

Potential Allergenicity of Novel Proteins in Murine Models

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Bioengineered crops represent an important advancement for farmers who want to avoid losses caused by insect infestations or adverse environmental conditions. However, the use of modern biotechnology has raised questions regarding the safety of bioengineered foods because of the potential allergenicity of proteins expressed by the newly introduced genes. Standard approaches for safety assessment of these foods are still evolving. Animal models have been suggested as a tool that could help evaluate the potential allergenicity of such compounds. Several investigators are developing animal models to evaluate novel proteins, but none of these have yet been validated. This article reviews the published murine models, rat and mouse in particular, and the different methods used to evaluate parameters related to allergy. It also addresses the factors involved in the development of a model. Finally, it raises some questions that should be considered by the international community so that financial and intellectual efforts can be addressed in a unified manner.

Agricultural production has changed with the introduction of transgenic crops that are more resistant to certain adverse factors that, in the past, have led to significant economic losses. For example, transgenic crops have been developed that confer resistance to important insect pests or drought, or aid in weed control. There are other properties that can more directly benefit the consumer, such as improved taste or nutritional value. The increasing concern of agricultural bioterrorism is opening another possible application for the use of transgenic crops. For example, producers could use pathogen-resistant varieties to preserve production with minimum losses in the case of an attack. Whatever the rationale for their development, all genetically modified plants contain new inserted genes that express proteins responsible for conferring to the plant the characteristics mentioned above. However, these novel proteins have raised a number of safety questions regarding their potential ability to elicit allergic reactions in some

susceptible individuals who consume the novel protein-containing food (1).

The issue of potential allergenicity of novel proteins expressed by bioengineered plants has been addressed by expert panels organized under the auspices of the International Life Sciences Institute (ILSI), the International Food Biotechnology Council (IFBC; 2), the Food and Agriculture Organization (FAO), and World Health Organization (WHO; 3). These experts recognized the need to establish criteria for potential allergenicity of novel proteins. In considering those criteria, there was general agreement that proteins derived from known allergenic sources or having properties similar to those of known allergens should be subject to careful scrutiny. Two properties indicating similarity to known allergens that are generally agreed upon are amino acid sequence similarity and resistance to pepsin digestion. However, because physical and biochemical properties do not fully define the sensitizing capacity of allergens, animal models, if appropriately validated, were considered to be an important potential tool to address the assessment of potential allergenicity of novel proteins (3).

Currently, a few animal models seem to be promising; however, none of them have been adequately validated. Validated animal models would play an important role in premarket evaluation of the potential allergenicity of genetically modified foods. The focus of validation would be to avoid false negatives and false positives when testing novel proteins that would lead to unexpected allergic reactions.

In an ideal animal model, exposure to a potential allergen would give rise to immune sensitization through mechanisms similar to those found in human food allergy.

Food allergies are abnormal responses of the immune system to particular dietary proteins (4). These reactions are mediated mainly by one isotype of immunoglobulin (Ig) known as IgE. Even though the mechanisms of food allergy are not fully understood, the participation of different types of cells of the immune system and, to some extent, the regulatory mechanisms that lead to the development of allergies are known (Figure 1). Any type of allergy comprises 2 stages. The animal or person has to be initially sensitized to a particular protein. Antigen-presenting cells (APCs) internalize and process the foreign protein, and the resulting peptide-major histocompatibility complex (MHC) class II complexes are presented to specialized helper lymphocytes (Th cells), which activate B lymphocytes (B cells). These interactions are mediated by cytokines produced by the different cells involved in the process.

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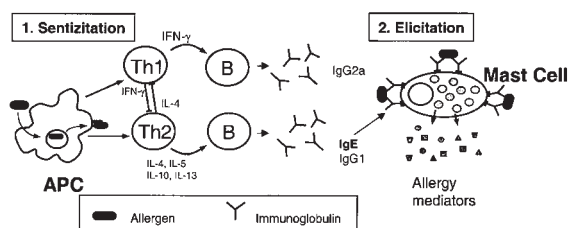


Figure 1. Mechanisms and factors involved in the development of an allergic reaction.

