

Auto IgG anti-IgE and IgG $\times$ IgE immune complex presence and effects on ELISA-based quantitation of IgE in canine atopic dermatitis, demodectic acariasis and helminthiasis.

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Abstract

Atopic dermatitis is a common allergic disease manifestation in dogs; however, there is no correlation between clinical disease and detectable total serum IgE. Auto antibodies of the IgG subclass against IgE may affect the detection of serum IgE by immunoassay and may be important in the regulation of IgE production by B cells. ELISA were developed to detect serum antibodies specific for IgE using a newly available canine monoclonal IgE of known antigen specificity, generated from a canine $\times$ murine heterohybridoma. To test for correlation of auto IgG anti-IgE levels with manifestation of atopic dermatitis, the sera from 101 atopic dogs were compared with sera from non-atopic dogs of various breeds, foxhounds manifesting clinical signs of demodectic acariasis and helminth parasitized random bred dogs for quantities of IgG anti-IgE measured in units/ml compared to a high titer standard serum. To test for serum effects on quantitation of IgE, known amounts of canine monoclonal IgE were added to various sera and measured by capture ELISA with detecting monoclonal antibodies specific for heat labile or heat stabile epitopes.

Abbreviations: Fc ϵ RI: high affinity receptor for IgE; Fc ϵ RII: low affinity receptor for IgE; RT: room temperature; PBS-T: Phosphate-buffered saline solution containing Tween-20; ABST: 2,2'-azino-di-(3-ethyl-benzthiazoline) sulfonate; mAb: monoclonal antibody; kD: kilodalton

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Unheated sera from dogs manifesting clinical atopic dermatitis and helminth parasitized dogs had levels of IgG anti-IgE that were significantly lower than various breeds of dogs not manifesting dermatologic lesions and foxhounds manifesting demodectic acariasis. Heating sera at 56°C for 3 h to denature the high affinity binding site on the IgE heavy chain caused a marked increase over non-heated sera in detectable IgG anti-IgE in almost all dogs. This increase was most profound in helminth-infected dogs and foxhounds manifesting demodectic mange with 7 fold increases each, respectively, and in atopic dogs with a 5 fold increase compared to 3 fold increases for clinically-normal springer spaniels and all soft coated wheaten terriers. The terriers demonstrated an association of lower heated serum values of IgG anti-IgE with manifestation of a familial syndrome of protein-losing enteropathy and protein-losing nephropathy. The ability of mouse anti-canine IgE monoclonal antibodies specific for either heat labile or heat stabile epitopes to detect canine monoclonal IgE added to sera in known amounts varied from serum to serum and at different concentrations of the same serum, but did not correlate with IgG anti-IgE values for these sera. The range of absolute levels of serum IgE in dogs showing little or no inhibition of detection of added IgE was < 0.5 ng/ml to 2 µg/ml. It was concluded that the increase in detectable IgG anti-IgE after heating sera indicates that IgG × IgE immune complexes are normally present in most dogs; however, the increase over uncomplexed IgG anti-IgE was most pronounced in dogs manifesting atopic dermatitis and demodectic acariasis. A quantitative comparison of IgG anti-IgE or IgG × IgE to total serum IgE was not made because the ability of monoclonal antibodies specific for either heat labile or heat stable epitopes on the IgE heavy chain to detect IgE added to serum, as well as innate serum IgE, was highly variable in different dilutions of serum from individual to individual. © 1997 Elsevier Science B.V.

Keywords: Auto anti-IgE; ELISA; Atopic; Helminth; Immune complexes; Demodectic acariasis

1. Introduction

Canine atopic dermatitis is similar to human atopic dermatitis (Schwartzman, 1984) and parallels the human disease on a number of features including: an incidence level of 10–15% in dogs seen by veterinarians (Griffin, 1993) compared to 10% in humans (Schultz-Larsen et al., 1986); skin lesions involving eosinophilia and mastocytosis (Nimmo Wilkie et al., 1990; Olivry et al., 1997, in press); breed and familial association indicating an inherited basis (Schwartzman, 1984; Schwartzman et al., 1983; Willemse, 1987); seasonal and regional variations in occurrence relating to allergen exposure (Willemse, 1987); and, intradermal skin test reactions to one or more allergens (Bevier, 1990; Willemse, 1987). The one feature that seems to distinguish dogs from humans manifesting atopic dermatitis is a lack of correlation between total serum IgE levels and disease (Hill et al., 1995; Schwartzman, 1984). Why this should be true is not known. Auto antibodies of the IgG subclass against IgE have been described in humans and demonstrated to mask detection of serum IgE (Vassella et al., 1990). It has been proposed that these auto antibodies against IgE may play a role in regulation of IgE production and may influence the manifestation of allergic disease (Shakib et al., 1995). The former proposal is being tested by the development of chimeric monoclonal antibodies against selected IgE epsilon chain epitopes responsible for binding to mast cells for therapeutic use in humans (Liu et al., 1995).

