The atopic dog as a model of peanut and tree nut food allergy

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Background: Animal models are needed that mimic human IgE-mediated peanut and tree nut allergy. Atopic dogs have been previously used in a model of food allergy to cow's milk, beef, wheat, and soy, with the demonstration of specific IgE production and positive oral challenges similar to those seen in human subjects.

Objective: We sought to sensitize dogs to peanut, walnut, and Brazil nut and to assess whether sensitization is accompanied by clinical reactions and whether there is cross-reactivity among the different preparations.

Methods: Eleven dogs were sensitized subcutaneously by using an established protocol with 1 μg each of peanut, English walnut, or Brazil nut protein extracts in alum first at birth and then after modified live virus vaccinations at 3, 7, and 11 weeks of age. The dogs were sensitized to other allergens, including soy and either wheat or barley. Intradermal skin tests, IgE immunoblotting to nut proteins, and oral challenges were performed with ground nut preparations.

Results: At 6 months of age, the dogs' intradermal skin test responses were positive to the nut extracts. IgE immunoblotting to peanut, walnut, and Brazil nut showed strong recognition of proteins in the aqueous preparations. Each of the 4 peanut- and the 3 Brazil nut–sensitized dogs and 3 of the 4 walnut-sensitized dogs reacted on oral challenge with the corresponding primary immunogen at age 2 years. None of the peanut-sensitized dogs reacted clinically with walnut or Brazil nut challenges. One of the walnut-sensitized dogs had delayed (overnight) vomiting to Brazil nut.

Conclusions: On the basis of measurements of the mean amount of allergen eliciting a skin test response in dogs, the hierarchy of reactivity by skin testing is similar to the clinical experience in human subjects (peanut > tree nuts > wheat > soy > barley). Cross-reactivity, which was not apparent between soy and peanut or tree nuts or between peanut and tree nuts, was slight between walnut and Brazil nut. The results give further support to the dog as a model of human food allergy. (J Allergy Clin Immunol 2002;110:921-7.)

Key words: *Canine, dog, food allergy, model, peanut, tree nut, walnut, Brazil nut*

In the United States allergy to peanuts or tree nuts affects approximately 1.1% of the population.[1] Of the tree nuts, walnuts and Brazil nuts have frequently been associated with triggering systemic allergic reactions, including fatalities.[1-6] Major allergens in these seeds have been described and include 2S albumin, vicilins, and legumin seed storage proteins.[3-6] It is not known why certain proteins are more likely than others to elicit food sensitivity and why, among foods, some tend to elicit more extreme degrees of an allergic response than others (eg, peanut in comparison with wheat). Rodent animal models have been important to ongoing research in mechanisms and modulation of food immune responses but only recently are being used to address questions such as the relative allergenicity of different foods.[7]

The dog is one of the few species other than humans in which allergies develop naturally on normal environmental exposure to a broad spectrum of allergens, including pollens, house dust mites, human dander, fleas, and foods.[8-10] The dog might thus have a role as a model in studying protein allergenicity.[11] Disease states include allergic rhinitis, conjunctivitis, atopic dermatitis, asthma, and IgE-mediated food hypersensitivity.[8-12]

After Portier and Richet's historic description of anaphylaxis,[13] it was recognized that dogs could be potentially useful in the study of IgE-mediated hypersensitivity. An atopic dog colony at the University of California has been under development as a model of food allergy.[14-16] The animals, a high IgE-producing inbred colony, were originally used as a model of airway hyperreactivity to ragweed and grass pollens.[17] Subsequently, they were successfully immunized with food proteins by using the same methodology used for airborne allergens.

Although the dogs have been sensitized to a variety of food allergens, they have not been tested with peanuts or tree nuts, which would be expected to be more allergenic than a food such as soy if dogs mimic the human condition. We have therefore undertaken experiments in this direction and now describe the successful sensitization of a small number of dogs with peanut, walnut, or Brazil nut extracts.

