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Invited review article

Common food allergens and their IgE-binding epitopes

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AA, amino acid; AAI, α-amylase inhibitor; IgE, immunoglobulin E; LMW-GS, low molecular weight glutenin subunits; LTP, lipid transfer protein; OM, ovomucoid; OVA, ovalbumin; WDEIA, wheat-dependent exercise-induced anaphylaxis

ABSTRACT

Food allergy is an adverse immune response to certain kinds of food. Although any food can cause allergic reactions, chicken egg, cow's milk, wheat, shellfish, fruit, and buckwheat account for 75% of food allergies in Japan. Allergen-specific immunoglobulin E (IgE) antibodies play a pivotal role in the development of food allergy. Recent advances in molecular biological techniques have enabled the efficient analysis of food allergens. As a result, many food allergens have been identified, and their molecular structure and IgE-binding epitopes have also been identified. Studies of allergens have demonstrated that IgE antibodies specific to allergen components and/or the peptide epitopes are good indicators for the identification of patients with food allergy, prediction of clinical severity and development of tolerance. In this review, we summarize our current knowledge regarding the allergens and IgE epitopes in the well-researched allergies to chicken egg, cow's milk, wheat, shrimp, and peanut.

Introduction

The prevalence of food allergy in Japan is estimated to be 5%–10% among infants (aged 0–6 years) and 1.5%–3% among school-aged children (aged >6 years), and the prevalence among adults is thought to be similar to that of schoolchildren.[1,2] The prevalence of food allergy among pediatric patients in the U.S. and Europe is reported to be 8% and 6.9%, respectively, which is similar to that in Japan. The most common foods that induce immediate-type food allergy in Japan are chicken egg (38.2% of food allergy patients), cow's milk (15.9%), wheat (8%), shellfish (6%), fruit (6%), buckwheat (5%), fish (4%), peanut (3%), and fish roe (3%).[1] The frequency of causative foods in the U.S. is different: peanut (25.2%), cow's milk (21.1%), shellfish (17.2%), tree nut (13.1%), chicken egg (9.8%), fin fish (6.2%), strawberry (5.3%), and wheat (5.0%).[3] In European patients, the most common causative foods in order of decreasing frequency are cow's milk, wheat, chicken egg, fish, soy, tree nut, shellfish, and peanut.[4] Despite these differences, there is a global trend toward an increased prevalence of food allergy.[5,6]

To understand the pathogenesis of food allergy and establish effective approaches for diagnosis, treatment, and prevention, detailed information on the allergen molecule is essential. Recently, a number of allergen molecules have been identified, and their three-dimensional (3-D) structures have been determined by advanced biological and analytical methods. The immunoglobulin E (IgE) recognition sites (IgE-binding epitopes) in allergens contribute to allergenicity. Therefore, it is important to identify the epitope structure for the development of new strategies for accurate diagnosis and allergen-specific immunotherapy of food allergy as well as the production of hypoallergenic foods. The benefit of component-resolved diagnosis of food allergy using epitope peptides[7–14] and effective immunotherapy for peanut allergy with IgE-binding epitope modified recombinant allergen[15] have been reported. Here, we provide an overview of the most common food allergen molecules from chicken egg, cow's milk, wheat, shrimp, and peanut, for which IgE-binding epitopes have been well characterized.

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Food allergens

Food allergy is an allergen-specific IgE-mediated type I response. Its pathogenesis is divided into two phases: 1) the sensitization phase in which the allergen enters the body through the gastrointestinal tract, skin, or mucosa, where it encounters a naïve immune system under Th2-dominant conditions, resulting in IgE production; and 2) the induction phase, which occurs following the oral intake of the same allergen as in the sensitization phase, and which elicits allergic symptoms such as urticaria, itching, wheezing, dyspnea, and abdominal pain. These responses are caused by the release of chemical mediators such as histamine and leukotrienes from activated mast cells and basophils. Cross-linking of IgE receptors with an allergen is required for the activation of these cells; therefore, more than two IgE antibodies are required to bind to one allergen molecule.

Food allergens include proteins or glycoproteins that have a molecular weight of 5–100 kDa and the ability to cross-link IgE receptors. Although many potential allergens are enzymatically digested and denatured by the acidic environment of the stomach, some are resistant to these conditions.[16] Intact food allergens have been detected in the serum after oral food administration in animals[17–19] and humans.[20–22] In addition, anti-ulcer agents that inhibit gastric digestion of the allergen have an effect on the sensitization and induction phases.[23]

A number of different forms of plant food allergies have been reported, including pollen-food allergy syndrome and latex-fruit syndromes, in which allergic symptoms are induced mainly in the mucous membranes after ingestion of the causative food.[24–26] The most comprehensively characterized allergen components associated with plant food allergies are profilins, seed storage proteins (2S albumins, 7S/11S globulins), and pathogenesis related proteins such as non-specific lipid transfer proteins (LTPs). These components, collectively named panallergens, are widely distributed among various plants and have cross-reactivity with related plant species.[27–29] Carbohydrate determinants have been also reported as cross-reacting IgE-binding motifs in several plant allergens.[30,31]

IgE-binding epitopes and T-cell epitopes in food allergens

IgE-binding epitopes can be divided into two types, linear (sequential) and conformational (discontinuous). Linear epitopes comprise continuous amino acid (AA) sequences, while conformational epitopes are formed by spatially adjacent AAs that are distantly located in the AA primary sequence of the proteins. Several methods of IgE-binding epitope mapping have been reported.[32] Arrays of overlapping peptides synthesized on a nitrocellulose membrane (SPOT membrane)[33] are frequently used to determine sequential epitopes.[10,34,35] Peptide microarrays, formed from hundreds of synthetic peptides printed on a glass slide, have been used to determine IgE-binding linear epitopes.[11,13,36–40] The determination of conformational IgE epitopes, however, requires sophisticated techniques, such as X-ray crystallography of allergens and immunocomplexes, nuclear magnetic resonance, mutant generation, and *in silico* analysis.[41,42] In such cases, IgE epitopes are predicted based on the 3-D structures of food allergens, which have been determined for lysozyme (Gal d 4), β-lactoglobulin (Bos d 5), latex (Hev b 6), birch pollen (Bet v 1), and peach (Pru p 3).[29,43,44] Recently, conformational epitopes recognized by monoclonal antibodies specific for almond (Pru du 6) and cashew (Ana o 2) allergens were mapped by hydrogen/deuterium exchange footprint analysis.[45–47] Although this new technique is not directly applicable to the identification of conformational epitopes recognized by human polyclonal IgE antibodies, the information obtained for the epitopes of monoclonal antibodies is useful to predict the conformational epitopes of human IgE antibodies.

