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Rapid identification of hyper-allergenic and hypo-allergenic properties in modified and processed peanut products through different mechanisms and methods

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Abstract

Peanuts contain a variety of proteins, with Ara h 1, Ara h 2 and 6, and Ara h 3 identified as major allergens responsible for triggering allergic reactions in most individuals. Their allergenicity arises from three primary factors: (1) Structural Stability, these proteins possess highly stable molecular structures, making them resistant to degradation by digestive enzymes, thus increasing their likelihood of entering the immune system intact and eliciting an immune response. (2) High protein content, the relatively high concentrations of Ara h 1, Ara h 2 and 6, and Ara h 3 in peanuts elevate the chances of human exposure. (3) Strong allergenicity, these proteins exhibit a high binding affinity to immunoglobulin E (IgE) antibodies, rapidly initiating allergic reactions upon entry into the body. Advancements in the rapid detection of peanut allergens and allergic reactions have seen significant progress in recent years. This review evaluates the hypoallergenic and hyper allergenic properties of modified and processed peanut products, along with innovative detection methods for applications in healthcare, clinical settings, and the food processing industry.

1. Introduction

Food allergy and oral tolerance are history questions unsolved even though we are understanding more and more as immunology and molecular biology make progress. The concepts of food allergy and oral tolerance are part of the complex interaction between the immune system and the environment, particularly dietary antigens. These mechanisms may indeed have evolutionary roots that predate mammalian life. For the protection or survival each species in the evolution may be unique, but the immune system has been ubiquitous, this is because the immune system is very accurate and large enough to cope and develop. The variant chains have been sure enough to build up 10^{12} of antibodies to track epitopes of different proteins once the encounter most frequently orally. There are some other mechanisms beside immune protection, one of the recent discovery is probably aryl hydrocarbon receptor (AHR), which recognize foreign molecules by different rate of binding affinity, and then the foreign molecules in cytosol will be docked to AHR, and then activate the transcription and translation as a transcriptor. One of well know examples is the dioxin binding with AHR because the mutation from alanine to Valine of AHR (**Fig. 1**) led the affinity of dioxin to AHR reduced 20 folds, so make much less toxicity to human around 20000 years ago, while Neanderthalesis remained the same amino acid, one of the hypothesis is that this was on the reaons led to the disappearance of Neanderthalesis.

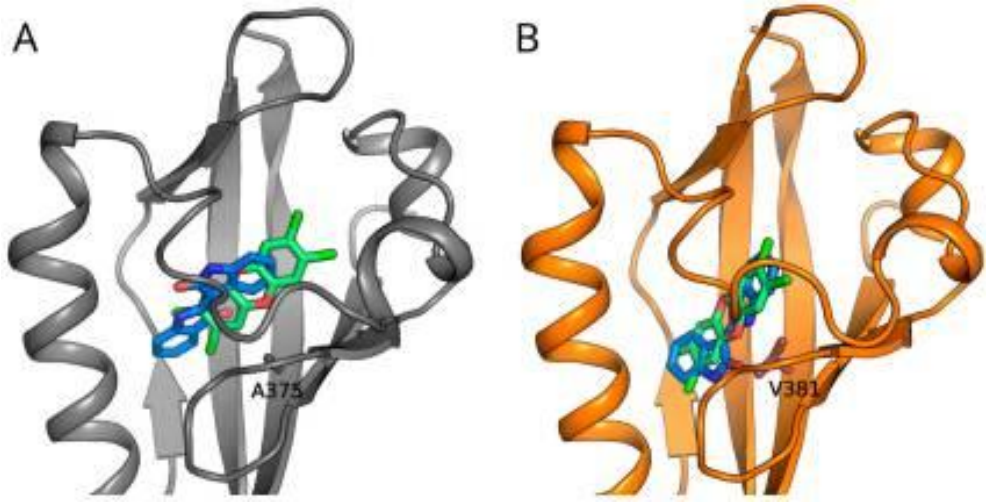

Fig. 1, Binding geometries of TCDD (light green) and indirubin (marine blue) predicted by molecular docking to the AhR PASB LBD homology models. (A) mAhR in grey and (B) hAhR in orange.

