

Food Allergy to Proteins

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Abstract

Food allergy is defined as an immune system-mediated adverse reaction to food proteins. Class 1 food allergens are represented by peanut, egg white, and cow's milk; they are heat- and acid-stable glycoproteins that induce allergic sensitization via gastrointestinal tract and cause systemic reactions. Class 2 food allergens are homologous to proteins in birch tree pollen and class 2 food allergy develops as a consequence of respiratory sensitization to the cross-reactive pollen. Class 2 food allergens are very heat-labile and tend to induce reactions limited to oral allergy symptoms. In contrast, plant nonspecific lipid transfer proteins are resistant to heating and tend to induce systemic reactions. Analysis of IgE-binding epitopes with SPOT membranes revealed that cow's milk-, egg- and peanut-allergic subjects without IgE antibodies against certain sequential epitopes of the major allergens were more likely to achieve tolerance than subjects whose IgE antibodies were directed against those epitopes. Subsequently, peptide microarray showed a correlation between reaction severity and the intensity of IgE binding and the number of epitopes recognized of patients' immune responses against peanut allergens. Taken together, these data suggest that the epitope recognition pattern and intensity of IgE binding are important determinants of severity and duration of food allergy.

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Introduction

Food allergy is defined as an immune-mediated adverse reaction to food protein [1]. Nonimmune adverse food reactions (i.e. lactose intolerance) can mimic food allergy but their pathophysiology and treatment are distinctly different from those of food allergy (table 1). It is estimated that 6–8% of young children and 3.5% of adults in the USA have food allergy; a similar prevalence was reported from many Western European countries [2]. Almost any food can cause an allergic reaction, but more than 90% of food allergy in infants

Table 1. Adverse reactions to foods mimicking food allergy

Condition	Symptoms	Mechanism	Treatment
Lactose intolerance	Bloating, abdominal pain, diarrhea (dose dependent)	Lactase deficiency	Lactase replacement or lactose-free milk
Pancreatic insufficiency	Malabsorption	Deficiency of pancreatic enzymes	Enzyme replacement
Food poisoning	Pain, fever, nausea, emesis, diarrhea	Bacterial toxins in food	Supportive
Scombroid fish poisoning	Flushing, angioedema, hives, abdominal pain	In spoiled fish histidine is metabolized to histamine	Supportive
Auriculotemporal syndrome (Frey's syndrome)	Facial flush in trigeminal nerve distribution associated with spicy foods	Neurogenic reflex, frequently associated with birth trauma to trigeminal nerve (forceps delivery)	None
Gustatory rhinitis	Profuse watery rhinorrhea associated with spicy foods	Neurogenic reflex	Avoidance of spicy food
Panic disorder	Subjective reactions, fainting upon smelling or seeing the food	Psychological	Pharmacologic treatment

and young children are caused by cow's milk, egg, peanut, soybean, wheat, tree nuts, fish and shellfish. In adults, shellfish, peanut, tree nuts and fish are most common, indicating that these food allergies are rarely outgrown by children. Allergies to cow's milk, egg, wheat and soy are typically outgrown by age 3–5 years; in contrast, peanut allergy may resolve by age 5 years in only about 20% of children [3, 4].

A recent US twin study estimated heritability of peanut allergy at 81%, but genetic factors alone cannot explain doubling of peanut allergy prevalence in the past two decades [5]. Peanut allergy increased up to 0.8% in the USA, UK and Canada, suggesting that environmental factors influence the expression of food allergy. The exact mechanisms by which the environment contributes to the development of food allergy are unclear; however, promoting peanut butter as healthy nutrition for pregnant and lactating women and for young children (increased exposure), presence of food ingredients in skin care

preparations (cutaneous sensitization), and liberal use of antacids (lowering gastric pH and decreased ability to digest food proteins) have been proposed as potential culprits.

Pathophysiology

Food allergy results from failed oral tolerance that is characterized by suppression of immune responses toward food proteins by the gut-associated lymphoid tissue [6].

In the past two decades many of the food allergens were identified and characterized, contributing to our understanding of how these proteins induce Th2-skewed immune responses. Traditional or class 1 food allergens induce allergic sensitization in the gastrointestinal tract and are responsible for systemic reactions. Recent data from experimental studies in mice as well as from epidemiological reports in humans suggest that cutaneous exposure to class 1 allergens (e.g. through inflamed skin of atopic dermatitis, AD) may also contribute to the development of allergy. Class 1 food allergens are typically heat- and acid-stable, water-soluble glycoproteins ranging in size from 10 to 70 kD, such as proteins in cow's milk, egg white, and peanut. In contrast, class 2 food allergens are heat-labile and susceptible to digestion. Class 2 food allergens are highly homologous with proteins in pollens (e.g. Mal d 1 in apple and Bet v 1 in birch tree pollen) and sensitization occurs in the respiratory tract as a consequence of sensitization to the cross-reactive pollen allergens (oral allergy syndrome) [7]. Class 2 food allergy affects approximately 50% of adults with birch tree pollen-allergic rhinitis. Cooking can reduce the allergenicity of fruits and vegetables by destroying conformational allergenic epitopes of pollen-homologous allergens. In contrast, high temperatures (e.g. roasting) can increase allergenicity of certain allergens such as peanut through the induction of glycosylated end-products and covalent binding that leads to new antigens or improved stability [8] (table 2).

