Evaluation of the clinical and allergen specific serum immunoglobulin E responses to oral challenge with cornstarch, corn, soy and a soy hydrolysate diet in dogs with spontaneous food allergy

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Abstract Fourteen dogs with known clinical hypersensitivity to soy and corn were maintained on a limited antigen duck and rice diet until cutaneous manifestations of pruritus were minimal (78 days). Sequential oral challenges with cornstarch, corn and soy were then performed. Subsequently, the dogs were fed a diet containing hydrolysed soy protein and cornstarch. Throughout the study period the dogs were examined for cutaneous manifestations of pruritus and, additionally, serum was collected for measurement of allergen-specific and total immunoglobulin (Ig)E concentrations. Intradermal testing with food antigens was performed prior to entry into the study and after 83 days. A statistically significant clinical improvement was measured between days 0 and 83. Significant pruritus was induced after oral challenge with cornstarch, corn and soy (P = 0.04, 0.002, 0.01, respectively) but not with the hydrolysed diet (P = 0.5). The positive predictive value of the skin test for soy and corn allergy was reduced after feeding a soy and corn free diet. Although increases in soy and corn-specific serum IgE concentrations were measured in individual dogs post challenge they were not statistically significant and could not be used to predict clinical hypersensitivity.

Keywords: dog, food allergy, hydrolysed protein, IgE, intradermal test.

INTRODUCTION

Most veterinary practitioners recognize a percentage of dogs in clinical practice which apparently have an adverse reaction to dietary components. The prevalence of true food hypersensitivity in the population is unknown. A recent study identified food hypersensitivity in 7.6% of all dogs presented to a referral dermatology practice over a one year period. This represented 32.7% of all dogs presenting with allergic skin disease.1

Diagnosis is difficult and requires that the clinical signs improve during a diet trial with a limited antigen diet and return upon provocative challenge. Traditionally, diet trials have relied on identifying a novel protein source (one to which the dog has had little or no previous exposure) which is fed as either a home cooked recipe or commercially available diet. As the variety of protein sources available in commercial pet foods has expanded, the choice of a novel protein source has become more limited. Consequently, there is strong interest in the development of balanced hydrolysed protein diets for diet trials and long-term feeding. In these diets the protein source is modified by hydrolysis to peptides which are less able to elicit an immune response. A major limitation to clinicians recommending these diets, however, is the lack of data documenting whether dogs allergic to the parent protein can tolerate the hydrolysed product.

The majority of confirmed food allergic human patients are infants with hypersensitivities to cows’ milk. Owing to specific nutritional requirements, management of these individuals has led to the development of partial and extensively hydrolysed milk casein diets which have a high tolerance and safety profile. However, even in the extensively hydrolysed products (molecular mass < 1500 Da) allergic or intolerant reactions have been documented, thus even highly processed diets in humans are associated with the potential for adverse reactions in some individuals.2

At North Carolina State University we have a colony of Maltese × Beagle dogs that spontaneously manifest clinical signs of pruritus, otitis and colitis in response to oral challenge with dietary components to which they have been previously exposed.3 Dogs within this
colony are known to have sensitivities to orally administered corn and soy.

The diet under evaluation in this study was a hydrolysed soy protein canine diet with an average molecular protein mass of 12,200 Da (PVD HA, Nestle Purina Petcare, St Louis, MO). This diet also contains cornstarch. A previous randomised blinded feeding study demonstrated a reduction in pruritus in soy and corn allergic dogs when fed this diet by 50 and 80%, respectively.

The purpose of this study was to document clinical and immunological reactivity to oral challenge with soy, corn, cornstarch and the hydrolysed soy protein diet in the Maltese × Beagle dogs. This was an open feeding study designed to mimic a clinical dietary trial. Our hypothesis was that the majority of dogs with a proven clinical response to soy or corn would tolerate the hydrolysed soy protein diet.

