

Food Allergy Animal Models

An Overview

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ABSTRACT: Specific food allergy is characterized by sensitization to innocuous food proteins with production of allergen-specific IgE that binds to receptors on basophils and mast cells. Upon recurrent exposure to the same allergen, an allergic response is induced by mediator release following cross-linking of cell-bound allergen-specific IgE. The determination of what makes an innocuous food protein an allergen in predisposed individuals is unknown; however, mechanistic and protein allergen predictive models are being actively investigated in a number of animal models. Currently, there is no animal model that will actively profile known food allergens, predict the allergic potential of novel food proteins, or demonstrate clinically the human food allergic sensitization/allergic response. Animal models under investigation include mice, rats, the guinea pig, atopic dog, and neonatal swine. These models are being assessed for production of IgE, clinical responses to re-exposure, and a ranking of food allergens (based on potency) including a nonfood allergen protein source. A selection of animal models actively being investigated that will contribute to our understanding of what makes a protein an allergen and future predictive models for assessing the allergenicity of novel proteins is presented in this review.

KEYWORDS: food allergies; animal models; IgE

IgE-mediated allergic reactions to food proteins may induce a variety of cutaneous, gastrointestinal, and systemic symptoms. A delineation of the control mechanisms regulating IgE-antibody synthesis and the characterization of food proteins as allergens are important considerations in determining the pathogenesis of allergic disease. The normal immune response to dietary proteins is usually an increased mucosal immunity associated with an active

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suppression of the systemic response. For reasons yet to be defined, however, genetically predisposed persons respond to innocuous food proteins in an abnormal manner resulting in food allergy.

The immunopathogenesis of food allergies may be governed by more than one immunologic mechanism, including immediate manifestations mediated by IgE; inflammatory reactions caused by immune complexes, lectins, and superantigens; and delayed hypersensitivity reactions associated with specific T lymphocytes. This discussion will only address IgE-mediated food allergy. Two separate and important issues need to be considered in food allergy: first, a comprehensive understanding of the mechanisms of IgE-mediated disease and, second, an approach to predicting the potential allergenicity of novel proteins using information regarding known food allergens. The characteristics of a food allergen and what determines that a common food protein will be recognized by the gastrointestinal immune system as an allergen are largely unknown. In the assessment of food protein allergenicity, characterization of the allergen should include an accumulation of physicochemical, immunochemical, and biochemical information that is central to immediate type of hypersensitivity. Included in this assessment is a greater understanding of the molecular events of what makes food proteins allergenic and of what contributes to the sensitization of an individual and the elicitation of an allergic reaction.

The traditional definition of an allergen is an antigen that is able to produce an allergic reaction by inducing IgE formation. In determining protein allergenicity, two other essential definitions merit attention: (1) sensitization, or the ability to induce the formation of IgE antibody, and (2) the allergic reaction—the property of that protein to elicit an allergic reaction (i.e., the ability to trigger symptoms upon exposure). The reader is referred to excellent manuscripts on the structural biology of allergens¹ and the toxicology of protein allergenicity.² What is in agreement is that an allergen has some or all of the following characteristics: solubility (in the case of food allergy, the ability to cross the gut mucosal barrier), stability (heat and digestive enzyme resistance), and structure (surface molecule exposure). What is apparent is the abundance of knowledge concerning food allergens and the lack of information regarding nonallergenic antigens with respect to the immune response. The difference between immunogenicity and allergenicity is the ability of allergens to induce specific IgE antibody of sufficient avidity/affinity to cause sensitization and, upon re-exposure, the ability to induce an allergic response. What triggers sensitization to food proteins in food allergy continues to be an unknown.

For ethical and efficacious reasons, sensitization studies in humans are not possible. Therefore, what is needed for the assessment of proteins as sensitizing allergens is a validated animal model that mimics the allergic responses in humans. At present, no validated animal model is available for determining the sensitizing threshold of food allergens that can be extrapolated to or be

TABLE 1. Comparison of allergen sensitization in mice

	Sensitizing dose	Boosting	Duration of sensitization	Age
Peanut	5 mg/mouse	Single	3 weeks	3 and 5–6 weeks
Cow's milk	10 mg/mouse	Multiple (5)	6 weeks	Only 3 weeks

predictive of the human allergic response. Therefore, the role of food allergy animal models into allergenicity should be to accurately assess the overall potential of a protein to induce the production of protein-specific IgE sensitization and the elicitation of an allergic response upon repeated challenge of the offending protein source.

