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Recent Advances in the Understanding of Egg Allergens: Basic, Industrial, and Clinical Perspectives

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The emergence of egg allergy has had both industrial and clinical implications. In industrialized countries, egg allergy accounts for one of the most prevalent food hypersensitivities, especially in children. Atopic dermatitis represents the most common clinical manifestation in infancy; however, the range of clinical signs is broad and encompasses life-threatening anaphylaxis. The dominant egg allergens are proteins and are mainly present in the egg white, for example, ovalbumin, ovomucoid, ovotransferrin, and lysozyme. However, egg yolk also displays low-level allergenicity, for example, α-livetin. Strict avoidance of the offending food remains the most common recommendation for egg-allergic individuals. Nevertheless, the omnipresence of egg-derived components in prepackaged or prepared foods makes it difficult. Therefore, more efficient preventive approaches are investigated to protect consumers from inadvertent exposure and ensuing adverse reactions. On the one hand, commercial kits have become readily available that allow for the detection of egg contaminants at trace levels. On the other hand, attempts to produce hypoallergenic egg-containing products through food-processing techniques have met with promising results, but the approach is limited due to its potentially undesirable effects on the unique functional and sensory attributes of egg proteins. Therefore, the development of preventive or curative strategies for egg allergy remains strongly warranted. Pilot studies have suggested that oral immunotherapy (IT) with raw or cooked preparations of egg may represent a safe alternative, immediately available to allergic subjects, but remains applicable to only nonanaphylactic patients. Due to the limitations of conventional IT, novel forms of immunotherapy are sought based on information obtained from the molecular characterization of major egg allergens. In the past decade, promising approaches to the treatment and prevention of egg allergy have been explored and include, among others, the production of hypoallergenic recombinant egg proteins, the development of customized peptides, and bacterial-mediated immunotherapy. Nonspecific approaches have also been evaluated, and preliminary trials with the use of probiotic bacteria have yielded encouraging results. The current understanding of egg allergens offers novel approaches toward the making of food products safe for human consumption and the development of efficient immunotherapeutic strategies.

KEYWORDS: Egg allergy; ovalbumin; ovomucoid; lysozyme; food processing; allergen detection; immunotherapy; immune tolerance

1. INTRODUCTION

The estimated prevalence of egg allergy varies between 1.6 and 3.2% and thus makes it the second most common cause of food allergies in children (*1–3*). In several industrialized countries, egg allergy has in fact been reported as the most prevalent food hypersensitivity in the pediatric population, exceeding that of cow's milk allergy (*4–8*). Currently, the most efficient approach for egg allergy is total avoidance of the offending compound. However, the omnipresence of egg-derived components in cooked or manufactured food products renders the approach difficult (*9*), and inadvertent exposure may lead to life-threatening anaphylactic responses. Furthermore, when egg allergy occurs in association with other food allergies, such as cow's milk, the risks of nutritional deficiency represent an additional hurdle for the patients (*10*). Therefore, the development of egg products safe for human consumption complemented with efficient forms of immunotherapy is highly warranted. After a clinical presentation of egg allergy, this review provides the most recent information on egg protein allergenicity and industrial practices currently available toward the detection of egg allergens and the use of food-processing methods to produce hypoallergenic egg-containing products. In addition, emphasis is placed on the current knowledge of the

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molecular properties of egg allergens and on how the information can be utilized in the context of specific immunotherapeutic approaches.

2. CLINICAL PRESENTATION OF EGG ALLERGY

2.1. Natural History of Egg Allergy. Egg allergy usually develops within the first two years of life and resolves by school age (*3, 8*). Clinical adverse reactions sometimes emerge prior to any egg ingestion (*11*). In these cases, sensitization may have occurred in utero or may result from the transfer of small doses of antigen into breast milk (*12–16*). However, there is currently no evidence that strict egg avoidance during pregnancy or lactation can decrease the risk of sensitization in infants (*17, 18*). A few studies have suggested that early childhood sensitization to hen's egg may favor the subsequent development of respiratory allergies (*19, 20*). In adults, occupational asthma has been associated with the inhalation of aerosolized dried egg powder, leading to the development of IgE-mediated food allergy upon egg ingestion (*21, 22*).

Earlier studies documented that about two-thirds of children outgrew their allergy by the age of five (*23*). A very recent study, involving a cohort of more than 850 egg-allergic patients 2−18 years of age, reported that outgrowth of egg allergy may in fact occur at a later stage than previously reported (*24*). The study highlighted that the median time to develop tolerance was significantly increased in children suffering from other atopic diseases such as asthma or allergic rhinitis and that a specific immunoglobulin (Ig) E level of ≥50 kU/L was a good marker of persistent egg allergy. It was also observed that concomitant sensitization with other food allergens (e.g., peanut or milk) tends to delay the outgrowth of egg allergy (*24*).

2.2. Management of Egg Allergy. Hen's egg is an important cause of childhood allergy. In combination with peanut and cow's milk allergies, they represent up to 80% of food allergy cases in infants (*25, 26*). At present, management of egg allergy focuses on strict avoidance of the offending food and administration of drugs to suppress severe symptoms, for example, epinephrine in cases of anaphylaxis. Currently, novel forms of specific immunotherapy are being investigated as detailed further.

2.3. Clinical Manifestations of Egg Allergy. In egg-allergic patients, the clinical signs and symptoms involve various organs such as the skin (e.g., urticaria, angiodema, and atopic dermatitis), the respiratory system (e.g., asthma, rhinoconjunctivitis), and/or the gastrointestinal system (e.g., vomiting, diarrhea, abdominal pain) (*27*). Severe anaphylactic reactions can also develop in patients with acute IgE-mediated sensitivity to hen's egg (*28*). However, in infancy, atopic dermatitis represents the main clinical manifestation (*3*). In fact, egg hypersensitivity has been reported in two-thirds of children and adolescents with atopic dermatitis (*29*). It was documented that egg allergy tends to persist in children with more severe reactions (e.g., respiratory symptoms, angiodema, and multisystemic reactions) or with positive skin tests (*30*). In some patients, although ingestion is tolerated, contact with egg can cause urticaria. In these patients, serum IgE antibodies were shown to recognize egg white epitopes unstable to the action of digestive enzymes (*31*). A recent study suggested that the profile of sensitization may be related to the nature and severity of clinical symptoms. It was indeed reported that ovotransferrin and ovomucoid were dominantly recognized by IgE antibodies from patients with a history of egg-induced anaphylaxis, whereas atopic patients' sera showed dominant binding activities to ovalbumin and ovomucoid (*32*).

2.4. Diagnosis of Egg Allergy. The diagnosis of egg allergy is often determined by skin prick tests or radio-allergosorbent (RAST) assays, but the gold standard remains the double-blind placebo-controlled food challenge (DBPCFC), which confirms the clinical diagnosis. Due to the occurrence of potential adverse outcome following oral challenges, diagnostic cutoff values have been proposed for food-specific serum IgE levels (CAP-FEIA, CAP−fluorescent enzyme immunoassay) and skin-prick test wheal diameters (*3*). The reliability of skin prick tests in the diagnosis of egg allergy has been supported by recent studies (*33*). Earlier studies demonstrated that a serum IgE level to egg of >6 kU_A/L (CAP-FEIA, Pfizer-Pharmacia) had a positive predictive value of >95% in children and adolescents (*34*). In groups of infants, a German study reported a 95% predictive cutoff level at 12.6 kU_A/L (*35*), whereas a Spanish study reported a significantly lower cutoff at >0.36 kU_A/L with a 94% predictive value (*36*).

In skin-prick tests, a wheal diameter size of ≥7 mm has been reported as 100% predictive of a positive egg challenge in children older than 2 years of age (*37*) against a wheal diameter size of >17.8 mm with 99% predictive value in a separate study (*38*). Diagnostic cutoff values for specific serum IgE values and SPT were recently reviewed (*3, 11*). Variations between studies can be attributed to factors such as clinical characteristics of the targeted cohorts (e.g., infant vs children, country of origin) and the nature of the allergen preparation (e.g., raw egg vs allergen extracts) used during the challenge.

2.5. Current Clinical Issues Associated with Egg Allergy. In recent years, egg hypersensitivity has raised other issues related to the potential development of anaphylaxis subsequent to the administration of viral vaccines. Indeed, the triple measles, mumps, and rubella (MMR), as well as influenza and yellow fever vaccines, classically use egg in their manufacturing processes. The MMR vaccines are classically prepared from viral strains multiplied in chick embryo fibroblasts and may contain only minute amounts of hen's egg allergenic components. On the other hand, yellow fever and influenza vaccines are prepared directly from egg embryos and may therefore have an immunologically more significant content of allergens, for example, ovalbumin and ovomucoid (*8*). Studies have provided evidence that the MMR vaccine was acceptably safe in populations at risk such as children with anaphylactic sensitivity (*39, 40*). Clinical reports pertaining to the safety of influenza vaccines revealed controversy; however, current recommendations strongly advocate its safe use, pending precautionary measures involving a two-dose administration procedure (a $^1/_{10}$ dose followed by a $^9/_{10}$ dose with a 30 min interval) using a vaccine containing no more than 1.2 μg/mL of egg protein (*41–43*). Yellow fever vaccine is much less routinely administered, and limited scientific evidence was found on the risks for egg-allergic patients, but current guidelines (e.g., Centers for Disease Control in the United States and the World Health Organization) contraindicate its administration in egg-allergic individuals.

The total absence of a specific allergen from food products is often difficult to achieve, mainly because of food-manufacturing practices (*44*). However, fatal accidents subsequent to ingestion of trace amounts of food have been reported (*45*), and knowledge of the amount of food required to induce a clinical reaction is therefore essential. Establishment of the so-called lowest observed adverse effect level (LOAEL) helps food industries establish efficient sanitation and vigilance programs and prevent costly exercises such as food recalls (*46*). In a recent study examining thresholds of clinical reactivity to egg, it was

identified that 16% of individuals ($n = 124$) with egg allergies reacted to 65 mg of egg as a solid food, equivalent to 6.5 mg of egg protein (*47*). The study reported that 0.8% of individuals with egg allergies reacted to 10 mg or less of solid food and that the lowest reactive threshold was <2 mg of egg, that is, 0.2 mg of hen's egg protein. More recently, a separate study also involving oral food challenges, conducted in France, has reported an even lower threshold, equal to only 0.13 mg of hen's egg protein (*44*).

2.6. Egg Sensitization upon Occupational or Respiratory Exposures. Hypersensitivity to egg proteins is mostly known to develop upon ingestion of eggs or egg-containing food products, but reports of sensitization to egg after inhalation have also emerged. Studies have indeed documented that in egg-processing premises (e.g., baking industries, confectionaries, or egg-breaking plants), workers were daily exposed to unusually elevated levels of airborne egg proteins in the form of dust or egg-laden aerosols (*48, 49*). This phenomenon has been shown to be the underlying cause of egg-induced respiratory manifestations such as asthma, a disorder hence named occupational or baker's asthma. Skin-prick tests, measurements of serum-specific IgE, and inhalation challenges determined that occupational asthma sufferers were sensitized to the same major egg allergens identified upon ingestion, that is, lysozyme, ovomucoid, ovalbumin, and ovotransferrin (*22, 49–51*). Studies have in fact reported that occupational allergy to egg proteins could lead to a consecutive ingestive allergy (*21*). Interestingly, these patients developed allergic manifestations upon oral challenges with raw or undercooked egg but were clinically tolerant to the ingestion of egg-containing food products (*22*). This suggests that the process of epitope recognition differs on the basis of the route of sensitization, that is, linear epitopes upon ingestion versus conformational epitopes upon egg protein inhalation.

A second form of egg hypersensitivity inducible by inhalation is the "bird-egg syndrome". The disorder is characterized by the development of respiratory and gastrointestinal symptoms upon exposure to bird antigens or upon egg yolk intake. Its etiology differs from that of baker's asthma, as bird-egg syndrome primarily results from the immunological cross-reactivity which can occur between egg yolk proteins and bird antigens found in feathers, serum, or meat (*52, 53*). More specifically, cross-reactivity to the yolk protein α-livetin was reported as a significant contribution to the onset of bird-egg syndrome (*54*).

3. MUCOSAL IMMUNE RESPONSE TO EGG ALLERGENS

IgE-mediated food allergy, also known as type I food allergy, accounts for the majority of food-induced allergic responses and is characterized by the presence of elevated titers of allergen-specific serum IgE antibodies. Currently, it remains unclear how the mucosal immune system is oriented toward sensitization versus immune tolerance when exposed to innocuous dietary antigens. However, it is suggested that upon ingestion, food proteins are capable of crossing the intestinal epithelial barrier and being captured by the underlying immune system. Food proteins are then processed into peptidic fragments by a class of specialized immune cells, known as antigen-presenting cells (APC). The peptidic fragments are displayed on their surface in association with major histocompatibility (MHC) class II molecules. The peptide−MHC II complexes can in turn be recognized by specific T-cell receptors (TCR) (*55*) and potentially lead to the development of a specific immune response. The usual nonpathogenic response to soluble dietary proteins is characterized as a status of immune hyporesponsiveness, also known as oral tolerance (*56*).

