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Nonmurine Animal Models of Food Allergy

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Food allergy can present as immediate hypersensitivity [manifestations mediated by immunoglobulin (Ig)E], delayed-type hypersensitivity (reactions associated with specific T lymphocytes), and inflammatory reactions caused by immune complexes. For reasons of ethics and efficacy, investigations in humans to determine sensitization and allergic responses of IgE production to innocuous food proteins are not feasible. Therefore, animal models are used *a*) to bypass the innate tendency to develop tolerance to food proteins and induce specific IgE antibody of sufficient avidity/affinity to cause sensitization and upon reexposure to induce an allergic response, *b*) to predict allergenicity of novel proteins using characteristics of known food allergens, and *c*) to treat food allergy by using immunotherapeutic strategies to alleviate life-threatening reactions. The predominant hypothesis for IgE-mediated food allergy is that there is an adverse reaction to exogenous food proteins or food protein fragments, which escape lumen hydrolysis, and in a polarized helper T cell subset 2 (Th2) environment, immunoglobulin class switching to allergen-specific IgE is generated in the immune system of the gastrointestinal-associated lymphoid tissues. Traditionally, the immunologic characterization and toxicologic studies of small laboratory animals have provided the basis for development of animal models of food allergy; however, the natural allergic response in large animals, which closely mimic allergic diseases in humans, can also be useful as models for investigations involving food allergy. *Key words:* allergens, atopic dog, gastrointestinal food allergy, swine. *Environ Health Perspect* 111:239–244 (2003). [Online 21 January 2003]
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Immunoglobulin (Ig)E-mediated allergic reactions to food proteins may induce a variety of cutaneous, gastrointestinal, and systemic symptoms in humans, rodents, pets, and farm animals. The normal immune response to dietary proteins is an increased mucosal immunity associated with an active suppression of the systemic response. Given the obligate exposure of the gastrointestinal tract to food proteins and potential antigens/allergens, it is not surprising that this organ system has evolved to provide defense mechanisms that prevent foreign, intact proteins from gaining entry into the body and access to the immune system.

In discussions of food allergy, the predominant hypothesis is that there is an adverse reaction to exogenous food proteins or food protein fragments, which escape lumen hydrolysis and are thus available for exposure to the gastrointestinal-associated lymphoid tissues (GALT). Food or food constituents are normally degraded by digestive enzymes in the gastrointestinal tract, beginning in the mouth and stomach and ultimately completed in the small intestine. Although the sensitizing mechanisms are unknown for food allergens, intact or fragments of proteins are proposed to be absorbed by the gut mucosa, processed by immunocompetent cells, and then presented to the immune system. In a polarized helper T cell subset 2 (Th2) environment, Ig class switching to allergen-specific IgE is generated. The mechanisms and what determines this class switch and ultimately an immediate hypersensitive food pathogenesis continue to be a major focus for research investigators.

Tolerance to Food Allergens

The defense against hypersensitivities to dietary proteins is a well-developed, multitier system dependent on an effective mucosal barrier in association with the oral tolerance generated by the cellular immune system of the GALT. The balance between tolerance (suppression) and sensitization (priming) depends on several factors, such as genetic background, nature and dose of antigen, frequency of administration, age at first antigen exposure, immunologic status of the host, and antigen transmission via breast milk (Strobel 2002). However, the neonatal period is particularly critical in terms of mucosal defense. The intestinal barrier and function provided by secretory antibodies and the immunoregulatory network are poorly developed for a variable period after birth (Brandtzaeg 2002).

In the healthy adult, the mucosal lining of the gastrointestinal tract provides a physical barrier, preventing uptake of large molecules. Small quantities of intact proteins that do reach the GALT are controlled by an active suppressor cell activity, producing oral tolerance. However, for reasons yet to be defined, individuals with a genetic predisposition to atopy respond to harmless food proteins in an abnormal manner, producing food allergy.

Food Allergy

Food allergy is commonly defined as clinical symptoms that result from an inappropriate immune response to food proteins or food additives (Burks and Stanley 1998). Sensitization and the subsequent allergic response to IgE-mediated food allergy are generally identified as a multifactorial process, involving a genetic predisposition (the atopic syndrome) combined with environmental factors, that generates allergenic determinants during food processing and/or intestinal digestion and antigen processing by the immune system. Normally, proteins are readily digested and absorbed, leading to oral tolerance; however, through some intrinsic property of the protein and/or a breakdown in the intestinal epithelial barrier, there is an enhancement of protein-induced antigenicity and allergenicity.