Antigen-specific responses of $CD4^+$ T-cells, especially helper T-cells and regulatory T-cells, contribute to the sensitization, desensitization, and tolerance induction of food allergy.[48] T-cell epitopes that bind to major histocompatibility class II molecules are at least 13 AAs long.[49] The mapping of T-cell epitopes and IgE-binding epitopes provides useful information for the design of peptide and/or recombinant protein-based immunotherapy for food allergy. T-cell epitopes can be identified using peptides of 10–20 residues that overlap the entire AA sequence of the candidate allergen and allergen-specific T-cell lines derived from peripheral blood mononuclear cells. Then, peptides that induce T-cell proliferation contain T-cell epitopes. T-cell epitope sequences can be also predicted by computer-based *in silico* analysis.[50] However, numerous synthetic peptides need to be assayed with T-cells from patients to confirm the biological reactivity of the predicted epitopes. Although studies to identify T-cell epitopes are behind those of IgE-binding epitopes, T-cell epitopes of milk (Bos d 5), egg (Gal d 1 and 2), peach (Pru p 3), and peanut (Ara h 1 and 2), among others, have been reported.[51–57]

Chicken egg

Chicken (*Gallus domesticus*) eggs consist of white and yolk. Ovomucoid (OM, Gal d 1), ovalbumin (OVA, Gal d 2), ovotransferrin (Gal d 3), and lysozyme (Gal d 4) have been identified as egg white allergens[58] (Table 1), whereas serum albumin (α-livetin, Gal d 5) and a fragment of the vitellogenin-1 precursor (YGP42, Gal d 6) have been reported as egg yolk allergens.[65,66] In studies of

Table 1
Chicken egg allergens.

Protein name	IUIS name	MW (kDa)	AA length	Accession no.	IgE-binding epitopes (amino acid number)	Year	Ref
Ovomucoid	Gal d 1	28	186	P01005 (Gal d 1.0101)	4–20, 46–59, 91–104	2013	37
					1–10, 11–20, 47–56, 113–122	2007	8
					32–42, 40–50, 56–66, 71–75, 80–90, 101–105, 121–130, 159–174, 179–186	2002	59
					1–14, 11–24, 31–44, 51–64, 61–74, 101–114, 121–134	2001	60
					1–20, 49–56, 85–96, 115–122, 175–186	1997	61
Ovalbumin	Gal d 2	44	386	CAA23682 (Gal d 2.0101)	126–135, 142–155, 160–173, 165–177, 189–199, 327–337, 371–386	2014	62
					39–50, 96–103, 192–201, 244–249, 252–261	2003	63
Ovotransferrin	Gal d 3	77	705		–	–	–
Lysozyme	Gal d 4	14	129	CAA23711 (Gal d 4.0101)	11–27, 57–83, 108–122	2014	64
Serum albumin	Gal d 5	69	615		–	–	–
Fragment of vitellogenin-1 precursor (YGP42)	Gal d 6	35	285		–	–	–

component-resolved diagnosis of chicken egg allergy, measurements of Gal d 1- and/or Gal d 2-specific IgE reactivity improved the sensitivity of a serum-based test.[67–69] The prevalence of specific IgE to each allergen was 43.5% for Gal d 1, 52.1% for Gal d 2, 13.0% for Gal d 3, 36.9% for Gal d 4, 4.3% for Gal d 5, and 18.5% for Gal d 6 in suspected chicken egg allergy patients.[65,67]

OM (Gal d 1)

OM accounts for 11% of egg white protein and has three structurally independent tandem domains (DI, DII, and DIII), which exert trypsin-inhibitory activity.[70] OM is highly heat-stable and protease digestion-resistant, and is the immunodominant allergen in chicken egg.[71,72] Significant OM-specific IgE antibody levels indicate a risk of clinical reactions to both raw and cooked egg[71,73,74] and OM-specific IgE also has some prognostic value in predicting which patients will outgrow egg allergy. The IgE-binding epitopes have been identified by enzyme-linked immunosorbent assay and microarray techniques (Table 1).[8,37,59–61] The number of epitopes and their locations differed among the studies, possibly because of the population studied, the overlapping peptide length, and the techniques used. Järvinen *et al.*[8] reported that patients with persistent egg allergy, but not patients who outgrew egg allergy, had IgE antibodies that recognized the AA 1–10, AA11–20, AA47–56, and AA113–122 in OM. These observations indicated that the presence of sequential epitope-specific serum IgE antibodies represents the basis of a screening method for persistent egg allergy. In a recent study using microarray techniques, two major B-cell epitopes (AA4–20 and AA46–59) that coincided with the epitopes described previously were identified,[37] indicating that AA4–20 and AA46–59 are clinically relevant epitopes. Although AA4–20 and AA46–59 were recognized by 24% and 32% of patients who tested positive for OM-specific IgE, one third of the patients did not recognize any linear epitopes of OM.[37] The 3-D conformation of OM, including carbohydrate modifications, is preferentially recognized by IgE in egg allergy patients. Thus, the detection of conformational epitopes is also of importance for the diagnosis and prognosis of egg OM allergy.