An allergic immune response is believed to be orchestrated by a class of $CD4^+$ T-lymphocytes or T-helper (Th) cells. Indeed, cytokines produced by $CD4^+$ T-lymphocytes mediate a wide range of pro-inflammatory and anti-inflammatory responses. Most $CD4^+$ T-cells belong to either a Th type 1 (Th1) or type 2 (Th2) subgroup, producing type 1 or type 2 cytokines, respectively. Interferon (IFN)-γ is the archetypal type 1 cytokine, whereas type 2 cells typically produce a range of cytokines including interleukin (IL)-4, IL-5, IL-9, and IL-13, contributing to the differentiation of B-cells into IgE-producing plasma cells and the recruitment of effector cells such as eosinophils, basophils, and mast cells (**Figure 1**) (*57*).

The mechanism of allergic sensitization is initiated by differentiation of naive antigen-specific Th0 cells into effector Th2 cells, leading to the differentiation of B-cells into antibody-secreting plasma cells and the binding of allergen-specific IgE to high-affinity Fc receptors (FcϵRI) present on the surface of mast cells and basophils. Upon subsequent exposure to the allergen, the cross-linking of IgE receptors leads to their degranulation. The release of vasoactive amines and cytokines and the synthesis of a variety of arachidonic acid-derived inflammatory mediators ensue (*58*) and lead ultimately to the manifestations of clinical signs and symptoms commonly associated with egg allergy (e.g., atopic dermatitis, urticaria, angiodema).

A distinct population of $CD4^+$ T-cells, known as T-regulatory (Treg) cells, is believed to control the balance between Th1- and Th2-biased responses. Regulatory T-cells encompass "natural" T-regulatory cells (e.g., $CD4^+$ $CD25^+$ T-cells) and "induced" or adaptive regulatory T-cells such as IL-10-producing Tr1 cells and TGF-β-producing Th3 cells (*59*). The manipulation of Treg cells functions in the prevention and treatment of (food) allergic disorders represents an attractive approach (*60*). A detailed discussion of the role of T-lymphocytes in the immunopathology of food allergy is beyond the scope of this paper; however, excellent reviews can be found (*59, 61, 62*).

4. ALLERGENIC COMPONENTS OF THE EGG

The chemical composition of hen's egg has been extensively investigated (**Table 1A**). The major egg allergens were found to be mainly proteins and have been implicated in food allergy as early as 1912 (*63*). Clinically relevant egg allergens have been identified in both the albumen (egg white) and the egg yolk fraction; however, reports have documented that the major egg allergens were mainly contained in the egg white (*64*). The molecular and biological characteristics of egg allergens are presented in **Table 1B**.

Early studies involving a cohort of 342 patients reported that the major egg allergens were, in decreasing order, lysozyme > ovomucin > ovalbumin > ovomucoid, based on skin tests (*65*). Using 13 egg-allergic patients, further studies documented ovomucoid (OVM) as the dominant egg allergen (*66*). On the basis of RAST assays and cross-radio-immunoelectrophoresis (CRIE), studies completed in the 1980s established that ovalbumin (OVA), OVM, and ovotransferrin (OVT) were the major egg allergens (*67, 68*) and, later, lysozyme (LYS) was also demonstrated to be a significant egg allergen (*69*). Allergy to lysozyme has also been described in sensitization cases after exposure via inhalation (*51*). Frequency of recognition in egg-allergic patients was reported to range from 6 to 67% for lysozyme (*70–72*) and to reach up to 22% for ovotransferrin (*70*).

The two major allergens ovomucoid and ovalbumin constitute about 11 and 54% of egg white proteins, respectively (*73*). Both

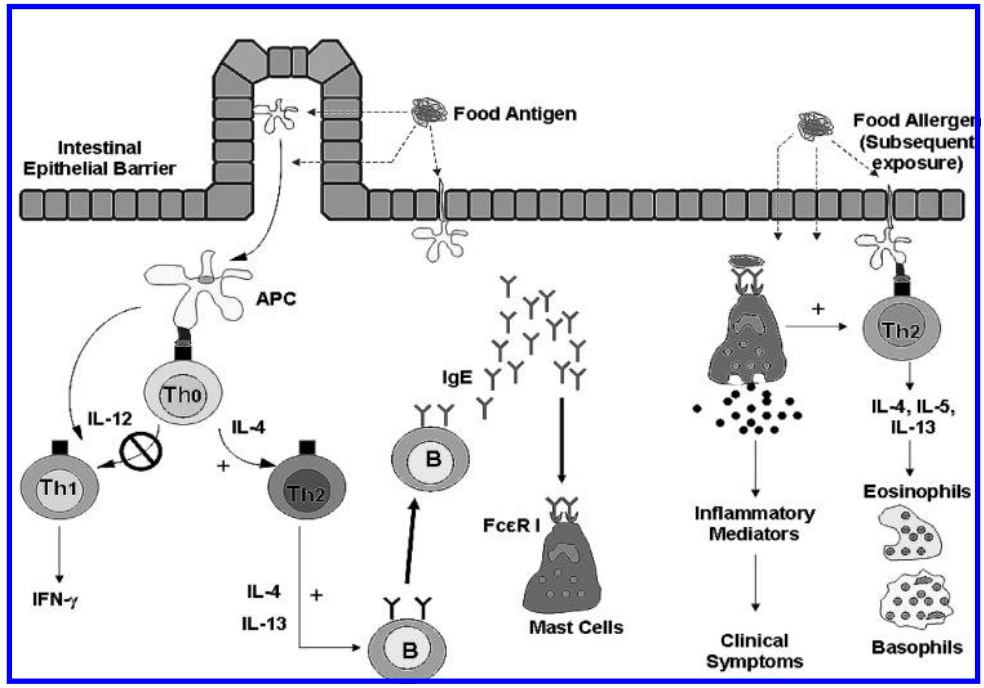

Figure 1. Schematic representation of cellular and molecular events underlying an allergic response. During the sensitization phase, it is believed that food antigens traverse the intestinal barrier and are captured by the underlying immune cells. They are subsequently processed and presented by a specialized class of immune cells known as antigen-presenting cells (APC) for presentation to T-lymphocytes. The cytokine environment encountered by a naive T-helper cell (Th0) plays a prominent role in determining whether Th0 will differentiate into Th1 or Th2. Allergic individuals will preferentially develop an interleukin (IL)-4-rich microenvironment, which drives the immune response toward a Th2 bias to the detriment of a Th1-biased response, characterized by cytokines such as IL-12 and IFN-γ. The activation of Th2 cells will lead to the production of molecules including IL-4 and IL-13, which both promote immunoglobulin E (IgE) production by B-cells. Allergen-specific IgE will then bind to high-affinity receptors (FcϵRI) present at the surface of mast cells. Upon subsequent ingestion of the food allergen, antigen presentation will lead to a rapid activation of Th2 cells, followed by the recruitment and activation of effector cells such as eosinophils and basophils. In the meantime, allergenic fragments (epitopes) may also bind to receptor-bound IgE present on mast cells, triggering the aggregation of the receptors and the subsequent release of inflammatory and vasoactive mediators such as histamine, directly responsible for the clinical signs and symptoms of food allergy. Reprinted with permission from ref *57*. Copyright 2005 Blackwell.

proteins are glycosylated, with as high as 25% of the mass of ovomucoid comprising carbohydrates. Debate flourished over the immunodominance of ovalbumin as the major egg allergen; however, studies showed that the use of contaminated commercial ovalbumin led to an overestimation of its dominance as a major egg allergen in egg-sensitive patients (*74*). Using egg-allergic patients' sera, further studies confirmed that OVM was the dominant allergenic components in egg white (*75–78*). In vitro analyses based on RAST and Western blot assays revealed minor egg allergens represented by metal-binding molecules that are ovomucin and phosvitin, both found in the egg white (*64, 79, 80*), and α-livetin present in the egg yolk (*53, 81*). In an effort to establish which egg proteins were truly major allergens, a very recent study investigated binding of specific IgE to eight purified egg white and yolk proteins (RAST analyses), using sera from 40 egg-sensitive children (*80*). The study confirmed that the major egg allergens originate primarily from egg white and include ovomucoid, ovalbumin, ovotransferrin, and lysozyme. However, the same study interestingly documented that 25% of the egg-allergic patients were significantly sensitized to apovitellenins I and VI and phosvitin, thereby supporting previous findings that egg yolk also contains allergenic proteins (*64, 79, 80*). Whereas major egg allergens have been the focus of a number of investigations, only a few studies have explored the immunological properties of minor egg allergens. The latter study strongly suggests that their clinical significance warrants further investigations. Other minor proteins such as ovoflavoprotein and ovoinhibitor were identified as antigenic, but lacked allergenic activity (*67, 68*).

5. MOLECULAR PROPERTIES OF EGG ALLERGENS

5.1. Biochemical Properties of Major Egg Allergens. *Ovomucoid (OVM or Gal d 1)* has a molecular mass of 28 kDa and represents 11% (w/w) of egg white proteins. It is a highly glycosylated protein comprising 186 amino acids and is known to exhibit trypsin inhibitor activity (**Table 1B**). The molecule consists of three structurally independent tandem homologous domains (domains Gal d 1.1, 1.2 and 1.3), possesses nine intramolecular disulfide bridges, and 20−25% of carbohydrates entities (*82*). Domain Gal d 1.3 was reported as the immunodominant fraction (*76, 83*).

Ovalbumin (OVA or Gal d 2) is a phosphoglycoprotein with a relative molecular mass of 45 kDa (**Table 1B**). It is the major protein in avian egg white, comprising 54% of its total protein content (*84, 85*). Its complete sequence of 385 amino acids has been determined (*86*). An interesting feature of ovalbumin structure is its homology with the serpin (serine protease inhibitor) superfamily, a group of protease inhibitors found in all eukaryote organisms (*84*). However, OVA was found not to exert any protease inhibitory activity. Besides its role as a major source of amino acid, no biological function has yet been attributed to this protein (*84*). Ovalbumin has been widely used not only as a standard model for studies of protein structural

Table 1

(**A**) Chemical Composition of Hen's Egg [Adapted from Kovacs-Nolan et al. (*73*)]

constituent	% (w/v)	major components (rel %, w/w)
egg shell	9.5 (including shell membrane)	inorganic salts (91.87) calcium carbonate (98.4) magnesium carbonate (0.8) tricalcium phosphate (0.8) proteins (6.4) water (1.7) lipids (0.03)
egg white	63.0	proteins (9.7−10.6): ovalbumin (54) ovotransferrin (12.0) ovomucoid (11) ovomucin (3.5) lysozyme (3.4) G2 globulin (4.0?) G3 globulin (4.0?) ovoinhibitor (1.5) ovoglycoprotein (1.0) ovoflavoprotein (0.8) ovomacroglobulin (ovostatin) (0.5) cystatin (0.05) avidin (0.05) lipids (0.03) carbohydrates (0.4−0.9) ash (0.5−0.6)
egg yolk	27.5	proteins (15.7−16.6): apovitellenin (I−VI) (37.3) lipovitellin apoproteins (40.0) α-lipovitellin β-lipovitellin livetins (9.3) α-livetin (serum albumin) β-livetin (α2-glycoprotein) γ-livetin (γ-globulin) phosvitin (13.4) biotin-binding protein (trace) lipids (32.0−35.0) triglycerol (66) phosphatidylcholine (PC) (24) phosphatidylethanolamine (PE) (2.8) lysophosphatidylcholine (LPC) (0.6) sphingomyelin (0.6) cholesterol (5.0) others (1.0) carbohydrates (0.2−1.0) ash (1.1)

(**B**) Molecular and Biological Properties of Identified Egg Allergens

protein name	M_r[a] (kDa)	protein family	biological function(s)	allergen epitopes reported	selected refs
			Egg White Proteins		
ovomucoid (*Gal d 1*)	28	Kazal-type serine protease inhibitor	serine protease inhibition activity	yes[b]	*69*, *79*, *80*, *131*, *254*
ovalbumin (*Gal d 2*)	45	serine protease inhibitor	storage protein?	yes[b]	*79*, *80*, *105*, *120*
ovotransferrin (*Gal d 3*)	76−77	transferrin	iron-binding capacity with antimicrobial activity	no	*79*, *80*, *131*
egg lysozyme (*Gal d 4*)	14.3	glycoside hydrolase family 22	antibacterial activity	no[c]	*72*, *79*, *80*, *122*
ovomucin	165	contains trypsin inhibitor-like domains	heavily glycosylated protein with potent antiviral activities	no	*79*, *80*, *95*
			Egg Yolk Proteins		
phosvitin	35	transferase?	metal-chelating agent	no	*79*, *80*, *96−102*
α-livetin (*Gal d 5*)	65−70	serum albumin	bind ions, fatty acids, hormones in physiological conditions	no	*53*, *54*, *81*
apovitellenins I	9.5	very low density lipoprotein	potent lipoprotein lipase inhibitor	no	*64*, *79*, *80*
apovitellenins VI (or apoprotein B)	170	unknown	lipid-binding activity	no	*64*, *79*, *80*

[a] M_r, relative mass. [b] See **Figures 2** and **3** herein. [c] Lysozyme served as a widely used model for investigations of paratope−epitope interaction, but to date, no epitope relevant to egg allergy has been reported.

and functional properties but also in experimental animal models of inhalant and dietary allergies.

