Forum

Workshop Overview: Approaches to the Assessment of the Allergenic Potential of Food from Genetically Modified Crops

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There is a need to assess the safety of foods deriving from genetically modified (GM) crops, including the allergenic potential of novel gene products. Presently, there is no single in vitro or in vivo model that has been validated for the identification or characterization of potential food allergens. Instead, the evaluation focuses on risk factors such as source of the gene (i.e., allergenic vs. nonallergenic sources), physicochemical and genetic comparisons to known allergens, and exposure assessments. The purpose of this workshop was to gather together researchers working on various strategies for assessing protein allergenicity: (1) to describe the current state of knowledge and progress that has been made in the development and evaluation of appropriate testing strategies and (2) to identify critical issues that must now be addressed. This overview begins with a consideration of the current issues involved in assessing the allergenicity of GM foods. The second section presents information on in vitro models of digestibility, bioinformatics, and risk assessment in the context of clinical prevention and management of food allergy. Data on rodent models are presented in the next two sections. Finally, nonrodent models for assessing protein allergenicity are discussed. Collectively, these studies indicate that significant progress has been made in developing testing strategies. However, further efforts are needed to evaluate and validate the sensitivity, specificity, and reproducibility of many of these assays for determining the allergenicity potential of GM foods.

Introduction and Overview of Food Allergy
(G. S. Ladics and W. Dong)

The prevalence of food allergy is approximately 1–2% in adults and 6–8% in children (Metcalfe et al., 1996; Sampson, 1997). Most food allergies are mediated by antigen-specific IgE and are characteristic of type-I reactions. It is such responses that are the focus of this paper (Fig. 1). Food allergic reactions occur typically in individuals who are genetically predisposed to allergy and who have been previously sensitized to the allergen (Sicherer, 2000). These reactions can range from effects on one or more of the following systems: gastrointestinal tract (nausea, vomiting, diarrhea); skin (urticaria, dermatitis, angioedema); and respiratory tract (rhinitis, asthma, bronchospasm). The potential consequences of food allergy can also be serious, with severe reactions (i.e., anaphylaxis) occurring in approximately 3 individuals per 100,000 a year (Burks and Sampson, 1997).

Overall, approximately 90 percent of all food allergies are associated with a small number of specific proteins represented by eight major allergens: peanuts, tree nuts, cows’ milk, hens’ eggs, fish, crustacea (e.g., shrimp), wheat, and soybeans (Metcalfe et al., 1996). Of the thousands of proteins in food, approximately 200, found among roughly 140 foods or food groups, are actually food allergens (Day, 1996; Hefle et al., 1996). The remaining 10 percent of food allergies are caused by less commonly allergenic proteins or minor allergens and affect a relatively small number of people (Hefle et al., 1996).

Importantly, food, whether developed by conventional means or through biotechnology, is a potential source of allergens. Nevertheless, there is a concern within the public, government, and industry that food allergens may be moved between foods or that proteins with no history of consumption may be proven to be allergenic when introduced into a food crop using biotechnology. Therefore, it is important that the allergenic potential of biotechnology-derived foods be evaluated. Additional information regarding the safety of genetically modified foods can also be found in a recently published position paper by the Society of Toxicology (2003).

Unlike respiratory allergies, such as hay fever, there are no
effective treatments for food allergy. Therefore, patients manage food allergy by avoiding the consumption of offending foods. Consequently, for biotechnology, allergy assessment has two main goals: (1) what assurances can be made that existing food allergens are not transferred into, or hidden in, new foods, and (2) what assurances can be made concerning the potential of an introduced protein to become a food allergen de novo. It is this second concern that was the focus of this workshop. The summaries that follow provide an overview of each speaker’s presentation.

