

CD4⁺ T cells participate in the nephropathy of canine visceral leishmaniasis

F.A.L. Costa^{1,2}, J.L. Guerra²,
S.M.M.S. Silva¹, R.P. Klein¹,
I.L. Mendonça¹
and H. Goto³

¹Departamento de Clínica e Cirurgia Veterinária, Centro de Ciências Agrárias, Universidade Federal do Piauí, Teresina, PI, Brasil

²Departamento de Patologia, Faculdade de Medicina Veterinária e Zootecnia, and

³Departamento de Medicina Preventiva, Instituto de Medicina Tropical de São Paulo, Universidade de São Paulo, São Paulo, SP, Brasil

Abstract

Renal involvement in visceral leishmaniasis (VL) is very frequent. The renal lesions of humans and dogs are similar but their pathogenesis has not been clearly elucidated. There is growing evidence that the cellular immune response is involved in the pathogenesis of immunologically mediated glomerulonephritis. Since T cells could participate in the pathogenesis of nephropathy, in the present study we investigated the possible involvement of CD4⁺ and CD8⁺ T cells in the nephropathy of canine VL. Six dogs naturally infected with *Leishmania (Leishmania) chagasi* from the endemic area in the Northeast of Brazil, the town of Teresina in the State of Piauí, were studied. An expressive inflammatory infiltrate of CD4⁺ T cells both in glomeruli and in interstitium was present in 4 animals and absent in 2. CD8⁺ T cells were detected only in one animal. CD4⁺ T cells alone were observed in 3 animals; when CD8⁺ T cells were present CD4⁺ T cells were also present. CD4⁺ T cells were observed in cases of focal segmental glomerulosclerosis, diffuse membranoproliferative glomerulonephritis, diffuse mesangial proliferative glomerulonephritis and crescentic glomerulonephritis. CD8⁺ T cells were present only in a case of crescentic glomerulonephritis. *Leishmania* antigen was detected in glomeruli and in interstitial inflammatory infiltrate in 4 animals and immunoglobulins were observed in 4 dogs. In this study we observed that T cells, in addition to immunoglobulins, are present in the renal lesion of canine VL. Further studies are in progress addressing the immunopathogenic mechanisms involving the participation of immunoglobulins and T cells in canine VL nephropathy.

Key words

- Visceral leishmaniasis
- Dog
- *Leishmania (Leishmania) chagasi*
- Nephropathy
- Immunoglobulin
- T cells

Correspondence

H. Goto
Laboratório de Soroepidemiologia e
Imunobiologia Celular e Molecular
IMTSP, USP
Av. Dr. Enéas C. Aguiar, 470
05403-000 São Paulo, SP
Brasil
Fax: +55-11-852-3622
E-mail: hgoto@usp.br

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In Brazil visceral leishmaniasis (VL) is caused by *Leishmania (Leishmania) chagasi* (1) and the dog is the most important reservoir of this parasite. Northeastern Brazil is an endemic area for VL. About 1600 dogs naturally infected with *Leishmania (L.) chagasi* are estimated to be present in

Teresina, State of Piauí. The dogs present lesions that are similar to those seen in human disease (2,3). The disease causes alterations in several organs and renal involvement is very frequent both in humans and in dogs (4-9). However, the pathogenesis of this nephropathy has not been clearly eluci-

dated. Until recently, the pathogenesis of renal lesion in both human and canine VL was mainly attributed to immune complex deposition as a probable mechanism of glomerular injury (10-12), but the scarcity of the antigen and the type of cell infiltrate are not fully compatible with this hypothesis. On the other hand, growing evidence that the cellular immune response is involved in the pathogenesis of some types of immunologically mediated glomerulonephritis has been recently obtained (13). The presence of T cells has been detected in cases of renal disease in humans and in experimental models (14-16). Since the nephropathy of canine VL has not been fully elucidated and T cells may participate in its pathogenesis, in the present study we investigated the possible involvement of CD4⁺ and CD8⁺ T cells in the nephropathy of dogs.