OVA (Gal d 2)

OVA is the most abundant heat-labile phosphoglycoprotein in egg white (approximately 54%) and is a dominant allergen in egg allergy.[58] Measurement of OVA-specific IgE is helpful for the diagnosis of egg allergy, especially in children with anaphylaxis.[68] The five IgE-binding epitopes of OVA, AA38–49, AA95–102, AA191–200, AA243–248, and AA251–260, have been mapped by Mine *et al.*[63] using SPOT membranes. A recent study using digested OVA with human and simulated gastroduodenal fluids showed that seven IgE-binding peptides, AA125–134, AA141–154, AA159–172, AA164–176, AA188–198, AA326–336, and AA370–385, remained after digestion.[62] In addition, the importance of AA370–385 was indicated by the observation that this peptide is bound by 80% of sera from egg allergy patients.

Ovotransferrin (Gal d 3)

Ovotransferrin, an iron-binding protein, is a heat-labile and digestible allergen[75,76] accounting for 12% of egg white proteins. Thirty-seven percent of egg-allergic patients were reportedly sensitized to this component.[67] Although the 3-D structure has been elucidated,[77] both linear and conformational IgE-binding epitopes remain to be identified.

Lysozyme (Gal d 4)

Lysozyme is well characterized physicochemically because of its use as an antimicrobial agent in clinical situations and as a food preservative. Jiménez-Saiz *et al.*[64] reported three possible IgE-binding peptides, AA11–27, AA57–83, and AA108–122, in immunoreactive fragments of lysozyme digested with simulated gastric and duodenal fluids. The lysozyme (AA24–129) fragment, which is linked by disulfide bonds to two immunoreactive peptides, AA57–83 and AA108–122, is resistant to digestive enzymes, and induces basophil activation. Thus, lysozyme contains linear IgE-binding epitopes that are involved in the clinical manifestation of chicken egg allergy.

Chicken serum albumin (Gal d 5)

Chicken serum albumin (α-livetin) was identified as the causative allergen of bird-egg syndrome, which causes respiratory symptoms following exposure to birds, with secondary symptoms of allergy after egg ingestion.[66] The prevalence of chicken serum albumin-specific IgE is relatively low in chicken egg allergy patients.[67,68]

YGP42 (Gal d 6)

A 35-kDa fragment of the vitellogenin-1 precursor, known as the YGP42 protein (Gal d 6), was also identified as an egg yolk allergen.[65] This protein is heat- and reduction-stable; however, IgE-binding of this protein is reduced by treatment with gastric fluid. To date, no B-cell epitopes have been identified.

Cow's milk

The physicochemical properties of allergens from cow's (*Bos domesticus*) milk are well characterized (Table 2). Fractionation of milk yields two fractions, caseins and whey. Caseins (Bos d 8) include α_{s1}- (Bos d 9), α_{s2}- (Bos d 10), β- (Bos d 11), κ- (Bos d 12), and γ-caseins. Whey also called milk serum consists of α-lactalbumin (Bos d 4), β-lactoglobulin (Bos d 5), bovine serum albumin (Bos d 6), immunoglobulin (Bos d 7), and lactoferrin. The prevalence of IgE reactivity to Bos d 8 (46.5%), Bos d 4 (27.6%), Bos d 7 (10.3%), and lactoferrin (10.3%) was demonstrated in patients with suspected cow's milk allergy.[67] These components, as well as cow's milk protein-specific IgE, are good prognostic markers of the persistence of cow's milk allergy.[86,87]

α-Lactalbumin (Bos d 4)

α-Lactalbumin, a calcium-binding milk protein, has as a key role in lactose biosynthesis.[88] Its high thermal stability was revealed by analysis of recombinant α-lactalbumin.[78] The prevalence of α-lactalbumin-specific IgE in milk allergy patients varies from 27.6% to 62.8% depending on the study population.[67,78,89] The IgE-binding epitopes of α-lactalbumin, AA1–16, AA13–26, AA47–58, and AA93–102, have been identified.[79] Hochwallner *et al.*[78] identified six IgE-reactive peptides using a panel of 19- or 20-mer overlapping peptides spanning the entire protein. Three of these peptides, AA1–19, AA15–34, and AA105–123, are located on the surface of the protein.

β-Lactoglobulin (Bos d 5)

β-Lactoglobulin is the most abundant protein in milk whey. The major IgE-binding epitopes of β-lactoglobulin, AA1–16, AA31–60, AA67–86, and AA127–152, have been identified.[79] Cerecedo *et al.*[38]

Table 2
Cow's milk allergens.

Protein name	IUIS name	MW (kDa)	AA length	Accession no.	IgE-binding epitopes (amino acid number)	Year	Ref
α-Lactalbumin	Bos d 4	14.2	123	AAA30615 (Bos d 4.0101)	1–19, 15–34, 45–64, 60–79, 90–109, 105–123	2010	78
					1–16, 13–26, 47–58, 93–102	2001	79
β-Lactoglobulin	Bos d 5	18.3	162	CAA32835 (Bos d 5.0101)	1–16, 56–70, 76–90, 136–150	2012	80
					58–77, 76–95, 121–140	2008	38
					1–16, 31–60, 67–86, 127–152	2001	79
Serum albumin	Bos d 6	67	583		–	–	–
Immunoglobulin	Bos d 7	160	–		–	–	–
Caseins (Bos d 9–Bos d 12)	Bos d 8	20–30	–		–	–	–
α_S1-Casein	Bos d 9	23.6	199	NP_851372 (Bos d 9.0101)	6–20, 11–35, 126–140, 171–185	2013	81
					16–35, 28–50, 73–92	2008	38
					17–36, 39–48, 69–78, 83–102, 109–120, 122–132, 139–154, 159–174, 173–194	2001	82
					181–199	1998	83
					19–30, 93–98, 141–150	1996	84
α_S2-Casein	Bos d 10	25.2	207	NP_776953 (Bos d 10.0101)	1–20, 13–32, 67–82, 106–125, 122–141, 157–182, 181–207	2008	38
					31–44, 43–56, 83–100, 93–108, 105–114, 117–128, 143–158, 157–172, 165–188, 191–200	2001	85
β-Casein	Bos d 11	24	209	XP_005902099 (Bos d 11.0101)	25–50, 52–74, 121–140, 154–173	2008	38
					1–16, 45–54, 55–70, 83–92, 107–120, 135–144, 149–164, 167–184, 185–208	2001	34
κ-Casein	Bos d 12	19	169	NP_776719 (Bos d 12.0101)	16–35, 34–53	2008	38
					9–26, 21–44, 47–68, 67–78, 95–116, 111–126, 137–148, 149–166	2001	34

also used a panel of overlapping peptides in a microarray-based immunoassay to analyze the IgE-binding epitopes of β-lactoglobulin. The identified IgE epitopes, AA58–77, AA76–95, and AA121–140, are recognized by more than 75% of milk allergy patients. AA58–77 is significantly associated with milk protein-reactive patients. Recent studies identified AA1–16, AA56–70, and AA76–90 as epitopes in Chinese patients.[80] These sequences are roughly in accordance with the epitopes detected in European patients described previously.