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Abbreviation used
TX-100: Triton X-100

METHODS

Dogs

The animals were descended from a colony of inbred, high IgE-producing spaniel/basenji dogs maintained at the Animal Resources Service, School of Veterinary Medicine, University of California, Davis. The animals, the seventh generation of the colony, were housed in facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care and were cared for according to Institute of Laboratory Animal Resources guidelines.[18] Puppies were nursed for 6 weeks and then weaned to Eukanuba Puppy Small Bites (Iams Company, Dayton, Ohio), as previously described.[16] After weaning, pups and adult dogs were fed commercial dry dog foods selected to avoid exposure to the experimental antigens to which a particular dog was sensitized. A veterinarian, an animal health technician, and student volunteers trained and socialized the animals.

Immunizations

Dogs were immunized on day 1 subcutaneously in the axilla with 1 μg of commercial extract in 200 μL of saline plus 200 μL of alum: peanut *(Arachis hypogaea)*, walnut *(Juglans regia)*, or Brazil nut *(Bertholia excelsa)* and either wheat *(Triticum aestivum)* or barley (*Hordeum vulgaris*; Hollister-Stier, Spokane, Wash). All dogs were immunized with soy *(Glycine max)*. Protein content was measured by using the BioRad protein assay kit (BioRad Inc, Hercules, Calif). At ages 3, 7, and 11 weeks, the puppies were vaccinated subcutaneously in the shoulder area with 1.0 mL of modified live canine distemper (at 3 weeks; Schering-Plough Animal Health, Omaha, Neb) or modified live canine distemper–adenovirus type 2–parainfluenza–parvovirus vaccine (at 7 and 11 weeks; Fort Dodge Laboratories, Fort Dodge, Iowa). Two and 9 days after the viral inoculations, the dogs were given the same allergen extracts they had received on day 1. Booster immunizations of the allergens in alum as above were administered bimonthly in the axilla by using 10 μg of allergen extract.

Skin testing

The ventral aspect of the abdomen was used for intradermal skin testing with the same extracts used for immunization.[16] Five minutes before the skin tests, 0.2 mL/kg filtered 0.5% Evans blue dye (Sigma Chemical Co, St Louis, Mo) was injected intravenously to help visualize the wheal response. Serial dilutions were prepared to determine the minimum concentration of protein needed to induce a wheal. Negative controls (saline, Hollister-Stier) were placed as well. A positive test response was defined as a wheal-and-flare reaction showing up as a blue area measuring greater than 5 × 5 mm when read at 20 minutes.

Statistical analysis

Comparisons were kept between litter mates that were identically inoculated when possible to compare the relative potency of the immunogens. Because the range of concentrations tested was quite broad, we applied the logarithm of the dose response for statistical analysis. To this end, we used the mean and SD of the logarithm obtained with the indicated number of dogs tested for the calculations by using the complete randomized block design method. The statistical significance of the differences between peanut and walnut (or Brazil nut) was determined by calculating the probability that the sensitivity of peanut over that of the other nuts can be tested by chance. The test was completed at a level of significance at which the P value was less than .05. The statistical significance of the differences between nuts and cereals or soy was determined by using the 1-tailed signed-rank test. The null hypothesis, assuming no difference in allergenic response between nuts (peanuts and tree nuts) and cereals or soy, was tested against the alternative hypothesis, assuming a difference between nuts and the other 2 seeds. The 1-tailed signed-rank tests were completed at the 0.1 level of significance (ie, $P < .1$ reflected statistical difference).