Six naturally infected dogs from the town of Teresina with positive sorology for VL and positive culture of *Leishmania* from bone marrow, spleen and/or popliteal lymph nodes and 2 non-infected controls were studied. Formalin-fixed and paraffin-embedded kidney samples were processed for histopathological and immunohistochemical studies. CD4⁺ and CD8⁺ T cells were detected using mouse monoclonal anti-canine CD4⁺ (VMRD, Pullman, WA, USA) and CD8⁺ (VMRD) antibodies diluted 1:500 in 0.01 M phosphate-buffered saline (PBS), pH 7.2, respectively, and a sensitive immunohistochemistry-catalyzed signal amplification (CSA) system (Dako Corporation, Carpinteria, CA, USA).

Of 6 naturally infected animals only 1 did not show any alteration. In 5 animals glomerular, interstitial and tubular changes were observed. Two control animals did not show any significant renal histopathological changes, and no CD4⁺ or CD8⁺ cells were observed. An inflammatory infiltrate of CD4⁺ T cells both in glomeruli (Figure 1A) and in interstitium was expressive in 4 infected animals and absent in 2. CD8⁺ T cells were only

detected in one animal both in glomeruli (Figure 1B) and in the interstitial infiltrate. CD4⁺ T cells alone were observed in 3 animals and when CD8⁺ T cells were present CD4⁺ T cells were also present. CD4⁺ T cells were observed in cases of focal segmental glomerulosclerosis, diffuse membranoproliferative glomerulonephritis, diffuse mesangial proliferative glomerulonephritis and crescentic glomerulonephritis. CD8⁺ T cells were present only in a case of crescentic glomerulonephritis. Of 2 cases in which CD4⁺ and CD8⁺ T cells were not observed, one did not present any renal lesion and the other presented chronic glomerulonephritis. To confirm that the renal changes were actually related to leishmaniasis, the presence of *Leishmania* antigen was determined using mouse polyclonal anti-*Leishmania (L.) amazonensis* antibody produced in our laboratory, diluted 1:1600 in 0.01 M PBS, pH 7.2, and the CSA system. *Leishmania* antigen was observed in the glomeruli (Figure 1C) and in the interstitial inflammatory infiltrate in 5 infected animals. The antigen was observed as characteristic diffuse dark brown peroxidase staining almost exclusively in phagocytic cells of glomeruli and in the interstitial mononuclear cell infiltrate, but whole parasites were not detected in any case. In 2 control animals *Leishmania* antigen was not detected in renal tissue.

Since in other studies on VL immunoglobulins have been always detected in renal lesions we also searched for the presence of IgG in our samples. IgG deposits were detected by streptavidin-peroxidase techniques in 4 dogs using commercially available goat monoclonal anti-dog IgG (Bethil Laboratories, Montgomery, TX, USA) antibody at the concentration of 10 µg/ml. Granular IgG deposits especially along the capillary walls (Figure 1D) were observed in 4 animals studied. Immunoglobulin deposits have also been observed in other studies on renal tissue of dogs with VL (8,17).

In the present study we used naturally

infected dogs as a model to study VL. Even though the parasite inoculation site has not been fully elucidated, we assumed that the disease of these naturally infected dogs in a way resembles more closely what occurs during the infection in man, with a spectrum of various organ specific lesion. In addition, the dog model has a great advantage in comparison to the well-known hamster model (18,19) since numerous reagents for cell markers and known parameters for an extensive immunological and pathological evaluation are available for this species.

The detection of CD4⁺ T cells both in glomeruli and in interstitium suggests the participation of these cells in the pathogenesis of the nephropathy in canine VL. Since CD8⁺ T cells were detected in only one case their participation in the pathogenesis of glomerulonephritis in canine VL is uncertain. These findings are similar to the requirement for CD4⁺ but not CD8⁺ T cells that has been shown in experimental crescentic glomerulonephritis in CD4⁻ and CD8⁻ mice (16). The predominance of CD4⁺ T cells over CD8⁺ T cells has also been ob-

served in different forms of glomerulonephritis (20), suggesting that activation of these cells leading to delayed-type hypersensitivity, cytolytic reactions, abnormal expression of major histocompatibility complex molecules, or B cell activation can result in renal injury (13).

