

Examination of serum total IgG₁ concentration in atopic and non-atopic dogs

In this study, serum immunoglobulin G₁ (IgG₁) concentrations were examined in atopic and non-atopic dogs receiving different levels of parasite control. Significantly lower serum total IgG₁ concentrations were found in non-atopic dogs receiving stringent parasite control than in atopic dogs or non-atopic dogs receiving less stringent parasite control. Examination of serum total IgG₁ concentrations of atopic dogs after six months of allergen specific immunotherapy (ASIT) showed a significant increase in serum total IgG₁ concentrations. It is proposed that serum total IgG₁ concentrations are affected by parasitism, atopic dermatitis and ASIT.

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INTRODUCTION

Most of the research into the role of antibodies in both human and canine atopic dermatitis has concentrated on immunoglobulin (Ig) E. A few authors have examined IgG and have suggested that it may be important (Djurup and Malling 1987, McHugh and others 1990, Hill and others 1995), but the exact role that IgG plays in atopic dermatitis has not been defined.

There is some disagreement as to the circumstances necessary for the production of specific IgG subgroups. It has been suggested that, in humans and animals, genetic background, type of antigen and the route or duration of exposure may all affect the subclass of IgG produced in an immune response (Mazza and others 1994, Day and others 1996).

As serum total IgG concentrations are influenced by parasitism, infection and allergic disease, the aim of this study was to compare serum total IgG₁ concentrations in atopic and non-atopic dogs, and examine the influence that different levels of parasite control have on these concentrations.

MATERIALS AND METHODS

A total of 78 atopic and non-atopic dogs was examined. A detailed breakdown of these dogs is given in Table 1. Atopic dogs

were defined as such using Willemse's criteria (1986) based on history, clinical findings, a negative response to an eight-week restriction diet, the demonstration of at least one positive reaction on intradermal skin testing and a negative response to flea allergen. Dogs were allocated to the non-atopic group retrospectively as the criteria for this required that they had not demonstrated multiple episodes of atopic-type skin disease by the time they had reached three years of age. Non-atopic dogs were further divided into those with pyoderma at the time of sampling and those without. They were not suffering from any other conditions likely to affect the immune system.

Guide Dogs for the Blind Association dogs, referred to in this study as working dogs, received stringent parasite control measures and were considered free from both endo- and ectoparasites. Parasite control began with the treatment of nursing bitches and their offspring with fenbendazole (Panacur; Intervet), at a dose of 1 g/2.2 kg, at two, five and eight weeks post-whelping. Subsequently, the offspring received a febantel/praziquantel/pyrantel combination (Drontal Plus; Bayer), at a dose of 5 mg praziquantel/kg (one tablet/10 kg) at 12 weeks, six months and 12 months of age. The dogs were then routinely wormed with Drontal Plus at six-monthly intervals. Faecal samples taken from all the dogs at eight to 10 months of age were uniformly negative for endoparasites. All the dogs were treated monthly with fipronil (Frontline; Merial) or permethrin (Exspot; Schering-Plough) spot-on to control ectoparasites and no parasites were found on clinical examination.

Blood samples were obtained from non-atopic working dogs undergoing routine haematological/biochemical analysis and from atopic dogs as part of a diagnostic work-up. Greyhounds were National Greyhound Racing Club registered and were treated three to four times a year with a proprietary anthelmintic. They were not suffering from any skin conditions at the

