Rare, medium, or well done? The effect of heating and food matrix on food protein allergenicity

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Purpose of review

To review recent advances in the area of food allergen processing and the effect on protein allergenicity.

Recent findings

Heating generally decreases protein allergenicity by destroying conformational epitopes. In peanut and shrimp, heat-induced Maillard reaction (glycation) may increase allergenicity. The majority of milk and egg-allergic children tolerate extensively heated (baked with wheat matrix) milk and egg. Introduction of extensively heated milk and egg proteins is associated with decreasing sizes of skin prick test wheals and increasing serum food-specific IgG4 levels.

Summary

Heating and other methods of food processing have different effects on food allergens, even those contained in the same complex food. Structural homology does not reliably predict the effect of processing on allergenicity, and individual food allergens have to be tested. Interactions with other proteins, fat, and carbohydrates in the food matrix are complex and poorly understood. Introduction of extensively heated milk and egg proteins into the diet of allergic children may represent an alternative approach to oral tolerance induction. Better characterization of these aspects of food allergy is critical for elucidation of food protein interactions with the gut-associated lymphoid tissue, the ability to induce IgE sensitization, the potential to trigger hypersensitivity reactions, and different clinical phenotypes of food allergy with regard to severity and persistence.

Keywords

allergenicity, conformational epitopes, egg allergy, food processing, milk allergy, sequential epitopes

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Introduction

It has been long recognized that the allergenicity of food proteins can be altered by food processing [1–6]. Thermal processing may involve dry or moist heat, whereas nonthermal processing includes germination, fermentation, proteolysis, ultrafiltration, storage, mechanical and enzymatic issue disintegration, pulping, peeling, mashing, and pasteurization treatment [7••,8]. The Maillard reaction, enzymatic browning or roasting, and all dry heat processes are most capable of modifying allergenicity of food proteins. Food processing can decrease protein allergenicity in several ways including destruction of predominantly conformational epitopes, with limited effect on sequential epitopes, and chemical reactions between proteins and fat and sugars in the food matrix that account for limited availability of protein to the immune system. Food processing might potentially increase protein allergenicity by formation of neoepitopes and by the effect of food matrix leading to decreased protein digestibility in the stomach and preservation of allergenic epitopes for interactions with the immune system in the intestine (Table 1). In general, the so-called classic, type 1 or complete food allergens (e.g. in cow's milk, egg white, peanut, and soybean) that have the capacity to induce IgE sensitization via the mucosa of the gastrointestinal tract are heat-stable and acid-stable, water-soluble glycoproteins ranging in size from 10 to 70 kDa. The class 1 food allergens (e.g. ovomucoid, Gal d 1 in egg white, or Ara h 2 in peanut) are less readily affected by food processing, although recent findings underscore the importance of conformational epitope modification in cow's milk and egg allergy. In contrast, type 2 (incomplete) food allergens are postulated to lack the capacity to induce IgE sensitization via the gastrointestinal tract exposures due to their susceptibility to thermal processing and gastric digestion. These proteins are believed to elicit symptoms only after primary sensitization with cross-reactive inhalant allergens and are referred to as 'nonsensitizing elicitors'. The classic examples are birch tree pollen allergen Bet v 1 cross-reactive proteins in apple (Mal d 1) and carrot (Dau c) that

