

Benefits and limits of different approaches for assessing the allergenic potential of novel foods

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The incidence of food allergy and the number of incriminated foods are increasing. Furthermore, the severity of the allergenic symptoms commonly observed is worsening. This is a major concern for both the food industry and those regulatory committees in charge of public health. Therefore, there is a need to predict and manage the allergenic risk of (novel) foods. There is also a pressing need to consider ways of creating new allergens or of increasing the expression/potential of existing ones and of unmasking hidden and therefore nonbio-available immunoreactive structures (1). This calls into question the development of new technologies for the production and processing of foods.

Insertion of a new gene into a plant could result in expression of new allergen(s) or increased expression of existing allergen(s). If the inserted DNA is well characterized, one can expect that only the trait protein (and possibly the marker gene product) will be expressed and will have to be assessed for allergenicity. However, the location where the transgene is inserted within the plant genome is not controlled, and the indirect pleiotropic effect by which a single genetic modification may interfere with the expression of other genes and affect numerous uncontrolled and nonrelated phenotypic characteristics is a specific issue that needs to be addressed.

It is worth first recalling that the sensitizing food is first degraded by digestive enzymes, absorbed by the gut mucosa, and processed in immunopotent

cells before being presented to the immune system. Therefore, the allergic reaction may be induced and/or triggered not only by the native form of a food protein but also by fragments of the molecule or by cross-reacting compounds which might share common allergenic structures.

Only recently have procedures/guidelines for testing allergenicity of food proteins been proposed for assessment of genetically modified plant products (2). They have been mostly endorsed by scientific/regulatory committees including FAO-WHO (3) and are applied in the applications submitted by manufacturers for marketing authorizations.

Recombinant vs natural allergens

If in a novel (transgenic) food, the introduced gene encodes a known allergen, it is very likely that the transgenic organism will express the protein with allergenic potential. This was the case when Brazil nut 2S storage albumin was engineered into soybean to raise levels of sulfur-containing amino acids so as to improve the biologic value of soybean meal proteins for animal feeding. Unfortunately, this protein is a potent allergen of Brazil nut. It provoked identical reactions of allergy, whether expressed in transgenic soybean or when naturally present in Brazil nut (4). Allergenicity was linked to this protein molecule, and not to the whole food, and was transferred through this protein after

genetic manipulation. Recombinant bovine β -lactoglobulin (b-Lg) expressed in *E. coli* also has the same immunologic and allergenic characteristics as the corresponding b-Lg secreted in milk (5).

In vitro immunochemical techniques using sera from allergic patients are a useful tool to assess the allergenicity of a genetically modified food when the transgene derives from a commonly allergenic source. However, it cannot be used on a large scale for immunoanalytic studies since serum banks built up from patients with known allergy are limited. *In vivo* tests on allergic patients are subject to ethical concerns.

Such assessments cannot be performed if the novel food has no history of causing allergy, or a limited history that precludes the availability of sera. No or very few cases of allergy have been reported for transgenic foods. This is probably due to the fact that most of the expressed proteins are bacterial, having no or low glycosylation levels, a fact which renders them poorly allergenic.

Therefore, complementary and/or alternative approaches are required, and they must be critically appraised and applied on a case-by-case basis.

Characterization of the test protein

The first critical point is the strict chemical identity of the foreign protein which is expressed in the genetically modified plant and of the test protein which is used for allergy assessment experiments. Indeed, the foreign protein is generally expressed at a very low level in transgenic plants, and the amount of material which is necessary to perform the experiments precludes extraction of the test substance from the plant. Large quantities of the protein are thus produced in genetically modified microorganisms in which the same corresponding transgene is introduced. However, slight structural changes may occur, depending on the producing organism. These can affect the primary sequence of the protein due to point mutations on a few amino-acid residues or can be due to post-translational modifications; i.e., variations in the glycosylation or phosphorylation levels. Such modifications may have an effect on the immunoreactivity of the protein.

Animal models

Some animal models, including guinea pig and rat, have been proposed as a mean of determining food allergens (6, 7). These provide important information for understanding the mechanisms of induction of specific IgE synthesis and of effector cell triggering. Although development of improved

animal models which mimic human IgE response and even clinical symptoms are in progress, none of these models has yet been validated for determination of human IgE-binding epitopes and thus for assessing and predicting the allergenic potential of particular proteins in humans (8, 9).

Structure analysis and comparison of sequences

The relationship between the primary structure of a protein and allergenicity is not established. However, computer programs for comparisons of the amino-acid sequences of food proteins and of some known allergens are available on the Internet.

