
HEPATIC STEREOLOGY

OF ACUTE SCHISTOSOMIASIS MANSONI

INFECTION IN C57BL/10 AND CBA MICE

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ABSTRACT

Previous studies have shown that the outcome of the experimental schistosomiasis mansoni infection depends on the genetic background of the host. To test whether *Schistosoma mansoni*-infected mice strains present different hepatic pathological pattern during acute infection, we examined liver alterations (hepatocytes, sinusoids, and fibrosis), using stereological approach. Changes in liver organization in adult inbred females C57BL/10 and CBA mice were examined 8 weeks postinfection. Volume density of hepatocytes Vv[h], sinusoids Vv[s] and hepatic fibrosis Vv[fh] were determined by point counting (M42 system-test) and video-microscopy, using light microscopy. In both mouse strains, Vv[h] and Vv[s] were significantly ($p < 0.05$) reduced in comparison to control group. The largest values of Vv[fh] were found in the C57BL/10 strain. We demonstrate that schistosome infection markedly reduced hepatocytes (CBA mice), sinusoids (C57BL/10), whereas fibrosis was higher in C57BL/10 strain in comparison to that in CBA/ strain.

KEY WORDS: *Schistosoma mansoni*. Stereology. C57BL/10 mice. CBA mice.

INTRODUCTION

Laboratory mice are commonly used for schistosomiasis studies because of their availability, low cost, susceptibility (Farah et al., 2000) and to model the

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pathophysiological features of the human infection (Abath et al., 2006). Susceptibility to experimental infection with *Schistosoma mansoni* has been shown to markedly vary among different strains of mice (Warren & Peters, 1967; Cheever et al., 2002). The host genetic heterogeneity seems to influence schistosome fecundity (Jones et al., 1989), susceptibility to infection (Incaci et al., 2001; Machado-Silva et al., 2005; Bin Dajem et al., 2008) and pathological syndromes during chronic infection (Henderson et al., 1993). Additionally, C57BL/6J mice had significantly smaller granulomas, less fibrosis and less mortality than C3H/HeN strain (Van de Vijver et al., 2006).

Because of numerous methodological advantages, stereology has been proved to be a useful tool for quantitative studies in both biomedical (Mandarim-de-Lacerda, 2003) and clinical studies (Franzen et al., 2005; Lazzarini et al., 2005). Also, liver injury during schistosomiasis mansoni infection and metabolic disorders has been investigated by stereological approaches. Zinc-deficient mice show smaller median liver volume and median granuloma volume than well-fed diet mice (Friis et al., 1998). We have shown previously that both high-fat diet (Neves et al., 2006) and low-protein diet cause damage in liver structure (Barros et al., 2009).

The hepatic injuries among inbred schistosomiasis mansoni infected-mice on stereological level appear not to have been investigated in detail. To address the effect of infection on liver structure (hepatocytes, sinusoids and fibrosis), we used inbred female C57BL/10 and CBA mice. We demonstrate that schistosome infection markedly reduced hepatocytes (CBA mice), sinusoids (C57BL/10), whereas fibrosis was higher in C57BL/10 strain in comparison to that in CBA/ strain.

MATERIAL AND METHODS

Host-parasite model

Adult (eight week-old) female C57BL/10 (n=5) and CBA mice (n=5) were obtained from the Laboratory Animals Breeding Center (Oswaldo Cruz Foundation, Brazil). Infections with 50 cercariae of *S. mansoni* (BH strain, Brazil) were performed as described by Freire et al. (2003). Five mice from each strain were kept uninfected as controls.

Mice were housed under standard caging conditions: polypropylene cages (40 x 33 cm) with stainless steel screened covers, temperature (21±1°C), humidity (60±10%), light (12h light and dark cycle), and permitted *ad libitum* consumption of water and pellet chow (Nuvilab CR-1, Paraná, Brazil). All animal experiments were conducted in accordance with valid international guidelines for animal experimentation (Ellery, 1985).

To confirm infection, we monitored fecal egg excretion. Following the onset of egg deposition, infection status was further confirmed at the time of necropsy (week 8 postinfection), by enumeration of eggs trapped in the small intestine (Machado-Silva et al., 2005) and adult worm recovery from the portal system and mesenteric veins (Freire et al., 2003).