Table 1 Effect of high temperature and food matrix on the common food allergens

Food	High temperature and/or matrix effect	Clinical implications
Beef	BSA and γ-globulin were heat labile, whereas six other protein fractions persisted after heating the beef extract for 2 h at 85°C [2]. Industrial homogenization and freeze-drying appear to eliminate allergenicity of beef more effectively than home cooking [6,8].	Reactivity to heat-labile proteins may explain why some patients tolerate well cooked beef but react to not well cooked beef. Patients with IgE antibodies to heat-labile beef proteins may not need to maintain a complete beef elimination diet.
Cow's milk	Casein is more resistant to heating than whey protein fractions. β-lactoglobulin forms disulfide bonds with other proteins in the food matrix resulting in decreased availability and decreased allergenicity.	Up to 70% of milk-allergic children tolerate milk baked with wheat matrix (muffin, waffle). Introduction of baked milk into diet was associated with decreasing milk skin prick test wheal size and increasing serum casein IgG4 antibody levels. Baked milk diet may represent a safer approach to oral tolerance induction.
Egg white	Ovomucoid is heat resistant, whereas ovalbumin is heat labile. Ovomucoid polymerizes and forms high-molecular weight complexes with gluten in baked foods.	Up to 70% of egg-allergic children tolerated egg baked with wheat matrix. Introduction of baked egg into diet was associated with decreasing egg white skin prick test wheal size and increasing serum ovomucoid and ovalbumin IgG4 antibody levels.
Fish	Codfish parvalbumin (Gad c 1) is very heat-stable. Canned tuna and salmon have significantly decreased IgE-binding capacity [3].	Some fish-allergic patients may tolerate industrially processed canned tuna and salmon.
Peanut	Dry roasting results in glycation and formation of Ara h 2 aggregates of increased allergenicity.	Dry roasting augments allergenic potential of peanut in contrast to boiling and frying. Dry-roasted peanut products prevalent in the westernized diet may be in part responsible for a greater rate of peanut allergy in westernized societies compared with Asian societies that have comparable peanut consumption but in the form of boiled, fried peanut, or peanut oil.
Shellfish	Boiling may result in the Maillard reaction (glycation) and formation of the neoepitopes; in some patients, boiled shrimp extract induced larger skin test responses than raw shrimp extract.	Frequently reported reactions following inhalational exposures to boiled/steamed shellfish may be explained by stability of shellfish allergens and by formation of neoantigens.
Soybean	Most soy proteins appear rather resistant to heating (boiling). Gly m 4, a Bet v 1 cross-reactive protein, is heat-stable.	Anecdotal evidence suggests that food matrix may decrease soybean allergenicity.
Wheat	α-amylase/trypsin inhibitors and gliadins are heat-stable.	Most of the allergic reactions are triggered by baked and extensively cooked wheat products. Baking may potentially decrease the exposure to wheat proteins in the carbohydrate matrix and results in resistance to digestion and increased allergenicity.
Apple	Birch Bet v 1 and Bet v 2 cross-reactive proteins, Mal d 1 and Mal d 2 are exquisitely heat sensitive.	Most patients with pollen–food allergy syndrome tolerate cooked apple products (pasteurized juice, apple sauce, and apple pie).

in the uncooked form cause immediate oral symptoms but following heating are readily tolerated [5].

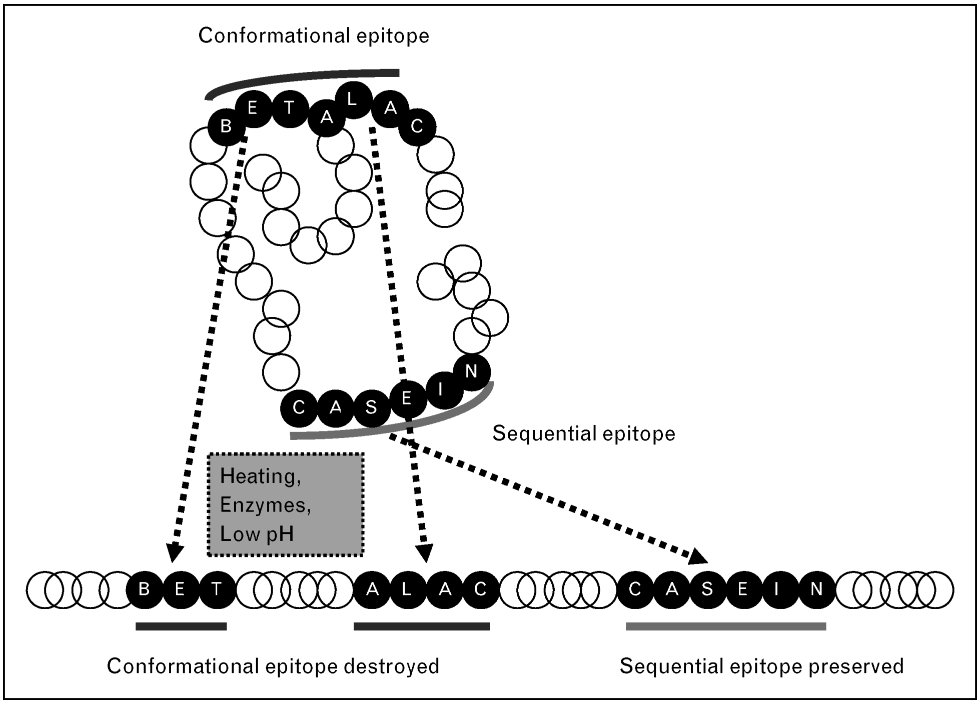
IgE-binding epitopes: conformational versus sequential epitopes

