Can We Have Allergen-Free Foods?

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Technology has been used to improve our food supply since primitive man first cultivated crops such as wheat and barley in Mesopotamia in 6,000 BC and domesticated animals such as sheep and goats in southwestern Asia over 10,000 years ago. Recently, improvement of our food supply has been achieved by the development of hybrids, notably of corn, and by breeding and selection which more than doubled wheat and rice crops in developing countries in the 1960s and 1970s (the "green revolution"). Breeding and selection has also increased the supply of domesticated animal species that are sources of foods; for example, chicken, one of the more expensive meats in the 1940s, is now one of the least expensive. Selection of certain traits in plants and animals did not occur without change, not only in the plant and animal species involved, but also in society.

What differentiates the so-called old technology described above from the new technology is genetic engineering, also referred to as molecular breeding. This technology facilitates the selection, identification, and transfer of genes encoding for specific proteins into the genome of another species. Molecular breeding precisely determines which proteins are introduced, where they are expressed and, in some cases, requires synthesis of only minute amounts of a protein in order to obtain the desired trait. Supporters of this technology believe that it will provide substantial benefits for mankind such as less expensive and healthier foods, foods that will help to eliminate diseases and aid in feeding the growing world population. Already, crops genetically modified for insect resistance are significantly reducing the use of synthetic organic insecticides in the United States. However, critics have raised concerns regarding environmental effects, such as gene spread from genetically-modified (GM) crops to indigenous relatives, and adverse effects on the health of mankind. A major health concern is the development of foods of greater allergenicity or containing novel allergens in new foods (Jacobson, 2002; Millis, 2002; NRC, 2002).

DEVELOPMENT OF FOOD-INDUCED ALLERGIC REACTIONS

Food may be a major cause of severe acute hypersensitivity reactions, including fatal anaphylaxis, in some individuals. Food allergy has been estimated to be the most frequent cause of anaphylaxis treated in emergency rooms (Yocum and Khan, 1994). Severe reactions to foods can occur at all ages, from infants (Ellis *et al.*, 1991; Saylor and Bahna 1991) to children, adolescents, and adults (Sampson *et al.,* 1992). Currently, the only means of managing severe acute food reactions is strict avoidance and the immediate availability of emergency medications. However, accidental or inadvertent exposure to food allergens can occur even for the most careful food-allergic patient. The unpredictability of accidental exposures and long periods of time during which patients at risk may not come in contact with the offending foods make it difficult to have medications available at all times, as is necessary to prevent a fatal reaction (Yunginger *et al.,* 1988; Sampson *et al.,* 1992).

The vast majority of acute, severe reactions to foods appear to be IgE-mediated, although non-IgE-mediated reactions also occur (Hill *et al.*, 1995). The presence of IgE antibodies as the likely cause for severe acute food reactions suggests the possibility of changing this allergic reactivity to a less noxious or even protective form of immune response through immunotherapy or of altering the reactivity of major food allergens with IgE antibodies.

The induction and provocation of an IgE-mediated hypersensitivity food allergy is summarized in Figure 1. When an individual is first exposed to a food
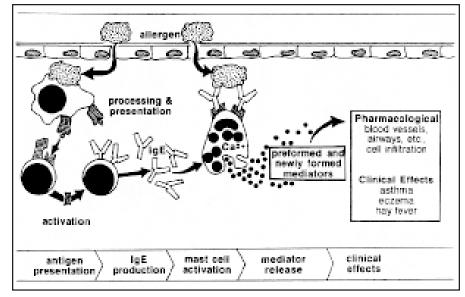

Figure 1. The induction and provocation of an IgE-mediated food-allergic response.

allergen, this molecule or a fragment thereof, crosses the mucosal barrier; following processing and presentation by macrophages and T lymphocytes, peptides (derived from allergens) can activate T and B lymphocytes. Interaction between these cells results in activated B lymphocytes that produce IgE antibodies that react specifically with the allergen that stimulated their production. These IgE antibodies (in addition to reacting to allergens) have the unique ability to fix to the surfaces of mast cells or basophils, cells that contain a number of potent pharmacologically active molecules called mediators. When an allergen cross-links two or more specific IgE antibodies bound to mast cell or basophil membranes, mediators are released that affect both local and systemic organ systems, resulting in the clinical effects seen in allergic reactions such as asthma, eczema, hay fever, and anaphylaxis.