Ovotransferrin (OVT or Gal d 3) is a monomeric egg white glycoprotein with a molecular mass of 77 kDa, divided into

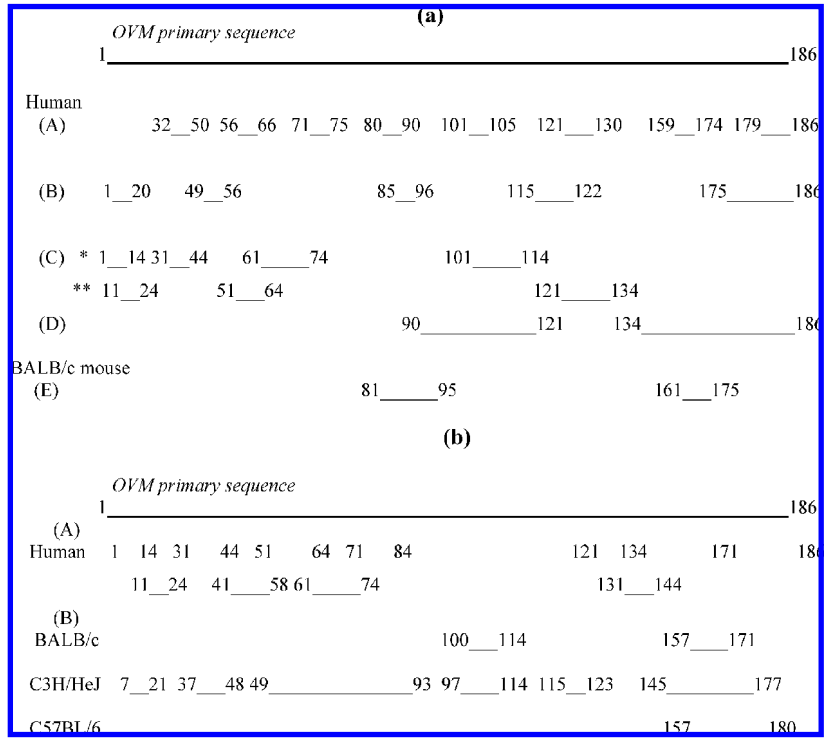

Figure 2. Schematic representation of IgE/T-cell epitope regions on ovomucoid (OVM), as reported by different groups. The numbers represent amino acid position along the primary sequence of the protein. (**a**) Ovomucoid IgE/B-cell epitopes (human and mouse) [(A) ref *32*; (B) ref *75*; (C) ref *108*; (D). ref *107*; (E) ref *254*] (∗, IgE isotype; ∗∗, IgG isotype). (**b**) Ovomucoid allergenic T-cell epitope (human and mouse) [(A) ref *108*; (B) ref *113*]. Reprinted with permission from ref *303*. Copyright 2008 Wiley.

two domains, an N domain and a C domain, with a short linkage region (*87–89*). Its main function is commonly accepted as iron transport molecule, and it belongs to the transferrin protein family. It shares the structural features of the hen serum transferrin, which differs by only its attached carbohydrate (*90*). Its antimicrobial activities have been well investigated on the basis of its iron-scavenging properties (*91, 92*), as well as its immunomodulating effects (*93*) and its antioxidant properties (*94*).

Lysozyme (LYS or Gal d 4) is a glycosidase containing four disulfide bonds, with a molecular mass of 14.3 kDa, well-known for its bacteriolytic activity against prokaryotic organisms. The protein represents only 3.4% of egg protein total content. It is a good example of naturally occurring enzymes used in the food industry to maintain product quality and reduce the incidence of spoilage.

Ovomucin is a minor egg white glycoprotein (3.5% w/w) with a molecular mass of approximately 165 kDa (*95*). It contains O-linked carbohydrate moieties that, upon formation of extensive hydrogen bonds with water, can give rise to a characteristic gel-like structure.

Present in the egg yolk, phosvitin has a molecular mass of 35 kDa with a very unique amino acid composition consisting of about 50% of serine residues (*89, 96*). It is the most highly phosphorylated protein known (*97*), with 3−10% phosphorus and 6% carbohydrates (*98*). Its unique primary sequence makes this protein one of the strongest metal-chelating agents. It has been estimated that >90% of the iron present in egg is found in the yolk, bound to phosvitin (*99*). Phosvitin has been investigated for both its antibacterial (*100, 101*) and emulsifying properties (*102–104*).

5.2. Epitope Mapping of Egg White Allergens. The immune response to food allergens consists of both antibody (IgE)-mediated and cell-mediated mechanisms. As mentioned earlier, food-allergic individuals mount a dominant Th2-biased response to the sensitizing food protein, resulting in the production of allergen-specific IgE by B-cells. However, B- and T-lymphocytes do not classically recognize the entire allergen molecule; instead, they tend to bind to restricted sites on the antigen or epitopes. Therefore, the mapping of epitopes encompasses the identification of the regions specifically recognized by IgE (B-cell) and T-cell receptors (TCR).

On the one hand, immunoglobulins primarily bind to conformational and sequential (or linear) epitopes based on the protein tertiary and primary structure, respectively. Recent investigations suggest that the sequences recognized by egg-allergic patients' specific IgE are mainly sequential (*32, 105*). The importance of linear epitopes was supported by clinical studies suggesting that egg-allergic children with predominant IgE binding activity to ovomucoid linear epitopes is a predictive factor for persistence of the disease (*106*). On the other hand, T-helper lymphocytes solely recognize small linear peptides presented in the cleft of class II MHC molecules by APC. The identification of these immunologically active regions is extremely useful in the design of hypoallergenic variants such as recombinant mutants, chemically modified molecules, and also T-cell epitope containing-peptides.

Ovomucoid. Findings that OVM was the immunodominant egg allergen have led to a number of investigations. Thus, it has been documented that a positive prognosis was associated with the absence or a decline in OVM-specific IgE titers (*74*). Studies have also shown that profiles of sensitization to OVM were variable among individuals, pertaining to their IgE- and IgG-binding sites. The same authors emphasized the importance of linear versus conformational epitopes and proposed that the recognition of linear epitopes was predictive of a persistent egg allergy. These observations were supported by a separate study

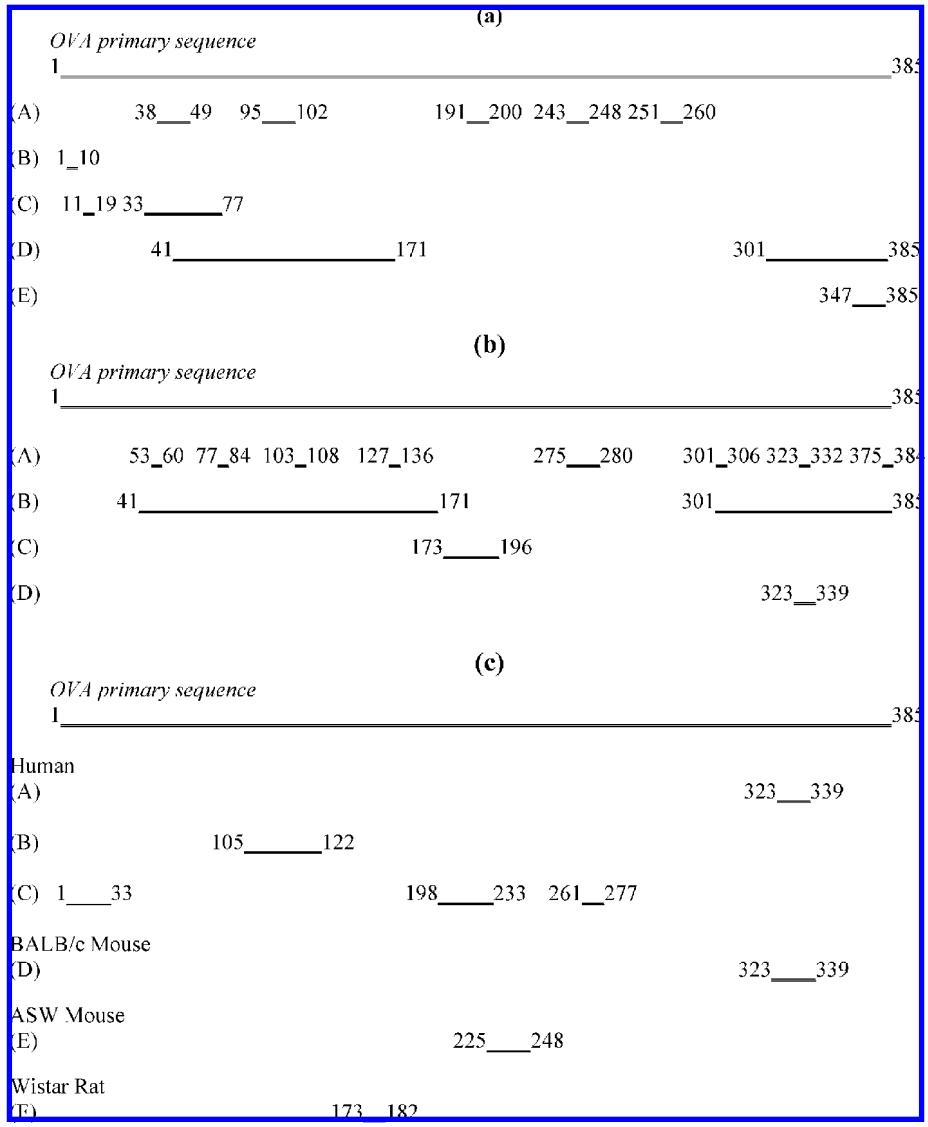

Figure 3. Schematic representation of IgE/T-cell epitope regions on ovalbumin, as reported by different groups. The numbers represent amino acid position along the primary sequence of the protein. (**a**) Ovalbumin allergenic (IgE) epitopes reported in human egg-allergic patients [(A) ref *105*; (B) ref *114*; (C) ref *115*; (D) ref *116*; (E) ref *117*]. (**b**) Ovalbumin allergenic (IgE) and antigenic (IgG1/IgM) epitopes reported in BALB/c mouse [(A) ref *120*, IgE isotype; (B) ref *116*, IgG1/IgM isotypes (monoclonal); (C) ref *118*, IgG1 isotype (monoclonal); (D) ref *125*, IgE isotype]. (**c**) Ovalbumin T-cell epitopes reported in human and BALB/c mouse [(A) ref *128*; (B) ref *122*; (C) ref *121*; (D) ref *127*; (E) ref *123*; (F) ref *119*]. Reprinted with permission from ref *303*. Copyright 2008 Wiley.

in which sera obtained from patients with persistent egg allergy had high IgE-binding activity to pepsin-treated ovomucoid (*78*).

Ovomucoid B- and T-cell epitopes have been reported in a number of studies in both human egg-allergic patients and murine experimental models. Earlier studies investigated at first human IgG- and IgE-binding epitopes. Probing with pooled sera from seven egg-allergic patients led to the identification of five IgE-binding epitopes and seven IgG-binding regions using enzymatic fragments and synthetic dodecapeptides (*75, 107*). Interestingly, the strongest IgE-binding activity was to OVM domain II, that is, residues 65−120. Another study determined instead that the strongest binding activity was to OVM domain III with immunodominant epitopes being mainly linear (*76*). In vitro experiments based on targeted chemical modifications determined that hydrophilic residues were more critical for IgG isotype binding, whereas hydrophobic residues were more essential for IgE isotype binding (*77*). Subsequently, a detailed study using pooled sera from eight egg-allergic patients and overlapping decapeptides synthesized on SPOTS (Simple Precise Original Test System, Sigma-Genosys) cellulose membranes allowed identification of three IgE epitopes in domain I, four IgE epitopes in domain II, and two IgE epitopes in domain III (*105*). IgG epitopes were also identified and were in general distinct sequences from IgE epitopes.

