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Immunological profile of resistance and susceptibility in naturally infected dogs by *Leishmania infantum*

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ABSTRACT

Visceral leishmaniasis has a great impact on public health, and dogs are considered the main domestic reservoir of *Leishmania infantum*, the causal parasite. In this study, 159 animals naturally infected by *L. infantum* from an endemic area of Brazil were evaluated through an analysis of cellular responses, using flow cytometry, and of the hematological parameters. The results confirmed that disease progression is associated with anemia and reductions in eosinophils, monocytes and lymphocytes. The investigation of the immune response, based on the immunophenotypic profile of peripheral blood, showed declines in the absolute numbers of T lymphocytes $CD5^+$ and their subsets ($CD4^+$ and $CD8^+$) and a drop of B lymphocytes in asymptomatic seropositive (AD-II) and symptomatic seropositive (SD) dogs. Neutrophils, when stimulated with soluble antigen of *L. infantum*, showed higher synthesis of interferon (IFN)-γ^+ in AD-II and SD groups, with decreased production of interleukin

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(IL)-4$^+$ in asymptomatic seronegative dogs positive for *L. infantum* infection based on polymerase chain reaction testing (AD-I group). In the AD-II and SD groups, subpopulations of stimulated lymphocytes (CD4$^+$ and CD8$^+$) also exhibited greater synthesis of IFN-γ$^+$ and IL-4$^+$ in culture. These results suggest that the animals of the AD-II and SD groups exhibited a mixed immune response (Type 1 and 2) and the AD-I group presenting an immune profile very similar to normal control animals.

1. Introduction

Visceral leishmaniasis (VL) caused by the protozoan *Leishmania* (*Leishmania*) *infantum*, is one of the most important zoonotic diseases affecting dogs and humans in South and Central America, the Mediterranean basin and parts of Asia (World Health Organization, 2010). Dogs (*Canis familiaris*) are the most important reservoir of the parasite in urban areas, especially those that have a high parasite burden in the skin and a high prevalence in this environment (Giunchetti et al., 2006; Coura-Vital et al., 2011b). It has often been observed that an increase in canine visceral leishmaniasis (CVL) cases precedes a rise in human cases (Fraga et al., 2012; Grimaldi et al., 2012).

CVL may evolve from a nonapparent infection to a severe and systemic disease, which usually culminates in death. Asymptomatic dogs can recover or develop clinical symptomatic disease, or they may remain infected for years, even lifelong, without clinical manifestation (Reis et al., 2009). Recently it has been shown that a high percentage of asymptomatic infected dogs are PCR positive but seronegative (Coura-Vital et al., 2011b). These animals, although their infection status is not detected by conventional serology, are more likely to seroconvert (Coura-Vital et al., 2013). They also apparently have a different type of immune response that seems to be related to resistance and is characterized by high proportions of CD4$^+$ T lymphocytes and CD21$^+$ B cells and high expression of IFN-γ (Reis et al., 2006b; Coura-Vital et al., 2011a; Menezes-Souza et al., 2011).

The components of innate and adaptive immunity engage in a range of interactions that is remarkably diverse and complex (Reis et al., 2009, 2010). The course of CVL is interconnected with the host immune response and the persistence and proliferation of the parasites throughout the skin and visceral organs. The innate immune response has a relevant role in protecting against the parasite in addition to switching on the adaptive response that can control the *Leishmania* infection without the development of a specific adaptive immunity (Moreno and Alvar, 2002). Studies indicate that the successful resolution of *Leishmania* infections depends on the ability of the host to mount a specific T-cell response, with the activation of macrophages mediated by cytokines derived from T cells (Carrillo and Moreno, 2009). Symptomatic dogs that develop severe disease already exhibit clear suppression of specific types of cell-mediated immunity, particularly CD8$^+$ T lymphocytes (Pinelli et al., 1994). Resistance to infection is associated with a Type 1 response, with a predominance of IL-12, IFN-γ, IL-2 and tumor necrosis factor (TNF)-α, which will increase the efficiency of phagocytic cells and cytotoxic lymphocytes, triggering a protective immune response. On the other hand, susceptibility to infection is associated with a Type 2 response, with predominance of IL-4, IL-5, IL-10, IL-13 and TGF-β (Pinelli et al., 1995, 1999a; Correa et al., 2007; Lage et al., 2007; Menezes-Souza et al., 2011).

In a previously reported study, our group showed that asymptomatic dogs (seronegative/PCR+ [AD-I] and seropositive [AD-II]) appear to have a dichotomous infection spectrum that influences the humoral and cellular immunological status in CVL (Coura-Vital et al., 2011a). The aim of the present study was to investigate immunological events in naturally infected dogs based on the dichotomy between asymptomatic groups (AD-I and AD-II) and to identify biomarkers associated with resistance and susceptibility to infection by *L. infantum*.

2. Materials and methods

2.1. Experimental design

The present study included 159 mongrel dogs of both sexes (81 male and 78 female) from an endemic area of Brazil. The mean age was 49.8 months (SD 37.8), and the median was 42 months (IQR 24; 66). The samples were collected from domestic dogs at the Zoonoses Control Centre of Belo Horizonte. Serological tests (immunofluorescence antibody test [IFAT] and ELISA) were performed following the manufacturer's instructions. Dogs with an IFAT titer <1/40 were considered seronegative, and dogs with IFAT titer ≥1/40 were considered seropositive and infected with *Leishmania* spp. The serological tests were performed in the Laboratory of Zoonosis at the Belo Horizonte Health Department. Because of the high prevalence of seronegative/PCR+ dogs in the endemic area (Coura-Vital et al., 2011b), the seronegative dogs were subjected to molecular testing. Molecular testing (PCR) was performed in buffy coat samples with primers from a conserved region of the *Leishmania* kDNA minicircle (P150–152) (Passos et al., 1999). A single PCR product of 120 bp was generated (Degrave et al., 1994). The reaction mixture was performed as described by Coura-Vital et al. (2011b), and the species of *Leishmania* was determined by RFLP-PCR (Volpini et al., 2004). In this study, the dogs were not submitted to tests against other canine vector-borne diseases.