IgE and IgG antibodies to food antigens in sera from normal dogs, dogs with atopic dermatitis and dogs with adverse food reactions

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Introduction

Adverse reactions to dietary components in dogs are believed to result from both immunological and non-immunological mechanisms, although the relative incidence of both is unknown. Dogs suffering from adverse food reactions generally present with dermatological or gastrointestinal signs, or a combination of both. The true incidence of such reactions is speculative, but they have been reported as comprising 6% of canine and feline dermatoses, and 15% of all allergic dermatoses in a referral practice, and as 19.6% and 30.6% of all dogs with non-seasonal pruritus. Concomitant atopic dermatitis is frequently seen. Although it is recognised that dermatological and gastrointestinal signs can be seen concomitantly, the proportion of cases in which this is documented may vary depending on the rigour with which these signs are observed and investigated. Thus, in one study involving 20 dogs with non-seasonal pruritus that were responsive to dietary change, only 4 of the owners had previously sought veterinary advice for gastrointestinal disease. However, on careful questioning, a further 11 were judged to have abnormal gastrointestinal function, as evidenced by increased frequency of defecation (more than three times per day), presence of faecal mucus or blood, or tenesmus. Dermatological signs are varied, and often indistinguishable from those associated with atopic dermatitis, although the presence of an unusual distribution or concomitant gastrointestinal signs may raise the index of suspicion for an adverse food reaction.

The definitive diagnosis of an adverse food reaction is dependent upon demonstrating resolution of the

Summary

Food antigen-specific IgE and IgG were determined in sera from three well-defined canine populations: normal dogs (group 1), dogs with atopic dermatitis that failed to respond to a home-prepared limited-antigen diet trial (group 2), and dogs with confirmed adverse food reactions (group 3). Positive IgE results were most frequently seen in group 3, followed by group 2, and then group 1. Multisensitivity was most often shown by dogs in group 3. IgE levels in group 3 were significantly higher than those in group 1 for 12/19 antigens, and than group 2 for 9/19 antigens, whereas IgE levels in group 3 were significantly lower than those in group 1 for 2/19 antigens, and than group 2 for 4/19 antigens. Results for IgG were arguably more discriminatory, with results in group 3 being significantly higher than in group 1 for 12/18 antigens, and than group 2 for 4/18 antigens.

The aetiological relevance of these results was not confirmed by antigen challenge studies, and indeed there are significant difficulties in such studies in the case of multisensitive dogs. Nonetheless, the results suggest that serology may be helpful in the management of dogs with suspected adverse food reactions by identifying suitable candidates for limited-antigen dietary trials, and in the selection of the most appropriate diet.
clinical signs following institution of dietary change, and relapse upon reinstitution of the original diet. However, as animals may be multisensitive, it may be necessary to attempt dietary restriction more than once. Although the commercial hydrolysed protein diets should, in theory, provide the ultimate hypoallergenic diet for diagnostic purposes, only 3/14 dogs from a colony of known food-allergic dogs were symptomatic when fed a hydrolysed soy protein diet. Use of a home-prepared limited-antigen diet is thus still favoured by many dermatologists for diagnostic purposes.

In man, the double-blind placebo-controlled food challenge is generally regarded as the gold standard for diagnosis. Nonetheless, recent studies comparing results of such food challenges with food-specific IgE levels have shown that high antibody levels have a > 95% concordance with responses to food trials, thus obviating the need for food trials in some instances.

Serological tests for food allergen-specific IgE have been evaluated as diagnostic aids for canine food hypersensitivity in a number of studies and reported to be unreliable. However, in earlier studies, the diagnosis was made by response to a 3-week limited-antigen diet trial, rather than the 6-8 week minimum that is now regarded as necessary in some cases.

The aim of this study was to assay sera for both IgE and IgG antibodies to food allergens under carefully controlled laboratory conditions, from three well-defined populations: normal dogs, dogs with atopic dermatitis who failed to respond to a limited-antigen diet trial, and dogs with confirmed adverse food reactions.

Materials and methods

Animals

Sera were obtained from the following animals:

- **Normal dogs (group 1):** group 1 comprised normal dogs owned by students and clients of the Hospital for Small Animals, University of Edinburgh, and sampled during the course of a study for which Home Office Approval was obtained.

