated processes, such as atopic dermatitis or eosinophilic gastroenteropathies may not be assessable with specific IgE tests, also warranting OFCs. This review provides an overview on the technique and interpretation of OFCs, use of food-specific testing to predict whether OFC is warranted and to predict OFC outcomes. Additionally, biomarkers that correlate with OFC outcomes will be discussed, as well as future diagnostic tests promising better predictive value.

1. Introduction

A food allergy is an adverse health effect arising from a specific immune response that occurs reproducibly on exposure to a food (Boyce et al., 2010). Additional adverse reactions may be attributed to a wide variety of causes including lactose intolerance, gastroesophageal reflux, toxins, gastrointestinal infections, and a host of other acute and chronic processes (Bruijnzeel-Koomen et al., 1995). Because the typical diet includes a frequent intake of foods and snacks of different types and origins throughout the day, any sudden adverse physiological event or chronic illness could incorrectly be ascribed to food. Once a patient incorrectly associates food and a symptom, it may be difficult to convince the patient otherwise.

Food allergies are broadly categorized on a pathophysiologic basis into IgE-mediated (immediate), non-IgE-mediated (cell mediated, delayed) and mixed processes. Acute allergic responses such as urticaria, vomiting, wheezing and anaphylaxis to food are due to IgE directed against various food allergens. The history of an immediate reaction consisting of typical allergic symptoms within 2 h of ingestion, supported by positive tests for specific IgE antibodies, is usually sufficient to establish a presumptive diagnosis for suspected IgE-mediated reactions. Either skin prick tests and/or in vitro tests for IgE are usually performed initially. Higher concentrations of food-specific IgE and larger skin test wheals correlate with an increased likelihood of a reaction upon ingestion. Unfortunately, these tests are not sufficiently sensitive or specific to establish a diagnosis

Abbreviations: DBPFCF, Double-blind, placebo-controlled food challenge; FPIES, Food protein-induced enterocolitis; OFC, Oral food challenge; SPT, Skin prick test.

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without supporting evidence. Furthermore, the fact that many foods are usually consumed at the same time, may obscure identification of the triggering allergen. Therefore, in the diagnosis of food allergy a convincing history of reaction is the key but only a well-performed OFC can give a definitive diagnosis.

Non-IgE-mediated food allergy, such as food protein-induced enterocolitis (FPIES) or proctocolitis, and mixed IgE and non-IgE mediated processes, such as atopic dermatitis or eosinophilic gastroenteropathies are particularly difficult to diagnose for lack of reliable *in vitro* tests. Food-specific IgE tests are expected to be negative if the symptoms do not suggest an IgE-mediated reaction. Atopy patch testing may provide additional information in these cases; this requires placement of the food under a cap or patch for over 24 h and assessing for a localized skin eruption over subsequent days. There are currently no standardized reagents, application methods, or guidelines for interpretation for atopy patch testing for foods, which cannot be recommended outside of research settings. The diagnostic procedure for these non-IgE mediated disorders relies heavily on elimination of the suspected food and oral food challenge.

Oral food challenge (OFC) is the gold standard for the diagnosis of food allergy. It is conducted to determine whether allergy to a food exists (initial challenge) or to determine if tolerance has developed. This review provides an overview on the technique and interpretation of OFCs, use of food-specific testing to predict whether OFC is warranted and to predict OFC outcomes. Furthermore, biomarkers during OFCs that may correlate with outcomes, and future diagnostic tests are reviewed.

2. Role of OFC

2.1. History of OFC

Reports on oral food challenges date back to 1950, when Graham and colleagues (Graham et al., 1950) performed experiments aimed to blind the patient to avoid subject-related bias. Nasogastric feedings were performed on subjects who strongly believed they had reactions to foods. They were told they were given the test food when water in fact was being instilled and *vice versa*. Reactions to the tests correlated with suggestion. Loveless performed masked ingestions in several studies in the 1950s (Loveless, 1950a, 1950b). In an editorial, Ingelfinger et al. (Ingelfinger et al., 1949a, 1949b) reviewed approaches in demonstrating food-allergic reactions in the intestine, including x-rays, gastroscopy, leukocytosis, eosinophilia and thrombocytosis and emphasized the need for blinded challenges to objectively demonstrate cause-effect relationships in food allergy. Double-blind, placebo-controlled oral food challenges (DBPCFCs) were introduced into routine clinical and research use by Charles May (May, 1976).

