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Hepatic extracellular matrix in BALB/c mice infected with *Leishmania donovani*

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Summary. The development of the extracellular matrix (ECM) in the liver of the BALB/c mouse infected with *Leishmania donovani* was observed by histology, immunocytochemistry and electron microscopy at 1, 2, 4, 8, 14 and 20 weeks after infection. Collagen III and proteoglycan were detected in granulomas and in the portal spaces from 4 to 20 weeks after infection. Laminin was not detected in granulomas but was observed in the basement membrane of new small blood vessels in the granulation tissue around the portal spaces from 8 to 20 weeks after infection. The ECM components in the space of Disse showed no changes in distribution throughout the entire period of study. Systemic fibrosis in the hepatic lobule was not evident in the BALB/c mice. This mouse strain does not appear to be an appropriate model to study the role of ECM in chronic visceral leishmaniasis.

Keywords: extracellular matrix, liver, BALB/c mice, *Leishmania donovani,* collagen III, laminin, proteoglycan.

The extracellular matrix (ECM) is a complex mixture of collagens (fibrillar and network), adhesion proteins (e.g. laminin, fibronectin, entactin), and proteoglycans (e.g. heparan sulphate, chondroitin sulphate). ECM is associated with all cells and cell–ECM interactions have major effects upon phenotypic features such as gene regulation, cytoskeletal structure, differentiation, and other aspects of cell growth control including intracellular signalling which can be triggered in a variety of situations by the aggregation of transmembrane glycoproteins (Martin & Kleinman, 1985, Ben Ze'ev *et al*., 1988, Schuppan *et al.*, 1992; 1993; Martinez-Hernandez & Amenta, 1993a).

In the liver, the interaction between the ECM and cells is essential for normal homeostasis and for the maintenance of lobular architecture; modification of the ECM results in deranged hepatic function. The ECM content of the liver has been shown to undergo quantitative and qualitative changes in hepatic fibrosis and cirrhosis (Rojkind *et al*., 1979, Grimaud 1970, Grimaud *et al*., 1987, Biagini & Ballardini, 1989, Schuppan, 1990, Friedman & Bissell, 1990, Chojkier, 1993, Martinez-Hernandez & Amenta, 1993b).

In particular, hepatic type IV collagen and laminin levels were reported to be significantly higher in all types of liver disease, and increased with the progression of fibrosis (Tsutsumi *et al.,* 1993). Collagenopoiesis also increased in intensity during the development of chronic persistent hepatitis to hepatic cirrhosis and in hepatic fibrosis (McGee & Patrick, 1972, Hahn *et al*., 1980, Maher & McGuire, 1990, Schuppan, 1990). Viral infection and alcohol are also potent aetiological

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Table 1. Course of *L. donovani* infection in BALB/c mice. Values are means ± s.e.m.

Weeks after infection	Liver weight (g)	*L. donovani* units
1	1.09 ± 0.01	52.58 ± 4.05
2	1.13 ± 0.01	434.48 ± 16.93
4	1.38 ± 0.02	909.12 ± 52.30
8	1.88 ± 0.04	181.34 ± 37.73
14	1.75 ± 0.05	93.68 ± 10.27
20	1.75 ± 0.23	65.97 ± 13.91

Uninfected mice. Liver weight (g)

1	0.83 ± 0.03
4	0.94 ± 0.01
20	1.03 ± 0.02

factors which stimulate collagenopoiesis (Burt *et al*., 1990; Inuzuka *et al*., 1990).

In human visceral leishmaniasis focal fibrosis at the site of granulomas and a systemic form of fibrosis in the sinusoids have been reported (Bogliolo, 1956, Rodrigues da Silva & De Paola, 1958, Andrade & Andrade, 1966, Goswami, 1970, Duarte & Corbett, 1987, Aggarwal *et al*.1990). The most extreme pattern of intralobular fibrosis is called 'Roger's cirrhosis' (Rogers, 1908). In mice, the pathology of hepatic visceral leishmaniasis has been described in relation to the dynamics of granuloma formation (Gutierrez *et al.,* 1984, McElrath *et al.,* 1988), the variable course of *Leishmania donovani* infections in inbred strains of mice of different susceptibility (Bradley & Kirkley, 1977) and their pathology (Barbosa *et al.,* 1987). However, the ECM components associated with the development of murine visceral leishmaniasis have not been fully characterized. This paper describes the histological and immunocytochemical changes of hepatic ECM components following the infection of BALB/c mice with *Leishmania donovani*.

Materials and methods

Parasites and Infection

Leishmania donovani amastigotes (strain MHOM/ET/67/L82) were isolated from the spleen of a hamster. BALB/c female mice (B & K Ltd,UK) were infected, via the tail vein, with 5×10^6 amastigotes in a volume of 0.2 ml. The animals were sacrificed in groups of 5 at 1, 2, 4, 8, 14 and 20 weeks after infection. Control groups of uninfected mice were sacrificed at 1, 4 and 20 weeks. The hepatic parasite burden in each mouse was determined from the ratio of amastigotes to organ cell nuclei (500 counted/mouse) multiplied by the liver weight (mg).