In man, naturally-occurring auto anti-IgE antibodies of isotypes IgG1 and IgG4 have been shown in vitro to block binding of IgE to both the high affinity receptor, $\text{Fc}\epsilon\text{RI}$, and the low affinity receptor, $\text{Fc}\epsilon\text{RII}$ for IgE on immune and inflammatory cells (Smith et al., 1995a,b). Simplistically, it would seem that the consequences of blocking IgE binding to these receptors would be decreased mast cell activation and decreased production of IgE, respectively (Shakib et al., 1995); yet, paradoxically, the highest levels of IgG auto anti-IgE have been found in people presenting with clinical allergic disease (Koike et al., 1989). From our results reported here, this paradox does not exist in the dog.

Because of the lack of clear in vivo evidence for mechanisms involving IgG anti-IgE in ameliorating clinical signs of allergic disease or down regulating IgE production, IgG isotype and epsilon chain epitope specificity of anti-IgE are being more closely examined to reveal correlations not apparent in measurements made on total IgG against intact IgE (Czech et al., 1995; Shakib et al., 1995; Smith et al., 1995a). This effort is encouraged by the potential for improving immunotherapeutic approaches to allergic disease (Bevier, 1990; Liu et al., 1995).

The objective of this study was to determine whether or not dogs have circulating IgG anti-IgE and if levels of these auto antibodies, undifferentiated by IgG isotype or epitope specificity, correlated with inhibition of ELISA quantitation of IgE. We demonstrated that IgG anti-IgE free in serum is present in all dogs in highly variable levels and is lowest in dogs manifesting allergic disease and helminth infection. Similarly, allergic and helminth infected dog serum IgG complexed with IgE at heat labile epitopes tends to be lower than in other dogs, but its relative proportion to uncomplexed IgG anti-IgE is much higher than in normal dogs. Pure monoclonal canine IgE added to various sera was not accurately measured in 5 of 11 sera tested using monoclonal antibodies specific for either heat stable or heat labile epitopes on the epsilon chain of IgE.

2. Methods

2.1. Dogs

One hundred and one dogs that presented at the Dermatology Service at the North Carolina State University College of Veterinary Medicine were judged to be atopic based on the criteria of greater than one year history of pruritic dermatitis and clinical signs including seborrhea, recurrent superficial bacterial pyoderma and *Malassezia* dermatitis. In addition, an important feature of diagnosis was exclusion of other causes of pruritic dermatitis in the dog. Dogs diagnosed as atopic and included in this study were evaluated for *Sarcoptes scabiei*, primary flea bite hypersensitivity, food hypersensitivity, insect hypersensitivity, intestinal parasite hypersensitivity, and contact hypersensitivity. The identification of allergens involved was made with a 70 allergen intradermal skin test and a 32 allergen in vitro (serologic) allergy test. All dogs included in this study showed positive responses to greater than 3 allergens on intradermal skin test and greater than one allergen on the in vitro testing.

Privately-owned dogs (Foxhounds, English Springer Spaniels and Soft Coated Wheaten Terriers), maintained on outdoor runs were bled with owner consent at the time

medical histories were obtained for these dogs. Forty-five Foxhounds manifesting chronic demodectic acariasis that were housed at a hunt club kennel and 38 English Springer Spaniels with no signs or history of allergic disease were included in this study. Twenty-six Soft Coated Wheaten Terriers were judged normal or abnormal for a familial syndrome of protein-losing enteropathy and protein-losing nephropathy associated with food hypersensitivity. Terriers were abnormal if they had proteinuria and/or enteric protein-loss manifested by panhypoproteinemia, histologic evidence of inflammatory bowel disease and/or increased concentrations of fecal α_1 protease inhibitor. All dogs were being treated with anthelmintics to prevent infection with *Dirofilaria immitis* (canine heartworm), ascarids and hookworms.

