

Antibody and blood leukocyte response in *Rhipicephalus sanguineus* (Latreille, 1806) tick-infested dogs and guinea pigs

Matias P.J. Szabó^{a,b,*}, Vanessa L. Aoki^a, Françoise P.S. Sanches^a,
Lúcia P.T.C.T. Aquino^{a,b}, Marcos V. Garcia^a,
Rosângela Z. Machado^a, Gervásio H. Bechara^a

^a FCAVJ, Depto. de Patologia Veterinária, Universidade Estadual Paulista, 14.884-900 Jaboticabal, SP, Brazil

^b Universidade de Franca, Franca, SP, Brazil

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Abstract

The dog is considered to be the natural host of *Rhipicephalus sanguineus* and is unable to develop appreciable resistance even after repeated feedings. The guinea pig develops strong resistance after one infestation with adult ticks. Antibody (IgG) titres against tick salivary gland antigens (SGAs) and blood leukocyte numbers in dogs and guinea pigs undergoing experimental *R. sanguineus* tick infestations were measured to detect a possible correlation with susceptibility or resistance of hosts. Since infested dogs develop an immediate hypersensitivity reaction to *R. sanguineus* antigens, total and anti-*R. sanguineus* SGA IgE levels were also measured in this host species. IgG and IgE antibody levels were determined by an enzyme-linked immunosorbent assay (ELISA) along three consecutive infestations of both hosts. Most dogs and guinea pigs displayed low IgG levels against *R. sanguineus* SGAs, though marked differences in individual response were observed. Although dog's total serum IgE levels increased significantly after infestations, no change in the amount of anti-salivary gland IgE was detected. Total and differential blood cell counts were determined in dogs and guinea pigs during primary and secondary infestation. In dogs, a tertiary infestation and a subsequent higher infestation level were also evaluated. Infested dogs did not display any alteration in blood leukocyte counts throughout the experiment. Guinea pigs, on the other hand, developed a significant basophilia during primary infestation which increased further during secondary infestation. These data reveal similarities and differences in the reactions of resistant and non-resistant hosts to ticks.

* Corresponding author. Fax: +55-16-3202-4275.

E-mail address: szabo@fcav.unesp.br (M.P.J. Szabó).

They contribute for the understanding of such host–parasite relationships and will hopefully aid in the development of immune control of ticks.

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1. Introduction

The hard tick *Rhipicephalus sanguineus*, commonly referred to as the “kennel tick” or “brown dog tick”, is a cosmopolitan parasite and probably the most prevalent of ixodid species (Pegram et al., 1987). *R. sanguineus* is widely distributed in the Americas, Europe, Africa, Asia and Australia. In Africa it is predominantly a parasite of carnivores but may also infest man and, probably rarely, his domestic livestock. Elsewhere, it is confined almost entirely to domestic dogs (Pegram et al., 1987). This tick species has been linked to such tick-borne diseases as spotted and boutonneuse fever and ehrlichioses in man, and babesiosis and ehrlichioses in dogs (Walker et al., 2000). In Brazil domestic dogs may suffer heavy *R. sanguineus* infestations and this tick species is a constant nuisance for the owners (Szabó et al., 2001).

The dog is considered to be the natural host of *R. sanguineus* and is unable to develop resistance even after repeated feeding (Garin and Grabarev, 1972; Szabó et al., 1995a), whereas guinea pigs, a commonly used laboratory host, develop strong resistance after one infestation of adult ticks (Szabó et al., 1995a).

To provide a rational basis for dog immunization protocols against *R. sanguineus* tick it would be desirable to distinguish between host responses that are effective against ticks from those which are not. Although information on immunopathological aspects of *R. sanguineus*–host relationships is accumulating, not much is known about antibody and blood leukocyte response in *R. sanguineus*-infested dogs and guinea pigs. Thus, the objective of the present work was to evaluate humoral immune response and blood leukocyte counts of dogs and guinea pigs repeatedly infested with *R. sanguineus*.

2. Material and methods

2.1. Ticks

Ticks (*R. sanguineus*) were reared in the laboratory as previously described (Bechara et al., 1995). Briefly, ticks were kept under a constant temperature of 29 °C, a relative humidity of 80 ± 5% and a 12:12 (L:D) photoperiod. A continuous tick supply was maintained by feeding nymphs and larvae on tick-bite naive guinea pigs and adults on dogs or tick-bite naive guinea pigs.