IgE immunoblotting

Sera were obtained when animals were 1, 2, and, for some of the dogs, 3 years of age. Peanut, walnut, and Brazil nut extracts were made in our laboratory by using previously published methods.[5] Electrophoresis and transfer of reduced protein extracts to 0.22-μm nitrocellulose membranes (MSI, Westborough, Mass) was carried out as previously described.[5] Blots were cut into strips containing approximately 25 μg of protein per 4 mm and then blocked for 1 hour at room temperature in PBS/3% nonfat dry milk/0.2% Triton X-100 (TX-100). Diluted sera from the immunized dogs or pooled sera from control nonatopic dogs (1:5 in PBS/3% nonfat dry milk/0.2% TX-100) were added to the strips and incubated overnight at 4°C. Additional strips were incubated with PBS/3% nonfat milk/0.2% TX-100 (buffer control to monitor nonspecific binding of the anti-canine IgE polyclonal antibodies). The strips were washed 3 times for 20 minutes in PBS/0.01% TX-100 and incubated overnight with anti-canine IgE–horseradish peroxidase (CMG, Fribourg, Switzerland) and then washed again and developed with the TMB Peroxidase Substrate Kit (Vector Laboratories, Inc, Burlingame, Calif). For comparison, strips from the same blots were used for IgE immunoblotting by using pooled sera from human patients with a history of anaphylaxis on ingestion of either peanut, walnut, or Brazil nut according to previously described methods.[5] Sera were obtained after informed consent and approval by the institutional review board.

Oral challenges

Dogs were monitored individually in their kennels for 3 days before food challenges to ensure normal appetite and the absence of diarrhea or vomiting.[16] On the day of challenge, food was withheld to decrease the risk of gastric torsion. Challenges with the freshly ground nut to which the animal had been sensitized were initiated at 1 g, followed by 4 g, 5 g, and, finally, 10 g at 20- to 30-minute intervals. The total dose was thus up to 20 g. Ground nuts were moistened with water to form a slurry, which was placed on the tongue and swallowed. Dogs were monitored for vomiting, swelling, nasoocular signs, pallor of the oral mucosa, lethargy, and diarrhea for 3 hours; rechecked at 5 and 7 hours; and then left overnight with stool-kennel checks 3 times per day for the next 3 days. Challenges were performed in all dogs at 2.5 years of age and again at 3.5 years in one dog that had not initially reacted.

RESULTS

Immunization

Two litters (7FB and 7FC) were immunized (birth date, January 1998). Four dogs from the 7FB litter were sensitized to peanut (ie, 7FB4, 7FB5, 7FB6, and 7FB9). Four dogs from a second (7FC) litter were sensitized to walnut (ie, 7FC1, 7FC3, 7FC4, and 7FC5), and 3 were sensitized to Brazil nut (ie, 7FC6, 7FC8, and 7FC9). The peanut- and walnut-sensitized dogs were also immunized to barley, and the Brazil nut–sensitized dogs were immunized to wheat. All dogs were immunized to soy.

TABLE I. Sensitivity of allergenic response to peanut and tree nut versus soy, as determined by means of skin testing

Sensitization	Dogs	Peanut	Walnut	Brazil nut	Soy
Peanut/soy	7FB4	0.02	2793	74	6600
	7FB5	0.002	2793	7400	660
	7FB6	0.2	NR	NR	66
	7FB9	0.002	NR	7400	66
Walnut/soy	7FC1	222	28	740	0.66
	7FC3	NR	279	740	66
	7FC4	NR	28	NR	660
	7FC5	2217	279	7400	660
Brazil nut/soy	7FC6	NR	NR	74	660
	7FC8	22	NR	0.74	660
	7FC9	NR	NR	7.4	6600
Mean dose in sensitized dogs		0.06	154	27	1518

Values are given in nanograms inducing a wheal response.
NR, Nonreactive to highest concentration used.

Skin testing

Dogs were skin tested to nuts at 6 months of age. All of the animals had positive responses to the commercial extracts used for sensitization. Responses to the saline negative controls consistently measured 0 × 0 mm. Skin tests were repeated at 14 and 26 months of age, and the latter results are summarized in Table I, which shows the mean minimum nanogram dose of protein eliciting a wheal. Among the nuts, peanut elicited the strongest response by a wide margin, followed by Brazil nut and walnut. On the basis of the mean amount of allergen eliciting a response, the hierarchy of activity was peanut > tree nuts > wheat > soy > barley. In general, this sequence was supported by statistics. Thus probability analysis confirmed that peanut was more allergenic than either Brazil nut or walnut, with respective *P* values of .029 and .014. Peanuts and tree nuts were significantly more potent allergens than soy or the cereals. Peanut was a stronger allergen than barley or soy ($P = .0625$), and walnut was stronger than wheat ($P = .0625$).