2.2. Types of OFC

There are various modalities of OFC that may be administered, including open, single-blind, or double-blind, placebo-controlled. The type of OFC to be chosen depends largely on how likely the risk for bias is (i.e. whether objective or subjective symptoms are anticipated) (Bindslev-Jensen et al., 2004; Bock et al., 1988; Niggemann et al., 2005; Sicherer et al., 2000; Nowak-Wegrzyn et al., 2009) but also practical considerations such as the age of the patient, the time available, and whether OFC is done for clinical or research purposes. An open OFC is an unmasked, unblinded feeding with a food in its natural form with an age-appropriate serving given gradually. This is the most common format used clinically and objective symptoms (urticaria, vomiting, angioedema, wheezing, etc.) are typically considered reliable. This format has the highest potential for bias (Bindslev-Jensen et al., 2004; Bock et al., 1988).

To reduce bias, blinded OFC include masking by mixing the challenge food in another food to hide taste and texture. They are typically done as placebo-controlled challenges, which include administration of the food in a form that would not allow its differentiation from the placebo and consists of 2 sessions, 1 with active food and 1 with placebo (Bindslev-Jensen et al., 2004; Bock et al., 1988; Vlieg-Boerstra et al., 2004). In the single-blind OFC, only the patient does not know when the food is being tested. In the double-blind, placebo-controled OFC, challenge material is provided by a third party, and the observer is also unaware of when the test food is given. A negative blinded challenge, is followed by an open feeding of the tested food in its natural form. A DBPCFC is the gold standard for the diagnosis of adverse food reactions in research studies and for selected cases in clinical practice (Bock et al., 1988). When the results of an open OFC are ambiguous (e.g., subjective symptoms) a DBPCFC may be used to provide a definitive result.

2.2. How to determine whether OFC is warranted

The decision to proceed to OFC is influenced by many factors including the patient's age, the severity and timing of past food reactions, comorbidities, dietary preferences, nutritional needs, and concomitant food allergies. A complete description is beyond the scope of this review but has been recently published (Nowak-Wegrzyn et al., 2009). The skin prick test (SPT) and serum food-specific IgE test results may be used to assist in predicting the likelihood of a food allergy and should be taken into consideration (see below) along with the medical history . Infants and young children may not cooperate with the feeding and may not be able to express the early and subjective symptoms. Unstable asthma is also a relative contraindication for an OFC. How long to wait since the last reaction until performing an OFC depends, in part, on the age of the subject, food involved, and test results. Children are more likely to develop tolerance to certain foods over time, and therefore waiting for 12–24 months since the last reaction may be considered appropriate but in adults' tolerance development is less likely. Other reasons to defer an OFC may include medical conditions that increase the risk for complications of a positive OFC, such as cardiovascular disease, and pregnancy, or increase the risk for difficult to treat allergic reactions such as treatment with beta-blockers and ACE-inhibitors. In addition, subjects with medical conditions that may obscure interpretation of the OFC, such as uncontrolled eczema and severe allergic rhinitis may not be good candidates for OFC until the disease is better controlled. In individuals with a previous history of a life-threatening reaction (i.e. previous intubation or ICU

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admission), OFCs should be approached with particular caution unless there is compelling evidence to suggest clinical tolerance at this point.

2.3. Technique of OFC

2.3.1. Location, food preparation, blinding

An OFC is performed after at least 2 weeks of complete allergen avoidance and may be administered in an office, or as an in hospital out-patient, in-patient, emergency room or intensive care unit procedure depending on the risk of an allergic reaction, level of monitoring and capacity to treat anaphylactic reactions. The challenge food is either brought from home for open office OFC, whereas for blind OFC, test material should be provided to ensure proper masking. For blinding purposes, opaque capsules are effective at hiding nearly any food. However, this modality is in disfavor because the timing of release of the allergen is unpredictable. Blinding may also be achieved by mixing the test allergen with various vehicles (such as applesauce, puddings, etc) (Bock et al., 1988). The placebo portion includes the vehicle without the challenge food.

2.3.2. Challenge dose and schedule

The total challenge dose which is equal to a full age-appropriate serving (8 to 10 g of the dry food, 16 to 20 g meat or fish, and 100 mL wet food) is administered in gradual increments every 15 min to minimize the risk of severe allergic reaction and detect the lowest provoking dose (Nowak-Wegrzyn et al., 2009; Modifi and Bock, 2004). A negative blinded OFC is followed by an age-appropriate serving of the food in its natural form. It may be served in small portions over a period of 30 to 60 min . Numerous dosing schedules have been proposed. One option is to administer 0.1% to 1% (10 to 100 mg) of total challenge food, followed by 4%, 10%, 20%, 20%, 20% and 24.4% of the total dose. In an open OFC performed according to a simplified protocol, the entire serving of challenge food is usually divided into 3–6 equal portions. Challenges to determine threshold doses may be done with smaller doses at longer intervals (Taylor et al., 2004).