Thirty helminth-infected dogs were obtained after euthanasia at a local animal shelter and necropsied to determine intestinal helminth and heartworm burden. These dogs were selected for a lack of skin lesions and the presence of *D. immitis*, *Ancylostoma caninum* (hookworm) and *Trichuris vulpis* (whipworm).

2.2. Serum samples and heat treatment

Venous blood was collected from the cephalic vein of live dogs or the right ventricle of euthanized dogs immediately after death. The blood was allowed to clot at room temperature, centrifuged at $800 \times g$ and the resulting sera collected and held at 4°C up to 12 h before freezing at 20°C. Frozen sera were thawed at room temperature (RT) and split samples were heated at 56°C for 3 h or held at 4°C before being serially diluted in phosphate buffered saline containing 0.05% Tween 20 (PBS-T) starting at 1/100.

2.3. Canine monoclonal IgE

Monoclonal canine IgE was isolated from canine $\times$ mouse heterohybridoma cell line 2.39 (Gebhard et al., 1995) supernatants by passage over protein A linked to agarose beads (Zymed, San Francisco, CA) and subsequently eluted with 0.5 M acetic acid pH 3.0. IgE was determined to be greater than 95% pure by SDS-PAGE and staining with coomassie blue.

2.4. Anti-IgE ELISA

Polystyrene 96 well microtiter plates (Dynatech Labs., Chantilly, VA) were coated overnight at 4°C with 50 μ l/well of canine monoclonal IgE at 5 μ g/ml in 10 mM carbonate/bicarbonate buffer, pH 9.6. Polystyrene microtiterplates were used to avoid high background binding of serum immunoglobulins associated with other types of plates. The plates were then washed one time with PBST. The wells were blocked with PBST containing 1% non-fat milk for 2 h at RT and washed once with PBST. Fifty microliters of serially diluted serum samples, assayed in duplicate, were added to the blocked wells and incubated for two hours at RT. The plates were then washed three times with three minute incubations between washes. Fifty microliters of peroxidase labelled goat anti-dog IgG (γ specific) (Kirkegaard and Perry Labs. KPL, Gaithersburg, MD) were added at 0.1 μ g/ml diluted in PBST containing 1% bovine serum albumin

and 50 ng/ml IgE to block non-specific binding of the labelled antibodies. The plates were incubated at RT for one hour and subsequently washed five times with PBST with three minute incubations between washes. Fifty microliters of ABTS substrate (KPL) was added to each well and absorbance at 405 nm was read after 30 min on a BT2000 ELISA plate reader (Fischer Scientific, Raleigh, NC) and the concentration of auto anti-IgE was calculated for each sample in relation to a high auto anti-IgE serum standard.

2.5. Capture ELISA for total serum IgE

Antisera specific for canine IgE was obtained by immunization of rabbits with canine monoclonal IgE. Cross reacting antibodies, in this antisera, to canine IgG and IgM were adsorbed out using IgG affinity purified on protein G sepharose (Zymed, San Francisco, CA) and IgM (Chemicon International, Temecula, CA) linked to Affi-gel beads. The IgG fraction of the rabbit anti-canine IgE antiserum was affinity purified on protein G beads and used at 50 μ g/ml to coat polystyrene microtiter plates overnight at 4°C. Coated plates were blocked with PBST containing 1% non-fat milk for 2 h at RT or overnight at 4°C. A standard curve of serial dilutions of monoclonal canine IgE, starting at 100 ng/ml, was run on each plate. Canine sera were serially diluted from 1/10 to 1/640 or more. All samples were run in duplicate. Monoclonal antibody D-9, specific for a heat-labile epitope on the IgE epsilon chain (Hill et al., 1995), or monoclonal antibody 5.91, specific for a heat-stable, papain-degradable IgE epsilon chain epitope as shown in Fig. 3, were used to detect quantities of captured IgE. The plates were developed with peroxidase labelled goat anti-mouse γ chain (KPL) diluted in PBST containing 1% BSA and 50 ng/ml canine monoclonal IgE followed by ABST substrate (KPL). Each step was preceded by five washes with PBST. Addition of protein G affinity purified canine IgG or canine IgM at 0.5 mg/ml to standard curve dilutions of canine IgE did not inhibit detection of IgE by either monoclonal antibody.