GENERAL PROPERTIES OF FOOD ALLERGENS

Although allergenic foods may contain over 10,000 different proteins, only a few (generally ten to twenty) elicit allergic reactions. The structural properties that are responsible for the allergenicity of a food protein are generally still poorly defined, although some broad characteristics of food allergens have been identified. These include abundance of a given protein in a particular food; physicochemical properties, such as acidic isoelectric point and glycosylation; and resistance to heat and digestion (Lehrer *et al.*, 2002). Although these characteristics have been associated with the allergenicity of proteins, some, if not all, of these properties characterize a vast number of non-allergenic proteins as well and, thus, are not unique to food allergens.

The portion of the allergen molecule that is recognized by, and interacts with, allergen-specific IgE is the allergenic epitope. Most allergens, as stated above, are resistant to heat; although heat denaturation may cause loss of the native protein's conformation, patients' IgE antibodies can still react with such denatured food proteins, suggesting that the allergens' epitopes are not dependent on the native conformation (Lehrer *et al.*, 2002). Thus, alteration of these epitopes is the focus of current technology—to reduce or abolish their reactivity with IgE, resulting in reduction of allergenicity.

Food allergens frequently account for a major fraction of the total protein content within a given food. For example, the major shrimp allergen, Pen a 1, accounts for about 20% of the total shrimptail-muscle protein (Daul *et al.*, 1994). An exception to this rule is the major allergen of codfish *Gadus callarias,* Gad c 1; this molecule, identified as parvalbumin, is not a dominant protein in cod muscle (Elsayed and Bennich, 1975). There are several aspects of molecular size that may contribute to a protein's allergenicity. First, the molecule must be large enough to elicit an immune response but small enough to cross the gut mucosal membrane barrier; second, it must be of sufficient size to contain at least two IgE binding sites to bridge mast-cell-bound IgE.

The ability of a food allergen to cross the mucosal membrane of the intestinal tract is most likely an important feature. As mentioned earlier, size is one parameter in this context; another may be a resistance to digestion. The results of one study, which used a gastric model of mammalian digestion to study the digestibility of food allergens, point in this direction (Fuchs and Astwood, 1996): allergens from egg, milk, peanut, soybean, and mustard resisted digestion for up to 1 hour, whereas nonallergens were digested within 1 minute. However, there is insufficient information to conclude that the resistance to enzymatic digestion is a property that distinguishes all food allergens from non-allergens, since some labile proteins can be allergenic and not all stable proteins are allergens.

PREDICTING FOOD PROTEIN ALLERGENICITY—DECISION TREE

Over the last 10 to 12 years, governmental agencies (FDA, EPA, USDA), industry organizations (ILSI, AII, IFBC) as well as international health organizations (FAO, WHO) have addressed the issue of the allergenicity of GM foods. Their discussions have resulted in developing a decision-making process to aid companies and regulatory agencies in assessing the potential allergenicity of products being developed. Theoretically, there are three potential alterations that could affect the allergenicity of a GM food. First, endogenous protein levels could be affected and if these proteins are allergens, this could result in enhanced allergenicity. Second, the protein whose gene is expressed in a GM food could be a known allergen if derived from an allergenic source. Third, novel proteins expressed from sources for which there is no prior human exposure may be allergens.

