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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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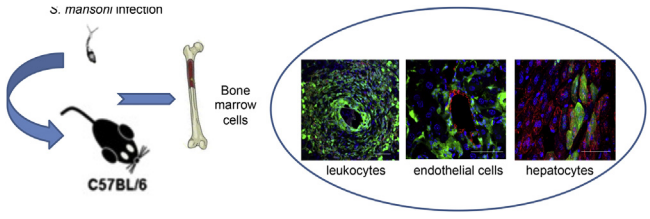
HIGHLIGHTS

- *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).

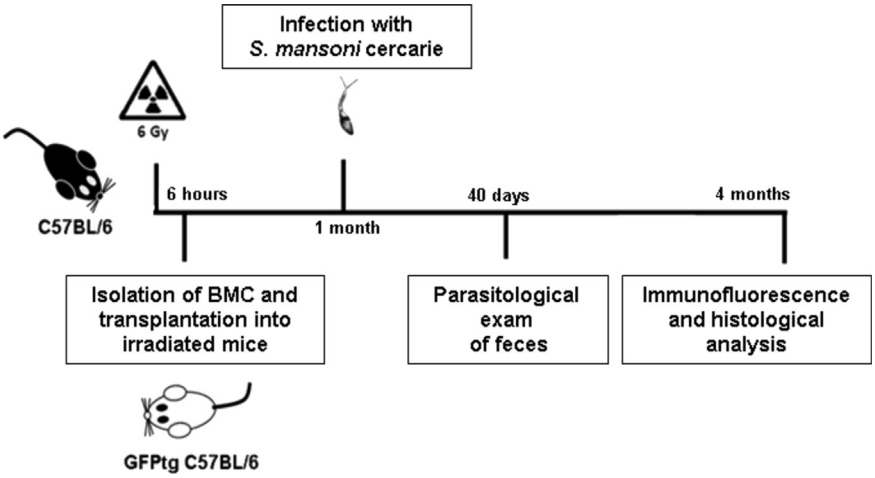

Fig. 1. Schematic illustration of the experimental design. C57BL/6 wild-type mice were infected with *S. mansoni* cercarie and euthanized 4 months after infection for peripheral blood analysis by flow cytometer. In parallel, naïve C57BL/6 female mice were used to generate chimeric mice. Animals were lethally irradiated and transplanted with BMC obtained from GFP^+ donors (10^7 cells/mouse). Chimeric mice were infected with *S. mansoni* and euthanized 4 months later. Control chimeric mice were evaluated at the same time points.

2.5. Immunofluorescence analysis

The animals perfused transcardially with 50 ml cold 0.9% saline, followed by 100 ml cold 4% paraformaldehyde (Merk, Darmstadt, Germany) in phosphate buffered saline, pH 7.2. Livers were excised and left lobes were fixed in 10% formaline and paraffin-embedded. After transcardiac perfusion with paraformaldehyde, non-left hepatic lobes were additionally fixed in 4% paraformaldehyde at 4 °C for 24 h. These samples were then incubated overnight in 30% sucrose solution in phosphate buffered saline at 4%, embedded in medium for congeal tissue and frozen at −70 °C. Ten μm frozen liver sections were used for detection of GFP^+ cells. The following primary antibodies were used: rabbit anti-albumin (1:400; Dako Denmark A/S, Glostrup, Denmark), rat anti-CD45 (1:200; BD Biosciences, San Diego, CA, USA), rabbit anti-collagen I (1:50; Novotec, Lyon, France), rabbit anti-cytokeratin 18 (1:50; Santa Cruz Biotechnology, Dallas, TX, USA), rabbit anti-collagen IV (1:50; Novotec), rabbit anti- GFAP (1:400; Dako), mouse anti-α-SMA (1:200; Dako) stained using M.O.M. kit (Vector Labs, Burlingame, CA, USA). Secondary antibodies anti-rabbit IgG Alexa Fluor 568 conjugated (Molecular Probes, Carlsbad, CA, USA) and anti-rat IgG Alexa Fluor 568 conjugated (Molecular Probes) were used. For biotinylated anti-αSMA stained sections, we used Alexa Fluor 568-conjugated streptavidin (Molecular Probes). Nuclei were counterstained with 4,6-diamidino-2-phenylindole (DAPI) (Vector Labs). Images were taken using a confocal microscope FluoView 1000 (Olympus, Tokyo, Japan). For quantification of GFP^+ cells, 10 random fields per liver section were captured and automatically counted using the software Image Pro Plus v.7.0 (Media Cybernetics, Bethesda, MD, USA).