Table 1. Description of dogs and serum total IgG₁ concentration

Dog number	Breed	Age at sampling (days)	Serum total IgG ₁ (mg/dl)	Dog number	Breed	Age at sampling (days)	Serum total IgG ₁ (mg/dl)
NON-ATOPIC WORKING DOGS WITHOUT SKIN DISEASE				39	Labrador × golden retriever	908	35
1	Labrador × golden retriever	261	25	40	Labrador × golden retriever	796	10
2	Labrador	615	20	NON-ATOPIC GREYHOUNDS (age in years as accurate ages not available)			
3	Labrador	684	10	41		3 years	425
4	Labrador	1005	130	42		3 years	325
5	Labrador × golden retriever	1833	10	43		3 years	925
6	Labrador	634	10	44		6 years	215
7	Golden retriever	457	10	45		6 years	430
8	Golden retriever	465	10	46		6 years	10
9	Labrador × golden retriever	1500	10	47		6 years	10
10	Labrador × golden retriever	691	35	48		6 years	205
11	Labrador × golden retriever	280	20	49		6 years	10
12	Labrador × golden retriever	793	20	50		3 years	760
13	Labrador	251	10	51		3 years	300
NON-ATOPIC WORKING DOGS WITH PYODERMA AT TIME OF SAMPLING				52		5 years	10
14	Labrador	554	10	53		12 years	80
15	Golden retriever	284	10	54		5 years	165
16	Golden retriever	215	10	ATOPIC DOGS (referred to the Glasgow University Veterinary School)			
17	Labrador × golden retriever	472	10	55	German shorthaired pointer	1266	240
18	Labrador	644	10	56	Dalmatian	750	25
19	Labrador × golden retriever	423	10	57	Labrador	915	240
20	Golden retriever	507	10	58	Yorkshire terrier	820	100
21	Labrador	401	10	59	Staffordshire bull terrier	2770	1520
22	Labrador × golden retriever	276	10	60	Crossbreed	1440	520
23	Labrador × golden retriever	1708	30	61	German shepherd dog	468	10
ATOPIC WORKING DOGS				62	Boxer	550	475
24	German shepherd dog	2689	1550	63	Terrier	545	200
25	German shepherd dog	1709	25	64	German shepherd dog × Labrador	2187	35
26	German shepherd dog	1325	135	65	Labrador	1865	360
27	Labrador × golden retriever	429	10	66	Bulldog	607	475
28	Labrador × golden retriever	565	10	67	Great Dane	388	970
29	Labrador	2687	50	68	German shepherd dog	305	165
30	Labrador	2892	520	69	Labrador	2521	1350
31	Labrador × golden retriever	1830	220	70	Irish wolfhound	498	10
32	Golden retriever	1820	345	71	Staffordshire bull terrier	1454	80
33	Labrador × golden retriever	630	60	72	German shepherd dog	719	365
34	Labrador × golden retriever	125	10	73	Labrador	1092	960
35	Labrador × golden retriever	784	40	74	Golden retriever	2166	280
36	Labrador	413	100	75	Cairn terrier	730	10
37	Labrador	1473	60	76	German shepherd dog	1640	640
38	Labrador × golden retriever	916	30	77	Staffordshire bull terrier	1939	330
				78	Boxer	288	685

time of sampling but were known to be regularly exposed to fleas. Blood samples were taken from these dogs as part of a routine health check.

Cases referred to the Dermatology Clinic at Glasgow University Veterinary School (GUVS) with clinical signs consistent with atopic dermatitis, no response to eight weeks on a restriction diet, at least one positive result on intradermal skin testing and a negative response to flea allergen were included in this study. These dogs were kept as household pets

and were exposed to both indoor and outdoor allergens. The parasite control measures before attending GUVS varied widely.

Blood samples of at least 5 ml were taken by jugular venepuncture, placed in a glass tube without any additives and allowed to clot at room temperature. Samples were then centrifuged at 2000 g for 10 minutes and the resultant serum was removed and divided into 0.5 ml aliquots. These were stored at -20°C until required for testing. Serum samples were then

allowed to defrost at room temperature and serum total IgG₁ concentrations assessed using a radial immunodiffusion assay (VetRID; Bethyl Laboratories). This assay is comprised of agar containing anti-IgG₁ antibody. The test serum containing IgG₁ was placed in a well in the agar and allowed to diffuse through the agar, resulting in the development of an antigen-antibody precipitin ring around the well. By applying known concentrations of IgG₁ it was possible to create a logarithmic graph from which unknown IgG₁ concentrations

Table 2. Breakdown of the clinical response to ASIT	
Good response	Dog required no, or only occasional, corticosteroid or antihistamine therapy to relieve pruritus
Fair response	Dog was still pruritic and required intermittent corticosteroid therapy
Poor response	There was no improvement in condition and the dog required continuous corticosteroid therapy

Table 3. Statistical analysis of serum total IgG ₁ concentration			
	Number of dogs	Mean IgG ₁ concentration (mg/dl)	Standard deviation
Non-atopic working dogs without pyoderma	13	24.6	32.6
Non-atopic working dogs with pyoderma	10	12.0	6.3
Atopic working dogs	17	188.8	377.2
Non-atopic greyhounds	14	276.4	283.8
Atopic GUVS dogs	24	418.5	419.3

GUVS Glasgow University Veterinary School

could be calculated according to the diameter of the precipitin ring.