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 (Helm and Burks 2000). Predisposing factors for food allergy include a genetic predisposition to atopy, the introduction of food before epithelial gastric mucosal barrier closure, an immature GALT immune system, feeding poorly digestible proteins, incomplete digestion, increased mucosal permeability, decreased IgA secretion, and deranged cell-mediated responses of the GALT.

True prevalence of adverse food reactions is unknown; however, a review of the literature indicates that approximately 6–8% of children and 1–2% of adults have some type of food

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allergy (Bock 1987; Burks et al. 1999). The most common food allergens in childhood are cow's milk, eggs, peanuts, soybeans, wheat, fish, and tree nuts. In adults, peanuts, tree nuts, fish, and shellfish are the most common sources of food allergy. Children by 3–5 years of age can outgrow their food allergy, typically to milk, eggs, soybeans, and wheat. Diet and geographic location are also considered to be factors contributing to food allergy in both children and adults (Burks 2002).

Diagnosis of Food Allergy

The diagnostic assessment of IgE-associated food allergy is based on clinical histories, skin prick tests, allergen-specific IgE, and the double-blind, placebo-controlled food challenge (DBPCFC) (Bock 2000; Sampson and Ho 1987). However, a proper clinical history in which the food can be identified is often sufficient to diagnose food allergy. Prick-puncture skin tests have been shown to have a high negative predictive accuracy for major foods compared with a low positive predictive value. This is often variable with age of the individual and quality of the food extracts. Similar to skin testing, *in vitro* testing for allergen-specific IgE is quite variable, especially with quality of food extracts and the interference from high total IgE and IgG. The DBPCFC is considered the gold standard for diagnosing adverse food reactions caused by any mechanism. In combination a good clinical history, skin tests, allergen-specific IgE levels, and the DBPCFC, a diagnosis of a food as the agent causing an adverse IgE-mediated event can be made (Sampson 1989).

Food Allergen Characterization

The traditional definition of an allergen is the ability of an antigen to produce an allergic reaction by inducing specific IgE antibody formation. Specifically, immunogenicity involves formation of antibodies (IgM, IgG, IgA) or cellular immunity, whereas 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. Allergens have been defined as either complete or incomplete. Complete allergens have the following distinct molecular properties: sensitization (the ability to induce the immune system to produce IgE antibody), elicitation (the ability to trigger allergic symptoms), and binding to allergen-specific IgE. An incomplete allergen is defined as a nonsensitizing protein that is able to elicit allergic symptoms (Aalberse 2000). In determining food protein allergenicity, two essential definitions must be considered: (*a*) sensitization—the ability to induce the formation of IgE antibody, and (*b*) the allergic reaction—the property of a protein to cross-link cell-bound specific IgE to elicit an allergic reaction by releasing mediators, for example, histamine (i.e., the ability to trigger symptoms upon re-exposure).

The characteristics and what determines that a common food protein will be recognized by the gastrointestinal immune system as an allergen are largely unknown. However, in the assessment of food protein allergenicity, characterization of the allergen should include an accumulation of physicochemical, immunochemical, and biochemical information based on known food allergen characteristics (Aalberse 2000; Kimber et al. 1999).

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). Allergens must contain B-cell epitopes to which IgE can bind, and T-cell epitopes capable of inducing type 2 T-lymphocyte responses. Although important to clinical responses, T- and B-cell epitopes alone are insufficient to endow a protein with allergenic potential. Careful consideration should also be given to post-translational glycosylation and function, for example, enzymatic activity, pathogenesis related-proteins, and contractile proteins (protein families).

Relative stability of proteins in simulated gastric fluid and simulated intestinal fluid can often correlate with allergenic activity (Astwood et al. 1996). Post-translational modification appears to enhance allergenicity by increasing uptake and detection by the immune system. Functional activity, particularly enzyme activity, may also enhance allergenicity by nonspecific activation of cells participating in the immunologic response, for example, the induction of inflammation. By better defining the limits within which these factors operate, we may be in a better position to identify and characterize the hazards and risks of allergic disease associated with novel proteins (Huby et al. 2000).