Bovine serum albumin (Bos d 6)

Bovine serum albumin is an important allergen in milk, meat, and epithelia allergy.[90–92] There is cross-reactivity between serum albumin from cow, sheep, deer, pig, dog, and cat (meat and/or epithelia)[90]; therefore, albumins are implicated as panallergens in mammals. Although bovine serum albumin is the major allergen in beef allergy,[93] bovine serum albumin-specific IgE is found in only 3.8% of patients with cow's milk allergy.[89] Structural modification by heat treatment and chemical denaturation does not affect the allergenicity of bovine serum albumin,[94] and several IgE-binding epitope sequences have been reported in beef allergy.[95,96]

Bovine gamma globulin (Bos d 7)

Bovine gamma globulin is also an allergen in cow's milk and beef allergy.[97] Approximately 10% of patients with cow's milk allergy are bovine gamma globulin-specific IgE positive.[67]

Caseins (Bos d 8–12)

Casein (Bos d 8) constitutes approximately 80% of the proteins present in cow's milk. These phosphorylated proteins are heat-stable but are susceptible to digestive enzymes.[98] Casein is composed of four major proteins, α_{s1}-casein (Bos d 9), α_{s2}-casein (Bos d 10), β-casein (Bos d 11), and κ-casein (Bos d 12), most of which exist in a colloidal particle known as the casein micelle. The molecular weight and AA length of caseins are listed in Table 2. The proportions of casein in milk protein are 37% for α_{s1}-casein, 37% for α_{s2}-casein, 13% for β-casein, and 13% for κ-casein.

α_{s1}-Casein (Bos d 9)

Approximately 50% of serum samples from patients with cow's milk allergy react with α_{s1}-casein, which has an unordered molecular structure.[89] Nakajima-Adachi *et al.*[83] identified AA181–199 in the C-terminal region as the immunodominant IgE-binding region. Three regions of α_{s1}-casein, corresponding to AA19–30, AA93–98, and AA141–150, were also identified as IgE-binding peptides.[84] In recent studies, several IgE-binding epitopes were identified by overlapping peptide mapping.[38,81,82]

α_{s2}-Casein (Bos d 10)

Busse *et al.*[85] identified 10 sequential IgE-binding epitopes of α_{s2}-casein, four of which AA83–100, AA143–158, AA157–172, and AA165–188, were detected in 77% of patients. Cerecedo *et al.*[38] mapped seven IgE-binding epitopes and showed that four, AA1–20, AA13–32, AA67–86, and AA181–207, were significantly associated with the reactive group of patients.

β-Casein (Bos d 11)

Six major and three minor IgE-binding epitopes of β-casein were identified by Chatchatee *et al.*[34] Cerecedo *et al.*[38] also identified four B-cell epitopes, three of which, AA25–50, AA52–74, and AA154–73, were found to be associated with allergic symptoms. Several IgE-binding epitopes remain as fragments of β-casein after gastrointestinal digestion.[99]

κ-Casein (Bos d 11)

Using overlapping peptide mapping, eight major IgE-binding peptides of κ-casein were identified, three of which, AA9–26, AA21–44, and AA47–68, were recognized by 93% of patients with cow's milk allergy.[34] AA16–35 and AA34–53 in κ-casein were also identified as IgE-binding dominant epitopes.[38] Thus, the N-terminal region (AA9–68) of κ-casein may play an important role in the allergenicity of this protein. The critical AAs for IgE-binding to linear epitopes of κ-casein were identified by Han *et al.*[100]

Time-related differences in the binding of IgE and IgG4 to epitopes of β-lactoglobulin, α-lactalbumin, and caseins between patients with early recovery or persistent cow's milk allergy have been investigated.[101,102] Furthermore, IgE and IgG4 epitope binding was also analyzed to predict the outcome of oral immunotherapy in cow's milk allergy using peptide arrays.[103] The results of this study showed that monitoring the binding of IgE and IgG4 to epitope peptides of caseins has predictive value for the development of tolerance to cow's milk or the outcome of oral immunotherapy.

Wheat

Wheat (*Triticum aestivum*) is an important allergen source that elicits various clinical types of food allergy, such as immediate wheat allergy, baker's asthma, wheat contact dermatitis, and wheat-dependent exercise-induced anaphylaxis (WDEIA). The total protein content of wheat flour ranges from 8% to 12%, and can be classified as water/salt-soluble proteins (albumin and globulin) and the insoluble protein, gluten. Wheat profilin (Tri a 12), non-specific LTP (Tri a 14), α-amylase inhibitors (AAI, Tri a 15, Tri a 28–30), wheat germ agglutinin (Tri a 18), thioredoxin (Tri a 25), thiol reductase homolog (Tri a 27), triosephosphate isomerase (Tri a 31), 1-cys-peroxiredoxin (Tri a 32), serpin (Tri a 33), glyceraldehyde-3-phosphate dehydrogenase (Tri a 34), dehydrin (Tri a 35), α-purothionin (Tri a 37), serine proteinase inhibitor (Tri a 39), thaumatin-like protein, peroxidase, and glutathione S-transferase in the soluble fraction have been identified as allergens in patients with baker's asthma, wheat food allergy and wheat contact urticaria (Table 3).[112–115] α/β-Gliadin (Tri a 21), γ-gliadin (Tri a 20), ω1,2-gliadin, ω5-gliadin (Tri a 19), low molecular weight glutenin subunits (LMW-GS, Tri a 36), and high molecular weight glutenin subunits (Tri a 26) in wheat gluten have been reported as allergens

Table 3
Wheat allergens.

