

The Dog as a Model for Food Allergy

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ABSTRACT: Research during the past decade has shown the dog to be an excellent model for human food allergies. Humans and dogs share many of the same allergies to foods. Furthermore, the dog model shows clinical symptoms typical of humans, that is, both experience vomiting and diarrhea. Present results suggest that the dog may provide a means to test genetically modified foods for unsuspected allergens.

KEYWORDS: β -lactoglobulin; bovine serum albumin; animal models; food allergy; oral challenges

INTRODUCTION

The renewed interest in food allergy in humans has been hampered by the lack of an appropriate animal model with similar comparative aspects of form and function. Therefore, we have been characterizing an inbred colony of high-immunoglobulin-E-producing dogs that were immunized subcutaneously with food antigen extracts in alum and that developed clinical manifestations of food allergy after oral challenges with food antigen.¹ The animals have appreciably high IgE antibody titer to specific food antigens, as measured by an enzyme-labeled immunodot assay. Skin test results for the food antigens are consistently positive, as evidenced by a wheal-and-flare reaction.

It is possible to measure gastroenteric food sensitivity through an endoscope by injecting allergenic food extracts into the gastric mucosa after intravenous injection of Evans blue dye. Mucosal changes include swelling, erythema, and hyperperistalsis. Examination of immediate-reaction biopsy

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specimens reveals edema and few inflammatory cells. Examination of 24+-hour late-reaction biopsy specimens shows increased eosinophil and mononuclear cell infiltrations typical of late-phase allergic inflammatory responses. Direct mucosal challenge with food extracts confirms the clinical and immunologic evidence of food allergy and suggests the usefulness of the sensitized dog as a model for food allergy. This model has the potential to detect hidden food allergies in unexplained inflammatory gastrointestinal tract diseases and in genetically modified food. In this article, we summarize recent work on wheat and milk in support of this conclusion. Details of these studies have been published previously.^{2,3}

MATERIALS AND METHODS

Animals

Dogs in this project are supplied by the Animal Resources Service, School of Veterinary Medicine, University of California, Davis. All animals are derived from a colony of inbred, high-IgE-producing, atopic spaniel/basenji-type dogs. The dogs in the atopic colony are selected for a genetic predisposition to allergy and have a long history of sensitivity to pollens and foods. The animals are cared for according to the principles outlined in the NIH Guide for the Care and Use of Laboratory Animals. Pups are nursed for 6 weeks and weaned onto Eukanuba Puppy Small Bites (IAMS Co., Dayton, Ohio). They are fed once per day, and water is available *ad libitum*. After 6 months the dogs are fed Eukanuba Puppy Large Bites (IAMS Co.). At 1 year of age they are fed Eukanuba Original (IAMS Co.).

Food Sensitization of Atopic Dogs

Newborn pups are injected subcutaneously at day 1 with 1 µg each of wheat, cow's milk, and beef extract (Miles) in 0.2 ml of alum. Pups receive 0.5 µl of distemper-hepatitis vaccine subcutaneously at 21, 49, and 77 days after birth. During this period the pups are boosted with the same food allergy regime used at day 1 (at 22, 29, 50, 57, 78, and 85 days) (FIG. 1); thereafter pups are boosted at bimonthly intervals using 10 µg of each allergen. At 3–4 months of age, the pups exhibit positive skin tests and have specific IgE antibodies to the foods determined by anti-canine IgE immunoblot assay.⁴ When fed 200 g of wheat flour gruel or cow's milk, the sensitized pups at 6 months of age exhibit vomiting and/or diarrhea (increased number of loose or watery stools for 2–4 days after feeding challenge).⁵ The animals are periodically monitored by IgE enzyme-labeled immunodot, skin, and gastroscopic testing. More complete details of the clinical symptoms have been