Genetically Modified Organisms and Food Allergy

The advent of bioengineered food crops in 1996 resulted in the recognition of the potential to introduce known food allergens or novel proteins that could be allergenic. Agribiotech officials and food safety regulatory agencies (U.S. Department of Agriculture, Environmental Protection Agency, and Food and Drug Administration) began to evaluate the safety of genetically modified foods that would end up in our food supply. Task forces, workshops, and food safety agencies took the initiative to develop decision trees to assess the potential allergenicity of (biotechnology-derived) novel proteins (Metcalfe et al. 1996; Taylor et al. 2002). The participants recognized that direct, indirect, or unintended effects of genetic modification in plants through classical or modern biotechnologic means should be evaluated to ensure the safety of our food supply. At present, there is no validated animal model or *in vitro* protocol for evaluating or predicting the allergic risk of proteins newly introduced into our food supply by either classical or modern biotechnology.

As identified by Wal (1999), the questions raised should be (*a*) whether it is possible to assess or predict an allergenic risk from any characteristic of the protein—for example, primary/secondary/tertiary structure, function, origin, physicochemical properties, and as yet unidentified characteristics; and (*b*) whether a "new" protein produced by modern biotechnology, such as recombinant proteins expressed by genetically modified organisms, can be intrinsically more allergenic than a "natural" conventional protein. It seems reasonable to state that allergenicity of a food protein cannot be predicted by the analysis of crude product, but that a physiologic basis of antigen transport and modification by the organism must be taken into account (Helm 2002a). Criteria are continually being re-evaluated and updated to help agribiotech officials determine potential allergenicity of novel proteins.

Animal Models

For reasons of ethics and efficacy, prospective sensitization studies in humans are not possible. Therefore, what is needed for the assessment of novel proteins, especially novel biotechnologically modified proteins, are animal models that mimic 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 predictive of the human allergic response. Animal models are being used to assess very different mechanisms: *a*) mechanistic approaches to the understanding of IgE-mediated disease, *b*) prophylactic and intervention therapeutic approaches to treat food allergy, and *c*) a qualitative approach to determine the allergenicity of novel proteins compared with known food allergens. Traditionally, the immunologic characterization and toxicologic studies of small laboratory animals have provided the basis for development of animal models of food allergy [mouse (Atherton et al. 2002), rat (Knippels and Penninks 2002), guinea pig (Piacentini et al. 1994)]. However, the natural allergic response in large animals—the dog (Buchanan and Frick 2002) and swine (Helm 2002b)—that closely mimic allergic diseases in humans can be useful models for investigations involving food allergy.

Criteria for Animal Models

The following information represents a summation of discussions from various workshops and national meetings concerning the issues of animal models, to which the author (R.M.H.) was a participant (NCFST 2000; Health Canada 2001; Society of Toxicology 2001; ILSI/HESI 2000; FAO/WHO 2001). Several methods have been introduced to bypass the state of tolerance and initiate a food hypersensitivity that reflects human IgE-mediated food allergy. In general, animal models of gastrointestinal food allergy have required nonphysiologic routes of antigen/allergen presentation and the use of adjuvants inducing Th2 cytokines. Most animal models, including small laboratory animals (mice, rats, and guinea pigs) and domestic animals (dogs, pigs, and calves), use intravenous, intraperitoneal, subcutaneous, or the intradermal route of administration with or without an adjuvant.

The degree of sensitization should take into consideration the following parameters: *a*) 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); *b*) that the allergen should be taken in context with the food source; *c*) 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; *d*) age of the animal (neonate, adolescent, adult), *e*) a genetic predisposition (high and low IgE responders); *f*) the use of adjuvants (natural or artificial—alum, cholera toxin, *Bordetella pertussis*, and carrageenan are known IgE-selective adjuvants); *g*) 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 with IgE); and *h*) the Th1/Th2 regulation/polarization (mice have very delineated Th1/Th2 polarization, whereas in humans 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 with what is seen in humans. The allergenicity profile should be comparable with what exists in the human response to different allergens, for example, anaphylactic episodes induced by peanut allergens or oral/pharyngeal symptoms elicited by fruit and vegetable allergens in the oral allergy syndrome. The simple production of IgE (analysis by *in vitro* methods, i.e., enzyme-linked immunoadsorbent assay) and binding of IgE to relevant proteins (sodium dodecyl sufate–polyacrylamide gel electrophoresis/immunoblot analysis) should be considered insufficient to establish a protein as an allergen without clinical relevance. Evidence of histamine release by skin prick test or using *in vitro* tests to support cross-linking of IgE to bound mast cells and basophils should also be considered. However, the demonstration of a positive test in any of these methods should raise warning signals as to the potential allergenicity of a novel protein.