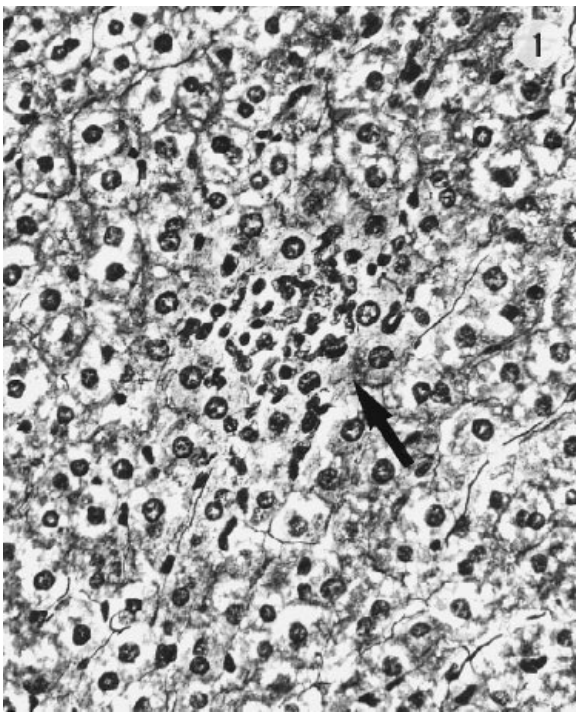
Histopathology and electron microscopy

Liver samples were fixed in a solution of 10% formal saline for 4 hours, dehydrated in ethanol and embedded in paraffin. Fixed specimens were cut and stained with haematoxylin–eosin, silver reticulum or Masson's trichrome. For electron microscopy 1 mm^3 samples were fixed in 3.0% glutaraldehyde in 0.075 M cacodylate buffer (pH 7.4) for 1 hour at 4°C. After the primary fixation, the fragments were washed in the same buffer and post-fixed in 1% OsO_4 for 2 hours at 4°C. Specimens were dehydrated in a methanol series, containing 2% uranyl acetate in the 30% methanol stage, and following intermediate stages in propylene oxide were embedded in TAAB resin (London Resin Co.,UK). One-μm sections were stained with toluidine blue and used for selecting samples for ultra-thin sectioning. Ultra-thin sections were mounted on copper grids and stained with 4% lead citrate and examined on a JEOL 100CX electron microscope at 80 kV.

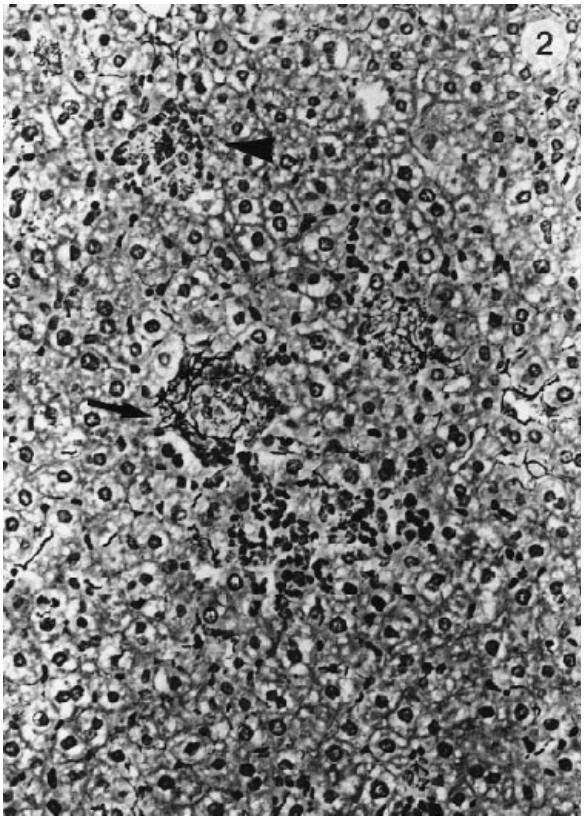
Table 2. Summary of the hepatic extracellular matrix immunohistochemistry in BALB/c mice infected with *L. donovani*

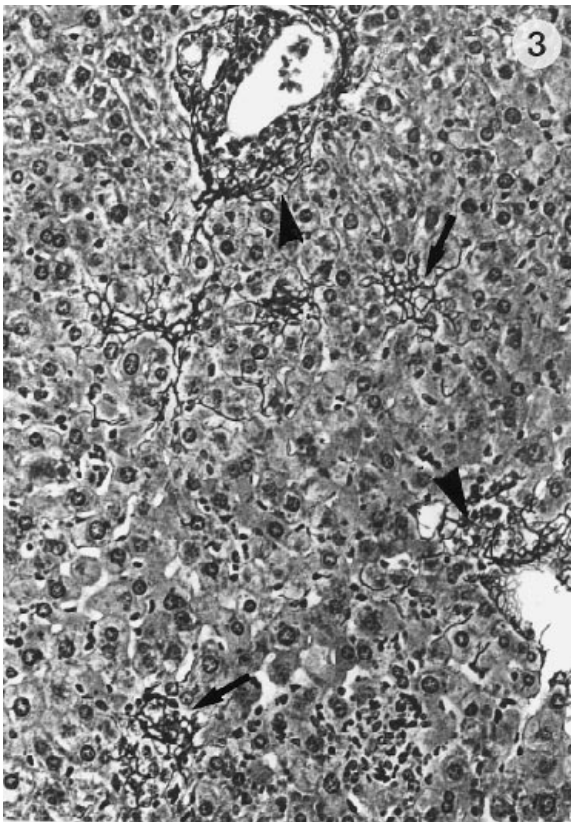
		Infected mice						Uninfected mice		
	Week	1	2	4	8	14	20	1	4	20
Collagen III	granulomas	–	–	++	++	+++	+++			
	sinusoids*	++	++	++	++	++	++	++	++	++
	portal spaces	+	+	++	++	+++	+++	+	+	+
Laminin	granulomas	–	–	–	–	–	–			
	sinusoids	+	+	+	+	+	+	+	+	+
	portal spaces	++	++	++	+++	+++	+++	++	++	++
Proteoglycan	granulomas	–	+	+	+	+	+			
	sinusoids	+	+	+	+	+	+	+	+	+
	portal spaces	+	+	+	+	+	+	+	+	+

* By immunofluorescence only.



Figures 1–3. Mouse liver infected with *Leishmania donovani (*Silver reticulum stain). **Figure 1.** One week after infection: immature granuloma (arrow) with macrophages and lymphocyte and no collagen in granuloma. **Figure 2 .** Four weeks after infection: granulomas with and without collagen (arrow and arrow head, respectively). The collagen begins to appear at the periphery of granulomas. **Figure 3.** Twenty weeks after infection: many granulomas randomly scattered throughout the lobule with the lobular architecture well preserved despite the presence of granulomas. Collagen observed in granulomas (arrow) and in portal spaces (arrow head).





