Molecular diagnosis of egg allergy

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Purpose of review

Allergy to hen's egg is common in infancy and childhood. Oral food challenges are often required to diagnose egg allergy, because of the limitation in the diagnostic accuracy of skin test and specific IgE to egg white. New molecular diagnostic technologies have been recently introduced into allergological research. In this article, we will review the recent literature regarding the potential value of these tests for the clinical management of egg-allergic patients.

Recent findings

Component-resolved diagnosis that can be combined with the microarray technology is promising as measurement of specific IgE antibodies to individual egg white components has been shown to predict different clinical patterns of egg allergy. Specific IgE to ovomucoid has been identified as a risk factor for persistent allergy and could indicate reactivity to heated egg. Ovomucoid and ovalbumin IgE and IgG4-binding epitope profiling could also help distinguish different clinical phenotypes of egg allergy. Particularly, egg-allergic patients with IgE antibodies reacting against sequential epitopes tend to have more persistent allergy.

Summary

Using recombinant allergens, IgE-binding epitopes, and microarrays, molecular-based technologies show promising results. However, none of these tests is ready to be used in clinical practice and oral food challenge remains the standard for the diagnosis of egg allergy.

Keywords

allergy, component, diagnosis, egg, microarray

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Introduction

After cow's milk, hen's egg allergy is the second most common food allergy in infants and young children [1–5]. A recent meta-analysis [6] of the prevalence of food allergy estimated that egg allergy affects 0.5–2.5% of young children. Egg allergy is closely associated with atopic dermatitis and was found to be present in about two-thirds of children with positive oral food challenges (OFCs) performed for allergy evaluation of atopic dermatitis [7]. The risks of sensitization to aeroallergens [8] and asthma [9] are also increased in egg-allergic children. The prognosis of egg allergy in young children is favorable and the majority of cases resolve within first years of life [10,11]. Today, the standard therapy for egg allergy is strict avoidance [12]. However, hen's egg is a versatile ingredient used in the cooking of many cultures, including a wide range of manufactured food products and the dietary avoidance of egg can thus be challenging [13].

Correct diagnosis of egg allergy is an absolute prerequisite for appropriate and potentially lifesaving preventive measures. The current tools available for diagnosis of egg allergy include the clinical history, physical examination, prick skin test and specific IgE to egg white. None of these parameters achieved sufficiently high predictive values and thus the majority of children still need to undergo clinician-supervised OFC to determine the clinical relevance of IgE sensitization. However, OFCs are resource-consuming and associated with a risk for severe anaphylaxis [14]. Although it has been shown that sensitivity and specificity of skin prick tests could be optimized using the end point titration approach [15], new testing methodologies are still needed for determining the presence and severity as well as the likelihood of resolution of egg allergy. Molecular diagnostic technologies have been recently introduced into allergological research as promising tools. Instead of measuring the IgE response to complex allergen extracts, specific responses on the level of individual allergens ('component-resolved diagnosis') or the epitopes of those allergens ('epitope mapping or profiling') are evaluated. We will discuss the potential role of these tools in the diagnosis of egg allergy.

Component-resolved diagnosis in egg allergy

The term component-resolved diagnosis has been coined to designate diagnostic tests based on pure allergen molecules which are produced either by recombinant expression of allergen-encoding cDNAs or by purification from natural allergen sources [16]. Measurement of specific IgE antibodies to individual egg white components could be of importance in predicting different disease manifestations in egg-allergic patients.

Allergenic components of egg white

Egg white of the domestic chicken (*Gallus domesticus*) represents the albumin fraction of the egg and contains more allergenic proteins than the yolk. Egg white contains more than 20 different glycoproteins, most of which have been purified. Ovomucoid (OVM) (Gal d 1, 11%), ovalbumin (OVA) (Gal d 2, 54%), ovotransferrin (Gal d 3, 12%) and lysozyme (Gal d 4, 3.4%) [17,18] have been identified as major allergens [19–21] (Table 1). Studies in humans utilizing Radio Allergo Sorbent Test (RAST) reported the order of allergenicity as ovomucoid > ovalbumin > ovotransferrin > lysozyme [22••]. In addition, two new allergen candidates in egg white have been identified recently: egg white cystatin and lipocalin-type prostaglandin D synthase (L-PGDS) [23].

