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Research brief

Rhipicephalus (Boophilus) *microplus*: Distinct acute phase proteins vary during infestations according to the genetic composition of the bovine hosts, *Bos taurus* and *Bos indicus*

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Abstract

Tick bites may trigger acute phase responses. Positive and negative acute phase proteins were measured in infested cattle genetically resistant and susceptible to ticks. During heavier infestations levels of haptoglobin increased significantly in susceptible bovines; levels of serum amyloid A increased in resistant bovines; levels of alpha-1-acid glycoprotein decreased significantly in resistant bovines; levels of transferrin decreased significantly in susceptible bovines.

In conclusion, tick infestations trigger acute phase responses and enhancement of specific acute phase proteins differs according to the genetic composition of hosts. Acute phase proteins may constitute useful biological signatures for monitoring the stress induced by tick infestations.

Index Descriptors and Abbreviations: Boophilus microplus, (Ixodidae) is a monoxene (one host) hard tick found in subtropical and tropical regions (Mexico, the Caribbean, Central and South America, parts of Africa, Madagascar, Taiwan, Southeast Asia, and Australia). It is found in wild and domestic herbivores, including cattle (*Bos taurus taurus*, *Bos taurus indicus*, and crossbreeds); ELISA, enzyme-linked immunosorbent assay; APR, acute phase reaction; APP, acute phase protein; SAA, serum amyloid A; Hp, haptoglobin; α-1AGP, alpha-1 acid glycoprotein; Tf, transferrin; Bovine; *Rhipicephalus* (Boophilus) *microplus*

1. Introduction

Vertebrate hosts mount immune responses against ticks that are implicated in the rejection of these ectoparasites. The outcome of the host/tick interface is much the same across a given species. In cattle, however, the level of infestation with ticks can vary according to breed and even after repeated infestations, susceptible hosts will still harbour significantly larger numbers of parasites than resistant breeds (O'Kelly and Spiers, 1976; Mattioli et al., 2000). It is well established that these distinct phenotypes are heritable, but the host genes involved in their expression have not been identified nor have the immune correlates been fully described. Mast cells and basophils are implicated in rejection of ticks (Schleger et al., 1976; Brown et al., 1984), but

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their exact role in these different phenotypes of infestation is not fully explained. While grazing in infested pastures bovines, even tick-resistant ones, are exposed to large numbers of questing larvae that attach themselves to the host skin by means of the cement cone produced by tick salivary glands. It is logical to assume that the acute phase response (APR) occurs during tick infestations because, in order to acquire a blood meal, ticks injure their hosts' skin and inoculate copious amounts of toxic saliva containing molecules that destroy extracellular matrix (Ribeiro et al., 2006; de Miranda Santos et al., 2004). These processes, together with the cement cone, constitute danger signals for the host (Matzinger, 2002), triggering production of acute phase proteins (APPs) in response to the cytokines IL-1, IL-6 and TNF-α released by resident and recruited phagocytes (Suffredini et al., 1999). APPs have an important role in the innate immune response and in the initiation of the acquired immune response, mediating or dampening inflammation and participating in tissue repair, a process which can facilitate or hamper the tick's blood meal. Furthermore, APPs have several roles in their biology as will be discussed. Plasmatic levels of APP correlate with the severity of infections and are biological signatures of inflammation (Suffredini et al., 1999). It is not known if production of APPs is affected during infestations with ticks. The present work examines whether tick infestations induce an APR in bovines and whether this response varies qualitatively and quantitatively in cattle of different genetic backgrounds presenting with different phenotypes of tick infestations. In cattle the most cytokine-sensitive positive APPs are haptoglobulin (Hp), serum amyloid A (SAA) and alpha-1 acid glycoprotein (α-1AGP). Transferrin (Tf), a negative APP, is also worth studying in tick infestations due to the anaemia caused by bloodfeeding of ticks and the role of this protein in haemostasis of iron.

2. Materials and methods

2.1. Experimental animals and infestations

Healthy, unrelated yearling bulls of a tick-resistant (R) breed (Nelore, *Bos taurus indicus*; $N = 5$) and of a tick-susceptible (S) breed (Holstein, *B. taurus taurus*; $N = 6$) were treated with acaricides until collection of the first blood sample for serum separation and from then on they were managed together in a pasture (Potirendaba, São Paulo state, Brazil) naturally infested with larvae of *R. microplus* and and new blood samples were collected at the time points indicated in the figures and that consider what is known about the half-life of APPs (Horadagoda et al., 1999). Sera were kept frozen until assayed together. The level of infestation of the experimental pasture varied during the experiment and was estimated by counting the number of engorging females larger than 4mm on one side of susceptible hosts (Kashino et al., 2005). At the time of the three sample collections indicated in the figures the ranges of engorging females were the following: 12–38, 20–65, and 38–148; resistant cattle were considered to be exposed to corresponding proportions of larvae and never presented with more than 20 engorging females larger than 4 mm on one side. Median numbers of female ticks were significantly ($P < 0.001$, Student's t test) lower on Nelore yearlings (6.7 ± 5.8) than on Holsteins (61 ± 26).

