REVIEW

What makes peanuts so allergenic?

ARND PETERSEN*, WOLF-MEINHARD BECKER and UTA JAPPE

Division of Clinical and Molecular Allergology, Research Center Borstel, Airway Research Center North (ARCN), Member of the German Center for Lung Research, Borstel, Germany

(Received 5 October 2012, revised 24 January 2013)

Abstract: Peanut allergy belongs to one of the most severe food allergies. So far 12 peanut allergens have been registered by the IUIS Allergen Nomenclature Subcommittee. The different peanut allergens and factors that contribute to allergenicity are described herein. Peanut contains several class I food allergens (especially Ara h 1, 2, 3) that are stable against heat denaturation and proteolytic digestion and represent storage proteins. These allergens are often associated with severe allergic reactions. Additionally, peanut contains class II food allergens (Ara h 5 and 8), where the IgE reactivity is caused by cross reactions to inhalant allergens. These allergens are mostly associated with mild to moderate allergic reactions. However, the severity of the symptoms may change by involvement of additional factors. The peanut matrix consists to about 50% of lipids, and allergen–lipid associations have been shown for several peanut allergens. Further factors influencing allergenicity depend on the peanut variety, geographical differences and alterations in food processing. Finally, the physiological function of allergens and the mechanisms by which they interact with the immune system are further modulating factors. Thus, the specific allergen structure, matrix, genetic variations, geographic alterations and further augmentation factors are important parameters that induce and influence allergenicity.

Keywords: allergenicity; augmentation factors; class I and II food allergens; epitope; IgE reactivity.

CONTENTS

1. INTRODUCTION
2. STRUCTURAL CHARACTERISTICS OF PEANUT ALLERGENS
   2.1. Characteristics of class I food allergens of peanut
   2.2. Characteristics of class II food allergens of peanut
3. ADDITIONAL FACTORS INFLUENCING ALLERGENICITY

*Corresponding author. E-mail: apetersen@fz-borstel.de
doi: 10.2298/JSC121105007P

321
1. INTRODUCTION

Peanut is one of the most hazardous sources of food allergens that can cause severe, sometimes even fatal, anaphylactic reactions. About 100 µg of peanut protein can provoke symptoms in allergic individuals. It is rather difficult to avoid peanuts, since they may occur as hidden allergens in exotic meals and as supplements, such as peanut oil in cosmetics. Due to their lipophilic matrix (peanuts contain about 50 % of lipids), peanut proteins are considerably adhesive and may be resistant to degradation. Possible sensitization routes are the gastrointestinal tract, the respiratory tract, the skin, as well as breast feeding.

To answer the questions concerning peanut allergenicity, the complexity of peanut allergens, co-factors and interaction with the immune system at different interfaces have to be taken into account.

Three topics should be considered:
1. the structural characteristics of peanut allergens;
2. additional factors that can increase allergenicity;
3. mechanisms of sensitization by peanut allergens.

2. STRUCTURAL CHARACTERISTICS OF PEANUT ALLERGENS

The molecular structure is obviously related to allergenicity. The fact that allergens exist only in 1.5 % of the 9318 protein families suggests a correlation between molecular structure and allergenicity. This was further confirmed by studies of da Costa Santiago et al., who demonstrated a correlation of allergenicity with structural uniqueness of proteins, while homologous proteins common in eukaryotic organisms and high amino acid conservation levels result in lower allergenicity or lead to immunological tolerance.

However, little is known about the typical structural characteristics of allergens. Allergens are glycoproteins or proteins with a molecular weight of 10 to 70 kDa. They must carry at least two IgE-reactive epitopes to enable cross-linking of IgE antibodies on effector cells to release mediators. The allergenic molecules must be stable to reach the immune cells. Interestingly, low dosages cause allergy, since low concentrations increase Th2 cell activation. Usually allergens are readily soluble. The physiological functions of allergens also have to be considered as factors promoting allergenicity, although few publications exist on this particular subject: some allergens have been identified as proteases, protease inhibitors, transport proteins, regulatory proteins, storage proteins and defence proteins.