Animals models are being used to assess to very different mechanisms: (1) a mechanistic approach to the understanding of IgE-mediated disease and (2) to determine the allergenicity of novel proteins. Several animal models have provided important information for understanding some of the mechanisms of allergenicity. Examples of animal models include the mouse, rat, guinea pig, and dog. As in humans, animal models have an innate tendency to develop tolerance to the myriad of proteins ingested, and it is difficult to generate valid food allergy models. A number of methods have been introduced to bypass the state of tolerance and initiate a food hypersensitivity that reflects human IgE-mediated food allergy and will be discussed with the model.

The degree of sensitization should take into consideration the following parameters: (1) The concentration of the allergen (high doses are known to induce tolerance; however, the high-dose tolerance/low-dose sensitization is relative to the host and the antigen/allergen source). (2) The allergen should be taken in context with the food source. (3) The route (feeding and/or gavage are the recommended avenues) and duration (time course may vary with respect to species and allergen) of allergen exposure. (4) Age of the animal (neonate, adolescent, adult).

For example, in comparing two well-known allergen sources—peanuts and cow's milk—differences in allergen sensitization were shown in mice³ that correlate with those in humans (TABLE 1).

Other considerations include the following: (5) A genetic predisposition (high and low IgE responders). (6) The use of adjuvants (natural or artificial—cholera toxin, *Bordetella pertussis*, and carrageenan are known IgE-selective adjuvants). (7) Isotype specificity response (mice respond with two anaphylactic antibodies, IgG1 and IgE; rats with IgG2a and IgE; guinea pigs with IgG1 and IgE; dogs with IgE; and pigs, likely to be IgE). (8) Th1/Th2 regulation/polarization. (Mice have very delineated Th1/Th2 polarization, whereas in the human polarization is not as discrete.)

In addition the animal model should demonstrate dose dependence to a wide range of food allergens without responding to nonallergens in the production of IgE comparable to what is seen in humans. The allergenicity pro-

file should be comparable to what exists in the human response to different allergens, for example, anaphylactic episodes induced by peanut allergens to oral/pharyngeal symptoms elicited by fruit and vegetable allergens in the oral allergy syndrome. The simple production of IgE and binding of IgE to relevant proteins should be considered insufficient without evidence of histamine release using *in vitro* tests to support cross linking of IgE to bound mast cells and basophils.

Ultimately, the question of allergenic sensitivity and the allergic response is not whether a novel protein is capable of sensitizing an animal, but at what concentration and which population is susceptible. For example, the Brown Norway rat, BLAB/c, and other strains of mice are good IgE responders. The C3H/HeJ mouse strain has a point mutation in Toll-like receptors that underlies a defect in the LPS-induced cytokine production by peritoneal macrophages,⁴ which may influence the response to specific allergens. These and other strain variations need to be taken into account when establishing a model representative of the human response to allergens. Consideration must also be given to the differential induction and regulation of chemokine production by different antigen-presenting cells between the species under investigation. Furthermore, allergenicity cannot be predicted by analysis of either purified or crude protein product; the physiological basis of antigen transport and modification by the respective organisms must be taken into account. Factors include the lumen hydrolytic environment and the degradation by digestive enzymes of the host and commensal flora, and the uptake, processing, and presentation of proteins by the immune systems under investigation. In this report, an overview of some of the representative animal models will be presented demonstrating the evidence in favor of animal models for determining food protein allergenicity. Additional models, which also contribute to our knowledge of food allergy, are available in the literature. The following represent animal models and those with which the author is closely involved.

MOUSE ANIMAL MODELS

Inbred strains of mice have been characterized as being either high or low IgE-responder animals for both inhalant and food allergens. As in humans, two separate events are required: the first event is a sensitization phase and, in the case of mice, the production of two anaphylactic antibodies, IgE and IgG1; the second phase is characterized as the allergic challenge following re-exposure to the allergen at the site of the response. In both inhalant and ingestant sensitizations, short-term daily exposures over a period of days or long-term exposure once a week over several weeks have been shown to induce allergen-specific IgE/IgG1. Differences in strain MHCs have been sug-

gested as a likely reason for differences in mouse models' responses to different allergens, which may parallel a genetic predisposition in humans.

