Strategies to Mitigate Peanut Allergy: Production, Processing, Utilization, and Immunotherapy Considerations

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Abstract

Peanut (*Arachis hypogaea* L.) is an important crop grown worldwide for food and edible oil. The surge of peanut allergy in the past 25 years has profoundly impacted both affected individuals and the peanut and related food industries. In response, several strategies to mitigate peanut allergy have emerged to reduce/eliminate the allergenicity of peanuts or to better treat peanut-allergic individuals. In this review, we give an overview of peanut allergy, with a focus on peanut proteins, including the impact of thermal processing on peanut protein structure and detection in food matrices. We discuss several strategies currently being investigated to mitigate peanut allergy, including genetic engineering, novel processing strategies, and immunotherapy in terms of mechanisms, recent research, and limitations. All strategies are discussed with considerations for both peanut-allergic individuals and the numerous industries/government agencies involved throughout peanut production and utilization.

INTRODUCTION

Anaphylaxis: systemic allergic response that may produce gastrointestinal, respiratory, and/or cardiovascular symptoms, which can result in complete airway obstruction, shock, and death

The peanut is an important crop worldwide, grown widely for edible oil production, as peanuts are typically comprised of 47–50% oil. Peanuts are also commonly directly consumed in many countries, including the United States, where the majority of the crop is used for peanut butter (45%), as snack nuts or in-shells (30%), or in candies and confections (25%) (American Peanut Council 2011). Peanuts and peanut butter are good, affordable sources of protein as peanuts are approximately 25% protein by weight. Peanuts are also rich in many vitamins and minerals, including vitamin E and folate, and contain primarily monounsaturated fats and no trans fat (Dean et al. 2009, McDaniel et al. 2012, Rychlik et al. 2007). Substantial epidemiological and clinical evidence shows that regular consumption of peanuts (and tree nuts) promotes cardiovascular health (Kris-Etherton et al. 2008) and weight management (Mattes et al. 2008). With their generally well-liked flavor and excellent shelf life, it is not surprising that peanuts and peanut butter are often recommended as healthy snacks for both children and adults.

For a small percentage of the population, however, an allergy to peanuts precludes their consumption and requires constant vigilance to avoid accidental consumption, which can have dangerous health consequences. An estimated 1% of the US population suffers from peanut allergy, and this number is slightly higher for children (Sicherer et al. 2010). Among the Big-8 ingredients (milk, eggs, fish, crustacean shellfish, tree nuts, wheat, peanuts, and soybeans) that trigger food allergies recognized by the US Food and Drug Administration (FDA), allergy to peanuts is often considered the most severe as it is the leading cause of fatal anaphylaxis and is rarely outgrown (Sicherer et al. 2010). As such, studies aimed at identifying new mitigation strategies for this allergy have permeated the literature in the past decade.

In this review, peanut allergy and mitigation strategies are considered from the perspectives of both (*a*) patients and their families living with peanut allergy and (*b*) peanut and related industries/government agencies that contribute to the production and delivery of this globally important oilseed. We discuss strategies being applied or under consideration related to all stages of peanut production and utilization, including breeding, through postharvest processing. We cover further novel processing methods aimed at mitigating peanut allergy, as well as antigen-specific and nonspecific immunotherapy approaches. All strategies are described in terms of mechanisms, recent studies, and limitations that hinder widespread applications.

WORLDWIDE IMPORTANCE OF PEANUTS

Peanut (*Arachis hypogaea* L.), one of the top five oilseeds in the world, is a legume grown primarily as a source of oil and protein (USDA 2013). The world's population is growing exponentially; the current (2013) population exceeds seven billion and is expected to eclipse nine billion by 2050 (US Census Bureau 2011). Such growth puts extreme demands on food supplies, especially protein, which on a basic level is a source of biological nitrogen necessary for human growth (food) and animal growth (feed) (Aiking 2011). In the future, substantially more protein must be produced using limited land, water, fertilizer, and energy resources ever more efficiently. Plant proteins are critical to meeting global protein needs, and legumes, such as peanut, are especially important as they require minimal nitrogen inputs in cropping systems (Sinclair & Vadez 2012). Nearly 25% of the peanut's dry weight is protein, and because peanuts are often directly consumed, unlike many other major oilseeds, they are an especially important plant source of dietary protein. In addition to peanuts being a staple in many parts of the world, roasted peanuts are also a primary component of ready-to-eat therapeutic foods for treatment of severely malnourished children, which has proved over recent years to be an incredibly successful and important intervention strategy for hunger in many developing countries (Ciliberto et al. 2005).

PEANUT ALLERGY: HISTORY AND PERSPECTIVE

The emergence of peanut allergy over the past approximately 25 years has profoundly affected the peanut and related food industries. It is important to put peanut allergy in some historical perspective of peanut production, utilization, and research, especially research related to characterizing peanut protein. Although numerous advances have occurred since its publication slightly more than 30 years ago, the book *Peanut Science and Technology*, edited by Pattee & Young (1982), provides a foundation on peanut production and utilization for the interested reader.

Peanuts are thought to have been domesticated for hundreds, if not thousands, of years, with origins in South America, as noted by several early European explorers who observed natives cultivating peanuts for personal use during the 1500s (Hammons 1982). From there, commerce spread peanuts to Europe and Africa, and with the colonization of North America by Europeans, peanuts were introduced to the New World, although the exact point(s) of introduction is not clear (Hammons 1982). By the American Civil War, roasted peanuts had become a favored food of many because of their popular flavor, convenience, affordability, availability, and extended shelf life. Peanut production within the United States increased extensively in the early 1900s, accelerated by extreme losses in cotton production resulting from boll weevil infestation. As a legume, peanut proved to be valuable in the rotation in cropping systems, and various advances in production and harvesting systems followed. At the outbreak of World War II, the US government deemed peanut, for its oil, an essential crop, and many research programs within the US Department of Agriculture (USDA) and public universities became dedicated to improving peanut production and quality through research in plant breeding, agronomics, harvesting, handling, and processing (Hammons 1982). Since that time, this research infrastructure, coupled with innovation in the private sector, has resulted in tremendous improvements to the US peanut industry, including development of advanced cultivars with record yields, high-efficiency planting/harvesting systems, and the premier postharvest processing infrastructure in the world.

By the late 1800s and early 1900s, research on peanut protein began appearing in the scientific literature. Using various salt solutions and ammonium sulfate precipitation techniques, Johns & Jones (1917) designated two primary globulins from peanut seed as arachin and conarachin. These terms persisted well into the 1980s as advances in chromatography and other characterization techniques led to more detailed understanding of the peanut seed storage proteins. This information was applied primarily to efforts at improving agronomic performance and postharvest quality. Significant research from the 1950s through the 1980s focused on adding value to the protein-enriched solids remaining after peanut oil extraction for both food and feed applications. This included development of technologies for isolating and producing enriched peanut protein ingredients, including concentrates, isolates, flakes, etc., and studies devoted to characterizing and optimizing the nutritional properties and functionality of these food ingredients, i.e., solubility, emulsification, foaming properties, etc. Substantial and important reviews on this era of peanut protein research are available (McWatters & Cherry 1982).