**MATERIALS AND METHODS**

Fourteen Maltese × Beagle dogs were entered into the study. The mean age was 2.4 years (range 1.5–3 years) and the group consisted of four neutered females, three intact females and seven intact males. The intact females were not in oestrus during the study. The dogs were housed in the laboratory research facility at the College of Veterinary Medicine, North Carolina State University and the experimental protocol was approved by the Institutional Animal Care and Use Committee.

As puppies these dogs were fed a diet containing chicken, corn, barley and soy (Science Diet Canine Growth, Hills Pet Nutrition, Topeka, KS) until 12 months of age. Thereafter, they were changed to a diet containing corn, pork and whole soybean protein (Diet 1: Prolab Canine 2000, Labdiet, Richmond, IN). Occasional treatments containing corn, wheat and soybean mill run were also given (Science Diet Light Dog Treats, Hills Pet Nutrition Inc) and all dogs also received monthly flavoured milkemben heartworm prophylaxis containing pork liver and soy (Interceptor, Novartis Animal Health, US Greensboro, NC).

The dogs were examined and each one assigned a cutaneous clinical score (CCS). Briefly, this assessed three criteria: erythema, excoriations and evidence of infection, which were graded on a scale of 0–3 (0 representing normal and 3 severely affected skin). Thirty-five different areas of the skin were examined and a maximum score of 315 could be achieved. This also included an otic examination at each scoring time and cytology was performed if any discharge was present. An adverse clinical event was defined as a minimum of a 50% increase in CCS. Clinical examination was performed by the same investigator (HAJ) on each occasion, previous scores were not provided at the time of examination.

Baseline data were collected on day 0 whilst the dogs were receiving Diet 1. Thereafter they were fed a duck- and rice-based diet (Diet 2: Hills d/d Duck and Rice diet, Hills Pet Nutrition) which was introduced gradually over a period of 5 days by mixing with Diet 1. Diet 2 was fed until day 147. All dogs were challenged orally with the following allergens at 200 mg kg⁻¹ each on two occasions 24 h apart: days 85 and 86 cornstarch, days 99 and 100 corn, and days 119 and 120 soy. They were maintained on Diet 2 throughout the challenge period. On day 147, Diet 3 was gradually introduced over a period of 5 days by mixing with Diet 2, it was then fed exclusively until day 160.

For six months prior to, and during the study, the dogs received a monthly flavoured heartworm prophylactic (Heartgard Tabs, Merial, Duluth GA).

The period between the challenges was determined by the clinical responses of the dogs. Resolution of clinical signs had to occur in the whole group before the next allergen was administered. Where necessary bacterial and Malassezia sp. infections of the skin and ears were treated with appropriate therapeutic agents. No glucocorticoids or other anti-inflammatory agents were administered during the study period.

Intradermal skin testing was performed on day 0 (Diet 1) and on day 83 (Diet 2). The positive control was a monoclonal antibody immunoglobulin (IgE) and the negative control was saline. Each dog was tested with three 10-fold dilutions of the soy and corn allergen starting with the concentrate 1/40 w/v (Greer Laboratories, Lenoir, NC). These were dialysed prior to intradermal injection to remove 50% glycerin from the product which had previously been shown to be irritant at this concentration in these dogs (data not shown). The diameter of each reaction was measured after 20 min and a positive result was considered to be one greater than or equal to 50% of the diameter of the positive control accompanied by induration and erythema.

Serum was collected by jugular venepuncture during the study, frozen at −70 °C and analysed at the end of the study for total and corn- and soy-specific IgE. A time line is illustrated in Table 1.