Using T-cell lines (TCL) derived from six hen's egg-allergic patients, Holen and co-workers characterized 10 distinct OVM T-cell epitopes (*108*). Among these sequences, six were recognized by IgE, but the remaining four were solely identified as T-cell epitopes. Similarly, several studies reported the isolation of T-cell clones (TCC) specific to ovomucoid from egg-allergic patients' peripheral blood mononuclear cells (PBMC) (*109, 110*). However, further investigations revealed that there was no match between their cytokine secretion patterns and the clinical manifestations of immediate hypersensitivity (*111*).

The mouse model has been and will continue to be an extremely useful tool for life sciences research. The fine mapping of OVM B-cell epitopes was recently reported in the inbred BALB/c mouse strain, a commonly accepted murine model of food allergy (*83, 112*). In line with the epitope results

obtained from egg-allergic patients' sera, the study showed that OVM domain III exhibited the highest IgE-binding intensity in BALB/c mice. Similarly, OVM T-cell epitopes have been characterized in three commonly used strains of mice, including BALB/c, C3H/He, and C57BL/6 (*113*). The T-lymphocytes from BALB/c mice recognized the two peptide sequences 100−114 and 157−171, whereas T-cells from C57BL/6 mice recognized only residue fragment 157−180. On the other hand, T-lymphocytes from C3H/He mice showed reactivity to residue sequences spanning the entire OVM molecule with the dominant regions being residues 49−93 and 97−114 and the subdominant regions being residues 7−21, 37−48, 94−96, 115−123, and 145−177. A summary of B- and T-cell epitope localization on the OVM molecule, as reported by different groups, is schematically presented in **Figure 2**.

Ovalbumin. OVA has been used as a surrogate antigen in a number of studies and, particularly, in experimental models of allergic diseases, mainly due to its ready availability. Studies involving both egg-allergic patients and murine model sera have examined both IgE- and IgG-binding sites present on OVA. Using a RAST assay, Elsayed and co-workers first reported human IgE-binding activity in the fragment 1−10 of the N terminus (*114*). Two subsequent papers showed that residues 11−19 and 34−70 (*115*) and also fragments 41−172 and 301−385 (*116*) exhibited human IgE-binding activity. Using synthetic overlapping peptides, in vitro experiments showed that peptide sequence 347−385 could specifically inhibit histamine release from human basophils (*117*).

The production of monoclonal antibodies (IgG1 and IgM isotypes) in BALB/c mice led to the identification of regions 41−172 and 301−385 as antigenic determinants (*116*). Irradiation of OVA led to the formation of a new binding site in the fragment 173−196, specifically recognized by monoclonal IgG1 mouse antibodies (*118*). The same fragment was reported to exert suppressive effects in a rat model of food allergy (*119*).

Recently, the fine mapping of IgE epitopes of the entire OVA molecule has been completed in both human (*105*) and mouse model (*120*). A total of five IgE epitopes were characterized in the human study involving egg-allergic patients' sera and eight distinct epitopes in the mouse model of orally induced egg allergy. Results from both studies are summarized in **Table 2**.

With regard to T-cell epitopes, residues 1−33, 198−213, and 261−277 were specifically recognized by T-cells obtained from atopic dermatitis egg-allergic patients (*121*). In a separate study, the region 105−122 was also identified as a dominant T-cell epitope of OVA, with no binding activity toward human specific IgE, thereby suggesting its potential use in peptide-based immunotherapy (*122*). Production of T–T hybridomas using ASW mouse strain led to the identification of OVA fragments 225−240 and 233−248 as immunodominant T-cell epitopes (*123*).

The OVA fragment 323−339 was shown to present binding activity not only to human IgE antibodies but also to OVA-specific IgE antibodies generated in BALB/c mice (*124, 125*). The same fragment had initially been identified as an immunodominant T-cell epitope (*126, 127*). It led to its widespread use in the context of peptide immunotherapy and immune tolerance induction (*128, 129*) and culminated in the creation of a transgenic mouse model coding for a unique T-cell receptor, which specifically recognizes OVA peptide 323−339 (*130*). A summary of ovalbumin B- and T-cell epitope mapping results, as reported by different groups, is schematically represented in **Figure 3**.

Table 2. Comparison of Human versus BALB/c Mouse OVA IgE Sequential Epitopes [Adapted from Mine and Yang (*120*)]

	human egg-allergic patients (105)	BALB/c mouse model of egg allergy[a] (*120*)
no. of epitope regions	5	8
epitope regions on OVA primary sequence	OVA 38–49 OVA 95−102 OVA 191–200 OVA 243−248 OVA 251−260	OVA 53–60 OVA 77−84 OVA 103−108 OVA 127−136 OVA 275−280 OVA 301−306 OVA 323−332 OVA 375−384
no. of residues per epitope	6−12 residues	6−10 residues
epitope physicochemical composition[b]	54.3% hydrophobic 23.9% polar 21.7% charged	53.1% hydrophobic 26.6% polar 20.3% charged
main residues critical for IgE binding activity	hydrophobic and charged	hydrophobic and charged
epitopes main secondary structures	β-sheets and β-turns	β-sheets and α-helices

[a] Oral sensitization to egg protein. [b] Percentage of total residues.

Ovotransferrin. The characterization of OVT epitopes relevant to egg allergy has not as yet been reported in human or animal experimental models. Earlier reports have, however, documented that there was allergenic cross-reactivity between OVT and components found in egg yolk (*131*). It was documented that elevated concentrations of OVT could partially inhibit IgE binding to chicken serum albumin in ELISA inhibition assays (*54*).

Lysozyme. Commonly referred to as hen's egg lysozyme (HEL), lysozyme is easily obtained in a purified form and has therefore been used as a model protein for the comprehensive study of protein dynamics, structure, and folding (*132*). HEL is one of the best understood folded proteins, and complexes of lysozyme with specific antibody binding sites have been finely examined (*133–137*). A number of crystallization and X-ray diffraction studies have revealed the binding sites of various Fab fragments and monoclonal antibodies (*138–140*). Similarly, HEL has served in the identification of peptide−MHC complexes (*141, 142*). In the BALB/c mouse, it has been reported that the T-cell response of HEL-sensitized mice was dominated by recognition of determinants located in regions 108−116 and a subdominant epitope in the region 9−25 (*143*). A number of studies have investigated HEL-specific T-cell epitopes in BALB/c, BALB.B, B10.A, CH3, and other inbred strains of mice (*144–146*). One report identified HEL-specific T-cell responses using PBMC from human egg-allergic patients (*122*), but no human T-cell epitope has yet been reported. The fine characterization of HEL epitope sequences relevant to egg allergy has yet to be initiated.

5.3. Stability of Egg White Proteins and Their Allergenicity. The allergenicity of egg proteins depends a great deal, but not exclusively, on their resistance to heat and digestive enzymes (*147*), reflecting their capacity to stimulate a specific immune response, that is, the presence of B- and T-cell epitopes (*3*).

A common approach to evaluating the stability of egg allergens is to examine alterations of their IgE/IgG-binding capacities upon physical, chemical, or genetic manipulations. In one study, the four major egg allergens were either physically

or chemically modified and their binding activities to egg-allergic patients' specific IgG/IgE and rabbit-specific IgG were examined (*32*). Thermal treatment was conducted for 15 min at 95 °C, in parallel with chemical modification using either 6 M urea or carboxymethylation. Urea-treated OVA, LYS, and OVT led to an increase in human IgG-binding activity, whereas carboxymethylation and thermal treatment of OVM and OVT led to a significant drop. Treatment with urea also enhanced the binding activity of rabbit IgG antibodies to OVT. Whereas carboxymethylation of OVM, OVT, and LYS decreased their allergenicity, IgE-binding activity to OVA was not affected. These findings suggest that OVA contains sequential IgE epitopes, whereas OVM and LYS may contain both sequential and conformational IgE epitopes (*32*).

Ovomucoid is characterized by its high heat stability and can also be resistant to other forms of denaturation (e.g., urea) (*148*), possibly related to the presence of its strong disulfide bonds (*70*). In fact, in vitro stability of OVM was reported in simulated intestinal fluid for at least 60 min (*149*). A separate study reported, in fact, that OVM could not be denatured by trichloric acid−acetase or 8 M urea procedures (*74*). The chemical stability of ovomucoid has been credited for its dominance as a major egg allergen and its role in the prognosis of egg allergy. In a study involving 38 patients with allergic reactions to freeze-dried egg white, it was shown that most of them also developed a positive response to heated egg white ($n = 21$). On the other hand, 17 of these 21 subjects were tolerant to a challenge with heated egg white depleted of OVM (*78*). Experiments pertaining to the allergenic stability of OVM led to contradictory results. Whereas some studies reported that the reduction of OVM did not affect its IgE-binding capacity (*70*), other studies reported a decrease (*75*) or an increase (*76*), using human patients' sera. It was documented that heating of ovomucoid at 100 °C for duration of 30 min could lead to irreversible conformational changes, as detected by monoclonal antibodies (*150*). However, the protein is noncoagulable upon heat application, and human-specific IgE were shown to bind to both native and denatured forms (*150*). Using egg-allergic patients' sera, an interesting study reported that the IC_{50} concentration for specific IgE inhibition was 1700-fold higher for denatured OVM compared to intact or oxidized OVM (*108*), suggesting that the allergenicity of OVM can significantly drop upon denaturation.

Interestingly, the allergenic and antigenic properties of OVM were shown to be maintained after peptic digestion (*151–153*). In vitro analyses showed that pepsin digestion of ovomucoid did not alter its IgE-binding capacity, as measured by ELISA using human egg-allergic patients' sera (*151*). The study also documented that pepsin digestion of OVM did not significantly affect its trypsin inhibitory activity. On the other hand, reduction of OVM enhanced its digestibility, indicating the importance of its disulfide bonds. These findings were supported by previous studies investigating IgE-binding activity of OVM after pepsin, chymotrypsin, and trypsin digestion using RAST inhibition and Western blot tests, with serum samples obtained from egg-allergic patients (*78, 107*). Finally, five genetic mutants of OVM Gal d 1.3 were analyzed and compared in vitro to their native counterpart. It was demonstrated that the genetic mutation of phenylalanine (F) at position 37 with an alanine (A) residue caused a drastic loss of binding activity to human allergic patients' IgE, attributed to a disruption of the α-helix structure. Furthermore, substitution of glycine (G) with methionine (M) at position 32 led to a synergistic effect on decreasing its binding activity (*154*).

Ovalbumin is easily denatured by urea and guanidinium salts (*74*). Furthermore, the protein is relatively heat labile, compared to OVM. Using egg-allergic patients' sera, reports have shown that the antigenicity of OVA could, however, resist heat treatment under certain conditions (*155*). As mentioned above, studies found that OVA can retain its human IgE-binding capacity after chemical treatment such as reduction and carboxymethylation, heat, or urea, indicating that IgE epitopes are linear and thermostable (*32*). On the other hand, an earlier paper indicated that exposure of OVA to a temperature of 80 °C for 3 min only had the effect of decreasing its IgE-binding activity by 90% (*156*). The disparities observed from one study to another may be explained by variations inherent to patient cohort characteristics, an idea that was supported by subsequent paper (*80*). Assessing resistance to enzymatic digestion represents an alternative manner to characterize egg allergen stability. It was documented that, in its native form, OVA is trypsin resistant but susceptible to pepsin digestion (*155*). Ovalbumin, along with phosvitin, was shown to be resistant to peptic digestion at pH 1.2 for >60 min (*147*). Finally, it was reported that OVA was relatively stable in simulated gastric and intestinal fluid; however, preheating could lead to a decrease of its resistance to proteolysis (*149*).

Lysozyme stability has been the object of a number of studies. A recent paper reviews the data obtained on LYS thermal stability (*157*) and demonstrates that polyols such as sorbitol exert a significant stabilizing effect on lysozyme in its native form. In adduct-free solutions, hen's egg lysozyme was reported most stable in the pH range of 3.5−5.0, with a denaturation temperature (T_m) between 75 and 80 °C, whereas at lower pH conditions its stability decreases rapidly and its T_m ranges within 52−56 °C at pH 2 (*157*). The mechanism of denaturation and unfolding of LYS has been finely characterized using the disulfide scrambling method (*158*). LYS has also been shown to be resistant to pepsin digestion and proteinase K after incubation at 37 °C for 60 min (*159*). To examine the relationship between the conformational stability of LYS and its allergenicity, BALB/c mice were sensitized to variants of lysozyme with different conformational stabilities, but similar three-dimensional structures (*160, 161*). Surprisingly, the least stable forms were associated with the strongest Th2-associated responses, as measured by IL-4 and specific IgE production.

Ovotransferrin is a heat-labile allergen, but it was reported that when coupled to bi- or trivalent metal ions, it could form heat-stable complexes (*148, 162*). As mentioned earlier, other egg white proteins, in particular ovomucin, were documented as minor egg allergens (*79, 80*), but their allergenic stability has not been been addressed in the current literature.