The late 1980s and early 1990s ushered in a new focus in peanut protein research: allergenicity. Food allergy is discussed in the scientific literature as early as 1912 (Schloss 1912). As the field evolved and, for example, improved understanding of the relevant immunological mechanisms, evidence emerged that a relatively small, but significant, portion of the population suffered from some type of true food allergy (i.e., an adverse IgE-mediated reaction) and that certain foods, including peanuts, were more likely than others to cause adverse allergic responses. A PubMed search for the term "peanut allergy" covering all years from 1975 to 2012 resulted in 1,387 publications. Although far from comprehensive, and not accounting for the general increase in scientific publications over recent years, **Figure 1** illustrates the trend of increased scientific

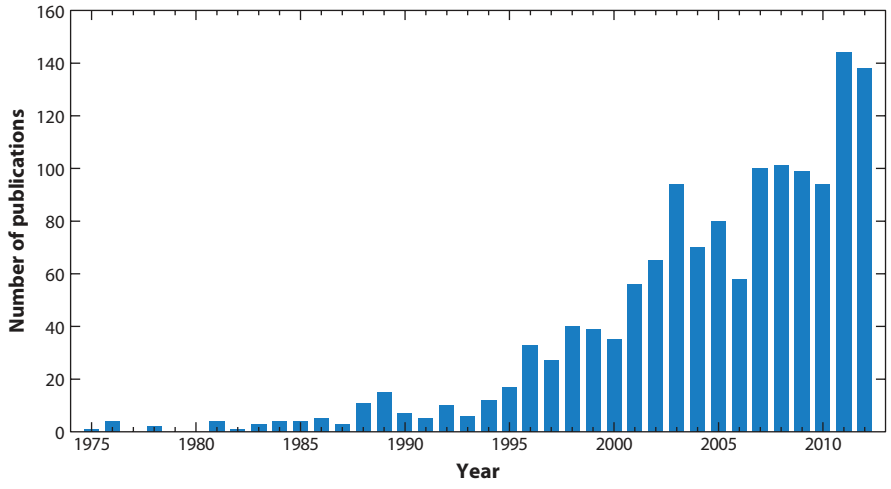

Figure 1

Number of scientific publications from 1975 to 2012 using the search term "peanut allergy" in PubMed conducted in May 2013.

research dedicated specifically to understanding peanut allergy. The sharp increase was driven by the fact that in the early 1990s several tragic deaths associated with anaphylactic shock resulting from peanut consumption catapulted food allergy, especially peanut allergy, to the forefront of public perception (Reading 2009). Accordingly, scientific research toward peanut allergy began in earnest around this time. In 1991, the first major peanut allergen was documented and named Ara h 1 (Burks et al. 1991).

Figure 2 is an oversimplification but provides an overview of stages involved in peanut production and utilization for reference when considering strategies to mitigate peanut allergy. First and foremost, peanut allergy is a critical issue for those individuals and families that are affected. However, peanuts are important worldwide as a source of food and feed, and their production and use involves numerous industry segments and regulatory agencies. Accordingly, research or development of new technologies aimed at improving production/agronomy, nutrition, processing/utilization, or allergenicity should consider all these stages for the highest level of successful

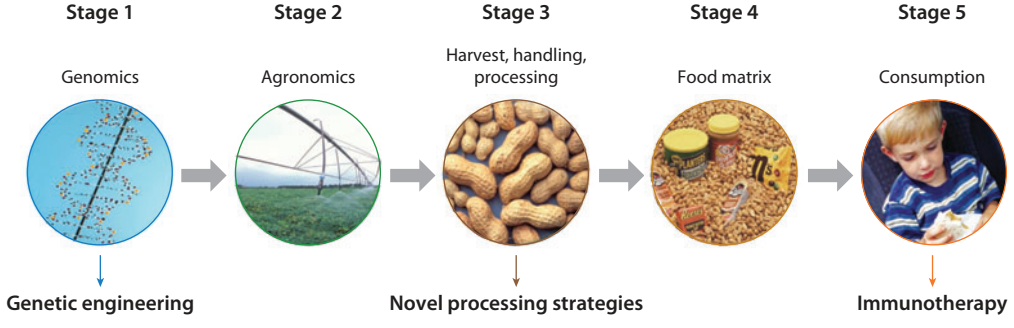

Figure 2

Overview of the stages involved in peanut production and utilization, with potential allergy mitigation strategies placed at the point in which they would be implemented.

implementation. From this perspective, we will be able to better understand the potentials and limitations of strategies aimed at addressing the critical issue of peanut allergy, while also considering current and future implications for the peanut and related food industries that produce this globally important oilseed.

OVERVIEW OF PEANUT ALLERGY

Pathogenesis of IgE-Mediated Food Allergies

Burks (2008) and Sicherer & Sampson (2010) have published in-depth reviews on this subject. Peanut allergy, as with most other food allergies, is an IgE-mediated reaction triggered by certain peanut proteins that the immune system mistakenly deems harmful. There are two stages in the development of an IgE-mediated food allergy. Sensitization occurs upon initial allergen exposure. Upon consumption of the allergen, gastric acid and digestive enzymes break down the conformational structures of the protein, resulting in small peptide fragments, which are then exposed to the mucosal immune system in the gastrointestinal tract (Burks 2008). A common characteristic of most allergenic proteins is that they are more resistant to digestive enzymes than nonallergenic proteins; therefore, larger peptide fragments reach the gastrointestinal tract intact (Astwood et al. 1996, Koppelman et al. 2010). This exposure causes plasma cells in allergic individuals to produce IgE specific for the target allergen (peptide fragment). The allergen-specific IgE molecules then attach to the surface of mast cells and basophils through high-affinity $Fc_{\varepsilon}RI$ receptors, ending the sensitization phase with no symptoms occurring. The second stage, allergic reaction, begins upon subsequent exposure to the allergen-containing food. The immune system now recognizes portions of the protein allergen known as epitopes, which can be defined by linear sequences of amino acids, structural motifs of the protein, or a combination of both. Through these epitopes, the allergen binds to and cross-links two or more IgE-$Fc_{\varepsilon}RI$ complexes that were deposited on the surface of the mast cells or basophils during the sensitization phase. This results in basophil or mast cell degranulation and subsequent release of various inflammatory mediators, such as cytokines, chemokines, lipid mediators, and histamines (Burks 2008). These chemicals cause a series of local symptoms, such as skin itching/hives, tongue swelling, and throat irritation, in addition to various systemic symptoms, such as airway obstruction, blood pressure depression, diarrhea, shock, and even fatal anaphylaxis.

