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Bone marrow-derived cells migrate to the liver and contribute to the generation of different cell types in chronic *Schistosoma mansoni* infection

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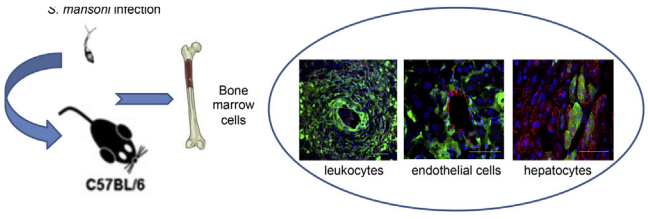
HIGHLIGHTS

- *S. mansoni* infection mobilizes endothelial progenitors and MSC to peripheral blood.
- Influx of bone marrow-derived cells is seen in the liver after *S. mansoni* infection.
- Most BMC-derived cells are myeloid and lymphoid cells present in the granuloma.
- BMC contributes to the generation of hepatocytes and endothelial cells.

GRAPHICAL ABSTRACT



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ABSTRACT

The main pathogenic event caused by *Schistosoma mansoni* infection is characterized by a granulomatous inflammatory reaction around parasite eggs and fibrosis in the liver. We have previously shown that transplantation of bone marrow cells (BMC) promotes a reduction in liver fibrosis in chronically *S. mansoni*-infected mice. Here we investigated the presence and phenotype of bone marrow-derived cells in livers of *S. mansoni*-infected mice. During the chronic phase of infection, C57BL/6 mice had an increased number of circulating mesenchymal stem cells and endothelial progenitor cells in the peripheral blood when compared to uninfected controls. In order to investigate the fate of BMC in the liver, we generated bone marrow chimeric mice by transplanting BMC from transgenic green fluorescent protein (GFP) mice into lethally irradiated wild-type C57BL/6 mice. *S. mansoni*-infected chimeric mice did not demonstrate increased mortality and developed similar liver histopathological features, when compared to wild-type *S. mansoni*-infected mice. GFP^+ bone marrow-derived cells were found in the liver parenchyma, particularly in periportal regions. $CD45^+GFP^+$ cells were found in the granulomas. Flow cytometry analysis of digested liver tissue characterized GFP^+ cells as lymphocytes, myeloid cells and stem cells. GFP^+ cells were also found in areas of collagen deposition, although rare GFP^+ cells expressed the myofibroblast cell marker α-SMA. Additionally GFP^+ endothelial cells (co-stained with von Willebrand factor) were frequently observed, while BMC-derived hepatocytes (GFP^+ $albumin^+$ cells) were sparsely found in the liver

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of chimeric mice chronically infected with *S. mansoni*. In conclusion, BMC are recruited to the liver during chronic experimental infection with *S. mansoni* and contribute to the generation of different cell types involved, not only in disease pathogenesis, but possibly in liver regeneration and repair.

1. Introduction

Schistosomiasis is the 4th most prevalent, neglected tropical disease worldwide (Hotez et al., 2007) and represents an important cause of human liver fibrosis. The pathogenesis of *Schistosoma mansoni* infection is associated with an inflammatory granulomatous response induced by deposition of parasite eggs, which recruits various cell types, including macrophages, eosinophils and lymphocytes (Weinstock and Boros, 1983).

In the pathogenesis of hepatic fibrosis, chronic inflammation is the element that drives the initiation and progression of fibrogenesis, a process in which hepatic stellate cells play a key role (Senoo et al., 2010). The activation of stellate cells is associated with a reduction of intracellular vitamin A storages, increased production of type I collagen and the expression of α-smooth muscle actin (α-SMA) (Senoo et al., 2010). Additionally, angiogenesis and fibrogenesis are closely associated in schistosomiasis (Andrade and Santana, 2010; Cassiman et al., 2002). Therefore, hepatic fibrogenesis in schistosomiasis is a process in which different cell types play a role, including stellate cells, endothelial cells, pericytes, and bone marrow-derived fibrocytes (Russo et al., 2006).