Consideration should also be given to the differential induction and regulation of cytokines (Th1/Th2 cytokine profile), immunoglobulins (IgA, IgG, and IgE), and chemokine production by different antigen-presenting cells between the species under investigation. Additionally, concentration and exposure of the food, the physiologic basis of the gastrointestinal tract environment, antigen transport, and modification of the food source (heat treatment, roasting) should be taken into account. These 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 the remainder of this article, an overview of the atopic dog and neonatal swine is presented, demonstrating the evidence in favor of these nonmurine animal models for determining food protein allergenicity. All animals used in the studies were maintained according to Animal Welfare Act and National Research Council regulations, as well as standards of the respective Institutional Animal Care and Use Committees. Advantages and disadvantages of large animal models are presented in Table 1.

Atopic Dog Model

In canine allergy, the most common clinical presentation is a nonseasonal generalized pruritis with or without lesions with papular eruptions in about 40% of cases (Reedy et al. 1997). Flea allergy and general atopy precede food allergy as the most common types of allergic disease in dogs (Reedy et al. 1997). About 8% of the canine population is affected by food allergy, with no age, sex, or breed being a predisposing factor (Baker 1974, 1990; Jeffers et al. 1991; White 1986, 1988). The incidence of spontaneous food and inhalant allergy in dogs is comparable with the approximately 10% incidence of allergy in humans. The clinical signs, which vary from gastrointestinal reactions (vomiting and diarrhea) to dermatologic reactions (pruritis, papules, and erythema), are symptoms of food allergy in both humans and dogs without predisposing factors such as age, sex, or breed of canines (Baker 1990; Jeffers et al. 1991; Halliwell 1992; August 1985; White 1986, 1988).

Food hypersensitivity may contribute to pruritis in up to 62% of dogs presenting with allergic skin disease (Leib and August 1989). It has been estimated that 10–15% of dogs with dermatologic signs may have concurrent gastrointestinal signs, which include emesis, diarrhea, bloating, and cramping (Carlotti et al. 1990). Multiple food allergies have been identified as presenting with primary gastrointestinal disease symptoms. Foods responsible for allergy in dogs include milk, soybean, wheat, oats, beef, eggs, chicken, horsemeat, cornmeal, pork, yeast, and commercial foods with varying protein bases.

Diagnosis in dogs can be made by dietary restriction and rechallenge similar to the DBPCFC (dogs have no preconception of food allergy) (Strombeck and Guilford 1990). In a frequency study of food allergy identified by single-ingredient provocation testing in 25 dogs with histories and cutaneous signs consistent with a food allergy, Jeffers et al. (1991) confirmed food ingredients in soy, chicken, milk, corn, wheat, and eggs provoked adverse cutaneous reactions. As in humans, the clinical signs in dogs sensitive to different foods were variable, including skin, gastrointestinal, respiratory, and central nervous system effects and combinations of these pathologic conditions.

A high IgE-producing spaniel/basenji dog colony with histories of sensitivity to pollens and foods has been used for studies into immunologic mechanisms (Ermel et al. 1993, 1994, 1997; Frick et al. 1994). The atopic dog model mimics a proposed mechanism involved in the development of food allergies in children, that of a viral infection combined with sensitization to other nonviral-related protein. In genetically predisposed individuals with an initial early infection or viral challenge, the

Table 1. Advantages/disadvantages of nonrodent animal food allergy models.