The non conserved internal residues A375/V381 are shown as sticks.[1]

In addition to evolution in the direction of reducing environmental toxicity, as far as we know that human has successfully developed a mechanism of oral tolerance, which reduce a large number of food allergies, through cyclooxygenase (COX)-2 dependent prostaglandins (PGs), this is largely because those PGs inhibit a number of cytokines that promote the inflammation while food antigens to the antigen presenting cells (APC) to the T-cells (**Fig. 2**)

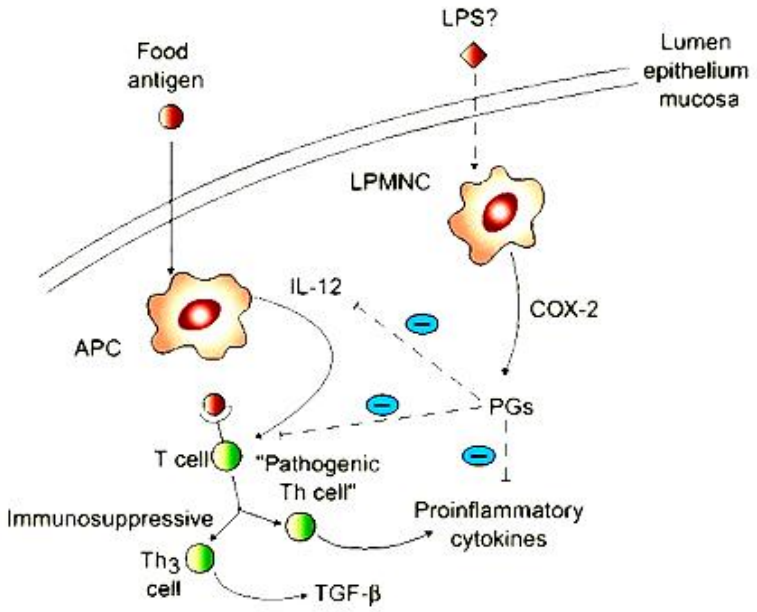

Fig. 2, COX-2 dependent prostaglandins promote oral tolerance.

Food Antigen: Represented by a red circle, it interacts with an Antigen-Presenting Cell (APC: shown as a cell with a nucleus, it presents the food antigen to a T cell). T Cell: Depicted as a cell receiving signals from the APC, it can differentiate into a "Pathogenic Th cell" or an immunosuppressive Th3 cell. "Pathogenic Th Cell": involved in the proinflammatory response. Th3 Cell: An immunosuppressive cell that produces TGF-β (Transforming Growth Factor-beta). TGF-β: A cytokine produced by Th3 cells, shown as an arrow pointing back to the Th3 cell. LPMNC (Lamina Propria Mononuclear Cell): Another immune cell type in the mucosa, potentially activated by LPS (Lipopolysaccharide). LPS: lipopolysaccharide shown as a diamond shape, frequently causes inflammation, or COX-2 inducer. PGs (Prostaglandins): Molecules that promote the production of proinflammatory cytokines. Proinflammatory Cytokines: Shown as blue circles, these molecules are involved in the inflammatory response. IL-12 (Interleukin-12): A cytokine produced by APCs, promoting the differentiation of T cells into "Pathogenic Th cells".[2]