Atopic dogs undergoing allergen specific immunotherapy (ASIT) were given an alum precipitated vaccine containing up to eight different allergens (ARTU Biologicals; the Netherlands) based on intradermal skin testing and/or serological test results. This ASIT vaccine was given subcutaneously. All dogs received the same injection regimen according to the manufacturer's directions. After six months of therapy, dogs were reassessed and examined for the presence of skin disease. During this period dogs had been treated by their local, first-opinion veterinary surgeon

and had not been examined by the authors. Owners were questioned as to how they felt about the dog's overall condition: whether there had been any improvement or not; whether the dog was still pruritic and, if so, was this any better than previously; and had any antipruritic treatment been prescribed for the dog at any time in the past six months. The dog's response was then scaled, based on these findings, and it was allocated to one of the groups described in Table 2.

Statistical evaluation of data required the examination of log_e serum IgG₁ concentrations in order to obtain results which were not distorted due to the skewed

nature of the data, as most of the results tended towards the lower range of concentrations. It was then possible to compare IgG₁ concentrations for the different groups using a one-way analysis of variance and a Newman-Keuls multiple range test (95 per cent confidence interval). A paired *t*-test was used to compare serum total IgG₁ concentrations before and after six months of ASIT.

RESULTS

Comparison of serum total IgG₁ levels in atopic and non-atopic dogs (Fig 1 and Table 3) revealed the highest mean value, of 418.5 mg/dl, in atopic pet dogs and the widest range of values, from 10 mg/dl to 1550 mg/dl, in atopic working dogs. The lowest mean value of 12.0 mg/dl, was observed in the non-atopic working dogs with skin disease, followed by a mean of 24.6 mg/dl in the non-atopic working dogs without skin disease. However, there was an area of overlap in all five groups.

A one-way analysis of variance of this data revealed that non-atopic working dogs with or without skin disease had significantly lower serum IgG₁ concentrations than atopic working dogs, non-atopic greyhounds and atopic pet dogs (*P*=0.000). There were no significant differences between any of the other groups. No significant difference was noted between non-atopic dogs with pyoderma and those without; indeed non-atopic working dogs with pyoderma had a lower mean serum IgG₁ concentration