Some limited cross-reactivity by skin testing was seen among the nut preparations. Two of the 4 peanut-sensitized dogs had positive skin test responses to walnut and 3 to Brazil nut but only with approximately 100,000 times the amount of protein compared with peanut. The 3 Brazil nut–sensitized dogs did not react at all to walnut, and only one reacted to peanut at a 30-fold higher dose of protein. Although 2 of the walnut-sensitized dogs showed no reactivity to peanut, the other 2 animals responded at a concentration 10-fold higher than that of the primary immunogen. Interestingly, 3 of the 4 walnut-sensitized dogs reacted to Brazil nut at approximately a 10-fold higher dose of protein. In addition, there was no apparent cross-reactivity between any of the nuts and soy; that is, although all animals were soy sensitive, they did not respond well to the individual nuts unless sensitized to a particular nut preparation. The influence of soy immunization on peanut titers is judged to be noncontributory because the walnut- and Brazil nut–sensitized dogs were similarly immunized and showed little IgE against peanut. Evidence of the lack of an effect of peanut or tree nut sensitization on soy sensitization is seen in the similar minimum doses of soy required to elicit a positive skin test response in both the peanut- and tree nut–sensitized dogs (Table I). As a result of a closer phylogenetic relationship, there was strong cross-reactivity between the cereals, wheat and barley (Table II). This finding prompts the question of whether responses of this type occur in human subjects, a factor of potential significance because of the common use of barley in infant food.

IgE immunoblotting

Fig 1 shows the canine IgE immunoblots with the 3 nut preparations obtained from dogs at 1 year of age. Fig 1, *A*, displays the IgE response to peanut. The peanut-sensitized dogs all showed sera IgE binding to Ara h 1 at approximately 60 kd and less extensively to multiple other polypeptides-proteins. Only faint IgE binding is visible to polypeptides in the region just below the 21.5-kd marker, the presumed location of Ara h 2. An unknown 45-kd band of varying intensity was observed with the peanut- and walnut-sensitized dogs. On the basis of these immunoblots, Ara h 1 appears to be the dominant protein eliciting an IgE response in the peanut-sensitized dogs. Walnut-sensitized dogs showed limited IgE binding to scattered peanut polypeptides but no response specifically to Ara h 1 or consistently to the other peanut allergens. Two of 3 Brazil nut–sensitized dogs (7FC8 and 7FC9) showed faint IgE binding to polypeptides at approximately 40 kd.

Fig 1, *B*, demonstrates that sera from the walnut-sensitized dogs bound to multiple walnut polypeptides but, interestingly, not to the large (approximately 7-9 kd) subunit of Jug r 1, a major human allergen.[4] All dogs showed IgE binding to presumed Jug r 2, the vicilin-like protein at approximately 43 kd. The peanut-sensitized dogs showed some cross-reactivity to multiple proteins and polypeptides, particularly 7FB5, with IgE binding to the presumed 43-kd vicilin-like protein, but this was not accompanied by clinical reactivity (see below). A prominent unknown band of approximately 40 kd was observed with 3 of the walnut-sensitized dogs (ie, 7FC3, 7FC4, and 7FC5).