Advantages	Disadvantages
Confirmed clinical/immunologic of natural food allergy	Limited species/strains
Anatomy/physiology/nutritional requirements similar to those of humans	Knockout strains not available
Immunopathogenic/mechanistic/therapeutic intervention strategies similar to those for humans	Lack of complete array of immunologic reagents
Repeated endoscopic analysis of gastrointestinal tract	Large size and smaller experimental animal numbers/group
Large size/numbers of primary and secondary immune organs/cells	Expensive to maintain colonies
Smaller concentration of sensitizing antigen/allergen per gram of body weight	

immune system is suggested to 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.

In the atopic dog model for food allergy (Ermel et al. 1997), newborn pups (day 1) were subcutaneously injected in the axillas with 1 μg of cow's milk, beef, ragweed, and wheat extracts in alum. Food antigen was again administered on days 22, 29, 50, 78, and 85. At ages 3, 7, and 11 weeks, all pups were vaccinated with attenuated distemper-hepatitis vaccine. Immunized pups responded with allergen-specific IgE by week 3 and peaked at week 26 of age. The IgE titer could be maintained with bimonthly injections of specific antigen in alum combined with a daily feeding of a maintenance diet containing minimal amounts of the allergen source.

Oral challenge with food antigens demonstrated clinical manifestations of food allergy. Nausea and vomiting were evident within 1 hr and mild to severe diarrhea within 12 hr that resolved within 3–4 days. Systemic clinical signs included pruritis, facial edema, conjunctivitis, dermatitis, and anaphylaxis. Intradermal skin tests were consistently positive when challenged with the relevant allergen and negative with an irrelevant allergen (nonimmunized allergen). Positive skin tests appeared to correlate with specific food antigen IgE titers, in that animals that had higher IgE titers had better responses to intradermal injections of specific food allergens. Serial dilutions of each food allergy were used to determine a relative end point as an indicator of true allergenicity. All clinical manifestations are consistent with infant, adolescent, and adult food allergy in humans.

Gastroscopic food sensitivity tests on animals receiving a hypoallergenic liquid maintenance diet were performed under general anesthesia (Ermel et al. 1997). Food allergen extracts were injected into the gastric mucosa, and the injection sites were monitored and videotaped. Observable responses were recorded within 3–5 min, ranging from mild to moderate to severe erythema and edema of the tissue sites. Gastroscopic food sensitivity test responses were consistent and reproducible in individual food-allergen–specific dogs. Endoscopic analysis of gastric food sensitivity testing and histologic tissue examination revealed acute-phase inflammatory responses with pronounced mucosal swelling and persistent erythema at the site of injection. Congestion, interstitial edema, gastric submucosal periglandular edema, and increased LTB4 and PGE2 were in evidence within 3–5 min postinjection. A late-phase inflammatory response with gastric submucosal epithelial vascular degeneration and neutrophil infiltration was identified at 24–48 hr postinjection. In addition, late biopsy specimens revealed evidence of eosinophil infiltration into the lamina propria and migration through the endothelium. Skin testing and oral challenges with peanut, walnut, Brazil nut, wheat, cow's milk, soy, and barley revealed an allergenic profile in the atopic dog model identical to that in humans. An allergic response profile indicates that peanuts and tree nuts caused the most significant and profound allergic responses, followed by wheat, cow's milk, soy, and barley, respectively.

The differences in skin test titration end points were also used to assess efficacy of different treatments of allergenic foods to determine their relative allergenicity (Buchanan et al. 1997; de Val et al. 1999). Thioredoxin treatment of proteins in foods reduces intrachain disulfide bonds, disrupting structural integrity. Wheat gliadins and glutenins, cow's milk β-lactoglobulin, soybean trypsin inhibitor, and glutenins treated with thioredoxin showed evidence of allergenic activity reduced 10–1,000-fold compared with native nontreated extracts by end-point skin test titration. Oral feeding challenges with thioredoxin-treated β-lactoglobulin or milk caused minimal or no gastrointestinal symptoms compared with native allergens. This suggests that structural integrity of some food allergens is necessary for sensitization.