Allergic disease is characterized by the activation of type 2 Th (Th2) cells, which produce the cytokines IL-4, IL-5, IL-9, and IL-13. IL-4 plays an important role in maintaining the Th2 response and down-regulates the activation of the type 1 Th (Th1) cells. Th1 response is characterized by the production of a different group of cytokines, IL-2 and IFN- γ , among others, which down-regulate the Th2 response. Th2 cells are able to activate B cells for antibody production. IL-4 appears to be required to induce isotype switching to IgE production, while other Th2 cytokines may also act synergistically with IL-4 in this process.

The second stage in the allergic process is called elicitation of the allergic response. Once the animal or person is sensitized and, after subsequent exposures to the same allergen, the allergen will cross-link with 2 IgE molecules bound to specific receptors for IgE (Fc ϵ RI) on the surface of mast cells. Mast cells are granular effector cells of immediate hypersensitivity reactions and allergic disease found in tissues. Their activation results in release of vasoactive mediators, like histamine, which are responsible for the symptoms of the allergic disease, as well as other chemotactic factors and cytokines.

Murine Animal Models

Current models involve at least 4 animal species: dog, swine, rat, and mouse. This article focuses on the use of murine models.

There are several reasons for using rodents as animal models in large-scale studies to predict the potential allergenicity of novel proteins. Rodents are usually well characterized with respect to immune properties. Also, their small size allows the use of increased numbers of animals per experiment because they are less expensive to maintain and to house. Moreover, there are a wide variety of specific immunological reagents available. For immunizing purposes, they require a smaller amount of the testing protein, a limiting factor because novel proteins are usually expressed in such small amounts that it is very difficult to purify sufficient amounts to carry out an experiment in a larger animal. If necessary, results in rodent studies could be confirmed in other more costly models using smaller numbers of animals.

It is important to define the requirements for an animal model. What is the application of the model? This is probably

the most relevant question, and it is possible to obtain different answers in the literature. Most investigators support the idea that the animal model should resemble human food allergy. Models that focus on development of clinical signs similar to human allergy may be most desirable for understanding the mechanisms of food allergy and to study possible treatments. For the purpose of safety assessment of novel proteins, the ideal animal model should be able to make evident the intrinsic allergenic or nonallergenic features of the novel protein. Understanding how humans or animals recognize and process the protein in the digestive tract is not necessarily the main goal of this model. Proteins that are allergenic in humans must be allergenic in the model and vice versa. The end point of the model should be a biomarker that correlates with the sensitizing properties of the novel protein. For example, an end point might be IgE production measured in serum, or the predominance of IL-4 and IL-5 in the cytokine response.

Several factors affect the development of an animal model: species, strain, gender, age, route of sensitization, use of adjuvants, dose and immunization schedule, form of protein used, and tests used to evaluate the model.

Species and Strain

The most common rodents used in food allergy studies are mice and rats (Table 1), although a few studies have been done with guinea pigs (5–7). Animal strains with a predominant high IgE production are preferred as animal models of food allergy because it is known that atopic humans have a genetic predisposition to develop IgE responses (atopy), and that prevalence of food allergic individuals within the atopic population is high (8).

The mouse is the animal species most frequently used for studies related to the pathology and mechanisms of food allergy (9, 10). The mouse has also dominated in studies of oral tolerance (11–13), immunoprophylaxis (14), and treatment of food allergies (15–18). With respect to studies on the response to known allergens, the BALB/c mouse has been the strain of choice (Table 1). The BALB/c mouse is, immunologically speaking, a well-characterized inbred strain. It has also been widely used to study induction of oral tolerance by food allergens. C3H/HeJ and, to a lesser extent C57BL/6 and B10A strains, also have potential for this type of study. Among rats, the Brown Norway (BN) has been shown to be the most suitable strain for inducing specific IgE responses when compared to Wistar, Hooded Lister, and PVG rats, which failed to produced IgE (19).

Gender

Either males or females are used in studies. However, females may be preferable because the incidence of anaphylaxis caused by food allergens is higher in women than men (20).

