Brazilian Journal of Medical and Biological Research (2001) 34: 539-543
ISSN 0100-879X Short Communication

Detection of immunoglobulin G in the lung and liver of hamsters with visceral leishmaniasis

R. Mathias[1], F.A.L. Costa[1,3] and H. Goto[1,2]

[1]Laboratório de Soroepidemiologia e Imunobiologia Celular e Molecular, Instituto de Medicina Tropical de São Paulo, and [2]Departamento de Medicina Preventiva, Faculdade de Medicina, Universidade de São Paulo, São Paulo, SP, Brasil
[3]Departamento de Clínica e Cirurgia Veterinária, Universidade Federal do Piauí, Teresina, PI, Brasil

Abstract

Several organs are affected in visceral leishmaniasis, not only those rich in mononuclear phagocytes. Hypergammaglobulinemia occurs during visceral leishmaniasis; anti-*Leishmania* antibodies are not primarily important for protection but might be involved in the pathogenesis of tissue lesions. The glomerulonephritis occurring in visceral leishmaniasis has been attributed to immune complex deposition but in other organs the mechanism has not been studied. In the current study we demonstrated the presence of IgG in the lung and liver of hamsters with visceral leishmaniasis. Hamsters were injected intraperitoneally with 2 x 10^7 amastigotes of *Leishmania* (*Leishmania*) *chagasi* and the presence of IgG in the liver and lung was evaluated at 7, 15, 30, 45, 80 and 102 days postinfection (PI) by immunohistochemistry. The parasite burden in the spleen and liver increased progressively during infection. We observed a deposit of IgG from day 7 PI that increased progressively until it reached highest intensity around 30 and 45 days PI, declining at later times. The IgG deposits outlined the sinusoids. In the lung a deposit of IgG was observed in the capillary walls that was moderate at day 7 PI, but the intensity increased remarkably at day 30 PI and declined at later times of infection. No significant C3 deposits were observed in the lung or in the liver. We conclude that IgG may participate in the pathogenesis of the inflammatory process of the lung and liver occurring in experimental visceral leishmaniasis and we discuss an alternative mechanism other than immune complex deposition.

Correspondence
H. Goto
Laboratório de Soroepidemiologia e Imunobiologia Celular e Molecular
IMTSP, USP
Av. Enéas C. Aguiar, 470
Prédio II, 4º andar
05403-000 São Paulo, SP
Brasil
Fax: +55-11-3062-3622
E-mail: hgoto@usp.br

Research supported by FAPESP (Nos. 98/15414-3 and 98/12092-5), CNPq (No. 300866/98-4 to H. Goto) and LIM/38 (HC-FMUSP).

Received April 12, 2000
Accepted February 16, 2001

Key words
- *Leishmania* (*Leishmania*) *chagasi*
- Visceral leishmaniasis
- Immunopathology
- Immunoglobulin G
- Liver
- Lung

Leishmaniasis is a disease caused by a protozoan of the genus *Leishmania*. Visceral leishmaniasis affects mononuclear phagocytes mainly in the spleen and in the liver where hyperplasia and hypertrophy of these cells occur, but other organs such as lung and kidney are affected during progression of the disease (1). Interstitial inflammation is observed in the lung, kidney and liver (1) but its pathogenesis has not been fully elucidated. Hypergammaglobulinemia occurs in humans and in hamsters with visceral leishmaniasis due to polyclonal activation of B lymphocytes (2,3). Antibodies have no evident protective effect in leishmaniasis (4) but may be involved in the pathogenesis of the lesions in

visceral leishmaniasis. In the kidney, immune complex deposition has been claimed as the mechanism of lesion (5-7). To study the immunopathogenesis of the lesions in the liver and lung, in the current study we evaluated the presence of IgG in these organs in hamsters with visceral leishmaniasis.