2.6. Flow cytometry analysis

Quantitative analysis of mesenchymal stem cells and endothelial progenitor cells was performed in peripheral blood of *S. mansoni*-chronically infected non-chimeric wild type mice ($n = 7$) and non-infected controls ($n = 5$) by flow cytometry. The cells were incubated 15 min at room temperature in the dark with the following rat anti-mouse conjugated antibodies from BD Biosciences: Sca-1-FITC, CD90-PE, CD45-PerCP/Cy5.5, CD31-APC, CD34-FITC, CD44-APC and CD73-PE. Then red cells were lysate with lysis solution for 10 min, then washed twice with phosphate buffered saline (PBS) and resuspended in 500 μL of PBS. To confirm the presence of GFP^+ cells in chimeric mice, blood samples of irradiated and reconstituted mice were evaluated one month after transplantation. Blood samples obtained from non-chimeric wild-type and GFP transgenic C57BL/6 mice were used as negative and positive controls, respectively. The acquisition was done using LSRFortessa (Becton Dickinson) cytometer and analysis was performed with software FACSDiva (version 6.1.3) from 10.000 events.

Chronically infected and uninfected chimeric mice were submitted to venipuncture, through the tail vein. About 300 μL of peripheral blood was collected and added to a tube containing 20 μL of 10% EDTA solution. Each sample was added to analysis tubes containing specific antibodies. After washing with 1 ml PBS, cells were resuspended in PBS for data acquisition using BDFACS Diva v6.1.3 software at a BD LSRFortessa SORP flow cytometer (BD Biosciences). Data analysis was performed by FlowJo v.7.6 FC software (TreeStar, Ashland, OR, USA).

Isolated liver cell samples were obtained by digestion with collagenase (Sigma), 1 mg/ml in Dulbecco's modified minimum essential medium (DMEM), as previously described (Oliveira et al., 2000). After obtaining a single cell suspension, samples were fixed by PBS 1% paraformaldehyde solution for 24 h at 4 °C. Prior to FC assay, samples were washed with PBS and added to analysis tubes containing specific antibodies (all from BD Biosciences) for immunophenotyping of T lymphocytes (CD3e-APC, CD4-PE-Cy5, CD8-PE), B lymphocytes (CD3e-APC, CD45R/B220-PE), monocytes/macrophages (CD45-PerCP, CD11b-APC, GR-1-PE-Cy7), progenitor cells (CD45-PerCP, CD90-APC, CD44-APC, CD117-APC, Sca1-APC-Cy7). Incubation, data acquisition and analysis were performed as described above for peripheral blood samples.

2.7. Statistical analysis

Results were expressed as means ± SEM. Statistical comparisons between groups were performed by Student's *t*-test, using Prim Software (version 5.0; GraphPad Software Inc., San Diego, CA, USA). Results were considered to be statistically significant when $p < 0.05$.

3. Results

3.1. S. mansoni chronic infection mobilizes bone marrow derived stem/progenitor cells to the peripheral blood

Non-chimeric wild type C57BL/6 mice infected with *S. mansoni* ($n = 7$) were assessed in order to evaluate whether the chronic

Table 1
Chronic infection with *S. mansoni* induces the mobilization of MSC and EPC to the peripheral blood.