The atopic dog model has also been used to test and compare the allergenicity of a transgenic corn line (corn genetically modified to produce a novel protein) with that of a nontransgenic corn (Buchanan BB. Personal communication). Peanut, soy, cow's milk, and ragweed allergens were used as positive controls, and the nontransgenic corn was used as a negative control for the genetically modified corn. Among the allergens tested, peanut provided the strongest IgE-mediated response, which developed rapidly early in the sensitization/allergic response and remained constant from 9 to 23 months of age. Responses to cow's milk and ragweed progressed slowly by 9 months and rose significantly by 18–23 months. Soybean showed the strongest response by 9 months, which became weaker by 18–23 months. These responses confirmed earlier responses demonstrating a similar allergenic potency profile in human food allergy; that is, soybean and milk allergy is common in infants and wanes in adolescence/adulthood, whereas peanut normally is considered a life-long food allergy.

The transformed corn extract caused no skin responses at 9 and 18 months, and minimal response by 23 months corresponding to 1/5,000th that of peanut, 1/9,000th that of ragweed, 1/700th of cow's milk, and 1/50th that of the soybean. Furthermore, after 23 months, the transgenic corn protein showed no evidence of skin reactions in dogs sensitized to transgenic corn extract when tested at 5–380-fold higher concentrations than the amount present in the parent corn extract.

Neonatal Swine Model

Swine present a number of important advantages compared with 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. Developing piglets have similar anatomy and nutritional requirements, a transient neonatal porosity to the gut to dietary proteins, a distribution and maturation of intestinal enzymes, and an enteric absorption of antibody that is similar to that of the developing infant. In addition, they are born immunocompetent, allowing for assessment of immune responses (Phillips and Tumbleson 1986).

Investigations in swine and calves demonstrate the induction of hypersensitivity responses that are similar to those of human allergic disease (Barratt et al. 1978). Studies in veterinary medicine have shown swine to have an IgE-mediated-like response to parasites, legumes, and pollens reminiscent of that in humans (Barratt et al. 1978; Bailey et al. 1994; Hankins et al. 1992; Heppel et al. 1989; Li et al. 1991; Rees et al. 1989; Wilson et al. 1989). Young piglets have been used as models for sensitization/tolerance to cow's milk and soy proteins, which parallel what is seen in young children (Bailey et al. 1994; Hankins et al. 1992; Heppel et al. 1989; Li et al. 1991; Rees et al. 1989; Wilson et al. 1989).

Moreover, gastrointestinal-associated adverse immunologic reactions include emesis, diarrhea, bleeding, and weight loss, which are also associated with human gastrointestinal allergy. 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 hypersensitivity to soybean proteins, glycinin, and β-conglycinin.

Considering these data and the similarities in digestive function in the neonatal pig and the developing child, a peanut food allergy model in young piglets was established to investigate gastrointestinal hypersensitivity to peanuts (Helm et al. 2002). The objective of the study was to evaluate gross physical appearance and a microscopic analysis to tissues from dissection and endoscopy, determine a histologic profile and obtain a molecular analysis from tissues for evidence of a Th1/Th2 cytokine profile, and perform immunologic tests to determine the relative state of the immune response.

A series of intragastric and intraperitoneal sensitizations followed by oral challenge with peanut materials was undertaken to achieve an

optimal sensitization/challenge regime. Preliminary investigation demonstrated that approximately 25% of intragastrically sensitized animals and 75–90% of intraperitoneally sensitized animals responded to an oral challenge of peanut meal. Thus, an optimal experimental regime using intraperitoneal administration of peanut extract and oral challenge with peanut meal was developed as follows. Outbred Large White/Landrace pregnant sows at day 108 of gestation were allowed to farrow and nurse under normal conditions on a soybean/peanut-free diet. Piglets at days 9–11, 17, and 25 of age were intraperitoneally sensitized with 500 µg of peanut extract with 100 µg cholera toxin. Two animals in each litter were selected to receive control treatment—phosphate-buffered saline (PBS) and PBS with 100 µg of cholera toxin, respectively. On an allergen/body weight basis, in both the atopic dog model and the swine model, this is far less than that used for smaller laboratory animals. Intragastric challenge with peanut meal or intradermal skin testing was performed on alternating weeks starting 2 weeks after the final sensitization. Blood was drawn at weekly intervals to assess immune responses to the sensitization protocol.