In recent years, plant nonspecific lipid transfer proteins (nsLTPs) were identified as major allergens in fruits and vegetables [9]. In contrast to pollen-related food allergy, sensitization to plant food nsLTPs occurs independently of birch pollinosis and frequently results in systemic and severe reactions. Plant LTPs belong to the prolamin superfamily and possess eight conserved cysteines that are stabilized by four intra-chain disulfide bonds. The compact structure of nsLTPs renders them remarkably stable by virtue of resistance to high temperature and relative inaccessibility to proteases such as pepsin and trypsin. LTPs were shown to retain their allergenicity in processed foods such as pasteurized peach juice, beer, baked or boiled apple and fermented products such as wine. LTPs are also abundant in the skin and peel of fruits and vegetables whereas they are present in significantly lower concentrations in the pulp. In view of the high prevalence of sensitization to nsLTPs in the

Table 2. Factors determining Th2 immune responses to food proteins

Factor	Examples
<i>Food protein-specific</i>	
Stability to high temperatures	Roasting of peanuts results in formation of Ara h 1 trimers of increased allergenicity and increases in Ara h 2 trypsin inhibitory activity; retained allergenicity of nsLTPs in plant foods
Stability to low pH and proteases	Egg ovomucoid and peanut Ara h 2 have trypsinogen inhibitor activity; nsLTPs are resistant to pepsin and trypsin due to their compact structure
Adjuvant activity	Ara h 1 from peanut is a ligand for DC-SIGN and may skew dendritic cells toward promoting Th2 responses
Homology to other food allergens	50% cross-reactivity among tree nuts
Homology to environmental allergens	Rosaceae fruits (apple, peach, almond, plum) cross-reactive with birch tree pollen major allergen Bet v 1 Alpha-livetin in egg yolk cross-reactive with bird proteins (chicken serum albumin) in bird egg syndrome in people exposed to birds (parakeets, pigeons) Avocado, chestnut, banana, kiwi, papaya, fig, melon, passion fruit, pineapple, peach, and tomato cross-reactive with latex
Environmental exposure to high levels of food proteins	Occupational bakers' asthma to inhaled wheat flour or to egg proteins; egg-egg syndrome in which adults previously egg-tolerant develop symptoms upon egg ingestion following development of egg asthma in the occupational setting
<i>Host specific</i>	
Atopy	Tendency to generate IgE antibody responses to non-pathogenic environmental and food proteins
Increased intestinal permeability	Developmental (infants, young children) Viral gastrointestinal infections Drugs: tacrolimus, aspirin Alcohol Exercise (decreased splanchnic blood flow)
Exercise	Activation of tissue transglutaminase and formation of high-molecular-weight complexes of n-5 gliadin (an alcohol-soluble fraction of gluten) that have increased allergenicity
Antiacids	Development of IgE to hazelnut and clinical reactions to ingestion of hazelnut in adults receiving antacid therapy

Mediterranean countries where no birch tree pollen is present, sensitization to nsLTPs likely occurs via the gastrointestinal route.

Considering that most food allergens are glycoproteins, attention has been turned to the role of food carbohydrate moieties in Th2 skewing. Complex carbohydrates are potent inducers of Th2 responses, and carbohydrate antigens can stimulate the production of different classes of glycan-specific antibodies,

including Th2-associated IgG and IgE. Biological effects of carbohydrate antigens are dependent on the recognition of these antigens by carbohydrate-binding proteins (lectins). Cell-surface C-type lectin receptors, such as DC-SIGN, L-SIGN, the mannose receptor, macrophage galactose-binding lectin, and other lectins, such as the soluble collectins and galectin-3, recognize particular glycan antigens of schistosomes and allergens. Shreffler et al. [10] proposed that the major allergen from peanut, Ara h 1 may be a ligand for DC-SIGN on dendritic cells. DC-SIGN bound to a 65-kD protein from peanut extract in a calcium-dependent manner, whereas there was no precipitation of proteins from chemically deglycosylated peanut extract. Mass spectrometry confirmed that DC-SIGN ligand from peanut was Ara h 1. Ara h 1 activated human dendritic cells as measured by phenotype and T cell stimulation. These preliminary results suggest that Ara h 1 is a ligand for DC-SIGN and may play a role in differentiating dendritic cells to promote Th2 responses to peanut.