Immunohistochemistry

Liver samples were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 2 hours and then washed, three times, in the same buffer containing 15% sucrose. The samples were frozen in Freon 12 and stored at –20°C or embedded in paraffin. Cryosections were mounted on glass slides and were processed using standard indirect immunofluorescence methods. Sections were incubated with rabbit antiserum anti-rat heparan sulphate proteoglycan (Dr M.G. Farquhar), rabbit antiserum anti-laminin (Sigma) or goat antiserum anti-collagen III (Southern Biotechnology Associate) at 4°C for 2 hours. After washing in PBS, sections were further incubated with anti-rabbit IgG FITC or anti-goat IgG FITC. Mouse kidney and skeletal muscle were used as positive controls for laminin and collagen III, respectively. Rat liver was used as positive control for proteoglycan. Paraffin sections were also stained using the labelled avidin-biotin technique: sections were first incubated with normal rabbit serum 1 : 10 (Dako), followed by goat antiserum anti-collagen III 1:200. Finally, sections were stained with rabbit anti-goat biotinylated IgG 1:200 (Dako) and avidin peroxidase conjugated 1 : 400 (Dako Ref. P 364) and the reactions were developed in a solution of 0.6% 3,3'-diaminobenzidine plus 0.03% H_2O_2.

Immunoelectron microscopy

Liver samples (1 mm^3) were fixed in 2% paraformaldehyde with 0.1% glutaraldehyde in PBS for 30 minutes. After washing with PBS the samples were quenched in NH_4Cl (50 mM, 30 min), and were then dehydrated in an ethanol series and embedded in LR White (London Resin Co.) at 50°C. Ultra-thin sections, mounted on nickel grids, were processed for immunogold staining. Sections were first incubated with rabbit antiserum anti-rat heparan sulphate proteoglycan, rabbit antiserum anti-laminin or goat antiserum anti-collagen III (for sources, see above) for 30 minutes at RT. Sections were then stained with antibody goat anti-rabbit IgG 5 nm gold conjugate or rabbit anti-goat IgG 20 nm gold conjugate (Bio-Cell Research Labs) for 30 minutes at RT. The silver enhancement procedure was sometimes used when working with 5-nm gold particles. In these cases the grids were then washed in water and incubated in IntenSE M silver enhancement kit (Amersham International, Inc.) at RT for 6 minutes. All sections were briefly contrasted with saturated methanolic uranyl acetate and 4% lead citrate.

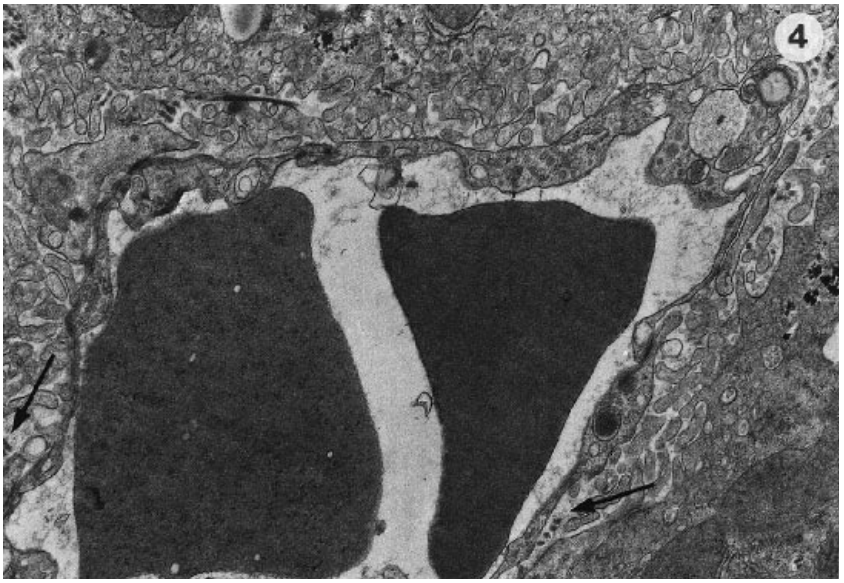
Results

Hepatomegaly was observed in mice at 1 week after infection. By 8 weeks the animals had significant hepatic enlargement which remained throughout the 20-week experimental period of infection. The parasite load in the liver reached a maximum after 4 weeks of infection. After this point the parasite load slowly declined (Table 1).

By week 1, histology of the mouse livers exhibited a small number of immature granulomas containing a few macrophages and lymphocytes (Figure 1). Over the period from week 2 to 20 after infection many granulomas were observed randomly scattered throughout the liver (Figures 2 and 3). By week 4, the granulomas contained intensely parasitized macrophages and lymphocytes. From weeks 8 to 20, the granulomas became larger as the parasite load was gradually reduced; amastigotes were sparse in sections by week 20. Kupffer cell hyperplasia was prominent throughout the infection period; by 14 and 20 weeks, the hyperplastic Kupffer cells were arranged in granulomas but rarely contained amastigotes. Lymphocytes had infiltrated the portal spaces by 1 week after infection and from the second week onwards there was a gradual rise in the number of these cells and an enlargement of the portal spaces. From 8 to 20 weeks after the infection, the portal spaces were greatly enlarged.

In granulomas, from 4 to 20 weeks after the infection, collagen was detectable by both the silver reticulum and Masson's trichrome stains (Figures 2 and 3). Collagen appeared first at the periphery and subsequently spread throughout the entire granuloma (Figures 2 and 3). The lobular architecture of the liver, as seen with haematoxylin–eosin, silver-reticulum and Masson's trichrome stains (this stain not shown), remained well preserved in infected mice, and was similar to that in control mice throughout the study despite the presence of granulomas (Figure 3). By electron microscopy collagen fibres were seen to increase in granulomas up to the 20th week of infection but no blood vessels were observed. By the