Ovomucoid

Although OVA is the most abundant protein in egg white, OVM has been shown to be the dominant allergen in egg [22••,24–26]. OVM is a highly glycosylated molecule containing 186 amino acid residues and is known to exhibit a trypsin inhibitor activity [27]. In two different studies [18,28••], children with persistent egg allergy had significantly higher specific IgE levels to OVM than children who outgrew their egg allergy. A favorable prognosis was associated with the absence or a decline in OVM-specific IgE titers [18]. The allergenicity of proteins depends mostly, but not exclusively, on their resistance to heat and digestive enzymes [29], reflecting their capacity to stimulate a specific immune response [17]. The importance of OVM in egg allergy may be due to its unique characteristics such as relative stability against heat [30] and digestion with proteinases [22••,31,32], compared with other egg white components. This is possibly related to the presence of strong disulfide bonds that stabilize the protein [26].

An earlier study concluded that IgE binding activity to pepsin-digested OVM was of diagnostic value for distinguishing the food challenge-positive patients from negative patients, and that patients with high IgE-binding activity to pepsin-treated OVM were unlikely to outgrow egg allergy [33]. From another point of view, gastric digestion has been demonstrated to reduce the allergenicity of OVM [34], which can explain why some patients have skin contact reactions to egg, but not ingestion reactions [35]. Significant differences in specific IgE to OVM were found in egg-allergic patients, depending on the reactivity to raw and heated egg, in which low levels of specific IgE to OVM were associated with tolerance to heated egg [22••]. Furthermore, it has been suggested that quantification of specific IgE to OVM could be useful in guiding the physician in the decision

Key points

- Although molecular-based technologies are promising to improve the diagnosis of egg allergy, oral food challenge will still be necessary for many patients.
- Measurement of specific IgE to individual egg white components has been shown to predict different clinical patterns of egg allergy.
- Specific IgE to ovomucoid, considered as the immunodominant allergen in hen's egg white, has been shown to be a risk factor for persistent allergy and indicates that neither raw nor heated egg is likely to be tolerated.
- Although specific IgG4 to OVM and OVA has been shown to have no value in the diagnostic of egg allergy, specific ratio IgE/IgG4 might be utilized as a marker in following the development of tolerance and resolution of egg allergy.
- Peptide-based microarray immunoassays are currently under development and epitope profiling of egg white allergens could radically improve the diagnosis of egg allergy.

Table 1 Major egg white allergens[a]

Allergen	Common name	Constitute (%)	Mw (kDa)	Carbohydrate (%)	IgE binding activity			
					Heat-treated	Digestive enzyme-treated	Allergenic activity	Test code[b] (in-vitro tests)
Gal d 1	Ovomucoid	11	28	25	Stable	Stable	+++	f233
Gal d 2	Ovalbumin	54	45	3	Unstable	Unstable	++	f232
Gal d 3	Ovotransferrin/conalbumin	12	76.6	2.6	Unstable	Unstable	+	f323
Gal d 4	Lysozyme	3.4	14.3	0	Unstable	Unstable	++	k208

[a] Reproduced with authorization from Benhamou AH, state of the art for egg allergy, Allergy 2010, 65:283–289.
[b] Test code for in-vitro UniCAP system.

whether to perform a challenge or not. Recently published data suggest that a concentration of specific IgE to OVM higher than approximately 11 kU_A/l (positive decision point based on 95% clinical specificity) indicates a high risk of reacting to heated egg as well as raw egg. At the same time, a concentration lower than approximately 1 kU_A/l (negative decision point, based on 95% clinical sensitivity) means that there is a low risk of reaction to heated egg, even if the patients might well react to raw egg [36••]. Lemon-Mule *et al.* [37•] investigated immunologic changes associated with ingestion of baked egg in children with egg allergy. Greater levels of specific IgE to OVM were found in children reacting to baked egg (baked with wheat flour in a form of a muffin or a waffle) compared with patients tolerant to baked egg and lightly cooked egg (e.g. French toast). However, in this study, only very high level of specific IgE to OVM (>50 kU_A/l) was highly predictive of heated egg reactivity. This might be explained by the so-called matrix effect [38,39], because egg was baked with wheat matrix. Kato *et al.* [40] previously showed a decreased solubility of OVM when egg was mixed with wheat flour and wheat gluten and heated, suggesting that OVM forms complexes with gluten leading to aggregation and insolubilization, and potentially decreased digestibility. Further studies are required to confirm the utility of specific IgE to OVM in predicting symptomatic egg allergy.

Ovalbumin

Ovalbumin is a phosphorylated glycoprotein with unknown biological function [41]. Its complete sequence of 385 amino acids has been determined [42]. Debate had flourished over the immunodominance of OVA as the major egg allergen; however, it has been shown that the use of contaminated commercial OVA led to an overestimation of its dominance as a major egg allergen in egg-sensitive patients [18]. A recent study [43] using experimental ImmunoCAP test confirmed that commercially available OVA contains a considerable amount of OVM as well as some ovotransferrin. In this study, a very sensitive affinity purification method with monoclonal chimeric antibodies was applied to reduce contamination with other allergens.

