DISCUSSION PAPER

EVALUATION OF ALLERGENICITY OF PROTEINS INTRODUCED INTO BIOENGINEERED FOODS

The Food and Drug Administration (FDA) published a notice in the **Federal Register** of May 29, 1992 (57 FR 22984), entitled "Statement of Policy: Foods Derived from New Plant Varieties" (the 1992 policy) (Ref. 1; http://www.cfsan.fda.gov/~acrobat/fr920529.pdf). The 1992 policy provides comprehensive scientific guidance to industry to aid in assessing the safety of foods derived from new plant varieties, including varieties developed using recombinant DNA technology (also referred to as bioengineering or genetic engineering). As discussed in the 1992 policy, evaluation of potential allergenicity of proteins that are introduced into food derived from new bioengineered plant varieties is an important consideration in assessing the safety of such foods for human consumption. Recently, FDA published a proposed rule in the Federal Register of January 18, 2001 (66 FR 4706), entitled "Premarket Notice Concerning Bioengineered Food" (the 2001 proposed rule) (Ref. 2; http://www.cfsan.fda.gov/~lrd/fr010118.html). In the 2001 proposed rule, FDA reiterated that this issue is important and stated its intention to publish a draft guidance for evaluating the potential allergenicity of proteins introduced into bioengineered foods.

This paper is intended to provide a brief background and summary of the issues regarding allergenicity assessment of new proteins that are before the scientific community, as well as to outline FDA's current approach and developments in the international community. The objective of this paper is to assist our new Food Biotechnology Subcommittee of the Agency's Food Advisory Committee in its discussions and deliberations over questions that will be posed to the Subcommittee at a public meeting.

The paper discusses the agency's current approach for assessing potential allergenicity of new proteins in bioengineered foods and explains how this approach was developed. Recently, an international committee, the Ad Hoc Intergovernmental Task Force on Foods Derived from Biotechnology (the Task Force), under the Codex Alimentarius Commission developed draft guidelines for assessing potential allergenicity of new proteins in foods derived from bioengineered plants[1] (Ref. 3; http://www.codexalimentarius.net/biotech/en/DNAPlant.htm). This draft guidance builds upon published work and represents a broad consensus among national governments and non-governmental organizations. Based on the types of proteins reviewed to date, the FDA believes that its current approach is generally consistent with the approach elaborated by the Task Force. The agency also believes that the Task Force's approach may provide certain additional scientific considerations that could improve FDA's current approach and that may be particularly useful for assessing potential allergenicity of proteins that have different characteristics from those that the agency has evaluated to date. This paper, therefore, also identifies these new scientific considerations, as well as other issues, with respect

[1] The Codes Alimentarius Commission (the Codex) was established in 1952 by the World Health Organization and the Food and Agriculture Organization to protect the health of consumers and to ensure fair practices in the food trade. The Codex establishes international guidelines, standards and codes of practice for the food supply. The Task Force has developed "Draft Principles for the Risk Analysis of Foods Derived from Modern Biotechnology" and a "Draft Guideline for the Conduct of Food Safety Assessment of Foods Derived for Recombinant-DNA Plants", including a Draft Annex on the Assessment of Possible Allergenicity (Proteins) (the allergenicity annex) and has recommend these documents for adoption by the Codex at its next meeting in 2003.

to assessment of potential allergenicity of new proteins that the agency intends to discuss with its new Food Biotechnology Subcommittee of the agency's Food Advisory Committee.

I. Background

i. The 1992 Policy

In the 1992 policy, FDA discussed the importance of evaluating the potential allergenicity of proteins introduced into foods derived from new plant varieties (Ref. 1). FDA also explained that the act requires that a food be labeled to inform consumers of possible consequences that result from the use of a product, such as the presence in food of an unexpected allergen (57 FR 22984 at 22991). FDA's principal concern regarding allergenicity is that proteins transferred from a donor plant source to the host food plant (introduced proteins) might confer on host plant food the allergenic properties of donor plant (57 FR 22984 at 22987). FDA stated that developers should initially assume that a protein derived from a food that commonly causes allergic reactions is an allergen and that labeling would be required to alert sensitive individuals, unless scientific evidence demonstrated that the introduced protein was not an allergen (57 FR 22984 at 22987 and 22991). FDA cited several examples of foods that commonly cause allergic reactions: milk, eggs, fish, crustacea, molluscs, tree nuts, wheat, and legumes (particularly peanuts and soybeans) (57 FR 22984 at 22987). Although not expressly addressed in the 1992 policy, FDA did not anticipate that labeling would be necessary in cases where the protein was not present in the finished food (e.g., refined vegetable oil). In addition, although not stated in the 1992 policy, in certain circumstances, labeling may not be adequate or practical to ensure that consumers are aware of the presence of unexpected allergens. FDA would likely consider such food containing an unexpected allergen to be adulterated within the meaning of section 402(a)(1) of the Federal Food, Drug, and Cosmetic Act (the act) because the unexpected allergen rendered the food possibly injurious to health.