Cell subpopulation	Naive	Infected	p value
$CD45^{-}Sca\text{-}1^{+}CD73^{+}CD31^{+}$	0.18 ± 0.04	2.54 ± 0.40	0.0004
$CD45^{-}Sca\text{-}1^{+}CD44^{+}CD90^{+}$	0.02 ± 0.004	0.08 ± 0.01	0.0034
$CD45^{lo}CD34^{+}$	1.02 ± 0.21	1.75 ± 0.26	0.065

Data represent the % and are expressed as means ± SEM of 5 naïve and 7 infected mice. Statistical analysis was performed using Student's *t* test.

infection causes the mobilization of stem and precursor cells from the bone marrow to the peripheral blood. A statistically significant increase in the frequencies of mesenchymal stem cells ($CD45^{-}Sca\text{-}1^{+}CD44^{+}CD90^{+}$) and endothelial precursor cells ($CD45^{-}Sca\text{-}1^{+}CD73^{+}CD31^{+}$) was observed in the peripheral blood of *S. mansoni*-infected mice, when compared to uninfected controls. Moreover, it was found a trend towards increased frequencies of hematopoietic stem cells ($CD45^{lo}CD34^{+}$) in peripheral blood of infected mice, when compared to uninfected mice, however lacking statistical significance (Table 1).

3.2. Chimeric mice chronically infected with S. mansoni present similar pathologic features to non-irradiated infected mice

Based on the previous data suggesting mobilization of stem/precursor cells to the periphery in infected mice, we conducted the following experiments in order to track the fate of mobilized bone marrow-derived cells. Bone marrow chimeric mice were generated and infected with *S. mansoni* in order to establish an experimental model for the study of migration and fate of bone marrow derived cells in the injured liver (Fig. 1).

In order to determine if lethal irradiation followed by bone marrow reconstitution could change the course and characteristics of the experimental model, chimeric and non-chimeric wild-type mice were compared regarding mortality rates and histological analysis after *S. mansoni* infection. Chimeric mice did not present increased mortality when compared to non-irradiated infected mice (33% and 30%, respectively). Liver sections of chronically infected chimeric mice presented areas with scattered granulomas (Fig. 2A and B) and periportal fibrosis (Fig. 2C and D), similar to those observed in non-irradiated controls. Moreover, morphometrical analysis showed similar degree of fibrosis and granuloma features when liver sections from chimeric and non-chimeric mice were evaluated (Table 2).

3.3. Migration and fate of bone marrow-derived cells in the liver of chronically infected chimeric mice

Bone marrow-derived cells were tracked in the liver by confocal microscopy analysis of GFP expression. Non-infected chimeric mice presented GFP + cells in association with sinusoids and larger vessels (Fig. 3A). In contrast, GFP + cells were found predominantly in periportal areas in the liver four months after infection (Fig. 3B). Chronic infection with *S. mansoni* significantly increased the number of GFP^{+} cells in the liver when compared to non-infected chimeric mice ($p < 0.0001$) (Fig. 3C).

In addition, GFP^{+} bone marrow-derived cells were frequently seen in association with collagen fibers, as shown by immunostaining for type I and IV collagens (Fig. 4A–F). However, co-localized staining between collagen and GFP was rarely observed. In order to evaluate the presence of bone marrow-derived stellate cells, fibrocytes or myofibroblasts, liver sections were analyzed by immunofluorescence. Rare GFP^{+} cells co-expressing α-SMA, a myofibroblast marker, were found inside granulomas (Fig. 4G–I). Moreover, cells double-positive for GFAP, a marker for liver resident stellate cells, and GFP were not found (Fig. 4J–L).

GFP^{+} cells composed the majority of the cells in granulomas in chronically infected chimeric mice. A high expression of CD45, a pan leucocyte marker, was found in the cells located in the periphery (Fig. 5A), as well as in the center of granulomas (Fig. 5B). We performed flow cytometry analysis of GFP^{+} cells isolated from