Oral challenges on days 39 and 53 with 10 or 20 g of peanut meal respectively resulted in symptoms in 75–100% of animals by the second oral challenge within 30–60 minutes of the challenge. A positive response included emesis, malaise, tremors, and convulsions with major and minor rashes. Physical evidence of respiratory distress and anaphylactic shock was identified in approximately 10–20% of sensitized animals. No animals were allowed to proceed to death as a result of anaphylactic shock. Treatment with epinephrine was administered when respiratory distress was evident to alleviate all symptoms. Repeated oral challenges up to day 90 resulted in sensitized animals that responded with increasing degrees of physical symptoms. Control animals receiving either PBS or PBS/cholera toxin challenged with peanut meal did not respond with any physical symptoms of gastrointestinal or systemic allergy. Peanut-sensitized animals challenged with soybean/peanut-free diet failed to show any symptoms.

Skin tests with crude peanut extracts on alternating weeks confirmed the persistence of the allergic state throughout a 14-week period. In addition, both the native and recombinant forms of the major peanut allergens, Ara h 1 and Ara h 2, induced a positive skin test compared with rice allergen extracts. Skin prick tests with 12–1,000 µg of peanut extracts intradermally were positive (wheal and flare > 5–15 mm); PBS and PBS/cholera toxin control administrations were negative (2 mm), and histamine was ≥ 15 mm in peanut-sensitized animals. In nonpeanut-sensitized animals, the histamine showed positive wheal and flares, whereas the peanut extracts showed < 3 mm wheal and flare reactions.

Immunologically, the animals were assessed for the production of antigen-specific IgG, IgE by passive cutaneous anaphylaxis, peripheral blood lymphocyte proliferative responses to antigen, and CBC/differentials. Peanut-specific IgG values measured in peanut-sensitized animals reached levels > 1,000 µg/mL (range, 26–7,700 µg/mL) by day 37 and maintained values of > 500 µg/mL (range, 51–1,500 µg/mL) at day 60. Peanut-specific IgG varied from litter to litter and within each litter; however, the course of the immune response to peanut was similar. Nonpeanut-sensitized animals had < 50 µg/mL antigen-specific IgG.

To confirm that IgE is the responsible isotype inducing the allergic symptoms, passive cutaneous anaphylaxis tests were performed in naive animals. One hundred microliters of nonheated and serial heat-inactivated serum from peanut-sensitized pigs was administered intradermally into the flank of naive (nonpeanut-sensitive) animals. Twenty-four hours later, 5 mg of peanut extract in 1.0 mL PBS was administered by intravenous injection, and the response was read 30 min later. Intradermal skin sites with the nonheat-inactivated serum responded with a wheal and flare > 10 mm at the site of injection, whereas heat-inactivated serum showed no reaction, confirming that native IgE was responsible for the reaction.

A substantial increase in neutrophil early band forms mirrored the recruitment of these cells seen in food-sensitive individuals after an oral challenge to the offending food. In proliferation assays, peripheral blood lymphocytes showed a 3–5-fold increase in tritiated thymidine uptake compared with media and rice protein controls.

Gastrointestinal tract analysis at the time of sacrifice after oral challenges revealed minor amounts of peanut meal in the stomach and intestine of peanut-sensitized animals that vomited compared with abundant quantities of soybean/peanut-free meal in peanut-sensitized animals or peanut meal in nonsensitized animals. The most prominent histologic findings were vascular congestion, hemorrhage, and epithelial denudation that occurred primarily in the proximal small intestine. The cellular phenotype in the small intestine consisted primarily of lymphocytes and plasmacytes. Other acute markers included mucus extrusion and submucosal edema in the stomach. The colon was normal in most piglets, with occasional vascular congestion and crypt abscesses.

Conclusion

Several animal 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 with nonallergen source material, is needed for controlled studies. Moreover, extrapolation to the human response to a profile of strong to weak food allergens and nonfood allergen proteins is incomplete. In both the atopic dog and the neonatal swine models for food allergy, substantial evidence and data for clinical and immunologic findings in hypersensitized animals support continued research. Both animal models are characterized by an underlying natural mechanism of allergy that contributes to their usefulness as models for further characterization of the immunopathogenesis of IgE-mediated food allergy and investigations aimed at predicting the allergenic nature of novel proteins in biotechnologically derived food sources. The continued research efforts in this area should include not only the ability of a protein to produce IgE but also clinical evidence of symptomology 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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