Protein name		IUIS name	MW (kDa)	AA length	Accession no.	IgE-binding epitopes (AA sequence or number)	Year	Ref
Water/salt-soluble protein								
Profilin		Tri a 12	14	–	–	–	–	–
Non-specific lipid transfer protein 1		Tri a 14	9	90	P24296	24–33, 37–46, 81–90	2011	104
α-Amylase inhibitors								
Monomeric	0.28	Tri a 15	12	–	–	–	–	–
Dimeric	0.19	Tri a 28	13	–	–	–	–	–
Tertameric	CM1/CM2	Tri a 29	13	–	–	–	–	–
	CM3	Tri a 30	16	–	–	–	–	–
	CM16/CM17	–	17	–	–	–	–	–
Agglutinin		Tri a 18	17	–	–	–	–	–
Thioredoxin		Tri a 25	13	–	–	–	–	–
Thiol reductase homologue		Tri a 27	27	–	–	–	–	–
Triosephosphate isomerase		Tri a 31	26	–	–	–	–	–
1-Cys-peroxiredoxin		Tri a 32	24	–	–	–	–	–
Serpin		Tri a 33	40	398	CAB52710	46–68, 76–92, 271–293, 364–384	2012	105
Glyceraldehyde-3-phosphate-dehydrogenase		Tri a 34	40–42	–	–	–	–	–
Dehydrin		Tri a 35	12	–	–	–	–	–
α-Purothionin		Tri a 37	12	111	AFQ60540	2–31, 42–71, 62–91	2014	106
Serine protease inhibitor-like protein		Tri a 39	9	–	–	–	–	–
Glutathione transferase		–	25	–	–	–	–	–
Thaumatin like protein		–	18	–	–	–	–	–
Peroxidase		–	36	–	–	–	–	–
Water/salt-insoluble protein								
α/β-Gliadin		Tri a 21	28–35	299	P04725	VRVPVPQLQP, QEQVPLVQQQ, VQQQQFPGQQ, QQQFPGQQQQ, YLQLQPFPQP, QILQQILQQQ, LQIPEQSQCQ, QEQKQQLQQQ, SFQQPQQQYP, LALQTLPAMC, YIPPHCSTTI	2011, 2005	104,107
γ-Gliadin		Tri a 20	28–35	280	BAN29066 (Tri a 20.0101)	QPQQPFPQ	2013	108
				308	P08453	QPQQPFPQ	2012	109
						QQX_3X_4PQ (X_3 = L, S, Q; X_4 = L, V, F)	2005	107
ω1,2-Gliadin		–	40	261	Q9FUW7	QPQQPFPQ	2012	109
						QQPX_5PX_6Q (X_5 = I, T, F; X_6 = V, Q, I)	2011, 2005	104,107
ω5-Gliadin		Tri a 19	65	420	BAE20328 (Tri a 19.0101)	QQX_1PX_2QQ (X_1 = L, F, S, I; X_2 = Q, E)	2011, 2005	104,107
						QQIPQQQ, QQFPQQQ, QQSPEQQ, QQSPQQQ, QQYPQQQ, PYPP, QQFHQQQ, QSPEQQQ, YQQYPQQ, QQPPQQ	2005, 2004	35,110
High molecular weight glutenin subunits		Tri a 26	88	827		QQPGQ, QQPGQGQQ, QQSGQGQ	2005	10
Low molecular weight glutenin subunits		Tri a 36	32–40	369	Q9ZNY0	HQQQPIQQQP, QQPIQQQPQQ, QQFPQQQPCS, PFVHPSILQQ, QCSPVAMPQS, LPQIPQQSRY, QSRYEAIRAI	2011	104
						QQQPP	1996	111

that cause WDEIA.[113,116,117] Gliadins and glutenins are allergens that also cause baker's asthma and common immediate wheat allergy in some cases.[118,119] In addition, LTP in the water/salt-soluble fraction was reported to cause WDEIA.[112,113] These findings demonstrate the comparative specificity of the causative allergens to each clinical type of wheat allergy, although some allergens contribute to the development of two or more clinical types of this allergy. It should be noted that the biological activity of some wheat allergens reported in this review have not been determined using techniques such as the skin prick test or the basophil histamine release test.

Water/salt-soluble wheat allergens

Tri a 12 is recognized by specific IgE antibodies in patients with baker's asthma, grass pollen allergy, and food allergy to wheat.[115,120–122] However, the prevalence of IgE reactivity to wheat profilin in patients with food allergy and respiratory allergy is low.[115] Tri a 14 was identified as an allergen in food allergy patients after ingestion of wheat products,[123–126] as well as in patients with baker's asthma[127] and WDEIA.[118] Monomeric AAI 0.28 (Tri a 15), dimeric AAI 0.19 (Tri a 28), tetrameric AAI CM1/CM2 (Tri a 29), AAI CM3 (Tri a 30), and AAI CM16 are allergens in pediatric patients with wheat allergy,[126] patient's with baker's asthma,[121,128] and those with work-related wheat dermatitis.[129]

Tri a 18, 25, 32–35, and 39 were identified as allergens in patients with baker's asthma and/or food allergy.[103,105,122,130–133] Four IgE-binding epitopes of Tri a 33, AA46–48, AA76–92, AA271–293, and AA364–384, were identified using overlapping peptide mapping,[105] and 3-D modeling showed that these epitopes are partially located on the surface of the protein. No differences were identified in the IgE-binding epitope peptides associated with food allergy and baker's asthma.[105]

Tri a 27 was identified as an allergen that causes wheat protein contact dermatitis and wheat food allergy.[114,134,135] It is considered that the N-linked glycan moieties with fucose residues contained in this allergen are involved in IgE-binding.[136] The highest frequencies of Tri a 27-specific IgE were reported in patients with baker's asthma.[121] Tri a 37 was recently reported as a novel wheat allergen specific for patients with wheat food allergy.[137] Twenty percent of patients with wheat food allergy had Tri a 37-specific IgE, whereas the frequency was only 3% among patients with baker's asthma.[137] The IgE-binding epitopes in this allergen have been mapped to three regions of the protein, AA2–31, AA42–71, and AA62–91.[106]

