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Microarray analysis of tick-infested skin in resistant and susceptible cattle confirms the role of inflammatory pathways in immune activation and larval rejection

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ABSTRACT

Tick bites promote activation of an inflammatory process that is influenced by bovine genetic composition and its history of previous exposure. Taurine and indicine breeds are known to differ on its immune response development against *Rhipicephalus microplus*. Nevertheless, further investigation about the complex molecular pathways involved in the development of immune response to tick infestation in cattle presenting the same genetic background is mandatory. The aim of this work was to access the early immune response triggered by *R. microplus* larvae attachment in previously selected resistant and susceptible animals in a bovine F2 population derived from Gyr (*Bos indicus*) × Holstein (*Bos taurus*) crosses. Microarray data analysis of RNA samples from tick infested skin was used to evaluate the gene expression at 0, 24 and 48 h after *R. microplus* larvae attachment. Our experimental design allowed us to deeply explore the immune response related to *R. microplus* infestation avoiding the innate differences between these breeds. The differentially expressed genes found reveal networks and pathways that suggest a key role of lipid metabolism in inflammation control and impairment of tick infestation in resistant animals. Acute phase response also seems to be impaired in susceptible animals. These results provide new insights about early immune response against ticks and raise the possibility of using immunomodulation processes to improve and develop novel tools for tick control.

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1. Introduction

Rhipicephalus Boophilus microplus is a blood-feeding parasite that acts as vector of virus, bacteria and protozoa to bovine cattle and, occasionally, to other non-specific hosts (Assadian and Stanek, 2002; Jones et al., 1992; Willadsen and Jongejan, 1999). This parasite is reported to be a highly adapted tick species which developed many strategies to counteract environmental conditions and its host immune response (de la Fuente et al., 2007; Francischetti et al., 2009; Kunz and Kemp, 1994; Ribeiro, 1989; Tonnesen et al., 2004; Wharton, 1967; Wikel, 1999). *R. microplus* infestation promotes great economic losses in bovine production due to the propagation of many diseases and additional costs for its control (Leger et al., 2013). Increase of acaricide resistance, which are widely used for tick control, leads to an arisen of endemic areas and contamination of food and pasture representing a great danger for animals, public health and environmental pollution (Kunz and Kemp, 1994). Immune correlates about tick–host interface have provided information to the development of sustainable tools for *R. microplus* control, but gaps still need to be filled in order to improve and develop new immunomodulation methods for tick control (de la Fuente et al., 2007; Parizi et al., 2012; Willadsen, 1999, 2006).

R. microplus bites promote activation of an inflammatory process which is influenced by bovine genetic composition, animal sex and history of prior exposure to ticks (Allen, 1994; de Castro and Newson, 1993; Jonsson et al., 2000; Kongsuwan et al., 2008; Pollock et al., 2012). It is well established that tick resistance is heritable and *Bos indicus* breeds are more resistant to *R. microplus* infestations than *Bos taurus* (Wambura et al., 1998). Additionally, variable levels of tick resistance are reported among individuals of the same host breed (Doube and Wharton, 1980; Ibelli et al., 2012; Wambura et al., 1998). Complex traits, such as tick resistance, are under control of many genes whose products function together in cellular processes or pathways that contribute to development of immune response which culminate in tick infestation control (Carvalho et al., 2010a, 2011; Francis and Ashton, 1967; Hewetson, 1972; Kongsuwan et al., 2008; Machado et al., 2010; Martinez et al., 2006; Piper et al., 2009; Rodriguez-Valle et al., 2013; Wambura et al., 1998). The recent sequencing of the bovine genome (Bovine Genome et al., 2009) has significantly facilitated the analysis of genetic divergence between cattle breeds although little is known about gene expression variation between subspecies (Bovine HapMap et al., 2009; Huang et al., 2012; Villa-Angulo et al., 2009). A recent report has demonstrated that taurine and indicine breeds display specific gene expression patterns whose regulation exists at multiple levels and is strictly related to the bovine subspecies (Huang et al., 2012). Therefore, the aim of this study was to investigate the bovine skin gene expression in response to *R. microplus* larvae attachment in F2 animals carrying the same genetic background (Gyr × Holstein) but contrasting phenotype of tick infestation. For that, we used microarray analysis that allowed in silico deconstruction of immune response development at the first 48 h after tick infestation pointing to possible molecules and key processes involved in modulation of immune response in susceptible animals bringing new targets to help unrevealing the mechanisms involved in tick–host interaction.

2. Materials and methods

2.1. Experimental animals

Bovine animals were selected from an F2 outbreed experimental population produced by crossing F1 animals (Gir × Holstein). Animals were kept at natural pasture conditions, in contact with ticks, until 10 months of age. Prior to experimental infestations with ticks, animals were bathed with acaricides to assure they were free of natural infestation on pastures. After quarantine, resistance phenotypes were determined in 376 F2 animals by individual challenge with an average of 10,000 *R. microplus* larvae. Detailed information about this population, including breeding schemes, tick phenotypical evaluation and the genetic value calculation for tick resistance and susceptibility was described by Nascimento et al. (2011). The counts of adult female ticks ranged from zero to 792 ticks per animal, showing extreme genetic variability within the F2 population. Additional traits that might interfere with tick resistance such as coat color, coat thickness, coat length and hair density were also evaluated in order to calculate the genetic values for tick resistance that was obtained throughout an equation solved by the system MTDFREML (Multiple Trait Derivative Free Restricted Maximum Likelihood) (Boldman et al., 1995; Nascimento et al., 2011). Based on results of individual genetic values for tick infestation phenotypes, animals with extreme breeding values for tick resistance/susceptibility were selected to generate the experimental groups that were formed by six tick-resistant and seven tick-susceptible animals.

2.2. Artificial infestation and sample collection

Pathogen free *R. microplus* larvae were obtained from adult engorged females fed in healthy animals and grown in laboratory controlled conditions. An artificial infestation challenge was made with an average of 10,000 *R. microplus* larvae on pre-selected tick-resistant ($n = 6$) and tick-susceptible ($n = 7$) F2 animals. Prior to challenge all animals were treated with acaricide and kept in quarantine, free of tick exposure. Skin biopsies were taken with 8 mm punch at the feeding sites before infestation (0 h), 24 and 48 h after tick attachment in each animal.

2.3. RNA extraction and microarray assay

Skin biopsies were treated with TRIzol® (Invitrogen, Carlsbad, CA, USA) and submitted to RNA extraction with Rneasy Mini Kit (Qiagen, Valencia, CA, USA). RNA quality and purity were analyzed by 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Microarray assays were performed using Affymetrix (Santa Clara, CA, USA) kits according to manufacturer's instructions. RNA from each skin biopsies was prepared with 3′ IVT Express Kit (Affymetrix, Santa Clara, CA, USA) and hybridization were performed on GeneChip® Bovine Genome Array