

Total serum IgE and IgA antibody levels in healthy dogs of different breeds and exposed to different environments

M. E. GRIOT-WENK*, A. BUSATO†, M. WELLE‡, B. P. RACINE†, R. WEILENMANN¶, P. TSCHUDI||, A. TIPOLD**,

**Institute of Animal Breeding and Small Animal Clinics, †Institute of Animal Breeding, ‡Institute for Animal Pathology, University of Bern, 3012 Bern, Switzerland, ¶Lab Animal Services, F. Hoffmann-La Roche, 4070 Basel, Switzerland, ||Klinik für Nutztiere und Pferde, **Institute of Animal Neurology, University of Bern, 3012 Bern, Switzerland.*

SUMMARY

Total serum immunoglobulin (Ig) E and A levels were analysed in 233 healthy dogs as basis for comparison with atopic dogs in future studies. They were measured by ELISA in a group of non-colonised dogs of various breeds (group A) and three groups of colonised dogs including one German Shepherd and two Beagle kennels (groups B–D). IgE levels from non-colonised dogs were significantly higher than the ones of German Shepherds and Beagles C ($P < 0.05$). IgA levels were alike in all groups except for the German Shepherds which displayed the lowest levels. Age and sex were not identified as common significant cofactors for IgE and IgA levels in all groups and IgE levels correlated negatively with IgA only in non-colonised dogs. In conclusion, IgE and IgA levels seem to be mainly influenced by genetic background. Thus, use of total serum IgE as a diagnostic tool in the atopic dogs requires extensive family data and therefore appears most suitable for research purposes within specific, well defined dog populations.
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IN THE DOG, atopy, a genetic predisposition to mount exaggerated immunoglobulin (Ig) E immune responses to common antigens, is a well-known disease entity. Atopic dermatitis represents the best characterized clinical expression of atopic syndromes (Scott et al 1995). Nevertheless, diagnosing atopy still remains a challenge because the full range of clinical signs is unknown and the diagnostic options are still limited.

In humans, increased total serum IgE levels with a heritability of 50 to 84 per cent represents a major empiric risk factor for development of atopy (Berg and Johansson, 1969; Dewar and Wheatley, 1996). In dogs, IgE levels have not been proven to be of similar diagnostic value (Wilkie et al 1990, Vriesendorp et al 1975, Schwartzman 1984, Hill et al 1995). In a previous study of healthy, colonised Beagles, we demonstrated that younger dogs displayed significantly lower total serum IgE levels than older ones, and that females had higher levels than males (Racine et al 1999). It is, however, unknown whether IgE levels in dogs of different breeds and exposed to various environments are also influenced by age and gender. Accounting for variation of total serum IgE due to breed, gender, and age influences might help to analyse an association with disease state in future studies in dogs as well.

Immunoglobulin A is the key Ig in the mucosal defense against entering antigens and has immunoregulatory functions (Schaffer et al 1991). For both humans and dogs, mild IgA-deficiency is in the majority of affected individuals not associated with clinical symptoms, but has, nevertheless,

been shown to predispose to allergy (Gershwin et al 1995, Schaffer et al 1991). To the authors' knowledge, no correlation has been found between serum IgE and IgA levels in healthy dogs.

In the present study we investigated the influence of breed, age, and gender on total serum IgE and IgA levels in different groups of healthy dogs living in various environments and determined whether a correlation between serum IgE and IgA levels exists in healthy dogs. The results can be applied in future investigations on the importance of IgE and IgA productions in the pathogenesis of developing clinical signs in atopic dogs.

MATERIALS AND METHODS

Dogs and serum samples

Test serum samples were obtained from four different groups of dogs including a total of 233 healthy animals. Group A consisted of dogs of various breeds presented to the veterinary school of the University of Bern for blood donations, Group B consisted of German Shepherds, and groups C and D were two groups of Beagles. Animals of groups B–D belonged to three different breeding colonies (F. Hoffmann-La Roche, Basel; Novartis AG, Stein and St. Aubin, Switzerland). To evaluate the difference in Ig levels at different time points, two serum specimens of group D obtained three days apart were investigated. Numbers of dogs per group and ratios of females to males are listed in Table 1. All dogs were considered healthy based on history and physical examination. Apart from dogs of group A, they were all exposed to similar environmental conditions,

including indoor and outdoor runs, and all underwent the same routine of vaccination and anthelmintic treatment. In the breeding colonies, dogs were related, but matings were carried out according to a special breeding scheme to maintain a low inbreeding coefficient; for group C, inbreeding coefficient was approximately 0.04 in the generation studied (Kocher 1992). Collected serum samples were stored in aliquots at -80°C until assayed.