IgE antibodies produced by B cells may be directed at sequential epitopes comprising sequential amino acids, or conformational epitopes comprising amino acid residues from different regions of the allergen brought together by folding of the protein (Fig. 1). As food allergens are subjected to extensive chemical and proteolytic digestion prior to absorption and uptake by the cells of gut-associated lymphoid tissue, it has been assumed that in class 1 food allergy, immune responses are directed predominantly against sequential epitopes [9]. However, the growing body of evidence regarding allergy to beef, cow's milk, egg white, and fish supports the importance of conformational epitopes in patients with class 1 food allergy (two, 10–12). Analysis of IgE-binding epitopes with the use of SPOTs membrane technology revealed that cow's milk and egg-allergic patients who lacked IgE antibodies against certain sequential epitopes of the major allergens were more likely to achieve tolerance to these foods than those whose IgE antibodies were directed against those epitopes [10]. Cooke and Sampson [11] evaluated IgE binding to linearized versus native ovomucoid and reported that pooled sera from egg-allergic patients showed no significant differences in native ovomucoid binding, although sera from several individual patients with transient egg allergy showed a marked reduction in IgE binding to linearized ovomucoid. Jarvinen *et al.* [12] found that IgE antibodies of children with persistent egg allergy recognized more linear ovomucoid epitopes than children with transient egg allergy, suggesting that different phenotypes exist among egg-allergic children. These studies suggested that ovomucoid conformational epitopes might be important in some egg-allergic patients.

Effect of heating on class 1 and class 2 food allergens

High temperature reduces allergenicity, presumably by altering the conformation of heat-labile proteins that results in loss of conformational epitopes (Fig. 1). This paradigm has been generally accepted for the pollen–food allergy syndrome to Bet v 1 cross-reactive proteins in Rosaceae fruit (e.g. apple, peach, and cherry). However, Bet v 1 cross-reactive protein in soybean, Gly m 4 retains

Figure 1 Effect of processing on the integrity of protein epitopes



Food processing such as high temperature, low pH, and enzymatic digestion may alter (destroy) conformational epitopes that are dependent on protein folding but generally do not affect sequential epitopes. ALAC, α-lactalbumin; BETALAC, β-lactoglobulin.

allergenicity in heat-processed foods, suggesting that thermostability is highly variable and food-specific, even for the food allergens from the same protein family [7••].

Each food is a mixture of allergenic proteins that differ in their physicochemical properties, stability to heat and digestion, and the potential to induce IgE sensitization and IgE-mediated hypersensitivity reactions. For example, plant foods contain heat-stable proteins such as lipid-transfer proteins that are recognized as a cause of systemic reactions and primary sensitizers, independent of exposure to pollen [5,7••]. In cow's milk, the caseins and serum albumin have higher heat stability than the whey proteins, α-lactalbumin, β-lactoglobulin, and lactoferrin. Casein bands were preserved in the SDS-PAGE gel even after 120 min of boiling at 100°C. Serum albumin band became progressively weaker after 10 min of boiling but was still visible at 120 min. In contrast, α-lactalbumin band disappeared after 30 min, β-lactoglobulin disappeared after 15 min, and lactoferrin disappeared after 10 min of boiling [13]. In egg white, ovomucoid is the dominant allergen. Although ovalbumin is the most abundant protein found in egg white, it is sensitive to thermal denaturation with resultant decrease in allergenicity. In contrast, ovomucoid is heat resistant and remains soluble after extensive heating; purified ovomucoid heated for 1 h at 100°C retained its antibody-binding activity.