FDA emphasized its concern regarding commonly allergenic foods with a hypothetical example of a tomato that contains a newly introduced peanut protein. The agency explained that, unless scientific evidence established that the introduced protein was not allergenic, labeling would be required for a new variety of tomato that contained peanut protein so that peanut-sensitive consumers could avoid the new food. In such circumstances, the presence of a protein derived from a food that commonly produces allergic reactions would be a fact whose omission would misbrand the new food under sections 201(n) and 403(a) of the act (21 U.S.C. 321(n) and 343(a)) (57 FR 22984 at 22991).

Since publication of the 1992 policy, there has been one instance in which an introduced protein was determined to be an allergen. The developer of a soybean variety introduced a protein (2s albumin storage protein) derived from Brazil nut to improve the protein quality of soybeans for use in animal feed. Prior to commercialization, the protein was shown to be an allergen in that the protein was demonstrated to cross react with the serum from individuals who are sensitive to Brazil nuts and to cause positive skin test reactions in these individuals (Refs. 4,5). The developer discontinued development of this variety of soybean, and it was never commercialized for any use.

In the 1992 policy, FDA also discussed allergenicity of proteins derived from non-food sources. The agency recognized that scientific procedures are not currently available to test directly

whether a protein will cause an allergic reaction and that it is not possible to conduct a definitive evaluation of food allergenicity if the source of the introduced protein has no history of use in food. Nevertheless, because most proteins introduced into bioengineered varieties to date are derived from non-food sources, FDA has taken several actions discussed below to provide guidance to industry to aid their assessment of potential allergenicity. FDA has also ensured the agency's policy regarding allergenicity is grounded on the most reliable, and most current, scientific information.

ii. The 1994 Allergenicity Conference

FDA, the Environmental Protection Agency (EPA), and the U.S. Department of Agriculture (USDA) hosted a public scientific conference on food allergy and transgenic crops (the 1994 allergenicity conference) (Ref. 6; Summary available at http://www.cfsan.fda.gov/~lrd/bioallrg.html). The goal of the 1994 allergenicity conference was to foster a dialogue among scientists on food allergy and new varieties of food crops developed by gene transfer to assess current information regarding the characteristic properties of food allergens and the methods available to assess allergenicity. The invited scientists presented and discussed papers on plant breeding and biotechnology, allergenic foods, exposure and allergic response, T cell and B cell antigenic determinants, *in vitro* and *in vivo* diagnostics, and animal models for assessing allergenicity. The scientists noted that allergic reactions to foods occur in a small percentage of the population but, nevertheless, affect a significant number of individuals. Life threatening reactions are rare, and greater than ninety percent of allergic reactions to food can be attributed to fewer than a dozen foods. Direct methods are available to assess the allergenic potential of proteins that are derived from sources to which consumers have reacted and for which serum from sensitive individuals is available.

The conference scientists acknowledged that there are no direct methods to assess allergenicity of proteins from sources that are not known to produce food allergy. However, they suggested that some assurance can be provided to minimize the potential that a new protein will cause an allergic reaction by evaluating its similarity to characteristics of known food allergens. (E.g., Does the new protein have amino acid sequences that are similar to sequences found in allergens? Is the protein resistant to simulated digestive conditions? Is it heat stable? Is its molecular weight typical of food allergens?). Because exceptions have been reported for the observed characteristics of allergens, no one factor is fully predictive, and the conference scientists recommended that an assessment of allergenicity should be based on the totality of available information.