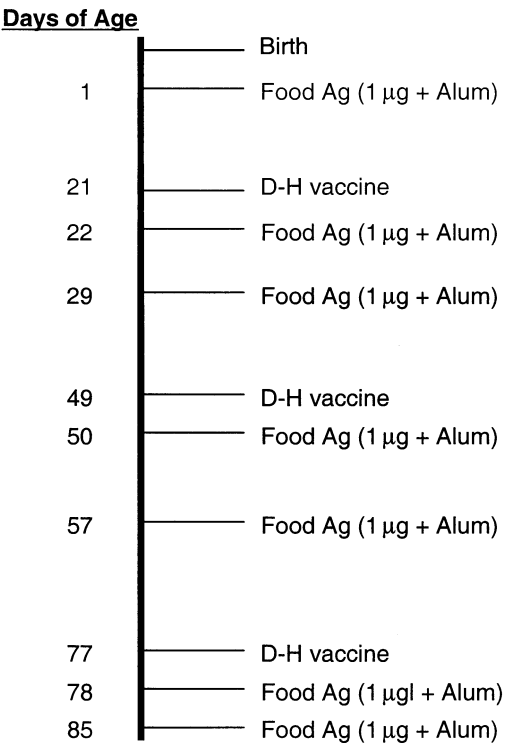


FIGURE 1. Immunization schedule for sensitizing dogs to food antigen extracts. Figure based on data from Ermel *et al.*¹

described previously.⁶ All of these tests support the utility of the animals as a model for food allergy.

Skin Tests

About 3 min before skin testing, each dog receives 4–5 ml of filtered 0.5% Evans blue dye solution (equivalent to 0.2 ml of 0.5% Evans blue dye per kg of weight) through a cephalic vein to enhance assessment of the cutaneous IgE antibodies.⁵ Serial dilutions of 100 µl of each sample are injected intradermally on the abdominal skin to establish the titer. After 15–20 min, the allergic response is determined by measuring the size of the blue wheal reaction (maximum length and width). An appropriate negative control (solution of glycerol, sodium myristate, or ethanol diluted in physiological saline buffer) is included for each animal tested.

Feeding Challenges

β -Lactoglobulin (BLG), 2.5 grams of a mixture of 80% pure A and B forms, is dissolved in water (final volume, 100 ml). BLG solution is mixed with a 12-oz can of D/D dog food (Hills) and fed to a dog. Unchallenged animals receive dog food alone. The observations and recording of vomiting and number and quality of stools are made blindly by an experienced investigator or veterinary technician. Changes in the upper (vomiting) and lower (stool quality) gastrointestinal tract are scored as in TABLE 1 and subjected to the statistical methods as indicated.

RESULTS

Experiments with Wheat: Skin Tests

Osborne Fractions

The proteins of wheat flour have classically been divided into four classes on the basis of solubility in the indicated solvent: albumins (distilled water) account for 15% of the total protein, globulins (salt water) for 5%, gliadins (ethanol) for 40%, and glutenins (acetic acid) also for 40%.⁷ Each of these fractions, which vary somewhat in abundance among different wheat cultivars, contains intramolecular disulfide bonds that are known to be effectively and in some cases specifically reduced by thioredoxin.^{8,9} The results of FIGURE 2 show that, while each fraction elicits an immunological reaction, there is a difference in response. In animals showing a range of sensitivity to wheat, gliadins are consistently the strongest allergens, glutenins are second, albumins third, and globulins fourth. On the basis of their abundance, the relative importance of the four flour fractions as food allergens in dogs follows the order gliadin > glutenin > albumin > globulin. It remains to be seen to what extent the activity observed with the glutenins as well as the albumins and globulins is due to contaminating gliadins.¹⁰

Gliadin Fractions

As the gliadin fraction of flour is the most active, it is of interest to determine the relative allergenicity of the purified components, which are made up of monomeric proteins of well-defined types. The principal types, α , β , and γ , contain intramolecular disulfide groups.¹⁰ The evidence available indicates that the α - and β -gliadin group contains three intrachain disulfide bonds per molecule and that the corresponding number for the γ -gliadins is four. The molecular mass of these proteins, which are devoid of interchain disulfides, ranges from 30 to 50 kDa. The less abundant ω -type lacks disulfides. Two of