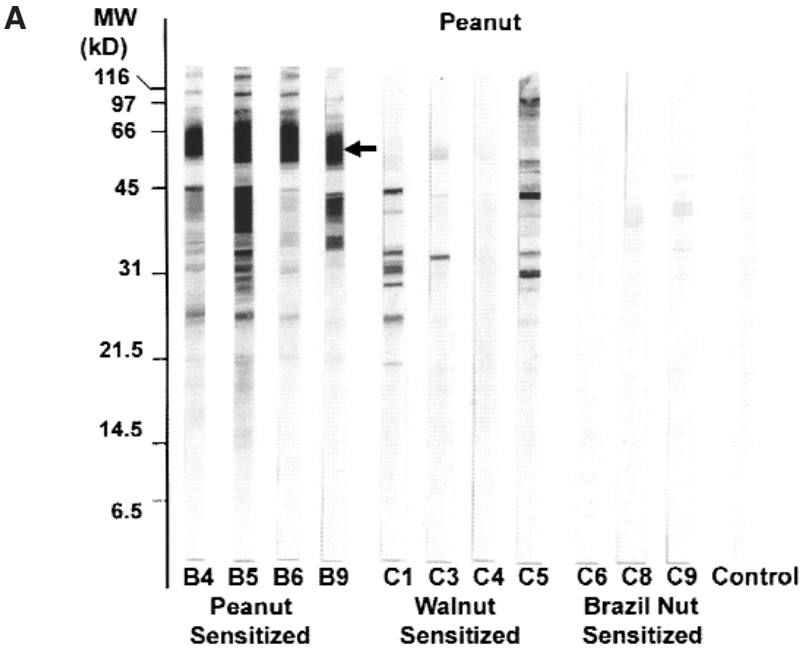

FIG 1. IgE immunoblots with canine sera. **A,** Peanut: the location of Ara h 1 is indicated with an *arrow.* **B,** Walnut: the location of Jug r 2 is indicated with an *arrow.* **C,** Brazil nut: the location of Ber e 1 is indicated with an *arrow.* The *wedge* denotes an unknown polypeptide at approximately 47 kd that strongly binds IgE.
(Figure continued on next page.)

Fig 1, *C*, shows that IgE from the Brazil nut–sensitized dogs specifically bound Ber e 1, the 2S albumin protein large subunit at 7 kd. All 3 dogs also had a strong response to an unknown protein of approximately 45 kd. None of the peanut- or walnut-sensitized dogs showed binding to the low-molecular-weight Brazil nut proteins, but 2 sera from peanut-sensitized dogs (7FB4 and 7FB5) and 3 sera from walnut-sensitized dogs (7FC1, 7FC3, and 7FC5) showed reactivity to proteins at approximately 36 to 40 kd and 45 to 47 kd, although the strength of the signal to the latter is not as strong as that seen for IgE binding from the Brazil nut–sensitized dog sera.

Fig 2 directly compares canine and human IgE reactivity. For peanut, there is general concordance of antigen recognition by IgE, except to the lower-molecular-weight proteins. This is also the case for walnut, for which the lack of IgE to Jug r 1 is noted. For Brazil nut, there is good concordance such that all polypeptides recognized as allergens by the dog sera used in this immunoblot are also human allergens.

Oral challenges

All peanut-sensitized dogs reacted on peanut challenge with vomiting and lethargy within 10 minutes of administering a provocative dose: 5 g in 1 animal, 10 g in 2 animals, and 20 g in 1 animal (7FB9, 7FB4, 7FB5, and 7FB6, respectively; Table III). Because proteins account for approximately 23% of the weight of peanut, this represents protein doses of approximately 1.2, 2.5, and 5 g, respectively. No peanut-sensitized animal reacted clinically to walnut or Brazil nut. All of the peanut-sensitized dogs recovered spontaneously without pharmacologic intervention.

Three of the 4 walnut-sensitized dogs reacted on walnut challenge at 2.5 years, whereas the fourth reacted 1 year later. One dog (7FC3) fed 5 g total had frank anaphylaxis with cyanosis and vomiting, with signs of hypovolemia observed; there was no obvious angioedema or wheezing. The dog responded to epinephrine, diphenhydramine, and intravenous fluids. Another animal (7FC1) had voluminous diarrhea and lethargy 1.5 hours into the challenge after a total dose of 10 g of walnut. The third animal (7FC4) had copious vomiting after a total dose of 20 g. The fourth dog (7FC5) failed to react to 20 g of walnut at 2.5 years but, when rechecked a year later, had severe emesis with 1.7 g of ground walnut and was given epinephrine and diphenhydramine. None of the walnut-sensitized dogs reacted to challenge with peanut on cross-challenge, but one, 7FC4, had a small amount of vomit in the kennel the morning after challenge with 20 g of Brazil nut. Sera from 7FC4 had no visible IgE binding to Brazil nut proteins at either 1 year (Fig 1, *C*) or 2 years of age (data not shown), and the dog was nonreactive to Brazil nut by means of intradermal skin testing at 26 months of age (Table I).