IgE antibodies produced by B cells may be directed at sequential epitopes comprised of sequential amino acids, or conformational epitopes comprised of amino acid residues from different regions of the allergen brought together by folding of the protein. Since food allergens are subjected to extensive chemical and proteolytic digestion prior to absorption and uptake by the cells of gut-associated lymphoid tissue, it has been assumed that in class 1 food allergy, immune responses are directed against sequential epitopes [1]. However, analysis of IgE-binding epitopes with the use of SPOTs membrane technology revealed that cow's milk-, egg- and peanut-allergic subjects who lacked IgE antibodies against certain sequential epitopes of the major allergens were more likely to achieve tolerance to these foods than subjects whose IgE antibodies were directed against those epitopes [11–14] (table 3).

In subsequent studies, Shreffler et al. [15] at the Jaffe Food Allergy Institute at Mount Sinai School of Medicine in New York utilized peptide microarray to characterize humoral responses to major peanut allergens. Reaction severity in patients correlated with the heterogeneity (intensity of IgE binding and number of epitopes recognized) of their immune responses against peanut allergens. In vitro sensitization of effector cells (basophils and rat basophil leukemia cell line SX38 transfected with human Fc ϵ RI) with more diverse IgE antibodies conferred greater reactivity to specific allergens. Taken together, these data suggest that the epitope recognition pattern (conformational vs. sequential, number of epitopes recognized) as well as intensity of IgE binding are important determinants of severity and duration of food allergy.

Classification of Food Allergy Disorders

Food allergy disorders may be classified based on the role of IgE antibody as IgE-mediated, non-IgE-mediated (cell-mediated) and mixed-IgE- and cell-mediated (table 4).

Table 3. IgE epitope recognition patterns in subjects with allergy to cow's milk, egg white and peanut

Study	Allergen	Patient population and methods	Results
Jarvinen et al. [11]	Cow's milk	10 patients with persistent CMA and 10 patients who subsequently outgrew their milk allergy; 25 decapeptides of α (s1)-casein, α (s2)-casein, κ -casein, α -lactalbumin, and β -lactoglobulin, comprising the core epitopes, synthesized on a SPOTs membrane; sera from individual patients were used for immunolabeling	Five IgE-binding epitopes (2 on α (s1)-casein, 1 on α (s2)-casein, and 2 on κ -casein) were not recognized by any of the patients with transient CMA but showed binding by the majority of the patients with persistent allergy. The presence of IgE antibodies against at least 1 of 3 epitopes (AA 123–132 on α (s1)-casein, AA 171–180 on α (s2)-casein, and AA 155–164 on κ -casein) identified all patients with persistent CMA
Jarvinen et al. [14]	Egg white	11 children with transient and 7 children with persistent egg allergy; the central decapeptides from each of the major IgE-binding epitopes of ovomucoid synthesized on a SPOTs membrane; immunolabeling was done with individual patients' sera	Both groups had a comparable range of egg-specific IgE levels, but none of the patients with transient egg allergy had IgE antibodies against these epitopes of ovomucoid: AA 1–10, 11–20, 47–56, and 113–122. In contrast, all 7 patients with persistent egg allergy recognized at least 4 of these immunodominant epitopes
Beyer et al. [12]	Peanut	15 patients with symptomatic peanut allergy and 16 patients who were sensitized but tolerant. Ten of these 16 patients had 'outgrown' their allergy. Eight peptides representing the immunodominant sequential epitopes on Ara h 1, 2 and 3 synthesized on SPOTs membranes and immunolabeled with individual patients' sera	Regardless of their peanut-specific IgE levels, at least 93% of symptomatic, but only 12.5% of tolerant patients, recognized 1 of the 'predictive' epitopes on Ara h 1 or 2. The cumulative IgE binding to the peanut peptides was significantly higher in patients with peanut allergy than in tolerant patients

Shreffler et al. [15, 41]	Peanut	77 patients with peanut allergy and 15 controls; overlapping 20-amino acid peptides covering the entire sequence of Ara h 1, 2 and 3 were used for microarray immunoassay	The majority of patients (97%) had specific IgE to at least one of the recombinant allergens, and 87% had detectable IgE to sequential epitopes. The analysis of individual patients revealed remarkable heterogeneity in the number and patterns of epitope recognition. High epitope diversity was found in patients with a history of more severe reactions
Lewis et al. [42]	Peanut	40 peanut-allergic patients underwent DBPC low-dose OFC to peanut; serum peanut IgE (CAP-FEIA) and IgE-binding patterns (Western blot) to peanut proteins were analyzed	Seventeen IgE-binding bands were identified between 5 and 100 kD with 8 bound by >50% of patients. The total number of bands correlated significantly with OFC score and peanut IgE. Cluster analysis failed to reveal any association between particular protein or pattern of proteins

AA = Amino acid; CMA = cow milk allergy; DBPC = double-blind placebo-controlled; OFC = oral food challenge.