In non–IgE-mediated FPIES with symptoms usually starting within 1 to 4 h after food ingestion, the total challenge dose equals 0.15 to 0.3 g protein/kg body weight (or as low as 0.06 g protein/kg body weight in those with a history of a severe reaction). The total maximum 3–6 g of food protein or 10–20 g total food may be administered gradually in 3 feedings over a period of 45 min (Nowak-Wegrzyn et al., 2009; Modifi and Bock, 2004) followed by a an appropriate single-serving amount in 2–3 h (Sicherer, 2005). In patients with atopic dermatitis and allergic eosinophilic gastroenteritis with detectable food-specific IgE antibody who may develop delayed symptoms several hours to days after ingestion of a food, an initial DBPCFC, under physician supervision, is followed by subsequent feedings with regular portions of the food over the following days or weeks (Bindslev-Jensen et al., 2004; Nowak-Wegrzyn et al., 2009). A suggested protocol for OFCs for food-dependent exercise-induced anaphylaxis is treadmill exercise starting 30 min after food ingestion until a target heart rate for maximal exercise is reached or until onset of symptoms (Nowak-Wegrzyn et al., 2009).

2.3.3. Evaluation of signs and symptoms

Before starting the OFC, baseline vital signs including respiratory rate, heart rate, and blood pressure should be recorded and physical findings are documented. Recording a peak flow and spirometry may be considered, especially for patients with asthma. A flow sheet may be used to record the doses administered, symptoms, signs, and physical findings as well as treatments during OFC (Bock et al., 2007).

At regular intervals and at the first symptom or sign of an allergic reaction, inspection of the skin and oropharynx and chest auscultation is performed. Vital signs may be measured including pulse, blood pressure, and oxygen saturation. The challenge is stopped at any objective finding of an allergic reaction, and treatment is initiated immediately. If there are respiratory symptoms, peak flow and/or spirometry may be obtained, if available, and compared with the baseline measurement, although pulmonary function may be normal in subjects having lower airway symptoms and increased airway hyperresponsiveness in a food challenge (James et al., 1996). At physician discretion, the challenge is also stopped for persistent subjective responses.

2.3.4. Interpretation of in vivo provocation test data

An OFC can either result in a clinical reaction (a positive or failed challenge), or no clinical reaction (negative or passed challenge). In addition to symptoms that develop during the OFC, change in vital signs and spirometry may give clues to a developing positive OFC.

The OFC is negative if the patient tolerates the entire challenge, including the masked and open portions of a blinded OFC and observation period (1–2 h for the immediate-type reactions and 4 h for FPIES before discharge followed by symptom record kept at home).

Positive challenges can elicit skin, respiratory, or gastrointestinal symptoms that may be mild, moderate or severe. Also, anaphylactic reactions can be elicited in OFCs. (Perry et al., 2004a; Jarvinen et al., 2009) Objective symptoms in IgE-mediated food allergy supporting positive OFC may include skin symptoms including pruritus, urticaria and erythematous rash, upper respiratory symptoms such as sneezing and nasal pruritus, nasal congestion, rhinorrhea, angioedema, oropharyngeal findings such as uvular edema and throat clearing, dry cough and inspiratory wheeze, as well as lower respiratory symptoms, wheeze and dyspnea, gastrointestinal objective symptoms as emesis and diarrhea, and cardiovascular symptoms (pale skin color, weakness, dizziness, tachycardia, mental status change, drop in blood pressure, collapse, unconsciousness, bradycardia).

In case of subjective complaints, interpretation of the challenge result is more challenging. For symptoms such as throat, mouth, skin itching, or nausea, a period of observation to allow for resolution of symptoms should be undertaken before administering a subsequent dose. In a single-blind OFC, administration of placebo food interspersed with active food clarifies whether subjective symptoms are truly allergic ones. A challenge may be considered positive if subjective symptoms follow 3 doses of test food but not placebo food (Nowak-Wegrzyn et al., 2009).

In FPIES, the criteria for a positive challenge have been suggested as follows: (1) emesis (typically in 2–4 h), diarrhea (typically in 5–8 h), or both, (2) fecal blood, (3) fecal

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leukocytes, (4) fecal eosinophils, (5) increase in peripheral neutrophil count >3500 cells/ml (peaking at 6 h),(Caubet and Nowak-Wegrzyn, 2011) Three of five criteria being positive will classify a positive reaction; however, in clinical practice typical symptoms (profuse vomiting and lethargy, possibly followed by diarrhea) establish a likely diagnosis of FPIES.