Mast cell: cell that is similar to a basophil and is found in tissues instead of blood

Basophil: white blood cell with high-affinity IgE receptors ($Fc_{\varepsilon}RI$) that functions to mediate allergic reactions of the immune system

$Fc_{\varepsilon}RI$ receptors: high-affinity receptors for a specific region of IgE molecules found on the surface of mast cells and basophils

Epitope: linear or conformational region of an antigen that can elicit a response from the immune system

Characterization and Allergenicity of Peanut Proteins

Proteins are the causative agent of peanut and other IgE-mediated food allergies. These proteins are also central to the physiology of growing seeds as nitrogen sources in the case of the seed storage proteins, defense proteins, or structurally important membrane proteins and hence are critical to agronomic performance. Peanut proteins are also necessary for the development of roasted peanut flavor through the Maillard and other related reactions (Newell et al. 1967, Oupadissakoon & Young 1984). Furthermore, from a consumer perspective, peanut proteins are a source of nutrition, as they provide both essential amino acids and calories. Beyond nutrition and flavor, proteins are also responsible for both allergenicity and bioactivity. Interestingly, many of the most common sources of food allergens are also good sources of biologically active peptides, i.e., peptides that confer health benefits beyond providing amino acids (Hartmann & Meisel 2007). The review by Foegeding & Davis (2011) highlights the similarities between protein allergenicity and bioactivity with respect to structural conformation, digestion, and food matrix effects.

The World Health Organization and the International Union of Immunological Societies Allergen Nomenclature Subcommittee currently recognize 12 allergenic peanut proteins, which

Table 1 List of major peanut allergens, including their biochemical names and MW as resolved by SDS-PAGE[a]

Allergen	Biochemical name	MW (SDS-PAGE)[b]
Ara h 1	Cupin (vicillin-type, 7S globulin)	64 kDa
Ara h 2	Conglutin (2S albumin)	17 kDa
Ara h 3	Cupin (legumin-type, 11S globulin, glycinin)	60 kDa, 37 kDa (fragment)
Ara h 4	Ara h 3.02 (no longer considered a separate allergen)	
Ara h 5	Profilin	15 kDa
Ara h 6	Conglutin (2S albumin)	15 kDa
Ara h 7	Conglutin (2S albumin)	15 kDa
Ara h 8	Pathogenesis-related protein, PR-10	17 kDa
Ara h 9	Nonspecific lipid-transfer protein 1	9.8 kDa
Ara h 10	Oleosin	16 kDa
Ara h 11	Oleosin	14 kDa
Ara h 12	Defensin	8 kDa (reducing), 12 kDa (nonreducing)
Ara h 13	Defensin	8 kDa (reducing), 11 kDa (nonreducing)

[a]Table adapted from allergen.org (**http://www.allergen.org/search.php?allergensource=peanut&searchsource=Search**).

[b]Abbreviations: MW, molecular weight; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

are termed Ara h 1 through Ara h 13 (**Table 1**). Ara h 4 is now termed Ara h 3.02 and is no longer considered to be a separate allergen. Structural, biochemical, and allergenic characterizations, including mapping the IgE-binding epitopes of the major allergenic proteins, have been performed and are extensively described in the literature (Burks et al. 1991, 1992; Rabjohn et al. 1999; Shreffler et al. 2004; Zhuang & Dreskin 2013). Structural comparisons of known food allergens have revealed important similarities in both primary amino acid sequence and three-dimensional folds, with various superfamilies and families of evolutionary-related allergens becoming apparent (Breiteneder & Radauer 2004). For example, Ara h 2, 6, and 7 fall within the prolamin superfamily and 2S albumin family of plant food allergens characterized by eight conserved cysteines that form disulfide linkages, which stabilize a core of alpha helices. Related 2S albumin proteins from various commercially important oilseeds have been identified in soy, buckwheat, walnut, sunflower seed, Brazil nut, and others. Ara h 1 and Ara h 3, which were previously classified as arachin and conarachin, respectively, fall within the cupin superfamily of plant food allergens and are further classified in the vicilin (Ara h 1) and legumin (Ara h 3) families. Again, these superfamilies of proteins are populated by homologous proteins from essentially all commercially important cereals and oilseeds. These comparisons across homologous plant food allergens, and related proteins that are not allergenic, are of great value as they facilitate systematic investigations into why certain food proteins are more intensely and/or commonly allergenic than related proteins, and hence provide insights into structural features generally important to food allergy (Radauer & Breiteneder 2007).

A major allergen is defined traditionally as one that binds allergen-specific IgE in the serum of greater than 50% of allergic individuals (Lowenstein 1978). According to this definition, Ara h 1, 2, and 3 are considered major allergens (Burks et al. 1991, 1992; Rabjohn et al. 1999). Recently, however, it has been suggested that the term major allergen be reserved for those proteins that are

responsible for a majority of the effector activity of a food rather than IgE recognition (Zhuang & Dreskin 2013). On the basis of this definition, Ara h 2 and Ara h 6 are considered major peanut allergens as they account for a majority of the effector activity found in crude peanut extracts (Porterfield et al. 2009).

Effector activity: total capacity of an extract to activate IgE-sensitized basophils or mast cells by cross-linking Fc_{ε} RI IgE receptors

Prevalence of Peanut Allergy

Reports of children with peanut allergy have increased in the Western world in recent years. The estimated prevalence in US children was 0.4% in 1997, 0.8% in 2002, and 1.4% in 2008 (Sicherer et al. 2010). The question of when to expose children to a potentially allergenic food is of special interest. Dietary avoidance of peanuts and other allergenic foods during pregnancy, breastfeeding, and early life was recommended in 2000 (American Academy of Pediatrics, Committee on Nutrition 2000); however, in 2008 this recommendation was withdrawn as ongoing research did not support the idea that early avoidance of an offending food minimized chances of developing a food allergy (Greer et al. 2008). In contrast, more recent evidence and recommendations strongly suggest that early exposure to a given food is preferable for food allergy prevention (Fleischer et al. 2013b). An important study comparing peanut allergy among similar populations of Jewish children in Israel and the United Kingdom showed that peanut allergy prevalence was approximately 10 times greater in the UK population than that in Israel, despite similarities in atopy, genetic background, and social standing (Du Toit et al. 2008). As part of their culture, Israeli infants commonly consume peanut products around the time of weaning, typically in the form of the extruded peanut snack Bamba. Having compared and controlled for other factors across the two populations, this early consumption of peanuts is hypothesized to account for these differences in peanut allergy prevalence. Most initial allergic reactions to peanuts are thought to be upon the patient's first consumption of a product containing peanuts, suggesting that sensitization in peanut-allergic individuals could be occurring through incidental environmental exposure, i.e., cutaneous or inhalation exposure (Brough et al. 2013). Early peanut consumption could counter this nonconsumption sensitization route by inducing tolerance, as has been shown in animal models (Strid et al. 2004, Lack 2008).