A decision process developed to address these issues (Figure 2) is based on the source of the gene: is it from an allergenic or non-allergenic source? If it is from an allergenic source, there are solid-phase, immune assays that, with sera from allergic individuals, can be used to determine the allergenicity of the molecule being expressed or the enhanced levels of endogenous allergens. If the expressed protein is from a source for which there has been no prior human exposure, the assessment of its allergenicity is more difficult. This assessment is based on a comparison of the properties of the molecule to properties of known food allergens, such as amino acid sequence similarity, stability to enzymatic digestion, and stability to processing. Such an approach, while not yielding absolute definitive answers, can help in assessing the potential allergenicity of the molecule in question (Metcalfe *et al.*, 2000). Clearly, as technology improves and our knowledge of food allergens increases, better assessment methods for allergenicity of novel proteins can be expected. Generally, the current risk assessment for allergens is reasonable, provides assurance of food safety and has worked well in avoiding the development of allergenic GM foods. Although risk assessment for known allergens is well delineated, risk assessment for novel proteins is more problematic and needs to be improved as our knowledge of food allergens increases.
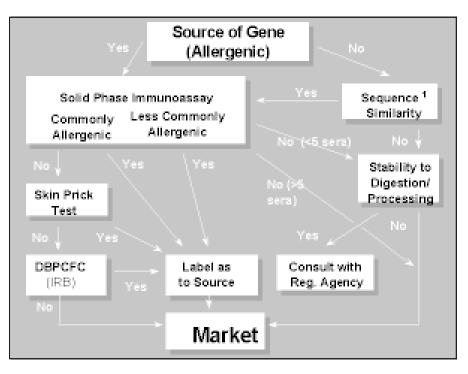
Figure 2. Assessment of the allergenic potential of foods derived from genetically engineered crops—International Life Sciences Institute (ILSI) decision tree.

USE OF BIOTECHNOLOGY TO DEVELOP HYPOALLERGENIC FOODS AND SAFER ALLERGENIC VACCINES

Genetic modification of plants and animals, may, in the future, improve the safety and quality of foods, by reducing allergenicity of known allergens. A number of major allergens have been identified in agronomically important crops such as soy and wheat as well as in less economically important crops such as peanut and tree nuts. Furthermore, important allergens have been demonstrated in a variety of animal products used for food, such as milk, eggs, fish and particularly shellfish (Bush and Hefle, 1996).

There are several approaches to reduce allergens in food. Traditional plant breeding has been used to identify strains with reduced allergenic activity. Food-processing methods have also been used in attempts to reduce or eliminate the allergenicity of various food products. Most recently, genetic engineering has been employed:
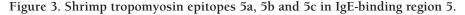
- post-transcriptional silencing to decrease the level of protein synthesized in a particular food;
- reduction of disulfide bonds to alter the structure of allergens to reduce their allergenicity;
- modification of genes encoding allergens.

For gene modification, extensive knowledge of allergen structure is needed, including amino acid sequences, as is gene-nucleotide sequence. Furthermore, the IgE binding sites—the portion of the allergen that actually binds IgE and is responsible for allergic reactions—must be determined (Bannon, in press).

Shrimp and peanuts are two foods that have been extensively investigated since they can induce severe anaphylactic reactions in sensitized children and adults that can result in death. The only major allergen in shrimp is the heat-stable muscle protein, tropomyosin, called Pen a 1 in *Penaus azectus*, which is studied in our laboratory. Tropomyosin has a rather intriguing, highly stable structure: a coiled coil composed of two identical tropomyosin polypeptide chains in alpha-helix formation coiled around each other. Using overlapping peptides, five major IgE-binding regions were identified in the tropomyosin molecule. Further analysis of these regions by overlapping peptides of shorter length identified the minimal sequence that binds IgE from sera of shrimp-allergic subjects. Individually recognized epitopes of region 5 are shown in Figure 3.

Figure 3. Shrimp tropomyosin epitopes 5a, 5b and 5c in IgE-binding region 5.

Region 5 is composed of three individual IgE binding epitopes (Lehrer *et al.*, 2002). A total of eight epitopes were identified in the five IgE binding regions. The epitope amino acid sequence, as defined by maximal IgE antibody reactivity, varies for some epitopes (*i.e.* epitope 3a) whereas is essentially identical for others (*i.e.* 5b). Alteration of these peptide epitopes by amino-acid

substitution was performed based on homologous amino acid sequences in other tropomyosin molecules (Figure 4). A number of amino-acid substitutions were demonstrated that completely abolished IgE antibody binding. These results are very encouraging since these amino-acid substitutions should not alter the structure of the protein molecule yet substantially abolish its allergenicity.