Allergy Assessment of Biotechnology Products: What Are the Issues Associated with the Use of Animal Models? (M. P. Holsapple)

In spite of extensive efforts to characterize the mechanisms of allergy at both cellular and molecular levels, we still have only limited understanding of the characteristics that allow a protein to induce a specific IgE response, and that render an individual susceptible to allergenicity. Because of these complexities, it has long been recognized that there is no single parameter that can address allergic potential, and instead, there is a reliance on a weight of evidence approach. Historically, the strategy to address allergenicity of biotechnology products has been captured in a “decision tree” (FAO/WHO, 2001; Metcalfe et al., 1996), which relies on the following parameters: source of the gene, sequence homology, human studies including serum testing, pepsin resistance, the prevalence of the trait (i.e., level of expression of the protein in the plant), and animal models (Fig. 2). The remainder of this section will focus on animal models by raising some specific questions that must be addressed.

- What is the most appropriate use of an animal model in a decision tree? Should all proteins be tested? Should an animal model be weighed equally to pepsin resistance, as conveyed in the 2001 “decision tree”?
- What is the most appropriate endpoint for an animal model? Should we rely on clinical signs reflecting human food allergy or can allergic potential be predicted by measuring protein-specific bioactive IgE?
- What constitutes a positive allergic response in an animal model? Should we rely on antibody titer or the number of responders? How will the response in an animal model be used to estimate the “probability of allergenicity” (i.e., from high to low), as indicated in the 2001 decision tree (FAO/WHO, 2001)?


• What is the most appropriate design for an animal model? Is a rodent (i.e., mouse or rat) the best choice or should a nonrodent model that can better mimic human clinical signs (i.e., pig or dog) be employed? What kind of dosing regimen should be used (i.e., number and timing of sensitization doses)? Should the dose-response relationship always be determined? Should adjuvant be used?

• What is the most appropriate route of exposure? The oral route may be the most relevant for food; but the complication of oral tolerance by prior exposure to the protein must be overcome. Should the potential for exposure via inhalation (i.e., through exposure to pollen or dust from food derived from biotechnology) be considered? Should intraperitoneal (ip) injection be considered for sensitization and/or elicitation? An ip injection may represent the most direct assessment of the allergic potential for a novel protein, and it has been demonstrated that ip injections may overcome the tolerance that would be manifested if the protein were administered orally.

• What form of the protein should be tested in an animal model? Should the pure (i.e., recombinant) form be tested or should the response between the pure form and a crude extract from the plant be compared?

• What factors should be considered in the validation of an animal model? How should the concordance of an animal model (i.e., the ability to correctly identify both positives and negatives) be determined? For example, should the results of an animal model be compared with the results from pepsin resistance studies? Should the validation of an animal model be based on a rank order of potency for a number of allergens that reflects what is seen in a clinical profile with humans? Should the strategy for validating an animal model be based on a pool of proteins, and if so, how many and what proteins should be selected as candidates for positive and negative controls? Should the establishment of a “validated” animal model be predicated on the use of a common protocol (i.e., including proteins from the same source)?

There is no doubt that a validated predictive animal model would help in the hazard identification of biotechnology products. The term “validation” can be viewed as a challenging opportunity or an insurmountable hurdle. In that context, it is reasonable to question the state of validation for the following parameters: sequence homology, serum testing, and pepsin digestibility. It is also reasonable to question if we should expect more from an animal model than we could currently derive from parameters presently used in the “decision tree,” in terms of the concordance of results and the level of predictability.

Factors Relevant to the Allergy Assessment of Foods Derived from Biotech Crops (J. D. Astwood, R. E. Goodman, A. Silvanovich, and G. A. Bannon)

Recent progress in the understanding of both the natural history of food allergy and the characteristics that define food and other allergens has led to new insights into the potential predictive value of in vitro tools, with a view towards preventing the development of new food allergies. The most advanced of these tools include (1) the adaptation of bioinformatics techniques and (2) the refinement of two factors that influence protein exposures via foods: stability to digestion and protein levels.

