Veterinary Parasitology 209 (2015) 157–163

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Histopathological findings and detection of toll-like receptor 2 in cutaneous lesions of canine leishmaniosis

L.O. Esteve[a,b,c], S.V. Saz[a], S. Hosein[d], L. Solano-Gallego[a,c,d,*]

[a] Department of Medicine and Animal Surgery, Veterinary School, Autonomous University of Barcelona, Bellaterra, Barcelona 08193, Spain
[b] Dermatological Service, Hospital Ars Veterinaria, c/Cardedeu, 3, Barcelona 08023, Spain
[c] Service of Veterinary Pathology, Veterinary School, Autonomous University of Barcelona, Bellaterra, Barcelona 08193, Spain
[d] Department of Pathology and Pathogen Biology, Royal Veterinary College, University of London, Hawkshead Lane, North Mymms, Hertfordshire AL9 7TA, UK

ARTICLE INFO

Article history:
Received 12 August 2014
Received in revised form 9 March 2015
Accepted 10 March 2015

Keywords:
Canine leishmaniosis
Leishmania infantum
Papular dermatitis
Granuloma
TLR2

ABSTRACT

A broad spectrum of clinical manifestations ranging from a chronic subclinical infection to a non-self-limiting illness has been described for canine leishmaniosis (CanL). This clinical variation is determined by a variable immune response, presumably genetically determined, against the infection. Although different types of adaptive immune response in dogs with CanL have been investigated in several studies, the mechanisms that underlie and determine this variability are still poorly understood. It is currently thought that innate immune response, and particularly the role of specific mediators of the innate immune system, such as toll-like receptors (TLRs), plays a central role in this polarization. However, there is limited data available concerning the role that TLRs play in canine *Leishmania infantum* infection.

The objective of this descriptive study was to characterize and compare the inflammatory pattern, the *Leishmania* burden and expression of TLR2 in skin lesions derived from dogs with different clinical stages of leishmaniosis and cutaneous lesions.

Routine histology, *Leishmania* and TLR2 immunohistochemistry assays were performed in 11 patients with papular dermatitis (stage I – mild disease) and 10 patients with other cutaneous lesions (stage II–III – moderate to severe disease).

A significantly higher frequency of granuloma formation was demonstrated in skin samples of dogs with stage I when compared with dogs of stage II–III. Although not statistically significant, a trend for a lower parasite burden was observed for skin lesions of dogs with stage I when compared with dogs of stage II–III. A lower expression of TLR2 in skin biopsies from dogs with stage I was statistically significant compared with stage II–III. The results obtained in this study indicated an association with TLR2 in the pathogenesis of canine cutaneous leishmaniosis. Further studies are required to fully elucidate these findings.

1. Introduction

A broad range of clinical manifestations and immune responses have been described for canine leishmaniosis (CanL). In fact, *Leishmania infantum* infection in the dog can manifest as a chronic subclinical infection, self-limiting disease, or non-self-limiting illness (Baneth et al., 2008;

* Corresponding author. Department of Medicine and Animal Surgery, Veterinary School, Autonomous University of Barcelona, 08193 Bellaterra, Barcelona, Spain. Tel.: +34 5868533.
E-mail address: laia.solano@uab.cat (L. Solano-Gallego).

http://dx.doi.org/10.1016/j.vetpar.2015.03.004
0304-4017/

Solano-Gallego et al., 2009). Therefore, a clinical staging system of CanL based on serological status, clinical signs, laboratory findings, and type of therapy and prognosis for each clinical stage has been proposed (Solano-Gallego et al., 2009). This clinical staging system ranges from stage I or mild disease to stage IV or very severe disease with different clinical outcomes, prognosis and treatment options. The two extremes of this clinical spectrum are characterized by: (1) "Resistant" dogs with a protective $CD4^+$ T-cell-mediated immune response characterized by production of Th1 cytokines such as IFN-γ, IL-2 and TNF-α, which induce anti-*Leishmania* activity by apoptosis of parasites in macrophages via nitric oxide metabolism and, thus capable of controlling infection, and (2) severally sick dogs which are characterized by a marked humoral immune response, reduced cell mediated immunity with a mixed Th1 and Th2 cytokine (TGF-β and IL-10) pattern and high parasite burden, which is detrimental to the animal (Baneth et al., 2008).