Age

It is estimated that food allergies affect approximately 6% of children under 3 years of age. Because some children can outgrow their allergies (most commonly, milk, egg, and soya),

Table 1. Published studies to evaluate allergenicity of known allergens/nonallergens in murine models

Strain/species ^a	Gender/age ^b	Allergen ^c	Route ^d	Adjuvant ^e	IgE determin. ^f	Ref.
BN rats	M, 6–8 wks	OVA, SSM	i.p.	Carrageenan	PCA	(36)
BN rats	M, 6–8 wks	OVA	oral	Carrageenan	PCA	(37)
BN rats	M, 4–6 wks	OVA	i.g.	No	ELISA	(42)
BN rats	M, 4–6 wks	OVA	i.g., i.p.	No, Alum	ELISA	(43)
BN, HL, PVG, and Wistar rats	M, 4–6 wks	OVA	i.g., i.p.	No, Alum	ELISA	(19)
BN rats	M, 4–6 wks	OVA, HEW, CM extract	i.g.	No	ELISA (OVA), PCA (HEW), PCA (CM)	(66)
BN rats	M, 4–6 wks	OVA, HEW, CM	i.g.	No	ELISA (OVA), PCA (HEW), PCA (CM)	(44)
BN rats, BALB/c, B10A, and ASK mice	F, 6 wks	OVA	i.g.	No	ELISA	(63)
BALB/c mice	F, 8–2 wks	OVA, BSA	i.p.	No	PCA	(68)
C3H/HeJ mice	F, 3 wks	CM	i.g.	Cholera toxin	PCA	(34)
BALB/c mice	F, 8–12 wks	OVA, BSA	i.p.	None or carrageenan	PCA	(62)
BALB/c mice	F, 4 wks	BLG	i.p.	Alum	ELISA	(61)
C3H/HeJ mice	F, 5 wks	CPE	i.g.	Cholera toxin	PCA	(24)
BALB/c mice	F, 6–8 wks	BLG, infant formula	i.p.	Alum	ICST	(64)
BALB/c mice	Rats: F, 8–10 wks, Mice: F, 8–12 wks	OVA, PAP, PA	Oral, i.p.	No	PCA	(27, 69)
BN rats, BALB/c mice		OVA, PAP, PA	Oral (rats and mice), i.p. (mice)	No	PCA	(31)
ASW, AJ, BALB/c, B10.M, C57BL/6, CBA/s mice	F, 6–8 wks	Bos d 2, (r)Bos d 2 and its peptides HEL	i.p., s.c.	Alum, CFA, IFA	No IgE, only IgG1, IgG2a	(70)
BALB/c mice	F, 6–8 wks	OVA, PPE	Oral, i.p.	No	PCA	(71)
BALB/c, C3H/HeJ mice	F, 3–5 wks	CM	i.g.	Cholera toxin	ELISA	(32)

^a BN = Brown Norway; HL = Hooded Lister; PVG = Piebald Virol Glaxo.^b M = Male; F = female.^c OVA = Ovalbumin; SSM = semi-skimmed milk; HEW = hen egg white protein extract; CM = skimmed cow's milk; BLG = β -lactoglobulin; CPE = crude peanut extract; PAP = potato acid phosphatase; PA = peanut agglutinin; (r)Bos d 2 = recombinant Bos d 2; HEL = hen egg lysozyme; PPE = crude potato extract; BSA = bovine serum albumin.^d i.p. = Intraperitoneal; i.g. = intragastric; s.c. = subcutaneous.^e CFA = Complete Freund's adjuvant; IFA = incomplete Freund's adjuvant.^f ELISA = Enzyme-linked immunosorbent assay; PCA = passive cutaneous anaphylaxis; ICST = intracutaneous skin test.

only 1–2% of adults have allergies, mainly to peanuts, nuts, fish, and crustaceans (8, 21). Allergic reactions in humans are mainly due to early sensitization (exposure) to the allergenic food. The exposure might occur as early as before birth (22) or during the lactation period (23). Young animals are usually used as animal models (Table 1). Oral mouse models have shown that peanuts can induce hypersensitivity at a later age than cow's milk, which cannot sensitize adult mice (24).