Figure 4. Control mouse liver. Electron microscopy of sinusoids showing sparse collagen fibrils (arrows) in Disse's space. × 13 200.
Figures 5–8. Electron microscopy of Disse's space in *Leishmania donovani* infection. Figures 5 and 6 (14 weeks after infection), and Figures 7 and 8 (20 weeks after infection), show only sparse collagen fibrils (arrows) or none in Disse's space (arrow heads). Figure 5. × 10 000; Figure 6. × 26 000; Figure 7. × 20 000; Figure 8. × 13 200.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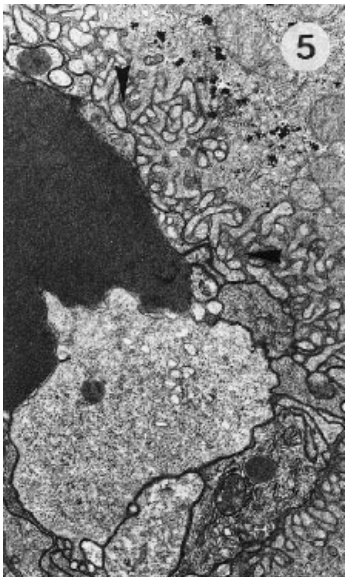
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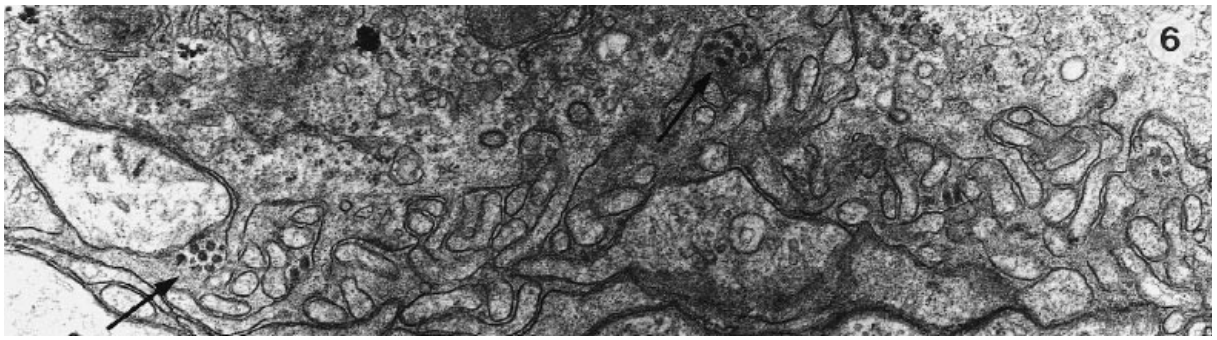
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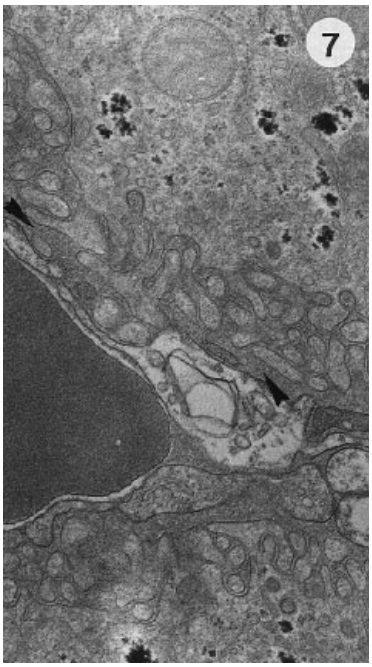
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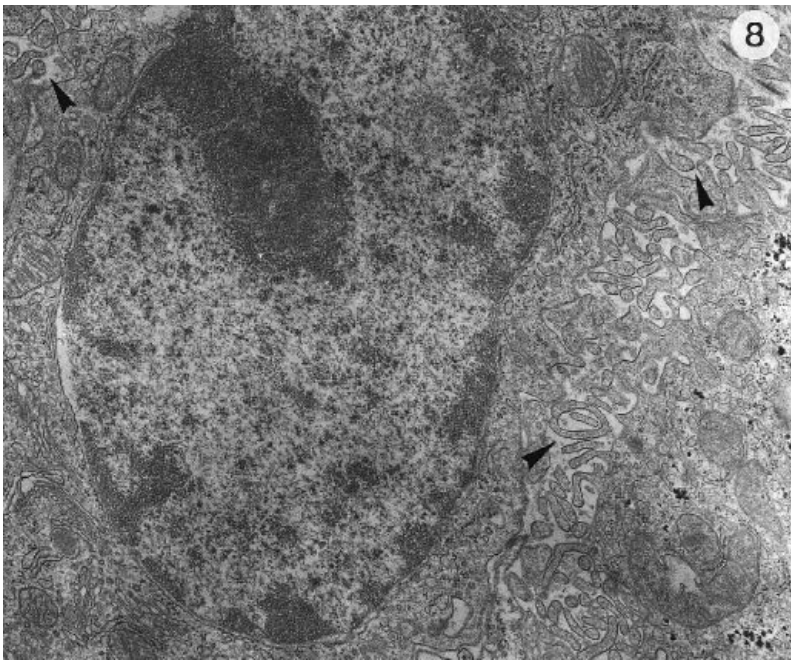
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fourth week the portal spaces contained many collagen fibrils. From 8 to 20 weeks after the infection, the portal spaces had not only inflammatory cells and collagen fibrils but also new small blood vessels. Few, or in some mice no, collagen fibrils were seen in the space of Disse at any time in the experiment (Figures 4–8).

A qualitative summary of the distribution of ECM as observed by immunohistochemistry is shown in Table 2. In the granulomas, collagen III was detected by immunofluorescence and by the avidin-biotin methods. The moderate, network-like distribution of collagen from the fourth week became gradually more intense until the twentieth week (Figures 9 and 10). In all infected mice the proteoglycan reaction was weak and laminin was not detected in the granuloma by immunofluorescence methods.

The immunofluorescence reaction for collagen III, in both infected and uninfected mice, was positive, moderate and discontinuous, in the sinusoids (Figure 9). However, by the avidin-biotin method the reaction was negative (Figure 10), possibly due to the fixation used in this method. The laminin and proteoglycan responses, by immunofluorescence, were weak in the sinusoids of infected (Figure 11) and non-infected animals.