The regenerative capacity of the liver is well known, and it occurs through different mechanisms, involving hepatocyte proliferation, activation of intra-hepatic progenitors known as oval cells, and migration of bone marrow-derived cells (Eckersley-Maslin et al., 2009). The relative contribution of each of these three compartments in liver regeneration and homeostasis vary, depending on the different pathophysiological settings (Jang et al., 2004; Muraca et al., 2007; Oh et al., 2007; Wang et al., 2003), and to date, it is not established to chronic schistosomiasis.

Elucidating the contribution of bone marrow-derived cells to liver regeneration and fibrosis can lead to further development of cell-based therapies for liver diseases. We have previously shown that transplantation of bone marrow mononuclear cells accelerates fibrosis regression (Oliveira et al., 2008). In the present study, we investigated the role of endogenous bone marrow-derived cells in schistosomiasis liver lesions, by using bone marrow chimeric mice chronically infected with *S. mansoni*.

2. Materials and methods

2.1. Animals

Six to eight weeks-old female C57BL/6 mice were used in the experiments. To generate chimeric mice, four week-old male C57BL/6 mice transgenic for enhanced green fluorescent protein (GFP) were used as bone marrow cells donors for reconstitution of irradiated wild-type C57BL/6 mice. The experiments were performed three times, in order to confirm the results obtained, using a total number of 61 mice. All mice were raised and maintained at the animal facilities at the Gonçalo Moniz Research Center, FIOCRUZ/BA, and provided with rodent food and water *ad libitum*. Animals were handled according to the NIH guidelines for animal experimentation. All procedures described had prior approval from the local animal ethics committee.

2.2. Generation of chimeric mice

C57BL/6 female mice (n = 46) were irradiated with 6 Gy for depletion of the bone marrow cells in a ^{137}Cesium source irradiator (CisBio International, Codolet, France). Bone marrow cells were obtained from femurs and tibiae from male GFP transgenic C57BL/6 mice and used to reconstitute irradiated mice. Bone marrow mononuclear cells were purified by centrifugation in Ficoll gradient at 1000 *g* for 15 min (Histopaque 1119 and 1077, 1:1; Sigma–Aldrich, St. Louis, MO, USA). After two washings in DMEM medium (Sigma–Aldrich), the cells were resuspended in saline for intravenous injection (10^7 cells/mouse in a volume of 200 μL) in irradiated mice. Thirty days after transplantation, mice were infected with *S. mansoni* (n = 28) or used as uninfected chimeric controls (n = 18) (Fig. 1).

2.3. Parasites and infection

C57BL/6 chimeric (n = 28) and non-chimeric mice (n = 10) were infected by transcutaneous route with 30 *S. mansoni* cercariae of the Feira de Santana strain (Andrade and Sadigursky, 1985). This strain was maintained through successive passages in laboratory-raised *Biomphalaria glabrata* snails. Two weeks later the mice were reinfected with 15 cercariae to increase the hepatic injury. Infected mice were selected 40 days after the primary infection by parasitological exam of feces. Only mice presenting viable eggs in the stools were used.

2.4. Morphological and morphometrical analyses

Groups of animals were euthanized 4 months after infection Morphological analysis was performed in sections of formalin fixed and paraffin embedded livers. The sections obtained (5 μm-thick) were stained with either hematoxylin–eosin for histological analysis or Sirius red to visualize fibrosis by images obtained from a Scanscope digital slide scanner (Aperio Technologies, Vista, CA, USA).

For morphometrical analysis, 5 μm-thick liver histological sections, stained with picro sirius-red for collagen, were examined using a semiautomatic morphometry processing and analysis image system (LEICA QWin V. 2.8 software; Leica Cambridge, Cambridge, England). For morphometric measurements, a total sectional area of 10.908 mm^2 per animal was evaluated. The following parameters were used: percentage of liver fibrous tissue, size, volume density and numerical density of granulomas. To estimate the percentage of fibrous tissue, red-stained sectional area was directly measured. All periovular granulomas that had *S. mansoni* eggshells were included and considered that the granuloma was sectioned in the center. Spherical shape was assumed for the granuloma volume. The granuloma volume density was calculated as the quotient of the total granuloma profile area to the total sectional area studied per animal. The number of granulomas per unit volume of liver was assessed by applying Weibel's formula, as previously described (Barbosa-Júnior, 2001).