ELISA for total serum IgE

Serum total IgE concentration was measured by ELISA, using two dog IgE-specific antibodies generated in our laboratories as capturing and detecting antibodies (Griot-Wenk et al 1998). The capture ELISA was carried out as described previously (Racine et al 1999). Immunoglobulin E levels were expressed as relative ELISA units (REU) compared to a reference serum pool arbitrarily assigned a value of 100 REU. To convert REU into actual protein levels, the range of IgE protein concentration was quantified by employing the recombinant IgE fragment of the second domain of IgE (Griot-Wenk et al 1998) in the ELISA.

ELISA for serum IgA

Serum IgA concentrations were measured by a capture ELISA as described previously and expressed in $\mu\text{g ml}^{-1}$ (Tipold et al 1994).

Determination of serum total globulin

Serum total globulin concentration was calculated from serum protein and albumin concentrations by an automated analyser (Hitachi 912, Roche Diagnostics AG, Rotkreuz, Switzerland).

Statistical analysis

Preliminary analysis of the data indicated skewed distributions of IgE and IgA values. For all subsequent statistical analyses, these values were transformed to natural logarithmic (ln) scales after addition of one to numbers of zero. The transformed values were then analysed using a linear model

containing the following parameters as explanatory variables: group (A–D), sex, and age. Least square means were compared and Bonferroni adjustments were applied in case of multiple comparisons. Spearman correlation coefficients (r) were evaluated within each group for the transformed IgE and IgA values with the following independent variables: lnIgE or lnIgA, and total globulin concentration. Proportions of variance of target variable explained by the models are shown (R^2). Finally, differences between IgE and IgA values obtained from two different time points of dogs belonging to group D were analyzed by ANOVA. The level of significance was set at $P < 0.05$, and all calculations were performed, using computer software (SAS 6.12, SAS Institute, Cary, NC, USA). Mean and median IgE and IgA concentrations were calculated applying nontransformed values.

RESULTS

An overview of medians and ranges of nontransformed IgE and IgA antibody levels and globulin concentrations is illustrated in Table 1. Data on IgE levels of group C were included in a previous study (Racine et al 1999). Both ELISA for IgE and IgA have been proven in previous studies to be reliable assays (Tipold et al 1994, Racine et al 1999).

Total serum IgE levels

In all four groups of dogs, a wide range of IgE levels (0–1,511 REU; Table 1) was observed. The range was particularly wide in group A including the non-colonised dogs, which also had the highest median total serum IgE level of 100.1 REU. Immunoglobulin E levels of all four groups were skewed to the left (Table 1), 95 per cent of dogs had IgE levels between 0–276.1 REU. Nevertheless, the median IgE level in the non-colonised dogs was statistically not different from the one of colonised Beagles of group D (median IgE level, 87.6 REU). In contrast, the German Shepherds (group B; median, 4.4 REU) and Beagles of group C (median, 20.5 REU) had significantly lower IgE levels than the ones of groups A and D ($P < 0.05$, $R^2 = 0.35$; Table 1). There were no significant differences in IgE levels between the samples obtained three days apart in dogs of group D ($P > 0.05$).

Table 1: Total serum IgE and IgA antibody levels and globulin concentration in four groups of healthy dogs of different breeds and environments.

Group	A	B	C	D	A-D
Breed	various	German Shepherd	Beagle	Beagle	
Origin	non-colony	colony	colony	colony	
Number of animals (n)	36	29	147	21	233
Females:males (n)	21:15	18:11	116:31	12:9	167:66
Age (year)	1.4*	1.4	3.8	4.6	3
	0.5–8†	0.8–6.2	0.8–8	2–6	0.5–8
IgE (REU‡)	100.1*	4.4§	20.5*	87.6	23.1
	1.2–1,511†	0–98	0–337	5.4–452	0–1,511
IgA ($\mu\text{g/ml}$)	64.9*	48.3	76.2	113.9	69.9
	10–508†	11.8–151.0	6.1–973	35.5–816	6.1–973
Total globulin (g/l)	31.4*	33.7	29	31.9	30.4
	20–54.0†	21.8–66.5	20.6–46.1	24.7–35.5	20–66.5

*median, †range, ‡relative ELISA Units.

§significantly lower than IgE values of groups A and D ($P > 0.05$).

||significantly lower than IgA values of group D ($P < 0.05$).

Converting IgE REU into absolute IgE concentrations, revealed an IgE protein range of 0 to 12 $\mu\text{g ml}^{-1}$.