Dearman *et al.*⁵ investigated the characteristics that confer allergenic activity on proteins by comparing the immune responses induced in BALB/c mice by BSA (limited potential to induce sensitization) and OVA (a common food allergen). Groups of mice received protein intranasally or intraperitoneally with or without carrageenan as adjuvant. Irrespective of the route of administration, OVA displayed a significantly greater potential to elicit IgE antibody production than BSA. Both OVA and BSA stimulated strong IgG and IgG1 antibody responses that clearly demarked the immunogenicity and allergenicity of the two proteins in BALB/c mice. The reciprocal ability of these two allergens to elicit IgE or IgG2a antibody production led the authors to suggest that the integrity of the immune responses is regulated by CD4 Th cell cytokines, which are clearly distinct in mice. Drawing all elements together, the authors proposed that variable responses to OVA and BSA were secondary to the development of divergent T-cell responses, which determine the antibody isotype production observed. The assumption was that the proteins had inherent physicochemical differences that resulted in different protein uptake, processing, and presentation by the immune system.

The following two examples of murine food allergy models are presented because they are under investigation to provide a better understanding of the mechanisms involved in food allergy and not so much as a measure of protein allergenicity. Using known allergens to sensitize and assess an IgE-mediated response is an essential element in determining whether we can optimize an animal model for determining the allergenic nature of novel proteins.

Cow's Milk Allergy

Cow's milk allergy (CMA) is a major cause of a transient food hypersensitivity in children. It involves the skin, respiratory tract, and gastrointestinal tract and can lead to systemic anaphylactic shock. In a study by Li *et al.*,⁶ several strategies were used to overcome oral tolerance in a mouse model and induce IgE-mediated cow's milk hypersensitivity. Three-week-old C3H/HeJ female mice were sensitized intragastrically with cow's milk plus cholera toxin as an adjuvant. Mice received 0.01 mg, 0.1mg, or 1.0 mg/g body weight with 0.3 µg/g CT as sensitizing doses and were boosted five times at weekly intervals. Six weeks after the initial sensitization dose, mice were fasted and intragastrically challenged with two doses of CM (30 mg/ml) given 30 minutes apart. Hypersensitivity responses were assessed based on symptom scores, vascular leakage, plasma histamine release, PCA, serum antibody titers, skin testing, and histological examination. Symptom scores were identified by independent observation of the physical features given in TABLE 2.

TABLE 2. Symptom score for peanut-allergic mice

Score	Symptoms
0	No symptoms
1	Scratching and rubbing around the nose and head
2	Puffiness around the eyes and mouth, diarrhea, pilar erecti, reduced activity, and/or decreased activity with increased respiratory rate
3	Wheezing, labored respiration, and cyanosis around the mouth and tail
4	No activity after prodding or tremor and convulsions
5	Death

The model exhibited characteristics of IgE-mediated cow's milk-induced food allergy. Elevated allergen-specific IgE levels were shown to be associated with systemic anaphylaxis, whereas levels of IgG1 were not; PCA reactions induced by serum from sensitized mice were eliminated by heating; and significant plasma histamine from mast cell degranulation was in evidence, all of which are important features of IgE-mediated food allergy. Serum casein levels after oral challenge were consistent with intestinal permeability studies and histologic examination revealed changes in both the GI and respiratory systems. The model was regarded to be useful for evaluating mucosal and systemic immunopathogenic mechanisms involved in IgE-mediated cow's milk allergy.

Peanut Allergy Model

The clinical features of persons with peanut-sensitive, IgE-mediated reactions predominantly express as immediate symptoms, which also involve the skin, gastrointestinal and respiratory tracts, and systemic anaphylactic shock in both children and adults. In the course of our studies in collaboration with Hugh Sampson's group at Mt. Sinai School of Medicine, we are investigating several strategies that will provide a reliable animal model of peanut allergy that will address the pathogenic mechanisms of this life-long, life-threatening food allergy.

As in the CMA model, to mimic the clinical and immunological characteristics of peanut allergy, C3H/HeJ mice were sensitized orally with freshly ground whole peanut and cholera toxin as adjuvant.³ Five-week-old female mice were sensitized by intragastric gavage with either a low dose (5 mg/ml of ground whole peanut equivalent to 1 mg of peanut protein) or high dose (25 mg equivalent to 5 mg of peanut protein) together with 10 µg cholera toxin on day 0 and day 7. Three and five weeks after initial sensitization, mice were fasted overnight and challenged intragastrically with 10 mg crude peanut extract divided into two doses at 30- to 40-minute intervals.