2.6. SDS-PAGE and Western Blot analysis

Monoclonal IgE from mouse $\times$ canine heterohybridoma clone 2.39, papain digested and undigested, was fractionated under reducing and non-reducing conditions as follows. Samples were added to buffer containing 0.5 M Tris-HCl, 10% glycerol and 2% SDS, with or without 5% 2-mercaptoethanol, for reducing and non-reducing conditions, respectively, heated at 100°C for 10 min and placed in wells of vertical 10% polyacrylamide slab gels run in 1% SDS in 0.2 M glycine-Tris buffer, pH 8.3. Staining for protein bands was done with 0.1% Coomassie blue, destained with 40% methanol and 10% glacial acetic acid. Western blots of SDS-PAGE gels were conducted by transfer to 0.2 μ m pore size nitrocellulose membranes (BioRad, Melville, NY) of protein bands using BioRad miniprotein transfer unit (BioRad). The nitrocellulose membrane was blocked with a 5% solution of non-fat milk in PBS for 2 h. Mouse monoclonal 5.91 and polyclonal mouse antisera against canine IgE were incubated with strips of the transblot-

ted membrane for 2 h at RT, washed and incubated with peroxidase-labelled goat anti-mouse IgG heavy chain-specific antiserum (KPL).

2.7. Statistical Analysis

Unequal groups lacking Gaussian distribution were compared by one-way analysis of variance using Kruskal–Wallis test to determine *p*-value for medians of groups. Comparison of clinically normal and abnormal Soft Coated Wheaten Terriers was made by the unpaired *t*-test.

3. Results

3.1. IgG anti-IgE in unheated sera

Sera from 101 atopic dermatitis dogs, 30 helminth-infected dogs, 45 Foxhounds with chronic demodicosis, 38 English Springer Spaniels and 26 Soft Coated Wheaten Terriers were assayed by ELISA for the presence of auto antibodies against IgE of the IgG subclass (Fig. 1). Both the atopic and helminth infected dogs had significantly less free

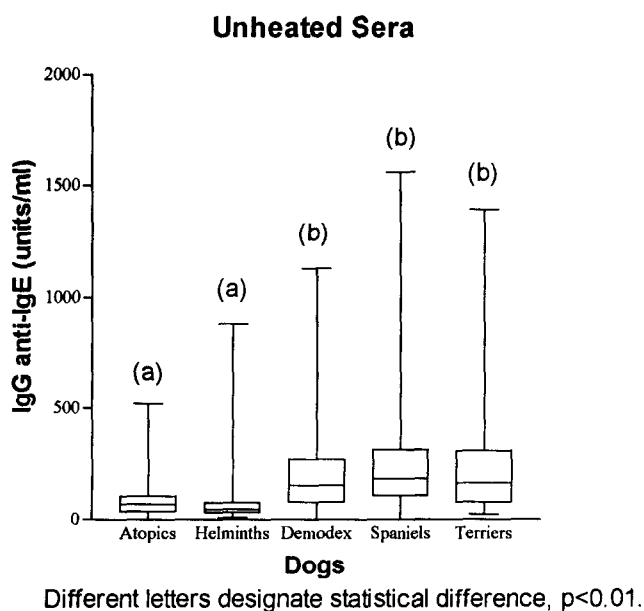


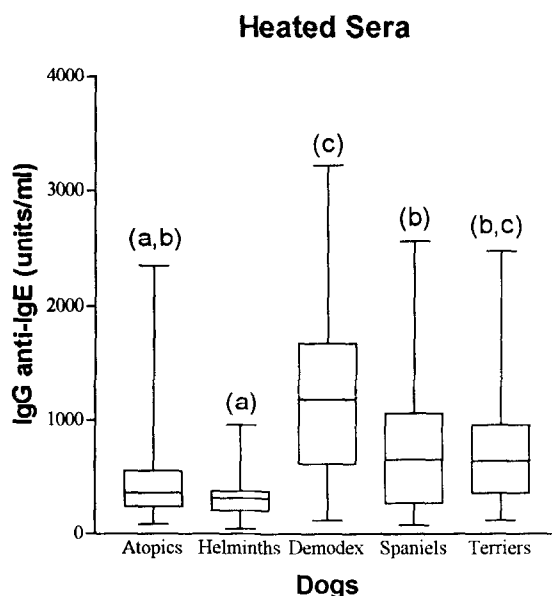
Fig. 1. Monoclonal antibody 5.91 reactivity with pepsin-digested and undigested non-reduced canine IgE, and reduced canine IgE distributed on SDS–PAGE. Reactivity with digest fragments were detected by western blot analysis. Gel A: protein stain of pepsin digested IgE, lane 1; reactivity of polyclonal mouse anti-canine IgE showing all fragments (intact IgE = 170–190 kD, Fc = 70–80 kD, Fab = 50 kD), lane 2; reactivity of mAb 5.91 with intact IgE only (not with Fc or Fab), lane 3. Gel B: protein stain of non-digested IgE, lane 4; reactivity of polyclonal mouse anti-canine IgE showing faint free epsilon chain in addition to intact IgE, lane 5; reactivity of mAb 5.91 with intact IgE, lane 6. Gel C: protein stain of reduced IgE showing epsilon chain = 70–80 kD and light chain = 20–25 kD, lane 7; reactivity of mAb 5.91 with the epsilon chain only, lane 8. Gel B showed a small amount of free light chain (protein stain) that reacted weakly with reagents used to detect mouse mAb.