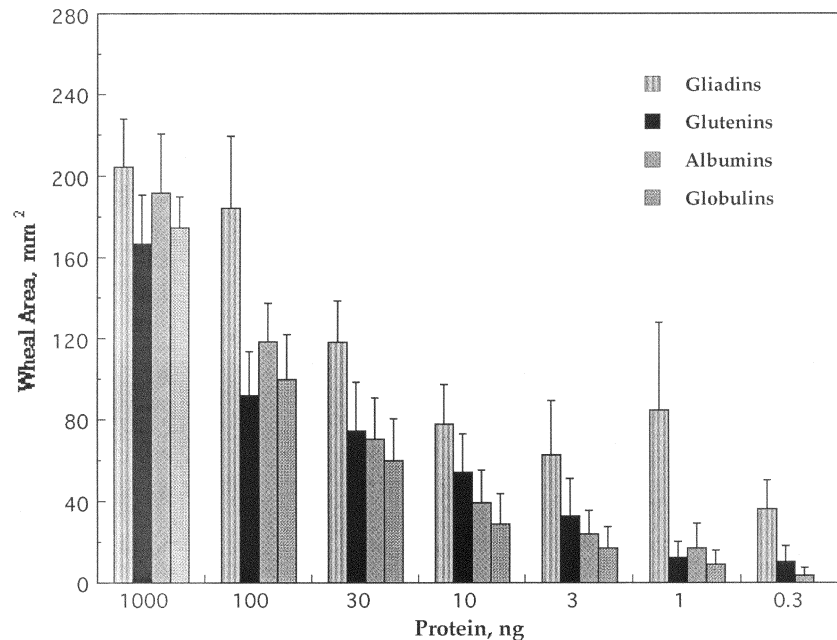


FIGURE 2. Relative allergenicity of wheat Osborne fractions. The full range of concentrations (1,000 to 0.3 mg) was tested with four dogs, three mildly and one highly sensitive. The concentration range from 100 to 3 ng of protein was tested with an additional four dogs, one mildly and three highly sensitive; response to this same concentration range was repeated with two mildly and one highly sensitive dogs. The data from all of these trials are incorporated. The error bars refer to standard error. (From Buchanan *et al.*² Used with permission.)

the fractions, α and β , originally believed to be different, are now considered to fall into a single group.^{10,11} On the basis of scanning acidic-PAGE gels, the relative abundance of the gliadins in our preparation was found to be 60% $\alpha + \beta$, 30% γ , and 10% ω . These figures fall within the range reported by others.¹² Highly and mildly sensitive dogs showed similar responses to the different gliadins: α similar to $\beta > \omega > \gamma$ (FIG. 3). Furthermore, of the total of 16 dogs showing sensitivity to wheat, 12 showed the strongest response to the α - and β -gliadins. On this basis, it appears that α - plus β -type gliadins represent the major allergenic forms. As determined by skin tests with our model, the α - and β -gliadins evoked the strongest response. This finding is of particular interest because it suggests a possible parallel between food allergy and celiac disease—a condition in which these same proteins appear to play a primary role.^{13,14} Significantly, the allergenicity of such disulfide proteins may be related to their heat stability, resistance to proteolytic digestions,¹⁵ and, for glycoproteins, carbohydrate content.¹⁶

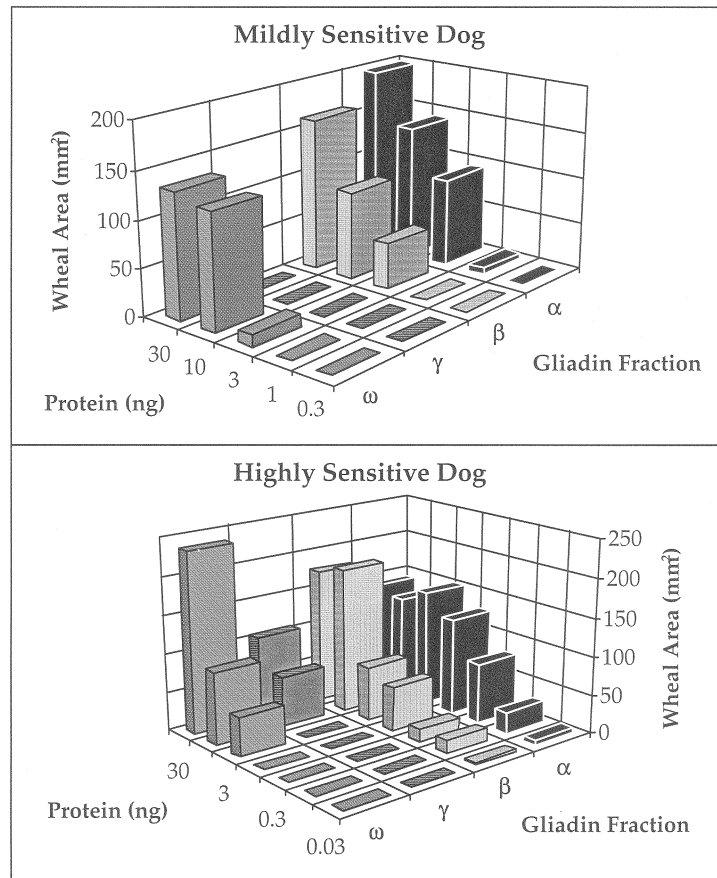


FIGURE 3. Response of highly and mildly sensitive dogs to gliadins. (From Buchanan *et al.*² Used with permission.)