2.3.5. Pitfalls

Preparation of foods for OFCs requires special attention. Thermal processing changes protein conformation which may result in a change in allergenicity in foods such as cow's milk or hen's egg, beef, fish, shellfish, fruits, and vegetables implicated in the pollen-food allergy syndrome (Thomas et al., 2007; Urisu et al., 1997). Also the matrix effect and fat content of the food preparation may affect allergenicity (Nowak-Wegrzyn et al., 2009). Proper blinding may also be difficult. Especially, masking foods with strong flavor, characteristic textures or raw fruits or vegetables can be challenging(van Odijk et al., 2005). Limitations in using capsules include problems in administering large amounts of unprocessed food and in passing early oral symptom. Delayed absorption may also be a problem.

Performing OFCs is dependent on participant's willingness to eat on demand. This may pose a special problem in children. Another challenging situation occurs with young children, who cannot express their symptoms and first symptoms of a positive reaction may include nonverbal clues such as ear picking, tongue rubbing, putting a hand in the mouth, or neck scratching; or a change in general demeanor, becoming quiet, becoming withdrawn, or assuming the fetal position. Similarly, isolated subjective symptoms in older patients, such as complaints of throat tightness or pruritus, nausea, abdominal pain, or general malaise, may represent a prodromal phase of a more severe reaction. A longer observation period before proceeding with a next dose or discontinuation of an OFC followed by treatment, depending on the level of the patient's discomfort and the physician's judgment, may be prudent.

OFCs for which delayed symptoms are anticipated and therefore home feedings with regular portions of the food over the following days or weeks performed may be subject to many confounding factors. Such OFCs are typically done for conditions are such atopic eczema for which factors other than foods (such as environmental allergens) may play a role outside hospital or clinic setting.

Guidelines for performing OFCs for exercise-induced anaphylaxis are lacking and OFCs are frequently negative, the reasons for which are not known.

3. Use of IgE testing to predict OFC outcomes (i.e. how to determine whether OFC is warranted)

Higher concentrations of food-specific IgE and larger skin test wheals correlate with an increased likelihood of a reaction upon ingestion. This principle has been applied in research studies to develop cut-off values that represent a very high (>95%) likelihood of a reaction. Predictive values for serum food-specific IgE levels and prick skin testing have been published for several of the major food allergens (cow's milk, egg, peanut, fish and walnut) to decrease the need for OFCs in selected patient populations (Sicherer et al., 2004; Sicherer, 2003) (Table 1).

Periodic follow-up with measurement of serum specific IgE levels and prick skin testing can help determine when oral food challenges would be appropriate utilizing the 95% predictive food-specific IgE levels. In follow up, the rate of decrease in food-specific IgE levels over time has been shown to have predictive value. (Shek et al., 2004) However, the laboratory test results need to be interpreted in the context of a clinical history of an individual patient and are never an absolute indication or contraindication to performing an OFC (Bindslev-Jensen et al., 2004). Serum food-specific IgE levels that are highly (90% to 95%) predictive of an allergic reaction on ingestion have been reported in children, primarily children with atopic dermatitis (Sampson, 2001). However, using 99% predictive values for deferring food challenges has also been advocated (Niggemann et al., 2005; Verstege et al., 2005) (Sampson, 2001). The mean diameter of the wheal on SPT has also been used for assessing the likelihood of a reaction, similarly based on selected patient populations (Verstege et al., 2005; Knight et al., 2006; Roberts and Lack, 2005). It must be appreciated that results of the "cut off" values have varied among studies (Table 1), which may be due to differences in the study populations (total IgE level, age range, presentation and severity of food allergy, degree of exposure to the food, duration of avoidance of the food at the time of testing) and methods of performing and interpreting the results of food challenges.

Unfortunately, the serum or skin test results are not accurately predictive of the nature or severity of reaction to the particular allergen. It is well established that the presence of food-specific IgE (i.e. sensitization) is not intrinsically di-

Table 1
Predictive values of specific IgE and prick skin testing for selected food allergens.