PEANUT ALLERGY AND THE FOOD INDUSTRY

Implications of Food Processing for Allergenicity

As mentioned previously, 12 peanut proteins have been recognized as allergens, including the seed storage proteins, Ara h 1, 2, 3, 6, and 7. The amino acid sequences of these proteins have been documented, and in most cases their secondary, tertiary, and quaternary structures are known. However, this structural information has been collected primarily on proteins derived from raw peanuts or recombinant proteins expressed in model systems, and peanuts are rarely consumed raw; they must undergo some form of postharvest thermal treatment to improve digestibility, ensure microbiological safety, and enhance peanut flavor and texture. Although structural information derived from raw/recombinant proteins is critical to understanding their allergenicity, to fully understand how structural characteristics contribute to allergenicity, the structure of proteins derived from processed peanuts must be understood, as, generally, one consumes processed peanuts. The relative lack of information on processed peanut proteins can be partially attributed to decreased solubility of the proteins after processing, as solubility is a prerequisite for many characterization measurements (Poms et al. 2004, Kopper et al. 2005, McDaniel et al. 2012). Research in recent years has begun to fill this knowledge gap and is discussed next.

During processing, protein modifications within peanuts and other foods are desirable and necessary for the quality of the final product, including color, flavor, and texture (Baker et al. 2003, Lee & Resurreccion 2006). However, changes to protein structure during food processing also have important implications for allergenicity (Mills et al. 2009). Studies on the effect of thermal processing on the IgE-binding capacity of peanut allergens are conflicting and often suggest that it depends on the processing method (Maleki et al. 2000, Beyer et al. 2001, Schmitt & Maleki 2004, Mondoulet et al. 2005). After curing, the primary postharvest thermal treatments applied to peanuts include dry roasting, oil roasting (frying), and boiling. Dry and oil roasting, which predominate for peanuts destined for consumption in North America and much of Europe, are similar in that peanuts are cooked in low-moisture environments, i.e., typically 7% moisture prior to roasting and less than 2% after. At these conditions, Maillard browning, which is facilitated by heat and involves condensation of reducing sugars with side-chain amino groups in proteins, causes specific modifications, primarily to lysine residues. Further thermal treatment results in protein cross-linking and the formation of a variety of advanced glycation end products (AGEs) (Chung et al. 2003, Hebling et al. 2013). High-molecular-weight (MW) AGEs satisfy one of the major criteria for food allergens as they are notably more resistant to digestive proteases compared to raw peanut proteins (Kopper et al. 2005, Lehrer et al. 2006). Ara h 1 is particularly predisposed to form large aggregates in response to heating in the presence of sugars, and these complexes have been shown to have enhanced effector activity (Vissers et al. 2011). In the same study, however, Ara h 2/6 displayed reduced IgE-binding capacity and effector activity after heating both with and without glucose. In contrast, model experiments involving thermal treatment of recombinant Ara h 2 with reducing sugars suggested that the Maillard reaction enhances IgE binding of Ara h 2 (Gruber et al. 2005). Because of their complexity, few attempts have been made to characterize specific roasting-induced protein modifications. Hebling et al. (2013) used a global proteomic screening approach to identify several AGEs in roasted peanuts, including carboxyethyllysine, carboxymethyllysine, and pyrraline. They observed that roasting resulted in the formation of high-MW, IgE-reactive complexes containing Ara h 1, Ara h 2, and Ara h 3. In the case of boiling, which takes place under high moisture, Maillard reactions are seemingly of minimal importance. The limited studies that have addressed boiling show that proteins do undergo modifications, including some unique aggregation, and boiling typically decreases the allergenicity of peanut proteins (Beyer et al. 2001, Blanc et al. 2011).

Labeling, Risk Analysis, and Detection of Food Allergens

As there is presently no cure for food allergies, strict avoidance of implicated foods and ingredients must be followed by allergic patients to prevent potential life-threatening allergic reactions (Gendel 2013). As such, food-allergic individuals rely heavily on accurate allergen labeling and ingredient disclosure to determine food choices. To help consumers with this effort, several countries have put forth legislation to govern allergen labeling. In 2004, the Food Allergen Labeling and Consumer Protection Act (FALCPA) was enacted in the United States to require food manufacturers to declare the source of ingredients derived from the Big-8 allergenic foods in an effort to remove hidden allergens in processed foods (FDA 2004). Additionally, in response to growing consumer concern over shared processing equipment and facilities, the food industry voluntarily includes allergen advisory statements on packaged foods (Hefle et al. 2007). Current US legislation, however, applies only to ingredients intentionally added to a food product; it does not define thresholds for food allergens or provide guidance for how the food industry should deal with cross-contact of allergens (Madsen et al. 2009). Consequently, there is intense international interest in developing new strategies and methodologies for risk assessment of allergenic foods

(Madsen et al. 2009, Food Standards Agency 2006, Threshold Working Group 2008). There is concern that the lack of defined thresholds and accurate testing methods coupled with the fear of litigation have resulted in advisory labels becoming too proliferative, and as a result, some allergic consumers could be ignoring them, thus putting themselves at risk for a life-threatening allergic reaction (Hefle et al. 2007). A study to determine the level of contamination in products containing advisory labels found that only 5 out of 112 products that contained peanut advisory labels actually contained detectable levels of peanut (Ford et al. 2010). Proliferative advisory labeling also places additional restrictions on the diet of allergic individuals who adhere to the labels, by preventing them from consuming a product that in all likelihood does not contain the allergen it advises against. Furthermore, food companies lose potential consumers when these labels are included on a product out of legal precaution. Although allergic consumers need to heed the warnings of advisory labels, this demonstrates that additional guidance is needed for allergen risk management in the food industry to provide consumers with accurate allergen labeling. Accordingly, an international study entitled Integrated Approaches to Food Allergen and Allergy Risk Management has been launched with the goal of establishing a standardized approach to allergen management for food manufacturers (Pendrous 2013).