serum IgG anti-IgE than all the other dogs ($p < 0.01$). Atopic dermatitis dogs were not significantly different from helminth infected dogs.

3.2. IgG anti-IgE in heated sera

Paired samples of the same sera assayed above as unheated were heated at 56°C for 3 h before ELISA for IgG anti-IgE. There was a profound increase in detectable IgG anti-IgE in atopic dermatitis dogs, helminth infected dogs and *Demodex* infected Foxhounds; whereas, there was a substantial, but less profound, increase in sera from the clinically normal spaniels and all terriers (Fig. 2). This was clearly evident when the ratio of heated to unheated IgG anti-IgE for each dog was calculated and the ratios compared by groups of dogs as shown in Table 1. Eighty percent of all dogs had a ratio of heated/unheated IgG anti-IgE that was greater than 2 (data not shown).

Soft Coated Wheaten Terriers had IgG anti-IgE values that were not significantly different from clinically-normal English Springer Spaniels. However, when normal ($n = 15$) and enteric disease manifesting ($n = 10$) terriers in this group were compared, the clinically abnormal terriers had significantly less IgG anti-IgE in heated serum, 545 U/ml and 965 U/ml respectively, at $p < 0.05$. The mean of unheated serum values was



Different letters designate statistical difference, $p < 0.05$.

Fig. 2. The amount of IgG anti-IgE autoantibodies in untreated sera of 101 dogs manifesting atopic dermatitis, 30 helminth-infected dogs, 45 Foxhounds manifesting demodicosis, 38 clinically-normal English Springer Spaniels and 26 clinically-normal Soft Coated Wheaten Terriers. Box and Whiskers graphics shows 75% of the values as the box and the median value as a line, whereas the bars shows the range of values.

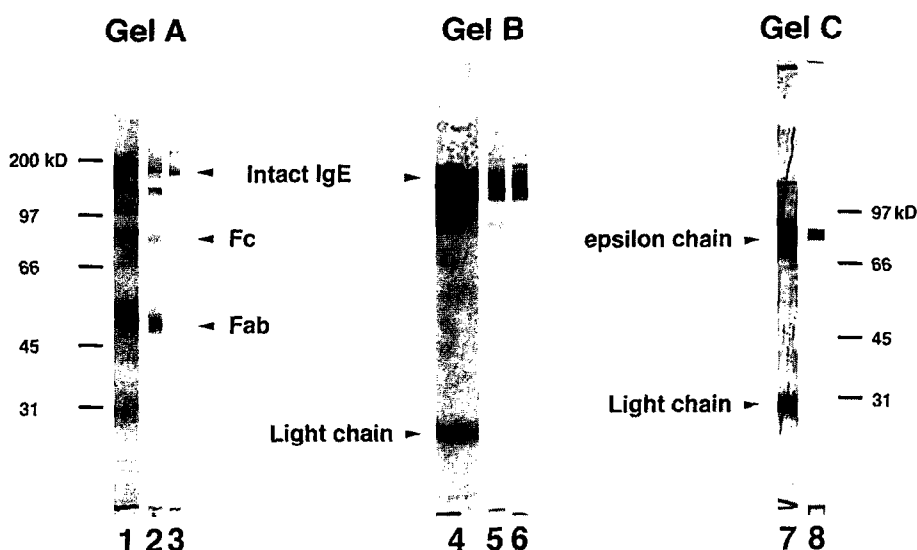


Fig. 3. The amount of IgG anti-IgE autoantibodies in heat-treated (56°C, 3 h) sera of 101 dogs manifesting atopic dermatitis, 30 helminth-infected dogs, 45 Foxhounds manifesting demadecosis, 38 clinically-normal English Springer Spaniels and 26 clinically-normal Soft Coated Wheaten Terriers. Box and Whiskers graphics shows 75% of the values as the box and the median value as a line, whereas the bars shows the range of values.

lower for abnormal terriers (206 U/ml) than normal terriers (442 U/ml) but not statistically different. Ratios of heated to unheated serum IgG anti-IgE were not significantly different between normal and abnormal terriers.