	>50% react	>95% react	>95% react (≤2 years of age)	References
Cow's milk	sIgE = 2 kIU/L	sIgE = 15 kIU/L SPT = 8 mm wheal	sIgE = 5 kIU/L SPT = 6 mm wheal	(Garcia-Ara et al., 2001; Perry et al., 2004b; Sampson, 2001) (Hill et al., 2004)
Hen's egg	sIgE = 2 kIU/L	sIgE = 7 kIU/L SPT = 7 mm weal	sIgE = 2 kIU/L SPT = 5 mm weal	(Perry et al., 2004b; Sampson, 2001; Boyano Martinez et al., 2001) (Hill et al., 2004)
Peanut	sIgE = 2 kIU/L (clear history) sIgE = 5 kIU/L (unclear history)	sIgE = 13–14 kIU/L SPT = 8 mm weal	SPT = 4 mm weal	(Perry et al., 2004b; Sampson, 2001; Maloney et al., 2008) (Hill et al., 2004)
Fish		20 kIU/L		(Sampson, 2001)
Walnut		18.5 kIU/L		(Maloney et al., 2008)

sIgE, food specific IgE level; SPT, skin prick test.

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agnostic of food allergy. Several studies have demonstrated that less than 40% of patients with positive PSTs or food-specific IgE have OFC-proven food allergy (Lemon-Mule et al., 2008). Additionally, reactions may occur in patients with negative test results presumably because the test may be missing some essential proteins or epitopes (Sicherer and Bock, 2006).

OFCs can be also used to establish the diagnosis of FPIES or to evaluate whether FPIES has been 'outgrown'. Follow-up challenges are usually recommended every 18–24 months in patients without recent reactions(Sicherer, 2005), although Korean investigators (Hwang et al., 2009) reported that majority of milk and soy-FPIES subjects developed tolerance by 10 months which they recommended as a suitable age for feeding test. Currently, there are no markers to indicate the probability of a positive OFC in FPIES or other food allergies of non-IgE-mediated etiology.

4. Biomarkers studied during OFCs to correlate with positive challenges

Whereas an anaphylactic reaction during an OFC is easily recognized and interpreted as a positive OFC, less subtle signs of an allergic reaction may pose a problem of interpretation. In clinical practice there are no markers of failed OFC that are used to differentiate negative from positive food challenges in IgE-mediated food allergy, but several approaches show potential. In order for a biomarker to be dependable and clinically useful it would need to come from studies with an independent, blind comparison with a reference standard and a patient sample including an appropriate spectrum of patients (Jaeschke et al., 1994). Furthermore, the reproducibility of the test would need to be satisfactory, and the test results would need to be applicable to the patient and result in improved patient care.

4.1. Markers of mast cell activation

In anaphylaxis, exposure to allergens leads to activation of the IgE receptor on mast cells or basophils. In mast cells, activation induces the release of preformed mediators such as histamine, tryptase, and chymase, as well as synthesis of cysteinyl leukotrienes, prostaglandin D_2, platelet-activating factor (PAF), and cytokines such as tumor necrosis factor α (TNF- α), although most of them are not involved in the induction of the observed allergic symptoms. To date, few mediators beyond histamine and tryptase, however, have been explored for their potential usefulness in supporting the clinical diagnosis of anaphylaxis (see below) and such markers have not been used to verify allergic reactions in routine OFCs. Recent studies on other granule mast cell mediators such as chymase and carboxypeptidase and acid arachidonic products such as prostaglandins and leukotrienes show these as potential markers of anaphylaxis (Nishio et al., 2005).

Currently, total tryptase level (pro, pro' and mature forms of α/ß mature tryptase) is most commonly measured to establish a diagnosis of anaphylaxis. Tryptase is a serine protease stored mainly in mast cell granules and is released from the granules at the onset of anaphylactic events. Tryptase levels increase immediately and peak at 1–2 h after the onset of anaphylaxis and return to baseline 24 h after resolution of symptoms. Levels are ideally obtained within 3 h of onset of symptoms and serial measurements may help establish a diagnosis of anaphylaxis. (Simons et al., 2007) In 19 cases of fatal anaphylaxis, elevated serum tryptase levels (12 ng/ml–150 μg/ml) were detected in 17 subjects, including 6 of 8 who died of food-induced anaphylaxis. Lack of tryptase elevation does not, however, rule-out the diagnosis of anaphylaxis, especially food-induced anaphylaxis. (Sampson et al., 1992; Sampson and Jolie, 1984). In a study by Sampson et al. (Sampson and Jolie, 1984) 4 out of 5 of patients with fatal and near-fatal food-induced anaphylaxis, in whom measurements were available, did not have detectable increases in serum tryptase. Sampson and colleagues also failed to demonstrate elevated tryptase levels in patients with symptoms of anaphylaxis undergoing food challenges even though samples were obtained in the ideal time frame (Sampson et al., 1992). There are several theories as to why tryptase levels are often not elevated in food-induced anaphylaxis (Simons et al., 2007). First, food-induced anaphylactic reactions tend to be slower in onset, more protracted and more likely to be biphasic as compared to anaphylaxis secondary to a systemic exposure, such as insect venom or intravenous medication. This may result in a slower release of tryptase and a decreased peak. Secondly, mucosal mast cells as well as those of the respiratory epithelium and alveolar wall (which are of MC_t type), the major effector cells in food-induced anaphylaxis, contain less tryptase compared to MC_{tc} mast cells found in skin, perivascular tissue, conjunctivae, heart, intestinal submucosa. Also, basophils, which do not contain tryptase, may play a significant role in food-induced anaphylaxis. Finally, tryptase may be quickly eliminated in some individuals. Nearly all of the α/ß tryptases spontaneously secreted by resting mast cells are in their pro form. Measurement of the mature ß-tryptase peaking at 1 hour which is elevated in hypotensive anaphylactic episodes and in hymenoptera sting anaphylaxis might also improve sensitivity (Schwartz et al., 2003; Caughey, 2006). However, measurement is not widely available.