The accuracy of allergen labeling also depends highly upon good manufacturing practices (GMPs) and an in-place Hazard Analysis and Critical Control Points (HACCP) program. Critical to an effective HACCP program is the availability of specific, sensitive, and cost-effective methods of allergen detection. There are currently a variety of detection methods used by the food industry to determine the presence of allergens in food products. Current methods target protein or DNA as indicators for the allergenic foods (van Hengel et al. 2006). Enzyme-linked immunosorbent assay (ELISA) is the most common technique because it can be high throughput and cost effective. ELISA targets individual proteins with protein-specific antibodies that produce a colorimetric response. ELISA kits targeting Ara h 1, Ara h 2, and total soluble peanut proteins are commercially available (Poms et al. 2005). Several limitations exist for the use of ELISA methods for accurate allergen detection and quantification, including inefficient protein extraction, cross-reactivity with matrix components, and lack of standard reference materials (Poms et al. 2004, 2005). Additionally, proteins undergo significant modifications during roasting, which affect both protein extractability and the effectiveness of ELISA kits targeted toward proteins from raw peanuts (Hebling et al. 2013). DNA-based methods use polymerase chain reaction to amplify specific fragments of peanut DNA; however, the presence/absence of DNA is not always an accurate indicator for the presence/absence of allergenic proteins (van Hengel 2007). Consequently, proteomics-based approaches have been evaluated recently for the simultaneous detection of trace-level contamination by multiple peanut allergens (Shefcheck et al. 2006, Chassaigne et al. 2007, Hebling et al. 2013). These approaches aim to improve protein extraction efficiency and identify peptides that can serve as allergen biomarkers for thermally modified peanut proteins, in an effort to improve allergen detection and quantification in complex food matrices (Hebling et al. 2013).

Allergenicity Considerations of Peanut Processing: Co- and By-Products

Edible peanut oil is the most common coproduct of the peanut industry, and in many places throughout the world, peanuts are grown primarily for oil production. Peanut-allergic individuals are often uncertain as to whether peanut oil will cause them to have an allergic reaction. The overwhelming majority of the scientific literature suggests that refined peanut oil does not pose a threat to peanut-allergic individuals (Crevel et al. 2000, Hidalgo & Zamora 2006, Hourihane et al. 1997a, Taylor et al. 1981). In an early study, peanut-sensitive patients fed 8 mL of peanut oil in capsules displayed no allergic reaction, suggesting that refined peanut oil does not pose a

threat to peanut-allergic individuals (Taylor et al. 1981). Additionally, Hourihane et al. (1997a) fed refined peanut oil to 60 individuals predisposed to peanut allergy in a controlled feeding challenge [double-blind, placebo-controlled (DBPC)], and none of the participants reacted to the refined peanut oil (up to 16 mL). Crude peanut oil, however, has been reported to elicit an allergic reaction in a small percentage of peanut-sensitive individuals (Crevel et al. 2000, Hidalgo & Zamora 2006, Hourihane et al. 1997a, Olszewski et al. 1998). Therefore, the refining process is critical for ensuring that enough protein is removed from the oil to render it essentially allergen free. For example, Crevel et al. (2000) reported the protein content of crude peanut oil to be approximately 190 μg/ml, and it was reduced during refining to a final protein content of ~2 μg/ml. Assuming that 100 μg of peanut protein is required to elicit a reaction from the most sensitive peanut-allergic individual, this person would have to consume approximately 50 mL of the oil that contained 2 μg/ml of protein to have an allergic reaction (Hourihane et al. 1997b, Crevel et al. 2000). Furthermore, FALCPA states that highly refined oils from the Big 8 are not major food allergens (FDA 2004).

Peanut skins (seed coats) are a major by-product of the peanut industry and have been recognized as a rich source of health-promoting polyphenolic compounds (Yu et al. 2006). As such, recent efforts have focused on identifying value-added applications for this readily available by-product (Constanza et al. 2012, Ma et al. 2013, Hathorn & Sanders 2012). However, because peanut skins are derived from peanuts and consist of approximately 18% protein (Constanza et al. 2012), they have the potential to elicit an allergic response in peanut-allergic individuals. White et al. (2013) used proteomics to compare the proteins present in peanut skins and seeds and found that peanut skins do contain many of the major allergenic peanut proteins. However, unless the proteins were isolated from the polyphenolic compounds, they did not bind peanut-specific IgE derived from peanut-allergic patients (White et al. 2013). This suggests that peanut skins have the potential to cause an allergic reaction in sensitive individuals, but the presence of polyphenolic compounds may attenuate an adverse response (Chung & Champagne 2009). However, further research is needed to fully understand this phenomenon.

EMERGING STRATEGIES TO MITIGATE PEANUT ALLERGY

Genetic Engineering to Reduce Allergenicity

Advances in plant biotechnology have greatly impacted food allergy research, as reviewed recently by Herman & Burks (2011). Genetic modification of peanuts (and other seeds, notably soybeans) has been proposed as a strategy to produce hypoallergenic seed by downregulating or silencing the genes coding for allergenic proteins, thus blocking the production of these proteins (**Figure 2**, stage 1) (Dodo et al. 2005). RNA interference (RNAi) was used to degrade mRNA derived from the peanut protein genes to produce transgenic peanut lines with suppressed expression of Ara h 2 and Ara h 6 (Chu et al. 2008). It is possible that suppression of one allergen may lead to unintended upregulation of other proteins, including other allergens; however, in this study, suppression of Ara h 2 and Ara h 6 did not affect expression of Ara h 1 or Ara h 3, and no differences in seed weight or germination data were observed (Chu et al. 2008). Additionally, there was concern that removal of Ara h 2 and Ara h 6, which are weak trypsin inhibitors, could increase the plant's susceptibility to fungal infection. However, transgenic lines showed no significant difference in *Aspergillus flavus* infection compared to nontransgenic controls in vitro, but additional studies need to be conducted to determine whether these findings translate to susceptibility in the field (Chu et al. 2008). RNAi was also used to produce transgenic peanut seeds that did not contain Ara h 2 and displayed decreased IgE-binding capacity (Dodo et al. 2008). However, individual-seed

analysis of the first transgenic generation revealed that often only one of the two seeds in a pod lacked Ara h 2.

Several limitations currently hinder the widespread use of genetic engineering to alleviate peanut allergenicity. The first consideration is that for the crop to be economically viable, genetic modification cannot sacrifice agronomic performance. As **Figure 2** shows, genetic modification of peanut affects all downstream stages, including agronomics, harvesting, handling, processing, utilization in food products, and most importantly, consumption. Many targeted allergenic proteins are required for normal plant function; thus it would not be feasible to remove all allergenic proteins (Singh & Bhalla 2008). However, removal of immunodominant allergens (such as Ara h 2) may not affect yield or growth, so the severity of allergic reactions may be reduced by eliminating them in transgenic peanuts without compromising agronomic performance (Singh & Bhalla 2008). Another consideration is that strict segregation of any seed with a specified trait(s), including hypoallergenic seed, is an extreme challenge for current infrastructure. Opportunities for cross-contamination are inevitable throughout production, including planting, harvesting, warehousing, processing, etc. To guarantee homogeneity in a hypoallergenic seed lot, the hypoallergenic trait would likely, in reality, have to be bred into all cultivars. Finally, as discussed previously, seed storage proteins make up a significant portion of the total peanut proteins, and their removal could be detrimental to both peanut nutrition (amino acid composition and availability) and development of the desirable roasted peanut flavor through the Maillard, and related, reactions. Although a highly promising strategy, these considerations are current economic and logistical barriers to the use of genetic engineering as a strategy to mitigate peanut allergy.