Table 4. Classification of food-allergic disorders based on the role of IgE antibody in the pathophysiology

Disorder	IgE-mediated	Mixed mechanism, IgE- and cell-mediated	Non-IgE-mediated
Generalized	Anaphylactic shock, food-dependent exercise-induced anaphylaxis		
Cutaneous	Urticaria, angioedema, flushing, morbilliform rash, acute contact urticaria	AD Contact dermatitis	Dermatitis herpetiformis
Gastrointestinal	Oral allergy syndrome, immediate gastrointestinal food allergy	AEE AEG	Allergic proctocolitis Food protein-induced enterocolitis syndrome Celiac disease Infantile colic
Respiratory	Acute rhinoconjunctivitis, bronchospasm	Asthma	Pulmonary hemosiderosis (Heiner's syndrome)

AD = Atopic dermatitis; AEE = allergic eosinophilic esophagitis; AEG = allergic eosinophilic gastroenteritis.

IgE-Mediated Food Allergy

Anaphylaxis represents the most severe form of IgE-mediated food allergy and is clinically defined as a food-allergic reaction involving two or more organ systems [16]. Symptoms start within seconds to 1–2 h following the ingestion and include feelings of ‘impending doom’, throat tightness, coughing or wheezing, abdominal pain, vomiting, diarrhea, and loss of consciousness. Cutaneous symptoms of flushing, urticaria, and angioedema are present in the majority of the anaphylactic reactions; however, the most rapidly progressive anaphylaxis may involve no cutaneous manifestations.

Peanut, tree nuts (i.e. almond, cashew, hazelnut, pecan, and walnut), fish, and shellfish are most often responsible for food-induced anaphylaxis in the USA.

Acute urticaria and angioedema are the most common manifestations of acute allergic reactions to ingested foods in children. Onset of symptoms may be rapid, within minutes of ingesting the responsible food. Skin involvement may be isolated or associated with other organ systems in food anaphylaxis. Acute IgE-mediated urticaria can be induced by skin contact with cow’s milk allergy, raw egg white, raw meats, fish, vegetables and fruits. Skin contact

reactions are typically local in nature but oral mucous membranes (e.g. kissing) or conjunctiva (e.g. eye rubbing) contact may lead to generalized reactions [17, 18].

Mixed-IgE-Mediated and Cell-Mediated Food Allergy Disorders

Food allergy is frequently seen in children with atopic dermatitis (AD). AD is a chronic inflammatory disease of the skin characterized by marked pruritus and a remitting and relapsing course. In a study of 64 patients with moderate to severe AD referred to a pediatric dermatologist in a tertiary medical center who underwent double-blind placebo-controlled food challenges, 35–40% of children were allergic to at least one food [19]. In adults with birch pollen sensitivity, ingestion of birch pollen-related foods (e.g. apple, carrot, celery) causes immediate and/or late eczematous reactions [20, 21]. Strict elimination of the causative food allergen results in significant improvement in dermatitis [19, 22].

Allergic eosinophilic esophagitis (AEE) and gastroenteritis (AEG) are characterized by infiltration of the gastrointestinal tract with eosinophils. Both, T lymphocytes and food-specific IgE antibodies are implicated in a subset of patients, especially infants and children; the role of food allergy in adults with AEE/AEG is controversial. Symptoms correlate with the extent of eosinophilic infiltration of the bowel wall [23]. AEE is seen most frequently in infants, children and adolescents and presents with symptoms of gastroesophageal reflux, such as nausea, dysphagia, emesis and epigastric pain, that fail to resolve with standard antireflux therapy. Patients typically have a negative pH probe; on esophageal biopsy more than 10–20 eosinophils per 40× high-power field are seen [24]. AEG can occur at any age, including young infants. Failure to thrive is common; in young infants AEG may cause gastric outlet obstruction with pyloric stenosis. Patients also present with abdominal pain, emesis, diarrhea, blood loss in the stool, anemia, and protein-losing gastroenteropathy.

Up to 50% of patients with these eosinophilic disorders are atopic and have detectable IgE sensitization to one or more foods; however, food-induced IgE-mediated immediate reactions are uncommon. Results of skin prick tests and serum allergen-specific IgE antibody tests correlate poorly with response to elimination of the food and thus must be interpreted with caution. Resolution of symptoms typically occurs within 3–8 weeks following the elimination of the responsible food allergen, frequently multiple foods, most commonly: cow's milk, soy, wheat, and egg. Because patients with AEE and AEG can react to single small peptides and trace amounts of the offending foods and testing may fail to identify all relevant allergens, a diet based on an amino acid formula may be necessary to achieve improvement [25–27].

Non-IgE-Mediated Gastrointestinal Food Allergy Disorders

Allergic proctocolitis typically starts in the first few months of life, with blood-streaked stools in otherwise healthy-looking infants and is considered a

major cause of colitis under age 1 year [28]. Unlike other forms of gastrointestinal food hypersensitivity, proctocolitis is highly prevalent in breastfed infants, with more than 50% of infants in the published reports being exclusively breastfed. Food protein-induced proctocolitis is typically caused by cow's milk and soybean protein. Pathologic findings are limited to the colon and include focal acute inflammation with epithelial erosions and eosinophilic infiltration of the lamina propria, the epithelium and lamina muscularis. Most infants respond well to casein hydrolysate and only few require amino acid-based formulas. After 9–12 months of age, the infants typically tolerate an unrestricted diet.

