

QUANTITATIVE ANALYSIS OF BOVINE CYTOKINE mRNA LEVELS IN RESPONSE TO TICK INFESTATION

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INTRODUCTION

Ticks cause economic losses to animal production in the order of billions of dollars annually (Horn, 1983). Alternatives for the control of these disease vectors are urgently needed due to the lack of efficacy and safety of the current methods that rely on chemicals (Pruett, 1999). Infestations with this parasite affect host immune responses, including several cytokines produced by T cells. The T cells, can be divided in T_H1 and T_H2. This classification is defined by the set of cytokines they secrete: T_H1 cells secrete *interleukin (IL)-1*, *IL-2*, *IL-8*, *IL-12*, interferon-gama (*IFN-γ*) and monocyte chemoattractant protein 1 (*MCP-1*), while T_H2 displays a cytokine secretion profile, including *IL-4*, *IL-5*, *IL-6*, *IL-9*, *IL-10* and *IL-13* (Cher and Mosmann, 1987; Else and Finkelman, 1998). T_H1 and T_H2 lymphocytes subsets provide immunoregulatory signal for generation of cell-mediated and antibody responses, respectively (Wikel, 1996).

IL-8 and *MCP-1* are chemokines and they act mainly like chemo-attractants for leukocytes, recruiting monocytes, neutrophils and other effector blood cells to infection sites. They are produced by monocytes, macrophages, fibroblasts and keratinocytes, and although both are produced by cells classified as T_H1, *MCP-1* has demonstrated to promote immunity T_H2 (Janeway et al., 2002). *IL-12* is secreted by macrophages as well as dendritic cells and is one of the cytokines produced early in several infections resulting in activation of natural killer cells (NK). *IL-2* is a growth factor that guide proliferation and differentiation of T cells, and is produced for the own T cell activated. In mice infested with *Rhipicephalus sanguineus* tick, *IL-2* and *IFN-γ* were found to be largely down-regulated, whereas *IL-4* and *IL-10* were up-regulated (Ferreira and Silva, 1999).

Many ticks modulate T-lymphocyte cytokine responses, resulting in a general pattern of down regulation of T_H1 and an increase in T_H2 cytokines (Schoeler and Wikel, 2001). Several methods exist that allow quantification of cytokine expression, such as real-time RT-PCR. In this sense, the present study used this methodology to quantify cytokines (*IL-2*, *IL-4*, *IL-8*, *IL-12p35* and *MCP-1*-monocyte chemoattractant protein) in two groups of Nelore calves: one infested (T) by ticks and other control (C), maintained free from infestation by ticks, in order to investigate their relationship with the immune response to *Boophilus microplus* infestations.

MATERIAL AND METHODS

Animals and tissue samples. A group (T) of five Nelore calves were submitted to artificial infestation with *Boophilus microplus* ticks at five months of age, and a control group (C) was constituted of five Nelore calves maintained free from infestation during the experiment. Biopsies from skin and lymph node were collected from all animals at 9th day post infestation.

Real-time PCR for quantification of cytokine cDNA. Real-time PCR was performed using a Light Cycler®, double-stranded DNA-binding dye Syber Green stain and the housekeeping

gene *RPL-19* for normalization. The expression of interleukin 2 (*IL-2*), interleukin 4 (*IL-4*), interleukin 8 (*IL-8*), α 35KDa subunit of interleukin 12 (*IL-12p-35*), and monocyte chemoattractant protein (*MCP-1*) were analyzed in skin and lymph node.

Statistical analyses. The comparative C_T method was utilized for relative quantification. Data were analyzed by one-way analysis of variance followed by Student's t-test. Differences between groups were considered significant if probability values of $P < 0.1$ were obtained.