In the connective tissue of the portal space of uninfected animals the collagen III reaction was positive by both immunofluorescence and avidin-biotin methods with a discrete network-like distribution. In the infected animals, the same reaction was also network-like, at first moderate (weeks 4 and 8), but gradually becoming more intense and extensive by weeks 14 and 20 after infection (Figures 9 and 10). In the connective tissue of the portal space the proteoglycan was positive, weak and network-like in control and infected groups throughout the experiment; the laminin reaction was positive in the basement membrane of the blood vessels and biliary ducts and was also positive in the basement membrane of new small blood vessels after 8 weeks of infection (Figure 11).

The distribution of ECM components in the space of Disse were examined by immunoelectron microscopy. Low levels of laminin and proteoglycan (with and without silver enhancement) and collagen III were detected in Disse's spaces (Figures 12 and 13) throughout the experiment. Skeletal muscle (endomysium) and tubular basement membrane were used as positive controls for collagen III and laminin, respectively (Figures 14 and 15) and rat liver for proteoglycan.

Discussion

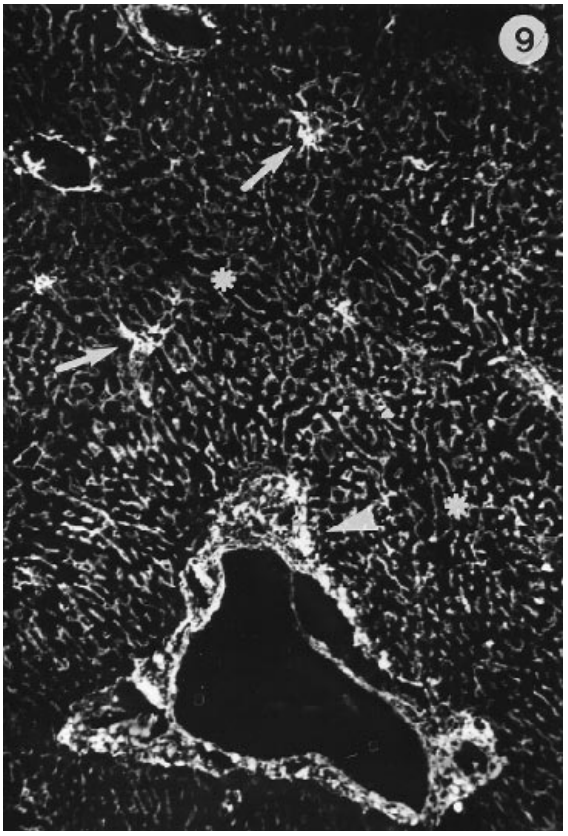
The BALB/c mouse strain has been used extensively in studies on visceral leishmaniasis in relation to immunology (Reiner, 1982), vaccination (Jaffe *et al.*1990), pathology (Reiner, 1982, Gutierrez *et al.*1984, McElrath *et al.*, 1988) and chemotherapy (Baumann *et al.*, 1991). In our study the hepatic pathology in BALB/c mice infected with *L. donovani* was similar to that previously described by Bradley and Kirkley (1977), Gutierrez *et al. (*1984) and McElrath *et al.* (1988). However, the aim of our study was to relate these changes to the distribution of components of the ECM in the infected mouse liver using an immunocytochemical approach. In the control mice livers, the distributions of collagen III, laminin and proteoglycan were similar to those described by Baranov *et al.* (1990) in BALB/c mice.

In infected mice the granulomas were found to include collagen III and proteoglycan. The absence of laminin in granulomas together with the lack of blood vessels in comparison to the positive reaction for laminin in the portal space indicates that in granulomas the stimulus for neovascularization is inhibited, as suggested by Lenzi *et al.* (1988).

The three ECM components studied were detected in the sinusoids by immunofluorescence, and in Disse's spaces by immunogold. There were no changes in distribution throughout the entire examination period in control or infected groups. In BALB/c mice systemic fibrosis in the hepatic lobule was non-existent. In contrast, in the C57BL/10 susceptible strain of mice, which is used less frequently than BALB/c in experimental leishmaniasis, changes in the liver granulomas but not in the sinusoids were detected using silver reticulum and Masson's trichrome stains (Barbosa *et al.*, 1987). Our light microscopy findings were similar to those of Barbosa *et al.* (1987) and further supported by immunocytochemistry and electron microscopy results.

In contrast, studies on the liver of *L. donovani* infected golden hamsters (*Mesocricetus auratus*) described early