Age was identified as significant cofactor for IgE ($P < 0.05$, Table 2) only in groups B ($R^2 = 0.26$) and C ($R^2 = 0.33$) with older animals displaying higher levels than younger ones. Additionally, in group C sex was associated with IgE levels with male animals displaying significantly lower levels (median, 3.6 REU) than females (median, 51.2 REU; $R^2 = 0.33$) (Racine et al 1999). In the Beagles of group D, a trend towards a comparable gender influence was observed ($P = 0.07$; $R^2 = 0.18$). Positive correlation for IgE values with serum globulin concentration was found in group C ($r = 0.35$; $P < 0.05$) with a similar trend observed in group A ($r = 0.32$, $P = 0.06$).

Total serum IgA levels

Similar to total IgE levels, a wide range of total serum IgA concentrations was observed in the four groups of dogs (0–973 $\mu\text{g ml}^{-1}$, Table 1). The values were also skewed to the left (Table 1); 95 per cent of animals had an IgA level between 6.1 and 443 $\mu\text{g ml}^{-1}$. The most narrow range of IgA was detected in group B, the German Shepherds (Table 1), which also displayed the lowest IgA (median, 48 $\mu\text{g ml}^{-1}$) as well as IgE (median, 4.4 REU) values. Their IgA level was significantly lower than the ones of group D (median, 113.9 $\mu\text{g ml}^{-1}$; $P < 0.05$, $R^2 = 0.06$); all other groups had similar IgA serum levels. Immunoglobulin A levels of group D obtained three days apart in group D were not different from each other.

Age represented a significant cofactor for IgA ($P < 0.05$) in group B ($R^2 = 0.50$, Table 2) with younger dogs exhibiting lower IgA levels than older ones. Additionally, sex influenced IgA levels in groups B ($R^2 = 0.50$) and C ($R^2 = 0.05$) (Table 2); in group B, females (median, 59 $\mu\text{g ml}^{-1}$) exhibited higher IgA values than males (median, 19.3 $\mu\text{g ml}^{-1}$) and in contrast in group C, females (median, 66.2 $\mu\text{g ml}^{-1}$) exhibited lower IgA levels than males (median, 112 $\mu\text{g ml}^{-1}$), respectively. No correlation could be identified between IgA levels and globulin concentrations.

Correlation analysis between IgE and IgA levels

In group A of non-colonised dogs, a negative correlation between IgE and IgA levels was encountered ($P < 0.05$, $r = -0.37$; Fig 1).

DISCUSSION

In the present study, we analysed total serum IgE and IgA levels in healthy dogs of different breeds and environments as a basis for their reassessment as diagnostic parameters in atopic dogs. In the four groups of dogs studied, total serum IgE and IgA levels differed between groups. Interestingly, IgE levels of non-colonised dogs were not uniformly higher than those of colony dogs. Lowest IgA but also IgE levels were observed in colonised German Shepherds. Age and gender were not uniformly significant cofactors for IgE and IgA levels in all of these four groups of dogs and a negative correlation between IgE and IgA levels was only evident for non-colonised dogs.

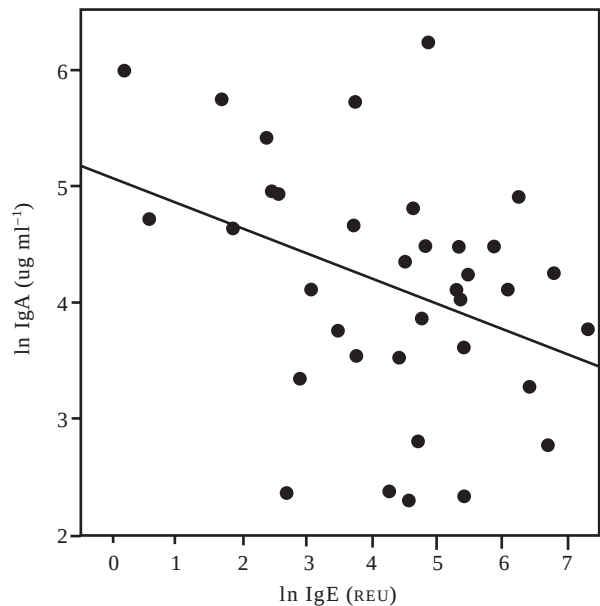


FIG 1: Association between total serum IgE and IgA (natural logarithm) in non-colonised dogs of different breeds ($r = -0.37$; $P < 0.05$).