2. **Immune mechanism of food allergy**

Food allergies are because of the food components with a protein specific or polysaccharide specific to their 3-dimensional unique structures. It is frequently multiple amino acids or polysaccharides that make the conformational epitopes which eventually select the antibodies (the majority, around 90%) from B cells, in turn these antibodies will neutralize foreign antigens when they encounter. Of course, linear epitopes can sometimes be part of larger conformational epitopes. As matter as facts the relationship between linear and conformational epitopes is not always straightforward, and their interplay can influence antibody binding in certain situations. In addition, food antigens can also trigger autoimmunity diseases, which may cause uncured chronic disease. It is also very difficult to detect these antigens. Peanuts contain a variety of proteins, with Ara h 1, Ara h 2, and Ara h 3 identified as major allergens responsible for triggering allergic reactions in most individuals. Their allergenicity arises from three primary factors: (1) Structural Stability: These proteins possess highly stable molecular structures, making them resistant to degradation by digestive enzymes, thus increasing their likelihood of entering the immune system intact and eliciting an immune response. (2) High Protein Content: The relatively high concentrations of Ara h 1, Ara h 2, and Ara h 3 in peanuts elevate the chances of human exposure. (3) Strong Allergenicity: These proteins exhibit a high binding affinity to immunoglobulin E (IgE) antibodies, rapidly initiating allergic reactions upon entry into the body[3].

In the food allergy, T cell epitopes or the specific peptide fragments presented by antigen-presenting cells (APCs) after processing antigens can be involved in certain allergic reaction. These peptides bind to major histocompatibility complex (MHC) molecules to form a pMHC complex, which is displayed on the surface of APCs. The pMHC complex is then recognized by T cell receptors (TCRs), triggering a T cell immune response. T cell epitopes are always linear, meaning they are continuous amino acid sequences and cannot bind to IgE antibodies. However, large evidences have been shown that CD4+ T cells play a crucial role in initiating and maintaining food allergies. When exogenous antigens (like food allergens) are processed by APCs and presented via MHC II molecules, they activate CD4+ T cells, which can differentiate into various subsets, including Th2 cells. Th2 cells secrete key cytokines such as:IL-4 and IL-13, which promote IgE production. IL-5, which recruits and activates eosinophils. Th2-driven immune responses are central to the pathogenesis of allergic reactions, including food allergies[4].

3. Food allergy and Food borne allergens

Food allergies can cause different symptom of diseases, depending on individuals, an allergen reaction can occur within minutes or hours, and symptoms can range from mild to life-threatening. The leading causes of food allergies identified are milk, eggs, fish, shellfish, tree nuts, peanuts, wheat, soybeans, and sesame. Peanuts are one of the leading sources of allergens in Europe and Asia, the allergy symptoms can range from mild (rash, itching) to severe (anaphylaxis, which can be life-threatening) and can affect various parts of the body. Here are some common symptoms: skin reactions: hives or itchy rash, eczema, swelling of the lips, tongue, throat, or face, also possible to respiratory Symptoms: coughing, wheezing, difficulty breathing, runny nose, sneezing, and gastrointestinal symptoms: nausea, vomiting, diarrhea, abdominal cramps, also possible dizziness, fainting, anxiety. In severe cases, a peanut allergy can trigger a life-threatening condition called anaphylaxis. However, foodborne allergen is a specific allergen found in food that triggers these allergic reactions. It is a type of foodborne hazard but different from foodborne illnesses caused by bacteria, viruses, or toxins. In essence, it refers to the actual allergenic protein present in food that causes the immune response in sensitive individuals (**Table 1**).

Table 1, the key differences of food allergy and foodborne allergen：

Aspect	Food Allergy	Foodborne Allergen
Definition	Immune reaction to a specific food protein	The allergenic protein in food causes allergy
Cause	Body’s immune response	Allergenic substance in food (e.g., peanuts)
Symptoms	Hives, swelling, anaphylaxis	Only in allergic individuals
Examples	Peanut allergy, shellfish allergy	Peanut protein, gluten (for celiac disease)

4. Peanut allergen and detection

When individuals are allergic to peanuts first come into contact with these proteins, the immune system will produce specific IgE antibodies, but usually will not cause allergic reactions, however, when exposed with peanut protein again, IgE antibodies will quickly recognize and bind to these proteins, activating the immune system to release a large amount of chemicals such as histamine.