The conference scientists also discussed whether glycosylation of a protein (the addition of carbohydrate groups to the protein) is useful for predicting allergenicity. They noted that allergens that have been characterized tend to be glycosylated. However, most glycosylated proteins that occur in foods are not allergens. Thus, the absence of glycosylation may provide some measure of assurance that a protein will not be a food allergen; however, the fact that a protein is glycosylated is not indicative of whether the protein would be an allergen.

iii. Suggestions from FDA's 1994 Advisory Committees

The agency also convened a meeting of FDA's Food Advisory Committee (FAC) (Ref. 7) in April 1994 and a joint meeting of the FAC and the Veterinary Medicine Advisory Committee

(VMAC) (Ref. 8) in November 1994. At these meetings of the FAC and VMAC committees, FDA discussed the approach that developers of bioengineered foods were then using to evaluate allergenicity.

Developers of bioengineered foods have assessed allergenicity of proteins derived from sources that are not known to be allergenic in a manner consistent with the principles discussed at the 1994 allergenicity conference. FDA discussed this approach and the findings with regard to specific examples with the FAC and the VMAC committees, including the newly expressed protein in the FLAVR SAVR™ tomato (Ref. 9; see also Ref. 7) and other new proteins in several bioengineered foods (see Ref. 8). Developers of these foods derived from bioengineered plants evaluated whether newly introduced proteins exhibit the characteristics of allergenic proteins. To date, in the cases discussed with the Committees where the food, as consumed, contained new proteins, the newly introduced proteins were shown to lack the characteristics of allergenic proteins, except that the new proteins had molecular weights that fell within the range attributed to allergens. Many other proteins in food also have molecular weights that fall in the same range. Committee members agreed that this approach to evaluating allergenicity was based on the latest and most reliable scientific information. Based on the lack of similarity of the new proteins to known food allergens and other information available, FDA concluded the proteins discussed at the FAC and VMAC meetings did not present a concern with respect to allergenicity and that special labeling with respect to allergenicity was not required for the new foods in question.

iv. The 1996 Consultation Procedures

In June 1996, FDA provided guidance to industry on procedures for consultations between industry and the agency to address proactively issues that are relevant to bioengineered foods (the 1996 procedures) (Ref. 10; http://www.cfsan.fda.gov/~lrd/consulpr.html)[2]. Under that process, FDA recommends that a developer who intends to commercialize a bioengineered food meet with the agency to identify and discuss relevant safety, nutritional, or other regulatory issues regarding the bioengineered food prior to commercial distribution. When the developer believes that it has accumulated adequate data or information to address any issues raised during the consultation, the developer begins the "final consultation" by submitting to FDA information that explains its scientific and regulatory assessment of the food. This information should be sufficient to permit agency scientists to understand the approach the firm has followed in identifying and addressing relevant issues. This assessment should include a discussion of any information regarding any known or potential allergenicity of the expression products and the basis for concluding that foods containing the expression products can be safely consumed. In addition, the assessment should also include a discussion of the available information that addresses whether the potential for the bioengineered food to induce an allergic response has been altered by the genetic modification.

v. The 2001 Proposed Rule

Recently, FDA published a proposed rule in the Federal Register of January 18, 2001, entitled "Premarket Notice Concerning Bioengineered Food" (Ref. 2). The 2001 proposed rule would

[2] In October 1997, FDA made administrative revisions to these procedures to reflect reorganization within the Office of Premarket Approval, CFSAN, and the Center for Veterinary Medicine (CVM). In this document, FDA refers to these procedures as "the 1996 procedures" to reflect the year the agency first made them available.

require submission to the agency of data and information regarding plant-derived bioengineered foods that would be consumed by humans and animals. FDA has proposed this mandatory notification to ensure that all market entry decisions by industry are made consistently and in full compliance with the law. In the 2001 proposed rule, FDA reiterates the importance of evaluating the potential allergenicity of proteins introduced into bioengineered foods. In addition, FDA proposes that in the new proposed premarket notification for bioengineered foods, a notifier should include a discussion of the available data or information that address the potential that a protein introduced into the food will be an allergen (proposed § 192.25(f)(4)) (66 FR 4706 at 4720). The proposed regulation is consistent with the 1996 procedures, which recommend that a notifier provide FDA with information regarding any known or potential allergenicity and a discussion of the available information about the potential for a bioengineered food to induce an allergic response.