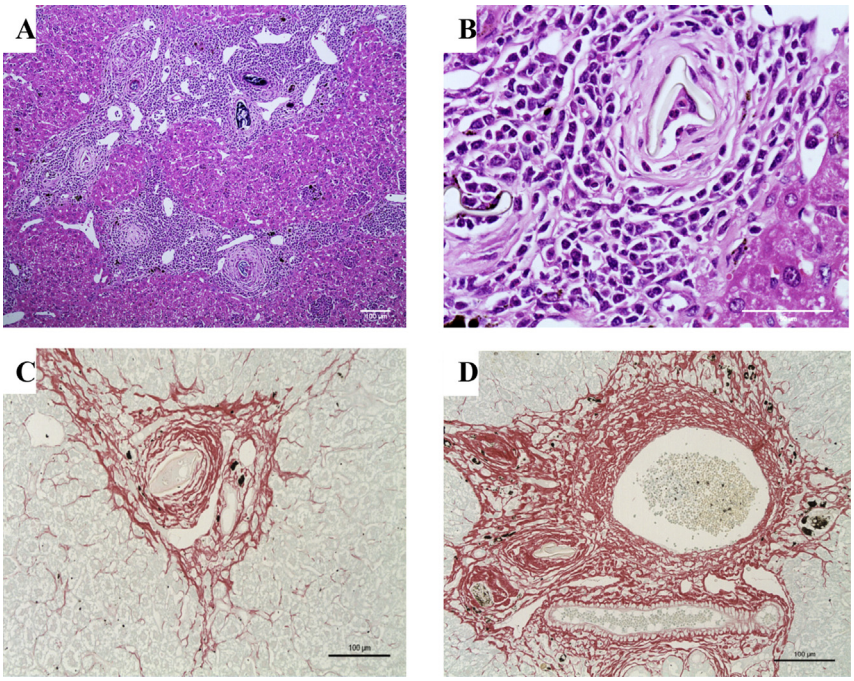

Fig. 2. Chronic infection histological patterns of chimeric mice. Liver sections of chronically infected chimeric mice present areas with scattered granulomas (A and B) or periportal fibrosis (C and D). Animals were euthanized 4 months after infection and liver sections were stained with H&E (A and B) or picrosiruis red (C and D).

Table 2
Chimeric and non-chimeric mice share similar liver alterations during chronic *S. mansoni* infection.

	Non-chimeric	Chimeric	p value
Fibrosis area (%)	11.66 ± 1.80	10.55 ± 1.08	0.5864
Granuloma mean volume (mm^3)	0.026 ± 0.006	0.033 ± 0.004	0.3852
Volumetric density	0.074 ± 0.014	0.083 ± 0.008	0.5651
Numerical density[a]	565.4 ± 92.13	528.2 ± 108.0	0.8098

Data represent the mean ± SEM of 5 (non-chimeric) and 7 (chimeric) mice.
[a] Number of granulomas/mm^3.

livers of infected chimeric mice (n = 7). The inflammatory infiltrate ($CD45^+$) was mainly composed by myeloid cells (71.0 ± 11.6% $CD11b^+$), B lymphocytes (20.9 ± 4.6% $CD45R^-B220^+$) and T lymphocytes (3.7 ± 2.6% $CD3^+$). Among myeloid cells, the majority were macrophages (89.7 ± 3.2% $GR1^-$). In addition, hematopoietic stem cells ($CD45^-Sca\text{-}1^+CD117^+$) and mesenchymal stem cells ($CD45^-Sca\text{-}1^+CD44^+CD90^+$) were detected among GFP^+ cells infiltrating the liver parenchyma at the frequencies of 0.007 ± 0.006% and 0.033 ± 0.014%, respectively.

We also observed GFP^+ cells in the walls of blood vessels, forming endothelial cells (expressing von Willebrand factor) in both infected (Fig. 5C) and non-infected (Fig. 5D) chimeric animals. Rare GFP^+ hepatocyte-like cells were found in *S. mansoni*-infected (Fig. 5E and F), but not in non-infected chimeric mice.

4. Discussion

The use of bone marrow chimeric mice has been a useful tool in the study of the pathogenesis of diseases and mechanisms of tissue regeneration (Alvarez-Dolado et al., 2003; Lagasse et al., 2000; Oliveira et al., 2012). In the present study we described the recruitment and homing of bone marrow cells to the injured livers of mice chronically infected with *S. mansoni*. We demonstrated that bone marrow-derived endothelial progenitors, hematopoietic stem cells and mesenchymal stem cells are recruited to the periphery after *S. mansoni* infection. Moreover, by using chimeric mice, we showed that bone marrow-derived cells were found in blood vessels, in areas of fibrosis and, as expected, composing the granuloma.

