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Peanut allergens

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ABSTRACT

Peanut allergens have the potential to negatively impact on the health and quality of life of millions of consumers worldwide. The seeds of the peanut plant *Arachis hypogaea* contain an array of allergens that are able to induce the production of specific IgE antibodies in predisposed individuals. A lot of effort has been focused on obtaining the sequences and structures of these allergens due to the high health risk they represent. At present, 16 proteins present in peanuts are officially recognized as allergens. Research has also focused on their in-depth immunological characterization as well as on the design of modified hypoallergenic derivatives for potential use in clinical studies and the formulation of strategies for immunotherapy. Detailed research protocols are available for the purification of natural allergens as well as their recombinant production in bacterial, yeast, insect, and algal cells. Purified allergen molecules are now routinely used in diagnostic multiplex protein arrays for the detection of the presence of allergen-specific IgE. This review gives an overview on the wealth of knowledge that is available on individual peanut allergens.

1. The role of peanut proteins in peanut allergy

Peanut allergy is one of the most severe food allergies which usually is not outgrown. Symptoms can be triggered by tiny amounts of allergens and even manifest as severe anaphylaxis. A survey made in the USA registered an increase of the prevalence of peanut allergy among children from a rate of 0.4% in 1997 to 1.4% in 2008 (Sicherer et al., 2010). This phenomenon was confirmed in the UK where an increase of the prevalence of peanut allergy was also registered (Venter et al., 2010).

The pattern of sensitization to peanut allergens varies among populations in different geographical regions (Vereda et al., 2011). The major peanut allergens Ara h 1, Ara h 2, and Ara h 3 are the main elicitors of allergic reactions in the USA and are often associated with severe symptoms. Spanish patients recognized these peanut allergens less frequently and were more often sensitized to the lipid transfer protein Ara h 9. Swedish patients detected Ara h 1 to 3 more frequently than Spanish patients but had the highest sensitization rate to Ara h 8, a cross-reactive homologue of the major birch pollen allergen Bet v 1. In a study involving peanut allergic subjects from 11 European countries sensitized to Ara h 1, Ara h 2 and Ara h 3 since childhood, Ara h 2 was identified as the sole major allergen (Ballmer-Weber et al., 2015). Geographical differences were observed for Ara h 8 and Ara h 9, which were major allergens for Central/Western and Southern Europeans, respectively. In a study of peanut allergic patients from the Netherlands, the most frequently recognized allergen was also Ara h 2 (Koppelman et al., 2004).

Peanut profilin, Ara h 5, is another allergen responsible for pollen-associated peanut allergy. IgE reactivity to Ara h 5 was shown in a Swedish cohort of peanut allergic individuals to be associated with that of the profilins from grass and birch pollen, Phl p 12 and Bet v 2, respectively (Cabanos et al., 2010a). In a study of individuals from the Swedish BAMSE birth cohort, children sensitized to both peanut and birch pollen were less likely to report symptoms to peanut than children sensitized to peanut but not to birch pollen at 8 years (Asarnoj et al., 2010). Sensitization to peanut oleosins was associated with severe systemic reactions (Schwager et al., 2017). No data are available on the prevalence or allergenic activity of Ara h 7. More studies are also needed to address the immunological properties of Ara h 12 and Ara h 13, the peanut defensins, which were recently found to be reactive with IgE from patients with severe peanut allergy (Petersen et al., 2015).

Abbreviations: CRD, component resolved diagnosis; DBPCFC, double-blind placebo-controlled food challenge; FPLC, fast protein liquid chromatography; HIC, hydrophobic interaction chromatography; HPLC, high performance liquid chromatography; IEC, ion exchange chromatography; MBP, maltose-binding protein; nsLTP, non-specific lipid transfer protein; OIT, oral immunotherapy; PBMC, peripheral blood mononuclear cells; PTM, post-translational modification; SEC, size exclusion chromatography; SLIT, sublingual immunotherapy; SPT, skin prick test

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2. Biological functions of peanut allergens

