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Topic 1: Overview of the Current Approach to Determine the Allergenicity of Genetically Modified Foods (Decision Tree Approach)

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FAO: http://www.fao.org/WAICENT/FAOINFO/ECONOMIC/ESN/gm/biotec-e.htm

WHO: http://www.who.int/fsf/GMfood/index.htm

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6.3 Allergenicity

6.3.1 Introduction

Food allergies are adverse reactions to an otherwise harmless food or food component that involves an abnormal response of the body's immune system to specific protein(s) in foods. True food allergies may involve several types of immunological responses (Sampson and Burks, 1996). The most common type of food allergies are mediated by allergen-specific immunoglobulin E (IgE) antibodies[1]. IgE-mediated reactions are known as immediate hypersensitivity reactions because symptoms occur within minutes to a few hours after ingestion of the offending food. IgE-mediated reactions can occur to pollens, mould spores, animal danders, insect venoms and other environmental stimuli as well as foods. IgE-mediated reactions affect perhaps 10-25% of the population in developed countries (Mekori, 1996), although food allergies represent a small fraction of all allergic diseases. IgE-mediated food allergies affect less than 2.5% of the population in developed countries (Anderson, 1996). Infants and young children are more commonly affected by IgE-mediated food allergies than adults; the prevalence among infants under the age of 3 may be as high as 5-8% (Bock, 1987; Sampson, 1990). True food allergies also include cell-mediated reactions which involve sensitized tissue-bound lymphocytes rather than antibodies (Sampson, 1990). In cell-mediated reactions, the onset of symptoms occurs more than 8 hours after ingestion of the offending food. The role of foods in cell-mediated reactions remains uncertain (Burks and Sampson, 1993) but, celiac disease, also known as gluten-sensitive enteropathy, affects 1 in every 300 to 3000 individuals in the population depending upon the specific geographic region.

[1] IgE, or immunoglobulin E, is a protein antibody that recognizes an allergen. It circulates in the blood, and becomes fixed on the surface of specific cells (basophils and mast cells). When IgE on the cell surface binds to allergen, this triggers the release of chemical mediators that provoke the symptoms associated with allergic reactions.

The Codex Alimentarius Commission has adopted a list of the most common allergenic foods associated with IgE-mediated reactions on a world-wide basis that includes peanuts, soybeans, milk, eggs, fish, crustacea, wheat, and tree nuts. These commonly allergenic foods account for over 90% of all moderate to severe allergic reactions to foods, although an extensive literature search has revealed more that 160 foods associated with sporadic allergic reactions (Hefle, 1996). Allergic reactions to fresh fruits and vegetables, the so-called oral allergy syndrome, are also rather common (Parker, 1990), but these foods are not included on the Codex Alimentarius Commission list because the symptoms are typically mild and confined to the oropharyngeal region and the allergens are unstable to heating and digestion. The list established by the Codex Alimentarius Commission also includes gluten-containing cereals (wheat, rye, barley, oats and spelt) that are implicated in the etiology of gluten-sensitive enteropathy.

The symptoms of IgE-mediated food allergies can range from mild to severe and life-threatening. Individuals display different thresholds for the offending food, but the most sensitive food-allergic individuals will experience reactions from exposure to trace quantities of the offending food. Life-threatening reactions usually involve exposure to larger doses of the offending food.

Gluten-sensitive enteropathy is a mal-absorbtion syndrome characterized by body wasting, anaemia, diarrhoea, and bone pain along with other symptoms. The threshold dose needed to provoke the symptoms of gluten-sensitive enteropathy are unknown, but also thought to be quite low.

Both IgE-mediated food allergies and gluten-sensitive enteropathy are treated with specific avoidance diets. Since in both cases, the threshold dose is quite low, great care must be taken in the construction of safe and effective avoidance diets.