Fatal or near fatal anaphylaxis occurred in 12.5% of orally challenged mice at 3 weeks. Symptoms following secondary challenges two weeks later were more severe, increasing the fatality rate to 21%. Peanut-protein-specific IgE titers were significantly increased at weeks 1–5, and IgG1 levels did not differ between low-dose and high-dose sensitizations, suggesting this antibody did not play a significant role in inducing anaphylaxis in this model. Heat-inactivated serum confirmed the anaphylactic response to be IgE-induced and not IgG1. IgG2a levels were significantly higher in the high-dose versus low-dose sensitization, suggesting that IgG2a was inversely related to the severity of peanut hypersensitivity. In mice sensitized to Ara h 2, mouse IgE was shown to bind to the same immunodominant region (amino acid residues 53–75) of Ara h 2 as human IgE from peanut-sensitive individuals.

In ongoing studies, again in association with Hugh Sampson's group, we are investigating the potential to modulate peanut allergy with plasmid Ara h 2 in mouse models.⁷ Three strains of mice, female C3H/HeSn (H-2^K), BALB/c (H-2^d), and male AKR (-2^k), six weeks of age were immunized by i.p. injections with 15 µg of pDNA-Ara h 2. Controls received mock DNA or were untreated. Mice received a single immunization or multiple immunizations (three daily injections followed by a booster immunization one week later). Antigen-specific IgE, IgG1, and IgG2a were measured by ELISA on a weekly basis. Systemic anaphylaxis following i.p. challenge with crude peanut extract was evaluated three weeks after the initial immunization. Other assessment measurements included vascular leakage with Evan's blue dye, plasma histamine release, histologic studies (mast cell degranulation of ear tissues following systemic reactions), PCA, and quantitation of cytokines from spleen culture. IgG2a levels were 10-fold higher than IgG1, and no Ara h 2-specific IgE was detectable in singly or multiply immunized mice. However, i.p. injection of either peanut extract or Ara h 2 resulted in anaphylactic reactions, which could be attributed to increased levels of IgG1. PCA analysis of heat-inactivated and non-heat-treated serum confirmed that the anaphylactic reactions were being induced by allergen-specific IgG1. Similar responses were identified using plasmid DNA encoding ovomucoid, demonstrating that pDNA immunization induced IgG1 antibody responses that were not unique to rAra h 2. In contrast to the C3H animal model, the AKR and BALB/c strains did not elicit any sign of anaphylaxis under identical conditions of immunization and challenge, clearly establishing that peanut allergen gene immunization, under these conditions and with these animal models, were strain specific. These results demonstrate that careful consideration must be given to the different strains of mice, allergens, and routes of administration during the development of models for human disease.

In summary, mice could prove valuable with respect to strain and allergen specificity in which related reactions may provoke information on the genetic basis of food allergen sensitization. Alternatively, interspecies extrapolation,

a transient response, and symptomology must be taken into consideration when trying to mimic the human response.

RAT MODEL

A number of rat strains have also been shown to produce IgE antibodies to food proteins. As the rat is routinely used for toxicological studies, the addition of a rat-based food allergy model would be particularly attractive.

GUINEA PIG MODEL

The allergenicity of cow's milk proteins has been assessed in a guinea pig model. In this study, the allergenicity of milk-based infant formula and cow's milk proteins were evaluated by examining altered intestinal permeability, intestinal anaphylaxis, and PCA after oral sensitization with cow's milk proteins and challenge with β -lactoglobulin.⁸ Colonic segments from cow's milk-sensitized or milk formula-sensitized animals were challenged with β -lactoglobulin in Ussing chambers. Cow's milk-sensitized animals responded with an antigen-induced, anaphylactically mediated elevation in the transmural short-circuit current as measured by net chloride secretion, whereas only 60% of animals fed infant formula responded to challenge. Bronchospasm developed in all animals fed cow's milk; however, only those animals fed infant formula that responded to intestinal challenge developed bronchospasm. The authors concluded that cow's milk-based infant formula had less sensitizing power than whole cow's milk and that the model was effective in testing allergenicity at the intestinal level. Both the isotype antibody responses, (anaphylactic IgG1 and IgE are produced) and the immune system of the guinea pig, which has yet to be completely characterized, differ from that of the human.