2.2. Hosts

Male and female mongrel dogs (*Canis familiares*) and albino female guinea pigs (*Cavia cutleri*) were used. Every animal was tick-bite naive at the beginning of the experiments.

Dogs were born and raised in a tick-free kennel at the Pathology Department of the School of Veterinary Science of Jaboticabal, University of the São Paulo State, UNESP. Guinea pigs weighed about 500 g at the beginning of the experiments and were supplied by São Paulo State University's bioterium (Botucatu, SP, Brazil). Water and commercial food were given "ad libitum" to animals.

2.3. Infestations

Each animal was subjected to 2–4 successive infestations, 1 month apart, with unfed adult ticks restricted to the host in feeding chambers as described elsewhere (Szabó et al., 1995a). Unless otherwise stated, every infestation consisted of four female and five male ticks in the case of rodents and 25 females and 30 males on every dog. Adult ticks used for infestations were 2–3 weeks old. Tick yield, female and egg mass weights and larval hatchability rates of ticks as described by Szabó and Bechara (1997) were used to evaluate resistance development in hosts.

2.4. Blood collection

Blood samples for hematology and serology were collected from the jugular vein of dogs and by cardiac puncture of guinea pigs. Blood for hematology (2 ml per dog and 0.8 ml per guinea pig) was collected in syringes containing ethylene diamine tetra acetate (EDTA). Serum was separated after centrifugation of blood (5 ml per dog and 1 ml per guinea pig) collected without anticoagulants and allowed to clot at room temperature for 1 h and another hour at 10 °C. Serum samples were stored at –20 °C until used.

2.5. Hematology

Guinea pigs ($n = 9$) were infested twice and dogs ($n = 6$) three times. Control hematological values were obtained from sham infested guinea pigs ($n = 8$) and dogs ($n = 6$). Since no hematological alteration could be detected in three consecutive infestations of dogs, they were submitted to a fourth, high intensity infestation with 110 male and 110 female ticks. Dogs were bled 24 h before each infestation; 1, 3, 5 and 7 days after beginning of infestations and 1, 2 and 3 days after the end of infestations. Guinea pig blood samples were obtained 24 h before infestation, and on days 3 and 7 after release of ticks on hosts and 6, 12 and 18 days after the end of each infestation. Romanovsky stained blood smears were prepared from each sample for white blood cell (WBC) differential counts. Complete blood cell (CBC) counts and hemoglobin (g/100 ml) were also determined. Packed cell volume (PCV) was determined by the microhematocrit method.

2.6. Antibody response

For this experiment other dogs ($n = 6$) and guinea pigs ($n = 8$) were infested three times. IgG titres were determined for both dogs and guinea pigs to evaluate humoral immune response to ticks. IgE levels of dogs were also determined since this host but not guinea pig was shown to develop a strong immediate hypersensitivity to *R. sanguineus* tick

antigens after infestations (Szabó et al., 1995b). Serum from all animals was obtained 1 day before infestations and every 7 days from the 14th day from dogs and every 10 days from guinea pigs. Serum was also obtained from both host species 30 and 60 days after the last infestation.

Enzyme-linked immunosorbent assay (ELISA) was employed to evaluate antibody response against ticks in sera of infested dogs and guinea pigs. For each of the enzyme immunoassays, optimal antigen concentration and serum dilutions were established testing four concentrations of the antigen (5, 10, 20 and 40 µg/ml) against six dilutions of guinea pig or dog reference sera (1:100, 1:200, 1:400, 1:800, 1:1600, 1:3200). Negative reference sera were obtained from animals prior to infestation and positive reference sera from animals inoculated with salivary gland extract and infested with *R. sanguineus* ticks. All immunoassays reactions were read photometrically at a wavelength of 405 nm in a microplate reader (MRX-DYNEX Technologies). Results were presented as absorbance data. For IgG titres cut-off point was determined at ELISA level two as described earlier (Machado et al., 1997). Tick salivary gland extract was prepared by salivary gland dissection of semi-engorged female ticks and sonicated as routinely done in the laboratory (Szabó and Bechara, 1997). Extracts were then centrifuged, the supernatant filtered and stored at –20 °C until used.