The presence of GFP^+ BM-derived MSCs in the liver, and increased mobilization of BM-derived progenitors to peripheral blood were observed during *S. mansoni* infection. Although the finding of increased mobilization and homing of stem/progenitor cells suggests a response to a myriad of inflammatory signals produced in the liver during schistosomiasis, the contribution of those progenitors to hepatic regeneration or fibrogenesis remains undetermined (Baba et al., 2004; Kisseleva et al., 2006; Paredes et al., 2012; Roderfeld et al., 2010).

As previously seen in wild-type C57BL/6 mice (Oliveira et al., 2008), chronically infected chimeric mice also presented areas with scattered granulomas or periportal fibrosis. The granuloma occurs around the *S. mansoni* eggs and involves different cell types, such as eosinophils, macrophages, lymphocytes and fibroblasts. In our study, flow cytometry analysis on digested liver showed the presence of lymphoid and myeloid GFP^+ cells, which, by composing the granulomas, might have an essential host-protective role (Pearce and MacDonald, 2002). These cells are recruited to the liver after the injury process starts, within a blood vessel (usually a pre-capillary), where the *S. mansoni* eggs are deposited. Thus, the vascular endothelium is the first target lesion and responds to the stimulus by proliferating (Andrade and Santana, 2010). Additionally, it has been previously demonstrated that endothelial cells are present in large numbers in the periovular schistosomotic granuloma (Lenzi et al., 1988), and their proliferation is induced by

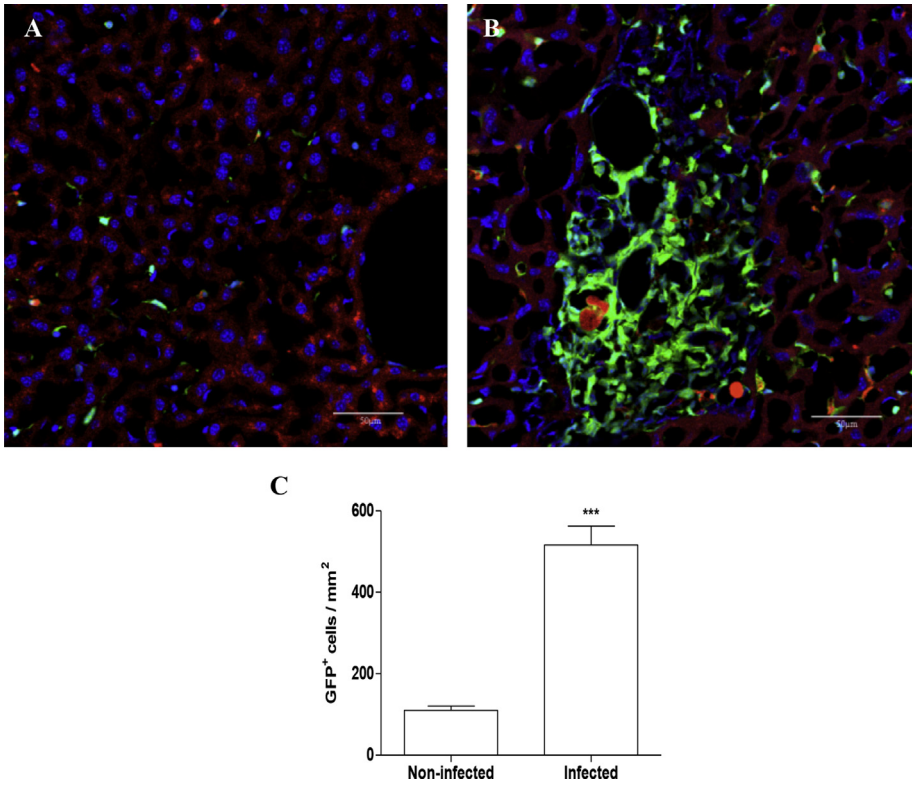

Fig. 3. Migration of GFP^+ cells to the liver of infected mice. Liver sections of non-infected (A) and infected (B) chimeric mice were analyzed to evaluate the influx of GFP^+ (green) cells. Sections were co-stained with CK-18 (red) and DAPI (nuclei in blue). C, Quantification of GFP^+ cells/area. ***, $p < 0.0001$. Scale bar = 50 μm.