In veterinary medicine, skin and gastrointestinal reactions to foods are commonly diagnosed in domestic animals such as pigs, calves, and dogs; however, the mechanisms have not been clearly established. Two animal models bear consideration, the atopic dog and a swine model.

ATOPIC DOG MODEL

A frequency of food allergy identified by single-ingredient provocation testing in 25 dogs with histories and cutaneous signs was consistent with food-induced allergic dermatitis.⁹ Although no IgE was measured, elimination diets and provocation tests confirmed that food ingredients in soy, chicken, milk, corn, wheat, and egg have ingredients that provoked positive

reactions. Ermel *et al.*¹⁰ reported the use of a colony of spaniel/basenji-type dogs that they selected with a genetic predisposition to allergy that had histories of sensitivity to pollens and foods. Newborn pups were subcutaneously injected into the axilla with 1 µg cow's milk, beef, ragweed, or wheat extracts in alum. At ages 3, 7, and 11 weeks, all pups were vaccinated with attenuated distemper-hepatitis vaccine. At 2 and 9 days after each vaccination, each animal received the same allergen they received as newborns. Immunized pups responded with allergen-specific IgE by week 3, peaked at week 26, and could be maintained with bimonthly injections of specific antigen in alum and daily feeding of a maintenance diet containing minimal amounts of the allergen source. Skin tests were consistently positive when challenged with the relevant allergen and negative with an irrelevant allergen (nonimmunized allergen). Endoscopic analysis of gastric food sensitivity testing and histologic tissue examination showed pronounced mucosal swelling and persistent erythema at the site of injection. In addition, late biopsy specimens revealed evidence of eosinophil infiltration into the lamina propria and migration through the endothelium.

This protocol, in atopic dogs, mimics the following proposed mechanism involved in the development of food allergies in children. In genetically predisposed individuals with an initial early infection or viral challenge, the immune system could be capable of responding to "bystander" antigens with more vigor. The inflamed gut becomes more permeable to proteins, thereby exposing the local immune system to more antigen sources. The symptoms and production of allergen-specific IgE suggest that the inbred allergic dog model could be useful for the characterization of the underlying mechanisms involved in the development of food allergy.

NEONATAL PIG MODEL

Pigs present a number of important advantages over other animal models for investigating the pathogenesis and immune responses to allergens: they closely resemble humans in gastrointestinal physiology and in the development of mucosal immunity. In addition, they are born immunocompetent, thus allowing for assessment of immune responses.¹¹ In this and other veterinary medicine studies, a reduced growth performance in pigs fed soybean proteins has been associated with reduced weight gain and protein digestibility, which may be caused by a transient hypersensitivity to soybean proteins, glycinin, and β -conglycinin.

Considering this data and the similarities in digestive function in the neonatal pig and man, this may be an attractive model to improve our knowledge of gastrointestinal hypersensitivity to peanuts. Pregnant sows, at day 109 of gestation, were fed a NRC-approved sow lactation diet consisting of a soy-

bean/peanut-free protein diet throughout lactation. Newborn piglets were allowed to suckle *ad libitum* for three weeks and then weaned to nursery-phase diets lacking soybean/peanut proteins meeting NRC regulations. Three-day-old piglets were divided into three groups and either intragastrically (i.g.) or intraperitoneally (i.p.) sensitized with crude peanut meal as follows. Group 1: 2 grams peanut on day 3, 4, and 5 i.g. with 100 μ l cholera toxin (CT). Group 2: 2 grams peanut on day 9, 10, and 11 i.g. with 100 μ l CT. Group 3: 500 μ g peanut on day 3, 4 and 5 i.p. with 100 μ l CT. Each group was subsequently boosted 1 week later with the respective peanut-sensitization protocol. Three weeks after sensitization, oral challenge with 10 grams of peanut meal resulted in symptoms ranging from erythematous rashes, cyanosis about the snout and ears, lethargy, vomiting, and respiratory distress in 25% of the piglets sensitized to peanut meal.