Each of the 3 Brazil nut–sensitized dogs reacted to ground Brazil nut. One (7FC9) had vomiting and lethargy after oral challenge with 1 g of Brazil nut (approximately 250 mg of protein). A second (7FC6) had vomiting after 10 g, and the third (7FC8) was given an incorrect dose of 20 g for its initial dose and nearly died, with vomiting and hematemesis beginning within 2 minutes of challenge, followed by cyanosis and collapse. The dog responded well to epinephrine and intravenous fluids with diphenhydramine and prednisolone sodium succi-

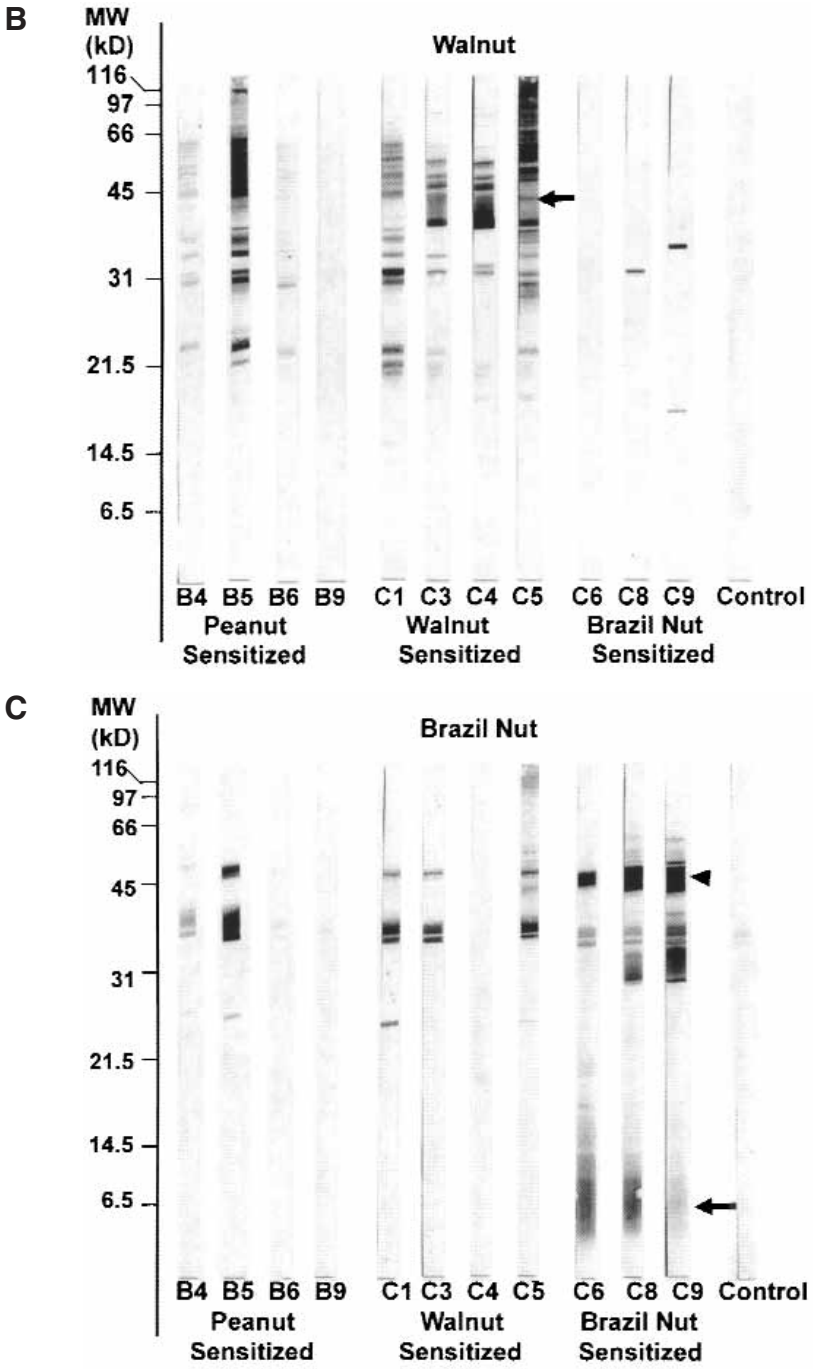

FIG 1. Continued from previous page.