Food protein-induced enterocolitis syndrome (FPIES) is most frequently seen in young infants who present with irritability, protracted vomiting, and diarrhea [29]. Vomiting generally occurs 1–3 h after feeding but continued exposure may result in bloody diarrhea, anemia, abdominal distention, and failure to thrive. FPIES is typically caused by cow's milk or soy-based formula but other foods such as grains (rice, oat), meats (turkey, chicken) and vegetables (pea) were reported [30, 31]. Patients rapidly recover with avoidance diets, but ingestion of the offending food proteins following a period of dietary elimination triggers subacute symptoms (median, 2 h) with an associated elevation of the peripheral blood polymorphonuclear leukocyte count.

Infantile colic is defined as unexplained paroxysms of irritability, fussing or crying that persist for more than 3 h per day, for more than 3 days per week and for at least 3 weeks. Prevalence of infantile colic has been recently estimated at 5–19%. Several studies demonstrated improvement of colic symptoms in a subset of babies fed with soy-based and extensively hydrolyzed hypoallergenic infant formulas, suggesting a possible role of an underlying transient hypersensitivity to one or several foods [32, 33].

It has been recently appreciated that up to 50% of gastroesophageal reflux symptoms in infants younger than 1 year is caused by hypersensitivity to dietary food proteins, mainly cow's milk and soybean [34].

Diagnosis

A careful medical history is crucial; however, it needs to be validated by laboratory tests and oral food challenges (OFCs), especially in chronic disorders such as AD or AEG. In such remitting and relapsing disorders, accurate identification of the offending food on the basis of history is particularly difficult and sometimes impossible [19].

Skin prick testing with commercial food allergen extract has a high negative predictive value >95%, whereas a positive skin test has only an average 50% positive predictive value. In infants and young children, a large skin prick test wheal (mean size 8–10 mm) is associated with a high >95%

likelihood of clinical reactivity to cow's milk, egg and peanut, confirmed by an OFC [35].

A number of laboratory immune assays (RAST, CAP system) have been developed for the detection of allergen-specific IgE antibody in the bloodstream. These assays have a similar performance to skin tests in that a negative test (specific IgE antibody <0.35 kIU/l measured by Pharmacia CAP system) has a high negative predictive value $>95\%$. Clinical decision points indicating $>95\%$ likelihood of reaction were established for the most common food allergens, including milk, egg, peanut, tree nuts, and fish [36]. For example, a child older than 2 years with milk IgE antibody level ≥ 15 kIU/l is highly ($>95\%$) likely to react during an oral milk challenge. Food-specific IgE antibody levels below the decision points indicate a decreasing likelihood of reaction that needs to be determined with OFC. Currently available diagnostic tests for IgE-mediated food allergy do not predict severity of reactions and chances of resolving food allergy with time. Considering data from studies in peanut, cow's milk and egg allergy on specific epitope recognition patterns correlating with severity of reactions and persistence of food allergy, these questions may be answered with novel approaches to diagnosis of food allergy that is based on a peptide microarray. This technique utilizes minute amounts of patient sera and can be highly automated; time and labor efficient. Hopefully peptide microarray will become incorporated into the clinical practice in the near future and allow for more precise and individualized diagnosis of food allergy.

Skin prick test and measurement of serum food IgE antibody concentration are not helpful in food allergy disorders with cell-mediated mechanism, such as FPIES, and have limited usefulness in disorders with mixed mechanism, such as AEE and AEG. Recently patch testing for the diagnosis of food allergy in children with AD and AEE has been investigated in a number of studies. Patch testing is typically used for the diagnosis of delayed contact hypersensitivity reactions in which T cells play a prominent role. In children with challenge-proven milk allergy, skin prick tests were positive in 67% of the cases with acute-onset reactions (under 2 h) to milk challenge, whereas patch tests tended to be negative [37]. Patch tests were positive in 89% of children with delayed-onset reactions (25–44 h), although skin prick tests were frequently negative. These results indicate that a combination of patch testing and detection of IgE could enhance the accuracy of the diagnosis of food allergy and eliminate the need for OFCs.

For gastrointestinal food allergy disorders such as AEE and AEG, the ultimate diagnosis is established by sampling of the mucosa and finding increased numbers of eosinophils. Noninvasive diagnostic tests are highly desirable but currently available laboratory techniques (e.g. peripheral eosinophil count, serum albumin and total protein level, fecal occult blood, fecal α_1 -antitrypsin) offer limited insight into these conditions. Experimental tests for AEE/AEG include peripheral T lymphocyte proliferation assays,

cytokine release upon food stimulation, inflammatory cytokines (interleukin-4, TNF- α) in serum and stool, as well as markers of eosinophil activation in stool (e.g. eosinophilic cationic protein). These tests require further evaluation and standardization before introduction into clinical practice.