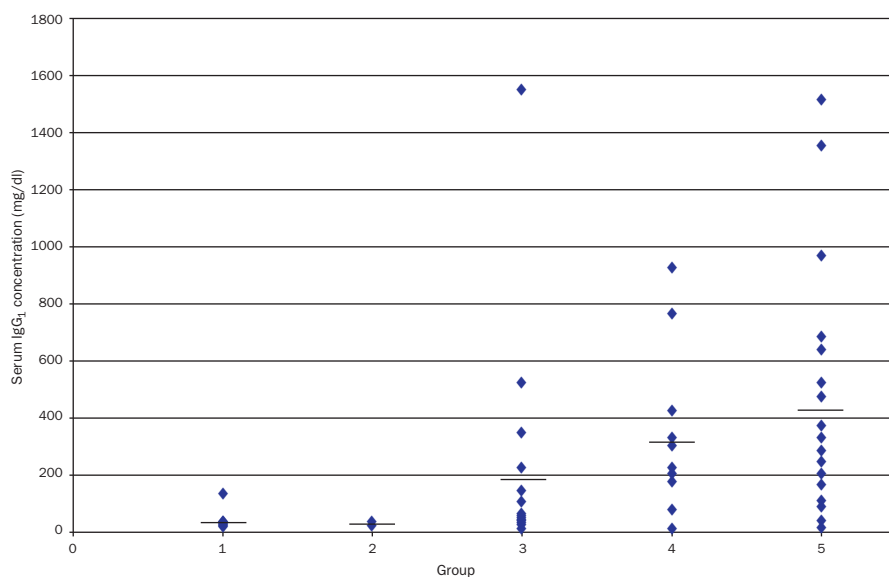


FIG 1. Serum IgG₁ concentrations in different groups of dogs. Bar shows mean value for that group. Group 1 Non-atopic working dogs without pyoderma, Group 2 Non-atopic working dogs with pyoderma, Group 3 Atopic working dogs, Group 4 Non-atopic greyhounds, Group 5 Atopic Glasgow University Veterinary School dogs

Table 4. Specific details of dogs receiving ASIT

Dog	Group	Breed	Initial IgG ₁ concentration (mg/dl)	IgG ₁ concentration after 6 months (mg/dl)	Difference between pre- and post-ASIT	Percentage increase	Clinical response
1	Working	Golden retriever × Labrador	30	205	175	583	Good
2	Working	Golden retriever × Labrador	35	215	180	514	Good
3	Working	Golden retriever × Labrador	40	230	190	575	Good
4	Working	Labrador	50	120	70	140	Good
5	Working	German shepherd dog	135	65	-70	-52	Poor
6	GUVS	German shepherd dog	165	250	85	51	Good
7	GUVS	Terrier	200	445	245	122	Good
8	GUVS	German shepherd dog	365	820	455	125	Good
9	GUVS	Dalmatian	25	120	95	380	Fair
10	GUVS	German shepherd dog × Labrador	35	150	115	328	Fair
11	GUVS	Labrador	240	215	-25	-10	Poor
12	GUVS	Labrador	960	975	15	1	Poor

ASIT Allergen specific immunotherapy, GUVS Glasgow University Veterinary School

than dogs without skin disease.

Serum IgG₁ concentrations of 12 atopic dogs after six months of ASIT (Table 4 and Fig 2) were significantly higher than before the initiation of ASIT (paired *t*-test, *P*<0.01).

In addition, seven of the 12 atopic dogs which demonstrated a good clinical response to ASIT, were found to exhibit an increase in serum total IgG₁ concentration varying from 51 per cent to 583 per cent of the original serum IgG₁ levels. This compares with three dogs that demonstrated a poor clinical response to ASIT, in which

serum IgG₁ concentrations after six months were similar to or lower than the initial levels. No correlation was evident between the degree of increase in serum IgG₁ concentration and the degree of clinical improvement shown by an individual dog, as the increase in IgG₁ ranged from 70 mg/dl to 455 mg/dl where a good response was found. However, comparison of the increase in serum total IgG₁ concentrations pre- and post-ASIT in dogs with a good response and those with a fair or poor response revealed a significant difference between the two groups (*P*<0.016).

DISCUSSION

Serum total IgG concentrations or allergen specific IgG levels have been shown to be increased by parasitism, infection and atopic dermatitis (Halliwell and Longino 1985, Hites and others 1989, Morales and others 1994, Hill and others 1995, Nuttall and Halliwell 2001). However, it is not known which, if any one, subclass of IgG is predominantly associated with each of these conditions.

Human IgG comprises four subclasses – IgG₁, IgG₂, IgG₃ and IgG₄ – based on the relative concentrations and electrophoretic activity of each. Mazza and others (1994) identified four subclasses of canine IgG, also described as IgG₁, IgG₂, IgG₃ and IgG₄, based on the same criteria as those described above for human IgG. However, as little work has been carried out to examine the functions of these subclasses, it is not possible to prove that human IgG₁ is functionally equivalent to canine IgG₁.

To confuse matters, a further subclass of IgG, identified as IgG_d, has been described in dogs (Willemse and others 1985). Although it has been stated that IgG_d, IgG₁ and IgG₄ may be the same subclass (Halliwell and DeBoer 2001), it has been shown that they are all different subclasses (Day and others 1996) and that IgG_d is not equivalent to IgG₁ (A. Willemse, personal communication).