Almost all food allergens are proteins, although the possibility exists that other food components may act as haptens[2]. Similarly, prolamin proteins from wheat, rye, barley, etc. are involved in the elicitation of gluten-sensitive enteropathy. While the crops from which staple foods are derived contain ten of thousands of different proteins, relatively few are allergenic. The distribution of these proteins varies in different parts of the plant and can be influenced by environmental factors such as climate and disease stress. Conventional breeding introduces additional protein diversity into the food supply. However, variations in the protein composition of our diets brought about through conventional crop improvement practices have had little, if any, effect on the allergenic potential of our major foods. In contrast, altered dietary preferences can have significant implications for the development of food allergies. For example, allergy to peanut (groundnut) occurs at a significant frequency in North America and Western Europe but not in other countries where peanuts are less commonly eaten. Also, recent food introductions such as kiwi fruit have proven to be additional sources of food allergens. These observations provide confidence that there are not a large number of potential allergens in the food supply, but show that new allergenic foods are sometimes introduced into the marketplace.

Because of the above, a clear need exists to pay particular attention to allergenicity when assessing the safety of foods produced through genetic modification.

[2] Haptens are small molecules which may interact with body proteins or food proteins and cause these proteins to become allergenic.

6.3.2 Evaluation of the Potential Allergenicity of Novel Proteins in Genetically Modified Foods

In 1996, the International Food Biotechnology Council and the Allergy and Immunology Institute of the International Life Sciences Institute developed a decision-tree approach (Metcalfe *et al*., 1996). This allergy assessment strategy has been widely adopted by the agricultural biotechnology industry. This strategy focuses on the source of the gene, the sequence homology of the newly introduced protein to known allergens, the immunochemical binding of the newly introduced protein with IgE from the blood serum of individuals with known allergies to the source of the transferred genetic material, and the physicochemical properties of the newly introduced protein (Metcalfe *et al*., 1996; Taylor, 1997). This study has been adapted by the Consultation for the assessment of the allergenicity of genetically-modified foods [Figure 1].

Since genetically modified foods usually contain novel proteins, their safety should include an assessment of the allergenicity of such novel proteins. The current decision-tree approach requires the examination of a number of parameters which are common to many food allergens. These characteristics facilitate the identification of potentially allergenic gene products, although no single criterion is sufficient to confirm allergenicity or the lack thereof. The relevant criteria used in the current decision tree include:

- Source of the transferred genetic material: Particular caution must be exercised if the source of this material contains known allergens.

- Sequence homology: The amino acid sequence of many allergens is readily available.

- Immunoreactivity of the newly introduced protein: If the novel protein is derived from a known allergenic source or if it has sequence homology with a known allergen, then the reactivity of this novel protein with IgE from the blood serum of appropriate allergic individuals is determined.

- Effect of pH and/or digestion: Most allergens are resistant to gastric acidity and to digestive proteases.

- Heat or processing stability: Labile allergens in foods that are eaten cooked or undergo other processing before consumption are of less concern.

The desirability of including other relevant criteria to improve the reliability of the allergy assessment decision-tree approach was discussed. When the genetically modified food contains genes selected from sources with known allergenic effects, then it must be assumed that the novel gene product is allergenic unless proven otherwise. The current decision-tree approach which advocates the assessment of the binding of the novel protein with IgE from the blood serum of individuals who are allergic to the source of the donor genetic material followed, if necessary by skin testing and blinded oral food challenges, was considered adequate and essential. The assessment of any unintended effects on the allergenicity of the host material after a genetic modification with genes from other sources, whether allergenic or not, was not considered necessary except in circumstances where the genetic modification could be predicted to alter the protein content of the host product significantly.

When the genetically modified food contain genes from sources with no history of allergenicity, the current decision-tree approach relies primarily upon sequence homology comparisons to known allergens and the stability of the novel protein to digestion and processing. It is widely recognized that these two criteria alone may not be sufficient to assess the potential allergenicity of genetically modified foods containing genes from sources with no history of allergenicity.