Bioinformatics. Many of the major food and respiratory allergens have been identified and cloned; and today’s databases (e.g., http://www.allergenonline.com) contain many hundreds of protein sequences for allergens. Therefore, it is possible to screen candidate proteins for similarity to known allergens very early in product development, even before plants are transformed, by using bioinformatics tools such as FASTA (Pearson and Lipman, 1988). Proteins that share a high degree of sequence similarity are often homologous and share three-dimensional folds (Pearson, 2000). Aalberse (2000) has noted that proteins sharing less than 50% identity over their entire length are unlikely to be cross-reactive, and only then do they share more than 70% identity, does cross-reactivity become common. To illustrate, the tropomyosin protein is found in a broad array of animal species, yet allergic reactions are common to only a subset of these proteins. By creating a phylogenetic cluster diagram using the amino acid sequences of tropomyosin proteins (Goodman et al., 2002), a clear bifurcation in allergenicity can be distinguished that is consistent with taxonomic considerations (Fig. 3). However, lack of similarity to existing allergens alone is insufficient evidence to conclude lack of potential allergenicity, and bioinformatic data should be evaluated in the context of other information such as overall structural and physicochemical similarity (Astwood et al., 2002).

There is some concern that the FASTA search might miss short regions within a protein that are identical or highly similar in sequence to an existing allergen and have the potential to bind IgE. IgE-binding epitopes, however, have only been identified for a few allergens. In the absence of complete descriptions of IgE epitopes for all known allergens, a theoretical database of all potential epitopes for these same allergens can be screened by scanning all overlapping peptides (in this case eight or more amino acids in length) of all the allergens of the database, and comparing them in pair-wise fashion to all same-size potential peptides of the test protein, using computer software or scanning manually. A recent FAO/WHO scientific panel recommended using a six amino acid window for this type of analysis (FAO/WHO, 2001). However, Hileman et al. (2002) showed that an amino acid window size of less than eight amino acids resulted in a high rate of false positives. Another consideration is that two IgE-binding epitopes on the same molecule are required to cross-link high affinity IgE receptors on mast cells and induce an intracellular signal. Therefore, a single match in this analysis may or may not be clinically significant.
Digestibility in vitro and protein abundance. Proteins that are highly digestible would be expected to have less opportunity to exert adverse health effects when consumed. Standardization of the pepsin digestibility assay conditions (i.e., pepsin concentration, pH, temperature, etc.) has been described in the U.S. Pharmacopeia and is sometimes referred to as simulated gastric fluid (SGF). The pepsin assay was not meant to precisely mimic the fate of proteins in vivo conditions, but rather to evaluate the susceptibility of the protein to digestion under fixed conditions in vitro, and to provide information that, together with other evidence such as abundance (i.e., exposure), would be useful in predicting whether a dietary protein may become a food allergen.

Stability to digestion, in vitro (using pepsin), of the major allergens found in the most common allergenic foods was the first factor to be studied systematically (Astwood et al., 1996). Under the conditions described in this study, food allergens were more resistant to pepsin hydrolysis than were common plant proteins. However, not all allergens from the most common allergenic foods were stable in the pepsin digestion assay. Stability of the whole protein or fragments from the allergens tested ranged from 8 to 60 min, whereas stability for the nonallergenic plant proteins was ≤15 s.

Subsequent studies repeating the pepsin digestion assay on these major food allergens have been performed (Besler et al., 2001). In general, the original findings that these allergens were stable to pepsin digestion relative to nonallergenic proteins were confirmed, but the length of time that either the whole protein or fragments of the allergen were stable did not always agree. These differences may be due to subtle changes in the pepsin digestibility assay or in the method by which the proteins of interest were detected. Indeed, there may be an overreliance on the use of the pepsin digestibility assay as a single decisive parameter, as the assay is not intended to mimic human digestion processes perfectly (Bannon et al., 2002) and alterations of the conditions of the assay can yield different results (Fu et al., 2002). ILSI has proposed a standardization process for the assay so that results from different laboratories can be directly compared (ILSI, Protein Allergenicity Subcommittee Simulated Mammalian Gastric in Vitro Ring Study, unpublished).