Novel Processing Strategies

Several novel processing strategies aiming to reduce the IgE-binding capacity of peanut proteins with the goal of reducing allergenicity have emerged in the literature (**Figure 2**, stage 3). Enzymatic treatment with digestive and commercial proteases has been investigated as a strategy to reduce the allergenicity of peanut proteins by hydrolyzing them to smaller peptides that no longer contain IgE-binding epitopes (Hong et al. 1999, Cabanillas et al. 2012b, Yu et al. 2011, Shi et al. 2013). Individual and sequential hydrolysis with the commercial proteases Alcalase and Flavourzyme decreased the IgE-binding capacity of roasted peanut protein extracts and decreased levels of Ara h 1, Ara h 2, and Ara h 3 (Cabanillas et al. 2012b). This was attributed to proteolysis of the whole proteins and breakdown of IgE-binding epitopes. However, our group has recently demonstrated that, although enzymatic hydrolysis reduces the IgE-binding capacity of peanut proteins, they retain the capacity to cross-link IgE in the basophil activation test, suggesting that they may not be hypoallergenic (Shi et al. 2013). Further work is warranted to understand the in vivo allergenicity of peanut protein hydrolysates.

Another strategy to reduce peanut allergy that is currently being investigated attempts to physically remove the allergenic proteins by precipitation. Researchers have used enzymes, such as peroxidase and polyphenol oxidase (PPO), or phenolic compounds, such as caffeic acid, to cross-link peanut proteins and render them insoluble (Chung et al. 2004, 2005). PPO and caffeic acid individually and in combination were applied to both raw and roasted peanut extracts, and competitive inhibition ELISA results revealed that all three treatments significantly reduced the IgE-binding capacity of peanut extract (Chung et al. 2005). Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot results indicated that both Ara h 1 and Ara h 2 were reduced, and this was attributed to the formation of insoluble, high-MW compounds containing dityrosine cross-links. Pulsed UV light processing has also been used to render peanut proteins insoluble and remove them from peanut extracts and liquid peanut butter (Chung et al. 2008).

Competitive inhibition ELISA revealed that, for both peanut extract and peanut butter, UV-treated samples displayed six- to sevenfold lower IgE binding than untreated controls, and Ara h 1 was particularly susceptible to the formation of insoluble aggregates (Chung et al. 2008). Autoclaving, which involves a combination of heat and pressure, has also been used to reduce the IgE-binding capacity and enhance the digestibility of allergenic peanut proteins (Cabanillas et al. 2012a). Circular dichroism of the autoclaved proteins suggested changes in the secondary structure of the proteins (Cabanillas et al. 2012a).

Each of the novel processing strategies discussed above involves either significant alterations to, or removal of, peanut proteins, which make up approximately 25% of the weight of a peanut; such changes would have significant implications for peanut flavor and nutrition. Furthermore, as outlined in **Figure 2**, stages 4 and 5, the potential ingredients produced by these processes would need to be incorporated into food products such that they would be acceptable to consumers. Also, none of the strategies have been shown to reduce allergen effector activity. All these concerns must be addressed if current novel processing strategies to mitigate peanut allergy are to be implemented. However, if a processing strategy were to reduce IgE binding but retain the capacity to stimulate T cells, it could be used to produce ingredients for immunotherapy, which is discussed next.

Immunotherapy

Immunotherapy for IgE-mediated allergies can effectively skew the immune response of an allergic individual, such that allergic symptoms no longer occur upon exposure to the allergen. This form of therapy is commonly injected subcutaneously and often referred to as allergy shots. Clinically, immunotherapy is often used in subjects with aeroallergen sensitivity (e.g., cat and dog dander, house dust mites, ragweed, or various pollens) or venom anaphylaxis (e.g., wasp allergy). However, there is no FDA-approved immunotherapy for food allergies.

In the 1990s, small studies were published describing results from clinical trials using subcutaneous immunotherapy (SCIT) with an aqueous extract of peanut proteins to treat peanut allergy (Oppenheimer et al. 1992, Nelson et al. 1997). The trials demonstrated improved clinical outcomes, but allergic side effects (including anaphylaxis) were common, and SCIT was ultimately deemed unsafe to continue larger trials. Currently, other routes of administering peanut immunotherapy are under investigation, and this section highlights the clinical trial outcomes from these allergen-specific studies. Additionally non-antigen-specific approaches, which aim to manipulate the allergic response to all allergens, have been tested in peanut-allergic subjects, and these therapeutic approaches are also discussed.

Antigen-specific approaches. Antigen-specific immunotherapy uses allergens from the source to which a patient is allergic. For example, a peanut-allergic patient would undergo antigen-specific immunotherapy with peanut proteins. An inherent risk to this approach is that a patient with a known peanut allergy is intentionally exposed to peanuts; thus, the amount of protein and the route of administration are critical to finding a balance between the risk of allergic side effects and the benefit of successful desensitization. The antigen-specific approaches currently under clinical investigation are oral, sublingual, and epicutaneous immunotherapy.

Oral immunotherapy. Oral immunotherapy (OIT) aims to deliver antigens (i.e., proteins that elicit allergic reactions) to the mucosal immune system via ingestion with absorption through the small intestine. The premise of OIT is that administration of low doses of antigen will not trigger severe reactions and can be carefully escalated over several months until the subject can tolerate large amounts of protein without adverse effects. Subjects reaching these larger doses are

considered desensitized. Typically, the active drug in peanut OIT studies is delivered in a daily dose of peanut flour (12% fat, light roast, 50% protein). OIT is administered in three phases: initial escalation, buildup, and maintenance. For example, subjects in a recent DBPC trial of peanut OIT were escalated from 0.1 mg to 6 mg of peanut protein on the initial escalation day (Varshney et al. 2011). Then, over the next approximately 10 months, these subjects underwent biweekly buildup before reaching a maintenance dose of 4,000 mg of peanut protein.