2.7. Anti-salivary gland IgG ELISA

ELISA procedure for the anti-salivary gland IgG was essentially as already described (Machado et al., 1997). Antigen protein concentration was adjusted to 10 µg/ml and test sera were diluted 1:400. Alkaline phosphatase conjugated anti-dog IgG (SIGMA A-6042) diluted 1:9000 and anti-guinea (SIGMA A-5062) 1:30 000 were used for the test.

2.8. Total serum IgE ELISA

Total serum IgE was quantified using a commercial kit and according to manufacturer's instructions (Bethyl Laboratories, Inc., E40-125). Briefly, microplates were coated with 10 µg/ml goat anti-dog IgE purified antibody diluted in carbonate–bicarbonate buffer pH 9.6. Phosphate-buffered saline (pH 7.4) with 0.05% Tween 20 (PBS-Tween) containing 5% rabbit serum was used as the blocking buffer and applied for 30 min at 37 °C. Dog serum samples were diluted 1:200 in blocking buffer and incubated for 2 h at room temperature. Goat anti-dog IgE conjugated with horseradish peroxidase diluted 1:20 000 was used as an indicator. After incubation for 2 h at room temperature, the reactions were visualized by the addition of the enzyme substrate 2,2-azino-bis-3-ethylbenz-thiazoline-6-sulfinic acid (ABTS) at 2% and read after 40 min.

2.9. Anti-salivary gland IgE ELISA

The protocol was the same employed for total serum IgE determination, but salivary gland was used to coat microplates (as described above). Only sera samples from animals displaying high total IgE levels were analyzed ($n = 4$).

2.10. Data analysis

Hematological parameters were evaluated by ANOVA and means were compared by the Tukey's test ($P < 0.05$). Hematological data were transformed using the $\log + 1$ transformation for analysis. Total serum IgE levels were evaluated by ANOVA and Dunnett's Multiple Comparison Test was used to compare means. Pre-infestation sera were used as control data. For anti-salivary gland antibody titres (IgG and IgE) serum cut-off point was determined at ELISA level two as described elsewhere (Machado et al., 1997).

3. Results

Guinea pigs but not dogs displayed strong resistance from second infestation on, in both experiments. Main features of resistance included low tick yield and decreased engorged female and egg mass weights for ticks from resistant guinea pigs. Biological parameters of ticks were very uniform within groups in each infestation (low standard deviation). Fig. 1 illustrates the decline of engorged female weight with each host infestation in guinea pigs compared to dogs, one of such alterations in tick biology.

Blood counts of tick-infested dogs remained within or close to normal physiological levels. No significant difference could be observed between control animals (carrying feeding chamber without ticks) and those submitted to first, second, third and high intensity infestations (data not shown). Most analyzed blood parameters of tick-infested guinea pigs also remained unaltered throughout infestations (data not shown), however significant basophilia was observed during first and especially during second infestations (Fig. 2 and Table 1).

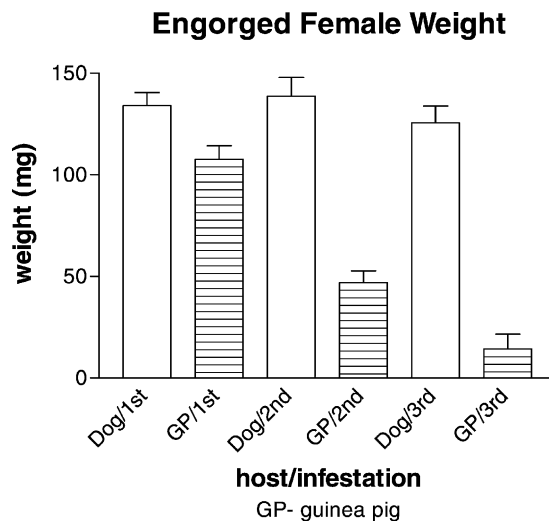


Fig. 1. Mean engorged female weight of *R. sanguineus* ticks feeding on dogs ($n = 5$) and guinea pigs ($n = 9$) during first, second or third infestation.