Tissue proceeding and stereological study

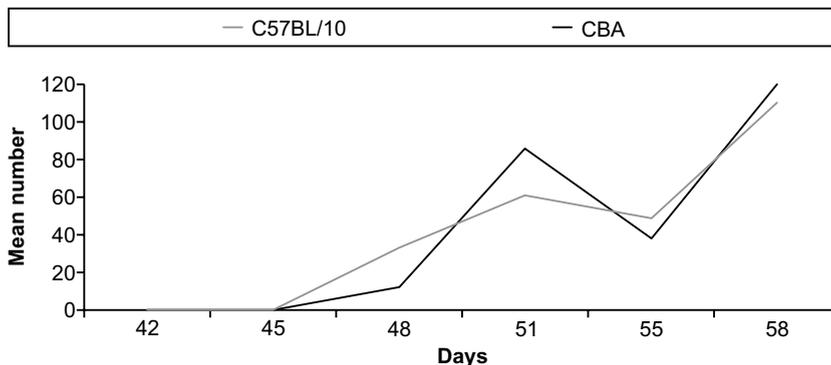
The liver was removed and liver volume was measured using immersion method (Scherle, 1970). Liver samples from infected mice were collected and fixed in Carson's modified Millonig's phosphate-buffered formalin, pH 7.4 (Carson et al., 1973). The tissue was paraffin embedded, sectioned at 3 μm thickness, and processed for Masson's trichromium staining using standard protocols (Strobel et al., 1981). Volume density of hepatocytes $Vv[h]$, sinusoids $Vv[s]$ and hepatic fibrosis $Vv[fh]$ were determined. The analysis used a video-microscopic system (Image Pro Plus, Media Cybernetics, USA) and the M42 test system (composed of 21 short lines, d , and 42 test points, PT ; the test-line length, LT is 21 d) (Gundersen, 1977; Mandarim-de-Lacerda & Pereira, 2003).

Statistical analysis

The values for each group were compared by Mann-Whitney using the Statistical Package for the Social Sciences (SPSS) version 9.0. P-values of ≤ 0.05 were considered statistically significant (Zar, 1999).

RESULTS

Although not statistically significant ($p < 0.05$), C57BL/10 yielded slightly higher worm recovery (14.8%) than CBA mice (12.4%). Microscopic examination of fecal specimens showed that both mice strains became infected (Figure 1). In this report, inbred female C57BL/10 mice showed highest intestinal egg density (4841) than CBA (3665) strain, although no statistical differences ($p > 0.05$) were found. C57BL/10 mice showed highest ($p < 0.05$) liver volume than CBA strain (Figure 2). All infected mice had fibrotic livers containing visible granulomatous lesions, composed of numerous



macrophages, eosinophilic granulocytes, lymphocytes, and fibroblasts (Figure 3).

Figure 1. Fecal egg-laying of *Schistosoma mansoni* in C57BL/10 and CBA mice.

Our stereological findings are summarized in Figures 4-6. The $V_v[h]$ was different between infected vs uninfected groups ($p<0.05$), C57BL/10 (11% vs 28%) and CBA (16% vs 31%). The $V_v[s]$ also differed between infected vs uninfected mice groups ($p<0.05$), C57BL/10 (6% vs 68%) and CBA (11% vs 69%). The $V_v[fh]$ was significantly larger ($p> 0.05$) in C57BL/10 mice (84%) than CBA mice (73%). The comparative analysis of data between mice strains showed that there were significant differences concerning $V_v[h]$ ($p<0.05$), $V_v[s]$ ($p<0.05$) and $V_v[fh]$ ($p<0.05$).

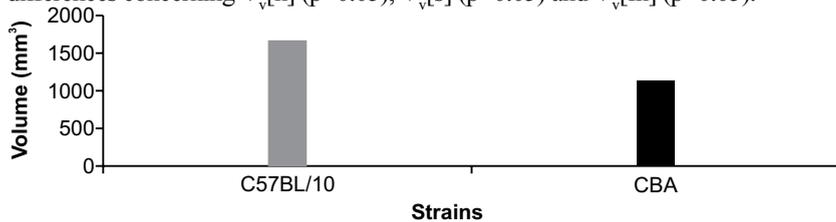


Figure 2. Livers volume of *Schistosoma mansoni*-infected C57BL/10 and CBA mice.