2.2. Quantification of APPs

APPs were quantified by means of commercial kits according to the instructions and with standards of the manufacturers. Kits for measurement of Hp and SAA were manufactured by Tridelta (Dublin, Ireland), for α-1AGP by Ecos Institute (Miyagi, Japan) and for transferrin by Bethyl Labs (Montgomery, TX, USA).

2.3. Statistical tests

Student's t test and the Mann–Whitney rank sum test were used to evaluate significance among group medians and a P-value < 0.05 was used to establish the level of significance. SigmaStat version 2.03 (SPSS Inc., Chicago IL) was used to perform the statistical tests.

3. Results and discussion

3.1. The levels of Hp increase during tick infestations in susceptible cattle

Levels of Hp peaked during heavy infestations with *R. microplus*, but the increase was significant ($P < 0.001$) only in susceptible bovines (Fig. 1a). A month later, when infestations had decreased, levels of Hp also decreased significantly ($P < 0.003$, Student's t test) in infested, susceptible bovines, but were still significantly ($P < 0.001$, Student's t test) higher than pre-exposure levels. Hp is the most abundant APP in cattle and the plasma protein with the highest binding affinity for haemoglobin. Hp defends the vasculature against haemoglobin and heme, which are removed by the monocyte/macrophage system through CD163, a scavenger receptor specific for the Hp–Hb complex. Once CD163 binds to the Hp–Hb complex, it mediates anti-inflammatory effects via the release of IL-10 and the induction of heme oxygenase-1 (Abraham and Drummond, 2006), which generates the immunosuppressive metabolites CO, Fe++ and biliverdin from heme. Therefore, it might establish a favourable milieu for the tick and down regulate the inflammatory immune response, leading to a Th2 pattern of immune response, which, at least for mice, is known to not be protective against ticks (Ferreira and Silva, 1999). In addition, Hp binds to mast cells and inhibits their growth (El-Ghmati et al., 2002), further hampering host resistance to ticks. Another study also compared the acute phase response between different breeds of cattle infected with the tick-borne protozoan, *Theileria annulata* (Glass et al., 2005). In accordance with this study, higher levels were also observed in taurine cattle.