Subsequently, different peanut allergens and factors that contribute to allergenicity are described. Peanut allergens were identified by 2D immunoblotting
using sera from peanut allergic individuals. The protein spots were identified by tryptic mass fingerprinting and sequence homology was searched for in databases.\(^6\) The complexity becomes evident when focusing on molecules of similar size but with different pI values. Many allergens consist of isoforms that differ from each other by exchange of one or a few amino acids and/or post-translational modifications.\(^7,8\)

The peanut allergens as they are listed by the IUIS Allergen Nomenclature Subcommittee are summarized in Table I. Twelve different allergens from peanut (\textit{Arachis hypogaea}) are known: Ara h 1–Ara h 13. The structure of the allergens varies from polypeptides (\textit{e.g.}, oleosins), small molecules stabilized by disulfide bonds (\textit{e.g.}, Ara h 2) to complex post-translation modified glycoproteins that oligomerize (\textit{e.g.}, Ara h 1). The physiological function of peanut allergens range from storage proteins, protease inhibitors, pathogenesis-related proteins (PR proteins for defence) to transport proteins.

<table>
<thead>
<tr>
<th>Allergen</th>
<th>MW / kDa</th>
<th>pI</th>
<th>Structure</th>
<th>PTM</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ara h 1</td>
<td>62–69</td>
<td>6.0–6.4</td>
<td>Cupin, vicilin type, 7S, trimer</td>
<td>glyc, S–S</td>
<td>Storage protein</td>
</tr>
<tr>
<td>Ara h 2</td>
<td>17–18</td>
<td>5.5</td>
<td>Conglutin type, 2S albumin</td>
<td>S–S</td>
<td>Storage protein, trypsin inhibitor</td>
</tr>
<tr>
<td>Ara h 3.01</td>
<td>56–59</td>
<td>5.4–5.6</td>
<td>Cupin, legumin type, 11S, hexamer</td>
<td>glyc, S–S, (\alpha)- and (\beta)-subunits</td>
<td>Storage protein</td>
</tr>
<tr>
<td>Ara h 3.02 (Ara h 4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ara h 5</td>
<td>14</td>
<td>4.6</td>
<td>Profilin</td>
<td>–</td>
<td>Profilin</td>
</tr>
<tr>
<td>Ara h 6</td>
<td>14</td>
<td>5.0</td>
<td>Conglutin type, 2S albumin</td>
<td>S–S</td>
<td>–</td>
</tr>
<tr>
<td>Ara h 7</td>
<td>16–17</td>
<td>5.6–7.5</td>
<td>Conglutin type, 2S albumin</td>
<td>S–S</td>
<td>–</td>
</tr>
<tr>
<td>Ara h 8</td>
<td>16–17</td>
<td>5.0</td>
<td>Bet v 1 homologous protein</td>
<td>–</td>
<td>PR-10, transport</td>
</tr>
<tr>
<td>Ara h 9</td>
<td>9</td>
<td>9.3–9.5</td>
<td>Lipid transfer protein</td>
<td>S–S</td>
<td>PR-14, lipid transport</td>
</tr>
<tr>
<td>Ara h 10</td>
<td>16–18</td>
<td>9.4–9.6</td>
<td>Oleosin</td>
<td>–</td>
<td>Lipid transport</td>
</tr>
<tr>
<td>Ara h 11</td>
<td>14</td>
<td>9.1</td>
<td>Oleosin</td>
<td>–</td>
<td>Lipid transport</td>
</tr>
<tr>
<td>Ara h 12(^{18})</td>
<td>5</td>
<td>7.7</td>
<td>Defensin, dimer</td>
<td>S–S</td>
<td>PR-12, defence</td>
</tr>
<tr>
<td>Ara h 13(^{18})</td>
<td>5</td>
<td>8.2</td>
<td>Defensin, dimer</td>
<td>S–S</td>
<td>PR-12, defence</td>
</tr>
</tbody>
</table>

In contrast to pollen allergens that enter the organism without structural changes, peanuts are usually processed by roasting before consumption and subsequently undergo digestion under acidic conditions by pepsin in the stomach.

Available online at shd.org.rs/JSCS/
and in basic conditions in duodenum by pancreatic enzymes, before fragments (single allergens) reach the immune system, a complex procedure, which influences their allergenicity.\textsuperscript{10}

Food allergy is divided into two classes as described in Table II.\textsuperscript{11} Class I food allergy is characterized by systemic and more severe reactions, which is associated with allergens resistant to heat, proteases and digestion, allowing these allergens to pass the gastrointestinal tract without structural changes. This category of allergens in peanut contains Ara h 1, 2 and 3, which are known to be responsible for severe anaphylactic reactions after peanut consumption.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Class I</th>
<th>Class II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitization route</td>
<td>Gastrointestinal</td>
<td>Inhalation</td>
</tr>
<tr>
<td>Characteristics</td>
<td>Thermally and proteolytically stable</td>
<td>Thermally and proteolytically unstable</td>
</tr>
<tr>
<td>Symptoms</td>
<td>Severe reactions</td>
<td>Often mild reactions</td>
</tr>
<tr>
<td></td>
<td>Anaphylaxis</td>
<td>OAS</td>
</tr>
<tr>
<td>Examples</td>
<td>Ara h 1, 2 and 3</td>
<td>Ara h 5 and 8</td>
</tr>
</tbody>
</table>

However, peanut also contains class II food allergens. In this case, the sensitization is usually raised against pollen allergens \emph{via} the respiratory tract. Due to cross-reactive IgE epitopes in peanut allergens, such molecules can cause allergic reactions as well. Usually these allergens are thermally and proteolytically unstable, and often only mild symptoms are provoked.