vi. Other Publications on the Evaluation of Allergenicity

Members of industry and academia have also taken steps to ensure that evaluation of allergenicity is predicated on sound scientific principles (Refs. 11-16). In 1996, the International Food Biotechnology Council (IFBC) in collaboration with the Allergy and Immunology Institute (AII) of the International Life Sciences Institute (ILSI) published a peer-reviewed report that proposes an approach to evaluating allergenicity of proteins in bioengineered foods (the 1996 ILSI/IFBC report) (Ref. 17). The approach taken by the scientists participating in this effort used a decision tree for the assessment of potential allergenicity. The 1996 ILSI/IFBC report recommends that bioengineered foods first be evaluated according to the allergenicity of the source of the donor gene. Proteins derived from allergenic sources are initially assessed by immunoassay using serum from individuals sensitive to the protein source. If serum testing proved negative, the 1996 ILSI/IFBC report recommended additional testing including skin prick and double blind placebo controlled food challenge (DBPCFC) to assure lack of allergenicity. Proteins derived from sources with no history of allergenicity are initially assessed for sequence similarity to known allergens. If sequence homology with an allergen is detected, immunoassay with serum from sensitive individuals is performed. If no sequence homology is found or if serum testing is negative, the 1996 ILSI/IFBC report recommended examining the stability of the proteins under simulated conditions of digestion and processing.

In 2000, the Food and Agricultural Organization of the United Nations (FAO) and World Health Organization (WHO) convened a Joint Expert Consultation on Foods Derived from Biotechnology and published a report on the safety aspects of bioengineered crops (the 2000 FAO/WHO report) which included a discussion of allergenicity (Ref. 18; http://www.who.int/fsf/GMfood/FAO-WHO_Consultation_report_2000.pdf). The 2000 FAO/WHO report supported the approach to allergenicity assessment described in the 1996 ILSI/IFBC report and adopted a slightly modified version of the 1996 ILSI/IFBC decision tree.

In January 2001, the Joint FAO/WHO Consultation on Foods Derived from Biotechnology was convened specifically to provide scientific advice in relation to the assessment of allergenicity of bioengineered foods (the 2001 FAO/WHO report) (Ref. 19; http://www.who.int/fsf/GMfood/Consultation_Jan2001/report20.pdf). The consultation focused on several items, including the general issues of allergenicity of bioengineered foods, the reevaluation of the decision tree for the assessment of allergenicity of bioengineered foods

developed in the 2000 FAO/WHO report, and the development of standardized procedures for consideration of the use of individual criteria used in the decision tree. After consideration of the current status of scientific information and extensive discussion, these scientists developed a new decision tree. This decision tree builds upon previous approaches to examining allergenicity but also includes several additional strategies. These strategies are targeted serum screening of proteins from sources with no known history of allergenicity in addition to no sequence homology to known allergens; the use of animal models; and the elimination of human testing.

In March 2002, the Codex Ad Hoc Intergovernmental Task Force on Foods Derived from Biotechnology agreed to recommend to the Codex Alimentarius Commission the "Draft Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants", including a Draft Annex on the Assessment of Possible Allergenicity (Proteins) (the allergenicity annex) (Ref. 3) for adoption at Step 8 of the Codex elaboration process. In the allergenicity annex, the Task Force acknowledges that there is no definitive test that can be relied upon to predict allergic response in humans to a protein new to the food supply, and recommended a "weight of evidence" approach. The assessment strategy outlined in the allergenicity annex begins with an initial data set consisting of the source of the newly introduced protein, any significant similarity between the amino acid sequence of the protein and that of known allergens, and its structural properties, including but not limited to, its susceptibility to enzymatic degradation, heat stability and/or, acid and enzymatic treatment. Specific serum screening is recommended for proteins that originate from a source known to be allergenic or have sequence homology with a known allergen. These data, as well as additional factors including absolute exposure and the effects of relevant food processing, contribute to an overall conclusion regarding human health risk. Targeted serum screening and animal models are also discussed in the allergenicity annex. However, neither is recommended for inclusion in an allergenicity assessment until fully developed and validated as predictive for human allergic response.