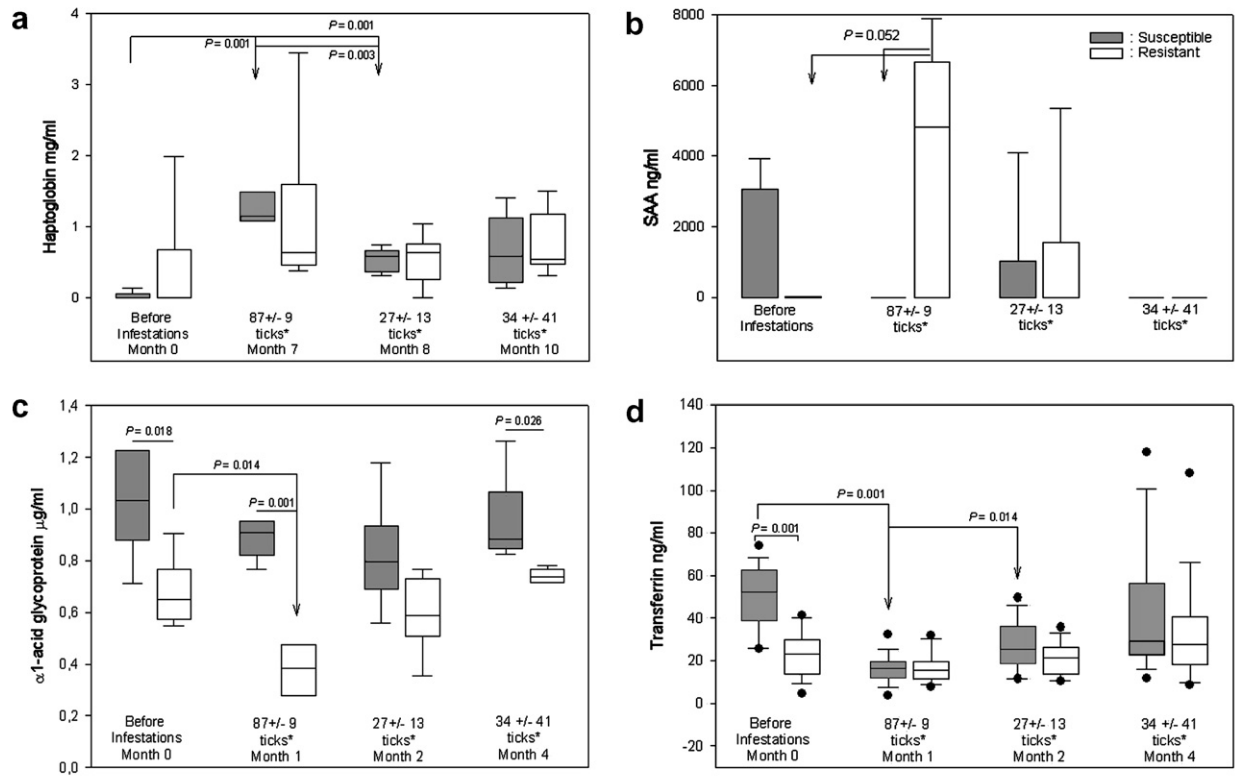

Fig. 1. Levels of three positive ((a) Hp, haptoglobin; (b) SAA, serum amyloid A; (c) α-1AGP, alpha-1 acid glycoprotein) and one negative ((d) Tf, transferrin) acute phase proteins were measured in sera of tick-resistant and -susceptible breeds of cattle. Animals were managed in a pasture undergoing different levels of natural infestations with the cattle tick, *R. microplus*, as indicated by the average number ± SD of ticks counted on one side of the S bovines (*) and sera were collected at the time points indicated. Grey bars indicate levels of APPs in tick-susceptible (S) bovines and empty bars indicate levels in resistant (R) bovines.

3.2. The levels of SAA do not vary during ticks infestations

Regarding SAA, in spite of significantly ($P < 0.001$, Student's t test) less ticks feeding on resistant hosts, during periods of exposure to heavily infested pastures, higher ($P < 0.052$, Mann–Whitney rank sum test) levels of this APP were found in their sera than in those from susceptible bovines (Fig. 1b). Levels did not differ between the two breeds during less intense exposures. While the difference between systemic levels of SAA produced by resistant and susceptible bovines did not reach significance, the observed trend suggests that local production of this APP by epithelial cells of the skin in the vicinity of the tick bite could be significantly augmented in resistant animals, but not enough to affect systemic levels. SAA is an agonist of formyl peptide receptors that are expressed on basophils, monocytes and neutrophils (Le et al., 2001; de Paulis et al., 2004) and attract these cells to the site of tissue damage suggesting a pro-inflammatory role in resistance to ticks. Indeed, resistant hosts react to ticks mainly with mononuclear cells, eosinophils and basophils, while, on the other hand, susceptible hosts react to ticks mainly with neutrophils (Brown et al., 1984; Gill and Walker, 1985; Gill, 1986; Szabo and Bechara, 1999; Ferreira et al., 2003). SAA also induces the expression of integrins in monocytes, neutrophils, and T lymphocytes (Xu et al., 1995). Lastly, SAA is a chemotactic factor for mast cells (Olsson et al., 1999), which are also involved in resistance to ticks (Schleger et al., 1976). Furthermore, tryptase from mast cells degrades SAA (Niemi et al., 2006) emphasizing the need to refine studies on the role of the acute phase response in tick infestations. In accordance with the study of the APR in *T. annulata*-infected taurine and zebuine cattle (Glass et al., 2005), there were no significant differences between the baseline levels of SAA in either breed. In spite of fewer parasites, whether protozoa or ticks, affecting zebuine cattle, the levels of SAA were higher in these animals than in taurines.