Twenty-eight outbred 45-60-day-old male hamsters (*Mesocricetus auratus*) from the Animal Breeding Facility of the Medical School of the University of São Paulo were maintained in the Animal Facilities of the Institute of Tropical Medicine of São Paulo during the experiment. Seventeen hamsters were inoculated intraperitoneally with purified 2×10^7 amastigotes of *Leishmania* (*Leishmania*) *chagasi* (MHOM/BR/72/strain 46) in RPMI 1640 medium (Gibco BRL, Gaithersburg, MD, USA), and sacrificed at 7, 15, 30, 45, 80 and 102 days postinfection (PI). Eleven control animals were injected with RPMI 1640 medium and sacrificed at the same times. At the time of sacrifice, spleen, liver, kidney and lung were obtained and the presence of IgG in the liver, kidney and lung was evaluated by immunohistochemistry using biotinylated goat anti-hamster IgG antibody (20 µg/ml) (Rockland, Gilbertsville, PA, USA) in formalin-fixed and paraffin-embedded tissue sections. Complement fraction C3 was detected in cryosections of the organs using a polyclonal rabbit anti-hamster C3 antibody produced by us (8). Anti-*Leishmania* antibody titer was determined by immunofluorescence using *Leishmania* (*L.*) *amazonensis* antigen. The parasite burden in the spleen and liver was calculated by counting the number of cells and parasites in each microscopic field of organ imprints up to 1000 cells or parasites. Parasite burden was calculated by the formula: (number of parasites/number of cells) x weight of the organ (mg) $\times 2 \times 10^4$.

Parasite burden in the spleen and liver and anti-*Leishmania* antibody titer in infected hamsters increased progressively during infection. Using immunohistochemistry we observed some background staining in control animals but in the liver of infected animals we clearly observed a weak deposit of IgG at day 7 PI that increased progressively until it reached highest intensity around 30 and 45 days PI, declining at later times (Table 1). IgG deposits outlined the sinusoids (Figure 1A). In the lung a deposit of IgG was observed in the capillary walls (Figure 1B) which was of moderate intensity at day 7 PI, but increased remarkably at day 30 PI and declined at later times of infection (Table 1). No significant C3 deposits were observed either in the lung or in the liver. In two preliminary experiments with 3-4 infected

Table 1 - Intensity of IgG deposits detected by immunoperoxidase staining using biotinylated goat anti-hamster IgG antibody in the liver, lung and kidney of hamsters infected with 2×10^7 amastigotes of *Leishmania* (*L.*) *chagasi* and non-infected control animals at different times of infection.

- = Negative. Intensity of positive reactions graded from + through ++++. Data are reported as the mean of 2-4 animals/group.

Days post-infection	Liver		Lung		Kidney	
	Control	Infected	Control	Infected	Control	Infected
7	-	+	+	++	-	+
15	+	++	+	+++	-	++
30	+	+++	+	++++	++	+++
45	+	+++	+	+++	+	++
80	++	++	+	+++	+	++
102	+	++	++	+++	-	+++

and control animals sacrificed at the same times, we observed similar results by immunofluorescence using polyclonal rabbit anti-hamster total immunoglobulin serum.

Since the absence of C3 deposits is not compatible with immune complex deposition in the pathogenesis of the disease and since an immune complex-mediated mechanism has been shown in the kidney in visceral leishmaniasis (5-7), as a control we examined kidney tissue from the same animals. In the kidney the deposit of IgG was observed outlining the capillary walls (Figure 1C). The intensity of the deposit was highest at 30 and at 102 days PI and slightly lower at other time points (Table 1). C3 deposits of moderate intensity were detected in the kidney throughout the experiment, with a slight increase in intensity at day 102. Thus, we conclude that the conditions for the presumed formation of an immune complex were present in the experimental animals. Furthermore, one of the factors for immune complex deposition is the incapacity to clear immune complexes due to dysfunction of the reticuloendothelial system (9), which is likely to occur in visceral leishmaniasis due to *Leishmania* parasite proliferation in mononuclear phagocytes.

The absence of C3 deposits in the liver and lung still contradicts the mechanism of immune complex deposition. However, the presence of IgG deposits in the liver and lung might indicate its role in the pathogenesis. IgG deposits in the lung have been reported in some situations such as the presence of anti-basement membrane antibody in the sep-