- **Atopic dogs (group 2):** group 2 comprised dogs with atopic dermatitis that were patients of the University of Edinburgh Hospital for Small Animals. All had clinical signs compatible with atopic dermatitis, showed positive intradermal tests to one or more antigens (Greer Laboratories, Lenoir NC, USA), and failed to respond to a 6-week home-prepared limited-antigen diet trial.

- **Vienna dogs (group 3):** group 3 comprised dogs with confirmed adverse food reactions that were patients of the Veterinary University of Vienna. All had dermatological signs that responded to an 8-week limited-antigen diet trial with capelin and tapioca (Waltham Centre for Pet Nutrition, Melton Mowbray, Leicestershire, UK), and relapsed upon reinstitution of the original diet. Seven had concomitant gastrointestinal signs that were likewise diet-responsive.

Antisera

Monoclonal anti-canine IgE (E6-71; Custom Monoclonals, Sacramento CA, USA), alkaline phosphatase-conjugated polyclonal sheep anti-mouse IgG (Fc-specific, and non-reactive with canine IgG; Chemicon International, Temecula CA, USA), and alkaline phosphatase-conjugated polyclonal anti-canine IgG (Fc-specific; Bethyl Laboratories, Montgomery TX, USA) were purchased for use in the assays.

Food antigens

Extracts of beef, lamb, chicken, turkey, pork, codfish, soybean, potato, corn, wheat, rice, catfish, milk, egg white, egg yolk and milk (Greer Laboratories) were purchased as standardised antigens (protein nitrogen units, PNU). Additionally, purified milk proteins (lactoferrin, β-lactoglobulin A, casein) and bovine serum albumin (Sigma, Poole, Dorset, UK) were purchased as standardised antigens (µg/ml).

Performance of the ELISAs

IgE and IgG ELISAs were performed as described previously for our laboratory. Briefly, microtitre trays (Thermo Lab Sciences, Basingstoke, Hampshire, UK) were coated with antigen at 200 PNU/ml, or 16 µg/ml in the case of the purified antigens. Plates were freshly coated for each assay, incubated with dilutions of sera for 2 hours at 37°C, and with each antiserum overnight at 4°C. Following addition of the conjugate, plates were incubated with substrate for periods ranging from 30 minutes to 2 hours, and read when optimal colour development was reached.
For each antigen, a serum was identified with a high level of IgE or IgG antibody, and dilutions were included on each plate to generate a standard curve. This was arbitrarily assigned a relative antibody unit (RAU) value of 1000. Sera were assayed at 1/10 dilution. In the event that the optical density (OD) fell above the midpoint of the standard curve, the assay was repeated at a higher dilution. In the case of the IgE assay, a positive was defined as > 1.5 times the OD of the end-point of the standard curve.

Statistical methods
Results are depicted as mean ranks; the data from all groups were listed in ascending order and then each data point was allocated its rank in that ordering. The mean rank for each group was then the arithmetic mean of the ranks for that group.

Overall differences for each antigen were assessed by Kruskal-Wallis test, and pairwise differences by Mann-Whitney test. Correlations between IgE and IgG antibody levels and between results for BSA and beef were assessed by Spearman’s rank correlation.

Results

Dogs
There were 24 dogs in group 1 with a mean age of 5 years (range, 9 months to 13 years), 32 dogs in group 2 with a mean age of 3.3 years (range, 9 months to 8 years), and 22 dogs in group 3 with a mean age of 5 years (range, 1 to 12 years).

IgE
Incidence of positive reactions
The IgE assay employed all 19 antigens. Overall, IgE results were positive to 3.51% of antigens in group 1, 7.07% in group 2 and 15.8% in group 3. Of the group 1 sera ($n = 24$), positive results to one or more antigen were seen in 11 sera (45.8%). The incidence was greater in group 2 with 19/32 (59%) sera positive to one or more antigens, and greater still in group 3 where 19/22 sera (86%) gave at least one positive reaction. When considering sera with three or more positive reactions, the distinction between the groups was greater, where 1/24 (4.2%) of group 1 sera, 5/32 (15.6%) of group 2 sera and 8/22 (36.4%) of group 3 sera were positive to three or more antigens (Fig. 1.4.1).

Comparison of sera from groups 1, 2 and 3
In comparing the IgE levels, the calculated RAU value was used. In many cases this approximated to the RAU value of the end-point of the standard curve. In the case of eight antigens, the levels were significantly greater in sera from group 2 than in those from group 1, and for eight antigens the reverse was the case. For three antigens, there were no significant differences (Fig. 1.4.2a and b, Table 1.4.1). In the case of nine antigens, levels were significantly greater in group 3 than in group 2, with the reverse situation in the case of four antigens. Levels in group 3 were significantly greater that those in group 1 for 12 antigens, with the reverse situation for two antigens.