Matrix effect

Protein interactions with other ingredients such as other proteins, fats and sugars in processed foods are also important, in general resulting in decreased availability of protein for interaction with the immune system. Heating of β-lactoglobulin results in the formation of intermolecular disulfide bonds and subsequent binding to other food proteins, making β-lactoglobulin less allergenic [7••]. A recent study by Kato *et al.* [14] demonstrated a marked decrease in the solubility of ovomucoid when egg white was mixed with wheat flour and wheat gluten and then heated at 180°C for 10 min, mimicking the process of bread making. Immunoblotting suggested that ovomucoid polymerizes and forms high-molecular weight complexes with gluten leading to aggregation and insolublization of ovomucoid.

Can heating enhance allergenicity?

High temperature may enhance allergenicity of peanut and shrimp as a result of glycation, the Maillard reaction between free amino acids, and aldehyde or ketone groups of sugars (Table 1). The Maillard reaction induces the formation of Ara h 2 aggregates that are more resistant to gastric digestion and bind IgE antibody more effectively than unheated Ara h 2. For shellfish, such reactions may create new epitopes. However, in the case of other foods, glycation may result in decreased allergencity; following pasteurization, the IgE-binding capacity of Pru av 1, the major cherry allergen, is significantly decreased [7••].

Diet containing baked milk and baked egg

As outlined in reviews in this section by Skripak and Wood, and Tey and Heine, the concept of introducing food allergens via the oral route to treat food allergy has generated significant research activity in recent years. Oral immunotherapy is a promising direction in food allergy research; however, it is clear that food oral immunotherapy may not be possible for some patients due to the high rate of adverse reactions and adherence to treatment. An alternative approach to introducing food protein modified by high temperature and by interactions with wheat matrix to the diet of children with milk and egg allergy was recently explored. Two studies [15,16] reported the results from nonrandomized clinical trials in which over 100 children allergic to milk and over 100 children allergic to egg were tested by oral food challenges to baked products that contained milk or egg. In both studies, about 70% of tested children were able to ingest baked products with milk or egg without any immediate symptoms. Children added baked products to their diet and tolerated them well, without adverse effects on growth, intestinal permeability, or the severity of the coexisting allergic disorders (e.g. asthma, atopic dermatitis, and allergic rhinitis). Introduction of baked products was associated with increasing levels of milk and egg-specific IgG4 antibody levels and decreasing skin prick test wheal sizes. There were no significant changes in the levels of specific IgE antibodies. In addition, children tolerant of baked milk had more regulatory T cells in the peripheral blood at baseline; following introduction of baked milk protein, the fraction of peripheral T regulatory cells decreased, suggesting possible migration of these cells to the sites of the contact with the dietary antigen (gastrointestinal tract) [17]. These observed humoral and cellular immunologic changes parallel the changes reported in the oral immunotherapy trials, suggesting that introduction of baked milk and egg products may represent an alternative pathway of accelerating development of tolerance. Follow-up randomized studies are underway.

Conclusion

Heating and other methods of food processing have different effects on food allergens, even those contained in the same complex food. Structural homology does not reliably predict the effect of processing on allergenicity, and individual foods have to be tested. Interactions with other proteins, fat, and carbohydrates in the food matrix are complex and poorly understood. Better characterization of these aspects of food allergy is critical for elucidation of food protein interactions with the gut-associated lymphoid tissue, the ability to induce IgE sensitization, the potential to trigger hypersensitivity reactions, and different clinical phenotypes of food allergy with regard to severity and persistence.

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