Total serum IgE levels differed among the four groups of dogs studied. Immunoglobulin E levels of non-colonised dogs displayed the highest range of values but revealed a significant difference only to IgE values of groups B and C (Table 1); Beagles belonging to group D had similar values to non-colonised dogs. It has been stated that total IgE levels in dogs are higher than these of humans due to widespread parasitism (Hill and DeBoer, 1994; Schwartzman, 1984). The strict anthelmintic prevention programme and confined environment similar in all three colonies makes augmented IgE levels due to parasitism less likely. Furthermore, both Beagle colonies originating from different genetic backgrounds were exposed to comparable environmental factors. Thus, serum total IgE levels in dogs appear to be primarily genetically determined and therefore reflect family-associated differences. In humans, in contrast to antigen-specific IgE, twin studies showed that total serum IgE levels had a heritability of 50 to 84 per cent (Marsh and Blumenthal, 1990). In group C including 147 Beagles, we have previously demonstrated a trend towards a genetic influence by heritability estimates ($R^2 = 0.67$, $P = 0.09$) (Racine et al 1999); heritability estimates could not be applied to the three other groups of this study due to limited availability of family members.

The reported actual range of IgE protein concentration in this study of 0–12 $\mu\text{g ml}^{-1}$ applying a recombinant dog IgE heavy chain fragment is comparable to previous reports (Hammerberg et al 1997; Hill and DeBoer, 1994). In dogs of group D, we demonstrated that there was no difference between IgE (and IgA) levels of serum samples taken 3 days apart. Therefore, it appears sufficient to measure total Ig levels of a single serum sample. This is underlined by findings in humans, in whom no significant difference was found in total serum IgE concentrations in repeated serum specimens obtained over the period of one month (Nye et al 1975).

A significant negative correlation between IgE and IgA levels was only observed in non-colonised dogs (group A,

Table 2: Significant age and sex influences on total serum IgE and IgA antibody levels are illustrated within four groups of dogs; the significance level was set at $P < 0.05$.

Group	A	B	C	D
Breed	various	German Shepherd	Beagle	Beagle
Origin	non-colony	colony	colony	colony
For IgE				
Age	—	0.008*	0.0001	—
Sex	—	—	0.0001	—
For IgA				
Age	—	0.03	—	—
Sex	—	0.0001	0.02	—

*P-value

Fig 1). In atopic and parasitised dogs, Hill and DeBoer (Hill et al 1995) have shown a similar negative correlation but not in their healthy control, animals. These findings suggest that increased IgE levels might represent an important immune response to reduced IgA levels and warrants further investigation. However, the immunoregulatory interplay between IgE and IgA only appears to be important in case of continuous and diverse antigen exposure such as in non-colonised dogs; in colonies, exposure to different and potentially novel antigens is more limited.

Our hypothesis of significant and uniformly applicable age and gender influences on IgE levels in different groups of dogs had to be rejected. Immunoglobulin E levels were influenced by age only in the German Shepherds and Beagles of group C (Table 2), which both also displayed lowest IgE levels (Table 1). There was not even a trend to significance observed in the other two groups indicating that the lower number of dogs studied of groups A and D are unlikely responsible for lack of significance. Sex was only a significant factor in group C with a similar trend observed in the Beagles of group D (Table 2). In humans, data on effects of age and gender on total IgE levels is also controversial (Villar and Holgate, 1994). Additionally, serum globulin concentration, a rough measure of Igs, correlated with IgE levels only in group C which may be due to the lower numbers of dogs examined in the other three groups. However, compared to serum IgG, total IgE as well as IgA levels contribute little to the total Ig amount which explains the overall lack of correlation; wide ranges of IgA and IgE levels were not paralleled by serum globulin concentrations (Table 1).

The German Shepherds displayed lowest IgA levels. As shown for other breeds such as Chinese Shar-Pei dogs (Rivas et al 1995), it is well known that German Shepherds have lower IgA levels (Whitbread et al 1984). This phenomenon is thought to be correlated with a predisposition to intestinal bacterial overgrowth (Batt et al 1991). As with IgE levels, total serum IgA levels seem to be mainly under genetic control. Previous observations of significant differences in IgA levels in two separate Beagle colonies support this notion (Glickman et al 1988). However, the proportion of variance for IgA explained by the model with the variables age, gender, and group was low ($R^2 = 0.06$). This suggests that yet unidentified factors are important determinants of IgA concentrations. Within groups of dogs, age was only a significant factor in group B, the German Shepherds, which displayed the lowest levels. Even though group A

dogs had the same median age value of 1.4 years, we could not confirm the observation found in German Shepherds. In a previous study, male Beagles had higher IgA levels than females (Glickman et al 1988), which was only observed in group C but in group D.

In conclusion, this report represents the first comparison of total serum IgE as well as IgA levels in different groups of colony and non-colonised dogs. Serum IgE and IgA levels vary among groups of dogs, which seem to be mainly influenced by genetic background. No common significant cofactor for IgE and IgA levels was detected. The negative correlation between IgE and IgA levels in non-colonised dogs might be important for the underlying immunoregulatory mechanisms and warrants further assessment. Difference in total serum IgE levels between groups illustrates that its measurement appears mostly suitable for research purposes within highly defined dog populations.

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