Cutaneous clinical signs are the most common manifestation of CanL and skin lesions are very pleomorphic from a clinical and histopathological point of view (Ordeix and Fondati, 2013). The causes of this clinico-pathological variability remain uncertain. However, as described for human leishmaniosis, it appears that the diversity of the immune responses against *L. infantum*, probably genetically determined, is the most important factor deciding the pathogenic mechanisms and the type of lesions observed (McCall et al., 2013). In fact, based on the recent clinical classification of CanL, a benign form characterized by a papular dermatitis is the only permissible dermatologic manifestation in stage I leishmaniosis, and therefore associated with a favourable prognosis (Solano-Gallego et al., 2009). Often dogs with papular dermatitis show no other clinico-pathological findings and the level of anti-*Leishmania* antibodies are negative or weakly positive. This dermatological problem is associated with a good specific immune cell-mediated response as well as the spontaneous resolution of the lesions within 3–5 months in some cases (Ordeix et al., 2005; Bottero et al., 2006; Lombardo et al., 2014).

In human and experimental murine leishmaniosis, caused by a variety of *Leishmania* species, the type of the inflammatory infiltrate in tissues reflects the profile of the host immune response to the parasite. This is considered as morphopathological marker of Th1- or Th2-type immune responses to *Leishmania* infection: resistance *versus* susceptibility to the disease (Lemos de Souza et al., 2000). Hence, the inflammatory infiltrate in lesions of resistant subjects is characterized by a mixture of lymphocytes, plasma cells, and epithelioid macrophages with granuloma formation and few parasites (Lemos de Souza et al., 2000). On the other hand, in susceptible individuals, the inflammatory infiltrate is composed almost exclusively of non-activated macrophages containing large cytoplasmic vacuoles burdened with parasites, and granulomas are not formed (Lemos de Souza et al., 2000). This aspect has not been fully investigated in canines.

It is important to highlight that although the adaptive immune response in dogs with a Th1- or Th2-type immune responses has been investigated in several studies, the mechanisms that underlie and determine these polarized responses in CanL are still poorly understood (Baneth et al., 2008). Interestingly, there is growing evidence that it is during the initial establishment of infection, when *Leishmania* species actively regulate adaptive T cell responses by confronting the innate immune system (Sacks and Sher, 2002). However, these findings arise from experimental infections in murine models with different *Leishmania* species and immune responses, and information regarding canine *L. infantum* infection is lacking.

Toll-like receptors (TLRs), which are transmembrane receptors found on the surface of cells of the innate immune system such as macrophages, mast cells and dendritic cells (Kawai and Akira, 2011), are one of the pattern recognition receptors that are activated when invading microbes confront the innate immune system. TLRs activation rapidly triggers a variety of anti-microbial immune responses like phagocytosis, maturation and microbicidal activity of phagosomes, induction of various inflammatory cytokines and development of pathogen-specific, long-lasting adaptive immunity through B and T lymphocytes (Kumar et al., 2011). Some studies confirm the importance of TLRs in the onset of leishmanial pathogenesis, susceptibility, and resistance in mice and human disease models (Tuon et al., 2008). While most reports on canine TLRs have been focused on chronic enteropathies, pyometra and osteoarthritis (McMahon et al., 2010; Chotimanukul and Sirivaidyapong, 2012), to date, TLRs have not been studied in canine *L. infantum* infection in detail (Amorim et al., 2011; Figueiredo et al., 2013). It is in this context that the characterization of TLRs needs to be clarified and further explored in CanL.

The hypothesis for this study is that dogs with papular dermatitis (mild disease, stage I) present a specific histopathological pattern in the lesional skin suggestive of a protective immune response. Moreover, these dogs with mild disease (stage I) express different levels and/or types of TLRs allowing them to better control *Leishmania* infection when compared with dogs with more severe disease. Therefore, the objective of this descriptive study was to characterize and compare the inflammatory pattern, the parasite burden and expression of TLR2 in skin lesions derived from naturally *L. infantum* infected dogs with different stages of diseases.

2. Materials and methods

2.1. Study population

Skin biopsy specimens from 21 dogs with CanL diagnosed by positive specific *Leishmania* immunohistochemistry reaction in cutaneous lesions were included in the study.

Eleven patients with mild disease (stage I) characterized by papular dermatitis with negative or low antibody levels, and 10 patients with moderate or severe disease (stage II–III) (Solano-Gallego et al., 2009) with any type of cutaneous lesions other than papular dermatitis and high antibody levels were enrolled.