Experiments with Milk: Skin Tests

A skin test comparison of the allergenicity of the major proteins of milk (α -lactalbumin, bovine serum albumin or BSA, BLG, and the α , β , and κ forms of casein) reveals that BLG is the major allergen of milk, accounting for 80% of the total activity in the dog model. Of the other proteins, only BSA and, to a lesser extent, casein (β form) show significant activity. Like BLG, milk elicits a strong skin test response in both highly sensitive (FIG. 4, upper panel) and mildly sensitive dogs (FIG. 4, lower panel).

TABLE 1. Allergic response of dogs fed β -lactoglobulin

Upper GI index: vomit scores		Lower GI index: stool scores	
Dog	Score	Dog	Score
6GCB3	7	5CBB6	1
6CGB7	6	6CGB1	0
6CGB1	5	6CGB4	2
6CGB4	9	6CGB6	0
6CGB6	8	6CGB8	3
		6CGB7	1
		6CGB9	2
		6GCB2	2
		6GCB3	4
		6GCB7	4
Mean:	7	Mean:	2

NOTE: Upper gastrointestinal (GI) index response was measured by following the vomit response after feeding 2.5 g of the thioredoxin-treated or untreated BLG to dogs. Upper GI index quotations were as follows: no vomit = 0, limited vomit (< 10 cm diameter) = 1, extensive vomit (>10 cm diameter) = 2, solid vomit = 1, liquid vomit = 2, liquid vomit with blood or bile = 3, delayed vomit = 1, and immediate vomit = 2 (maximum value = 9). Lower GI changes were followed on the challenge day and the following day. They were scored by the quality of the stools as follows: no change = 0, solid to semisolid = 1, semisolid to liquid = 2, and constipation = 2. Scores reflect multiple vomits with numbers greater than 7. The number 6 indicates that the dog is a member of the sixth generation of the colony.

Experiment with β -Lactoglobulin: Feeding Challenges

An upset in the gastrointestinal tract leading to vomiting and diarrhea is a clinical symptom that accompanies the ingestion of food allergens. A minority of dogs consistently shows constipation rather than diarrhea. The severity of these symptoms provides a clinical measure of the strength of allergens that complements skin testing. To obtain evidence on the gastrointestinal response, we supplemented the food fed to milk-sensitive dogs with BLG (TABLE 1). Each of the five dogs tested vomited after eating BLG. Of the 10 animals fed BLG, six showed diarrhea, two constipation, and two were unaffected. In all experiments, the unchallenged dogs (fed dog food alone) consistently failed to vomit and showed no loose stools.

DISCUSSION

Canine IgE-mediated food hypersensitivity is a relatively common presenting chief complaint in veterinary practice, ranging from atopic dermatitis to nausea/vomiting, diarrhea, and anaphylaxis. The information from a dog model of food allergy may, therefore, more closely mimic the situation in humans than rodent counterparts in which allergies do not occur naturally. Ev-