The first study to convincingly demonstrate the efficacy of OIT was reported in 2009, when Jones et al. 2009 described 29 subjects that had completed OIT and were ingesting 300 mg of peanut protein daily. Following OIT, these subjects underwent an oral food challenge (OFC), and 27 out of 29 (93%) subjects were able to consume the entire 3,900-mg protein challenge without allergic symptoms. A follow-up randomized, DBPC trial definitively demonstrated that peanut OIT can induce desensitization (Varshney et al. 2011). In this trial, subjects underwent a 5,000-mg OFC after 12 months of receiving either peanut flour (treatment) or oat flour (placebo). All 16 subjects receiving treatment were able to consume the entire 5,000-mg protein challenge without allergic symptoms, and no subjects receiving placebo were able to ingest 5,000 mg (median dose eliciting allergic symptoms: 280 mg). Similar desensitization effects of peanut OIT have also been shown by researchers in Germany (Blumchen et al. 2010) and England (Clark et al. 2009, Anagnostou et al. 2011).

Although these peanut OIT trials have demonstrated desensitization, the long-term outcomes following OIT remain unclear. Can permanent tolerance, which would allow subjects to stop daily ingestion of peanut while remaining unreactive to subsequent peanut ingestion, be induced? A small cohort has been tested for tolerance, which was defined as the ability to stop OIT for at least four weeks and remain unreactive to an OFC (Burks et al. 2012). Eleven of nineteen (58%) subjects were deemed tolerant. Though this outcome was promising, larger studies are needed to assess whether OIT must be a lifelong therapy or if it can be stopped without loss of allergic suppression.

Peanut OIT is immunomodulatory, and one of the major findings of the above studies is suppression of mast cell and basophil activation. Studies have shown a decrease in skin prick test (SPT) size in response to peanut antigens (Blumchen et al. 2010, Jones et al. 2009, Varshney et al. 2011), and a study of OIT effects on basophils also demonstrated hyporesponsiveness to peanut antigens ex vivo in whole blood (Thyagarajan et al. 2012). SPTs assess skin mast cells' ability to release histamine in response to peanut. The test is conducted by placing a drop of peanut extract on the skin and then using a needle-like device to scratch the skin, thus allowing peanut proteins to come in contact with mast cells. These studies indicate that mast cells and basophils release only small quantities of allergic mediators that cause allergic reactions upon exposure to peanut. Considering that the first event leading to activation of mast cells and basophils is the cross-linking of antigen-specific IgE, it is important to note that the suppression of effector cells is disconnected from the amount of peanut-specific IgE in the serum of these subjects. For example, all subjects receiving treatment in Varshney et al. (2011) passed the final OFC and showed suppressed SPT and basophil activation at the time of the challenge; however, peanut-IgE levels did not significantly decrease over this period. With more time receiving treatment, peanut-IgE levels do decrease significantly, which likely plays a role in tolerance (Burks et al. 2012), but this decrease is apparently not immediately necessary for desensitization. This disconnect may be explained in part by noninflammatory, blocking peanut-specific IgG and IgG4 antibodies shown to increase during OIT and successfully compete with IgE for the binding of peanut proteins (Jones et al. 2009). Additionally, experiments have shown that plasma from subjects undergoing OIT can successfully inhibit the activation of basophils from allergic subjects, and peanut-specific IgG in the plasma has been postulated as the mechanism (Burk et al. 2012).

Langerhans cells: dendritic cells found in skin and mucosa that take up antigen and present it in a recognizable form to other immune cells

Long-term effects of OIT, as with other types of allergen immunotherapy, are thought to target T cells. It has been shown that proallergic, Th2-type cytokines such as IL-4, IL-5, and IL-13 are decreased in cell cultures from OIT subjects (Blumchen et al. 2010, Varshney et al. 2011). Interestingly, regulatory or suppressive T cells, termed Tregs, increased over 12 months but returned to baseline levels thereafter.

The results of published studies of peanut OIT are encouraging for the prospects of developing a treatment for the millions of peanut-allergic children around the world. It has been repeated in multiple forums, however, that OIT is not ready for widespread use and is still an experimental treatment for food allergy (Thyagarajan et al. 2010).

Sublingual immunotherapy. Sublingual immunotherapy (SLIT) aims to deliver antigens to the oral mucosa by placing protein solutions under the tongue. It is believed that oral Langerhans cells take up the antigen in a tolerogenic manner to downregulate the allergic response. Typically, subjects are instructed to hold several drops of solution under their tongue for 1–2 min and then swallow. The active drug in peanut SLIT studies is delivered in an aqueous extract of peanut proteins combined with 50% glycerol saline solution preserved with 0.2% phenol. Similar to OIT, subjects begin at a low dose and undergo buildup until a maintenance dose, but all doses in SLIT are several orders of magnitude smaller than in OIT.

The first peanut SLIT study was reported in 2011 (Kim et al. 2011). In this randomized controlled trial (RCT), children ages 1 to 11 were escalated to 250 ng of peanut protein (or an equivalent volume of placebo) on the initial escalation day and underwent 6 months of biweekly buildup to a maintenance dose of 2 mg. After 12 months of peanut SLIT or placebo, subjects underwent a 2,500-mg OFC. The median tolerated dose for the peanut SLIT subjects was 1,710 mg of peanut protein, which was significantly different than the 85-mg median tolerated dose for the placebo subjects. It is important to note that, of the subjects undergoing peanut SLIT, not all tolerated more protein than the placebo group during the OFC. However, it is very encouraging that this significant desensitization effect could be demonstrated after only 12 months of low-dose SLIT.

A second, much larger RCT evaluating peanut SLIT was recently reported by the Consortium of Food Allergy Research (Fleischer et al. 2013a). At five different centers, subjects ages 12–17 (median 15 years old) were tested at baseline for eliciting doses of peanut through OFC and placed on peanut SLIT or placebo. By 44 weeks, 14 of 20 (70%) subjects undergoing treatment had a substantial increase in the eliciting-dose threshold compared to only 3 of 20 (15%) subjects in the placebo group. These outcomes demonstrate that SLIT can effectively desensitize peanut-allergic subjects.

Epicutaneous immunotherapy. Epicutaneous immunotherapy (EPIT) is a relatively new modality in allergen immunotherapy. This technology uses spray-dried allergens on a membrane applied to a subject's skin. Once on the skin, naturally produced moisture should solubilize the antigens for uptake by skin Langerhans cells (Mondoulet et al. 2010). One possible advantage of EPIT is the safety aspect. Though still in development, it should be well tolerated, with only local inflammation, devoid of systemic allergic side effects sometimes seen in OIT or SLIT.

So far, mouse models of peanut allergy have been used to evaluate EPIT and have shown that the therapy downregulated proallergic cytokine responses and modulated the IgE/IgG ratio (Mondoulet et al. 2010). In humans, small EPIT trials in milk-allergic and grass pollen–allergic subjects (Dupont et al. 2010; Senti et al. 2011, 2012) have inspired clinical trials of EPIT for peanut allergy, which will begin in the near future (DBV Technologies). Though EPIT has just begun to be investigated, it is exciting that desensitization through the skin may be a possible therapeutic.