Complete food allergens (having the capacity to both sensitize and elicit allergy) have several biochemical characteristics in common, including their abundance in food and the ability to promote IgE production and elicit IgE-mediated clinical symptoms (Aalberse, 1997). Another significant characteristic of complete food allergens is that they are stable to the proteolytic and acidic conditions of the digestive tract. However, incomplete allergens (having the capacity to elicit allergy, but not to sensitize) would not be expected to be stable to pepsin digestion (Bannon et al., 2002). Pepsin digestion stability is believed to impart on the allergen an increased probability of

![Tropomyosin phylogenetic tree and allergen cluster. Evolutionary distance based on AA sequence similarity to shrimp tropomyosin; shaded portion of tree indicates reported allergens.](image-url)
reaching the intestinal mucosa intact where absorption of significant quantities may lead to sensitization as well as impact the exposure rate of this protein to susceptible populations, an important variable in any risk assessment paradigm. While these are characteristics of most major food allergens, there are exceptions. Patatin (Sol t 1), the major allergen of potato (Seppala et al., 1999), is one notable exception. However, patatin represents >40% of total protein in potato, which equals about 5 g of patatin per serving. The emerging story is that stability and abundance together appear to be important factors, where stability may be a contributor to overall exposure. In the case of patatin, high exposure is achieved in the absence of digestive stability, and with the effect of sensitization.


Research in this laboratory with respect to food allergy has two main objectives. The first of these is to define the characteristics that confer on proteins the ability to induce allergic sensitization (Huby et al., 2000). The second, more pragmatic objective, is to develop a method for assessment of the inherent allergenic potential of proteins that could be used for the purposes of hazard identification and characterization.

Specifically, the aim is to develop an animal model that would provide a holistic evaluation of sensitizing activity, and which could be used in concert with other information to inform the safety assessment process (Kimber and Dearman, 2001; Kimber et al., 2000). To this end, we have chosen to examine immune responses provoked in BALB/c mice, a strain that is predisposed to mounting strong IgE antibody responses. The strategy is to distinguish among allergenic proteins and those that, despite being immunogenic, lack the potential to cause allergic sensitization. In this context, overall immunogenicity is measured as a function of the vigor of induced IgG antibody responses (measured by enzyme-linked immunosorbent assay; ELISA), while allergenicity is defined on the basis of IgE antibody production (homologous passive cutaneous anaphylaxis [PCA] assay) (Dearman and Kimber, 2001).

Our chosen experimental approach is to administer test proteins to mice, using intraperitoneal (ip) injection. Although it might appear more appropriate to use oral exposure for examination of the allergenic potential of food proteins, this is not necessarily the case. Thus, dietary exposure of rodents to proteins is known not to result in robust IgE responses, even to known allergens, probably due to the induction of oral tolerance. Gavage administration may be somewhat better, but (in our experience at least) is less sensitive than ip injection with regard to the production of IgE antibody (Dearman et al., 2001).

Presently, the standard protocol is as follows. Groups of mice (n = 5 or 6) receive various concentrations of the test protein in 0.25 ml of phosphate-buffered saline by ip injection. Seven days later this treatment is repeated. At various periods following the initiation of exposure, mice are exsanguinated by cardiac puncture, and individual and pooled serum samples are prepared for subsequent analyses. Using this approach, it has been possible to demonstrate that under conditions of exposure, where proteins display a comparable ability to elicit IgG antibody responses; there are marked differences in the vigor of IgE antibody production (Dearman et al., 2000, 2001). To illustrate this point, a representative experiment is shown in Figure 4 where responses induced by a known allergen (ovalbumin; OVA) have been compared with those induced by a crude potato protein extract (PPE) that is believed to be nonallergenic, or only weakly allergenic. The results displayed in Figure 4 reveal that although both proteins are of comparable immunogenicity (IgG responses), IgE, responses induced by exposure to OVA are much more vigorous. Experience of a relatively limited number of proteins has revealed that there is a strong correlation between what is known of the allergenic potential of proteins among exposed human populations and their ability to induce IgE antibody production in mice. Clearly such correlations, and the sensitivity and selectivity of this approach, require further evaluation and confirmation with a wider range of proteins that are known to be allergenic and proteins that are known or suspected of lacking the potential for sensitization. Such investigations are currently in progress.

Our view currently is that the failure to elicit IgE antibody production under conditions of exposure where immunogenicity is evident (on the basis of IgG responses) may, together with other sources of information, provide some reassurance.