Water/salt-insoluble wheat allergens

Gliadins and glutenins are water/salt-insoluble wheat proteins that have been identified as causative allergens in patients with wheat food allergy, baker's asthma, and WDEIA.[10,35,107,113,117] ω5-Gliadin (Tri a 19) is a major allergen in WDEIA[138,139] and children with immediate allergy to ingested wheat.[140] Because of the presence of a repetitive domain, there are many IgE epitope sequences in ω5-gliadin; however, the consensus sequence comprising QQX_1PX_2QQ (X_1 = L, F, S, I; X_2 = Q, E, G) has been identified as the IgE-binding epitope in Japanese and European patients.[35,104,107,110] The measurement of specific IgE to ω5-gliadin and its IgE-binding epitopes in the diagnosis of patients with wheat food allergy including WDEA has been reported.[10,141–145] However, the Tri a 19-specific IgE test alone could not be used to diagnose all patients with wheat food allergy.[146,147] γ-Gliadin (Tri a 20) is a causative allergen in patients with food allergy to wheat and those with baker's asthma.[113,117] Furthermore, γ-gliadin-specific IgE was also detected in Japanese patients with WDEIA following transdermal sensitization with hydrolyzed wheat protein.[108,148] The IgE-binding epitope sequence of QPQQPFPQ in γ-gliadin identified in Japanese patients sensitized via the dermal route was identical to the epitope sequence identified in European patients sensitized orally with hydrolyzed wheat proteins.[109] α/β-Gliadin (Tri a 21) and α1,2-gliadin are also important causative allergens in wheat food allergy, WDEIA, and baker's asthma.[104,107,113,146] The IgE-binding epitopes for these allergens were identified in patients with wheat allergy[104,107] and in addition, the sequences of QQPFP and PQQPF in gliadin were epitopes in atopic dermatitis-related wheat allergy.[149]

LMW-GS (Tri a 36) was identified as an allergen that induces wheat food allergy[123,150,151] and WDEIA[152] in European patients. There are several types of LMW-GS encoded by genes located on group 1 chromosomes at the Glu-A3, Glu-B3, and Glu-D3 loci,[153] and patients with wheat allergy exhibited IgE-reactivity to different LMW-GS.[150] Although the LMW-GS GluB3-23, B16, and P73 epitopes are well characterized,[147,150,152] only GluB3-23 is defined as Tri a 36 in the IUIS allergen database.[147] Tanabe *et al.*[111] identified the QQQPP motif in LMW-GS as a major IgE-binding epitope responsible for atopic dermatitis in patients sensitized with wheat.

High molecular weight glutenin subunits (Tri a 26) has been identified as an allergen responsible for WDEIA in Japanese patients and its IgE-binding epitopes, QQPGQ, QQPGQGQQ, and QQSGQGQ, have been fine-mapped.[10] We showed that the combined detection of IgE antibodies specific to epitope peptides or recombinant proteins of Tri a 26 and Tri a 19 improved the sensitivity and specificity of the diagnosis of WDEIA.[10,154]

Shrimp

Seafood allergy is a common food allergy. The prevalence of fish and shellfish allergies is estimated at 0.2%–0.3% and 0.6%, respectively.[155] Allergy to crustacean shellfish, which include shrimp, prawns, lobsters, and crabs, seems to affect school-aged children and adults predominantly.[1] Crustacean-allergic patients can show cross-reactivity to different types of shellfish as well as molluscan shellfish, such as gastropods (abalones, limpets, snails), bivalves (scallops, oysters, mussels), and cephalopods (squid, octopus).

Studies of shrimp allergens are the most advanced among the shellfish allergies.[156] There are various types of edible shrimp worldwide, with White Pacific shrimp (*Litopenaeus vannamei*), black tiger (*Penaeus monodon*), kuruma prawn (*Marsupenaeus japonicus*), and Japanese spiny lobster (*Panulirus japonicas*) the most commonly eaten in Japan. The allergens responsible for crustacean allergies are also well studied. Tropomyosin, arginine kinase, sarcoplasmic calcium-binding protein, myosin light chain, troponin C, and triosephosphate isomerase have been identified as allergens in *Penaeus monodon*, *L. vannamei*, *Crangon crangon* (North Sea shrimp), *Homarus americanus* (American lobster), and some crabs (Table 4).[156,159–161]

Tropomyosin (group 1)

Tropomyosin is associated with the actin and troponin components of muscle cells, and isoforms of tropomyosin were identified in several shrimp species, including *Pandalus borealis* (Pan b 1), *Penaeus monodon* (Pen m 1), *Penaeus aztecus* (Pen a 1), *Penaeus indicus* (Pen i 1), *Metapenaeus ensis* (Met e 1), *L. vannamei* (Lit v 1), *Crangon crangon* (Cra c 1), and *H. americanus* (Hom a 1). Although tropomyosins from crustaceans share high homology (up to 98%), the AA sequence identity of crustacean and molluscan tropomyosin is much lower (approximately 60%).[160] The major continuous IgE epitopes were identified in Pen a 1[157] and Lit v 1[158] using overlapping peptide mapping. Nine IgE-binding peptides in Pen a 1 were identified, five of which, AA37–63, AA82–105, AA115–150,

Table 4
Shrimp allergens.