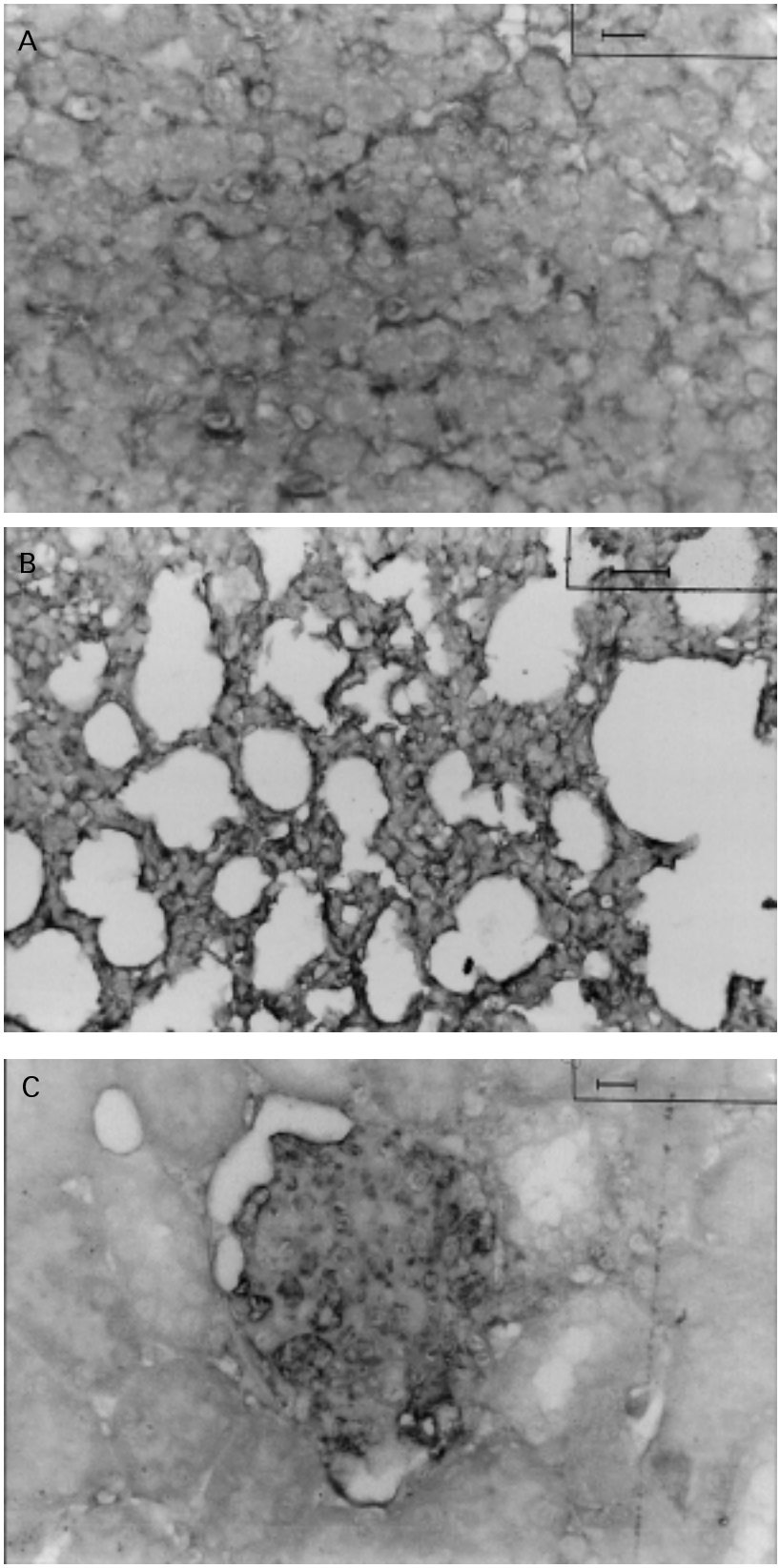

Figure 1 - Detection of IgG deposits in the liver, lung and kidney of hamsters infected with 2 x 10^7 amastigotes of *Leishmania* (*L.*) *chagasi* by immunoperoxidase staining using biotinylated goat anti-hamster IgG antibody. *A*, IgG deposits along the sinusoid are seen in the liver of infected hamsters at 45 days of infection. *B*, IgG deposits in the capillary wall of the lung septum from infected animals at 30 days of infection. *C*, IgG deposits outlining the capillary wall in the glomeruli of infected hamsters at 45 days of infection. Bar: 25 µm.

tum in Goodpasture syndrome (10) and in acute and chronic immune complex-mediated disease models (11). In the present study, we observed the presence of IgG deposits in the lung mainly in the vessel wall, in agreement with findings for rabbits with chronic serum sickness, in which interstitial pneumonitis develops (12). In addition, in visceral leishmaniasis the presence of *Leishmania* antigen has been observed in inflammatory foci in the lung (13). In immune complex-mediated diseases and in animal models of acute and chronic serum sickness, a known model of immune complex-mediated disease (11), no liver lesion has been described. Furthermore, in diseases involving an immune mechanism such as viral hepatitis and primary biliary cirrhosis, the antibodies are directed at the hepatocytes and bile ducts, respectively (14,15). Therefore, to our knowledge, this is the first observation of IgG along the sinusoids.