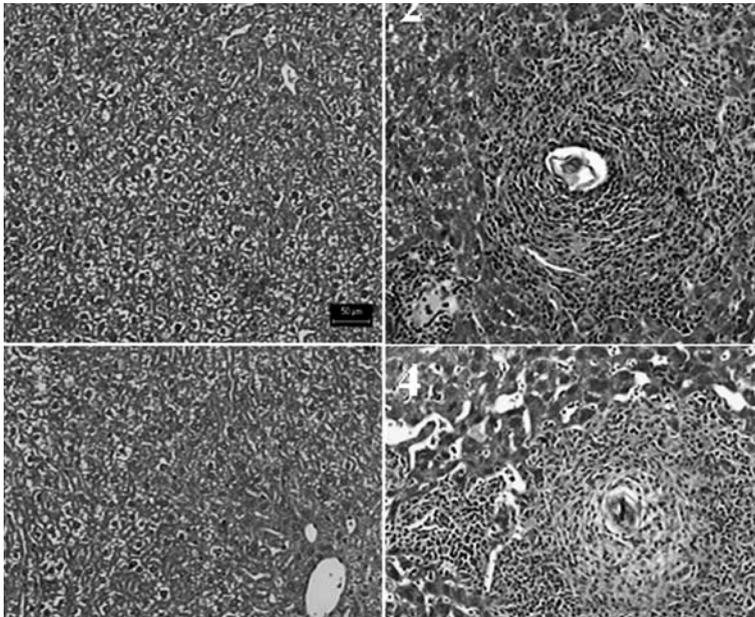


Figure 3. Photomicrographs of the hepatic structure in C57BL/10 (1 and 2) and CBA (3 and 4) mice, stained with Masson's trichrome. 1 and 3: panoramic view of a normal mice liver; 2 and 4: view of a typical schistosomal hepatic showing concentric arrangement of fibers and normal.

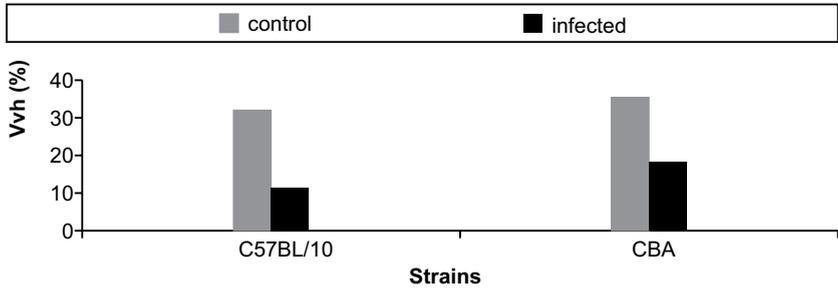


Figure 4. Density of volume of hepatocytes (%) in uninfected and *Schistosoma mansoni*-infected C57BL/10 and CBA mice.

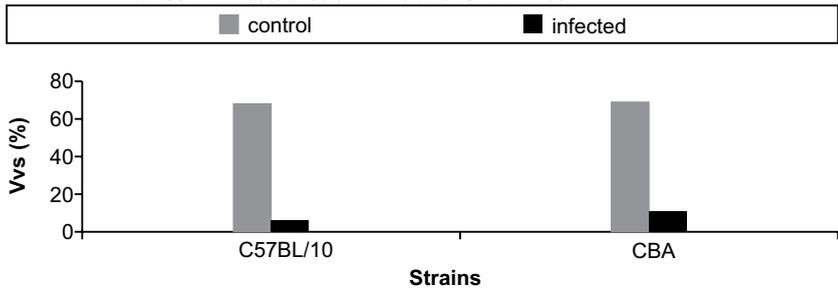


Figure 5. Density of volume of sinusoids (%) in uninfected and *Schistosoma mansoni*-infected C57BL/10 and CBA mice.

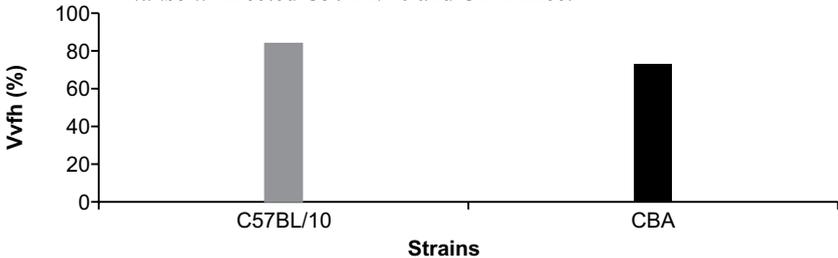


Figure 6. Volume density of hepatic fibrosis in C57BL/10 and CBA mice.

DISCUSSION

In this study, C57BL/10 yielded slight higher worm recovery than CBA mice, which might suggest that both mice strains provide favorable micro-environments for schistosomes. The obtained results showed that laid eggs mature within both C57BL/10 and CBA mice tissues, subsequently pass through the intestinal wall and then exit the host through the feces. As in other studies, the onset

of fecal egg output was characterized by an initial low egg releasing, peaking around 7-9 weeks pi (Rocha et al., 1995; Martinez et al., 2003), which coincides with an intestinal granulomatous inflammation, eosinophilia, and a Th2 response that becomes maximal at 8 weeks of infection (Fallon et al., 2000; Cheever et al., 2002). In addition, results suggest that C57BL/10 mice may ease egg output on account of more effective intestinal inflammatory response through immune mediators even in short-term infection.

In this report, inbred female C57BL/10 mice showed highest intestinal egg density than CBA strain, although no statistical differences ($p>0.05$) were found. Unlike our previous results using C3H mice strain (Freire et al., 2003) or SW mice strain (Neves et al., 2007), duodenum concentrated greatest egg counting, suggesting differences in the wandering capacity inside the portal system vessels in mouse model (Valadares et al., 1981).