Spot	Amino Acid Sequence								
	266	267	268	269	270	271	272	273	
1	K	Y	K	S	I	T	D	E	WT
2	•	•	•	F	•	•	•	•	
3	•	•	•	•	•	C	•	•	
4	•	•	•	•	•	•	•	D	
5	•	•	•	F	•	C	•	•	
6	•	•	•	F	•	•	•	D	
7	•	•	•	•	•	C	•	D	
8	•	•	•	F	•	D	•	D	
9	•	•	•	•	•	S	•	•	
10	K	Y	K	S	I	T	D	E	WT

Figure 4. Amino-acid substitutions in epitope 5c that alter IgE binding.

Advances have also been achieved using a similar approach to modify peanut allergens. Three major peanut allergens have been identified: Ara h 1, Ara h 2, and Ara h 3 (Burks *et al.*, 1991, 1992; Eigenman *et al.*, 1996). Four other proteins have been identified as peanut allergens and designated Ara h 4–7. With the exception of Ara h 5, they all show significant homology with either Ara h 1, 2, or 3 (Kleber-Janke *et al.*, 1999). Ara h 5 is a member of the profilin family, but is only recognized by IgE from a small fraction (13%) of the peanut allergic population (Kleber-Janke *et al.*, 1999). As discussed for shrimp tropomyosin, the linear IgE-binding epitopes for the major peanut allergens were mapped using overlapping peptides and serum IgE from patients with documented peanut hypersensitivity. Twenty-one different linear IgE binding epitopes were identified throughout the length of the Ara h 1 molecule (Burks *et al.*, 1997). Ten IgE-binding epitopes were identified in Ara h 2 and 4 in Ara h 3 using the same methods (Stanley *et al.*, 1997; Rabjohn *et al.*, 1999). The

epitopes ranged in length from six to fifteen amino acids, but no obvious sequence motif was shared by all peptides. Four of the Ara h 1 epitopes appeared to be immunodominant IgE-binding peptides in that they were recognized by serum from more than 80% of the patients tested and bound more IgE than any of the other Ara h 1 IgE-binding epitopes. Similarly, three of the Ara h 2 IgE-binding epitopes and one of the Ara h 3 epitopes were determined to be immunodominant.

Each of the IgE-binding epitopes for the three major peanut allergens was subjected to site-directed mutational analysis. Single amino acid changes within these peptides had dramatic effects on IgE-binding characteristics. One or more amino acids within each epitope were found to be critical for IgE binding. Substitution of one of these critical amino acids led to loss of most of the IgE binding (Burks *et al.*, 1997; Shin *et al.*, 1998; Stanley *et al.*, 1997). Analysis of the type and position of amino acids within the IgE-binding epitopes that had this effect indicated that substitution of hydrophobic residues in the center of the epitopes was likely to lead to loss of IgE binding (Shin *et al.*, 1998). These results have been used to develop recombinant forms of these allergens for use in immunotherapy. The engineered hypoallergenic peanut protein variants display two characteristics essential for recombinant allergen immunotherapy: they have a reduced binding capacity for serum IgE from peanut-hypersensitive patients and they stimulate T-cell proliferation and activation (Bannon *et al.*, 2001; Rabjohn *et al.*, 2002; Bannon, in press).

CONCLUSION

The studies reviewed are representative of investigations using genetic modification to alter allergenicity of food proteins. In spite of the initial success of such studies, significant challenges remain. The simultaneous expression of modified allergen genes with repression of wild-type allergen genes needs to be further developed, particularly in animal species. It is important that any altered allergens developed be demonstrated not to contain potentially new allergenic epitopes. Only further studies over time can delineate this. However, in spite of these issues, the impact of biotechnology on the future production of hypoallergenic foods appears to be bright.

The discovery and characterization of existing food allergens and their genes has occurred at a very rapid rate due primarily to progress in technology. In addition, numerous methods are being developed for enhancing allergy-diagnostic technologies and allergen therapy. One of these approaches is the development of hypoallergenic foods. It is to be hoped that, in the not too distant future, foods will be developed that will substantially reduce the number and severity of allergic reactions for already sensitized subjects while reducing sensitization of others. In addition, extracts of these foods will be important in developing safer vaccines for future treatment of food-allergic subjects.

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