![Figure 4](https://example.com/figure4.png)

**FIG. 4.** Differential antibody responses provoked by systemic exposure to ovalbumin (OVA), or to a crude potato protein extract (PPE) containing acid phosphatase activity. Groups of BALB/c mice (n = 5) received 0.25 ml of OVA or PPE (5% and 10% solutions) in phosphate-buffered saline by intraperitoneal injection, with the same treatment repeated 7 days later. Fourteen days after the initiation of exposure, mice were exsanguinated and individual and pooled serum samples tested for protein-specific IgG antibody by enzyme-linked immunosorbent assay (a), and for protein-specific IgE antibody by PCA assay (b). IgG antibody titers are displayed as mean and SE of IgG reciprocal titer (log2) for each treatment group. IgE antibody titer is recorded as the highest dilution of pooled serum resulting in a positive PCA reaction. In every experiment, sera from naive (untreated) animals were tested concurrently and were uniformly negative (data not shown). ND, not detectable.
that the protein lacks a significant potential to provoke allergic sensitization. It must be acknowledged, however, that the induction of IgE antibody production under these circumstances will not necessarily translate into a risk of sensitization when the protein is experienced via dietary exposure. Notwithstanding this consideration, the first imperative is to provide a robust method for hazard identification that can be used in concert with considerations of resistance to proteolysis, structural homology, and immunological identity to provide a reliable safety assessment. Our contention on the basis of investigations to date is that systemic exposure of BALB/c strain mice to test proteins represents the most suitable experimental approach if false negative results are to be avoided.

Finally, it is appropriate to look to the future and consider what opportunities may exist for the development of alternative strategies for characterizing allergenic potential. Although most animal models proposed for evaluation of the sensitizing activity of proteins are based on measurement of serological responses (and in particular the elaboration of IgE antibody), it may be relevant to consider alternative endpoints. For instance, it is clear that the initiation and maintenance of IgE responses are dependent upon the selective development of type-2 T helper (Th) 2 cells, is required for IgE production, whereas interferon γ (IFN-γ), a cytokine secreted by Th1-type cells, antagonizes IgE responses. Preliminary investigations in this laboratory have shown that known protein allergens (including peanut allergens) provoke selective Th2-type immune responses in mice, characterized by the selective secretion of IL-4 and other type 2 cytokines, following a challenge of draining lymph node cells with the relevant protein in vitro. It remains to be seen whether it may be possible to distinguish between protein immunogens and protein allergens as a function of divergent cytokine expression patterns.

Determination of Protein Allergenicity: Studies in a Brown Norway (BN) Rat Food Allergy Model (L. M. J. Knippels and A. H. Penninks)

For the safety evaluation of genetically engineered crops, the potential allergenicity of the newly introduced protein(s) has become an important issue. However, validated animal models to study the allergenicity of food proteins are not available yet. When developing an animal model, several aspects such as selection of species and strain, route of exposure for sensitization and challenge, as well as the use of adjuvants are important criteria to consider (Penninks et al., 2001; Taylor et al., 1996). We have developed an oral sensitization protocol in BN rats as this is a high-immunoglobulin (particularly IgE) responder rat strain (Table 1). We believe that for the evaluation of the intrinsic allergenic potential of new proteins, oral application is preferred and that the presence of an adjuvant is to be avoided.

We showed that upon daily intragastric administration of 1 mg ovalbumin (OVA) for 42 days, without the use of adjuvants, the animals developed OVA-specific IgG and OVA-specific IgE responses, as measured by both enzyme-linked immunosorbent assay and PCA assay (Knippels et al., 1998a). Although in general, 80% of the animals developed a response, sometimes no detectable OVA-specific IgE responses were induced. This was probably due to preexposure of the animals to OVA through their diets, which induced tolerance in the animals. In previous oral sensitization studies with soy proteins, we have shown that an important factor that influences the results of oral sensitization studies is unscheduled dietary preexposure of test animals or their parental generation to the antigen under investigation (Knippels et al., 1998b). In subsequent studies with BN rats, the sensitizing potential of hen egg white (HEW) and cows’ milk (CM) proteins was examined. Although antigen-specific IgG responses were found upon daily gavage dosing of the animals with different concentrations of HEW or CM, only a limited number of IgE responders was observed as measured by PCA. However, immunoblotting experiments with these rat sera demonstrated the presence of specific IgE antibodies against both HEW and CM proteins (Knippels et al., 2000). Moreover, both IgG and IgE antibodies present in sera of rats sensitized orally to HEW or CM and in sera of HEW- or CM-allergic patients recognized a comparable profile of allergens in these food products. These results indicate that the specific protein recognition of induced antibodies in the BN rat is comparable with that observed in sera from