Schistosomiasis mansoni has the liver as the major focus of its histological lesions, physiopathological alterations and clinical manifestations (Neves et al., 2007). *S. mansoni* eggs laid in the postcapillary venules of the mesenteric tract are carried by the bloodstream into smaller blood vessels, where they are trapped in the sinusoids. This process leads to marked inflammation, tissue eosinophilia, collagen neof ormation and, ultimately, fibrous expansion of the portal spaces and intrahepatic portal-vein obstruction (Abath et al., 2006). C57BL/10 mice showed higher ($p<0.05$) liver volume than CBA strain, which may have been due to higher density of liver eggs and/or hepatic fibrosis. All infected mice had fibrotic livers containing visible granulomatous lesions, composed of numerous macrophages, eosinophilic granulocytes, lymphocytes, and fibroblasts in agreement with previous studies (Andrade & Azevedo, 1987; Lenzi et al., 1998; Baptista & Andrade, 2005).

The histological lesions in murine models have been reported at morphometrical level (Costa-Silva et al., 2003; Silva et al., 2004). In addition, quantification of liver injury based on stereology has proven to be a useful tool for schistosomiasis mansoni studies (Friis et al., 1998; Neves et al., 2006; Barros et al., 2009).

The results presented here clearly demonstrate that infection causes damage in liver structure because hepatocytes and sinusoids are reduced in number. Also, significant differences were evidenced between both mice strains concerning such hepatic alterations. Marked differences in granuloma volume and in hepatic fibrosis suggest that its modulation and features differ among different mice strains (Cheever et al., 2002). In addition to granuloma modulation, it has been reported that granulomas size are also unequal between mice strains (Swiss Webster and C3H/He) (Lenzi et al., 1995). It appears that CBA mice are more protected from infection than C57BL/10 mice since hepatocyte and sinusoids were less affected, while hepatic fibrosis was greater in this later host.

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RESUMO

Esterologia hepática da infecção esquistossomótica aguda em camundongos das linhagens C57BL/10 e CBA

Estudos prévios mostraram que o curso da infecção esquistossomótica experimental depende da constituição genética do hospedeiro. A fim de testar se linhagens de camundongos infectados por *Schistosoma mansoni* apresentam diferenças nos padrões de patologia durante a fase aguda, examinamos possíveis alterações nos hepatócitos, sinusóides e quantidade de fibrose, utilizando uma abordagem estereológica. As mudanças na organização hepática de camundongos fêmeas, adultas, das linhagens C57BL/10 e CBA, foram examinadas oito semanas após a infecção. A densidade de volume de hepatócitos Vv[h], sinusóides Vv[s] e fibrose Vv[fh] foram examinadas por determinação do ponto de contagem (sistema-teste M42) e video microscopia, por microscopia de luz. Em ambas linhagens de camundongo, Vv[h] e Vv[s] foram significativamente reduzidas ($p < 0,05$) em relação ao grupo controle. Os maiores valores de Vv[fh] foram encontradas na linhagem C57BL/10. Demonstramos que a infecção esquistossomótica reduziu acentuadamente hepatócitos (linhagem CBA), sinusóides (linhagem C57BL10), enquanto a fibrose foi maior em C57BL10 do que CBA.

DESCRITORES: Esterologia hepática. Esquistossomose mansoni. Camundongo isogênico.

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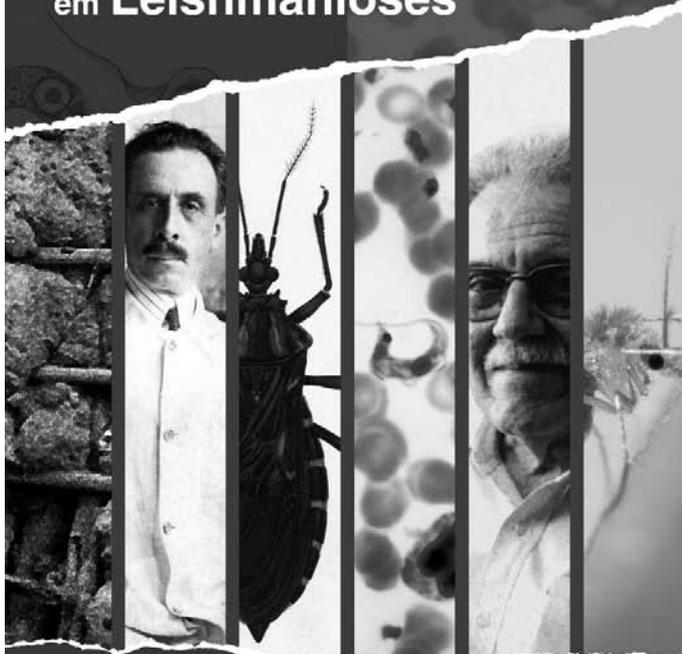
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