Figure 9. Immunofluorescence for collagen III at 20 weeks after infection. Intense positive reaction is present in granulomas (arrow) and in portal spaces (arrow head); in the sinusoids (asterisk), the positive reaction is moderate and discontinuous. **Figure 10.** Labelling avidin-biotin for collagen III in 20 weeks after infection: intense positive reaction in granulomas (arrow) and in portal spaces (arrows head) whereas in the sinusoids the reaction was negative. **Figure 11.** Immunofluorescence stain for laminin at 14 weeks after infection. Moderate, positive reaction seen in the basement membrane of structures of portal spaces, blood vessels and biliary ducts (arrow) and in new small blood vessels (arrow head). In the sinusoids, the reaction is weak.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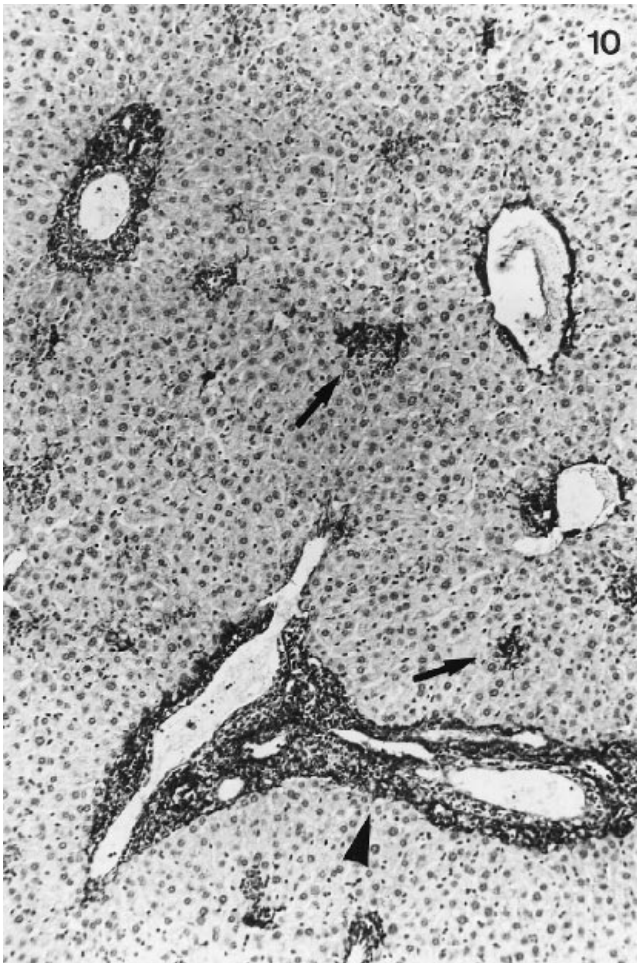
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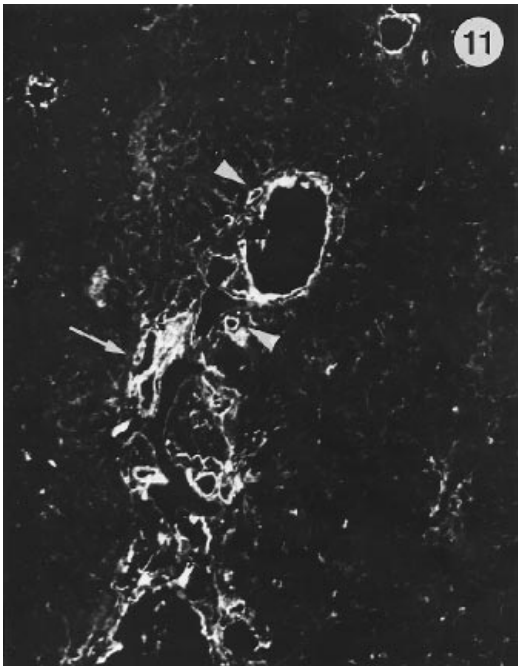
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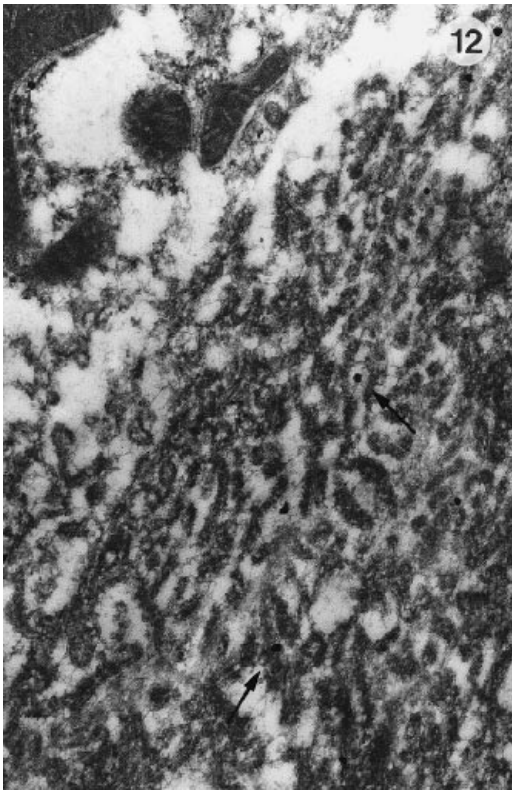
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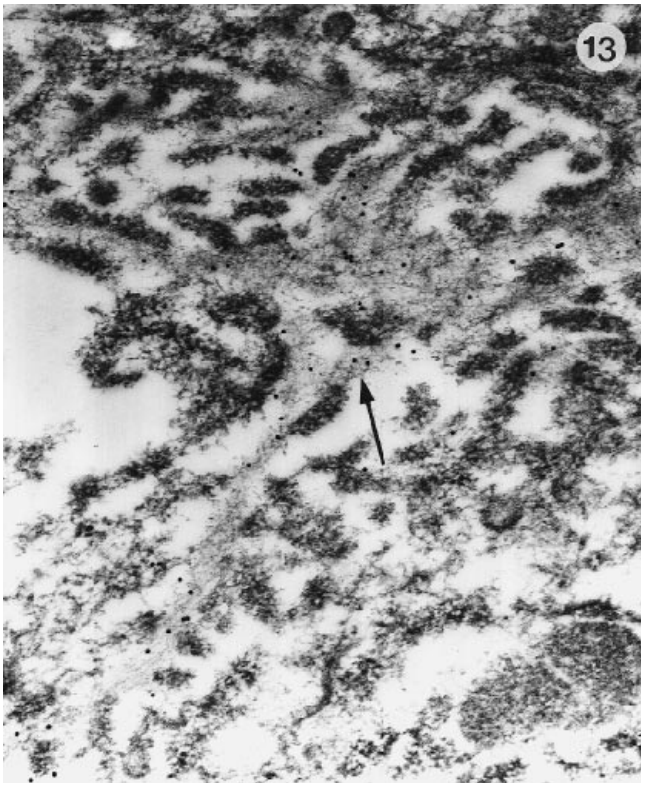
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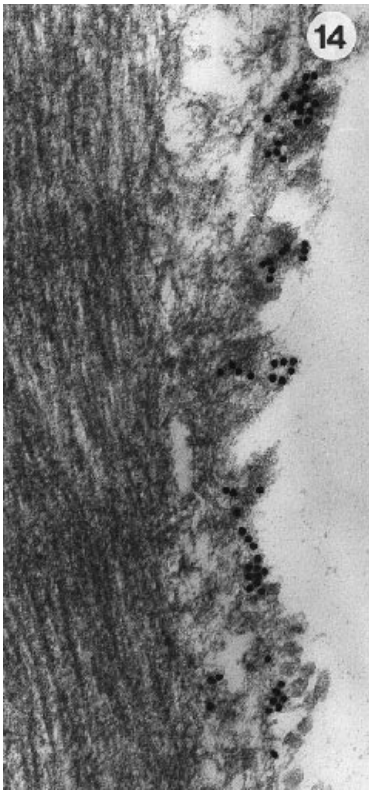
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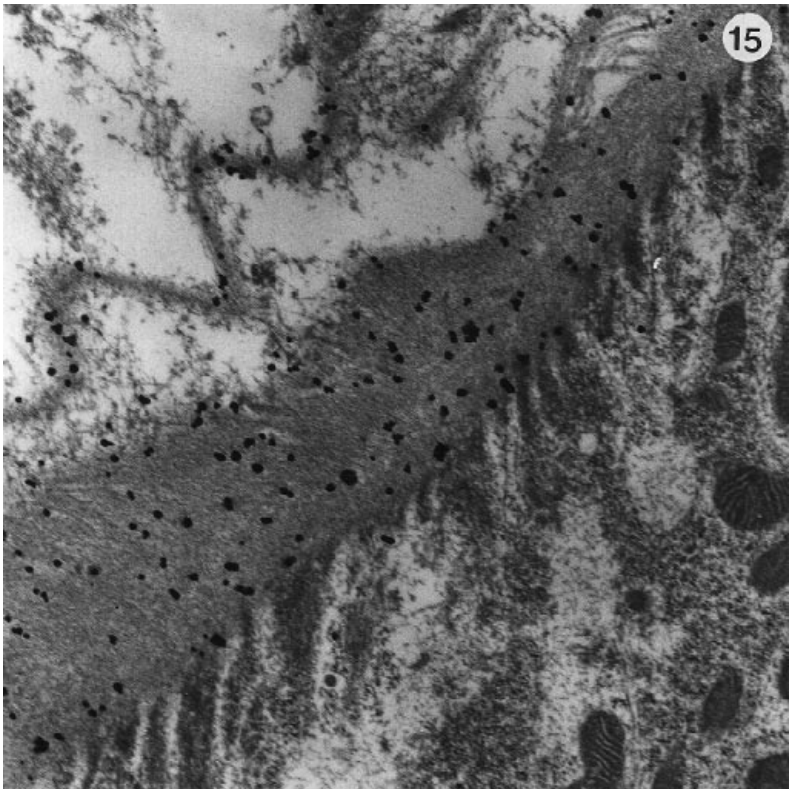
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histopathological changes characterized by hyperplasia and hypertrophy of the Kupffer cells with discrete parasitism (Wilson *et al.*, 1987, Duarte *et al.*, 1988). In the late stages of infection there was was also an increase in hyperplasia and hypertrophy and parasitism similar to the lesions observed in human infections by Duarte and Corbett (1987) and Corbett *et al.* (1993).