Another laboratory marker of anaphylaxis is serum histamine. Histamine levels typically peak within 10 min of onset of symptoms and decrease to baseline by 60 min. This is not a clinically useful marker of anaphylaxis in the field as patients do not present to the emergency room in time to capture the histamine peak. However, urinary histamine metabolites remain elevated for up to 24 h after anaphylaxis and may be helpful in establishing the diagnosis.

Previously, poor correlation between histamine and total tryptase levels has been reported in individuals with nonhypotensive anaphylaxis, and it has been observed that measurement of both histamine and total tryptase improves sensitivity of testing and ability to confirm the clinical diagnosis of anaphylaxis (Lin et al., 2000). It might also be useful to measure a panel of other mast cell mediators to aid in the diagnosis as follows.

Chymase, a serine protease, is stored mainly in secretory granules of human mast cells and has been detected to be elevated in anaphylactic deaths. (Nishio et al., 2005) Because is it quite stable in serum and its level positively correlates with that of tryptase, further studies are required to determine if it could be useful markers for food-induced anaphylaxis (Brown et al., 2011).

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Mast cell carboxypeptidase A3 levels measured in serum or plasma are elevated (>14 ng/mL) in individuals with a clinical diagnosis of anaphylaxis, but not in healthy blood donors or individuals with asthma or other IgE-mediated allergic diseases. In patients with anaphylaxis, mast cell carboxypeptidase A3 and tryptase seem to appear at different rates in the circulation, and the serum levels of these mediators do not necessarily correlate with each other. Mast cell carboxypeptidase A3 levels remain elevated longer than total tryptase levels, and high serum carboxypeptidase A3 levels have been detected in individuals with clinically diagnosed anaphylaxis who did not have elevated total tryptase level and in drug-induced reactions (Brown et al., 2011; Zhou et al., 2006).

Platelet activating factor (PAF) is secreted by other cells such as macrophages and monocytes as well as by mast cells and basophils. PAF levels have been shown increased and PAF acetylhydrolase (PAF-AH), the enzyme that inactivates PAF, levels decreased in fatal cases of peanut-induced anaphylaxis as compared with healthy controls, patients with non-fatal peanut allergic reactions and non-anaphylactic fatalities (Vadas et al., 2008). It has not been assessed as a marker of OFCs.

4.2. Basophil activation

Analysis of the expression of basophil markers is known as the basophil activation test (BAT). These techniques offer interesting alternatives in the diagnosis of anaphylaxis. The basophil activation test provides important advantages in patients with anaphylaxis to beta-lactams, non-steroidal anti-inflammatory drugs, neuromuscular blocking agents and drugs where there is no technique otherwise available to measure specific IgE. In addition to confirming sensitization to allergen, this test is being assessed for its utility to confirm allergen challenge in allergic rhinitis (Saini et al., 2004) and might be useful in confirming the diagnosis of an allergic episode when performed on basophils collected within a few hours of onset of symptoms. The practical reason that this test is not widely used is that it requires fresh blood that is difficult to collect and ship to the laboratory in sufficient time (e.g, 1 to 2 days) for analysis.