Protein name	IUIS name	MW (kDa)	AA length	Accession no.	IgE-binding epitopes (amino acid number)	Year	Ref
Tropomyosin							
Penaeus aztecus (Brown shrimp)	Pen a 1	37	284	AAZ76743 (Pen a 1.0101)	1–36, 37–63, 61–81, 82–105, 115–150, 142–162, 157–183, 190–210, 246–284	2002	157
Litopenaeus vannamei (White shrimp)	Lit v 1	37	284	ACB38288 (Lit v 1.0101)	43–57, 85–105, 133–148, 187–202, 247–284	2010	158
Pandalus borealis (Northern shrimp)	Pan b 1		–	–	–	–	–
Metapenaeus ensis (Greasyback shrimp)	Met e 1		–	–	–	–	–
Penaeus indicus (Indian white shrimp)	Pen i 1		–	–	–	–	–
Penaeus monodon (Black tiger shrimp)	Pen m 1		–	–	–	–	–
Crangon crangon (North sea shrimp)	Cra c 1		–	–	–	–	–
Arginine kinase	Lit v 2	40	356	ABI98020 (Lit v 2.0101)	1–18, 25–42, 64–96, 121–141, 142–159, 160–192, 232–255	2010	158
	Pen m 2		–	–	–	–	–
	Cra c 2		–	–	–	–	–
Myosin light chain 2	Lit v 3	20	177	ACC76803 (Lit v 3.0101)	13–30, 22–48, 49–66, 58–90, 79–99, 118–141	2010	158
	Pen m 3		–	–	–	–	–
Sarcoplasmic calcium–binding protein	Lit v 4	20	193	ACM89179 (Lit v 4.0101)	10–36, 49–72, 130–147	2010	158
	Pen m 4		–	–	–	–	–
	Cra c 4		–	–	–	–	–
Myosin light chain 1	Cra c 5	18	–	–	–	–	–
Troponin C	Cra c 6	20	–	–	–	–	–
	Pen m 6		–	–	–	–	–
Triosephosphate isomerase	Cra c 8	28	–	–	–	–	–

AA190–210, and AA246–284, corresponded with the epitopes of Lit v 1. The sequences LEX_1X_2L or LEX_1X_2N (X_1 = D, E, N or K, X_2 = D or E) in AA1–36 (LEKDN), AA37–63 (LENDL), AA82–105 (LEEDL), and AA246–284 (LEDLE) were identified as common IgE-binding motifs in Lit v 1.[158] The immunodominant regions of Pan b 1 were also mapped by Myrset *et al.*[162]

Arginine kinase (group 2)

Arginine kinase was identified in three types of shrimp (Cra c 2,[163] Lit v 2,[164] Pen m 2[165]). Eight IgE-binding epitopes were identified in Lit v 2,[158] with the highest diagnostic efficiency shown in the test for AA232–255 peptide-specific IgE.[13] Recent studies showed that Pen m 2 is an important allergen recognized by Th2 cells in patients with shrimp allergy.[166]

Myosin light chain (group 3 and Cra c 5)

Myosin light chain (Lit v 3, Pen m 3) was first identified in *L. vannamei*[167] and *Penaeus monodon*.[168] Cra c 5 was also confirmed as a novel type of IgE-binding myosin light chain with only 13% AA sequence identity to Lit v 3.[163] Six IgE-binding sequences were identified in Lit v 3.[158]

Sarcoplasmic calcium-binding protein

Sarcoplasmic calcium-binding protein was identified as an allergen in three types of shrimp (Cra c 4,[163] Lit v 4,[169] and Pen m 4[170]). Three IgE-binding regions were identified in Lit v 4.[158]

Troponin C (group 6) and triosephosphate isomerase (Cra c 8)

Troponin C (Cra c 6[163] and Pen m 6[171]) and triosephosphate isomerase (Cra c 8[163]) were identified as allergens in patients with shrimp allergy but have not been reported in *L. vannamei* and *Penaeus monodon*, suggesting they are minor or local shrimp allergens.

Several studies conducted to investigate component-resolved diagnosis using recombinant shrimp allergens and IgE-binding epitope peptides showed that measuring IgE binding to tropomyosin and sarcoplasmic calcium-binding protein is useful for the prediction of clinical reactivity in patients with shrimp allergy although the measurement of IgE specific for other allergens and epitopes is necessary for the accurate diagnosis of shrimp allergy.[7,13]

Peanut

Twelve allergens have been identified in association with peanut (*Arachis hypogaea*) allergy (Table 5). These are designated Ara h 1 to Ara h 13, with the exception of Ara h 4, which has been renamed Ara h 3.02.[176] Ara h 2, Ara h 6, and Ara h 7, which belong to the 2S albumin family, are seed storage proteins and have homologous AA sequences and secondary structures.[177] Ara h 2 and Ara h 6 are the most clinically relevant allergens in peanut allergy.[178,179] Ara h 1 (7S globulin) and Ara h 3 (11S globulin) are seed proteins belonging to the cupin superfamily, which contains a conserved barrel domain.[180,181] Ara h 5 is a member of the profilin family with 72% AA sequence identity to birch pollen profilin (Bet v 2), and is a class II type labile food allergen.[182] Ara h 8, a pathogenesis related protein-10 (Bet v 1) homolog, is also a class II food allergen. It is a major allergen and produces allergic symptoms in birch pollen-allergic patients after the ingestion of peanuts.[183,184] Ara h 9, a non-specific LTP, was identified as a peanut allergen in patients who have peach allergy and are positive for Pru p 3-specific IgE.[185,186] Most of these patients did not have IgE antibodies specific to Ara h 1, Ara h 2, and Ara h 3, indicating that Ara h 9 is an important allergen in peanut allergy. Oleosins (Ara h 10 and Ara h 11) are obtained from peanut oil bodies.[187,188] In a recent study, the IgE-binding epitopes of Ara h 10 and Ara h 11 were identified using

Table 5
Peanut allergens.