In conclusion, the non-fatal, slow cure BALB/c mouse model for visceral leishmaniasis did not show systemic fibrosis in the hepatic lobule and is an inappropriate model to study Disse's space fibrosis and the role of the ECM in chronic visceral leishmaniasis.

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References

Aggarwal J., Wali J.P. & Chopra P. (1990) Liver in Kala-azar. *Indian J. Gastroenterol.* **9,** 135–136.

Andrade Z.A. & Andrade S.G. (1966) Alguns novos aspectos da patologia do calazar (estudo morfológico de 13 casos necropsiados. *Rev. Inst. Med. Trop. São Paulo* **8,** 259–266.

Barbosa Jr A. A., Andrade Z. A. & Reed S. G. (1987) The pathology of experimental visceral leishmaniasis in resistant and susceptible lines of inbred mice. *Braz. J. Med. Biol. Res.* **20,** 63–72.

Baranov V., Rescan P.Y., Rissel M., Grimaud J.A. & Guillouzo A. (1990) Ultrastructural localization of the major components of the extracellular matrix in normal mouse liver. *J. Submicrosc. Cytol. Pathol.* **22,** 497–506.

Baumann R.J., Mccann P.P. & Bitonti A.J. (1991) Suppression of *Leishmania donovani* by oral administration of a bis (benzyl)-polyamine analog. *Antimicrob. Agents Chemother.* **35,** 1403–1407.

Ben Ze'ev A., Robinson G.S., Bucher N.L.R. & Framer S.R. (1988) Cell-celland cell–matrix interactions differentially regulate the expression of hepatic and cytoskeletal genes in primary cultures of rat hepatocyte. *Proc. Nat. Acad. Sci. USA* **85,** 2161–2165.

Biagini G. & Ballardini G. (1989) Liver fibrosis and extracellular matrix. *J. Hepatol.* **8,** 1124–1125.

Bogliolo L. (1956) Nova contribuição ao conhecimento da anatomia patologica da leishmaniose visceral. A proposito de um caso brasileiro e com especial referencia a fibrose hepatica. *Hospital (Rio de J.)* **1,** 393–440.

Bradley D.J. & Kirkley J. (1977) Regulation of *leishmania* populations within the host. I The variable course of *Leishmania donovani* infection in mice. *Clin. Exp. Immunol.* **30,** 119–129.

Burt A.D., Griffiths M.R., Schuppan D., Voss B. & Macsween R.N.M. (1990) Ultrastructural localization of extracellular matrix proteins in liver biopsies using ultracryomicrotomy and immunogold labelling. *Histopathology* **17,** 521–527.

Chojkier M. (1993) Pathogenesis of hepatic fibrosis. In *Extracellular Matrix: Chemistry, Biology, and Pathobiology with Emphasis on the Liver* . Eds M.A. Zern & L.M. Reid. New York: Marcel Dekker, pp. 541–557.

Corbett C.E.P., Duarte M.I.S. & Bustamante S.E. (1993) Regression of diffuse intralobular liver fibrosis associated with visceral leishmaniasis. *Am J. Trop. Med. Hyg.* **49,** 616–624.

Duarte M.I.S. & Corbett C.E.P. (1987) Histopathological patterns of the liver involvement in visceral leishmaniasis. *Rev. Inst. Med. Med. Trop. São Paulo* **29,** 131–136.

Duarte M.I.S., Laurenti M.D., Andrade Jr H.F. & Corbett C.E.P. (1988) Comparative study of the biological behaviour in hamster of two isolates of leishmania characterized, respectively, as *L. major*-like and *L. donovani. Rev. Inst. Med. Trop. São Paulo* **30,** 21–27.