The following description of the class I and II allergens of peanut is based on the review of Becker \emph{et al.}\textsuperscript{9}

2.1. Characteristics of class I food allergens of peanut

Some of the peanut allergens reveal many sequential IgE-binding epitopes, \emph{e.g.}, Ara h 1 possesses 23 linear epitopes.\textsuperscript{12} Even after partial cleavage during digestion, some IgE-binding epitopes remain on the fragments and probably cause histamine release.

Ara h 1 and Ara h 3 belong to the cupin superfamily with a molecular structure formed by a $\beta$-barrel core domain and two additional conserved sequence motifs. Ara h 1 is a 7S globulin and belongs to the vicilin type. Hydrophobic patches are located at the ends of the molecule; they are the binding edges for the formation of Ara h 1 trimers.\textsuperscript{13} Ara h 3 tends to form hexamers. Its monomers are composed of an acidic and a basic subunit linked by a disulfide bond.\textsuperscript{14} Ara h 3 is an 11S globulin and belongs to the legumin type.

Ara h 2, 6 and 7 are members of the prolamin superfamily. They are alcohol soluble proline and glutamine rich storage proteins that show a specific, highly conserved cysteine pattern (C–Xn–C–Xn–C–Xn–C–Xn–C–Xn–C–Xn–C–Xn–C),
whereby Ara h 6 contains 10 cysteines. Ara h 2, 6 and 7 belong to the 2S albumins. Ara h 2 is the most frequently recognized peanut allergen. Studies by Maleki et al. showed that the stability of this molecule is due to the four disulfide bonds. Roasting and denaturating conditions with 8 M urea did not alter the secondary structure, as demonstrated by CD spectroscopy. However, the conformational structure is destroyed by reducing procedures.

Ara h 9 also belongs to the prolamin superfamily, but to the subgroup of the non-specific lipid transfer proteins (LTP). The LTPs are largely distributed in fruits and vegetables and cause allergic reactions, especially in individuals of the Mediterranean region. The underlying molecule is stabilized by four disulfide bonds and belongs to the pathogenesis-related proteins (PR12-protein). Two further families of peanut allergens are associated with lipids: the oleosins (Ara h 10 and 11) and the defensins (Ara h 12 and 13). Oleosins are amphiphilic molecules that form a monolayer on oilbodies and peanut defensins, isolated from lipophilic fractions, have recently been identified as IgE-reactive components. Plant defensins are small, highly stable, cysteine-rich peptides that constitute a part of the innate immune system, primarily directed against fungal pathogens.

2.2. Characteristics of class II food allergens of peanut

Class II food allergens in peanut are Ara h 5 and 8. Ara h 5 is a profilin. Profilins are detectable in all eukaryotic cells and are involved in the formation of the cytoskeleton. Since they also exist in human cells, immune responses can only be elucidated to structurally and phylogenetically less related plant profilins. Nevertheless, the structural differences to plant profilins are small. Clinical relevance seems to be negligible. The symptoms, if any occur, are usually only mild.

Ara h 8 is a Bet v 1-homologous protein (PR-10 protein) and a class II food allergen, since the sensitization (especially in Northern Europe) is caused by the major birch pollen allergen Bet v 1. This plant family is rather large and consists of many members of fruits and vegetables from Rosaceae and Apiaceae, respectively.

Even though the protein sequences can differ by 60% among different members, the underlying 3D structure and surface identity (important for the antibody binding) are more similar. The characteristic 3D structure is formed by 7 antiparallel $\beta$-sheets and 2 short $\alpha$-helices. These allergens are unstable to heat and digestion and, therefore, often cause only mild reactions, e.g., the oral allergy syndrome (OAS).
3. ADDITIONAL FACTORS INFLUENCING ALLERGENICITY

For allergy development, the specific structure of the allergen/s in the context of allergen exposure and the genetic background of the individual are of profound importance.

Allergenicity can vary considerably due to the variety of the allergen sources, the form of protein extraction and the further processing.