![Fig. 1.4.1 Frequency of positive IgE results to 1, 2, 3, 4 and > 4 of 19 food allergens in normal dogs (group 1), atopic dogs (group 2) and Vienna dogs with confirmed adverse food reactions (group 3). Positive reaction is defined as a relative antibody unit level of at least 1.5 times the end-point of the standard curve.](image-url)
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Fig. 1.4.2 (a, b) Mean rank of allergen-specific IgE antibody levels for normal dogs (group 1), atopic dogs (group 2) and Vienna dogs with confirmed adverse food reactions (group 3). Asterisks indicate allergens for which significant differences between groups were detected. Significant differences between groups ($p < 0.05$) for each allergen are indicated by different letters; groups with the same letter were not significantly different.

IgG

Incidence of positive reactions

The IgG assay employed 18 antigens, with rice being omitted. Further, results were obtained in respect of 16 sera from group 1, 16 from group 2 and 13 from group 3, as some sera had become depleted. The incidence of positive reactions in the IgG assay was far greater, with positive values resulting from most sera to most antigens. However, levels were often low or undetectable in the case of potato, egg white and lactoferrin.

Comparison of sera from groups 1, 2 and 3

The IgG results showed a similar trend to those in the IgE assay, but with greater distinction between groups (Fig 1.4.3a and b, Table 1.4.2). The levels of IgG for group 3 were significantly higher than group 2 in respect of four antigens, and than group 1 for 12 antigens. group 2 results were significantly greater group 1 in the case of five antigens. For all other antigens there was no significant difference. In contrast with the results for IgE, for no antigens were the results for groups 1 or 2 significantly greater than those for group 3.

Correlation between the levels of IgE and IgG

Overall, the levels of IgE and IgG were significantly correlated ($p = 0.001$). When the results for each antigen were examined, significant correlations were seen in respect of lamb ($r = 0.39; p = 0.008$), chicken ($r = 0.44; p = 0.002$), soybean ($r = 0.34; p = 0.024$), catfish
Correlation between the levels of anti-BSA and anti-beef antibody

In the case of IgG, BSA and beef antibody levels were highly correlated ($r = 0.5164; p = 0.001$), whereas there was no significant correlation for IgE ($r = 0.0854; p = 0.469$) against these two antigens.

Discussion

The results for each antigen are reported by mean ranks, which enables adjustment to be made for the varying RAU levels for each antigen. Assessment by median RAU levels gave very similar results, with the significant differences identical in each case (data not shown). In the case of IgE antibody, the mean ranks in group 3 were higher than those in group 1 for 14/19 allergens, and significantly so in the case of 12/19. The levels for the group 2 dogs fell generally in between the other two groups. IgG antibody levels appeared to be even more discriminatory. The mean ranks for group 3 were higher that those for group 1 in all cases, and significantly so in the case of 12/19. Again, the levels in sera from group 2 fell in between the two. Similarly, when a positive reaction was defined as an OD $>1.5$ times the end-point of the standard curve, the incidence of positive reactions was greatest for group 3, with group 2 being intermediate. Although positive results were indeed seen in some normal dogs, multisensitivity was more frequently encountered in group 3.

In the case of the dogs with atopic dermatitis (group 2), a contribution from adverse food reactions had been excluded by failure to respond to a single home-prepared limited-antigen diet trial. Obviously, although chosen by reference to the current diet, it is possible that there were indeed dietary components to their disease, and that the use of two, or even three, different single source protein diets might have identified them. It is also possible that they may have been multisensitive in respect of their adverse food reactions, and react to both protein antigens and also to antigens that are predominantly carbohydrate (e.g. rice, potato). Thus the removal of one offending allergen might not have induced clinically evident improvement. The use of dietary trials can also be confused by cross-reacting antigens. It is, of course, possible that the presence of the generally higher levels of IgE and IgG antibody of different specificities in this group may merely be a reflection of generalised hyper-reactivity of the immune response in the dogs with atopic dermatitis.