Such sequence comparisons may provide an indication of the allergenic potential of a particular protein when a sequence identity of at least eight contiguous identical or chemically similar amino-acid residues is found (2). However, this method is limited by the paucity of information currently available on specific epitopes of most allergens. Moreover, these criteria are very restrictive and are limited to linear epitopes. It is worth noting that in the spatial configuration of the protein, short homologous sequences comprising fewer than eight identical amino-acid residues can be brought close together by the folding of the molecule and thus participate in the formation of conformational epitopes which could mediate allergenic reactivity. When applied to some food allergens, three-dimensional structure analysis reveals data on the structure-activity relationship of some protein families known to include allergens, such as enzymes, storage proteins, pathogenesis-related proteins (stress proteins), calcium-binding proteins, lipocalins, or profilins.

Predicted vs identified antigenic/allergenic structures

Analysis of available crystallographic structures, computer analysis, and molecular structure modeling can be used to predict which are the most likely immunoreactive regions within a protein molecule. Many reports have dealt with the theoretic prediction of protein antigenic determinants from their amino-acid sequences, assuming that antigenic sites are surface features of the proteins and should be related to regions of high hydrophilicity along the polypeptide chain (10). However, IgE often appear to be specific to destructured and denatured parts of food allergens, such as milk allergens. Epitopes are often located in hydrophobic parts of the molecule, inaccessible to IgE antibodies in the native conformation of the protein, but becoming bioavailable after digestive processes (11, 12).

Prediction of allergenic potential according to physicochemical and biologic characteristics of the food or food components

This approach is based on the hypothesis that allergenic compounds share common physicochemical and biologic characteristics, although no relation between structure, function, and allergenicity of a protein has been clearly and unequivocally established. In fact, allergenicity is often considered to be mainly due to the whole intact protein. Thus, for sufficient amounts of the protein to reach the gut, be absorbed through the intestinal mucosa, and then be presented to the immunopotent cells, a prerequisite for food protein allergenicity would be stability in acidic gastric conditions and resistance to digestion by the proteolytic enzymes present in the gut. An *in vitro* test has been proposed (13) to simulate gastric and intestinal digestion; however, the kinetics of decrease of pH and of release of enzymes is difficult to reproduce, as they occur in physiologic conditions. The stability of proteins under conditions encountered during industrial processing, particularly heat denaturation, is also considered to be an important property of food allergens. However, it is not always necessary to have intact protein in order to elicit food allergy. Denatured molecules and/or short peptide fragments may be as potently allergenic as the native molecule (11, 12); Haddad et al. (14) showed that digestion of b-Lg increased allergenicity by unmasking new epitopes which were hidden in the native spatial configuration of the molecule.

Other considerations

Another consideration relates to the host organism. Allergens which naturally occur in foods have a multigenic origin. The introduced transgene may provoke pleiotropic effects on the host and in particular may deregulate the expression of other genes, i.e., those encoding endogenous allergens, thereby increasing the amount or modifying the composition of endogenous allergens present in transgenic organisms compared to those naturally present in classical strains. With this in mind, Burks & Fuchs (15) used electrophoretic techniques to check that the introduced transgene encoding the protein which confers tolerance to the herbicide glyphosate in transgenic soybeans does not alter the qualitative or quantitative composition of endogenous soybean allergens, as compared with the corresponding commercialized varieties.

Conclusions

Allergenic foods and constituents of food responsible for allergenicity seem to be widespread and unlimited in number. New allergens are constantly appearing, and it is not easy to provide a simple, universal, and definite answer to the question, "What makes an allergen an allergen?" (16). Moreover, IgE immunologic cross-reactivity may occur between related or unrelated compounds which share analogous sequences, and no reliable test is as yet available for definite detection of minor allergens or the allergenic potential related to minor allergens. Those considerations hamper a coherent approach to predicting allergenicity. As a consequence, and even if no evidence has yet suggested that novel foods, and particularly recombinant proteins expressed by genetically modified organisms, would be more (or less) allergenic than the corresponding conventional foods, care should be taken in interpreting and applying the results of the different available predictive procedures.

- 1) These procedures and the rules governing the licencing, labeling, and marketing of novel foods should be subject to case-by-case critical appraisal and approval by national and/or European committees in charge of consumer protection and food and environmental safety. These procedures should also be ratified by the various agribusiness sectors (producers, processors, distributors) and by consumers.
- 2) The predictive approach to determining the allergenic potential of novel foods should be allied to mandatory implementation of monitoring of the potential postmarketing impact of these new foodstuffs on public health. Such a postmarketing surveillance can only be achieved through the development and organization of procedures ensuring the traceability of novel food products as part of a quality assurance policy applicable to all agribusiness sectors.

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