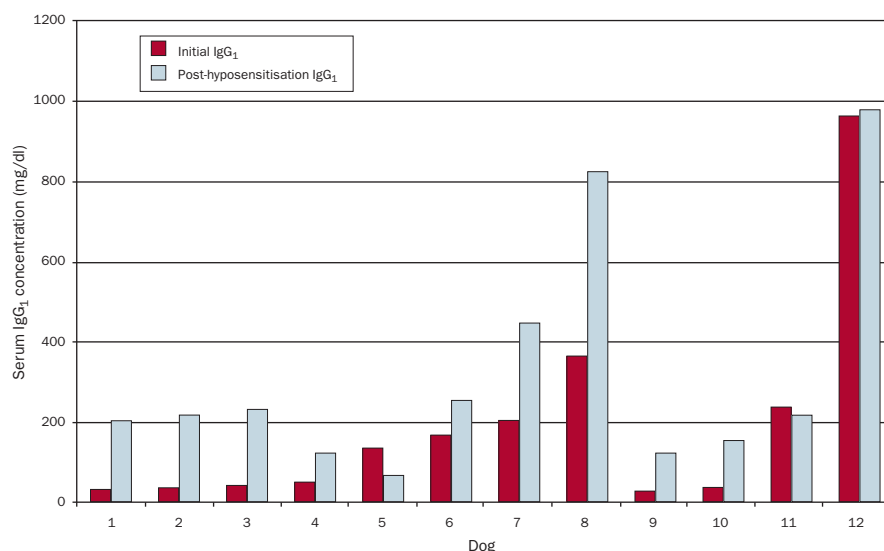


FIG 2. Serum total IgG₁ concentration (mg/dl) before and after hyposensitisation therapy

The aim of this study was to compare concentrations of serum total IgG₁ in atopic and non-atopic dogs. A commercial assay (VetRID; Bethyl Laboratories) was used as the author's hoped to identify a cost-effective, easily accessible method which could be included as another test in the diagnosis of atopic dermatitis.

Mazza and others (1993) claimed that this commercial assay was not specific for canine IgG₁, but did not provide any evidence to substantiate this. Following communication with Bethyl Laboratories it was shown that the antibodies used in the current work were different to those described by Mazza and others (1993) (J. Billings; Bethyl Laboratories, personal communication) and that this commercial assay is specific for the canine IgG₁ subclass.

A wide variation in serum total IgG₁ concentrations was observed between all five groups of dogs. Comparison of individual groups showed that the atopic working dogs had a significantly higher concentration of serum total IgG₁ than non-atopic working dogs. As these dogs are kept in identical conditions and receive the same parasite control therapy, this suggests that atopic dermatitis must have an effect on serum IgG₁ concentrations.

It is interesting also to note that the presence of pyoderma in the non-atopic working dogs did not influence IgG₁ levels, suggesting that infection is not the reason for elevated IgG₁ in the atopic dogs.

Increased concentrations of *Malassezia pachydermatis* specific IgG have been found in atopic dogs (Nuttall and Halliwell 2001, Chen and others 2002). It is possible that this may be part of the reason for the increased IgG₁ concentrations in these atopic dogs, although no work was carried out in this study to examine levels of *M. pachydermatis*.

Comparison of non-atopic dogs receiv-

ing different levels of parasite control therapy also showed that dogs receiving intensive levels with no or minimal flea exposure had significantly lower IgG₁ concentrations than those dogs receiving intermittent therapy and known to be exposed to fleas. This suggests that IgG₁ concentrations may be affected by a parasite burden and implies that any future work examining serum total IgG₁ concentrations should be carried out in dogs known to be free of parasites. It is also possible that greyhounds as a breed have higher levels of IgG₁ and further work is required to identify any breed variations in IgG₁ concentrations.

Only a small group of dogs receiving ASIT was examined. However, they did show a significant increase in serum total IgG₁ concentrations following six months of ASIT vaccination. Variations in serum IgG subclasses have been shown in humans and dogs undergoing ASIT (Hites and others 1989), and these results suggest that similar processes may be taking place in dogs, although further work is required to evaluate the exact nature of this process.