Non antigen–specific approaches. Non antigen–specific therapies for food allergy do not involve the administration of the allergens that would cause an allergic reaction. For example, a peanut allergic patient would not undergo therapy with peanut proteins but would instead receive therapy to broadly neutralize the ongoing allergic disease. A potential benefit of this approach is that patients with allergies to several foods could be treated for multiple allergies with a single treatment course.

Anti-IgE therapy. Because IgE is critical in the allergic manifestation of food allergy, it was hypothesized that the removal of circulating IgE would decrease or eliminate reaction to food allergens. A monoclonal antibody against human IgE, TNX-901, was developed, and after "humanizing" the antibody, it was tested for efficacy in human subjects (Leung et al. 2003). The trial demonstrated that, at the highest dose tested, subjects increased from tolerating 178 mg of peanut protein at baseline to an eliciting dose of 2,805 mg after anti-IgE therapy.

A second anti-IgE therapy for peanut allergy trials was conducted using the antihuman IgE monoclonal Xolair (omalizumab), which is FDA approved for the treatment of asthma (Sampson et al. 2011). This trial showed evidence of efficacy but was terminated early, so only a limited number of subjects were evaluated. Even if shown to be effective, anti-IgE therapy may be impractical due to the likely necessity of expensive, lifelong injections to continually deplete the circulating IgE.

Another potential use of anti-IgE therapy is in combination with other forms of allergen immunotherapy, such as OIT. The aim of such an approach is to decrease the initial side effects of OIT (Hofmann et al. 2009), allowing for more rapid dose escalation before beginning conventional OIT. In a study testing this combination, 9 of 10 milk-allergic subjects pretreated with Xolair for several weeks before beginning milk OIT were able to tolerate 1,992 mg of milk protein during the initial escalation day (Nadeau et al. 2011), a level much higher than others reported with conventional milk OIT (Skripak et al. 2008). A small study in peanut-allergic subjects also demonstrated an increase in tolerated peanut doses on initial escalation in subjects pretreated with Xolair (Henson et al. 2012).

Immunomodulatory phytochemicals. Many studies have shown the impact of phytochemicals (small molecules from plants) on immune cells relevant to allergic disease (Singh et al. 2011). These findings have led to investigation into phytochemicals as a non-antigen-specific food allergy treatment. In 2001, a group of researchers working with traditional Chinese medicine for asthma developed the food allergy herbal formula-1 (FAHF-1), a mixture containing 11 herbal extracts that had possible therapeutic implications for peanut allergy. This formula was shown to prevent allergic reactions in peanut-allergic mice challenged immediately after seven weeks of treatment with FAHF-1 (Li et al. 2001). Next, the researchers developed FAHF-2, which contains nine herbal extracts. Mice treated with this formula had decreased peanut-specific IgE; increased peanut-specific IgG2a; directly suppressed mast cells and basophils; and decreased proallergic, Th2-type responses from cultured cells (Srivastava et al. 2005). FAHF-2-treated mice also did not react to oral challenges given by gavage up to five weeks after finishing treatment with the formula, whereas mice given placebo experienced severe anaphylaxis. Since these studies, this non-antigen-specific formula has also been used in mice to treat other food allergies such as egg and fish (Srivastava et al. 2012).

An extended phase I study of FAHF-2 showing safe and well-tolerated dosing has been conducted in humans (Patil et al. 2011). Food-allergic subjects were dosed with six tablets of FAHF-2 (or placebo) three times a day for six months, and suppressed basophil activation was shown in subjects receiving treatment. Because the consumption of 18 pills per day may be difficult for

some patients, especially children, the research group is working to decrease the active therapeutic dosage using improved extraction methods (Srivastava et al. 2011). The efficacy of FAHF-2 in humans has not been reported.

CONCLUSIONS

Over the past 25 years, peanut and other food allergies have emerged from being highly uncommon and essentially unknown to the general public to now affecting conservatively more than 1% of the US population; this is fairly representative of many other countries as well. Food allergy is a dangerous condition for those affected and it detracts significantly from quality of life. Fortunately, there has been an explosion in research and technology development to understand and mitigate peanut and other food allergies. The causative agents of peanut allergy, largely the seed storage proteins, are now fairly well characterized on a structural level using many of the most advanced analytical tools available. Additionally, recent work has focused on understanding various thermal processing–induced protein modifications. From an immunology perspective, relevant biochemical mechanisms responsible for allergic reactions have been an area of intensive study. For the food industry, the emergence of food allergy has significantly impacted production and utilization chains with an emphasis on labeling, HACCP, GMPs, and risk management. Clear and consistent legislation, policies, practices, etc., are needed for the consumer and producer alike to mitigate food allergy, and these continue to emerge. Of the different mitigation strategies being practiced and/or considered for peanut allergy, immunotherapy is of special interest as it targets the source of the problem, adverse immune responses, and therefore minimizes unintended effects on peanut production and utilization. Current immunotherapy trials, including OIT, are being conducted on relatively small patient sets but are showing excellent promise. Clinical trials are currently underway and larger trials are expected to begin soon. However, additional research studies are needed, and FDA approval will be required before any immunotherapy can be used as treatment.

SUMMARY POINTS

1. Exponential population growth and subsequent increased demand for protein; the importance of legumes in cropping systems; and the excellent nutritional properties, flavor, shelf life, and affordability of peanuts all highlight their importance worldwide.
2. The emergence of peanut allergy over the past 25 years has significantly impacted both those living with the allergy as well as the peanut and related food industries.
3. Thermal processing induces structural modifications to peanut proteins that have important implications for allergenicity and allergen detection.
4. Various strategies aimed at mitigating peanut allergy, including genetic engineering to reduce/silence expression of peanut allergens, novel processing to reduce IgE binding of peanut proteins, and immunotherapy to modulate the immune system of peanut-allergic individuals, have emerged.
5. For any mitigation strategy to be effective, it must consider all stages of peanut production and minimize any unintended consequences to agronomic performance, processing/handling, utilization, or consumer acceptability.
6. Various immunotherapy strategies, including OIT, to induce tolerance in peanut-allergic individuals are showing excellent promise for peanut allergy mitigation.

FUTURE ISSUES

1. Although several interesting hypotheses are being explored, the cause for the rapid emergence of peanut and other food allergies remains unclear. It is likely not one particular factor but a confluence of factors. Regardless, such an understanding is critical to ultimately preventing this condition in the future.
2. Obtaining more detailed structural characterizations of peanut proteins (and other food allergens) in their processed format, i.e., after various thermal processes used to prepare peanut-based foods, is quite challenging but ultimately critical to understand the allergenic properties of peanut.
3. Current efforts aimed at developing immunotherapy strategies are showing excellent promise, but additional research studies are needed, and FDA approval will be required before any immunotherapy can be used as treatment.

DISCLOSURE STATEMENT

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