5.4. Role of Egg Protein Glycosylation. The primary role of protein glycosylation pertains to the functional properties of the protein. For instance, they notably contribute to the gelling properties of ovomucin present in the egg white. However, there has been evidence that glycosylation was not always essential to the function of egg proteins. For example, deglycosylation of avidin does not affect its affinity for biotin. Furthermore, both glycosylated and unglycosylated forms of OVM domain III were shown to be equally inhibitory toward proteases (*163*). The presence of N-glycosylation usually has a rigidifying or stabilizing effect on protein structure, thereby conferring an enhanced resistance to denaturation (*164*). It was reported that human IgE binding to OVM domain III was greatly reduced by deglycosylation (*165*) but that mouse anti-ovomucoid IgG could still bind to deglycosylated OVM domain III (*166*).

The contribution of glycosylation to egg allergenicity has therefore been explored and yielded mixed results. On the one hand, some studies suggested that the carbohydrate moieties did not contribute to the IgE or IgG binding activity of human patients' sera (*75, 107*). Furthermore, carbohydrate moieties were found not to be important for antigen presentation of ovomucoid to T-lymphocytes (*113*), as assessed in murine models. On the other hand, subsequent studies provided evidence that OVM carbohydrate chains may in fact have inhibitory effects on specific IgE-binding activity (*76*). Recent in vitro studies using recombinant glycosylated variants of OVM domain III indicate that a masking effect exerted by *N*-glycans could contribute to a reduced allergenicity of OVM (*167*). This hypothesis was tested in vivo using a BALB/c mouse model (*167*). A recombinant isoform of OVM domain III (P-Gly) was expressed in a *Pichia pastoris* yeast system. Groups of mice were subcutaneously injected with either P-Gly or native OVM third domain (DIII). Results based on OVM-specific IgE levels and cytokine secretion profiles showed that P-Gly is a hypoallergenic variant of DIII. Further structural analyses determined that P-Gly carbohydrate chains present in residue position 28, but not in position 45, may be involved in the hypoallergenicity of P-Gly. Although the specific role and effect of carbohydrates on egg allergenicity remain an issue of debate, it has been suggested that the structure as well as the location of carbohydrate moieties present on a protein may exert immunomodularoty effects, associated with the stimulation of carbohydrate-specific innate receptors (*168*).

5.5. Cross-Reactivity of Egg Proteins. Studies have determined that cross-reactivity could occur between egg white proteins and egg yolk proteins. Earlier studies showed cross-reactivity between ovotransferrin and ovalbumin, as well as between apovitellenin I and ovalbumin (*169*).

Although in Western countries, hen's eggs are the most commonly consumed, eggs from duck, quail, and goose are sometimes consumed in other countries (*170*). In this regard, serological cross-reactivity has been described between hen's egg proteins and those of other bird eggs such as turkey, duck, goose, and seagull (*64, 131*). Mouse monoclonal IgG raised against Japanese quail ovomucoid was capable of binding to both hen and duck ovomucoid (*171*). A case of duck and goose egg allergy has also been reported, but the patient was tolerant to hen's egg (*172*). A recent study also reported a case of quail's egg anaphylaxis in a child affected by hen's egg allergy (*173*). The bird-egg syndrome, discussed earlier, is a typical example of the cross-reactivity that may exist between egg yolk proteins and bird antigens (*52, 53, 81*). Sequence data today available for egg proteins from many avian species, complemented by computational methods, could help predict such IgE cross-reactivity (*174, 175*).

5.6. Egg Yolk Allergenicity. It was initially believed that egg yolk was void of allergenic components (*176*). However, as previously mentioned, subsequent studies reported the existence of significant IgE-binding activity against egg yolk components (*64, 79, 80, 169*). Clinical reports of the binding activity of human-specific IgE to egg yolk were first documented in the late 1980s (*177, 178*). As indicated earlier, α-livetin, identical to chicken serum albumin, has been identified as a minor allergen and is believed to be significantly involved in the pathogenesis of bird-egg syndrome (*27, 54, 179, 180*). A report documented in fact that heating of α-livetin could significantly reduce, but not totally eliminate, its allergenicity (*54*). Similarly, a recent study determined that apovitellenins, a class of apoproteins representing up to 37% of total egg yolk proteins (*73*), also showed IgE-binding activity, as determined by RAST assays. The proteins were specifically identified as apovitellenins I and VI. Surprisingly, it has also been reported that egg-derived phospholipids (or lecithins) may have residual allergenic properties. However, their presence in manufactured food products was reportedly insufficient to elicit any adverse effects (*3, 181*).

6. EGG ALLERGY AND INDUSTRIAL PERSPECTIVES

The emergence of egg allergy has had various implications for the food industry, affecting their manufacturing and labeling practices. Because no efficient therapy currently exists for egg allergy, strict avoidance of the offending food remains the preventive method of choice. However, the omnipresence of egg-derived components in prepackaged food products represents a significant hurdle for allergic consumers. Therefore, regulatory bodies, food industrials, and research scientists have made joint efforts to implement preventive measures encompassing (a) the production of hypoallergenic egg-containing products using chemical or physical processing methods and (b) the development of commercial kits for robust and sensitive detection of egg-derived contaminants.

6.1. Use of Food Processing in the Making of Hypoallergenic Egg Products. Food-processing methods may alter the allergenicity of food proteins in various ways. In general, food proteins present in a processed food will be in a denatured state, aggregated in protein networks, or interacting with carbohydrates (e.g., Maillard reactions) and lipids, leading either to the reduction of allergen content—and therefore potentially reducing its sensitizing potential—or to the formation of new epitopes (or neoepitopes) (*182*). With the intention to develop approaches for reducing egg allergenicity in food products, a number of studies have investigated the use of food-processing methods such as heat application, enzymatic fragmentation, irradiation, or high-pressure treatments.

Heat Application. Thermal processing is usually carried out to enhance texture and flavors or to ensure microbiological safety, but it is not primarily used to reduce allergenicity. However, the basis for this approach was demonstrated in a clinical study during which patients allergic to freeze-dried egg white did not react to cooked egg white (*78*). Another case report described two patients who developed anaphylactic reactions upon raw egg ingestion, whereas no reaction was observed after cooked egg consumption (*183*). Similarly, earlier in vitro studies showed that thermal treatment of egg white at 90 °C for 10 min led to a >50% decrease of RAST binding intensity using 16 egg-allergic patients' sera (*64*). Recently, it was documented that heating of α-livetin at 90 °C for 30 min led to an 88% reduction of its IgE reactivity (*54*). On the other hand, other studies reported that residual IgG-binding activity against ovalbumin and ovomucoid could still be detected in both soft-boiled (100 °C, 3 min) and hard-boiled (100 °C, 20 min) eggs, as tested by radio-immunoelectrophoresis (*68*).

Enzymatic Fragmentation. Enzymatic processing represents a more specific approach than thermal processing and was reported to be efficient in the production of milk-based hypoallergenic formulas (*184, 185*). Stability of the major egg allergens to enzymatic digestion was discussed in section 5. However, epitopes present on most food allergens are believed to be sequential, so that reduction in egg allergenicity would only be expected when egg allergen epitopes are eliminated by the enzymatic fragmentation (*186*). Another major hurdle associated with the use of enzymatic hydrolysis is to maintain the unique functional properties of egg proteins, for example, foaming and gelling. Interestingly, a nine-step process involving a combina-

tion of thermal treatments and enzymatic hydrolyses was recently described, in which a hydrolyzed liquid egg product with 100 times less IgE-binding activity than the starting material was obtained. Analyses were based on in vitro analysis (Western blot and EAST inhibition assay) using pooled sera obtained from 11 egg-allergic patients (*187*). More importantly, the authors reported that functional properties of the technologically modified egg preparation, such as flavor and texturizing properties, were not altered when incorporated into various food products. This suggests the potential for food industrials to manufacture customized products accessible to egg-allergy sufferers.

γ-Irradiation. Radiation technology has been explored in a number of studies for the modification of egg and other food allergens, such as shrimp and milk allergens (*188, 189*). In the food industry, treatment doses up to 3 kGy ensure a bacteriological quality of acceptable standard for liquid, frozen, or dehydrated egg white preparations (*148*). One study has investigated the use of γ-irradiation in the confection of hypoallergenic egg-based cakes (*190*). ELISA assays using egg-allergic patients' sera and OVA-specific rabbit IgG revealed a significant decrease in OVA content after γ-irradiation treatment. The same authors reported that γ-irradiation of OVA led to a hypoallergenic form of the protein (*191*). Results showed that γ-irradiation superior to 10 kGy altered the structure of OVA and led to a significantly decreased immunogenicity. Immunization trials in BALB/c mouse revealed that the titers of OVA-specific immunoglobulins and OVA-specific T-cell-mediated responses were significantly lowered in groups of mice sensitized to irradiated OVA. Furthermore, the combination of γ-irradiation and heat treatment has been shown to be efficient in reducing the IgE-binding properties of OVM, the immunodominant egg allergen (*192, 193*). These findings suggest that structural alteration of protein allergens may offer great opportunities for the development of desensitization approaches in the context of egg allergy.

Other Food-Processing Methods. A main obstacle to the use of food processing in reducing egg allergenicity is the risk to alter the unique functional attributes of egg proteins. Therefore, alternative and milder approaches have been proposed. A recent review suggests that novel food-processing methods, such as high pressure and pulsed electric field, possibly combined with physical and biochemical treatments, hold great promise for the development of hypoallergenic food products (*194*). No such treatment has yet been reported for the reduction of egg allergenicity. On the other hand, the production of egg food products with low ovomucoid content by solvent extraction has been explored. Ethanol (20%) precipitation successfully removed 70% of the OVM content from egg white powder, without affecting the whipping ability and foam stability of the remaining egg white proteins (*195*).

6.2. Detection of Egg Allergens in Food Products. Egg-derived components are very often added to processed food for a very specific purpose, for example, as emusilifiers or gelling agents. Quantification methods in food analyses are necessary to comply with legal requirements such as food labeling but also, more importantly, to ensure the protection of food-allergic consumers. Ideally, analytical methods aimed at the detection of egg and other food allergens should provide specific (e.g., reliable detection in a wide range of matrices), sensitive (e.g., at levels relevant to thresholds reported in allergic populations), and rapid (e.g., suitable for routine testing and large volume production) analyses (*196*). Lists of commercially available detection kits and challenges faced in the development of analytical tools can be found in recently published reviews (*197, 198*).

Diffusion-in-Gel Methods. Diffusion-in-gel methods, such as radio-immunoelectrophoresis (RIE) and cross-radio-immunoelectrophoresis (CRIE), were the first methods to be used in the study of egg allergens. They led to the egg content analysis of various processed food products including pastes, mayonnaises, ready-to-cook mixtures, ice creams, and pie fillings (*199*) and allowed the investigation of the clinical importance of ovalbumin and ovomucoid as major egg allergens (*67, 68, 200*). However, their lack of practicality for the food industry, as well as their low sensitivity, led to the preferential use of immunoenzymatic techniques such as ELISA.

Antibody-Based Methods: ELISA and Biosensors. A number of immunoassays have been described for the detection of egg proteins in processed food products. Currently, the methods mostly employed in the detection of egg allergens are based on immunological methods such as ELISA. Currently, commercial ELISA kits for the detection of egg allergens are mostly based on the detection of ovalbumin and ovomucoid (*177*). As yet, only a few studies have investigated the detection of egg yolk proteins in food products (*201*).

For instance, earlier studies reported the quantification of ovalbumin in canned foods (*202*) and dairy-based foods (*203*), as well as the detection of other egg proteins in a wide range of egg-containing processed foods (*204, 205*) using ELISAs. A relevant study reported the successful development of a sandwich ELISA method for the detection of residual ovalbumin in pasta products, a method deemed to be useful for routine monitoring in industrial cleanup operations (*206*). It seems, however, that most immunoassays have so far been based on the binding properties of specific IgG isotypes, thus detecting antigenic components rather than providing a reliable indication of allergenicity (*177*). One of the first reports to identify the presence of egg white allergens in processed foods using serum IgE from egg-sensitized patients in an ELISA assay was completed by Leduc and co-workers (*207*).

Other antibody-based techniques have also been examined. An array biosensor has been developed for the detection of ovalbumin as an indicator of egg contamination and was shown to reach a detection limit as low as 13 ppb in pasta extracts (*208*). Using a similar approach, a very recent study reported the successful use of resonance optical biosensors in the detection of multiple food allergens, including eggs, with a sensitivity of 1−12.5 ppm (*209*). Specific detection of ovomucoid by surface plasmon resonance (SPR) technology (Biacore, Biacore AB, Uppsala, Sweden) has also been investigated (*177, 210*).