Route of Sensitization

A relevant issue of food allergy studies in animals pertains to the route of sensitization. There is a strong rationale for the development of an orally sensitized animal model (25) because it is thought to reflect more accurately the actual route of sensitization in food-allergic individuals. One of the difficulties of developing oral murine models is their innate tendency to develop oral tolerance to ingested antigens (26). Moreover, the oral approach may not possess the sensitivity and reliability to provide an initial assessment of allergenic potential (27). This results in a risk of false negatives owing to the development of tolerance to a particular protein, a situation that would not resemble events in the human allergic responses. This is a major concern when working with novel proteins of unknown allergenicity.

The BALB/c model has become controversial because these mice have been shown to be hyporesponsive to oral protocols (28, 29). However, Kimber and Dearman (30) were able to induce IgE in orally sensitized BALB/c without the use of adjuvant, even though responses are generally much less robust than those provoked by intraperitoneal administration of the same protein (31). Fritsche (26) was also able to sensitize BALB/c mice orally to β -lactoglobulin using cholera toxin as adjuvant. In contrast, Morafo et al. (32) studied the induction of Th1/Th2 responses in C3H/HeJ and BALB/c mice following oral sensitization. C3H/HeJ had a typical Th2 profile, whereas BALB/c resulted in a Th1 response. However, the study design was previously optimized for C3H/HeJ and the same conditions were used to evaluate BALB/c as an oral model. Because, though the 2 strains are likely to respond differently because of genetic background, it is possible that the protocol (dose and immunization schedule) is not suitable for BALB/c and would require further optimization.

The alternative to oral route of sensitization is parenteral administration of the allergen. This approach avoids tolerance problems and evidences the inherent ability of proteins to induce biomarkers of allergenicity, such as IgE. However, induction of an IgE response after parenteral administration of the test protein will not necessarily translate into allergic reaction after oral exposure. This could be due to differences between the biochemical and immunological environments of the digestive system and the peritoneal cavity, which might affect the physical-chemical properties of the proteins and their further presentation to the immune system.

Use of Adjuvants

Adjuvants induce a nonspecific inflammatory response that enhances the immune response to a particular antigen given simultaneously. Moreover, adjuvants can polarize the cellular response toward Th1 or Th2 type. The use of Th2-boosting adjuvants has been suggested in order to sensitize rodents, particularly mice (24, 26, 33, 34). Cholera toxin is the most common adjuvant used in oral sensitization because it induces Th2 responses and the production of IL-4, in particular (35). Aluminum hydroxide and carrageenan, used parenterally, also induce type-2 polarization (36–39). However, it is not known whether adjuvants can modify the inherent properties of proteins to induce IgE responses or whether weak allergens or nonallergenic proteins, when used with certain adjuvants, could induce an IgE response, resulting in false positives (40). Consequently, the avoidance of adjuvants has been advocated by some investigators (19, 41–45). In the case of testing food extracts, it is possible that some food components could act as adjuvants. It has been suggested that some hydrolyzed milk formulas may have adjuvant-like activity (46).

Number of Mice per Group and Ratio of Responders to Nonresponders

Responses of individual animals to allergens may be variable within the same study. Because there is no standardized protocol for conducting animal trials to assess allergenicity, there is no agreement on the minimum number of animals per experiment required to give a minimum level of confidence. There are also no data indicating the minimum percentage of responders necessary to draw conclusions about the allergenicity of the protein. Is a single responder within a group enough to classify the studied protein as a potential allergen?