3.3. Detection of IgE added to atopic dog sera

In attempting to quantitate total serum IgE by capture ELISA using the adsorbed rabbit anti-IgE, it was found that many, up to 60%, of the atopic, helminth-infected and Foxhound sera failed to yield linear dilution curves for three consecutive points in

Table 1
Medians of individual dog ratios of heated to unheated serum IgG anti-IgE

Dog group	Median heated/unheated IgG anti-IgE	Differences compared to Spaniels
Atopic <i>n</i> = 101	4.9	<i>p</i> < 0.01
Helminth infected <i>n</i> = 30	7.7	<i>p</i> < 0.01
Foxhounds (demadecosis) <i>n</i> = 45	7.1	<i>p</i> < 0.001
Spaniels <i>n</i> = 38	2.8	n.s.
Terriers <i>n</i> = 26	2.9	n.s.

dilutions ranging from 1/10 to 1/320 (data not shown). Various detecting systems were used in an attempt to overcome this perceived technical problem, including biotinylating the detecting monoclonal antibodies D-9 and the rabbit anti-IgE antisera, as well as the assay system reported here. This lack of linear response did not correlate with levels of IgG anti-IgE.

To investigate this problem further, known amounts of pure IgE were added to a few selected sera to test the ability of capture ELISA systems to detect IgE in the presence of IgG anti-IgE. Two different monoclonal antibodies were used to detect 75 ng/ml of IgE in various dilutions of sera (Table 2). Mouse mAb D-9 is reported to be specific for a heat labile epitope (Hill et al., 1995). Mouse mAb 5.91 elicited an immediate type skin reaction upon intradermal injection at 0.2 μ g/ml in dogs and was specific for a heat stable, papain labile epitope on the canine epsilon chain (Fig. 4) and showed no binding to canine IgG, IgM or IgA (data not shown). Seventy-five ng of IgE were added to 1 ml of each dilution of serum 30 min before the serum dilutions were applied to the ELISA plate. The values of IgE detected are reported for two broad ranges of serum dilutions (1/10 to 1/80 and 1/160 to 1/640), as the values for both total autologous IgE and IgE added to these dilutions were often distinctly different depending upon the serum dilution. It was found that 3 of 9 dog sera inhibited the detection by mAb D-9 at all serum dilutions tested when a constant amount of IgE was added to different concentra-

Table 2

Detection of a constant amount of IgE added to various dilutions of atopic dog sera

Atopic dog No. dilution range	Total IgE/ml serum mAb D-9 detector	Total IgE/ml serum mAb 5.91 detector	75 ng IgE added ^a mAb D-9 detector	75 ng IgE added mAb 5.91 detector
64 1/10–1/80	80 ng/ml ^b	780 ng/ml ^b	> 50 ng/ml ^c	> 50 ng/ml ^c
1/160–1/640	1120	800	> 50	24
77 1/10–1/80	105	1620	25	50
1/160–1/640	< 0.5	1000	10	20
76 1/10–1/80	168	630	35	20
1/160–1/640	30	1810	12	10
49 1/10–1/80	65	20	> 50	25
1/160–1/640	240	< 0.5	> 50	10
7 1/10–1/80	28	365	> 50	> 50
1/160–1/640	50	< 0.5	13	16
16 1/10–1/80	80	700	> 50	> 50
1/160–1/640	426	1490	> 50	32
35 1/10–1/80	100	420	> 50	28
1/160–1/640	320	310	> 50	9
93 1/10–1/80	19	10	30	30
1/160–1/640	< 0.5	< 0.5	9	15
98 1/10–1/80	66	710	> 50	> 50
1/160–1/640	240	1546	25	27

^a 75 ng of canine monoclonal IgE was added to 1 ml of each dilution.

^b Value relating to a standard curve, generated on each plate with pure IgE, multiplied by dilution factor.

^c Value relating to a standard curve, generated on each plate with pure IgE (not multiplied by dilution factor).