Oral Food Challenges

OFCs remain the most accurate method for diagnosing food allergy, for both IgE-mediated as well as for non-IgE-mediated food allergy and for determining the threshold dose of food. Many protocols were developed; in one approach, during an OFC for an IgE-mediated food allergy, a premeasured amount of food (typically 8–10 g of dry food or 80–100 ml of liquid food) mixed with a masking food is administered in small increments every 10–15 min over 90 min. In a placebo-controlled challenge, two sessions (one with real food, one with placebo food) are separated by a 90-min break and completed on a single day or each session may be done on separate days. Double-blind, placebo-controlled food challenge is considered a gold standard for the diagnosis of food allergy and is preferred in the research setting. OFCs are stopped at the first sign of an objective reaction such as hives, rhinorrhea, sneezing, coughing, or vomiting. OFCs are always conducted under physician supervision in a controlled environment. Patients with AEE or AEG, whose food-induced symptoms are delayed and more insidious, may require prolonged challenges over several days.

Management of Food Allergy

Management of food allergy currently focuses on avoidance, prompt recognition and treatment of food-allergic reactions, and nutritional support.

Avoidance of food allergens focuses on dietary avoidance but attention must also be paid to exposure via skin (e.g. peanut oil in cosmetics), mucous membranes (e.g. kissing) or inhalation (e.g. peanut dust, steaming milk or fish). Accidental reactions are common; in children with peanut allergy, 50% reported reactions to peanuts despite avoidance over a 2-year period [38]. Individuals with a history of immediate allergic reactions, anaphylaxis, those with asthma, and those with allergy to foods typically associated with severe reactions (i.e., peanut, tree nuts, fish, shellfish) should be prescribed an epinephrine self-injector.

Children with food allergy, particularly those with multiple food allergies, are at risk of nutritional protein and calorie deficiency due to restricted diets and may require a hypoallergenic formula. Hypoallergenic formulas available in the US are either based on extensively hydrolyzed casein derived from cow's milk (Pregestimil, Nutramigen, Mead & Johnson; Alimentum, Ross) or

on a mixture of single amino acids (Neocate, SHS; Elecare, Ross). Hypoallergenic formulas are well tolerated by children with IgE-mediated and with cell-mediated food allergy [25, 27]. Hypoallergenic formulas are also recommended for prophylaxis of food allergy in infants at risk of atopy.

Based on the observation that children with IgE-mediated immune responses directed predominantly at conformational epitopes are more likely to outgrow egg and cow's milk allergy, clinical trials of diets containing baked egg and baked milk (in which conformational epitopes are destroyed by high temperatures) are underway at the author's institution. Children undergo OFC to baked egg/milk to confirm tolerance and are followed prospectively for maximum 48 months or until they achieve tolerance to uncooked egg/milk. Clinical (weight, body fat, intestinal permeability, symptoms of AD, asthma, rhinitis, acute allergic reactions) and immunological parameters (allergen-specific IgE and IgG4 antibody levels, skin prick test) are monitored. The inclusion of baked egg/milk products results in substantial liberalization of the diet and improved nutrition. In addition, ingestion of baked egg/milk might promote tolerance and resolution of egg/milk allergy.

Oral immunotherapy for milk allergy and sublingual immunotherapy for hazelnut allergy have been reported but it is unclear whether such therapies result in a transient state of desensitization or permanent oral tolerance [39, 40].

The most promising future approaches to food allergy therapy include anti-IgE monoclonal antibody (TNX-901), Chinese herbs, vaccines containing heat-killed *Escherichia coli* expressing modified peanut proteins, and chimeric molecules with allergen and Fc γ . Considering a variety of approaches that are being investigated, effective prophylaxis as well as potentially curative therapy for food allergy seem to be within reach and bring hope to patients for whom no effective therapy is currently available.

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Discussion

Dr. Lentze: When you talk about the nonimmunological food allergens you mention a disease called fructose deficiency. I think you probably mean sucrase isomaltase deficiency because we don't have an enzyme called fructase.

Dr. Nowak-Wegrzyn: This is correct, thank you.

Dr. Wahn: You were explaining to us how important the IgE pattern is with regard to the epitopes of the allergen as far as the prognosis is concerned. We usually consider cow's milk allergy as an infantile allergic manifestation to food but occasionally it may become manifest only in adulthood. My personal prejudice is that the prognosis in adults is much worse than in children. They will never outgrow it apparently. Is there anything known about the epitopes relevant in adults? I always felt that casein plays more of a role.