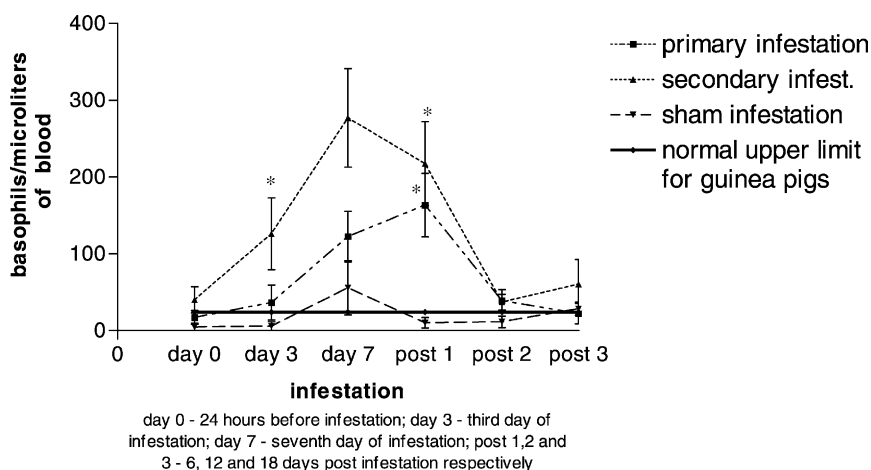


Fig. 2. Mean blood basophil counts per microliter of blood in tick-infested ($n = 9$) and control (sham infested) guinea pigs ($n = 8$). Significant differences between infested and control guinea pigs at the same infestation date (* $P < 0.05$).

IgG antibody response of dogs to *R. sanguineus* infestations (Fig. 3) were low and only one animal presented high titres following second and third infestation. Guinea pig IgG response was variable within the experimental group. Three out of eight guinea pigs reacted with high titres to second infestation and only one reacted to third infestation (Fig. 4). Dog total serum IgE levels increased significantly after the first infestation and fell to non-significant levels after the end of parasitism. Second infestation significantly increased IgE levels again which decreased to non-significant levels only 60 days after the third (last) infestation (Fig. 5). In

Table 1

Variation in mean circulating basophil numbers (cells $\times 10^3 \mu\text{l}^{-1}$) of guinea pigs during and after primary or secondary infestation with *R. sanguineus* ticks or sham infestation (controls)^a

Infestation	First day prior infestation	Third day of infestation	Seventh day of infestation	Six days post-infestation	Twelve days post-infestation	Eighteen days post-infestation
<i>Primary</i>						
Mean (9)	0.017 aA	0.037 aAB	0.122 abA	0.163 bA	0.039 abA	0.022 aA
S.D. ^b	0.027	0.068	0.099	0.124	0.043	0.040
<i>Secondary</i>						
Mean (9)	0.040 aA	0.126 aA	0.277 aA	0.218 aA	0.037 aA	0.061 aA
S.D.	0.051	0.140	0.193	0.163	0.030	0.086
<i>Sham</i>						
Mean (8)	0.005 aA	0.006 aB	0.056 aA	0.010 aB	0.011 aA	0.029 aA
S.D.	0.014	0.016	0.100	0.019	0.021	0.025

^a Means in a column followed by the same capital letter do not differ significantly ($P < 0.05$). Means in a row followed by the same lower case letter do not differ significantly ($P < 0.05$).

^b Standard deviation.