Dose and Immunization Schedule

The choice of dose to be administered to the animal is crucial, because high doses can induce tolerance (47, 48). Lower doses have been shown to induce more severe reactions than higher sensitizing doses (24). However, very low doses fail to induce IgE responses in an oral mouse model (24). There may also be differences in optimal dosing regimens for different strains of mice. For instance, BALB/c mice required a higher sensitizing dose to elicit Th2-type response than C57BL/6 after nasal sensitization with ovalbumin (OVA; 49). The optimal dose also depends on the type of protein to be studied. The induction of peanut-specific IgE required a lower sensitization dose, fewer sensitizing doses, and a shorter sensitization period than milk proteins in an oral mouse model (24, 34). This result suggests that the less allergenic the protein is, the higher sensitizing dose and more injections are required to induce an IgE response. A comparison of dosing schedules that are required for sensitization is one way to assess the relative allergenicity of proteins. An important factor that influences sensitization studies is unscheduled dietary pre-exposure of test animals,

i.e., animals exposed earlier in life to a particular protein can become nonresponsive to the same protein in later studies (36). This issue can be extended to the parental generation exposed to the antigen under investigation. This exposure can cause oral tolerance in their descendants that are used in oral sensitization studies, even though they are under an allergen-free diet (41).

Form of Protein

(a) Purified protein vs extracts.—Is it more appropriate to study the overall ability of a food extract to induce allergic reactions, or should we study the purified protein and attempt to evaluate its contribution to the overall allergenicity of the food? Purified proteins have been more commonly used in animal models of food allergy, mainly in those of parenteral sensitization (Table 1), whereas food extracts have been used more extensively in oral sensitization studies. The primary advantage of using food extracts for sensitization in a model is that it more faithfully mimics the situation in normal dietary exposure in allowing for the possible augmenting effects of other foods' components. Additional complicating factors associated with the use of food extracts are the potential to develop responses to multiple allergens (e.g., both *Ara h 1* and *Ara h 2* may be present in peanut extracts) and the possible effects of other food components on the response.

A related concern could arise when assessing the allergenicity of a novel protein introduced in an already allergenic food. This is important when considering the potential allergenicity of bioengineered foods. For example, soybean and corn are reported to be allergenic and are even able to induce anaphylactic reaction in very sensitive individuals (50, 51). They have also been engineered to contain novel proteins introduced in the food chain. It may be possible that other components or properties of these foods contribute to sensitization of allergic individuals and might influence the response to a bioengineered protein. In fact, the possible adjuvant-like effects of nonallergenic components of foods is itself an important question to be addressed when animal models are used.

(b) Recombinant vs native form of the protein.—Studies involving bioengineered proteins usually use the recombinant form of the protein because the native form is present in the crop in very low amounts. Recombinant proteins are easy to obtain in the larger amounts needed for testing purposes. One of the main concerns derived from the use of recombinant proteins produced in prokaryotic organisms is that of post-translational differences, e.g., glycosylation, with respect to eukaryotic organisms. Glycosylation is one post-translational modification that some of the known allergens seem to share. It has been reported that glycosylated moieties might play a role in the allergenicity of a protein (52). However, because this is not a unique feature of allergens and not all of the known allergens carry this post-translational modification, this parameter should not be used to discriminate between allergens and nonallergens.

End Points for the Model

Much of the diagnosis of food allergy and many research studies rely on the measurement of IgE as a unique marker of food allergy, even though the presence of specific IgE is not necessarily predictive of an allergic reaction after exposure to the offending food. Therefore, it would be desirable to confirm the IgE response with other humoral and/or cellular parameters.

(a) Study of the humoral response—antibody production.—IgE has been used as a biomarker to measure the level of sensitization to a particular allergen (53, 54). Several laboratories that have developed animal models to evaluate the allergenicity of food proteins have used different techniques to measure IgE (Table 1). The absence of standardized methods among studies makes it difficult to compare models and to define whether an animal is suitable as a model to predict allergenicity. In addition, false negatives are possible if the technique used is not sufficiently sensitive or specific.

Several possible techniques are used to measure antigen-specific IgE. The most popular test is the enzyme-linked immunosorbent assay (ELISA) performed in antigen-coated microplates. Immunoblotting is another type of ELISA, in which proteins are previously separated in a polyacrylamide gel by electrophoresis and transferred to a nitrocellulose, nylon, or polyvinylidene difluoride (PVDF) membrane. This approach has the advantage of distinguishing the number and approximate molecular weight of the proteins recognized by IgE. Determination of IgE by ELISA and immunoblotting is limited by the ratio of IgG:IgE. Because IgG can be 10 000 times more concentrated than IgE in serum, it can effectively inhibit binding of IgE in situations where the same epitope is being recognized. In the case of ELISA and immunoblots, there are a relatively small number of available epitopes, making this competition a significant potential problem that could result in false negatives for IgE. However, this problem can be overcome by limiting the number of booster doses and by optimizing the assay. Knippels et al. (44) found that the ELISA method they developed was only suitable for detecting antigen-specific IgE raised against single proteins, not for protein extracts.