The current criteria used to determine significant sequence similarity, a match of at least eight contiguous, identical amino acids (Metcalfe *et al*., 1996) has been criticized. Suggestions have been made that sequence similarity should instead require a match of a smaller number of contiguous, identical amino acids, perhaps as few as four amino acids. The use of a match of eight contiguous, identical amino acids appears to have some relevance based upon the minimum peptide length for a T cell-binding epitope[3] (Metcalfe *et al*., 1996). Also, it is recognized that the criterion cannot identify discontinuous or conformational epitopes that depend upon the tertiary structure of the protein (Metcalfe *et al*., 1996). However, the stability of food allergens to heat processing argues for greater significance of linear, continuous epitopes for these particular allergens. International scientific consensus should be sought on the use of sequence homology in the assessment of the allergenicity of genetically modified foods.

The use of digestive stability appears to be a rather useful criterion in the assessment of the allergenicity of genetically modified foods. Simulated gastric and intestinal digestive models of mammalian digestion have been used to assess the digestive stability of known food allergens and proteins introduced into foods through genetic modification (Astwood *et al*., 1996). While the usefulness of this criterion is apparent, consensus is needed on the ideal protocols for assessment of digestive stability. It is recognized that novel proteins may exist that are stable to digestion but will not become allergens. Additional testing is needed to assess the allergenic potential of such proteins.

The desirability of the development of additional tests to assess the allergenic potential of foods containing genes from sources with no history of allergenicity has been widely expressed. Two additional tests seem to show some promise for addition to the decision-tree approach.

The level and site of expression of the novel protein is an important component of the assessment of allergenicity. Novel proteins expressed at comparatively low amounts in the food would have limited potential for allergic sensitization. Major food allergens are usually major proteins in commonly consumed foods. Thus, greater scrutiny should occur with genetically modified foods containing novel proteins at significant levels in the product. New proteins expressed in non-edible portions of plants are not a concern in terms of food allergy.

[3] Epitopes are groups of amino acids within proteins that can bind to either T cells (T cell epitopes) or IgE antibodies (IgE-binding epitopes). Epitopes can be either linear or conformational.

Consideration of the function of the novel protein should also form part of the decision-tree assessment of allergenic potential. Certain classes of proteins are well known allergens. For example, the 2S, high-methionine albumins from Brazil nut, walnut, sunflower seed and mustard are major allergens from those sources. Thus, other 2S, high-methionine albumins should be scrutinized very carefully for allergenic potential. Many of the pathogenesis-related proteins of plants display allergenic activity, therefore, the entire class of proteins would also merit close examination. International consensus should be sought on a list of functional proteins with allergenic potential. Certainly, other proteins not on such a list must also be evaluated but this aspect could be a useful part of an overall assessment strategy.

The potential for the use of animal models for the assessment of the allergenicity of genetically modified foods was also discussed. Unfortunately, reliable, well validated animal models for the assessment of the allergenicity of genetically modified foods do not presently exist, although further research on the development of animal models is encouraged.

Other attributes, such as molecular weight and degree of glycosylation, were also discussed. However, it was felt that these attributes were not sufficiently discriminatory to be very helpful.

The novel proteins present in genetically modified foods should also be evaluated for any possible role in the elicitation of gluten-sensitive enteropathy. Clearly, if the desired gene is obtained from wheat, rye, barley, oats, or related cereal grains, the possible role of the novel protein in provoking gluten-sensitive enteropathy must be carefully considered. Furthermore, if genetic modifications are conducted on these cereal grains, possible unintended effects on the gluten proteins should be considered. International consensus should be sought on an appropriate decision-tree approach to the assessment of the role of genetically modified foods and their novel proteins in gluten-sensitive enteropathy.

6.3.3 Reduction or Elimination of Allergens through Genetic Modification

Genetic modification offers the opportunity to decrease or eliminate the protein allergens that occur naturally in specific foods. An example is the development of a genetically modified rice variety developed through anti-sense technology, which dramatically reduced levels of the major rice allergen (Matsuda *et al*., 1995). Further efforts of this type should be encouraged.

Assessment of the Allergenic Potential of Foods Derived From Genetically Modified Crop Plants