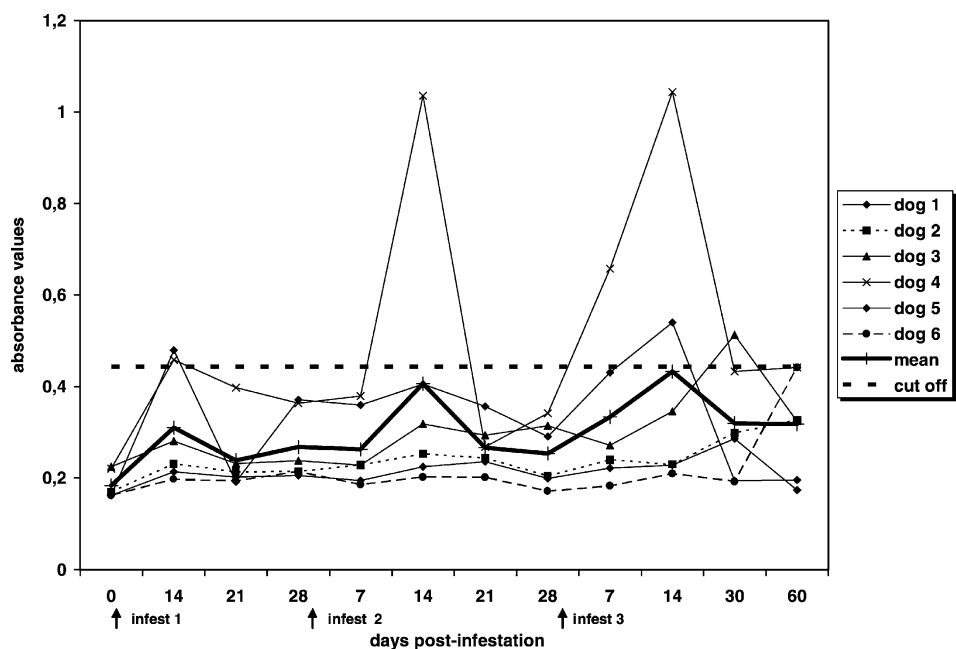


Fig. 3. Absorbance values of anti-salivary gland IgG ELISA of sera from six dogs experimentally infested with *R. sanguineus* ticks.

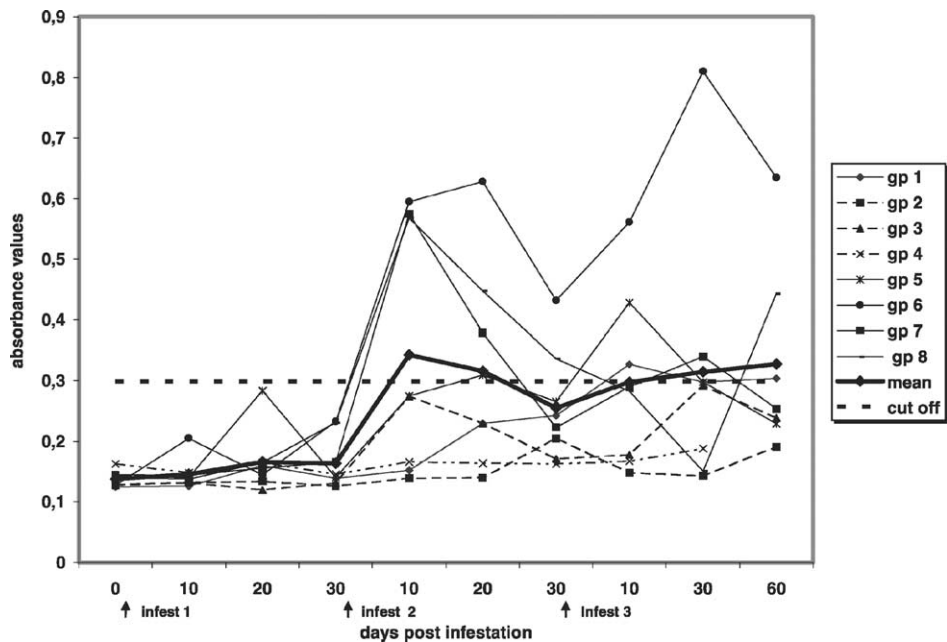


Fig. 4. Absorbance values of anti-salivary gland IgG ELISA of sera from eight guinea pigs experimentally infested with *R. sanguineus* ticks.