| TABLE 1 | Characteristics of the Brown Norway Rat Model |
|-----------------------------------------------|
| **Advantages** | **Disadvantages** |
| Oral sensitization without adjuvants | Fairly large amounts of protein needed for sensitization |
| Clinical signs upon oral challenge (i.e., increase in permeability of the gastrointestinal tract, decrease in breathing frequency, decrease in blood pressure) | Relatively long dosing time needed for sensitization |
| Relative allergenicity of tested food proteins fits the human situation | To obtain naïve animals for the sensitization studies, the BN rats need to be bred and raised for at least two generations on a diet free from the protein(s) under investigation |
| Induced IgE antibodies are directed against the same proteins as compared to the IgE antibodies present in food-allergic patients |  |
allergic patients (Knippps et al., 2000). In a more recent study, BN rats were sensitized with different doses (0.01–10 mg) of either crude raw peanut extract or roasted peanut extract. Although no clear differences were observed in the sensitizing potency of crude, raw, or roasted peanut extracts, as measured by Th2-mediated IgG2a production, a marked difference in response was observed following either intraperitoneal or oral exposure. After oral sensitization, IgG2a antibodies were directed against all three major peanut allergens (Ara h1, Ara h2, and Ara h3); however, following intraperitoneal sensitization, IgG2a antibodies were mainly directed towards Ara h2 and to a lesser extent against Ara h1 and Ara h3.

More recently, the relative allergenicity of selected allergenic and nonallergenic proteins, based on human experience, has been investigated in the BN rat. In these studies, we used Ara h1 purified from peanut, Pen a1 (tropomyosin) purified from shrimp or beef tropomyosin, and Sol t1 (patatin) purified from potatoes. Preliminary results indicated marked differences in the two identical sensitization studies performed with these purified proteins. It was found that the rats in the first study had been unexpectedly preexposed in the diet to one allergen used for sensitization and to a cross-reacting allergen, and it is assumed that this affected the results. In the second study, the oral sensitizing potential decreased in the following order Ara h1 > Pen a1 > Sol t1, with no sensitization to beef tropomyosin in either study.

In the BN rat food allergy model, in addition to oral sensitization, oral challenge reactions were also investigated (Knippps et al., 1999). In previously sensitized animals, increased gut permeability was observed after an oral challenge. An oral challenge with OVA did not induce clear systemic effects on the respiratory system or blood pressure in the majority of animals. However, this low incidence is considered to be in accordance with clinical observations in food allergic patients.

In conclusion, the results obtained to date indicate that the BN rat might be a useful animal model for studying the potential oral allergenicity of “novel” food proteins. However, further testing with either whole food or with additional purified nonallergenic, weakly allergic, and strongly allergic proteins is needed to evaluate this BN rat model further.

Nonrodent Animal Models for Assessing Protein Allergenicity (R. M. Helm)

Among domestic animals that develop allergy, the atopic dog model (Ermel et al., 1997) and the swine peanut allergy model (Helm et al., 2002) are appropriate animal models that will provide a comprehensive understanding of IgE-mediated disease mechanisms and may predict potential allergenicity of novel proteins. Similar anatomy, physiology, and nutritional requirements of these animals combined with gastrointestinal and immune system maturation and enteral absorption of antibody are fundamental features of these animals that make them attractive to gastrointestinal food allergy investigations.

The natural food allergies and the physiological/immune maturation of the gut provide large animal models that mimic and extrapolate to clinical food allergy as characterized in the human (Table 2).