Several studies found higher specific IgE to OVA in egg-sensitized and egg-allergic patients compared with non-allergic controls [36••,37•,43]. In contrast to OVM, OVA is heat-labile and undergoes conformational changes to form more stable, and possibly less allergenic, S-ovalbumin upon exposure to elevated temperature [41,44]. This means that the IgE-binding epitopes on OVA might be destroyed after heating, suggesting that children who have specific IgE primarily to OVA are likely to tolerate heated egg [22••,32,45]. A recent study [30] investigated the T-cell immunogenicity of chemically glycated ovalbumin termed advanced glycation end products (AGEs), produced by the Maillard reaction that occurs between reducing sugars and proteins during thermal processing of foods. The glycation structures of AGEs are suggested to function as pathogenesis-related immune epitopes in food allergy. Interestingly, T-cell immunogenicity of OVA was enhanced by the Maillard reaction, indicating a critical role for thermal processing in allergenicity of OVA.

Ovotransferrin and lysozyme

Ovotransferrin (also called conalbumin) is a nonheme, iron-binding, acute-phase glycoprotein in egg white [27]. As OVA, ovotransferrin is a heat-labile allergen, but it was reported that when coupled to bivalent or trivalent metal ions, it could form heat-stable complexes [46]. However, little scientific evidence is available currently regarding the direct relationships of the heavy metals in egg and egg allergy. Although ovotransferrin is considered to be a major allergen in egg white, the role of specific IgE antibody to ovotransferrin in the diagnosis of egg allergy has not been determined. Lysozyme is a glycosidase commonly used as a food preservative due to its antibacterial properties, in some pharmaceuticals and foods (e.g. eye drops and cheese) [27]. Egg-allergic individuals sensitized to lysozyme may therefore react when exposed to such products [47,48]. Moreover, being widely used in the food and pharmaceutical industry, lysozyme is also considered an important occupational allergen, causing asthma via the inhalation route [49,50].

Allergenic components of egg yolk

The main allergen in egg yolk, chicken serum albumin, also called alpha-livetin (Gal d 5), is thought to be involved in the bird-egg syndrome [51,52]. In this syndrome, the primary sensitization is to airborne bird allergens with the secondary sensitization or cross-reactivity with albumin in egg yolk (Gal d 5). These patients experience respiratory symptoms such as rhinitis and/or asthma with bird exposure and immediate allergic symptoms with egg ingestion [52,53]. Testing-specific IgE to Gal d 5 might therefore confirm the diagnosis of bird egg syndrome. Several other potential allergens have been identified in egg yolk, including vitellenin (apovitellenin I) and apoprotein B (apovitellenin VI), although their roles in egg allergy remain unclear.

Microarray-based component-resolved diagnosis

Protein microarray has recently become available for measuring specific IgE and commercialized in the form of the ImmunoCAP-ISAC or Immuno Solid phase Allergen Chip (VBC Genomics-Vienna, Austria; Phadia, Uppsala, Sweden) [54,55]. It currently has 103 native/recombinant component allergens from 43 allergen sources and includes nGal d 1, nGal d 2, nGal d 3 and nGal d 5. This technology has two main advantages: it assesses simultaneously specific IgE to different

components and requires small amounts of serum, which is especially relevant in children. Moreover, ImmunoCAP-ISAC can be considered as a cost-efficient approach, as it delivers results for over 100 components. Ott *et al.* [56••] published the first study on the clinical performance of a component-based microarray with respect to the outcome of the OFC in suspected egg allergy. No advantage was found compared with the current diagnostic tests, that is skin prick test and specific IgE to egg white. However, a recent study [57•] suggested that the protein microarray has a good ability to predict the OFC results in egg-allergic children and could be used as a second-level assay, if the ImmunoCAP-specific IgE to egg white is less than 95% clinical decision points. In this study, this led to a decrease in the number of OFCs to be performed, as well as of positive OFCs with a subsequent decrease in severe reaction risk. Discrepancies between these two studies are probably due to difference in patient selection [57•]. Further large-scale studies are warranted before the protein microarray can be introduced into routine management of patients with egg allergy.