To correlate the lesion with the IgG deposits histopathological analysis was performed. In the liver, a progressive increase of diffuse hyperplasia and hypertrophy of Kupffer cells were observed from 7 to 80 days PI, when hyperplasia became less pronounced. Foci of mononuclear cells were observed from 7 days PI, initially in periportal and centrolobular spaces, but later more disseminated with no preference for any particular zone. In the lung, foci of septal thickening due to congestion, edema and mixed infiltrate of polymorphonuclear neutrophils and mononuclear cells were observed at 7 days PI. Inflammation increased progressively and the polymorphonuclear neutrophils were gradually replaced by mononuclear cells in the infiltrate, and at 30 days PI only mononuclear cells were practically seen. In the kidney, hypercellularity and an increase in mesangial matrix were observed at 30-45 days PI followed by a decrease at 45 days, and at 102 days PI a deposit of an amorphous eosinophilic substance was seen. The lesions in different organs were progressive, with noticeable changes in their features during evolution. In the liver, the mononuclear cell inflammatory infiltrate became more prominent than hyperplasia of Kupffer cells at later times and in the lung the cell population in the interstitial infiltrate changed during the course of infection. The changes in the characteristics of the lesion and the occurrence of more intense IgG deposits around 30-45 days PI in the lesion might suggest that participation of IgG in the pathogenesis occurs at a certain time during the evolution of the infection.

Concerning the mechanism of lesion, the presence of IgG deposits in the liver which has not been shown in immune complex-mediated diseases and the absence of C3 deposits both in the lung and in the liver led us to consider alternative mechanisms. We have some evidence that pathogenic mechanisms other than immune complex-mediated ones participate in the lesions of visceral leishmaniasis. We recently observed the presence of T cells in the kidney in canine visceral leishmaniasis (16). In addition, in systemic lupus erythematosus it has been shown that immunoglobulins might participate in the pathogenesis of glomerular lesions by a mechanism distinct from immune complex deposition (17,18). We have been working with the hypothesis that internalization of immunoglobulins by endothelial cells might have some role in the pathogenesis of visceral leishmaniasis. We observed *in vitro* internalization by endothelial cells of serum IgG from visceral leishmaniasis patients (19) and we preliminarily detected by transmission electron microscopy the presence of IgG in endothelial cells in the liver of hamsters with visceral leishmaniasis processed for immunodetection using anti-hamster immunoglobulin antibody and protein A-gold (20). If this alternative mechanism is present in visceral leishmaniasis, it might play a role in the early phase of infection when IgG deposition is more evident. This mechanism then might trigger processes leading to mi-

gration of mononuclear cells including T cells to the lesion, as observed in the nephropathy of canine visceral leishmaniasis (16). Our findings in the liver and lung are compatible with this hypothesis and studies are in progress for a better understanding of the immunopathogenesis of visceral leishmaniasis.

Acknowledgments

We thank the biologist Edite H.Y. Kanashiro for technical support and the Histopathology Laboratory of the Faculdade de Medicina da Universidade de São Paulo for the histopathological preparations.