nate but was thereafter extremely anxious when the investigators were doing food challenges. The animal had tolerated 20 g of walnut before the erroneous dose of Brazil nut. Several weeks later, the same dog was given 1 g of peanut, and it promptly vomited. After another break, it was given 1 g of peanut, followed by 4 g of peanut, which were tolerated. Doses of greater than 5 g were not tried in this dog because of its continuing agitation at challenges. The other 2 Brazil nut–sensitized dogs (7FC6 and 7FC9) did not react to either walnut or peanut.

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, rarely, anaphylaxis. The information from a dog model of food allergy might therefore more closely mimic the situation in human subjects than in rodent counterparts, in which allergies do not occur naturally. Evidence for this conclusion is provided by recent

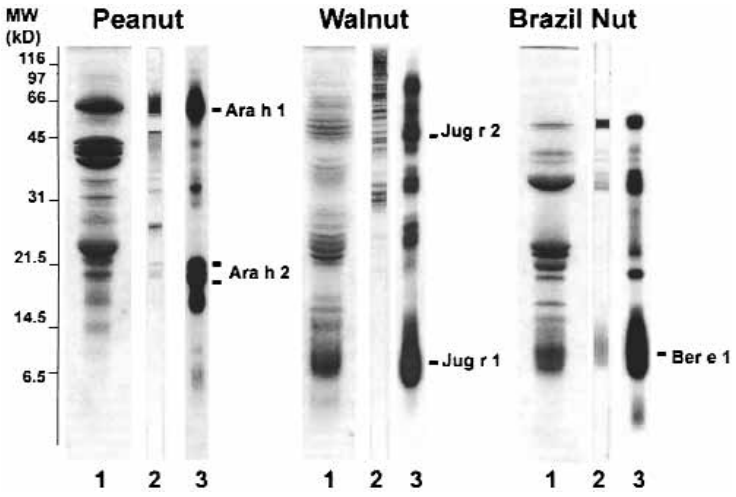

FIG 2. Comparison of canine and human IgE in reaction to peanut, walnut, and Brazil nut extracts: *lane 1,* Coomassie Brilliant Blue–stained gel corresponding to the blots; *lane 2,* IgE immunoblot with dog serum; *lane 3,* IgE immunoblot with human pooled sera.

TABLE II. Cross-reactivity of cereal allergens in wheat- and barley-sensitized dogs, as determined by means of skin testing

Sensitization	Dogs	Wheat	Barley
Wheat	7FC6	177	143
	7FC8	177	143
	7FC9	1767	1425
Barley	7FB4	1767	1425
	7FB5	ND	143
	7FB6	17,667	143
	7FB9	177	143
	7FC1	177	1425
	7FC3	176,667	1425
	7FC4	17,667	14,250

Values are given as nanograms inducing a wheal response.
ND, Not done.

studies with wheat[15] and milk.[16] The results presented herein showed that the dog model was applicable to other allergenic foods (ie, peanut and tree nuts) and could be used to investigate cross-reactivity. More species might evidence spontaneous atopy under the right conditions than previously recognized, however, because work by Knippels et al[19] demonstrated that the Brown Norway rat produced IgG and IgE of similar specificity to the human subject on oral antigen sensitization without adjuvants.