Similarly, examination of serum total IgG₁ concentrations following six months of ASIT and the clinical response did show some correlation. It is acknowledged by the authors that only a small group of dogs was examined but this does show some interesting results worthy of further investigation.

Conclusions

As further work is required on the functions of canine IgG subclasses, the exact role played by IgG₁ in allergic disease and parasitism is not known. However, these findings suggest that serum total IgG₁ concentrations in the dog are influenced by allergic skin disease, the presence or absence of parasites and the administration of ASIT.

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References

- CHEN, T. A., HALLIWELL, R. E. W. & HILL, P. B. (2002) Immunoglobulin G responses to *Malassezia pachydermatis* antigens in atopic and normal dogs. In: *Advances in Veterinary Dermatology*. Eds K. L. Thoday, C. S. Foil and R. Bond. Blackwell Science, Oxford. pp 202-209
- DAY, M. J., CORATO, A. & SHAW, S. E. (1996) Subclass profile of allergen specific IgG antibodies in atopic dogs. *Research in Veterinary Science* **61**, 136-142
- DJURUP, R. & MALLING, H.-J. (1987) High IgG₄ antibody level is associated with failure of immunotherapy with inhalant allergens. *Clinical Allergy* **17**, 459-468
- HALLIWELL, R. E. W. & DEBOER, D. J. (2001) The ACVD task force on canine atopic dermatitis (III): the role of antibodies in canine atopic dermatitis. *Veterinary Immunology and Immunopathology* **81**, 159-167
- HALLIWELL, R. E. W. & LONGINO, S. J. (1985) IgE and IgG antibodies to flea antigen in differing dog populations. *Veterinary Immunology & Immunopathology* **8**, 215-223
- HILL, P. B., MORIELLO, K. A. & DEBOER, D. J. (1995) Concentrations of total serum IgE, IgA and IgG in atopic and parasitised dogs. *Veterinary Immunology and Immunopathology* **44**, 105-113
- HITES, M. J., KLEINBECK, M. L., LOKER, J. L. & LEE, K. W. (1989) Effect of immunotherapy on the serum concentrations of allergen specific IgG antibodies in dog sera. *Veterinary Immunology and Immunopathology* **22**, 39-51
- McHUGH, S. M., LOVELLE, B., KEMENY, D. M., SADANAH, P. & EWAN, P. W. (1990) A placebo-controlled trial of immunotherapy with 2 extracts of *D. pteronyssinus* in allergic rhinitis comparing clinical outcome with changes in antigen specific IgE, IgG and IgG subclasses. *Journal of Allergy and Clinical Immunology* **86**, 521-531
- MAZZA, G., DUFFUS, W. P. H., ELSON, C. J., STOKES, C. R., WILSON, A. D. & WHITING, A. H. (1993) The separation and identification by monoclonal antibodies of dog IgG fractions. *Journal of Immunological Methods* **161**, 193-203
- MAZZA, G., WHITING, A. H., DAY, M. J. & DUFFUS, W. P. H. (1994) Development of an enzyme-linked immunosorbent assay for the detection of IgG subclasses in the serum of normal and diseased dogs. *Research in Veterinary Science* **57**, 133-139
- MORALES, C. A., SCHULTZ, K. T. & DEBOER, D. J. (1994) Anti-staphylococcal antibodies in dogs with recurrent staphylococcal pyoderma. *Veterinary Immunology and Immunopathology* **42**, 137-147
- NUTTALL, T. J. & HALLIWELL, R. E. W. (2001) Serum antibodies to *Malassezia* yeasts in canine atopic dermatitis. *Veterinary Dermatology* **12**, 327-332
- WILLEMSE, A. (1986) Atopic skin disease: a review and a reconsideration of diagnostic criteria. *Journal of Small Animal Practice* **27**, 771-778
- WILLEMSE, A., NOORDZIJ, A., RUTTEN, V. P. M. G. & BERNADINA, W. E. (1985) Induction of non-IgE anaphylactic antibodies in dogs. *Clinical and Experimental Immunology* **59**, 352-358