Current Approach to Assessing Potential Allergenicity

To date, with one exception[3], the proteins introduced into bioengineered foods that have been evaluated by the agency[4], are primarily metabolic enzymes, derived mainly from microorganisms or food plant sources. Enzymes are naturally occurring proteins that are ubiquitous in living organisms. A wide variety of enzymes has always been present in human food. In addition, many enzyme preparations derived from organisms have been used as processing aids and thereby become components of food that have been safely consumed as part of the human diet (Ref. 22). Using the recommendations of expert scientists, developers have

[3] The only new protein introduced into food through bioengineering reviewed for safety by FDA that is not an enzyme is Barstar. Barstar is the specific inhibitor of the enzyme Barnase, a ribonuclease. Barnase has been used in genetically engineered plants to generate plants that do not produce pollen and are therefore sterile. Barstar specifically inhibits only the function of the Barnase enzyme, thereby restoring fertility (Ref. 20; http://www.cfsan.fda.gov/~acrobat2/bnfM032.pdf; Ref. 21; http://www.cfsan.fda.gov/~acrobat2/bnfM057.pdf).

[4] FDA is the primary Federal agency responsible for ensuring the safety of commercial food and food additives, except meat and poultry products. FDA works closely on food safety matters with the U.S. Department of Agriculture (USDA), which regulates meat and poultry products, and with the U.S. Environmental Protection Agency (EPA), which regulates pesticides (including pesticidal substances engineered into food) and sets tolerances for pesticide residues in food.

assessed the possibility that these new proteins might cause allergic reactions in some individuals who consume the bioengineered foods containing them. The approach recommended by scientists at the 1994 Allergenicity Conference relies upon a comparison of the characteristic properties of the new proteins to those of food allergens[5]. The characteristics that are considered relevant to this assessment include source of the protein (i.e., is the source known to cause allergic reactions and if so are patient sera available?), sequence similarity to known food allergens, heat stability, stability under simulated conditions of gastric and intestinal digestion, glycosylation, and expression levels in food. Because exceptions have been reported for the observed characteristics of allergens, and no one factor is fully predictive, the conference scientists recommend that an assessment of allergenicity be based on the totality of available information. Conference scientists recommended that allergenicity assessments for bioengineered foods should be based on all available data, using a preponderance of the evidence to determine the likelihood that an expressed protein would be a food allergen.

The proteins introduced into food through bioengineering that FDA has reviewed for safety to date are similar in structure or function to other proteins currently found in food, and do not exhibit the characteristic properties of food allergens. Although FDA is confident of its existing approach to the assessment of potential allergenicity, we believe it is important to reexamine this issue in light of current scientific data and information and the possibility that proteins introduced into foods via bioengineering in the future may present new scientific challenges.

Current Allergenicity Issues

FDA recognizes that the scientific methods to assess allergenic potential are evolving. Recent reports on the assessment of potential food allergenicity, including the allergenicity annex, have reevaluated earlier approaches and recommended some new strategies based on recent scientific opinions on this issue. FDA is presently evaluating these recommendations regarding sequence homology, serum testing, digestibility/stability, and use of animal models. Below, the agency discusses some of the considerations related to these issues, including advantages and limitations. The agency is considering whether new scientific information would warrant changes in FDA's current recommended approach to assessing potential allergenicity of new proteins in bioengineered foods, and if so, what changes should be made. The following is a brief synopsis of the issues currently under debate by the scientific community.

Sequence Homology

Amino acid sequence analysis is an important consideration for identifying similarity between a new protein and a known allergenic protein (Refs. 1-3,17-19). However, there are not standardized "rules" that can be applied to how sequence comparisons are performed, nor are all allergenic proteins included in databases. Searches are also limited to examining linear sequence homology with known allergens that have already been sequenced, while it is known that discontinuous epitopes exist[6].

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[5] See for example, 59 FR 26702, May 23, 1994, for the agency's discussion of the safety assessment of the enzyme, aminoglycoside 3'-phosphotransferase II (APH(3')II), used in the development of bioengineered crops (Ref. 23).

[6] Epitopes are small regions of proteins that bind specific components of the immune system which, in the context of this discussion, refer to IgE antibody.

Further, the usefulness of sequence homology comparison is limited by a number of other factors, including the algorithms and strategies used for the search, criteria for evaluating the search, in addition to the composition, completeness, and design of the database. For sequence homology comparison to provide more compelling and reliable information in the assessment of potential allergenicity, the development of an unrestricted specialized allergen database is required. In addition, research is needed to define the minimal epitope required for sensitization and elicitation of an allergic response, to determine the effects of amino acid substitutions, to determine the significance of overall sequence homology, and to determine the importance of discontinuous epitopes. Finally, when sequence homology to a known allergen is detected, additional evaluation of that bioengineered food should be performed (unless product development is discontinued), including immunoassay using sera from individuals sensitive to the allergen in question.