4.3. Infrared thermography

Infrared thermography, which has been shown to detect small increases in temperature associated with positive SPTs, areas of atopic dermatitis, and histamine or allergen-induced rhinitis has been assessed as a novel, objective and sensitive indicator of challenge outcomes in egg challenges (Clark et al., 2007). In positive challenges, nasal temperatures showed an early transient rise at 20 min, irrespective of whether nasal symptoms were present or not, preceding objective symptoms by more than an hour, and was not seen in negative OFCs (91% sensitivity and 100% specificity and predicted challenge outcome in 96%). A short, low dose challenge using thermography to define outcome could be an attractive alternative to a full oral challenge, especially for subjects with severe allergy, where there is anxiety about large doses of allergen and risk of severe symptoms.

4.4. Leukocytosis and thrombocytosis

Leukocytosis and thrombocytosis have been reported in acute FPIES reactions(Mehr et al., 2009) and may be due to presence of pro-inflammatory cytokines and chemokines such as TNF-alpha. Leukocytosis with a left shift has long been recognized as a common finding for patients presenting with acute FPIES and has been included in the diagnostic criteria proposed by Powell (Powell, 1978). In the Powell study, peripheral blood neutrophil counts were elevated in all positive challenges, peaking at 6 h with a mean increase of 9900 cells/ml, confirmed by further studies (Mehr et al., 2009; Sicherer et al., 1998). Neutrophils have also been found in stool mucous of FPIES patients.

Thrombocytosis has also recently been reported in more than half of episodes(Mehr et al., 2009) and may be due to acute phase reactants such as IL-6 and other hematopoietic growth factors as seen in infections or due to endogenous epinephrine induced by stress (Nowak-Wegrzyn and Muraro, 2009).

In addition, Hwang et al. (Hwang et al., 2008) proposed gastric juice analysis as an additional confirmatory test in the equivocal oral challenges. Gastric juice leukocytes higher than 10 cells/ hpf were observed in 15 of 16 positive milk challenges after 3 h, including two infants without emesis or lethargy, whereas none of the eight age-matched control infants had gastric juice leukocytes over 10 cells/hpf. This observation needs to be validated in larger groups of patients.

Nowak-Wegrzyn and Muraro included increase in peripheral neutrophil count >3500 cells/ml peaking at 6 h, fecal leukocytes and eosinophils and/or gastric juice leukocytes >10 cells/hpf in the diagnostic criteria for FPIES (Nowak-Wegrzyn and Muraro, 2009).

5. Future diagnostic tests to better relate to outcomes

Although allergy tests correlate with the likelihood of reactivity to foods, there is great overlap in food-specific IgE concentrations between subjects who are reactive and those who are not. Furthermore, food-specific IgE concentration or skin test wheal size do not correlate with the severity of reactions (Steckelbroeck et al., 2008). Measurement for epitope-specific IgE against food allergens shows promise in better differentiating subjects with favorable outcomes of food allergy. It has been demonstrated that patients with persistent egg and milk allergy recognize a greater number of sequential (linear) egg- or milk-protein epitopes as compared with patients who had developed clinical tolerance to egg or milk ("outgrown" their food allergy) (Jarvinen et al., 2002, 2007). Microarray technology utilizing the presence and affinity of such epitope-specific IgE has been applied to identify those patients who will likely develop clinical tolerance versus those patients with persistent food allergy (Wang et al., 2010; Savilahti et al., 2010; Ayuso et al., 2010) and could be used to target patients for food challenges as well as for immunotherapeutic interventions in the future. It has also been shown that children possessing IgE antibodies directed at more numerous epitopes on major peanut allergens had history of more severe peanut-induced reactions than the children with IgE antibodies directed at fewer epitopes (Shreffler et al., 2004). In their study, greater

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diversity of recognized allergenic epitopes was associated with more efficient cross-linking of the IgE receptors and effector cells' degranulation.

Protein microarrays allow specific IgE against multiple molecules to be detected making molecular diagnosis or component-resolved diagnostics (CRD) possible. Nowadays, growing number of recombinant and purified molecules can be evaluated. The ISAC was the first protein microarray commerzialized by ThermoFischer Scientific (ImmunoCAP ISAC-CDR 112, ThermoFischer Scientific, ImmunoDiagnostics Division, Portage, MI). This approach may provide a diagnostic and prognostic platform for identifying subjects food allergy from those with sensitization based on cross-reactivity, prediction of severity of reactions based on sensitization to proteins with greater risk of severe reactions, and prediction of natural history (Sanz et al., 2011). Nicolaou et al. (Nicolaou et al., 2010) compared sensitization profiles between children with peanut allergy and peanut-tolerant children by using a microarray with 12 pure components (major peanut and potentially cross-reactive components, including grass allergens). By performing oral challenges, they estimated the prevalence of clinical peanut allergy among sensitized subjects as 22.4%. The peanut component Ara h 2 was the most important predictor of clinical allergy. In subjects with egg and milk allergies, the performance characteristics have not so far been found superior to those of existing methodologies (Fiocchi and Nowak-Wegrzyn, 2011). However, it has been reported that in children with milk and/or egg allergy the ISAC CRD-89 (Phadia, Uppsala, Sweden) could be used as an additional assay if the ImmunoCAP sIgE is less than 95% clinical decision point (D'Urbano et al., 2010). Parallel determination of different antibody isotypes using microarrays also allows for determination of mean allergen-specific IgE/IgG4 levels, which were shown to be higher in patients with positive DBPCFCs to milk, egg, wheat, and soybean than in those with negative OFCs (Noh et al., 2007).