Measurement of allergen-specific serum IgE

Corn- and soy-specific serum IgE was measured using enzyme-linked immunosorbent assay (ELISA). Prior to coating the microtitre plates the food allergens were denatured by heating to reveal linear epitopes. IgE was detected with a biotinylated mouse monoclonal antibody 5.91 specific for a heat-stable IgE epitope located in the C2 domain of the epsilon chain (unpublished data). Soy and corn allergen protein preparations (Greer Laboratories) were dialysed in phosphate-buffered saline, pH 7.4 (PBS) to remove glycerin before being boiled for 5 min in the presence of 5% 2-mercaptoethanol. After centrifugation the resulting denatured protein solution was dialysed against PBS and adjusted to 1 mg mL⁻¹ concentration. Stock denatured allergens were diluted to 50 µg mL⁻¹ in 0.1 m sodium carbonate bicarbonate buffer, pH 9.0, for coating microtitre styrene U-bottom plates (Dynex, Chantilly, VA) with 50 µL per well overnight at 4 °C. Plates were
Hydrolysate diet and canine food allergy

Table 1. Timeline of procedures performed during the study

<table>
<thead>
<tr>
<th>Day of study</th>
<th>Diet</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>S/CCS/IDT</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>S/CCS</td>
</tr>
<tr>
<td>36</td>
<td>2</td>
<td>S/CCS</td>
</tr>
<tr>
<td>58</td>
<td>2</td>
<td>S/CCS</td>
</tr>
<tr>
<td>83</td>
<td>2</td>
<td>CCS/IDT</td>
</tr>
<tr>
<td>85</td>
<td>2</td>
<td>Cornstarch</td>
</tr>
<tr>
<td>86</td>
<td>2</td>
<td>Cornstarch</td>
</tr>
<tr>
<td>87</td>
<td>2</td>
<td>S/CCS</td>
</tr>
<tr>
<td>99</td>
<td>2</td>
<td>S/CCS/Corn</td>
</tr>
<tr>
<td>100</td>
<td>2</td>
<td>Corn</td>
</tr>
<tr>
<td>101</td>
<td>2</td>
<td>S/CCS</td>
</tr>
<tr>
<td>106</td>
<td>2</td>
<td>CCS</td>
</tr>
<tr>
<td>111</td>
<td>2</td>
<td>CCS</td>
</tr>
<tr>
<td>118</td>
<td>2</td>
<td>S/CCS</td>
</tr>
<tr>
<td>120</td>
<td>2</td>
<td>Soy</td>
</tr>
<tr>
<td>121</td>
<td>2</td>
<td>S/CCS</td>
</tr>
<tr>
<td>125</td>
<td>2</td>
<td>S/CCS</td>
</tr>
<tr>
<td>146</td>
<td>2</td>
<td>S/CCS</td>
</tr>
<tr>
<td>147</td>
<td>2 &amp; 3</td>
<td>Diet 3</td>
</tr>
<tr>
<td>149</td>
<td>2 &amp; 3</td>
<td>S/CCS</td>
</tr>
<tr>
<td>153</td>
<td>3</td>
<td>S/CCS</td>
</tr>
<tr>
<td>160</td>
<td>3</td>
<td>S/CCS</td>
</tr>
</tbody>
</table>

IDT, intradermal test; S, serum; CCS, cutaneous clinical score; Diet 1, Prolab Canine 2000; Diet 2, Hills Duck and rice d/d; Diet 3, PVD HA.

washed three times with PBS containing 0.05% Tween-20 (PBST) prior to addition of 200 µL per well of 0.5% gelatin (Sigma, St. Louis, MO) in PBS for 2 h at room temperature. Gelatin-blocked plates were washed once before the addition of 50 µL per well of sample. Serum samples were run in triplicate at dilutions of 1:5 and 1:10 in PBST. After 2 h incubation with samples, plates were washed five times with PBST and 50 µL per well of biotinylated mouse monoclonal 5.91 anti-IgE at 1 µL ml⁻¹ in PBST containing 10% mouse serum was added and incubated for 1 h. Plates were washed five times with PBST before the addition of 1:1000 dilution of Zymax streptavidin–horseradish peroxidase (Zymed Laboratories, San Francisco, CA) and incubated 30 min. Finally, ABTS (Kirkegaard & Perry Laboratories, Gaithersburg, MD) was added after five washes and plates were read at 450 nm on an Elx 800 microtitre plate reader (Bio-Tek Instruments, Inc., Winooski, VT) after 30 min, 60 min or overnight for colour development. Standard curves for units of antigen level were developed on each plate using the same high IgE titre dog serum diluted serially from 1:5 to 1:40.