Dr. Nowak-Wegrzyn: I am not aware of any studies that have been done on epitope recognition in adults with food allergy. All the data are from children and I agree with you that there could be different epitopes in adults. In adult food allergy mechanisms may be different. The primary failure to develop oral tolerance due to immaturity of the gastrointestinal tract, the inability to break down the proteins and increased intestinal permeability that is implied in childhood food allergy are unlikely to underlie food allergy in adults. So if somebody develops allergy at an older age there must be special circumstances. An example that comes to mind in adults is in the setting of heavy occupational exposure. For instance people who work in bakeries and are exposed by inhalation to aerosolized wheat may develop so-called baker's asthma, but some of them may go on to develop symptoms following ingestion of wheat. I don't think anybody is looking at the epitopes specific to adult food allergy and it definitely would be very interesting to see whether those are different.

Dr. Vandenas: There are large differences in the prevalence of eosinophilic esophagitis between North America and Europe. You mentioned that 50% of the refluxing babies have eosinophilic esophagitis. If I exaggerate a little bit, I could say it does not exist in Western Europe. Certainly in our center, we are below 5%. Knowing that feeding is the same, reflux medication is the same, and that the condition is relatively easy to diagnose, does it not mean that 'environment' is by far the most important factor and that all the other factors we are studying are in fact only of minor importance? Can you speculate about that?

Dr. Nowak-Węgrzyn: Actually I did not include gastroesophageal reflux in the same category as allergic eosinophilic esophagitis (AEE). These are two separate disorders. AEE is an example of a mixed pathogenesis food allergy, and it is more common in older children, adolescents especially with pollen allergy [1]. The comment on gastroesophageal reflux was made in reference to non-IgE-mediated food allergy, specifically cow's milk allergy in infants younger than 1 year. We don't necessarily have biopsy data from those young infants to confirm that indeed it has anything to do with AEE. The studies that have focused on AEE emphasized that although the symptoms are similar, the patients with AEE fail to respond to standard antireflux therapy but improve with amino acid-based elemental formula [2, 3]. You made a very good point; there are animal models that show that inhalation of pollen produces AEE, and there are reports that show that patients with this disorder suffer exacerbation in the high pollen season, so there may be an environmental component involved [4, 5].

Dr. B. Koletzko: In your paper you referred to different methodologies or heat treatment and changes of antigen. In the table you presented you also refer to proton pump inhibitor treatment in relation to hazelnut allergy in adults. You are probably aware of the Vienna studies both on proton pump inhibitors and allergy. I wonder whether you have any comments to offer on the plausibility of the concept and the mechanisms behind it. Is it likely that this observation is simply related to an acid-induced denaturation of food allergens that reduce the allergenicity? If that would be so, is there also a potential for therapeutic or preventive use, for example considering fermentation of foods where acidity is somewhat enhanced even though to a lesser degree than in the fasting stomach? Are there any data that a fermented cow's milk product offers a lower allergy risk?

Dr. Nowak-Węgrzyn: There are data from animal models that if you use antacids the animals are more likely to develop hazelnut IgE antibody [6]. Adult patients with reflux have documented a new development of IgE antibody and the clinical reactivity to hazelnut [7]. I think that it is plausible; in the animal models when we encounter difficulties with sensitizing animals we actually add antacids to decrease the gastric pH. How close we are to using this as a principle for treatment I am not sure and many more studies are necessary to evaluate the utility of fermented foods.

Dr. B. Koletzko: Are there any studies looking at antigens in fermented vs. nonfermented foods, for example cow's milk products? Are you aware of any such investigations?

Dr. Nowak-Węgrzyn: No.

Dr. Fuchs: Can I follow up a little bit on the discussion that Dr. Vandenplas raised with regard to the role of allergy-related gastroesophageal reflux and esophagitis? If I may be so presumptuous as to speak for North America, we don't see 50% of children with reflux as having eosinophilic esophagitis. It is fairly rare, but I think most of us are convinced that it is a little bit more difficult to make a distinction between reflux esophagitis and eosinophilic esophagitis. There are certainly children that don't have reflux and still have eosinophilic inflammation of the esophagus. There are also children who have reflux esophagitis and eosinophilic inflammation which resolves completely with standard antireflux therapy. So clearly we have some phenotypic common expression, yet mechanisms that are really very different from one another. What I heard you say though, is not eosinophilic esophagitis but reflux, 50% of reflux, is related to the allergic response and that is clearly different from our current conceptual framework. I don't think we will find many North American gastroenterologists that would describe this sort of rate. I think there is evidence that about 10–20% of young infants with reflux seem to respond to an amino acid-based formula, but the precise mechanism has yet to be determined.

Dr. Nowak-Węgrzyn: A review article postulated that in up to 50% of infants younger than 1 year of age, gastroesophageal reflux may be associated with cow's milk

allergy [8]. Maybe it is an exaggerated estimate, but it points out that there may be more reactions to the food than is being appreciated at this point. Considering a very aggressive use of antacids for treatment of gastroesophageal reflux in infants, this could be another potential mechanism why we see an increased prevalence of food allergy.

Dr. Fuchs: I think that is really a speculation at this point. Those of us who are more focused on reflux and esophagitis would be concerned if those were withheld for concern about precipitating allergy.

Dr. Wahn: Recently there was a study from Vienna confirming exactly what you said and they found a strong association between this kind of medication and the development of food allergy.