To the best of our knowledge, this is the first report on the presence of T cells in kidney in cases of VL. Recent studies have shown the presence of T cells in glomeruli, especially in cases of crescentic glomerulonephritis (16), membranoproliferative glomerulonephritis (15), and anti-glomerular basement membrane glomerulonephritis in rats (14). In the present study we detected the presence of T cells in several patterns of glomerular lesion but not in chronic glomerulonephritis.

The detection of *Leishmania* antigen in glomeruli and in renal interstitium in the animals with infection was also an important finding since we used naturally infected dogs. In these animals the *Leishmania* infection was ascertained by parasitological and serological tests but other subclinical infections

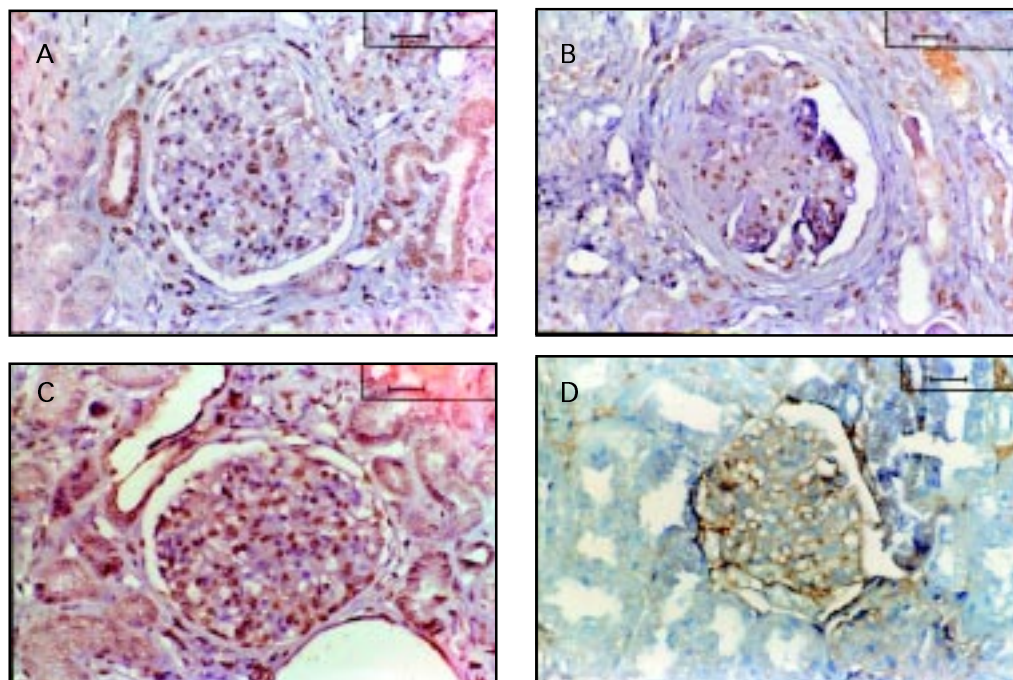


Figure 1 - Detection of T cells, *Leishmania* antigen and immunoglobulin in glomerular lesions in canine visceral leishmaniasis by immunoperoxidase staining. A, Presence of CD4⁺ T cells of severe intensity in focal segmental glomerulosclerosis. Bar: 25 µm. B, Presence of CD8⁺ T cells of moderate intensity in crescentic glomerulonephritis. Bar: 25 µm. C, Presence of *Leishmania* antigen of moderately severe intensity in focal segmental glomerulosclerosis. Bar: 25 µm. D, Presence of an immunoglobulin G deposit of moderate intensity in diffuse mesangial proliferative glomerulonephritis. Bar: 25 µm.

or diseases could be present inducing a T cell inflammatory infiltrate in the kidney. However, the fact that the control non-infected dogs from the same endemic area did not present any lesion and the presence of *Leishmania* antigen in the studied animals strongly indicate that the presence of T cells is related to the presence of *Leishmania* infection. Furthermore, in the single case in which *Leishmania* antigen was not detected T cells were not present.

We observed that T cells besides immunoglobulins are present in the renal lesion of canine VL. Further studies are in progress

addressing the immunopathogenic mechanisms involving the participation of immunoglobulins and T cells in canine VL nephropathy.

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