Comparing peanuts from different varieties and origins, a peanut species from Bali that showed nearly no Ara h 1 was evidenced by 2D PAGE and immunoblotting.\(^{26}\) It might be speculated that this was a hypoallergenic peanut variant. However, when the allergenicity of the peanut extract was determined, it was found to be comparable to those other varieties, which is probably due to compensation with higher amounts of the other peanut allergens. This is a natural example that demonstrates that the elimination of a single allergen does not necessarily result in a lower overall allergenicity.

The extraction conditions also play an important role. Under alkaline conditions (carbonate buffer, pH 8), which mirrors the physiological conditions of peanut uptake in man, Ara h 1 and Ara h 3 are especially detectable, while under acidic conditions (acetate buffer, pH 5) oleosins and LTP (Ara h 9) appear more prominently.\(^{27}\)

When an attempt was made to improve the isolation of Ara h 8 from an extract,\(^{28}\) it was observed that lipophilic extraction by use of chloroform and methanol\(^{29}\) produced a more efficient isolation, resulting in pure Ara h 8. It would not have been possible to identify the sera of Ara h 8-reactive patients in the usually isolated extract (carbonate buffer, pH 8). However, by application of the lipophilic extraction method, Ara h 8 could clearly be detected. This is a fact that has to be considered for diagnosis.

A further interesting phenomenon was observed in these experiments: extracts prepared from roasted peanuts revealed a stronger IgE reactivity when compared to the extract from unroasted peanuts (personal communication). This may be due to roasting as Maillard products are formed\(^{30}\) by the reaction of carbohydrates and allergens, which was demonstrated for the peanut allergens Ara h 1 and 2.\(^{10,13}\)

A lipophilic matrix can also have an important influence on the allergenicity of the molecules. Sancho et al.\(^{31}\) demonstrated that phosphatidylcholine induced conformational changes in the apple allergen Mal d 1 and allowed this allergen to penetrate the phosphatidylcholine vesicle. By this, the digestion of the Mal d 1 was retarded, and the allergenicity was retained to a large extent. Since peanut consists of up to 50 % of lipids,\(^{2}\) evidence was found that lipids also interfere with the IgE binding and digestibility of Ara h 8. After roasting, some covalent Ara h 8–lipid oxidation products might have been generated. Recombinant Ara h
8 without lipids was degraded rapidly, while Ara h 8 isolated from roasted peanuts was resistant in gastrointestinal digestion experiments.\textsuperscript{28}

Not only Ara h 8, but also several other peanut allergens are associated with lipids. Thus, the lipid transfer protein Ara h 9, the oleosins, which float on the surface of oilbodies,\textsuperscript{19} Ara h 10 and 11, and finally plant defensins were identified as by-product when Ara h 8 was isolated by lipophilic extraction.\textsuperscript{18}

Besides the specific structure, the quantity of allergens can influence sensitization as well. Fox \textit{et al.}\textsuperscript{32} reported that high levels of environmental exposure to peanut during infancy caused higher rates of sensitizations. On the other hand, du Toit \textit{et al.}\textsuperscript{33} showed that early consumption of peanuts in infancy seems to be associated with a low prevalence of peanut allergy. They determined a 10-fold lower prevalence for peanut allergy in Jewish children in Israel than in Jewish children in the U.K. Israeli children consume peanuts in high quantities in the first year of life, while U.K. infants avoid peanuts. Other probable factors arising by atopy, social class, genetic background and peanut allergenicity were ruled out in the study.\textsuperscript{33} This indicates that a window of opportunity might exist in early life that causes tolerance induction.\textsuperscript{34}

Furthermore, the route of the allergen exposure seems to be decisive. In peanut allergic patients, the proliferation of peanut-specific memory T-cells with the skin-homing factor CLA+ (cutaneous lymphocyte antigen) predominates, while peanut-tolerant groups have a mixed skin- and gastrointestinal-homing factor composition CLA+/α4β7+.\textsuperscript{35} These results further support the hypothesis that the initial route of allergen exposure in early life is crucial: allergic sensitization might occur \textit{via} the skin, while passage \textit{via} the gut induces tolerance.\textsuperscript{35}

Geographical parameters and environmental factors can also influence the occurrence of allergic reactions to specific allergens. Concerning peanut allergens, a clear distribution of the North European population exposed mainly to birch pollen results in reactivity to the Bet v 1-homologous Ara h 8, while in the Mediterranean countries, the reactions are especially based on the lipid transfer proteins. This is probably caused by exposure to the marker allergen Pru p 3 from peach, which cross-reacts with Ara h 9.\textsuperscript{17,36}

Food processing habits can also influence sensitization. It is interesting to note that peanuts are usually cooked in China, while they are mainly roasted in other countries.\textsuperscript{37} Although peanut consumption is high in China, peanut allergy does not seem to be as prevalent as it is in the U.S.A. The reason might be due to the food processing. By cooking peanuts, some allergens may be washed out, while roasting can generate new stable allergenic epitopes, \textit{e.g.}, Maillard products.\textsuperscript{38}

Last but not least, factors such as alcoholic drinks, non-steroidal anti-inflammatory drugs (NSAIDs), exercise and menses can increase food allergic symptoms.\textsuperscript{39,40}
4. WHAT ARE THE MECHANISMS OF THE SENSITIZATION BY PEANUT ALLERGENS?