Protein name	IUIS name	MW (kDa)	AA length	Accession no.	IgE-binding epitopes (amino acid number)	Year	Ref
Cupin (Vicillin-type, 7S globulin)	Ara h 1	64	626	AAB00861 (Ara h 1.0101)	139–147, 175–183 (most dominant)	2008	12
					26–33*, 48–57, 66–73*, 90–97*, 98–104, 108–115, 124–131, 134–143, 144–151, 295–302, 312–319, 325–334, 345–352, 361–385, 393–402, 409–418, 463–468, 498–507*, 525–534, 539–548, 551–560, 559–568, 578–587, 598–605 (* dominant epitope)	2004, 1997	11,172
Conglutin (2S albumin)	Ara h 2	17.5	160	ABQ96212 (Ara h 2.01)	31–36, 43–53, 63–68, 92–103, 106–118, 120–128, 130–139, 140–152	2014	173
					31–36 (most dominant)	2008	12
					30–39, 44–59, 62–67, 70–80, 92–103, 110–116, 130–136	2005	39
					18–27, 24–31, 30–39, 42–51, 52–59, 62–67, 68–75, 120–125, 130–135, 146–152	1997	174
	Ara h 6	15	124	ABQ96216 (Ara h 6.0101)	1–13, 23–33, 41–47, 58–70, 73–85, 97–106, 107–119	2014	173
	Ara h 7	16–17	–	–	–	–	–
Cupin (Legumin-type, 11S globulin, Glycinin)	Ara h 3	60	510	AAC63045 (Ara h 3.0101)	301–309 (most dominant)	2008	12
					30–44, 237–251, 276–290, 300–312	1999	175
Profilin	Ara h 5	14	–	–	–	–	–
Pathogenesis-related protein 10 (PR-10)	Ara h 8	17	–	–	–	–	–
Non-specific lipid-transfer protein type 1	Ara h 9	9.1	–	–	–	–	–
Oleosin	Ara h 10	18	–	–	–	–	–
	Ara h 11	14	–	–	–	–	–
Defensin	Ara h 12	5.2	–	–	–	–	–
	Ara h 13	8.4	–	–	–	–	–

in silico methods.[189] In addition, Kobayashi *et al.*[190] identified the IgE-binding epitope of peanut oleosin 3, which differs from those of Ara h 10 and Ara h 11. Ara h 12 and Ara h 13, which are defensins directed against fungal pathogens, were identified as allergens by Petersen *et al.*[191] Although they have been listed as peanut allergens by the WHO/IUIS Allergen Nomenclature Subcommittee, the details have not been reported. The prevalence of IgE binding to Ara h 10–13 has not yet been elucidated. From the viewpoint of component-resolved diagnosis of peanut allergy, Ara h 1, 2, 3, 6, and 9 are significant allergens that correlate with reactivity and/or severity, although the sensitivity and specificity of each component is population-dependent.[192–194]

7S globulin (Ara h 1)

Ara h 1 is a glycosylated seed storage protein. IgE binding to Ara h 1 has a low sensitivity to heating and digestion with pepsin.[195,196] Twenty-three IgE-binding epitopes were identified throughout the protein by overlapping peptide mapping,[172] and a further epitope, AA361–385, was reported by Shreffler *et al.*[11] Six of these epitopes, AA90–97, AA98–104, AA108–115, AA124–131, AA134–143, and AA144–151, are pepsin-resistant.[197] Bøgh *et al.*[198] identified five conformational epitope motifs in Ara h 1 by competitive immunoscreening of a phage-displayed random peptide library, and epitope mimics were found to cluster in three areas of Ara h 1. Furthermore, phage-display technology was used to analyze the difference between IgE and IgG epitopes of Ara h 1.[199] Elucidation of IgE and IgG epitope recognition patterns could be a valuable tool for the diagnosis of peanut allergy.

11S globulin (Ara h 3)

Ara h 3 is post-transcriptionally cleaved to form two protein fragments by an asparaginyl endopeptidase after the formation of an intermolecular disulfide bond. Thus, 23-kDa and 36-kDa spots are observed by 2D-PAGE under reducing conditions.[181] Analysis of the IgE-binding epitope sequences in Ara h 3 in three different overlapping peptide array experiments showed that four epitopes, AA30–44, AA237–251, AA276–290, and AA300–312, were important in peanut allergy.[12,175]

2S albumin (Ara h 2 and 6)

Ara h 2, which consists of two isoforms, Ara h 2.01 and Ara h 2.02, is highly resistant to boiling and proteolytic digestion.[200,201] Seven to ten IgE-binding epitopes were identified in Ara h 2 using overlapping peptide immunoassays.[12,39,173,174] In addition, post-translational proline hydroxylation of Ara h 2 contributes to linear IgE epitopes.[202]

Ara h 6 shares 55% identity with the AA sequence of Ara h 2 and some of the IgE epitopes of Ara h 6 are cross-reactive with those of Ara h 2.[173] As with Ara h 2, the resistance of Ara h 6 to heat and digestive enzymes was reported.[201] Seven IgE-binding epitopes were identified for Ara h 6 by Otsu *et al.*,[173] and the linear IgE-binding epitopes of Ara h 2 and Ara h 6 were useful in predicting clinical reactions in patients with peanut allergy.

Protein structures of Ara h 1–3, 5, and 8 were elucidated by X-ray crystal structure analysis.[181,203–206] B-cell epitopes of Ara h 1–3, 5–13 were predicted using *in silico* methods, and the predicted epitope sequences of Ara h 1–3 correlated with the epitopes identified experimentally,[189] suggesting that computational prediction of epitopes may contribute to improved accuracy of peanut allergy diagnosis.

Conclusions

Advances in molecular biology and analytical chemistry in the past three decades have facilitated the identification of food allergens and their sequential IgE-binding epitopes. The production of recombinant allergens and the use of 3-D structural analysis have also contributed to this progress. The results of these studies have enhanced our understanding of the mechanisms of food allergy at the molecular level. Although there are a number of limitations associated with studies of food allergen components and epitopes, the detection and quantification of serum IgE antibodies specific to allergen components and epitope peptides are useful for the diagnosis and prognosis of food allergy. In addition, clarification of the sensitization patterns of allergen components in individual patients might facilitate allergen-specific immunotherapy of food allergy. However, numerous issues remain to be investigated. For example, conformational IgE epitopes and T-cell epitopes have not yet been identified for most food allergens, even those for which the 3-D structure has been determined. Furthermore, many allergens remain unidentified because of biodiversity, especially in seafood, and the disposition and digestion of food allergens after ingestion have not been clarified. Future studies should focus on full IgE and T-cell epitope mapping in individual patients to improve the diagnosis, therapy, and prevention of food allergy.

Conflict of interest

The authors have no conflict of interest to declare.

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