These chemicals can cause a series of reactions such as vasodilation and smooth muscle contraction, leading to allergic symptoms such as skin itching, urticaria, difficulty breathing, and even anaphylactic shock. Differences in sensitization of different allergens Ara h 2: Studies have shown that Ara h 2 is one of the strongest allergens in peanuts. More than 95% of peanut allergy patients have specific IgE antibodies to Ara h 2 and 6 (**Fig.3**). Ara h 1: Ara h 1 is also highly allergenic, but slightly lower than Ara h 2. About 50% of peanut allergy patients react to Ara h 3.

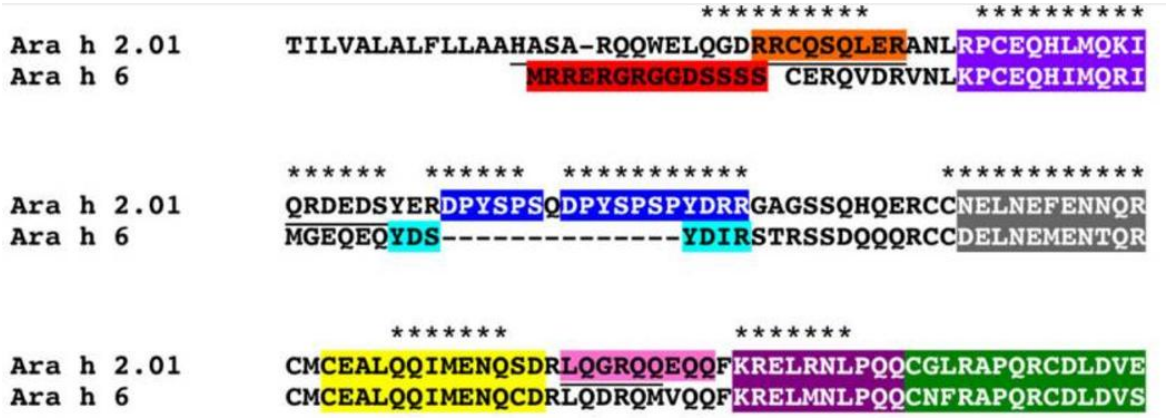

Fig. 3, Ara h 2 and Ara h 6 share homologous linear epitopes, adapted from [5].

IgE is the largest among the immunoglobulins in terms of molecular weight, the size makes it significant for binding to receptors on mast cells and basophils, triggering histamine release during allergic responses, that is why peanuts allergy is potency of allergens: Certain peanut proteins can bind to IgE even more effectively, some can maintain their allergenicity even after cooking. Peanut allergens can sometime mimic other allergens, exacerbating immune responses. (**Fig. 4**). Ara h 1, Ara h 2 and 6, and Ara h 3 are the main allergens in peanuts. A deep understanding of their structure, function, and sensitization mechanism is of great significance for the prevention and treatment of peanut allergies.