Two methods used in human IgE detection, the radio allergosorbent test (RAST) and CAP[®] RAST (Pharmacia, Kalamazoo, MI), circumvent the problem of IgG competition for epitopes by using paper disks or sponges as an antigen-binding matrix. These matrixes contain a very large number of potential epitopes relative to ELISA methods, thus reducing the impact of IgG inhibition. The use of these methods for murine IgE, however, has been very limited. Radioimmunoassay (RIA) techniques, which use sensitive radioactive labels, have also been successfully used for the detection of rat IgE from rats infested with nematodes (55).

Passive cutaneous anaphylaxis (PCA) is a functional test that measures biologically active IgE. In this test, the serum from a sensitized mouse is injected intradermally in a naïve mouse. Subsequently, the animal is administered Evans Blue

and the allergen of interest. Cutaneous reactions will be identified by the blue spots at site of injection. It has been considered as one of the most important *in vivo* models of anaphylaxis in local allergic reactions (56). IgE is measured based on the inflammatory response resulting from the antigen-IgE binding. However, it is labor-intensive and not as sensitive as ELISA. In addition, murine IgG1, at higher concentrations than IgE, can also trigger PCA responses. However, IgG1 and IgE responses can be distinguished because of their different biochemical characteristics. IgE is heat-labile and active after 48 h of sensitization, whereas IgG1 is heat-stable and active only up to 2 h. The guidance for industry regarding "Immunotoxicology Evaluation of Investigational New Drugs" edited by the U.S. Food and Drug Administration, Center for Drug Evaluation and Research (57) does not recommend the use of PCA or related tests for routine safety evaluation of investigational new drugs, because of the occurrence of false-negative findings, which should not be interpreted as indicating that an experimental drug cannot produce anaphylactic reactions (58). ELISA and immunoblotting have been demonstrated to be more sensitive than PCA in determining specific IgE (42, 44).

The rat basophilic leukemia (RBL) assay is another functional test that allows determination of the allergenic potency of extracts of purified proteins (59). It uses the basophile leukemia (RBL)-2H3 cell line (60). Serum from the test mouse is incubated with RBL cells. In the presence of a specific antigen, if the serum contains antigen-specific IgE, RBL cells will release β -hexosaminidase, among other mediators of the inflammatory response. The activity of this enzyme is measured and correlated with the presence of IgE. Measurement of histamine released after challenge is another parameter related to the presence of IgE.

Allergen-specific IgE could also be used to characterize protein and epitope recognition patterns. For safety assessment purposes, an animal model ideally should be able to recognize the same allergenic proteins recognized by humans. IgE produced by orally sensitized BN rats recognized the same egg and milk allergenic proteins as human IgE from allergic patients (44). BN rats also recognized the same milk allergens as human allergic serum when the rats were sensitized intraperitoneally (36). More detailed information can be obtained by epitope mapping, which describes continuous epitopes recognized by IgE. C3H/HeJ recognized the same Ara h 2 epitopes as human sera (24).

How relevant is it that human IgE and the model IgE bind the same epitopes? It might be important in the process of evaluating bioengineered proteins or other novel allergens because one of the parameters for evaluating novel proteins is sequence similarity, which translates into continuous epitopes. The characteristics that define an allergen are not fully understood. It is possible that other unknown properties can influence the inherent allergenicity of proteins. Individual variation in immune response may also play a role, accounting for the observation that there is some variation in the epitopes recognized by human allergic sera in response to the same

allergen. More information will be required before conclusions can be made about the importance of shared IgE epitopes between an animal model and the allergic individual.