Friedman S.L. & Bissell D.M. (1990) Hepatic fibrosis: New insights into pathogenesis. *Hosp. Pract.* **25,** 43–50.

Goswami B.M. (1970) A study of cirrhosis of the liver due to chronic Kala- azar. *J. Indian Med. Assoc.* **54,** 315–320.

Grimaud J.A. (1970) Cell matrix interactions in schistosomal portal fibrosis: a dynamic event. *Mem. Inst. Oswaldo Cruz Rio de J.* 82 (Suppl. 4), 55–65.

Grimaud J.A., Druguet M. & Hartmann D. (1987) Quando a fibrose hepatica se torna doença. *Rev. Soc. Bras. Med. Trop.* **20,** 189–192.

Gutierrez Y., Maksem J.A. & Reiner N.E. (1984) Pathologic changes in murine leishmaniasis (*Leishmania donovani)* with special reference to the dynamics of granuloma formation in the liver. *Am.J. Pathol.* **114,** 222–230.

Hahn E., Wick G., Pencev D. & Timpl R. (1980) Distribution of basement membrane proteins in normal and fibrotic human liver: collagen type IV, laminin and fibronectin. *Gut* **21,** 63–71.

Inuzuka S., Ueno T., Torimura T., Sata M., Abe H. & Tanikawa K. (1990) Immunohistochemistry of the hepatic extracellular matrix in acute viral hepatitis. *Hepatology* **12,** 249–256.

Jaffe C.L., Rachamin N. & Sarfstein R. (1990) Characterization of two proteins from *Leishmania donovani* and their use for vaccination against visceral leishmaniasis. *J. Immunol.* **144,** 699–706.

Figures 12 and **13.** Mouse liver 20 weeks after infection with *Leishmania donovani.* Figure 12. Immunogold product reaction for laminin is present, in small amount (arrow), in Disse's space using 5 nm gold particles and silver enhancement. × 26 000. Figure 13. Immunogold product reaction for collagen III is present, in small amount (arrow), in Disse's space using 20 nm gold particles. × 40 000. Figure 14. Immunoelectron microscopy. Skeletal muscle with positive reaction for collagen III in endomysium control. 20 nm gold particles. × 52 000. Figure 15. Immunoelectron microscopy. Laminin detected in tubular basement membrane control. 5 nm gold particles and silver enhancement. × 52 000.

LENZI H.L., ARARIPE J.R. & KERR I.B. (1988) Intrinsic vascularization of granuloma in experimental paracoccidioidomycosis. *Braz. J. Med. Biol. Res.* **21,** 945–947.

MAHER J.J. & MCGUIRE R.F. (1990) Extracellular matrix gene expression increases preferentially in rat lipocytes and sinusoidal endothelial cells during hepatic fibrosis *in vivo*. *J. Clin. Invest.* **86,** 1641–1648.

MARTINEZ-HERNANDEZ A. & AMENTA P.S. (1993a) The hepatic extracellular matrix. I. Components and distribution in normal liver. *Virchow's Arch. A Pathol. Anat. Histopathol.* **423,** 1–11.

MARTINEZ-HERNANDEZ A. & AMENTA P.S. (1993b) The hepatic extracellular matrix II. Ontogenesis, regeneration and cirhosis. *Virchow's Arch. A Pathol. Anat. Histopathol.* **423,** 77–841.

MARTIN G.R. & KLEINMAN H.K. (1985) The extracellular matrix components in development and disease. *Semin. Liver Dis.* **5,** 147–156.

MCELRATH M.J., MURRAY H.W. & COHN A.Z. (1988) The dynamics of granuloma formation in experimental visceral leishmaniasis. *J. Exp. Med.* 167, 1927–1937.

MCGEE JO'D. & PATRICK R.S. (1972) The role of perisinusoidal cells in hepatic fibrogenesis: an electron microscopic study of acute carbon tetrachloride liver injury. *Lab. Invest.* **26,** 429–440.

REINER N.E. (1982) Host-parasite relationship in murine leishmaniasis: pathophysiological and immunological changes. *Infect. Immun.* **38,** 1223–1230.

RODRIGUES DA SILVA J. & DE PAOLA D. (1958) O problema das fibroses hepáticas na leishmaniose visceral americana. *Rev. Assoc. Med. Bras.* **4,** 8–21.

ROGERS L. (1908) A peculiar intralobular cirrhosis of the liver produced by protozoal parasite of kala-azar. *Ann. Trop. Med. Parasitol.* **2,** 147–152.

ROJKIND M., GIAMBRONE M.A. & BIEMPICA L. (1979) Collagen types in normal and cirrhotic liver. *Gastroenterology* **76,** 710–715.

SCHUPPAN D. (1990) Structure of the extracellular matrix in normal and fibrotic liver: collagens and glycoproteins. *Semin. Liver Dis.* **10,** 1–10.

SCHUPPAN D., SOMASUNDARAM R. & JUST M. (1992) The extracellular matrix: a major signal transduction network. In *Cellular and Molecular Aspects of Cirrhosis*. Eds. B. Clement & A. Guillouzo. INSERM/J Libbey, pp. 115–134.

SCHUPPAN D., HERBST H. & MILANI S. (1993) Matrix, matrix synthesis, and molecular networks in hepatic fibrosis. In *Extracellular Matrix: Chemistry, Biology, and Pathobiology with Emphasis on the Liver*. Eds M.A. Zern & L.M. Reid. New York: Marcel Dekker, pp. 201–254.

TSUTSUMI M., URASHIMA S., NAKASE K., TAKASE S. & TAKADA A. (1993) Type IV collagen and laminin contents of liver from patients with alcoholic liver disease. *Alcohol-Alcohol* **(Suppl. 1 A)**, 45–52.

WILSON M.E., INNES D.J., SOUSA A.Q. & PEARSON R.D. (1987) Early histopathology of experimental infection with *Leishmania donovani* in hamsters. *J. Parasitol.* **73,** 55–63.