soluble egg antigens (Freedman and Ottesen, 1988; Loeffler et al., 2002). In our study we observed a significant increase in endothelial progenitor cells in the peripheral blood of infected animals, and detected the presence of endothelial cells derived from bone marrow chimeras in livers of infected mice. These data suggest that the bone marrow is a source of precursor cells to the angiogenesis process that occurs in schistosomiasis.

Although GFP + cells have been found in areas of collagen deposition, few bone marrow-derived cells were positive for α-SMA, a marker widely used to characterize myofibroblasts, while GFP + cells co-expressing GFAP, a marker for liver resident stellate cells, were not observed. Myofibroblasts are the main source of extracellular matrix in the injured liver, but their origin is still debated (Lemoinne et al., 2013). Russo et al. (2006) demonstrated the presence of high numbers of bone marrow-derived myofibroblasts in the fibrotic liver in model. However, others demonstrated that bone marrow derived cells do not contribute significantly to collagen production in the experimental model of liver fibrosis induced by CCl_4 (Higashiyama et al., 2009). Our data do not give support to an extensive contribution of bone marrow cells to the generation of myofibroblasts in schistosomiasis, suggesting that the fibrogenesis is mainly dependent on liver resident stellate cells or fibrocytes.

Bone marrow-derived hepatocytes were only found in chimeric mice when infected with *S. mansoni*, although this was a rare finding. Similarly, in a previous study we observed in chimeric animals with chronic liver injury induced by CCl_4 administration, few cells co-expressing GFP and albumin in the hepatic parenchyma, while none was found in non-injured controls (Oliveira et al., 2012). The finding of bone marrow-derived hepatocytes has