The supplementation of food products with lysozyme as an antimicrobial agent also led to the development of a sandwich ELISA detection test (*211*). More recently, a study using lysozyme as a food allergen model has reported the development of an automated system, based on the use of antibody-conjugated bacterial magnetic particles deemed to reach a turnover of 24 samples per hour (*212*). Many efforts have been made to develop ELISAs for egg allergen detection in various matrices (*213*), and their performances have been carefully validated through interlaboratory evaluations (*214*).

Electrophoretic and Blotting Methods. Electrophoretic and blotting methods may be useful for the detection of egg components in raw or slightly cooked products, but may fail in the analyses of highly cooked or sterilized products because of the denaturation and protein aggregation processes undergone during thermal treatment (*177*). Isoelectric focusing techniques coupled to immunoblotting techniques were described to determine the presence of egg white byproducts in raw and

heat-pasteurized meat pastes (*207*). However, electrophoretic and blotting methods are most commonly used to study the IgE- and IgG-binding abilities of modified or native egg allergens (*172, 177, 215*) and have been rarely used for the quantitative determination of egg allergen content.

PCR-Based Detection Methods. The use of Polymerase Chain Reactions (PCR) for the detection of egg allergens has not been reported as yet. In general, the use of PCR in food analysis is afflicted by a number of hurdles, including DNA fragmentation ensuing food processing, the presence of PCR inhibitors, and low extraction yield. Unless combined with protein-based specific detection methods, a PCR-based approach may not allow chicken-derived DNA to be distinguished from egg-derived DNA and may not therefore represent the best alternative. Furthermore, PCR-based methods are more likely to generate false-negative results, for example, contamination of food products with DNA-free egg allergens (*196*).

Limitations of Current Detection Methods. There are obstacles to the quantification of egg contaminants in food matrices. As mentioned above, the efficiency of egg protein extraction has a direct correlation on the interpretation of a test, and a low extraction yield can lead to false results (*216*). Moreover, it should be emphasized that the immunoreactivity (i.e., antibody-binding activity) of egg proteins can be altered during food processing. Denaturation or aggregation of food proteins during processing or matrix effects due to lipids or Maillard reactions may influence the detection test results (*177*). Robust methods capable of detecting residual contaminants in raw as well as in processed food products are still warranted. One study investigated the IgG- and IgE-binding properties of ovomucoid in a pasta-like model of wheat flour mixed with egg white (*217*). The authors reported that heating of the pasta model caused the formation of aggregates with wheat proteins and may therefore explain the almost complete abrogation of rabbit IgG- and human IgE-binding activity against ovomucoid (*217*).

Clinical Assessment of Processed Foods. Due to their inherent limitations, the use of allergen detection tests in processed foods may sometimes be insufficient to ensure that the allergic consumer is safe. Contamination with egg components is a very common source of hidden allergens. Therefore, it could be argued that the analyses of allergens in food products should be supplemented with diagnostic tests such as the skin-prick test, radio-allergosorbent tests, or inhibition assays and validated with double-blind placebo-controlled food challenges. It has been suggested that clinical studies be conducted by involving referral populations with distinct profiles (e.g., countries, age, symptoms, and sensitization profile) and validated with appropriate numbers of patients showing convincing allergic reactions toward the allergen of interest (*218*). Alternatively, the biological activity of egg-containing food products could be assessed by bioassays based on their capacity to degranulate IgE-bound basophils or mast cells and the quantification of vasoactive mediators such as histamine. A number of cellular systems have been described for this purpose; they, however, remain reserved for diagnostic or research purposes (*177*).

7. CURRENT PREVENTIVE AND THERAPEUTIC OPTIONS FOR EGG ALLERGY

7.1. Exclusion or Avoidance Diet. Upon established diagnosis of egg allergy, the most medically advised approach is that of a preventive intervention consisting in complete exclusion of the offending food from the patient's diet. However, the main issues with elimination diets remain those of compliance, potential for nutritional deficiencies, and social hurdles, for example, the time required purchasing food or preparing meals and the impossibility of eating at restaurants or at school (*58*). More importantly, exclusion diets do not annihilate the risk of inadvertent exposure to egg components, which may sometimes be life-threatening. It has in fact been reported that 22% of allergic reactions caused by eggs were due to contamination with "hidden" egg allergens (*219*). Interestingly, a six-month study involving a cohort of 90 egg-allergic children has compared the effect of an exclusion diet to that of an oral desensitization regimen with egg and egg-containing products. At the end of the trial period, SPT wheal diameters and specific IgE levels were monitored after a single-blind placebo-controlled food challenge (SBPCFC) with a high dose of raw egg white. Surprisingly, mean wheal diameter sizes and specific IgE levels were significantly lower in children undergoing the desensitization protocol. The report concluded that an avoidance diet may not represent the safest alternative for egg-allergic patients and may, in fact, have a negative impact by lowering the patient egg reactivity threshold (*220*). Additional studies supported the fact that regular intake was necessary to maintain the status of tolerance (*26*).

7.2. Oral Immunotherapy (OIT) or Specific Oral Tolerance Induction (SOTI). As mentioned above, avoidance diets are primarily preventive and present several hurdles as they are difficult to comply with, they may lower threshold reactivity, and they often leave the natural history of egg allergy unchanged (*220*). Therefore, an active and therapeutic approach seems to be more appropriate. Among them, oral desensitization procedures with egg components have been largely investigated (**Table 3**). In the context of food allergy, the terminology associated with a good prognosis remains controversial. It is commonly referred to as *desensitization*, *hyposensitization*, or *SOTI* (*221*). Earlier studies using mouse models established the possibility of inducing a status of immune tolerance by the administration of ovalbumin with either microdoses (100 μg/dose for 14 days) (*222*) or low-concentration diet (5 vs 20% protein content) (*223, 224*). In a BALB/c mouse model, Kjaer and Frøkiaer demonstrated that there was a microgram threshold (100 μg of OVM/day for 40−50 days) above which a Th2-mediated response could be reversed depending on the length of the feeding period (*225*). The mechanisms of tolerance induction were characterized by increased levels of TGF-β and IL-10 cytokines in animal models such as the atopic dogs (*226*).

Recent attempts at desensitizing human egg-allergic patients using oral desensitization or OIT have ensued. The potential to use a low-dose challenge to facilitate the reintroduction of egg in allergic children has been examined (*227*). Using raw egg in a sublingual administration setting, several attempts to standardize the procedures of oral desensitization have been reported (*228–230*). Encouraging results were obtained from a pilot study in which egg-allergic subjects were documented to develop partial tolerance to egg ingestion (*230*). A larger study was subsequently conducted by the same authors, in which >90% of a patient cohort ($n = 17$) successfully developed immune tolerance upon challenge with egg yolk or albumen and an additional two patients developed partial tolerance (*231*).

In a pilot study involving seven egg-allergic children, it has been reported that OIT was successful in raising the children's reactivity threshold (*232*). The major benefit of increasing the threshold dose for egg reactivity is to reduce the risk of severe reactions upon accidental egg allergen exposure. The study is ongoing, involving an increasing number of egg-allergic children and aiming to conclude whether the specified OIT protocol is capable of not only increasing the reactivity threshold but also, ultimately, inducing a status of clinical tolerance (*233*). Similar

Table 3. Immunotherapeutic Approaches Investigated in the Context of Allergy to Egg or Egg White Proteins

immunotherapeutic approaches	exptl models	egg allergens	setting	outcome/comments	refs
oral immunotherapy/oral tolerance induction	human clinical trial ($n = 17$)	whole egg or egg albumen	therapeutic	egg-allergic children (<16 years old) subjects underwent sublingual desensitizing treatment; control group followed an elimination diet; successful desensitization in 12 patients and partial tolerance in 2 patients	*231*
	human trial ($n = 18$)	whole egg or albumen	therapeutic	increasing doses of allergen were ingested; successful desensitization in 13 patients, among which were 10 children aged <16 years old	*230*
	human trial ($n = 5$)	whole egg	therapeutic	aim of study was to standardize oral desensitization program; subjects who completed the 4−5 month treatment could tolerate egg-containing food products	*229*
	human trial ($n = 8$)	whole egg	therapeutic	treatment successful in 6 patients showing egg allergy; side effects (urticaria, angiodema, rhinitis) were reported in 3 subjects during treatment	*228*
	human cases ($n = 2$)	hen egg proteins	therapeutic	two patient cases showing that oral tolerance is transient when a successful desensitizing treatment is followed by a period of allergen avoidance	*26*
	human trial ($n = 11$)	hen egg proteins	therapeutic	permanent tolerance achieved in about one-third of subjects and partial tolerance in two-thirds of the cohort; treatment could successfully increase reactivity threshold	*234*
	human trial ($n = 39$)	egg white proteins	therapeutic	aim of study was to develop a model of low-dose food challenge to facilitate gradual reintroduction of egg in young children outgrowing their allergy	*227*
	human trial ($n = 90$)	whole egg	therapeutic	first randomized study of oral tolerance in food-allergic children; comparison of avoidance (A) vs oral desensitization (OD) diet: OD diet resulted in improved recovery rates and lowered sensitivity	*220*
	human trial ($n = 7$)	whole egg or egg white	therapeutic	study aimed at decreasing the threshold reactivity in nonanaphylactic children; 2 subjects showed oral tolerance; maintenance phase with daily doses improved reactivity threshold in most patients	*232*
oral immunotherapy/oral tolerance induction	atopic dog ($n = 6$/group)	OVA	therapeutic	daily feeding with OVA (10 mg) for 28 consecutive days; mechanisms of oral tolerance involved increased levels of TGF-β and IL-10	*226*
	transgenic mouse for $OVA_{323-339}$	OVA	therapeutic	daily oral microfeeding with 100 μg/dose of OVA for 14 days; suppression of Th2 allergic response is mediated by down-regulation of costimulatory molecules (B7 family) on APC and TCR expression	*222*
	BALB/c mouse	OVM	prevention	microdoses of OVM in drinking water: oral tolerance induction obeys to a threshold mechanism (cutoff value at 100 μg/day), selective for the inhibition of Th2-dependent response	*225*
chemically or physically or chemically modified allergens	BALB/c mouse	OVA	comparative study	mice immunized to either intact OVA or γ-irradiated OVA (10−100 kGy): significantly lower OVA-specific IgE and reduction of both specific Th1 and Th2-cytokine secretions	*191*

Table 3. Continued

immunotherapeutic approaches	exptl models	egg allergens	setting	outcome/comments	refs
	BALB/c mouse	LYS	comparative study	conjugation of OVA with copolymer of maleic anhydride and *N*-vinylpyrrolidone (VMA) results in preferential stimulation of Th1-like response	*244*
	in vitro	OVM	in vitro analysis	combination of heat and irradiation can reduce human IgE-binding activity (ELISA) to OVM	*193*
	in vitro	OVA, OVM, LYS, OVT	in vitro analysis	impact of heat denaturation, reduction, carboxymethylation on human IgE- and IgG-binding properties from 8 egg allergic patients sera; importance of sequential vs conformational epitopes is highlighted	*32*
	BALB/c mouse	LYS	comparative study	RCM (reduced and carboxymethylated)-HEL generated a skewed Th1-response, independent from dose or adjuvant	*247*
	human trial ($n = 38$)	egg white/OVM	food challenge study	21/38 patients with positive challenge to freeze-dried EW have negative response to heated EW; 16/17 subjects with positive response to heated EW did not respond to the heated OVM-depleted EW challenge	*78*
peptide-based	Sprague−Dawley rats	OVA	prevention	orogastric administration of OVA-peptides (pepsin digestion) have prophylactic effects in neonatal rats	*259*
	BALB/c transgenic mouse DO11.10	OVA	therapeutic	12 peptide analogues (single alanine substitutions) of $OVA_{323-339}$ were tested; peptide analogues with Th1-skewing properties can efficiently modulate an ongoing Th2-response	*260*
	Wistar/Brown Norway rats	OVA	prevention	oral gavage with 173−196 fragment (CN-Br cleavage derived) inhibits Th2-like response by promoting oral tolerance to OVA and reducing specific IgE responses	*119*
recombinant hypoallergens	BALB/c mouse	OVM	therapeutic	inhibition of OVM-specific immune response and shift toward a Th1-type bias, by alteration of immunodominant IgE epitope (i.e., GMFA molecule)	*83*, *112*, *254*
	BALB/c mouse	OVM	comparative study	mutant glycosylated form of OVM shows significantly lower serum-specific IgE and reduced levels of Th2-type cytokines	*167*
novel allergen delivery systems	transgenic mouse DO11.10	OVA	therapeutic	study shows that antigen delivery by oral administration of genetically engineered bacteria led to antigen-specific tolerance; significant upregulation of FOXP3 and CTLA-4; also known as bacterial encapsulated-allergen immunotherapy	*278*
	BALB/c mice	OVA	therapeutic	intradermal administration of OVA-containing microparticles (poly-ϵ-caprolactone) led to lower levels of serum histamine, higher survival rate, and weaker anaphylactic responses, compared to OVA-alum group	*276*
oral administration of probiotics	children ($n = 26$)	OVA	prevention	population of children with atopic dermatitis: a transient decrease in IL-13 responses to OVA during probiotics supplementation period (PBMC)	*283*