Total IgE was captured with monoclonal antibody 5.91 and quantified against standard curves of absolute quantities of monoclonal canine IgE generated for each plate using purified products of the heterohybridoma cell line 2.395 (Bethyl Laboratories, Montgomery, TX).

Statistics

Statistical analysis was performed using SAS software (Cary, NC). Correlation between variables was determined using a Pearson rank coefficient calculation. Determination of the significance of treatments (cornstarch, corn, soy and Diet 3) on the readout variables (clinical score, serum allergen specific and total IgE concentrations) was performed using longitudinal data analysis. Post-treatment variables were compared with day 83 when the dogs had been on Diet 2, the limited antigen diet exclusively for 78 days. To determine the effect of Diet 2, a comparison was made between day 83 and day 0.

The relationship between a positive clinical response to allergen and any (corn or soy) specific serological response was examined with a Fisher's exact test.

The data were also analysed to determine whether the age and gender of the dogs affected the responses and whether any carryover effect between treatments was present.

The positive predictive value of the skin test anticipating a clinical response to allergen was calculated when the dogs were on Diet 1 and compared with the predictive value when they were fed Diet 2.

RESULTS

Clinical response

All treatments had an effect on the CCS (Fig. 1). Diet 2 resulted in a statistically significant reduction in CCS (P = 0.001) at all time points evaluated. Oral challenges reached significance for corn on day 101 (P = 0.009) and soy on days 121 and 125 (P = 0.0028 and 0.0001, respectively).

Oral challenge with cornstarch resulted in an adverse reaction in 3/14 (21%) dogs. All three of these dogs subsequently reacted to corn. Ten of the 14 dogs (71%) reacted to corn, 11/14 dogs (78%) reacted to soy. Three dogs reacted adversely to Diet 3, all of these had developed cutaneous signs of pruritus (excoriations and self-induced hair loss) after both the soy and corn challenge. Two of these three had also reacted to cornstarch.

An increase in clinical scores was measured within 48 h of receiving the first dose of allergen in all cases in which there was a positive reaction although the peak response, defined as the highest CCS received after each challenge, in some dogs did not develop until 7 days post challenge. In the three dogs which reacted to Diet 3 the peak response was measured at 14 days post challenge.

Total and allergen-specific IgE measurements

A significant increase in total (P = 0.005), soy-specific (P = 0.015) and corn-specific (P = 0.013) IgE concentrations was seen when serum collected on day 83 was compared with day 0 (Figs 2 and 3). An adverse clinical response to orally administered allergen in individual dogs was commonly associated with an increase in serum allergen-specific IgE post challenge. However, the specificity of the response could not be predicted by the allergen administered orally. That is, when the dogs were fed corn some had a corn-specific,
and/or soy-specific allergen response. The initial oral challenge with cornstarch resulted in a significant increase \((P = 0.007)\) in total IgE concentrations and this increase was maintained throughout the challenge period (Fig. 4).

**Correlation**

A positive statistically significant correlation was determined between the cutaneous clinical scores and corn-specific serum IgE concentration \((P = 0.001)\). Also, there was a positive relationship between serum

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**Figure 1.** Mean cutaneous clinical scores (CCS) throughout the study period. Error bars represent the standard deviation from the mean. At time 0 the dogs were receiving Diet 1. CS = cornstarch, C = corn, S = soy. †; A significant reduction at day 83 compared with day 0 \((P = 0.001)\). *Significant increases post challenge \((P = 0.009, 0.0028, 0.0001)\), compared with day 83.

**Figure 2.** Mean serum soy-specific IgE concentrations throughout the study period. Error bars represent the standard deviation from the mean. At time 0 the dogs were receiving Diet 1. *Significant increase after feeding Diet 2 \((P = 0.015)\). Compared with day 0. CS = cornstarch, C = corn, S = soy.