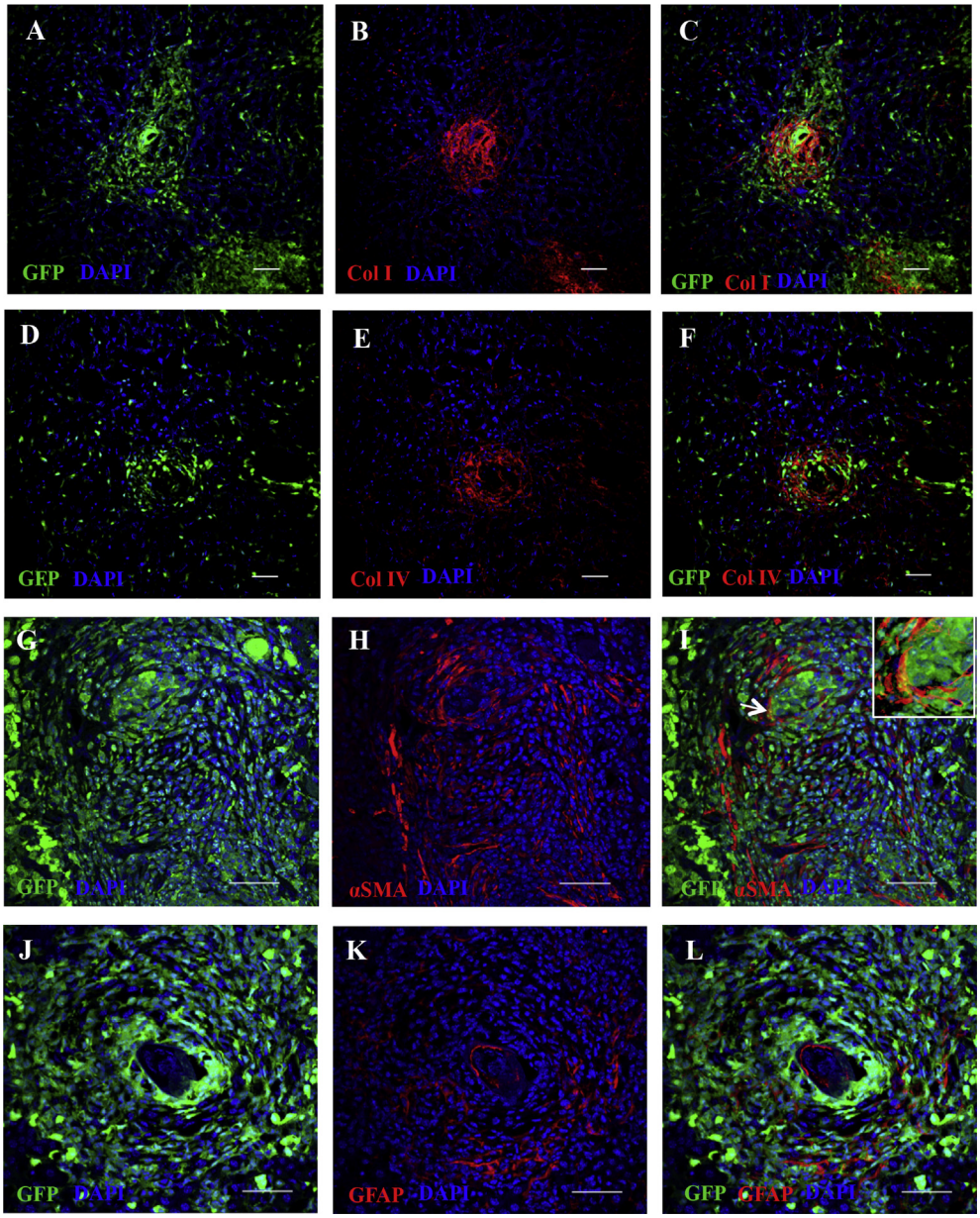

Fig. 4. Distribution of bone marrow-derived cells in liver of infected chimeric mice. GFP^+ cells (green) were found within fibrotic areas. GFP^+ cells in fibrosis area confirmed with collagen I (red; A–C) or collagen IV (red; D–F) staining. G–I, Most GFP^+ cells do not express the myofibroblast marker αSMA (red). Arrow indicates a GFP^+ $αSMA^+$ cell, shown also in detail in the inset. J–L, GFP^+ cells do not express the stellate cell marker GFAP (red). Nuclei were stained with DAPI (blue). Scale bar = 50 μm.