Seed storage proteins are present as one or more groups of proteins in high amounts in seeds to provide a store of amino acids for use during germination and seed growth. Ara h 1 and Ara h 3 are bicupin seed storage proteins. They belong to the cupin superfamily, a functionally highly diverse protein superfamily (Dunwell et al., 2004) which contains at present 61 member families. In legumes, such as the peanut, the globulin type seed storage proteins are present in two forms, the 7S trimeric vicilins (e.g. Ara h 1) and the 11S hexameric legumins (e.g. Ara h 3). Experiments performed by Viquez and colleagues revealed that Ara h 1 had trypsin inhibitory activity indicating that the protein might play a role in plant defense against insects (Viquez et al., 2003). Interestingly, the peptide that is cleaved off at the N-terminus to yield mature Ara h 1 contains six cysteine residues that might stabilize its structure against digestive denaturation (Wichers et al., 2004). The peptide resembles a class of antifungal oligopeptides from plant seeds such as Rs-AFP2, a defensin isolated from radish seeds (Terras et al., 1995).

Ara h 2, Ara h 6, and Ara h 7 are 2S albumin seed storage proteins (Mylne et al., 2014) which are members of the prolamin superfamily (Shewry and Tatham, 1990). Non-specific lipid transfer proteins (nsLTPs) form another family of the prolamin superfamily. They are present as type 1 (~9 kDa) and type 2 nsLTPs (~7 kDa) in plants and involved in stabilization of membranes, cell wall organization, signal transduction, and plant growth and development as well as in resistance to biotic and abiotic stress (Liu et al., 2015). Ara h 9 and Ara h 17 are type 1 nsLTPs while Ara h 16 is a type 2 nsLTP.

Plants contain actin-binding proteins which regulate the supramolecular organization and function of the actin cytoskeleton, including the monomer-binding profilins (McCurdy et al., 2001). Profilins regulate cytoskeletal dynamics and membrane trafficking. The peanut allergen Ara h 5 is a member of the profilin family. The major birch pollen allergen Bet v 1 is the founding member of the Bet v 1 family of proteins. Bet v 1 isoforms show an individual, highly specific binding behavior for differently glycosylated flavonoids, the physiological ligands of Bet v 1 (Seutter von Loetzen et al., 2015). Isoform and ligand mixtures have been suggested to act as fingerprints of the pollen from distinct trees and thus to play an important role in recognition processes during pollination. Ara h 8, the Bet v 1 homologous allergen from peanut, was shown to bind the isoflavones quercetin and apigenin as well as resveratrol with high avidity (Hurlburt et al., 2013).

Lipids are stored in oil seeds in specialized intracellular structures called oil bodies which are involved in various aspects of lipid and energy metabolism. They consist of a core of neutral lipids surrounded by proteins embedded is a phospholipid monolayer (Jolivet et al., 2013). Oleosins, amphiphilic structural proteins, are the most abundant oil body proteins. Ara h 10, Ara h 11, Ara h 14, and Ara h 15 are the peanut oleosins.

Plant defensins are small, cysteine-rich peptides that possess biological activity towards a broad range of organisms, their activity being primarily directed against fungi (Vriens et al., 2014). Ara h 12 and Ara h 13 are allergenic peanut defensins. The antimicrobial activity of the amphiphilic peanut defensins Ara h 12 and Ara h 13 is solely antifungal (Petersen et al., 2015). The peanut defensins showed inhibitory effects on mold strains of the genera *Cladosporium* and *Alternaria*.

3. Sequences of peanut allergens

To date, the WHO/IUIS Allergen Nomenclature Sub-Committee (http://www.allergen.org), the only body of experts authorized to assign official allergen designations, recognizes 16 peanut allergens (Table 1). The allergen Ara h 4 was renamed Ara h 3.02 and the number 4 is not available for future peanut allergen designations to avoid confusions with the already existing literature (Radauer et al., 2014).