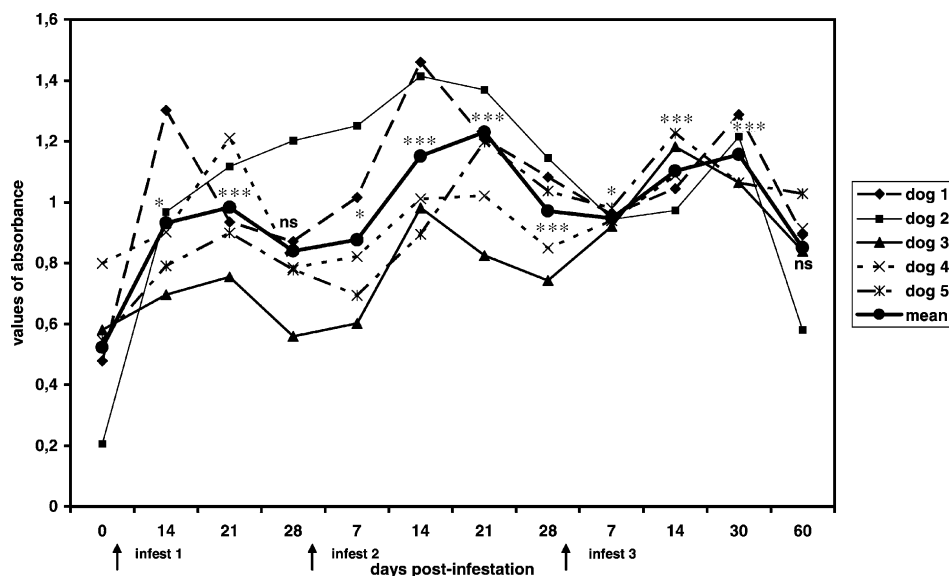


Fig. 5. Absorbance values of total serum IgE ELISA from five dogs experimentally infested with *R. sanguineus* ticks. All mean values are compared to pre-infestation values. Significance of differences is indicated by: * $P < 0.05$; *** $P < 0.001$; ns: non-significant.

the case of anti-salivary gland IgE no differences could be detected in sera from both pre- and post-infested dogs (data not shown).

4. Discussion

Immunization of hosts against ticks has long been considered a promising alternative to the use of acaricides and much effort is being spent to understand tick–host immune relationships to aid the development of an efficient product. Mechanisms of naturally acquired immunity to ticks, however, are poorly understood (Willadsen and Jongejan, 1999) and protective responses can be confounded by tick-induced immunosuppression and by host responses irrelevant to tick rejection. Dogs and guinea pigs are, respectively, susceptible and resistant hosts of the *R. sanguineus* tick, and provide a model for comparison of inefficient to efficient immune mechanisms.

It is already known that the local inflammatory reaction at the tick feeding sites differs between dogs and guinea pigs. The skin reaction of dogs presents a prominent infiltration of neutrophils throughout all infestations while guinea pigs react with basophils and eosinophils (Szabó and Bechara, 1999). In addition, repeatedly tick-infested guinea pigs were shown to develop both a small immediate and an intense 18–24 h delayed type hypersensitivity (DTH) cutaneous reaction to a tick extract. Conversely, dogs, which are naturally susceptible to ticks, developed a much more intense immediate but, not an evident DTH cutaneous reaction after repeated infestations with ticks (Szabó et al., 1995b). Before the

hematological analysis presented in this paper, neutrophilia, due to local neutrophilic reaction to *R. sanguineus*, or an eosinophilia, as commonly occur in parasitic diseases of dogs (Carroll and Grove, 1986; Yoshida et al., 1997) was expected. The present work has shown that 55–220 adult *R. sanguineus* feeding ticks do not significantly alter dog hematological parameters, indicating a low interference of this tick parasitism on such parameters. We cannot rule out, however, that much higher infestation levels might induce detectable alterations.

Infested guinea pigs presented a significant increase in basophil counts as the sole hematological alteration. Such an alteration has already been described in guinea pigs infested with *Dermacentor andersoni* Stiles larvae (Gordon and Allen, 1979) and point to basophils as the cell that might be involved in anti-tick immune mechanisms. In fact basophils might form 73% of the cellular infiltrate at *R. sanguineus* tick attachment sites on guinea pigs (Szabó and Bechara, 1999). It has also been shown that anti-eosinophil and mainly anti-basophil serum reverses immune resistance of guinea pigs to *Amblyomma americanum* (L.) ticks (Brown et al., 1982). This cell's exact role is not known but it is considered hallmark of a type of delayed hypersensitivity known as cutaneous basophil hypersensitivity (Dvorak and Dvorak, 1993) which was associated to expression of resistance of laboratory animals to ticks (Wikel, 1996). A more recent review indicated that basophils in parasitic reactions are associated with immediate type hypersensitivity reaction induction mechanisms (Falcone et al., 2001) and thus controversy is established.