Table 3. Continued

immunotherapeutic approaches	exptl models	egg allergens	setting	outcome/comments	refs
	human study ($n = 13$)	whole egg	therapeutic	enhanced production of Th1 and Treg cytokines was observed in vitro with PBMC cultures; however, specific IgE levels and SPT wheal sizes remained unchanged after probiotics challenge period	*284*
	BALB/c mouse	OVM	therapeutic	*Lactococcus* lactis G50 can enhance Th1-type immune response; total IgE, specific IgG1, and IgE significantly decreased after G50 administration; probiotic strain G50 can be potentially used for suppression of Th2-mediated hypersensitive reactions	*282*
DNA-based immunotherapy (ISS and gene vaccination)	mouse W/W^v	OVA	comparative study	oral administration of CpG-OVA induced antigen-specific Th1 responses (immunoglobulins and cytokines), compared to control groups; potential use of CpG- OVA as an oral vaccine	*275*
	BALB/c mouse	OVA	therapeutic	mouse model of allergic asthma: subcutaneous administration of CpG-like motifs is capable to prevent allergic airway hyper-responsiveness, with a decrease in specific IgE and IL-4 levels	*262*
	BALB/c mouse	OVA-cytokine fusion DNA	prevention/therapeutic	intramuscular administration of OVA−interleukin 18 fusion plasmid can prevent but also reverse an established airway hyper-responsiveness	*265*
	C57BL/6 mouse	OVA-cytokine fusion DNA	comparative study	OVA−interleukin 18 fusion plasmid efficiently induced OVA-specific Th1-response and inhibited in vitro production of OVA-induced IL-4	*264*
	C57BL/6 mouse	plasmid DNA-OVA	comparative study	one of the first studies to show that administration of plasmid DNA encoding for a specific allergen induced a differentiation of naive T-helper cells toward a Th1-phenotype	*263*
immunomodulatory food components	BALB/c mouse	OVA	therapeutic	*Quillaja* saponin suppresses IgE-mediated immune response to OVA characterized by a shift from Th2- to Th1-biased response	*296*
	BALB/c mouse	OVA	impact of dietary components	studies report that a low-protein diet (5% casein) can favor induction of oral tolerance (OVA oral feeding) by modulation of the Th1/Th2 balance toward a nonallergic response	*223*, *224*
	Brown Norway rats	OVA	impact of dietary components	feeding of enzyme-treated wheat flour lowered ovalbumin concentrations detected in plasma; inhibition of ovalbumin absorption may explain the delayed sensitization observed	*293*

results were obtained from an independent study involving egg and cow's milk allergic children (*234*). The authors reported earlier a study involving three cases of children allergic to cow's milk and egg. The results suggested that the tolerance status induced by SOTI may only be transient and that regular intake of the offending food may in fact be essential to maintain the tolerance status (*26*). The above studies and their outcomes are summarized in **Table 3**.

OIT approaches seem to hold promising perspectives for egg-allergic patients but require further optimization and validation with larger cohorts of patients with varied clinical profiles. Furthermore, in most studies, the most sensitive patients were carefully excluded because of the potentially life-threatening reactions associated with the oral challenges. Therefore, alternative approaches to OIT remain warranted for highly sensitive egg-allergic patients such as anaphylactic individuals.

8. NOVEL IMMUNOTHERAPEUTIC APPROACHES FOR EGG ALLERGY

Immunotherapeutic approaches are usually divided into specific and nonspecific approaches. Specific immunotherapy (SIT) targets the individual allergens responsible for the patient's

disease, whereas nonspecific immunotherapy aims at modulating the immune system in an allergen-independent manner (*235*).

8.1. Principle and Mechanisms of Allergen-Specific Immunotherapy (SIT). The principle of SIT is based on administration of an allergen to which the patient has been sensitized, in an attempt to reduce or abolish the clinical responses associated with exposure to that allergen (*235*). Under healthy physiological conditions, a dynamic balance is maintained between Th1 and Th2 responses. Egg allergy is regarded as a Th2-weighted imbalance; therefore, novel immunotherapeutic approaches have often aimed at redirecting a Th2-biased toward a Th1-biased response. In the past few years, recent findings have drawn attention to the immunomodulatory functions of another class of lymphocytes, known as regulatory T-cells, capable of regulating both Th1- and Th2-associated responses. The mechanisms underlying allergen-SIT are currently the focus of intense investigations, which are beyond the scope of this review. Comprehensive information can, however, be found in recent papers (*236–238*). In brief, a successful SIT is believed to result from diverse mechanisms including (a) immune deviation from a Th2- to a Th1-skewed immune response, (b) activation of T-regulatory cells, (c) pro-inflammatory T-cell anergy, and/or (d) apoptosis of allergen-specific T-cell clones.

There has been strong evidence suggesting that conventional SIT, using pure allergens or allergen extracts, could be efficient in allergic diseases caused by inhalant allergens (*239*). However, conventional SIT is not usually encouraged in egg and other food allergies due to unacceptably high rates of adverse responses (*240*). Therefore, alternative and safer SIT approaches have been investigated.

8.2. Current Options for Immunotherapeutic Approaches. **Table 3** provides an overview of the different immunotherapeutic approaches investigated as yet in the context of egg allergy.

Chemically and Physically Modified Egg Allergens. A number of attempts were made to chemically modify allergens for use in specific immunotherapy. Glutaraldehyde and formaldehyde were among the first reagents to be used (*241*), followed by carbamylation strategies (*242, 243*). The concept behind the production of chemically modified allergens, or allergoids, lies in the elimination (by masking or by removal) of the IgE-binding determinants while retaining T-cell reactivity, thereby preserving immunogenicity (*244*). Allergoid preparations have been well established for allergen-specific immunotherapy against inhalant allergens (*245–248*), but not many papers have been found on food allergens. Several conjugates of OVA with the copolymer *N*-vinylpyrrolidone and maleic anhydride (VMA) were evaluated in a murine model of allergy (*244*). Passive cutaneous anaphylaxis, histamine release, and RAST inhibition assays determined that the conjugate OVA (20%)−Acp−VMA had a significantly decreased IgE-binding capacity compared to its native analogue. The administration of the conjugate to OVA-sensitized mice led to a significant decrease of OVA-specific IgE accompanied by a greater production of OVA-specific IgG, suggesting its potential use in therapeutic applications.

A recent study finely characterized the T-cell responses induced by the native versus chemically modified form of the hen's egg white lysozyme in a mouse model (*247*). HEL was reduced in an 8 M urea solution, followed by a treatment with iodoacetic acid, resulting in a reduced and carboxymethylated form of HEL (RCM-HEL). An enhanced Th1 response was induced by RCM-HEL compared with native HEL independent of the dose or type of adjuvant used. A lower production of IL-4, combined with an enhanced IFN-γ production, and a drastic drop in specific IgG1 titers were suggestive of a skewing toward a Th1 bias.

Food-processing treatments have also been examined in the context of immunotherapy. For example, the use of heated and OVM-depleted egg white preparation has been proposed as a desensitizing approach in egg-allergic patients (*78*). Animal studies have suggested, however, that cooked egg white may lose its desensitizing potential (*249*). Groups of mice were sensitized to either intact OVA or irradiated OVA. Results revealed that γ-irradiated OVA led to a hypoallergenic form of the protein, as evidenced by a significant decrease in both antibody-mediated response (decrease in OVA-specific IgE and IgG titers) as well as T-cell-mediated response with a decline in both Th1- and Th2-type cytokine production (*191*). Extrapolation of these results to clinical applications warrants further investigations.

Genetically Engineered Egg Allergens. Genetic engineering consists in introducing a number of modifications into a protein to alter its biological function or biochemical properties in a targeted manner (*250*). Clones of cDNA have been isolated and reported for an increasing number of food allergens and were expressed in prokaryotic and eukaryotic systems as recombinant molecules. Studies have reported the successful expression and purification of recombinant ovomucoid and ovalbumin, two major egg allergens, using histidine tag in an *Escherichia coli* expression system (*215, 251*). In theory, recombinant allergens with therapeutic potential show a reduced capacity to bind IgE—thus ensuring lower risks of IgE-mediated side effects—while retaining their T-cell immunogenicity and their propensity to induce so-called *blocking IgG* molecules (*252, 253*).

Site-directed mutagenesis can disrupt the structural motifs involved in IgE-binding activity, also known as B-cell epitopes. The substitution of residues occurring in position 32 (glycine to methionine) and position 37 (phenylalanine to alanine) in ovomucoid resulted in a drastic loss of IgE- and IgG-binding activities by human egg-allergic patients sera, as evidenced by ELISA and Western blot analyses (*154*). The isoform was coined GMFA recombinant domain III. GMFA mutant was assessed in vivo for its allergenicity compared to its native analogue (*83*). Intraperitoneal injections of either GMFA recombinant or native OVM domain III were administered to distinct groups of BALB/c mice. In the GMFA-sensitized mice, increased levels of IFN-γ and low levels of IL-4 were detected in splenocyte culture supernatants. Similarly, OVM-specific IgE titers were significantly lower and specific IgG2a titers higher, suggesting that GMFA isoform is a hypoallergenic form of the antigen and favored the development of a Th1-skewed immune response (*83*). The potential use of GMFA recombinant as desensitizing agent has therefore ensued.

Using a BALB/c mouse model, GMFA mutant was administered to mice previously sensitized to OVM domain III (*254*) or to full OVM (*112*). In the first study, administration of GMFA mutant to OVM domain III-sensitized mice caused a significant reduction of hypersensitivity responses. A decrease in both serum histamine and specific IgE titers, accompanied by elevated concentrations of IFN-γ in spleen cell cultures, suggested a deviation toward a Th1-dominant response. In addition, increased concentrations of cytokine IL-10 in spleen cell supernatants suggested the ability of GMFA to activate regulatory mechanisms (*254*). Similarly, in the second study, full OVM-sensitized mice subsequently exposed to GMFA showed significant improvement in their overall allergic response when compared to the positive control group (*112*). The mechanisms underlying the desensitizing effect of GMFA remain to be

elucidated, but it can be hypothesized that the point mutations present on GMFA may have resulted in a differential antigen presentation to immune cells, resulting in a switch of the specific immune response toward a Th1-dominant response. Recently, a mouse study has documented the hypoallergenicity of a genetically glycosylated form of OVM domain III, using the *P. pastoris* yeast expression system (*167*), and results were discussed in section 5.4. The results suggest that genetically glycosylated OVM could be investigated as a desensitizing molecule in egg allergy.

The expression of recombinant molecules for other major egg allergens, such as ovotransferrin and egg lysozyme, have also been reported in *P. pastoris* (*255, 256*). However, their antigenic and allergenic properties have not been explored in a context relevant to food allergy. Collectively, these data provide strong support for the potential use of recombinant forms of egg allergens in immunotherapeutic approaches.

Peptide-Based Immunotherapy (PIT). The concept behind PIT is to induce a protective immune response while avoiding potential undesirable side effects. The principle is based on the use of non-anaphylactic (synthetic or not) peptides, unable to cross-link IgE molecules present at the surface of mast cells (*250*). One of the most promising forms of PIT uses peptide fragments containing T-cell epitope sequences. It is suggested that T-cell epitope-containing fragments can modulate the activity and functions of T-lymphocytes via diverse mechanisms involving T-cell anergy, induced-apoptosis, or regulatory functions (*257*). Clinical studies have shown the applicability and potential efficacy of PIT in the context of respiratory or insect venom allergies (*258*). However, no investigations have yet reached the same extent in food allergy.

One of the major egg allergens, ovalbumin, has served as a surrogate allergen in the development of peptide-based approaches. In a neonatal rat model, an earlier study suggested that the enteric administration of OVA−pepsin-digested fragments led to a significant decrease of serum-specific IgG levels, in a preventive manner (*259*). The study was one of the first to emphasize (a) the importance of fragment sizes with regard to their immunomodulatory effects and (b) the differential effects due to the route of peptide administration. Following identification of the immunodominant T-cell epitope OVA323−339, an interesting in vivo study showed that analogue peptides, designed with an MHC binding affinity similar to that of the fragment 323−339, could skew the allergic response toward a Th1 response (*260*). These effects were mainly attributed to the altered interaction between the MHC-peptide and the TCR complex. This emphasizes yet again that appropriate structural modifications have a predefined impact on the efficacy of immunotherapeutic interventions in food allergy.

More recently, the fragment 173−196 of ovalbumin, identified as a T-cell epitope, has been tested for it capacity to suppress ovalbumin-induced responses in a rat model of egg allergy (*119*). The oral administration of the T-cell epitope fragment prior to ovalbumin administration resulted in a lower titer of OVA-specific IgE and a decrease in the proliferation rate of inguinal lymph nodes upon in vitro stimulation with native OVA.