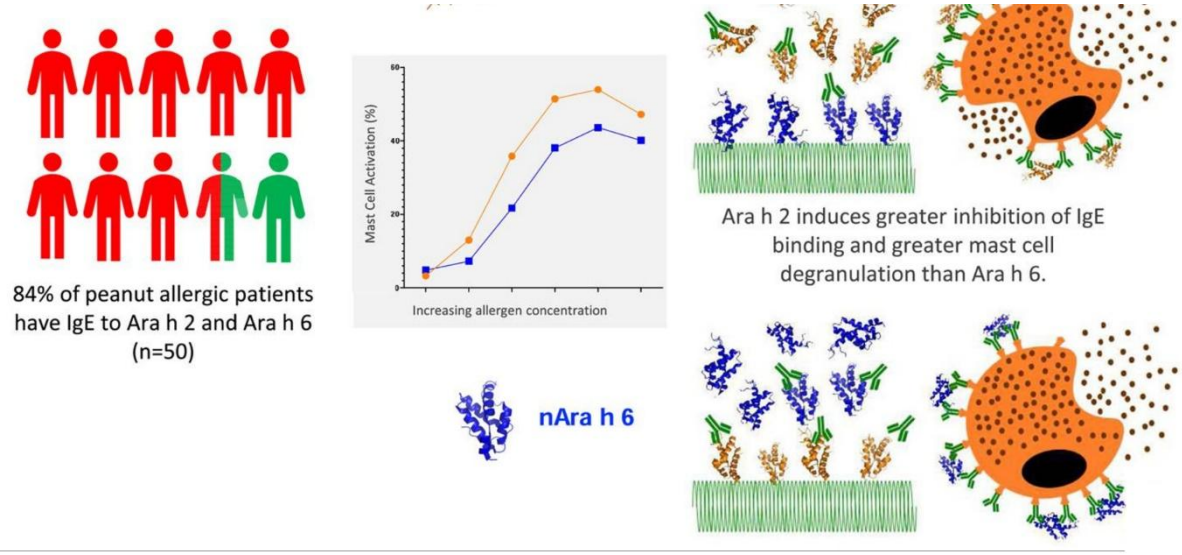

Fig. 4, Peanut allergy is a prominent IgE-mediated food allergy that has garnered substantial clinical attention owing to the severity of reactions and the increasing prevalence and rates of anaphylaxis. Adapted from [6].

5. Development of hypoallergenic or non-allergenic peanut products

The food industry has been using different approaches to prepare hypoallergenic or non-allergenic peanuts products, most frequently and effectively using heat or enzymes to denature proteins or reduce their allergenicity. Boiling peanuts or enzymatic hydrolysis, or high-pressure processing (HPP), pulsed light, or irradiation to alter allergen structures, some of them have shown some promise in term of economical consideration as well. Some companies have developed peanut butters with reduced allergen content through selective processing [7]. In 2023, RNA interference (RNAi) was used to knock down allergen expression in peanut, which silenced immunodominant allergen Ara h 2 and led to its significant reduction and a decrease in peanut allergenicity [8].

6. Accurate testing and precise diagnosis of peanut allergens

By testing the level of IgE antibodies to different allergens, peanut allergies can be tested in food processing industry and diagnosed more accurately in clinic. For trace levels of peanut proteins, or peanuts allergens have been tested by using biosensors and Lab-on-a-Chip Technologies or antibodies or molecularly imprinted polymers. Some portable devices, such as the Nima Peanut Sensor provide on-the-spot testing for peanut allergens within minutes (**Fig. 5**).

Fig. 5, Nima, or Nima peanut sensor is based on immune method of ELISA, *a food sample can be placed in a one-time-use capsule, which is inserted into the Nima sensor.* Once the test begins, the food is mixed with proprietary antibodies and analyzed by the sensor in under 5 minutes. Nima delivers a binary result for the sample tested for gluten and peanut. In addition to antibody based immune assay, some biomarker testing, mainly blood tests assess histamine or tryptase levels to confirm allergic reactions. For large samples for instance in the case of school, large samples may be analyzed by high-throughput screening, such as AKITA device [9] . Artificial Intelligence (AI)-driven applications including the images-based motion and emotional recognition to analyze the allergic symptoms have also been established [10].

7. Accurate testing and precise diagnosis of peanut allergens

Near-Infrared (NIR) spectroscopy is a non-destructive technique that has shown significant potential for rapid and accurate detection of food allergens, including peanuts. By analyzing the interaction of near-infrared light with a sample, NIR spectroscopy can identify specific molecular vibrations associated with different compounds, including proteins. Traditionally NIR spectroscopy needs a complicated sample procedure, so transmission spectral can be collected after NIR pass through the sample. However, today the linear variable filter replace the traditional prisma and attenuated total reflectance (ATR) replaces the transmission, such portable NIR spectrometer can be used to analysis the antigens in real time in a non invasive way (**Fig. 6**) because each molecule, or each scan of a single molecule or a mixture will generate a specific spectrum, which can be used for comparison locally or remotely in databases.