Guinea pigs are also known to react to *R. sanguineus* ticks with up to 38% of eosinophils at the tick attachment cellular infiltrate (Szabó and Bechara, 1999) and blood eosinophilia would not have been unexpected in the present work. For example, blood eosinophilia and basophilia has been previously observed in guinea pigs infested with *A. americanum* ticks (Brown et al., 1982). We are unable to provide an explanation for the lack of eosinophilia in our work, but it is possible that the higher number circulating baseline blood eosinophils of guinea pigs were enough to supply *R. sanguineus* tick attachment sites without detectable blood increases. It is worthwhile mentioning that *R. sanguineus* ticks are tiny and thus differences in stimulus intensity when compared to *A. americanum* ticks might be expected. Another explanation would be probable differences between salivary components of the two tick species and thus differential stimuli for leukocyte production.

Another observation from the present work was, apart from one animal, a low IgG response to salivary gland antigens (SGAs) of tick-infested dogs. In this regard, it was already shown that *R. sanguineus* infestations of dogs diminish IgG antibody production against rabbit serum albumin (Inokuma et al., 1997). Such a low reaction could occur due to a humoral immunosuppression caused by tick saliva as described for bovines infested with *Boophilus microplus* (Canestrini) (Inokuma et al., 1993) and might represent an evasion mechanism of ticks. On the other hand, guinea pigs, hosts which displayed strong resistance to ticks, also had a low IgG response although some individual variations were observed. Such a situation suggests dissociation between IgG antibody levels and capacity to develop resistance to ticks. In fact, previous observations involving sheep and *A. americanum* ticks showed an inverse relationship between resistance expression of the host and antibody formation (Barriga et al., 1991).

The immediate hypersensitivity displayed by dogs to *R. sanguineus* antigens (Szabó et al., 1995b) suggests that IgE may be preferentially involved in the reaction to ticks and

should also be investigated. Total serum IgE levels increased to significant levels after each infestation and decreased to non-significant levels only 60 days after the last infestation. This observation suggests an increased production of IgE immunoglobulins induced by *R. sanguineus* infestation in dogs. At the same time we were unable to detect anti-salivary gland IgE in the sera of infested dogs. It is possible that higher levels of anti-salivary gland IgG antibodies displaced IgE from binding sites in the ELISA microplates. It is also possible that specific anti-salivary gland IgE antibodies are in very low levels and beneath the sensitivity of the test. In this last situation the increase in total IgE levels could have been associated to the inducement, by the *R. sanguineus* tick infestation, of an immune environment favorable for IgE immunoglobulin production against other unrelated antigens as well. In this regard, it was already observed in immunohistochemical assays that draining lymph nodes of *R. sanguineus*-infested dogs display high numbers of IgE positive cells (Szabó and Bechara, unpublished results).

Tick biological parameters were very uniform within groups in each infestation and thus individual variations in antibody and blood leukocyte response could not be correlated with resistance. This observation suggests that response of dogs and guinea pigs to *R. sanguineus*, as measured in the present work, follow a host specific pattern.

As observed from the results of this work, differing and some common blood-borne reactions to *R. sanguineus* infestations can be seen in the susceptible dog and resistant guinea pig. *R. sanguineus* infestation induces circulating basophilia only in guinea pigs but a weak IgG antibody response in both dogs and guinea pigs. IgE seems to be involved in the reaction of dogs to *R. sanguineus* ticks. It has already been shown that ticks tend to polarize host immune response towards a Th2 cytokine profile (reviewed by [Wikel and Alarcon-Chaidez, 2001](#)) which is particularly extreme in the case of mice susceptible to *R. sanguineus* ticks ([Ferreira and Silva, 1999](#)). On the other hand, much less is known about immune response of resistant hosts to ticks. The role of basophils and delayed hypersensitivity to ticks in the reaction to *R. sanguineus* ticks is a worthwhile matter for investigation. Such knowledge would undoubtedly contribute for the development of tick control methods based on host immune responses.

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