Issues:

?? Current guidelines recommend amino acid sequence comparison of the new protein to known allergens. Which parameters of sequence homology (overall or segments of contiguous identical amino acids) are relevant to identifying potential allergens? Are there sufficient data to indicate what level of homology is meaningful in identifying potential allergens?

Serum Testing

Immunoreactivity with sera from sensitized individuals is another measure used to evaluate the potential allergenicity of bioengineered foods. Serum testing provides several benefits. Specific IgE against food antigens is often, but not always, associated with clinical allergy. Positive results of IgE reacting to a new protein provides a strong indication to proceed cautiously with the development of a bioengineered crop. This is based on the fact that IgE is implicated in immediate hypersensitivity reactions that are consistent with food allergies. In addition, serum testing is a minimally invasive and low risk procedure. Serum testing is an important element of current approaches for allergenicity assessment (Refs. 3,17-19).

To date, serum testing has been recommended for proteins derived from known allergenic sources or for proteins exhibiting sequence homology with known allergens. Under these circumstances, it has been possible to obtain sera from individuals sensitive to the particular allergenic substances in order to conduct the testing. Positive consistent results among several individual sera may indicate that further study is necessary to determine the clinical significance of the reaction. The occasional, in contrast to multiple, initial positive serological results in an enzyme-linked immunosorbent assay (ELISA) may be confirmed by other methods such as competitive inhibition with free antigen or Western blot. However, while positive results are suggestive of potential allergenicity, the presence of IgE antibodies does not always correlate with clinical allergy. In addition, negative results may be inconclusive. Furthermore, human sera for less commonly allergenic substances may not be available. Despite its limitations, specific

serum testing is currently one of the most useful methods for screening proteins derived from known allergenic sources or for proteins exhibiting sequence homology with known allergens.

In the case of proteins derived from non-allergenic sources, the choice of which sera to use is problematic. In that situation, no known human serum is available to serve as a positive control and the results of such testing would be difficult to interpret. A negative result would always be subject to the question of whether a sufficient number of sera were used. In addition, the criteria for the quality and quantity of 'normal' sera to be used have not been established.

Issues:

- ?? Are testing procedures including guidelines, protocols, reagents, and interpretation of results sufficiently standardized for screening of proteins for immunoreactivity to sera from sensitized individuals?
- ?? Are there sufficient data on the efficacy of targeted serum screening in the identification of potential allergens to warrant recommendation as part of an allergenicity assessment of bioengineered food?

Digestibility/Stability

Stability to digestion, in addition to stability to heat and other food processing conditions are among the characteristic properties that were first considered to be common among food allergens. Simulated gastric and simulated intestinal fluids are used as *in vitro* models for assessing the digestibility of proteins (Refs. 3,17-19). Proteins rapidly broken down into single amino acids and peptides smaller than 3.5 kDa are considered to be readily digestible (Ref. 19). The rationale for the use of digestion stability is based on initial information that food allergens were stable to digestion (Ref. 24). However, more recent studies comparing the digestion stabilities of food allergens and other proteins have shown that some allergens are labile to digestion and allergens are not necessarily more stable than non-allergenic proteins. Furthermore, the use of digestibility as a parameter to rank protein allergenicity is not fully supported by published data that demonstrate that major food allergens are not necessarily more stable to digestion than minor allergens (Ref. 12).

Another rationale to support the use of digestion stability as a criterion for allergenicity assessment is that stability to the conditions of the human digestive system is a key requirement to sensitize for food protein allergenicity. One published study suggests that the intactness of a protein may be necessary to sensitize a person (Ref. 25). However, degradation of food allergens may not prevent elicitation of allergic reactions in individuals that have previously been sensitized (Refs. 26-27). While there are few data in the literature to support the assertion that stability in human digestive tract is required for food allergenicity, a consensus exists among scientists that the likelihood of small peptides to sensitize an individual is low. Because allergic reactions involve the interaction of a protein with various components of the immune system and certain epitope structures are necessary for this interaction, the easier a protein is degraded by digestion, the less likely the 3-D structure necessary for binding is retained. In this respect, stability to digestion may be one parameter to measure the likelihood of a protein to interact with

the immune system when ingested, and therefore its ability to sensitize. However, it may not be useful to prevent entry into the marketplace of those proteins that cause human sensitization through routes other than ingestion (e.g., respiration or skin contact) or those proteins that cross-react with existing proteins that have already resulted in the sensitization of some individuals. In these cases, rapidly digested proteins, or previously degraded proteins, may still elicit allergic responses in individuals who have already been sensitized (Ref. 27).