Measurement of food-specific IgG levels has not been proven in the diagnosis of allergy and its use has been discouraged (Boyce et al., 2010). Specific IgG antibody production reflects antigen exposure and is a normal physiologic phenomenon to ingestion of food proteins (Barnes, 1995). Specific IgG levels of egg- or peanut-allergic and healthy controls overlap (Barnes, 1995; Tay et al., 2007). Hence, food-specific IgG measurements do not appear to be of diagnostic nor prognostic value. Among IgG subclasses, allergic children have been shown to have higher levels of food-specific IgG1 and IgG4 to peanut, milk and egg than age-matched healthy children (Scott-Taylor et al., 2010 Scott-Taylor et al., 2010), although this was disputed by another study (Ito et al., 2012). Despite the correlation of high IgG4 with food allergy, IgG4 has no pathological role in food allergy. However, a decline in the ratio of IgG1 to IgG4 was inverted with subjects with resolution of food allergy (Scott-Taylor et al., 2010). In another study, the concentrations of IgG1- and IgG4-specific antibody and the IgE/IgG1 and IgE/IgG4 ratios for milk proteins were significantly less in the patients losing milk allergy (James and Sampson, 1992). Monitoring these ratios may therefore have some prognostic value in the natural history of food allergy. Consistently, it has been observed that an increase in the intensity of IgG4 binding to cow's milk epitopes occurred concurrently with a decrease in IgE-binding intensity among patients who recovered early from cow's milk allergy (Savilahti et al., 2010). Food-specific IgG4 antibodies increase during successful immunotherapeutic interventions for food allergy (see below).

6. Immunological biomarkers used in monitoring oral or sublingual immunotherapy efficacy

A recent study on peanut immunotherapy provides insight into which biomarkers could be used to monitor the efficacy of oral (or sublingual) immunotherapy (Jones et al., 2009). By 6 months of OIT, titrated skin prick tests and activation of basophils decreased significantly. Peanut-specific IgE decreased by 12 to 18 months whereas IgG_4 increased significantly. Serum factors inhibited IgE-peanut complex formation in an IgE-facilitated allergen binding assay. Secretion of IL-10, IL-5, IFN-gamma, and TNF-alpha from peripheral blood mononuclear cells increased over a period of 6 to 12 months. Peanut-specific forkhead box protein-3 (Fox P3) –positive T cells increased until 12 months and decreased thereafter. In addition, T-cell microarrays showed down-regulation of genes in apoptotic pathways. Measurement of food-specific IgG4 in addition to IgE may provide additional insights on the probability of tolerance.

7. Summary

The OFC is an invaluable tool in the initial diagnosis and follow-up of natural history of food allergy. Higher concentrations of food-specific IgE and larger skin test wheals correlate with an increased likelihood of a reaction upon ingestion and provide a basis for clinical decision points to predict the likelihood of reactivity. Unfortunately, the diagnostic utility of the skin and serum tests is limited. Furthermore, there are currently no markers to predict the probability of a positive food allergy in non-IgE-mediated food allergy and food allergy of mixed etiology. In an OFC, subtle and/or subjective signs of an allergic reaction may pose a problem of interpretation, although DBPCFC is the least subjective to bias and is the gold standard for food allergy diagnosis in research and in selected clinical settings. Mast cell mediators, basophil activation and facial thermography have been assessed as objective and sensitive indicators of challenge outcome, but none are currently validated for clinical practice. Measurement of presence and affinity of epitope-specific IgE as well as component resolved diagnostics may provide a diagnostic and prognostic platform for differentiating subjects with food allergy from those with sensitization based on cross-reactivity, for prediction of severity of reactions based on sensitization to proteins with greater risk of severe reactions, and prediction of natural history.

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