Table 3

IgE added to serum of atopic dog No. 18 followed by serial dilution

Dilution	0 ng IgE added	5 ng IgE ^a added	10 ng IgE added	100 ng IgE added
1/10	5 ng/ml ^b	14 ng/ml ^b	21 ng/ml ^b	> 50 ng/ml ^b
1/20	3	14	19	> 50
1/40	8	13	17	40
1/80	3	6	12	18
1/160	3	8	6	18
1/320	2	3	4	6
1/640	3	3	4	4
1/1280	< 0.5	1	1	3
1/2560	< 0.5	2	< 0.5	2
1/5120	< 0.5	< 0.5	< 0.5	1

^aAmount of canine monoclonal IgE added to 1 ml of 1/10 diluted serum.^bValues corresponding to standard curve (not multiplied by dilution factors).

tions of sera. An additional 2 sera inhibited detection of added IgE only at high serum dilutions. Detection with mAb 5.91 was even more consistently inhibited in its detection of added IgE. This table also shows that there was little agreement between the two different anti-IgE mAb used to detect IgE and that there was no consistency by either detecting mAb at quantitating IgE in different dilutions of the same serum.

In an another approach to attempt to validate the ELISA system, 100, 10 and 5 ng of canine monoclonal IgE were added to 1 ml of 1/10 dilutions of two different atopic dog sera and these were then serially diluted to 1/5120. Dog #18 had an IgG anti-IgE value of 340 U/ml in heated serum and 45 U/ml in unheated serum and was strongly skin test positive for house dust mite antigen but was negative for antigen specific IgE in serum; whereas, dog #25 had an IgG anti-IgE value of 850 U/ml in heated serum and 63 U/ml in unheated serum and was skin test and serum IgE positive for pine pollen.

Table 4

IgE added to serum of atopic dog #25 followed by serial dilution

Dilution	0 ng IgE added	5 ng IgE added ^a	10 ng IgE added	100 ng IgE added
1/10	2 ng/ml ^b	3 ng/ml ^b	4 ng/ml ^b	43 ng/ml ^b
1/20	0.5	3	3	24
1/40	< 0.5	2	1	14
1/80	1.5	2	1	10
1/160	< 0.5	1	1	3
1/320	< 0.5	< 0.5	< 0.5	2
1/640	1	1	2	2
1/1280	1.5	2	1	3
1/2560	3	3	3	3
1/5120	1.5	3	2	1.5

^aAmount of canine monoclonal IgE added to 1 ml of 1/10 diluted serum.^bValues corresponding to standard curve (not multiplied by dilution factors)

Table 3 shows that when mAb D-9 was used as the detecting antibody and developed with peroxidase labelled rabbit anti-mouse γ , dog serum No. 18 enhanced the detection of added IgE at the three levels, whereas dog serum No. 25 allowed detection of approximately half of the added IgE at all three levels Table 4. Thus, as in Table 2, there appears to be no correlation between levels of IgG anti-IgE and inhibition of detectability of IgE.

4. Discussion

This is the first report to our knowledge of auto anti-IgE antibodies in dogs. It is also the first report of total serum IgE values quantitated against known quantities of pure IgE. The levels of IgG anti-IgE detectable with a monoclonal canine IgE based ELISA can be inferred to be relatively higher in dogs than man by comparing the working dilutions of sera used in essentially the same ELISA configuration. For dogs, sera were diluted from 1/100 to 1/800, whereas assays on human sera usually start with dilutions of 1/20 (Wilson et al., 1987). The range of values for total serum IgE reported here were from < 0.5 ng/ml to approximately $2 \mu\text{g/ml}$, when evidence from added IgE indicated that the influence of IgG anti-IgE was minimal. This parallels the reported higher levels of total IgE found in dogs than in man (Schwartzman, 1984). However, early measurements of canine serum IgE using immunoprecipitation and showing levels far exceeding those reported here (Vriesendorp et al., 1975) may not be accurate, given the lack of pure myeloma IgE with which to standardize those assays. Our unambiguous evidence for levels of IgE being relatively high in dogs compared to man and the presence of auto antibodies directed against IgE, indicate that studies designed to elucidate roles for auto antibodies in immunoregulation and immunopathogenesis can be readily conducted in dogs.