3.1. Cupins: Ara h 1, Ara h 3

Ara h 1 is a bicupin storage protein of the vicilin type. The cDNA sequences of two Ara h 1 encoding clones, 41B and P17, were published in 1995 (Burks et al., 1995). Both clones showed a sequence identity of greater than 97% and encoded proteins of around 68 kDa. Both proteins have an N-terminal 25 amino acid residue signal peptide and a single glycosylation site (NAS) at amino acid positions 521–523. A genomic Ara h 1 clone, capable of giving rise to the mRNA for the cDNA of clone 41B, consisted of four exons and three introns (Viquez et al., 2003). Its open reading frame encoded a protein of 626 residues. The first report of an N-terminal sequence of mature Ara h 1 indicated that, depending on the length of the isoallergen, 78 or 84 residues in total are cleaved off at the N-terminus during post-translational processing of Ara h 1(de Jong et al., 1998). In SDS-PAGE, the two Ara h 1 isoforms (P43237 and P43238) appear as two closely spaced bands at 69 and 66 kDa (Wilson and Tan-Wilson, 2015). These sizes are consistent with the removal of a 25-residue signal peptide as well as the removal of an N-terminal propeptide. Ara h 1 is translated as a pre-pro-protein. The signal peptide directs the nascent protein to the storage vacuole where the propeptide is cleaved off to yield the mature Ara h 1 found in peanuts (Hurlburt et al., 2014). The cleaved-off N-terminal propeptide contains three allergenic epitopes, of which two are major (Burks et al., 1997). The Ara h 1 monomer, which forms stable trimers held together by non-covalent interactions, occurs in peanuts as larger oligomers (van Boxtel et al., 2006).

The bicupin storage protein Ara h 3 was originally identified as a 14 kDa peanut protein by Eigenmann and coworkers (Eigenmann et al., 1996). Its N-terminal sequence was determined (Burks et al., 1998) and used to design degenerate oligonucleotides for screening a peanut cDNA library (Rabjohn et al., 1999). The open reading frame of the Ara h 3 cDNA identified in this screen coded for a protein of around 60 kDa. The 14 kDa protein appeared to be an N-terminal breakdown product of the larger allergen. A genomic clone encoding Ara h 3, AF10854, revealed the presence of four exons. The deduced protein of 538 amino acid residues has a calculated molecular mass of 61.7 kDa. Ara h 3 has a leader peptide of 20 amino acid residues that is important for protein translocation to the storage vacuole. The deduced amino acid sequence showed 93% and 91% identity with the peanut allergens Ara h 3 (Rabjohn et al., 1999) and Ara h 4 (Kleber-Janke et al., 1999) indicating that these proteins were in fact variants of the same gene. Ara h 4 was later renamed Ara h 3.02 (Radauer et al., 2014). Ara h 3 is post-translationally cleaved into a 43 kDa acidic and a 28 kDa basic subunit that are covalently linked by a disulfide bond (Schmidt et al., 2009). In summary, several fragments of Ara h 3 (14, 25, 42 and 45 kDa) can be observed, even under extraction conditions that inhibit proteases (Koppelman et al., 2003). This illustrates that Ara h 3 is proteolytically processed in peanuts. An additional isoform, iso-Ara h 3, only shares 70–85% sequence identity with the other reported Ara h 3 isoforms (Boldt et al., 2005). In fact, five different genes were described to encode isoforms of Ara h 3 (Yan et al., 2005).

3.2. 2S albumins: Ara h 2, Ara h 6, Ara h 7

Ara h 2, a 2S albumin, can be purified as a doublet as described by Burks et al. (Burks et al., 1992) and de Jong et al. (de Jong et al., 1998), both bands having the same N-terminal sequence. An almost complete cDNA sequence of Ara h 2.01 was published in 1997 by Stanley et al. (Stanley et al., 1997), and in 2003 complete cDNA sequences for both Ara h 2.01 and Ara h 2.02 were made available by Chatel and colleagues (Chatel et al., 2003). The isoform Ara h 2.02 is characterized by a 12 amino acid residue insertion at position 75 in comparison to the isoform Ara h 2.01.The deduced amino acid sequence of a full length intron-free genomic clone of Ara h 1.01 comprises 207 residues and includes a signal peptide of 21 residues (Viquez et al., 2001). The two isoforms of Ara h 2 are expressed from different genes. Furthermore,