**Figure 3.** Mean serum corn-specific IgE concentrations throughout the study period. Error bars represent the standard deviation from the mean. At time 0 the dogs were receiving Diet 1. *Significant increase after feeding Diet 2 \((P = 0.013)\). Compared with day 0. CS = cornstarch, C = corn, S = soy.

**Figure 4.** Mean serum total IgE concentrations throughout the study period. Error bars represent the standard deviation from the mean. At time 0 the dogs were receiving Diet 1. *Significant increase after feeding Diet 2 \((P = 0.005)\). **This increase in IgE is significant at \(P = 0.007\) compared with measurements taken on day 83. CS = cornstarch, C = corn, S = soy.
Table 2. The number of positive intradermal test reactions obtained on days 0 and 83. On day 0 the dogs were consuming a diet containing corn and soy, on day 83 they were on a corn- and soy-free diet.

<table>
<thead>
<tr>
<th>Dilution of 1:40 w/v solution</th>
<th>Soy day</th>
<th>Corn day</th>
<th>Soy day</th>
<th>Corn day</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.1</td>
<td>9</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>0.01</td>
<td>9</td>
<td>2</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 3. The value of intradermal testing with food allergens in predicting a positive clinical response to oral ingestion of that allergen is higher in Maltese × Beagle dogs when they have been recently ingested that allergen (Diet 1) compared with no ingestion for 78 days (Diet 2).

<table>
<thead>
<tr>
<th>Diet</th>
<th>Soy</th>
<th>Corn</th>
<th>Soy</th>
<th>Corn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet 1</td>
<td>90%</td>
<td>69%</td>
<td>20%</td>
<td>44%</td>
</tr>
<tr>
<td>Diet 2</td>
<td>0%</td>
<td>100%</td>
<td>0%</td>
<td>17%</td>
</tr>
</tbody>
</table>

No statistically significant carryover effect between treatments was measured.

Intradermal skin test results
The results of intradermal skin testing are represented in Table 2.

More positive reactions at all dilutions were seen in all dogs on day 0 when still receiving a diet containing corn and soy (Diet 1) as compared with a corn- and soy-free diet (Diet 2). The data were then evaluated for the predictive value of any positive reaction to the intradermal skin test in determining the cutaneous clinical reactivity to oral ingestion of that protein. Results were compared between Diets 1 and 2 and are illustrated in Table 3.

Gender and age effects
A statistically significant increase in total and corn-specific IgE responses was seen with increasing age (P = 0.04 for both variables). In addition, a greater response was seen in male dogs compared with intact or neutered females for the clinical score (P = 0.06), corn-specific IgE (P = 0.02) and total IgE (P = 0.03). There was no significant difference between the mean ages when genders were compared.

DISCUSSION
This population of soy- and corn-allergic dogs demonstrated a significant reduction in CCS after being fed a corn- and soy-free diet (Diet 2) exclusively for 78 days. Subsequent provocative oral challenge with whole corn and soy proteins resulted in the rapid development of pruritic skin disease and otitis, clinical signs which are consistent with our current understanding of a clinical diagnosis of canine food allergy. In people the gold standard of diagnosis is a double-blinded, placebo-controlled challenge. Owing to the small number of dogs in this colony it was not possible to incorporate this into the study design and remains a limitation of this report.

The majority of dogs with confirmed adverse reactions to soy (11/14) and corn (10/14) did not exhibit an increase in CCS when the hydrolysed soy protein diet (Diet 3) was added to the regime. Three of fourteen dogs did have an adverse cutaneous reaction to the introduction of Diet 3 which is consistent with the performance of hydrolysed milk formulas in infants with cows’ milk allergy. Because all three of the dogs that did not tolerate the diet had previously demonstrated clinical reactivity to both corn and soy, and two of them additionally to cornstarch it is impossible within this study design to determine which component of the diet was responsible for the adverse reactions.

With regard to the three dogs that had an adverse cutaneous reaction to cornstarch, there are two possible explanations. First, the cornstarch itself may have contained some corn protein or alternatively, the carbohydrate may be allergenic in these individuals as reported previously in people.