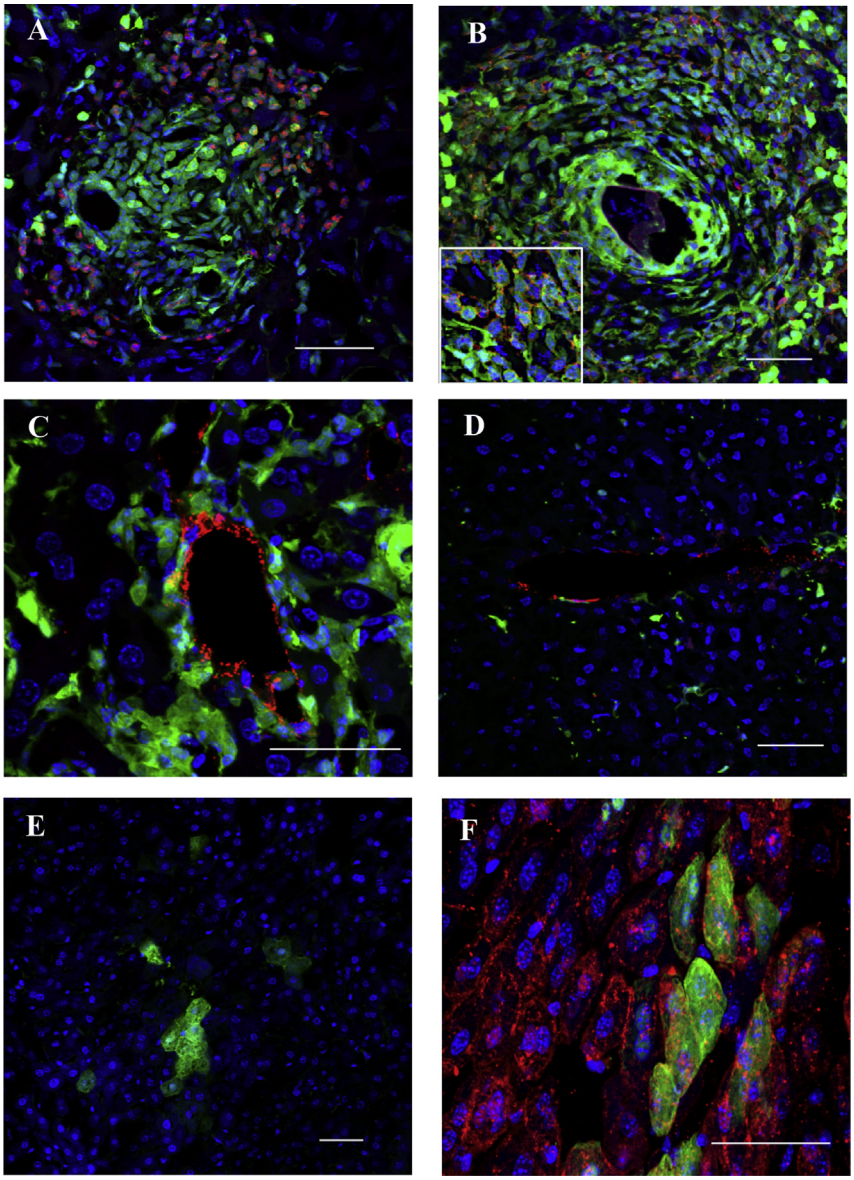

Fig. 5. Presence of different cell types involved in liver injury and tissue repair derived from bone marrow cells. GFP^+ cells (green) co-stained with CD45 (red) in periphery (A) and in the center (B) of granulomas. GFP^+ cells co-stained with wVF (red), an endothelial cell marker, in infected (C) and non infected (D) chimeric animals. E and F, Bone marrow-derived GFP^+ hepatocytes (green) co-stained with albumin (red; F). Nuclei were stained with DAPI (blue). Scale bar = 50 μm.

been previously explained both by the occurrence of cell fusion or transdifferentiation, which was not addressed in the present study. In the fusion hypothesis, stem cells could fuse with injured hepatocytes and undergo reprogramming to express specific proteins of the resident cell (Vassilopoulos et al., 2003). Most studies that suggest the occurrence of fusion processes used mice deficient for the enzyme fumarylacetoacetate hydrolase ($Fah^{-/-}$) as an experimental model that simulates the tyrosinemia type I (Vassilopoulos et al., 2003; Wang et al., 2003; Willenbring et al., 2004). In this model there is a high proliferative pressure of hepatocytes (Fausto, 2004). In contrast, *in vitro* (Jang et al., 2004) and *in vivo* (Jang et al., 2004; Theise et al., 2000) studies have demonstrated the transdifferentiation of bone marrow cells by cytogenetic analysis of chromosomes X and Y and expression of hepatocyte-specific markers such as albumin. Despite the possible mechanisms for hepatocyte formation, since in schistosomiasis the loss of functional hepatocytes is not a critical issue, the little contribution of bone marrow cells to the appearance of newly formed hepatocytes may result from the lack of a selective pressure, as seen in the model of $Fah^{-/-}$ mice.

In conclusion, our results indicate that bone marrow-derived cells are recruited to the liver in chronic experimental infection by *S. mansoni* and give rise to hepatocytes, inflammatory cells of the granuloma, as well as stromal cells, such as endothelial cells and fibrocytes. Further studies are needed to clarify their roles both in the pathogenesis and in regeneration of the injured liver.

Acknowledgments

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