With 2 grams of peanut meal plus cholera toxin (50 or 100 μ g) given intragastrically, three of eight piglets responded with vomiting and lethargy (not easily prodded to action), tremors, and convulsions. All the piglets showed a positive peanut-specific IgG with titers ≥ 500 mg/ml by day 50 following sensitization. In proliferation assays, peripheral blood lymphocytes showed 3- to 5-fold increases with peanut extracts above a rice protein control. Skin prick tests with 1–50 μ g peanut extract were all positive (5–15 mm with pseudo-pods); cholera toxin and rice controls did not react (>2 mm); histamine levels were 15 mm and greater. With 250–1000 μ g plus cholera toxin (20–100 μ g) given intraperitoneally, four of sixteen animals responded with gastrointestinal symptoms (coughing, gagging, evidence of stomach contractions, reduced activity) and positive skin tests. In these two groups responding to oral challenge, two piglets had skin responses that included flushing (erythematous rashes) of the ears and in the trunk of the animals. Control animals challenged with 10 grams of peanut meal did not respond with any of the symptoms associated with peanut-sensitized animals. Skin test results one week later showed wheal and flare skin test reactions typical of immediate type allergen skin tests in sensitized piglets with no response in control animals.

In the course of our investigations into a neonatal pig peanut animal model, we have completed additional studies that support the basis for this food allergy model. Intraperitoneal injection with 500 μ g of peanut extract in conjunction with 100 μ g of cholera toxin at days 9, 10, and 11 and boosted on days 17 and 24 have increased our allergic model to 75–100% of sensitized animals responding to an intragastric challenge. In 75% of sensitized animals, emesis and respiratory distress were in evidence, whereas in the remaining 25%, lethargy and skin manifestations were in evidence. Allergic symptoms could be reversed or diminished with subcutaneous administration of epinephrine and steroid treatment in the most severely responding animals. We have been able to reproduce these symptoms in symptomatic animals in two follow-up intragastric challenges spaced at two-week intervals, substantiating the longevity of the allergic response out to 14 weeks of age.

TABLE 3. CBC/differential counts in piglets

	Peanut-sensitive			Control
	#5	#6	#7	#10
WBC	16,100	14,900	15,100	10,100
Mono	13%	10%	10%	10%
Lymph	48%	39%	37%	59%
Seg	24%	37%	32%	29%
Bands	15%	14%	21%	2%

Skin tests with crude peanut extracts on alternating weeks confirm the persistence of the allergic state throughout the 14-week period. In addition, both the native and recombinant forms of the major peanut allergens, Ara h 1 and 2, will induce a positive skin test when compared to rice allergen extracts. To confirm that IgE is the responsible isotype inducing the allergic symptoms, PCA tests were performed in naïve animals. One hundred microliters of non-heated and serial heat-inactivated serum from two peanut-sensitized pigs were administered intradermally into the flank of naïve animals (non-peanut sensitive). Twenty four hours later, 3 µg of peanut extract was administered by intravenous injection, and the response read 30 minutes later. Only the non-heat inactivated serum sites responded with a grade 1–2 skin test response (wheal and flare > 15 mm). We have also determined CBC/differentials for evidence of changes in circulating peripheral blood lymphocytes following intragastric challenge (TABLE 3).

The substantial increase in white blood cells, which can be attributed to bands, mirrors the recruitment of these cells seen in food-sensitive individuals following an oral challenge to the offending food.

To clarify the specificity of our oral challenge procedure, we selected four peanut-sensitive animals for subsequent challenge with peanut meal or regular pig diet consisting of corn meal less peanut and soybean meal. Only the pigs challenged with peanut responded by vomiting and presented with other symptoms characteristic of a peanut allergic state. The two animals receiving the corn meal diet did not show any evidence of allergic symptoms. This data provides additional convincing evidence for our pig model for investigations into the mechanisms of IgE-mediated peanut allergy.

CONCLUSIONS

Several animals models are being used to determine the mechanisms of IgE production; however, a validated animal model for determining which proteins are allergenic, that is, what characteristics or traits of novel proteins are predictive for the induction of IgE compared to nonallergen source mate-

rial, are incomplete. Moreover, extrapolation to the human response is incomplete. The continued research efforts in this area not only should include the ability of a protein to produce IgE, but also should demonstrate some clinical evidence of symptoms characteristic of the human allergic response, a ranking of the allergens with respect to potency of proteins in food allergy, and comparative studies with nonfood proteins.

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