Other types of immunoglobulins are used to characterize the humoral immune response. The measurement of total specific IgG indicates only that the protein is immunogenic, i.e., it is recognized by the immune system as a foreign protein, but does not indicate that the protein is allergenic. Additional information on the allergenicity of a particular protein can be derived from characterizing IgG subclasses, IgG1 and IgG2a. IgG1 is characteristic of a Th2 response and is usually elevated in allergic individuals. On the other hand, high values of IgG2a are indicative of Th1 responses. Th1 responses to protein antigens generally predominate in nonallergic individuals. Studies should include the parallel evaluation of IgE, IgG1, and IgG2a. Milk proteins, like OVA and β -lactoglobulin have been reported to produce high IgG1 responses in orally or parenterally sensitized mice and rats (61–64). Several less-allergenic food proteins (e.g., bovine serum albumin) were shown to induce high IgG2 titers (62). IgG1 and IgG2a measurements as indicators of Th2 and Th1 responses are, in general, strain, route of sensitization, and antigen dose-dependent (27, 49). These measurements are valuable as complementary end points, but are not an adequate substitute for IgE measurement.

(b) Cellular response.—The cellular component of the allergic response is also used to better characterize the immune response and to complement information obtained from the evaluation of the humoral response. Cytokine profiles are potentially highly relevant to the sensitizing properties of novel food proteins. Lymphoid cells are isolated from sensitized animals and are incubated with the test protein or allergen. Cytokines in culture supernatants are measured by ELISA. It is expected that animals producing IgE and IgG1 will also produce Th2-associated cytokines, i.e., IL-4, IL-5, IL-9, and IL-13, whereas animals that respond with high levels of IgG2a will produce IFN- γ , a marker for Th1 responses. A few studies have investigated the cytokine profile induced by allergenic and nonallergenic food proteins. Peanut lectin, a minor peanut allergen, has been shown to induce the production of Th2 cytokines in BALB/c (65). Milk proteins also induced the same type of response in C3H/HeJ mice (34). Cytokine production usually is accompanied by the study of lymphoid cell proliferation in response to antigen.

Physiological Response to Challenge

Another way to confirm whether an immunized animal has been sensitized to a dietary antigen is to perform a challenge test. Several parameters such as respiratory function, blood pressure, gut permeability, mast cell degranulation, vascular leakage, and hives, among others, can indicate whether the animal underwent an allergic episode after exposure to the test protein (19, 34, 37, 43, 66–71). A negative response in the challenge does not necessarily mean that the protein is not allergenic. The protein can possess the ability to sensitize the animal but be incapable of eliciting the allergic response (52). These proteins are considered incomplete allergens; allergens

that are able to both sensitize and elicit an allergic reaction are called complete allergens. It is quite frequent that skin-prick tests are positive and further oral challenges, such as the double blind placebo control food challenge (DBPCFC), are negative. It is also possible that positive results may not indicate a type I hypersensitivity reaction mediated by IgE if some component of the food or test material has inherent inflammatory properties. Challenge tests can augment the model, but they may not substitute for measurements of cellular and humoral sensitization, especially measurement of IgE.

Validation of Models

There is no consensus on the criteria for validating an animal model of food allergy. A validation protocol should include the evaluation of the robustness and reliability of the methods used to test the different parameters that define food allergy. A given animal model must be evaluated for its ability to produce allergen-specific IgE and to induce Th2 responses, and whether it can discriminate between allergic reactions caused by allergenic proteins and the lack of reactivity of nonallergenic proteins. Protocols, such as determination of IgE, associated with that particular animal model should be standardized, and the ranges for parameters (doses, number of immunizations, etc.) should be established.

Conclusions

There is still a long way to go to have a validated animal model. There is a need to validate the model, and additional efforts are needed to standardize the methodology used to evaluate the different parameters associated with food allergy, such as methods for the determination of IgE. The demonstration of allergen-specific IgE production and/or the induction of Th2 responses should be taken as an indication of potential hazard, which must be re-evaluated, taking into account physical and biochemical characteristics of the protein as described in WHO/FAO guidance (3).

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