Fig. 6, The desktop NIR spectroscopy needs to prepare the sample into a compressor, the spectrals are collected after the transmission of NIR (left). The portable NIR spectrometer is based on the modern linear variety filter (LVF) and ATR technology, which can directly scan the same and the NIR spectrals can be analyzed locally and remotely.

Conclusions, outlook and challenges

Recent years have seen significant advancements in the rapid detection of peanut allergens and allergic reactions. Genetically modified peanuts that are allergen-free have also emerged in several plant physiology laboratories, prompting further research on harmfulness testing. However, the complete removal of peanut allergens is quite challenging, and the allergen dose can vary greatly, so caution is still advised.

References

[1] Faber S.C. et al 2018, Int. J. Mol. Sci. 2018, 19(9), 2692; https://doi.org/10.3390/ijms19092692)
[2] Morteau, O. COX-2: promoting tolerance. Nat Med 5, 867–868 (1999). https://doi.org/10.1038/11301
[3] Becker WM et al Peanut allergens: new consolidated findings on structure, characteristics, and allergome Allergol Select. 2018 Sep 1;2(1):67–79. doi: 10.5414/ALX01418E
[4] Koenig JFE, Bruton K, Phelps A, Grydziuszko E, Jiménez-Saiz R, Jordana (M. Memory Generation and Re-Activation in Food Allergy. Immunotargets Ther. 2021 Jun 9;10:171-184. doi: 10.2147/ITT.S284823.

[5] Otsu K, Guo R, Dreskin SC. Epitope analysis of Ara h 2 and Ara h 6: characteristic patterns of IgE-binding fingerprints among individuals with similar clinical histories. Clin Exp Allergy. 2015 Feb;45(2):471-84. doi: 10.1111/cea.12407
[6] Hemmings O, Du Toit G, Radulovic S, Lack G, Santos AF. Ara h 2 is the dominant peanut allergen despite similarities with Ara h 6. J Allergy Clin Immunol. 2020 Sep;146(3):621-630.e5. doi: 10.1016/j.jaci.2020.03.026
[7] Jianmei Yu, Nona Mikiashvili, Effectiveness of different proteases in reducing allergen content and IgE-binding of raw peanuts, Food Chemistry, 307:125565, 2020, https://doi.org/10.1016/j.foodchem.2019.125565
[8] Dodo HW, Konan KN, Chen FC, Egnin M, Viquez OM. Alleviating peanut allergy using genetic engineering: the silencing of the immunodominant allergen Ara h 2 leads to its significant reduction and a decrease in peanut allergenicity. Plant Biotechnol J. 2008 Feb;6(2):135-45. doi: 10.1111/j.1467-7652.2007.00292.x.)
[9] Nguyen HT, Rissanen SL, Peltokangas M, Laakkonen T, Kettunen J, Barthod L, Sivakumar R, Palojärvi A, Junttila P, Talvitie J, Bassis M, Nickels SL, Kalvala S, Ilina P, Tammela P, Lehtonen S, Schwamborn JC, Mosser S, Singh P. Highly scalable and standardized organ-on-chip platform with TEER for biological barrier modeling. Tissue Barriers. 2024 Oct;12(4):2315702. doi: 10.1080/21688370.2024.2315702.
[10] Haoyu Chen, Xin Liu, Xiaobai Li, Henglin Shi, and Guoying Zhao. 2019. Analyze spontaneous gestures for emotional stress state recognition: A micro-gesture dataset and analysis with deep learning. Proceedings of the 14th IEEE International Conference on Automatic Face and Gesture Recognition