Potential role of ovalbumin and ovomucoid-specific IgG4 in the diagnosis of egg allergy

It was recently demonstrated that specific IgG4 does not add additional value to IgE measurement in the diagnostic procedure of egg allergy [58•]. This is in contrast to immunotherapy trials in which increase in specific IgG4 levels is associated with acquisition of tolerance. Protective or blocking functions for this subclass are assumed [59,60]. Because the balance between allergen-specific IgE and IgG4 production may have an impact on whether clinical allergy or tolerance develops, the determination of the ratio of specific IgE/IgG4 antibodies might be superior to the absolute amount of IgG4 for assessing an ongoing status of egg sensitization. Measurements of specific ratio IgE/IgG4 to OVA and/or OVM have been shown to be useful in following the development of oral tolerance and outgrowing egg allergy in the research studies [37•,61]. However, measurement of specific IgG4 has not been validated sufficiently to be used in clinical practice.

Role of epitope mapping in the diagnosis of egg allergy

Food allergens must at least partially survive digestion and absorption from the gastrointestinal tract to be immunogenic. This fact has led to the hypothesis that individuals who generate IgE antibodies recognizing a greater number or a specific pattern of sequential epitopes (e.g. those not easily destroyed by denaturation and partial digestion) are more likely to have clinical allergy rather than asymptomatic IgE sensitization [62]. There have been a few studies on the IgE-binding epitopes in OVM, and the reported binding sites resemble each other (Table 2) [26,27,28••,63]. Egg white-specific IgE antibodies that recognize sequential or conformational epitopes of OVM and OVA can distinguish different clinical phenotypes of egg allergy. It has been shown that egg-allergic patients with IgE antibodies reacting against sequential epitopes tended to have persistent allergy, whereas those with IgE antibodies primarily reacting against conformational epitopes tended to have transient allergy [26,28••]. In the study by Jarvinen *et al.* [28••], seven patients with persistent egg allergy had IgE that recognized four sequential epitopes of OVM. In contrast, none of the 11 children with transient egg allergy had specific IgE to these epitopes. These observations were supported by a separate study [22••] in which sera obtained from patients with persistent egg allergy had high IgE-binding activity to pepsin-treated OVM.

In the past, epitope mapping was mainly performed using SPOT membrane-based immunoassays [64–66] in which the peptides were synthesized on the nitrocellulose membrane and then incubated with the patient's sera. However, synthesis of large numbers of peptides is relatively error-prone, time-consuming, labor-intensive and expensive, and has limitations because of the specific chemistry of the method. A large volume of serum is

Table 2 Sequential IgE-binding epitopes of ovomucoid (Gal d 1)

	Ref.	Ref. no.	Year	IgE epitope				
Domain 1	Cooke and Sampson	[26]	1997	AA 1-20				AA 49-56
	Jarvinen *et al.*	[28••]	2007	AA 1-10	AA 9-20			AA 47-56
	Holen *et al.*	[63]	2001	AA 1-14	AA 11-24	AA 31-44		AA 51-64
	Mine and Wei Zhang	[67]	2002			AA 32-42	AA 40-50	AA 56-66
Domain 2	Cooke and Sampson	[26]	1997		AA 85-96		AA 115-122	
	Jarvinen *et al.*	[28••]	2007				AA 113-124	
	Holen *et al.*	[63]	2001	AA 61-74		AA 101-114	AA 121-134	
	Mine and Wei Zhang	[67]	2002	AA 71-75	AA 80-90	AA 101-105	AA 121-130	
Domain 3	Cooke and Sampson	[26]	1997		AA 175-186			
	Jarvinen *et al.*	[28••]	2007					
	Holen *et al.*	[63]	2001					
	Mine and Wei Zhang	[67]	2002		AA 159-174	AA 179-186		

AA, amino acid; Ref., reference.

required, and there is also a limitation of the number of targeted peptides. With the development of microarray technology and evolution in peptide synthesis techniques, peptide microarray-based immunoassays for epitope mapping of egg allergens could be the next step. Indeed, analyzing epitope-specific binding with this assay may further increase the positive predictive value of laboratory tests, provide information on the natural history of egg allergy, that is whether the patients may outgrow their allergy, and perhaps provide information on the potential severity of the allergic reaction to egg. Also, characterization of IgE epitopes of egg allergens is of fundamental importance in the design of immunotherapeutics.

Conclusion

Molecular diagnosis technologies will improve diagnosis of IgE-mediated egg allergy. Measurement of specific IgE antibodies to individual egg white components has been shown to predict different clinical patterns of egg allergy. Component-resolved diagnosis based on a microarray platform is especially promising. However, a better purification of individual allergens is required in order to avoid contamination and overestimation of specific IgE level to different egg allergens (components). On the basis of data from other food allergens, peptide microarray-based immunoassay could facilitate determination of egg allergy phenotypes. This test is currently under development. None of these molecular-based tests is ready to be used in clinical practice and an oral food challenge will still be necessary in many patients for the diagnosis of egg allergy.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 270–271).

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