Food allergy is the third most common type of allergic disease in dogs, with approximately 8% of canines of all ages and breeds and both genders being affected. Clinical presentation of naturally occurring allergy is nonseasonal, with a generalized pruritis of varying degrees of severity and distribution. Ten to fifteen percent of dogs present with dermatological signs concurrent with gastrointestinal symptoms, including vomiting, diarrhea, bloating, and cramping. Utilizing this knowledge, Ermel and coworkers (1997) established a high IgE-producing spainel/basenji dog colony that responded with IgE antibodies to specific food allergens. Sensitized dogs, orally challenged with the specific food, presented with clinical manifestations of nausea and vomiting within 60 min, followed by loose mud pie diarrhea by 12–18 h later. Intradermal skin testing with relevant food allergens (wheat, milk, or beef) and a nonrelevant food allergen (soybean) revealed positive wheal and flare reactions to only the sensitizing food allergen. Gastroscopic food sensitivity tests (GFST), initiated by injection of food allergen into the gastric mucosa, revealed a wheal response at the site with mild to severe erythema and edema. Histologic analysis of tissues revealed acute inflammatory responses (interstitial and submucosal, periglandular edema) within 3–5 min, followed by a late-phase inflammatory response (epithelial vascular degeneration with cellular infiltration) 24–48 h later.

Swine have a natural IgE-mediated disease-like response to parasites, legumes, and pollens reminiscent of that in human allergy. In the veterinary literature, the introduction of soybean protein prior to weaning and positive skin tests to Ascaris suum antigen suggested an immediate type hypersensitivity response to food allergens could be established in young piglets. Helm et al. (2002) demonstrated that young piglets could be sensitized with peanut extract and, upon oral challenge with peanut meal, a moderate to severe gastrointestinal food allergy could be established. Peanut-sensitized piglets presented with a physical appearance that included whole-body or localized rashes, vomiting, and respiratory distress (stridor) within 60–90 min, followed by diarrhea 18–24 h later. Skin tests with peanut-

<p>| TABLE 2 |</p>
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<th>Nonrodent Animal Models</th>
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<td>Advantages</td>
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<td>Confirmed clinical and immunological evidence of natural food allergy</td>
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<td>Similar anatomy, physiology, and nutritional requirements to that of human</td>
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<td>Immunoapathogenic, mechanistic, and therapeutic intervention strategies</td>
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sensitive animals showed positive wheal and flare responses to peanut extract and the two major peanut allergens, Ara h 1 and 2. In the absence of an antisenine IgE antibody, passive cutaneous anaphylaxis with heat-inactivated serum failed to induce a wheal and flare reaction compared to non–heat-inactivated serum from peanut-sensitive animals, confirming an IgE-mediated response. Histological assessment of tissues from inflamed areas showed evidence of mucosal edema, enlarged goblet cells, and extrusion of mucus into the lumen when compared to stomach tissues from non–peanut-sensitized piglets. The small intestine showed marked edema, mucus secretion, epithelial denudation, and vascular congestion with hemorrhage in piglets responding to peanut challenge. Cytokine analysis of cell supernatants revealed minor increases in IL-4. IL-10 mRNA was increased in peanut-challenged small intestine.

The atopic dog and peanut allergy swine models provide ample opportunity for mechanistic studies of hypersensitivity, investigations into therapeutic strategies, and possibly prediction of protein allergenicity. The major concern lies in the large size and husbandry needed for these investigations and the associated cost. Ultimately, these animal models will provide valuable information characterizing the underlying mechanisms of food allergy, immunopathogenic studies, and therapeutic intervention studies that should translate well to human food allergy disease.

Workshop Summary

Advances in the science of allergy assessment for foods and feeds derived from biotechnology-derived crops include the availability of expanded and robust databases of known allergen sequences, refinements in bioinformatics criteria, and multicenter validation of in vitro digestibility assays such as the pepsin assay. As the needs for a more comprehensive risk assessment become apparent, appropriate public policy measures will likely include an evaluation of threshold doses and the need for a better understanding of how much allergen is too much (Taylor et al., 2002). Several animal models are also currently under development in both rodent and nonrodent species, which evaluate different endpoints, routes of exposure, and dosing regimens. Significant progress has been made in developing testing strategies to assess the allergenic potential of proteins; however, further efforts are needed to evaluate and validate the sensitivity, specificity, and reproducibility of many of these assays

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REFERENCES