RESULTS AND DISCUSSION

The real-time PCR was used to assess cytokine profiles in cells extracted from skin and lymph nodes from Nelore calves. *RPL-19*, a housekeeping gene, was used for normalization because it did not have the expression altered by the treatment in both tissues studied (Table 1). In skin (Figure 1), *MCP-1*, *IL-2*, *IL-8* and *IL-12p35* quantification were not statistically different between the groups and no *IL-4* transcription was detected in both groups. In lymph nodes (Figure 2), *IL-2* and *MCP-1* showed lower levels of mRNA ($P < 0.05$ and $P < 0.1$, respectively) in treated group, being *IL-2* 0.5-fold and *MCP-1* 0.3-fold less abundant in treated group in relation to control group. It is interesting to notice that *IL-4*, although not statistically significant ($P < 0.15$), was 3-fold more abundant in treated group. No statistical differences were observed for *IL-12p35* and *IL-8* quantification from lymph nodes. Recent investigation has revealed that cytokines play a critical role in the prevention or progression of diseases. In this sense cytokine analysis is critical to research on disease resistance and on development of vaccine against several pathogens. Several previous reports showed that ticks modulate T-lymphocyte cytokine responses, resulting in a general pattern of down regulation of T_H1 and an increase in T_H2 cytokines (Ferreira and Silva, 1999; Schoeler *et al.*, 2000; Mejri *et al.*, 2001; Schoeler and Wikel, 2001). Kovář *et al.* (2002a,b) in a study about the impact of *Ixodes ricinus* tick salivary gland extract (SGE) on proliferation of mouse and human lymphocyte, found an impaired T-lymphocyte proliferation and a clear modulation of the immune response towards the T_H2 pattern. They found that (SGE) increased the levels of *IL-4* and reduced the levels of *IL-2*, a principal autocrine growth factor of T_H1 lymphocytes, at both the protein and mRNA levels, similar to other data obtained in experiments with mouse cells (Ferreira and Silva 1999; Schoeler *et al.* 1999, 2000). In the early stages of infection, levels of *IL-4* and *IL-12* determine whether the cellular (T_H1) or humoral (T_H2) immune response will be activated (Wang *et al.*, 1999). The results obtained in the present work mostly indicate an inhibition of the T_H1 pattern, with the reduction of *MCP-1* and *IL-2* and a possible enhancement of T_H2 through increase in *IL-4* mRNA levels.

Table 1. Analysis of RPL-19 control gene. (S): Skin, (LN): Lymph node, (C): Control group, (T): Treated group. EP: Standard Error, CV: Variation coefficient.

Tissue	Groups	Mean \pm EP	N	CV(%)	P-value
S	C	19,33 \pm 0,47	5	5,74	0,97
	T	19,35 \pm 0,47	5	5,04	
LN	C	22,07 \pm 0,60	4	5,47	0,95
	T	22,01 \pm 0,53	5	5,25	

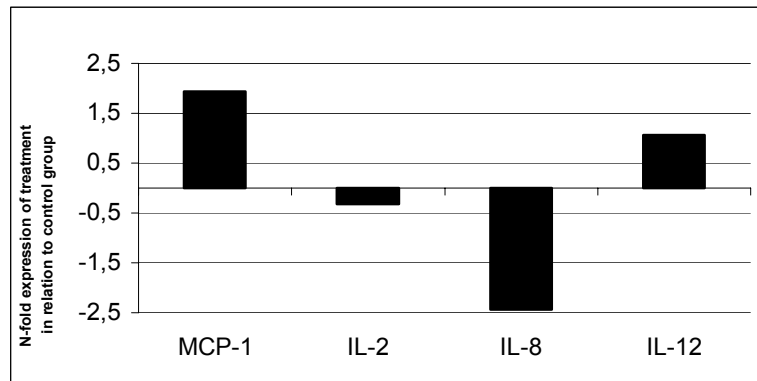


Figure 1. Ratio of cytokine expression between treatment and control group in skin, normalized for a reference gene (*RPL-19*). Bars represent the means of cytokine mRNA abundance, expressed as the *n*-fold differences between groups (C and T) in relation to the calibrator (control group).

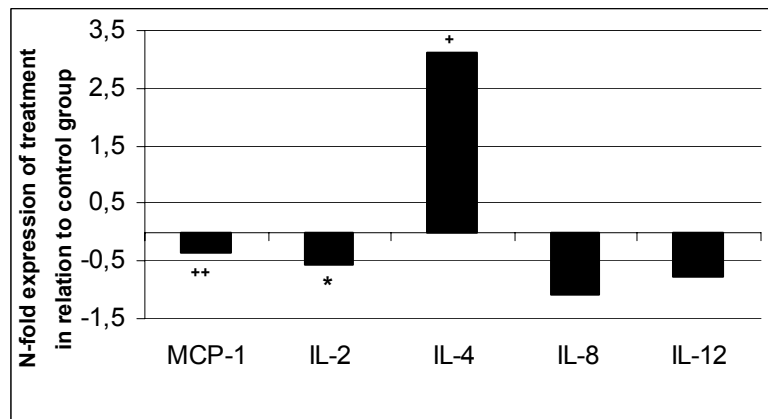


Figure 2. Ratio of cytokine expression between treatment and control (T/C) group in lymph node, normalized for a reference gene (*RPL-19*). Bars represent the means of cytokine mRNA abundance, expressed as the *n*-fold differences between groups (C and T) in relation to the calibrator (control group). *: $P < 0.05$; **: $P < 0.10$; +: $P < 0.15$

CONCLUSION

The results showed that cytokines produced by T_H1 response cells (*IL-2* and *MCP-1*) had their expression down-regulated in lymph nodes from animals submitted to artificial infestation with *Boophilus*, while *IL-4*, a cytokine from T_H2 response cells, although not statistically significant, was up-regulated in lymph nodes from treated animals, demonstrating a possible tendency for T_H2 response. Additional studies are being accomplished to better understand the immune response of bovine host to ticks, the immune-regulatory cytokines and their potential use for diagnosis, prophylaxis and immune therapy.

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