To understand the effect of allergenicity, it is important to investigate how specific allergens interact with the epithelium and the immune response-triggering cells.

Several food allergens have been identified as proteases, e.g., actinidin of kiwi (Act d 1), and Cavic et al. were able to demonstrate similar morphological changes and desquamation of epithelial cells after exposure to Act d 1 and with the house dust mite allergen Der p 1. Although no allergenic protease have hitherto been identified in peanut, it might be speculated that, for example, subtilisin, a serine protease in peanut kernels, disrupts the tight junctions, thereby enabling peanut allergens passage through the epithelial barrier.

The allergens Ara h 2, 6 and 7 show molecular similarities to the soybean trypsin inhibitor. This might result in incomplete digestion of allergens, which can consequently elicit allergic reactions. Shreffler et al. demonstrated a binding of the glycoprotein Ara h 1 via its carbohydrate moiety (β-glucan) to CD209 on dendritic cells, the DC-SIGN (dendritic cell-specific intercellular adhesion molecule 3 grabbing non-integrin). As a consequence, a shift towards Th2 activation occurs, as shown in human in vitro experiments. The results correspond to those obtained in studies of Royer et al., which showed that several native allergens with a carbohydrate moiety (among them Ara h 1) can bind to the mannose receptor on dendritic cells (C-type lectin) causing Th2 cell polarization.

Khodoun and co-workers proved that components from peanut extract can activate complement in mice in vivo and in vitro and they also demonstrated this effect in vitro in man. C3a and to a lower degree Fcγ receptors were identified as contributing to peanut extract-induced anaphylactic shock. This was shown to occur without the involvement of the adaptive immune system. To date, the elucidating components from peanut extract have not been identified.

Furthermore, the association of proteins with lipids and lipophilic molecules can have structural and functional effects. Karp hypothesized that intrinsic adjuvant activity may be provided for by the association of the proteins with their lipid cargo and that this underlies their immunogenicity and/or allergenicity. In this context, Ara h 8 and other lipophilic allergens may support or enhance the binding to receptors of the innate immune system, such as the Toll-like receptors TLR2 and TLR4 that bind lipopeptides and lipopolysaccharides (LPS), respectively. Dependent on the LPS concentration, the immune responses can vary considerably. In an animal model with ovalbumin, a dose of <1 ng LPS led to tolerance, a dose of 100 ng LPS to sensitization with a Th2 immune response and a dose of 100 µg to Th1 inflammation.
For the Bet v 1-homologous peanut allergen Ara h 8, a binding to lipid rafts and allergen uptake via caveolae was demonstrated. Interestingly, this binding was only detected in birch pollen allergic individuals, but not in healthy controls.49 The receptor, however, has not yet been identified.

5. CONCLUSIONS

The presented data provide evidence for a clear relationship between molecular structure and allergenicity, although many further influencing factors may be involved. Besides the allergen structure, the variety of the source of allergens, the matrix, the manner of processing, and with regards to the consumer, the genetic background, the qualitative and quantitative exposure, the route of exposure (gastrointestinal tract, respiratory tract, skin), and host-associated additional factors. Preliminary investigations have been performed to better understand these interactions. Since the development of an allergic phenotype depends on many factors, it is important to dissect and study the different factors.

Furthermore, the clinical relevance of the sensitization should be determined, since sensitization does not necessarily result in clinical symptoms. Clinical problems arise from inter- and intra-individual variability of the level triggering allergic reactions.

A first step is to switch from prick test with mixtures from allergenic sources, which can only differentiate between sensitized or non-sensitized, to a component-resolved diagnosis, which allows discrimination between the different allergens and, hopefully, to find marker allergens (e.g., Ara h 2) that allow severe symptoms to be predicted.50
рекло и други појачавајући фактори су важни параметри за индукцију и даљи ток алергијске реакције.

(Примљено 5. октобра 2012. ревидирано 24. јануара 2013.)

REFERENCES