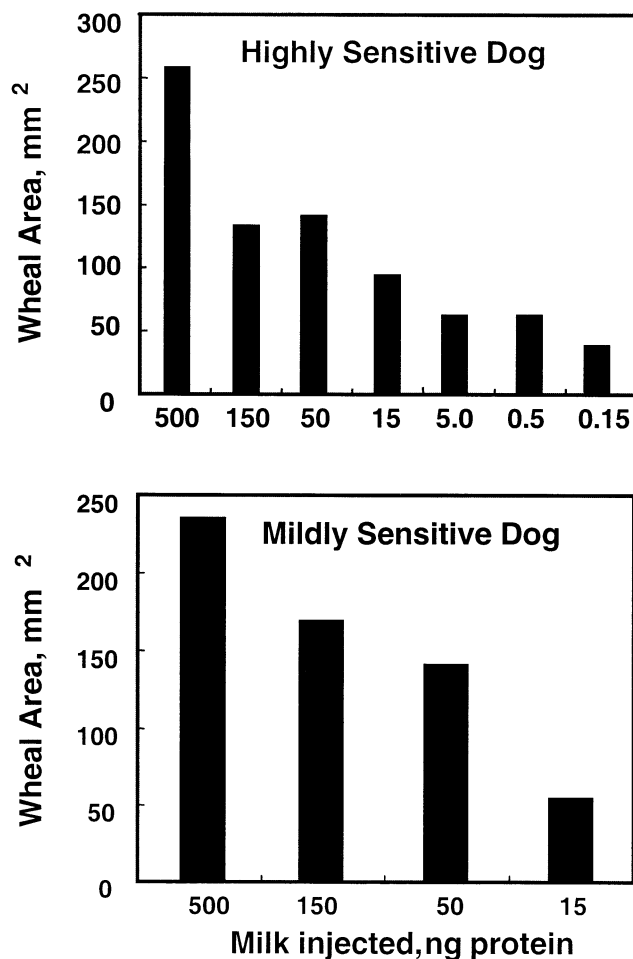


FIGURE 4. Skin test response of dogs of differing sensitivity to milk. The type I hypersensitivity reaction was determined by the wheal area (2 mm) induced by 100 μ l of intradermal injections of milk solution of known concentration. Physiologic-buffered saline control was negative for each of the nine dogs selected from the colony of 12. The remaining three dogs were inconsistent reactors. Data taken from del Val *et al.*³

idence for this conclusion is provided by the recent studies with wheat² and milk³ discussed above. It will be of interest to compare results obtained with the present colony of basenji-spaniel dogs to the soft-coated wheaten terrier model being developed to test food allergies.¹⁷

The characterization of the underlying mechanisms and the development of possible therapeutic strategies for human gastrointestinal tract hypersensitivities have been greatly enhanced by the use of animal models for food al-

lergy research. Domestic animals such as pigs, calves, and other large animals have all been used for food allergy research. For example, there is evidence that local cell-mediated immunity can be detected in the intestines of pigs after oral immunization with infective agents and soluble protein antigens.^{4,5} Other domestic animals can develop allergic reaction to food, as evidenced by calves that react to artificial milk in a similar fashion to human infants drinking cow's milk. Feeding preruminant calves with milk replacers containing >30% soya can lead to diarrhea, weight loss, and even death^{18,19} and is associated with a high titer of circulating antibody specific for soya bean antigens.²⁰⁻²²

In addition, models are being developed with laboratory animals—rats and mice. Knippels *et al.* are developing a Brown Norway rat model.²³ They demonstrated that the rat produces IgG and IgE of similar specificity to the human upon oral antigen sensitization without adjuvants. In another study, Miller *et al.* also have shown that the Brown Norway rat produces IgE that recognizes epitopes of cow's milk antigens similar to human sera.²⁴

Other investigators are developing promising models, including one in which the mice (DBA/2) were sensitized orally without adjuvant.²⁵ The use of cholera toxin co-administered orally with antigen has also been effectively used to induce an IgE response.^{26,27} Li *et al.* showed that mice sensitized orally with freshly ground peanut developed IgE responses to the major peanut allergens, Ara h 1 and Ara h 2, similar to humans, including reactivity with the major human IgE epitopes on Ara h 2.²⁸

Another type of mouse model (Balb c) is also under development.²⁹ Intraperitoneal administration of food antigens in the mice, with or without adjuvant, showed some evidence of a hierarchy response—a feature desirable in an animal model—suggesting a potential application in the evaluation of genetically modified foods.³⁰

From our studies of food allergen-immunized dogs, we have documented the feasibility and usefulness of the atopic dog as a model for food allergy. These inbred, allergic dogs easily make antibodies (IgE) to food antigens after immunization with a live virus vaccine, followed immediately by a subcutaneous injection of food antigen extract and alum. The immunized animals respond within 3 to 4 weeks by producing IgE antibodies that are specific to the food allergens (wheat, milk, and beef). Skin test results for the food antigens are consistently positive, as evidenced by a wheal-and-flare reaction. The characterization of the underlying mechanism involved in the development of food allergy in these dogs is important as a model for the study of this condition in humans. The atopic dog model might also be useful in detecting hidden food allergies in unexplained inflammatory gastrointestinal tract diseases of humans and animals as well as in the testing of genetically modified foods. This understanding would facilitate development of better diagnostic tests and aid in the development of pharmacologic agents for the treatment and/or prevention of food allergy and, at the same time, help ensure food safety.

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