Because the correlation between the allergenicity and the digestibility of a protein has not yet been established, it is difficult to relate, with confidence, the outcome of the stability/digestibility studies to the allergenic potential of a protein. The rapid digestibility of a protein does not offer any guarantee for the non-allergenic status of a protein. However, a protein that degrades rapidly is less likely to interact with the immune system and thus less likely to be allergenic. A protein that is resistant to digestion is more likely to interact with the immune system but is not necessarily an allergen.

For the purpose of increasing the predictive value of digestibility and stability in the assessment of potential allergenicity of bioengineered foods, a database of the digestibility of known food allergens and non-allergenic proteins should be developed. This database will enable scientists to determine the range of stability exhibited by food allergens and non-allergenic proteins and thus evaluate the predictive value of digestion stability for protein allergenicity assessment. Furthermore, scientists need to clarify the relationship between the resistance to degradation of a protein and its potential for sensitization and elicitation of allergic responses. Finally, standardized and validated assay conditions should be established.

Issues:

- ?? Currently, manufacturers test for digestibility using assays that simulate gastric and/or intestinal conditions. Is any particular pepsin resistance assay superior to other current tests that simulate digestive conditions for the purpose of allergenicity assessment?
- ?? Are there other relevant tests in addition to pepsin resistance for assessing protein degradation?

Animal Models

Because no one characteristic property of food allergens is predictive of potential allergenicity of novel proteins, there has recently been much emphasis on the development of an animal model. Several animal models of food allergy are currently under development, including Brown Norway rats (Ref. 28); Balb/c mice (Ref. 29); C3H/HeJ mice (Ref. 30); and Beagle dogs (Ref. 31). These models differ with respect to route of sensitization, route of challenge, symptoms exhibited, and responses evaluated. Many of the rodent models have been developed in strains that are genetically disposed to react with specific serum IgE to various test proteins. In the context of protein allergenicity, the development of specific IgE is the most relevant response in the vast majority of instances. The genetic predisposition of these animals also mimics the susceptible human population.

In addition to providing important information for understanding the mechanism of allergenicity, animal models may be useful in the prediction of the allergenicity of a novel protein and in risk assessment. With appropriate positive and negative controls, animal models may be important in determining the threshold of sensitization, the dose-dependent effects in sensitization and challenge, as well as the ranking of allergenic potency among proteins not associated with allergic reactions in addition to those proteins known to cause allergic responses.

There are numerous limitations to current animal models for the assessment of food allergenicity. Because no animal models have been validated at this time, assessment of protein allergenicity can be made only in association with other data and information. Few models have been subjected to ranking of allergenic potency of proteins using strong, medium, weak, and non-allergens. In addition, one factor common to both human and animal allergenicity is genetic predisposition. However, it is unlikely that a single animal model can adequately reflect the genetic variability of humans, in particular, the predisposition to respond to all potential allergens. Furthermore, published data show that the diet of animals must be free of the test protein for at least two generations prior to testing for potential allergenicity (Ref. 32).

For the purpose of developing a predictive test for the potential allergenicity of novel proteins, all promising animal models should further be characterized using proteins with a range of allergenic potency. All studies should employ both positive and negative control proteins. Research should develop fully animal models for food allergenicity that exhibit an immune response typical of an allergic reaction, a profile and specificity of food proteins, and a dose response to a wide range of food allergens. Additional models that exhibit clinical responses similar to humans, skin test reactivity, and use oral routes of sensitization and challenge should also be developed. Finally, the experiments should be easy to conduct and highly reproducible.

Issues:

- ?? Are any current animal models sufficiently developed to warrant recommendation as a part of an allergenicity assessment of bioengineered food?

Research

- ?? Are there areas of scientific research not currently addressed in the scientific community that might advance current approaches to assessment of possible allergenicity of proteins?

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