Dietary challenge clearly had a rapid effect of increasing serum allergen-specific IgE concentrations but it was not possible to predict clinical sensitivity from this reaction in individual dogs. Oral challenge with corn, for example, was often accompanied by a rise in both corn- and soy-specific serum IgE. It is unlikely that there are cross-reactive proteins between soy (a legume) and corn (a cereal) so we therefore speculate that when these dogs are exposed to antigen by the oral route, T-cell-dependent stimulation of previously committed B cells is initiated and production of IgE of multiple specificities occurs.

In man, elimination of a target allergen from the diet is associated with a reduction in the concentration of serum allergen-specific IgE concentrations and an improvement in clinical signs, and this has been suggested as a means of monitoring dietary compliance. In contrast, we measured an increase in serum allergen-specific and total IgE concentrations during the period the dogs were consuming a corn- and soy-free diet. The reason for this is not immediately apparent. One possibility is that under conditions of chronically reduced antigenic stimulation less IgE is complexed with IgG anti-IgE in the serum leading to increased measurements of free total IgE such as is seen in dogs with no history of allergic skin disease. This hypothesis remains to be tested.

After the initial oral challenge with cornstarch a significant increase in total serum IgE concentration was measured and this was maintained throughout the challenge period. Although total IgE has not been shown to be predictive of allergic disease in the dog, we propose that within an allergic population it may be influenced by cumulative and chronic allergen exposure as demonstrated in this study.

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The value of monitoring skin prick tests (SPT) during dietary allergen elimination in man has not been studied, however, a decrease in positivity to titrated SPT is associated with remission with allergen immunotherapy for atopic dermatitis. Intradermal testing (IDT), which is used in the dog in place of SPT, has been evaluated as a measure of clinical reactivity to dietary components by multiple authors but was found to be of poor predictive value. Clearly, our results demonstrate that the predictive value of the IDT in anticipating a clinical sensitivity to soy and corn allergens in this population of dogs with known hypersensitivities is dependent on recent oral exposure to that allergen and skin test reactivity is greatly decreased after 78 days on a corn- and soy-free diet. It should be noted, however, that we did not control for other variables such changes in the essential fatty acid content of the diet which may have also influenced our test results.

A novel finding was that of the effect of gender and age on not only the total and allergen-specific IgE responses to oral allergen challenge, but also the clinical cutaneous response. The male dogs had a greater clinical cutaneous response and corn-specific and total IgE serological response when compared with intact or neutered females. There was also an increase in total and corn-specific IgE responses with increasing age. One explanation for this might be that the older dogs had been exposed to Diet I for longer prior to receiving a limited antigen diet. However, this does not explain the gender effect we observed.

Other investigators have looked at the influence of gender and age on total serum IgE concentrations and found conflicting evidence. In one population of Beagle dogs, total IgE levels increased until the age of 4 years. It has also been suggested that allergen-specific IgE responses are genetically determined in the dog, a factor which has been exploited in the establishment of colonies for the study of atopic dermatitis. As the pedigree of this colony is well documented, further studies are under way to determine whether these observations have an hereditary basis.

The following conclusions can be drawn from this study. The soy hydrolysate diet was well tolerated by the majority of dogs with confirmed cutaneous reactivity to corn and soy and may be an appropriate choice for the long-term management of individuals allergic to soy or corn protein. Three of the fourteen dogs in this study had an apparent adverse reaction to the diet but whether this is an intolerance of the hydrolysed soy protein or the carbohydrate component is not clear from the study design and warrants further investigation.

There is strong evidence in this colony of dogs with known hypersensitivities that the diagnostic value of the intradermal test for food allergens in predicting clinical sensitivity is related to recent oral exposure to that allergen. This is similar to observations in atopic dogs regarding aero-allergen exposure. However, the predictive values of the IDT found in this study do not approach a level at which the authors would endorse this diagnostic test for specific food hypersensitivities in this or any other population of dogs. Likewise allergen-specific serological responses lack reliable specificity in these Maltese × Beagles.

ACKNOWLEDGMENTS

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REFERENCES