References

1. Duarte MIS & Corbett CEP (1994). Patologia das principais doenças tropicais no Brasil. Leishmaniose visceral. In: Brasileiro Filho G, Pitella JEH, Pereira FEL, Bambirra EA & Barbosa AJA (Editors), *Bogliolo-Patologia*. 5th edn. Guanabara-Koogan, Rio de Janeiro, RJ, Brazil.
2. Bunn-Moreno MM, Madeira ED, Miller K, Menezes JA & Campos-Neto A (1985). Hypergammaglobulinemia in *Leishmania donovani* infected hamsters: possible association with a polyclonal activation of B cells and with suppression of T cell function. *Clinical and Experimental Immunology*, 59: 427-434.
3. Galvão-Castro B, Sá-Ferreira JA, Marzochi KF, Marzochi MC, Coutinho SG & Lambert PH (1984). Polyclonal B cell activation, circulating immune complexes and autoimmunity in human American visceral leishmaniasis. *Clinical and Experimental Immunology*, 56: 58-66.
4. Sacks DL, Louis JA & Wirth DF (1993). Leishmaniasis. In: Warren KS (Editor), *Immunology and Molecular Biology of Parasitic Infections.* Blackwell Scientific Publications, Boston, MA.
5. Brito T, Hoshino-Shimizu S, Amato Neto V, Duarte MIS & Penna DO (1975). Glomerular involvement in human kala-azar: a light, immunofluorescent and electron microscopic study based on kidney biopsies. *American Journal of Tropical Medicine and Hygiene*, 24: 9-18.
6. Weisinger JR, Pinto A, Velazquez GA, Bronstein I, Dessene JJ, Duque JF, Montenegro J, Tapanes F & Rousse AR (1978). Clinical and histological kidney involvement in human kala-azar. *American Journal of Tropical Medicine and Hygiene*, 27: 357-359.
7. Sartori A, Roque-Barreira MC, Coe J & Campos-Neto A (1987). Immune complex glomerulonephritis in experimental kala-azar. *Parasite Immunology*, 9: 93-103.
8. Laurenti MD, Corbett CEP, Sotto MN, Sinhorini IL & Goto H (1996). The role of complement on the acute inflammatory process in the skin and on host parasite interaction in hamsters inoculated by *Leishmania* (*Leishmania*) *chagasi. International Journal of Experimental Pathology*, 77: 15-24.
9. Lawley TJ & Frank M (1980). Immune complexes and immune complex diseases. In: Parker CW (Editor), *Clinical Immunology.* WB Saunders Company, Philadelphia.
10. Sturgill BC & Westervelt FB (1965). Immunofluorescence studies in a case of Goodpasture's syndrome. *Journal of the American Medical Association*, 194: 172-174.
11. Cochrane CG & Dixon FJ (1978). Immune complex injury. In: Samter M (Editor), *Immunological Diseases*. Little, Brown and Company, Boston.
12. Brentjens JR, O'Connell DW, Pawlowski IB, Hsu KC & Andres GA (1974). Experimental immune complex disease of the lung. *Journal of Experimental Medicine*, 140: 105-125.
13. Duarte MIS, Matta VLR, Corbett CEP, Laurenti MD, Chebabo R & Goto H (1989). Interstitial pneumonitis in human visceral leishmaniasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 83: 73-76.
14. Eddleston ALWF (1980). Immunology and the liver. In: Parker CW (Editor), *Clinical Immunology.* WB Saunders Company, Philadelphia.
15. Krams SM, De Water JV, Coppel RL, Esquivel C, Roberts J, Ansari A & Gershwin ME (1990). Analysis of hepatic T lymphocyte and immunoglobulin deposits in patients with primary biliary cirrhosis. *Hepatology*, 12: 306-313.
16. Costa FAL, Guerra JL, Silva SMMS, Klein RP, Mendonça IL & Goto H (2000). $CD4^+$ T cells participate in the nephropathy of canine visceral leishmaniasis. *Brazilian Journal of Medical and Biological Research*, 33: 1455-1458.
17. Itoh J, Nose M, Takahashi S, Ono M, Terasaki S, Kondoh E & Kyogoku M (1993). Induction of different types of glomerulonephritis by monoclonal antibodies derived from an MRL/lpr lupus mouse. *American Journal of Pathology*, 143: 1436-1443.
18. Ono M, Yamamoto T, Kyogoku M & Nose M (1995). Sequence analysis of the germline VH gene corresponding to a nephritogenic antibody in MRL/lpr lupus mice. *Clinical and Experimental Immunology*, 100: 284-290.
19. Goto H & Nose M (1997). Ingestion of hamster and visceral leishmaniasis serum IgG by endothelial cells as pathogenetic mechanism of interstitial inflammation. *XXII Congresso da Sociedade Brasileira de Imunologia*, Mangaratiba, RJ, Brazil, September 28-October 1, 1997, 11.17: 115.
20. Mathias R, Sesso A & Goto H (1999). Detection of immunoglobulins in the endothelial cells and Disse space in the liver in visceral leishmaniasis. *Acta Microscopica*, 8 (Suppl C): 421-422.