The recent development of a colony of beagle/Maltese-cross dogs with food hypersensitivity has provided interesting data on this condition. In two reports, levels of serum allergen-specific IgE have been reported, as well as dynamic changes following challenge studies. These animals show sensitivity to a number of dietary components, including soybean and corn, manifested by both dermatological and gastrointestinal signs. In the first report, institution of a hydrolysed protein diet led to a significant fall in the level of corn-specific IgE, which reversed upon reinstitution of the original corn-containing diet. There was no change in the level of wheat-specific IgE, an antigen absent from both diets. Institution of the hydrolysed protein diet was also accompanied by a fall in milk-specific IgE. These animals had never knowingly been exposed to oral cows' milk proteins. Challenge of the test dogs with milk
induced a gastrointestinal response, which could be a result of the known relative lactase deficiency in adult dogs. Two, however, developed a yeast-associated otitis externa. None showed an increase in milk-specific IgE, although it should be pointed out that the milk was only fed on two single-dose occasions in four of five dogs, and only once in the remaining dog that manifested profuse vomiting. Thus, although there were dynamic changes in allergen-specific IgE in association with the diet change (albeit not with the milk challenge), these changes were not always antigen-specific.

The second report showed rather different results. In this study, a greater number of dogs were included (14 vs 5 dogs). Institution of a diet of duck and rice actually resulted in a gradual rise of both corn- and soy-specific IgE, antigens that were supposedly absent from the diet. The authors suggested that this could have resulted from less IgE being complexed with IgG during times of lesser antigen exposure, but it is nonetheless hard to reconcile the different response in the two trials. Further, although challenge with corn and soy often induced an increase in allergen-specific IgE,
the increase was not always antigen-specific, i.e. when the dogs were fed corn, some had a corn-specific and/or soy-specific allergen response. Limited studies on this aspect have been conducted in our laboratory. In general, levels of allergen-specific IgE fall upon institution of a hypoallergenic diet. Indeed in one case that was the subject of a long-term study, institution of a hypoallergenic diet led to a marked fall in the levels of allergen-specific IgE in the case of 15/18 allergens tested. However, genetic differences are viewed as unlikely, in that among the 22 dogs with adverse food reactions, 10 breeds were represented, and there were six mixed-breed dogs. In the normal group, eight breeds were represented, and there were four mixed-breed dogs.

The relevance of high levels of allergen-specific IgE or IgG was not assessed by allergen challenge studies, but then neither is this ordinarily undertaken in studies with atopic dogs, where the value of assaying for allergen-specific IgE is generally accepted. In considering whether such studies would be useful, or meaningful, one must also recognize that animals may be multisensitive, and that subclinical multisensitivity could also exist, as is believed to be the case in atopic dermatitis. Thus the patient could only become symptomatic when fed two or more of the offending antigens.

Surprisingly, the levels of IgG were generally a better predictor of an adverse food reaction than were the levels of IgE. Although it is generally assumed in man that food hypersensitivity usually results from IgE-, rather than IgG-mediated reactions, there are no definitive data to support this contention. It is possible that the changes in both IgE and IgG are not causative of the disease, but merely reflections of it. It is certainly logical to conclude that if there is gastrointestinal disease, with resulting increased intestinal permeability, increased serum levels of any food antigen-specific antibody could result, either of the IgE and/or IgG class.

Correlation studies showed that, overall, the IgE and IgG antigen-specific responses were highly correlated. Significant correlations were also seen in respect of 7/18 antigens. Although to our knowledge there have been no previous studies that have addressed this issue, the results are not surprising. Examination of the correlation between anti-BSA and anti-beef were undertaken to ascertain a possible role for BSA as a major allergen to which the anti-beef response is directed. Paradoxical results were obtained with significant correlations in respect of IgG antibody, but none were evident for IgE. It is not therefore possible to draw any conclusions on this question.

It is possible that genetic or environmental differences might have contributed to the differences between the normal dogs and those with adverse food reactions. However, genetic differences are viewed as unlikely, in that among the 22 dogs with adverse food reactions, 10 breeds were represented, and there were six mixed-breed dogs. In the normal group, eight breeds were represented, and there were four mixed-breed dogs.

In conclusion, these studies have shown that populations of normal dogs and of dogs with confirmed adverse food reactions show different responses in respect of food allergen-specific IgE, and more particularly IgG. Although the levels of IgG antibody appeared to be the more discriminatory, multisensitivities in terms of the IgE response was seen more frequently among animals with adverse food reaction. It is thus suggested that, while not diagnostic, assays of food-specific IgE and IgG may be of value in identifying patients in which dietary trials are warranted, and in identifying suitable allergens for exclusion in any subsequent trials.
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References


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