Dr. Vandenplas: Just to follow up on your comment; I think that the more the proteins are hydrolyzed, the more rapid their gastric emptying is. This has clearly been shown. So, if children have less reflux on extensive hydrolysates or amino acid formula, it may be because of the change in gastric emptying. In other words, the improvement obtained with extensive hydrolysates or with amino acid formula in reflux is not proof that a pathophysiologic mechanism is involved.

Dr. Heine: You dissected the IgE-mediated pathways very nicely. In gastroenterological food allergy often we don't find any increase in IgE, at least on serological testing, implying cell-mediated immune mechanisms. Is there any progress in identifying food protein epitopes that could explain why, for example, gastrointestinal food allergies are often transient?

Dr. Nowak-Węgrzyn: I am not aware of any studies in non-IgE-mediated food allergies looking at epitope recognition.

Dr. Heine: Do you feel that non-IgE food allergy is likely to align with the same recognition sites as IgE-mediated forms, or could this involve different parts of the protein?

Dr. Nowak-Węgrzyn: I think it could be different because the natural history seems to be so different.

Dr. Martaadmadja: I am a practitioner, not a researcher, so I would like to ask about the use of hypoallergenic formula given to allergic babies. Some of them have frequent defecation after having been given hypoallergenic formula which stops immediately after soy formula is used. Is there any other allergic factor in the hypoallergenic formula?

Dr. Nowak-Węgrzyn: Most of the children who are allergic to cow's milk tolerate soy-based formula. It is estimated that about only 12% will have problems when exposed to a soy formula [9].

Dr. Sorensen: It is commonly said that people in India and China have less peanut allergy because of different food preparation but there may also be a big difference in hygiene and the hygiene hypothesis may be the other explanation for it. What do you think is the most important part, the way the food is prepared or hygiene?

Dr. Nowak-Węgrzyn: This is a million dollar question. It is rather unlikely that a single factor can explain this discrepancy. Differences in food processing may account for part of it, but in addition the tendency of a population to develop an atopic type of reaction is crucial. So I agree with you that differences in hygiene are very important.

Dr. Rivera: At what point can we say that the so-called reflux in the newborn and low birth weight baby, which continues after the age of 6 months, is really a true reflux due to nonmaturity of the gastroesophagus or allergy?

Dr. Nowak-Węgrzyn: One practical way of approaching this issue is to focus on atopy risk factors such as atopic dermatitis and/or a family history of atopy in siblings or parents. When these risk factors are present, I would definitely consider evaluation for food allergy and/or an empirical trial of hypoallergenic infant formula. In contrast,

in a child with symptoms of mild reflux and good weight gain, and no atopic predisposition, treatment with antacids is appropriate.

Dr. S. Koletzko: We are all puzzled by the results of the group from Vienna. They showed that increasing the intragastric pH with acid-suppressive drugs increases the risk of sensitization and allergic manifestation in adults. They could show this for cod fish and hazelnut. As pediatricians, we wonder if we increase the risk of cow's milk allergy when we treat bottle-fed infants with acid-suppressive drugs. From the physiological point of view, I have my doubts because after feeding the acid is buffered for at least 2 or sometimes 3 h, and by that time a lot of milk has already left the stomach. So the question is whether nature is prepared for that or not? Did you look in vitro if there is pH-dependent denaturation of these epitopes of cow's milk allergens.

Dr. Nowak-Wegrzyn: The short answer is no, and actually pH studies are not something we do and I am not aware of anyone looking at that recently.

Dr. Lack: I was intrigued to hear your comments about work on T cell responses to fruits even after denaturation, and I guess it is translated into a simple clinical question. What do you tell your patients with oral allergy syndrome and allergy to fruits to do? Do you tell them to continue eating cooked fruits that don't cause symptoms or do you recommend avoidance? On the one hand gastrointestinal inflammation or disactive T cell epitopes could potentially be caused, or pollen allergy could be maintained; on the other hand you might argue that by getting patients to eat a lot of raw and cooked fruit you might be inducing tolerance. Which way should we be going clinically?

Dr. Nowak-Wegrzyn: The data I presented came from a very recent study [10]. It is too early to make clinical recommendations but it is a very interesting insight into the pathophysiology. My current recommendation is based on clinical symptoms, so if a patient is asymptomatic with baked fruits I do not recommend their avoidance.

Dr. Saavedra: You mentioned that casein fractions in milk may be associated or be more predictive of long-term persistence of allergic symptoms. Why caseins? Should we be more concerned with bovine casein than with bovine whey from the point of view of atopic persistence? Does that have any potential preventive or therapeutic implication?

Dr. Nowak-Wegrzyn: It is not clear at this point why IgE antibodies against caseins would be a marker of more severe and/or more persistent milk allergy. I think they are more resistant to heating, and compared to whey proteins they have a better defined secondary structure. Maybe this makes casein an important allergen in terms of the differences in epitope recognition.

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