3.3. Despite being a positive APP, the levels of α-1AGP decrease during tick infestations

The levels of a third APP, α-1AGP, are consistently and significantly ($P < 0.05$) higher in susceptible bovines in the absence or presence of feeding ticks on these hosts (Fig. 1c). Although α-1AGP is a positive APP, it was interesting to observe that levels of α-1AGP decreased in relation to baseline levels in all animals undergoing tick infestations. However the decrease was significant ($P < 0.014$) only in resistant animals undergoing exposure to heavily tick-infested pastures. α-1AGP is an immunocalin and, as such, binds components of biological fluids and dampens inflammatory responses of leukocytes as a feedback mechanism of control of the inflammatory cascade (Hochepied et al., 2003). Another relevant aspect of α-1AGP, including bovine α-1AGP (Ceciliani et al., 2007), is that it is extensively glycosylated with sialyl LewisX and the oligosaccharide moiety increases in inflammatory states, inhibiting the sLeX/E-Selectin interaction that is fundamental for adhesion of leukocytes to sites of inflammation (Jorgensen et al., 1998). These properties may favour the tick and support our finding that levels of the protein are higher in susceptible animals. Moreover, in accordance with our data, the study that examined the APR in *T. annulata*-infected zebuine and taurine cattle also found that baseline levels of α-1AGP were significantly higher in taurine animals; however in accordance with the role of α-1AGP as a positive APP, infection with the protozoan parasite induced an increase of this APP in taurines. At present we have no explanation for the decrease in the positive APP, α-1AGP, during tick infestations and can only speculate the following: skin γδ T cells have an important role in tissue repair through local secretion of growth factors including insulin-like growth factor-1 (IGF-1; Jameson and Havran, 2007). Levels of IGF-1 increase in tick-infested skin when compared to normal skin and they are significantly higher in tick-infested skin of resistant cattle when compared to susceptible cattle (de Miranda Santos, unpublished results). IGF-1 is a selective chemotactic factor for basophils (Hartnell et al., 2004), which are involved in resistance to ticks and, of relevance to the present results, it also decreases serum levels of Hp and α-1AGP in animals with burned skin when compared with controls (Jeschke et al., 2000).

3.4. The levels of transferrin decrease during tick infestations

Since hemoglobin is the major nutrient for ticks, the fourth component of the APR to be studied was transferrin (Tf), a negative APP (Beard, 2001) and a regulator of levels of iron. Baseline levels were significantly higher ($P < 0.001$, Student's t test) in susceptible than in resistant bovines (Fig. 1d). After animals were exposed to heavily infested pastures, levels decreased in both breeds, but relative to baseline levels, this decrease was significant ($P < 0.001$, Student's t test) only in susceptible animals. When infestations declined, levels of Tf increased significantly ($P < 0.014$, Student's t test) in susceptible animals. It is noteworthy that at the beginning of the experiment all measurements of haematological parameters related to red blood cells and iron (haematocrit, red blood cell counts and haemoglobin) were significantly higher in resistant bovines, regardless of the level of infestations (haematocrits: $26.083\% \pm 3.942$ for Holsteins and $35.091\% \pm 2.548$ for Nelores, $P < 0.001$, Student's t test; red blood cell counts: $4.8 \times 10^6/\mu l \pm 0.8$ for Holsteins and $5.8 \times 10^6/\mu l \pm 0.3$ for Nelores, $P < 0.002$, Mann–Whitney rank sum test; haemoglobin: $8.53\ g\% \pm 2.05$ for Holsteins and $13.66\ g\% \pm 0.73$ for Nelores, $P < 0.001$, Student's t test). The difference observed between basal levels of Tf in resistant and susceptible animals may reflect the fact that more free iron is available in the former. Tf affects the immune response by regulating the amount of iron available to cells of the immune system. Since IL-6 and IL-1, which induce APRs, also induce nitric oxide (NO), which in turn modulates iron homeostasis, and because Tf induces inducible nitric oxide syntase (Takenaka et al., 1995) we measured this mediator in the sera of the tick-infested bovines. During exposure to ticks, systemic serum levels of NO were higher in resistant than in susceptible animals, however, a significant difference was found only in animals undergoing a low intensity of exposure to ticks and no significant correlations between levels of NO and Tf were found (data not shown).

4. Conclusions

This is the first study that shows that tick infestations induce production of distinct APPs and that levels vary according to the genetic composition of the bovine host. In humans, genetic factors affect the levels at which APPs are produced (Ajiro et al., 2006; Langlois and Delanghe, 1996; Dente et al., 1987). Our results indicate that the same seems to be the case for bovines. Our results also suggest that genetically resistant bovines control ticks at a cost. Even though they harbour few feeding ticks, they gain less weight when they are exposed to the parasites (Jonsson, 2006). This loss in weight gain cannot be explained solely by the blood lost to feeding ticks, since the amount is negligible. Even in susceptible breeds, ticks account for only one quarter of the total loss in weight gain (Jonsson, 2006). Indeed, the APR induces production of the appetite suppressant, leptin (Matarese et al., 2005) and suppression of appetite is a relevant cause of lost production in cattle during tick infestations (Jonsson, 2006). One component of the cement cone induces strong local cutaneous reactions in immunized guinea pig hosts subsequently challenged with *R. appendiculatus* (Trimnell et al., 2005), the guinea pig being considered a host resistant to several species of ticks. APPs, therefore, could be useful markers to screen which tick candidate antigens can elicit potentially detrimental APRs before they undergo more extensive evaluation in vaccines. APPs could also be used to monitor the level of tick infestations that are tolerable for bovine hosts in order to determine the level of efficacy that an anti-tick vaccine must achieve.

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