DNA-Based Immunotherapy. The potential of using DNA vaccination (or gene vaccination) represents an innovative approach that is being actively investigated in the context of infectious diseases. Gene vaccination classically involves the intramuscular or intradermal administration of plasmid DNA encoding an antigen. Gene vaccination has only recently been recognized as a potential strategy for the treatment or prevention of food allergy (*261*), and the number of papers related to its use in egg or food allergy remains meager. However, promising results were obtained from DNA vaccination studies, using ovalbumin as a surrogate allergen, aiming at modulating allergen-induced IgE synthesis and airway hyper-responsiveness (*262*). In a mouse model of late-phase allergic response to OVA, gene vaccination was shown to down-regulate a pre-existing Th2 response as well as inhibit the production of specific IgE (*263*). Furthermore, to enhance the efficacy of DNA vaccination, various strategies were developed, such as the fusion of allergen plasmid with regulatory molecules (e.g., cytokine) or the use of polymer vectors for targeted delivery of the allergen (e.g., mucosal sites). For instance, an interesting study assessed the efficacy of a fusion plasmid containing the cDNA coding for OVA combined with the cDNA of Th1-driving cytokine such as IL-18 (*264, 265*). On the other hand, chitosan particles containing plasmid coding for Ara h 2, a major peanut allergen, were orally administered to mice. Chitosan presents the main advantages of being a biocompatible mucoadhesive polymer and a nonviral vector (*266*). How the plasmid DNA is transcribed, translated, and presented to the immune system remains obscure (*267–269*). Elucidation of these mechanisms may, however, encourage further investigations into the therapeutic or prophylactic use of DNA vaccination in egg allergy.

The immunomodulatory effects of DNA vaccines results primarily from the presence of immunostimulatory CpG motifs present within the bacterial plasmid backbone. These CpG motifs or immunostimulatory sequences (ISS) have the capacity to deviate an allergen-driven Th2 immune response toward a nonallergic Th1 response (*250*). The molecular and cellular events triggered by CpG motifs are primarily mediated by innate receptors known as Toll-like receptors (TLR) (*270*). This information led to a number of studies in which oligonucleotides containing ISS sequences were conjugated to various allergens for their potential use in SIT. Reports using ovalbumin as a model allergen have investigated the potential therapeutic use of ISS in asthma mouse models (*271–273*). One study in particular has investigated the synergetic effects of OVA conjugated to CpG motifs, as opposed to OVA alone or OVA mixed with CpG motifs (*274*). A dramatic increase in IFN-γ secretion upon in vitro stimulation of lymph node cells, in concomitance with decreased levels of IL-4 and IL-5, gave reason to believe that a switch from a Th2- to a Th1-dominant response occurred. Another study has supported the potential use of CpG-conjugated OVA as an oral vaccine using an allergic mouse model (*275*).

Immunotherapy Using Novel Antigen Delivery Systems. Nanoparticles and liposomes are examples of drug-delivery systems that represent innovative strategies for the administration of drugs and bioactive molecules. Studies using ovalbumin as a surrogate allergen have demonstrated that the substitution of conventional adjuvants (e.g., aluminum hydroxide) with micro- or nanoparticles could not only simplify immunization schemes (e.g., decrease the number of administrations) but also render the immunotherapeutic approach safer and more efficient (*276, 277*).

Besides the use of synthetic particles, the increased knowledge of microorganisms and their controlled genetic manipulation has allowed the introduction of a new type of delivery system: bacterial microorganisms. The food grade bacterium *Lactococcus lactis* was engineered to secrete OVA and was orally administered to a T-cell receptor (TCR)-transgenic mouse model (DO11.10). Measurements of delayed-type hypersensitivity responses and cytokine secretion upon OVA stimulation as well as adoptive transfer experiments were conducted to assess the

tolerogenic potential of OVA-secreting *L. lactis*. Results determined that OVA-secreting *L. lactis* could lead to antigen-specific tolerance, characterized by suppression of OVA-specific T-cell responses. This suppression was shown to be mediated by induction of $CD4^+$ $CD25^+$ Treg cells, in a TGF-β-dependent manner, accompanied by up-regulation of the transcription factor FOXP3 and surface CTLA-4 expression (*278*).

Nonspecific Immunotherapeutic Approaches. Nonspecific immunotherapy is also termed immunomodulation (*235*). Nonspecific immunotherapy is usually envisaged when other forms of treatment are unavailable or as a complementary intervention during the implementation of SIT.

(i) Use of Probiotics and Prebiotics. Understanding of the relationships between intestinal microflora and immune disorders is increasing. For many years now, probiotic bacteria have been shown to confer health benefits to humans, mainly through their immunomodulatory capacities. One of the first studies to assess the immunomodulatory effects of probiotics bacteria on ovalbumin-induced allergic response reported that oral feeding of heat-killed *Lactobacillus casei* strain Shirota could inhibit the production of OVA-specific IgE in BALB/c mice, in line with results obtained from Th1- and Th2-type cytokine secretion patterns (*279*). Probiotic bacteria from different genera (e.g., *Lactococcus*, *Lactobacillus*, *Bifidobacterium*) have been assessed in a number of studies involving murine models. For example, probiotic bacteria were administered into C3H/HeJ mice either in a preventive or in a curative setting or concomitantly to ovalbumin sensitization. In the preventive setting, oral administration of probiotics *Bifidobacterium bifidum* BGN4 and *L. casei* 911 led to decreased levels of total and OVA-specific IgE, as well as specific IgG1 (*280*). A similar study showed that *B. bifidum* G9-1 (BBG9-1) could also exert allergy-inhibiting effects in OVA-sensitized BALB/c mice (*281*).

Strains of *Lactococcus* microorganisms are commonly used as starter bacteria in the manufacture of many types of fermented dairy products. Ovomucoid-sensitized BALB/c mice were orally challenged with *L. lactis* subspecies *lactis G50* (Napiergrass) strain in a daily fashion. The total IgE antibody level in the G50-treated group was significantly lower than that of the control group. Similar observations were found in measurements of OVM-specific IgG1 and IgE titers, suggesting that *lactis* subspecies *lactis G50* may be used as a probiotic strain for the suppression of egg hypersensitivity mediated by Th2-skewed responses (*282*).

Egg hypersensitivity was documented in two-thirds of children with severe atopic dermatitis (*29*). The use of probiotics in the prevention or treatment of atopic dermatitis has therefore been explored. One clinical study involved 56 children with moderate to severe atopic dermatitis, among which 71% tested positive for specific IgE RAST to a food mixture containing egg white, milk, cod, wheat, peanut, and soybean (*283*). Participants received *Lactobacillus* fermentum VRI003 PCCTM (Protract Probiomics, Eveleigh, NSW, Australia) as a freeze-dried powder twice daily for 8 weeks. The ingestion of the probiotics strain led to a significant improvement in atopic dermatitis severity; however, no changes were observed on allergen-specific responses, that is, specific IgE and skin prick wheal sizes. Elucidation of the mechanisms underlying probiotics immunomodulatory effects is ongoing (*284*).

Studies using OVA-sensitized mouse models have demonstrated that the antiallergic effects of probiotics bacteria may be due to the presence of specific immunostimulatory DNA sequences, identified as ODN-BL07 (5′-GCGTCGGTTTCG-GTGCTCAC-3′) in the case of probiotic strain *Bifidobacterium longum* BB536 (*285–287*), and characterized by the activation of regulatory mechanisms involving TGF-β-mediated responses in the case of *Lactobacillus acidophilus* strains (*288*).

In parallel with the use of probiotics, compounds known as prebiotics have also been assessed in an experimental model using ovalbumin as surrogate allergen. The beneficial immunomodulatory properties of galacto- and fructo-oligosaccharides have been demonstrated in experimental models of allergy and recently reviewed (*289*). For instance, the potential use of fructo-oligosaccharides as a preventive measure against food allergy has been demonstrated in an OVA-sensitized mouse model (*290*).

(ii) Use of Specific Cytokine Therapy. The recent discovery of a new category of T-lymphocytes, coined T-regulatory cells, led to investigation of cytokine-mediated immunotherapy. Regulatory T-cells are believed to accomplish their functions by secretion of anti-inflammatory cytokines, such as IL-10 and TGF-β. In this regard, a recent study reported that the oral administration of TGF-β, a regulatory cytokine, could enhance the induction of oral tolerance to OVA in a BALB/c mouse model, as evidenced by a decrease in OVA-specific IgE and IgG1, T-cell reactivity, and immediate-type skin reactions (*291*).

(iii) Food Immunomodulatory Components. Antioxidant compounds naturally present in foods may have potential immunomodulatory effects in favor of allergy inhibition. A study reported that dietary supplementation with both vitamin E and β-carotene could suppress the production of OVA-specific IgE upon OVA exposure (*292*). Other types of dietary interventions were also investigated in the context of immunomodulation. For instance, concentrations of ovalbumin in plasma were lowered by administration of enzyme-treated wheat flour to Brown Norway rats, compared to a control group fed untreated wheat (*293*).

(iv) Alternative Medicine. Botanical active molecules have been widely investigated for their therapeutic values in humans and trialed as a form of alternative treatment for immunological conditions such as asthma or allergy (*294*). A particular group of steroid compounds, known as saponins, was documented to exert a wide range of biological activities, including immunomodulatory functions (*295*). A recent paper reported that *Quillaja* saponin could suppress IgE-mediated allergic response in a mouse model of IgE-mediated allergy (*296*). Oral administration of *Quillaja* saponin to OVA-sensitized BALB/c mice suppressed the anaphylactic reactions observed in positive control groups. Titers of IgE and IgG1 were markedly inhibited by oral administration of *Quillaja* saponin, whereas serum IgG2a levels were increased. Similarly, steroid-like compounds extracted from ginseng root (also known as ginsenosides) were examined for their antiallergic effects. Using an in vitro model of rat mast cell activation, one study showed that a ginseng extract could inhibit histamine release in a dose-dependent manner, accompanied by a significant decrease in tumor necrosis factor (TNF)-α and IL-6 secretions (*297*). A subsequent study examined the potential antiallergic properties of compound K, a major ginsenoside metabolite produced by the action of human intestinal microflora. It was demonstrated that compound K had an inhibitory effect on passive cutaneous anaphylaxis (PCA) reactions in mice (*298*), suggesting its potential use in the alleviation of allergic symptoms. Adverse effects were reported upon consumption of ginseng, but such cases were deemed to be rare, and symptoms were mild and transient (*299*).

(v) Anti-IgE Therapy. One of the most popular forms of nonspecific immunotherapy is anti-IgE therapy. It consists in neutralizing circulating IgE molecules and thereby preventing their binding to high-affinity receptors present at the surface of mast cells. A number of studies, including clinical trials, have shown that anti-IgE-mediated therapy may represent an efficient form of nonspecific immunotherapy. No report on anti-IgE therapy in the context of egg allergy has yet been found in the literature, but it has been extensively investigated in the context of peanut allergy (*300*–*302*). It is important to emphasize that although such intervention has for its primary goal the alleviation of the allergic symptoms, it may also represent a valuable approach when combined with efficient forms of specific immunotherapy.

9. ABBREVIATIONS USED

APC, antigen-presenting cell; CAP-FEIA, CAP-fluorescence enzyme immunoassay; CD, cluster of differentiation; CRIE, cross-radio-immunoelectrophoresis; CTLA, cytotoxic T-lymphocyte antigen; DBPCFC, double-blind placebo-controlled food challenge; EAST, enzyme allergosorbent test; ELISA, enzyme-linked immunosorbent assay; EW, egg white; Fab, antibody-binding fragment; GMFA, mutant isoform of ovomucoid; HEL, hen egg lysozyme; IFN, interferon; Ig, immunoglobulins; IL, interleukin; ISS, immunostimulatory sequences; LYS, lysozyme; MHC, major histocompatibility complex; MMR, measles, mumps, rubella (vaccine); OIT, oral immunotherapy; OVA, ovalbumin; OVM, ovomucoid; OVT, ovotransferrin; PBMC, peripheral blood mononuclear cells; PCA, passive cutaneous anaphylaxis; PCR, Polymerase Chain Reaction; PIT, peptide-based immunotherapy; RAST, radio-allergosorbent test; SBPCFC, single-blind placebo-controlled food challenge; SIT, specific immunotherapy; SOTI, specific oral tolerance induction; SPOTS, Simple Precise Original Test System (Sigma-Genosys); SPR, surface plasma resonance; SPT, skin-prick test; TCC, T-cell clones; TCL, T-cell lines; TCR, T-cell receptor; TGF, transforming growth factor; Th, T-helper cell; TLR, toll-like receptor; TNF, tumor necrosis factor; Treg, T-regulatory cell.

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