One of the major differences between canine and human atopic dermatitis is the lack of correlation between detectable levels of total serum IgE and clinical disease seen in the dog (Hill et al., 1995). There is an association between total IgE and atopic disease in man but there is not a strong correlation; and, it has been proposed that auto antibodies against IgE are responsible for this lack of a strong correlation (Vassella et al., 1990). It has been published that there is no correlation between atopic dermatitis and total serum IgE in dogs (Hill et al., 1995; Vriesendorp et al., 1975). Measurement of total serum IgE against a standard of known quantities of IgE should have revealed what proportion of IgE in circulation is bound in complexes and what part is unbound. Unfortunately, attempts to accurately quantitate serum IgE using monoclonal antibodies specific for IgE in ELISA showed highly variable results depending upon the serum dilution tested in a particular assay. This has lead us to conclude that serum IgG auto-antibodies against IgE affect the quantitation of serum IgE, although this effect may either enhance or diminish detection signals in ELISA.

In addition, serum IgM anti-IgE may also play a role in the ability of ELISA-based detection systems to accurately quantitate serum IgE. We have found that IgM anti-IgE is present in dog sera. Upon selective removal of IgG by protein G adsorption from sera, IgM anti-IgE remains in the sera at titers greater than 1/1000 in some dogs (unpub-

lished data). A preliminary study of sera from only 4 atopic dogs lacking linearity on dilution for quantitation of IgE showed that following protein G adsorption at low pH, when IgM anti-IgE was low, linearity became apparent; whereas, when IgM anti-IgE was high linearity was not apparent (unpublished data).

Although not tested in this study, it may well be that a more accurate determination of total serum IgE would be obtained by combining several monoclonal antibodies of different IgE epitope specificity in order to minimize the potential masking effect of IgG or IgM anti-IgE. The potential for quantitation errors giving falsely high IgE values, as we demonstrated here, may still exist and would be revealed by addition of pure IgE to such sera or by the removal of both IgG (protein G adsorption) and IgM (low salt precipitation) under conditions that dissociate immune complexes.

Increased levels of IgG anti-IgE observed in heated human sera have been assumed to be due to the release of the IgG antibody from immune complexes upon denaturation of IgE (Czech et al., 1995). The existence of IgG $\times$ IgE immune complexes involving heat stable epitopes were not quantitated in the study reported here; however, such immune complexes have been reported in humans (Czech et al., 1995). Circulating immune complexes may be involved in the modulation (Shakib and Smith, 1994; Vassella et al., 1994) or enhancement (Stadler et al., 1991) of pathogenesis during allergic disease. It is also possible that they either upregulate (Shakib et al., 1993; Smith et al., 1995b) or downregulate (Haba and Nisonoff, 1994) IgE production by blocking or stabilizing, respectively, the binding of the low affinity receptor (Fc ϵ RII or CD23) for IgE on B cells (Sutton and Gould, 1993). These dual roles in both pathogenesis and IgE production regulation would be dependent on auto anti-IgE antibody epitope specificity as already demonstrated to occur in humans (Miescher et al., 1993).

The very high level of IgG $\times$ IgE immune complexes detected in a kennel of Foxhounds manifesting demodicosis was surprising. The reasons for the high level could be many, including; allergen processing and presentation in the skin compared to helminth allergen in deep tissue or lumen sites, or genetic predisposition of this line bred group of dogs to production of IgG specific for heat labile epitopes of IgE linked or not to predisposition to demodicosis. Both of these possibilities are testable either experimentally in the case of the former or epidemiologically in the case of the latter.

In attempting to elucidate the role of anti-IgE antibodies in regulatory mechanisms controlling IgE production, it may be enlightening to compare dogs responding to helminth infection, as the classical stimulator of IgE production, with dogs manifesting atopic disease. Helminth infected dogs had the lowest levels of IgG anti-IgE in both heated and unheated sera of all dogs. Because we did not quantitate IgG $\times$ IgE involving heat stable epitopes, there is the possibility that induction of IgE production in helminth infections elicits IgG anti-IgE directed towards these epitopes. If there are auto anti-IgE regulatory mechanisms operating during elevated IgE, then these may well be different in atopic disease compared to helminth infection. This would not be unexpected given that most individuals are susceptible to elevation of IgE in response to helminth infection, whereas a minority of both dogs and humans suffer atopic disease. Future studies will be directed toward quantitating IgG anti-IgE by epitope specificity and IgG isotypes. In addition, it will be important to know the amount of IgG $\times$ IgE immune complexes involving heat stable epitopes.

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