There has been concern that a mouse model of food allergy might have problems because of the ease of induction of tolerance by oral antigen feeding,[20] but several investigators have developed promising murine models, including one in DBA/2 mice in which the mice were sensitized orally without adjuvant.[21] Li et al[22] showed that mice sensitized orally with freshly ground peanut, using cholera toxin as an adjuvant, had IgE responses to Ara h 1 and Ara h 2 similar to those of human subjects, including reactivity with the major human IgE epitopes on Ara h 2. Helm et al[23] have also successfully used cholera toxin and peanut extract administered by means of intraperitoneal injection at birth to induce IgE sensitization and clinical anaphylaxis in a swine model of peanut allergy. The dogs described herein were immunized and boosted with commercial preparations rather than freshly prepared extracts, a factor that might explain the lack of a strong response to allergens such as Ara h 2 and Jug r 1.

Intraperitoneal administration of food antigens in mice, with or without adjuvant, has shown some evidence of a hierarchy response, a feature desirable in an animal model, suggesting a potential application in the evaluation of genetically modified foods.[7,24] On the basis of the present and previous[15,16] findings suggestive of a hierarchy response closely resembling the human clinical experience, dogs might also provide a potential model to test the allergenicity of genetically modified products. With a small number of dogs, we observed that, on the basis of skin tests, peanut proteins elicited an unprecedented IgE response, followed sequentially by tree nuts, wheat, and then soy and barley. This is the first demonstration in an animal model of hierarchy responses within the same animal because of the ability to immunize with more than one antigen. In addition, as in human subjects, the mere presence of IgE, as determined by means of skin testing or in vitro immunoblotting to nuts other than the primary immunogen, did not predict a clinical response.[25] Skin test data demonstrated that relative to peanut, the allergenicity values range from 1/500th with Brazil nut to 1/50,000th with barley. This hierarchy makes the dog uniquely situated as a model to test the hypothesis that proteins not considered allergenic in human subjects will only weakly stimulate IgE production, whereas those known to be potent allergens will be expected to elicit high titers of IgE. This possibility is currently under investigation. In future studies, it will also be of interest to compare results obtained with the present colony with those obtained with the soft coated wheaten terrier model, which is also being developed to test food allergens.[26]

The dog has additional advantages as a potential model, despite its increased cost and the time needed to achieve stable IgE responses with the current sensitization protocol. Its large size permits the performance of gas-

TABLE III. Response of peanut- and tree nut–sensitized dogs to oral challenge

Sensitization	Dogs	Walnut	Brazil nut	Peanut
Peanut	7FB4	NR	NR	Vomit, 10 g
	7FB5	NR	NR	Vomit, 10 g
	7FB6	NR	NR	Vomit, 20 g
	7FB9	NR	NR	Vomit, 5 g
Walnut	7FC1	Diarrhea, 10 g	NR	NR
	7FC3	Cyanosis/collapse, 5 g	NR	NR
	7FC4	Vomit, 20 g	Delayed emesis (>8 h later), 20 g	NR
	7FC5	NR,* 20 g	NR, 10 g	NR
Brazil nut	7FC6	NR	Vomit, 10 g	NR
	7FC8	NR	Cyanosis/hematemesis, 20 g	NR,† 5 g
	7FC9	NR	Vomit, 1 g	NR

NR, Nonreactive to 20 g of whole ground nut unless other amount noted.
*This dog vomited after 1.7 g of walnut at 3.5 years of age.
†This dog initially vomited after 1 g of peanut but, on a later date, tolerated 5 g.

trointestinal studies, such as sampling of mucosa under endoscopy, without killing the animal. Resuscitation of an animal with profound anaphylaxis is also possible (as demonstrated on 2 occasions during this study), allowing for the possibility of preimmunomodulatory and postimmunomodulatory therapy oral challenges without loss of animals used in the initial challenges. Finally, in contrast to other models, in which sensitization has been successful only with single allergens, it is possible to immunize one dog simultaneously with a number of foods.

In summary, a small group of dogs was successfully sensitized to peanut, walnut, Brazil nut, soy, wheat, and barley proteins in a manner reflective of the human response, thus strengthening the use of a canine model for the study of food allergy and also positioning the canine model as a potential additional screening tool for assessing the allergenicity of genetically modified foods.

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