Differentiation and Homing of Transplanted Bone Marrow Cells in Livers of Murine Schistosomiasis: Pilot Study

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Abstract: Recent reports have demonstrated the capacity of bone marrow (BM) stem cells to transdifferentiate into hepatocytes in vivo and its importance in physiopathologic processes is still debated. The present study was designed to highlight part of the potential capabilities of bone marrow cells and their role in cell based therapy in hepatic diseases, we used the murine model of schistosomiasis. Using the murine model of schistosomiasis as a chronic liver injury, unfractionated male mice (BMC's) were transplanted through intravenous (IV) injection into tail vein of non irradiated gender mismatched \textit{Schistosoma mansoni} infected female mice on their 16th week post infection (PI). Two weeks after BM transplantation, the sacrifice schedule started: Mice were sacrificed at one week interval for a period of 10 weeks (from 18th - 28th week post infection) and their livers were fixed in standard buffered formalin and processed for both the light, electron and immunofluorescence (IF) microscopic studies. The morphological changes of the liver tissue were studied by light and electron microscopes (EM). Tracing of male donor derived cells was done by the detection of Y chromosome by FISH and also Y-chromosome protein (CDYL) by indirect immunofluorescence (IF) in female injured livers. Transformation into hepatocytes was studied by indirect IF using antibodies directed against mouse albumin within BM cells in a test vitro culture for 7 days. Histopathological and electron microscopic examinations of the liver sections of infected female recipients; transplanted (Tx) with BMC's, revealed focal appearance of small scattered hepatocyte like cells exhibiting small rounded centrally located nuclei as well as oval cells with relatively large nuclei facing the sinusoids and scattered in hepatic parenchyma. A marked proliferation of primitive and incomplete small groups of bile ductules lined by epithelial cells with abundant effaced faint pink cytoplasm and central small nuclei at the perigranulomatous areas at the portal tracts were also observed. A relative increase in the newly formed small blood vessels especially surrounding the periportal schistosomal granulomas was observed. These cells were only observed in the infected Tx female mice group. They were neither observed in the non Tx schistosoma mansoni infected control mice group, nor in the other control group which was Tx with BMC's without being infected. With increased post transplantation time; starting from 4th week, post transplantation, an increase in the number of these newly formed bile ductules and small hepatocyte like cells and blood vessels was noticed. Donor derived cells showing Y chromosome by FISH and also CDYL protein by IF were recovered in the female infected Tx with male BMC's livers. Cells were mainly localized in the periphery of schistosoma granuloma, hepatic parenchyma and periportal areas. Most of the donor derived engrafted cells seemed to be vascular, inflammatory cells and bile ductules together with few hepatic like cells. Only few donor derived cells appeared within the hepatic parenchymal tissue, and showed positivity for Y chromosome and Y protein and for albumin secretion by IF. Unfractionated BMC's can repopulate the injured schistosomiasis liver without immuno suppression. The extent of liver injury, the timing of cell administration, the threshold number of transplanted cells and the interplay of cytokines in the liver pathology are all important variables in the process of engraftment and differentiation that still need to be further defined. However, the transformation of bone marrow derived stem cells into viable hepatocytes sustains the idea of in vivo cell transplantation to be a successful therapeutic approach.

Key words: Bone Marrow Cells, Murine schistosomiasis, liver, Y chromosome, CDYL protein, FISH.
INTRODUCTION

After malaria, schistosomiasis is the second most prevalent tropical disease in the world. In some parts of the world, especially Egypt, it is known as bilharzia in honor of the German scientist, Theodor Bilharz, who first identified the etiological agent for Schistosomiasis in Egypt in 1851. The clinical manifestations depend on the species of parasite, intensity of worm burden, and immunity of the person to the parasite. World Health Organization reports (WHO, 2002, Mahmoud and Wahab, 1990) estimated that 500-600 million people in 74 tropical and subtropical countries are at risk for schistosomiasis. Over 200 million people in these countries are infected. Of these, 120 million are symptomatic, with 20 million having severe clinical disease (Chen and Mott, 1988, El-Garem, 1998).

Egypt has possibly the highest chronic HCV prevalence in the world; 10%–20% of the general population are infected and HCV is the leading cause of HCC and chronic liver disease in the country (Habib et al., 2001, Hassan et al., 2001, El-Zayadi et al., 2001) and aslo Egypt has a high incidence of hepatic injury due to chronic hepatitis B (Bassily et al., 1979) and chronic hepatic schistosomiasis (El-Garem 1998), which represents a serious health care problems leading to hepatic failure.

The prognosis of patients with hepatic failure is poor, although liver transplantation is a successful treatment for end-stage. A tremendous gap between the organs offered for transplantation and those needed is the major limitation of liver replacement therapy. In addition, major surgery, immune rejection, operative damage, long-term immunosuppression, and high cost which are the risks of liver transplantation, do not come without serious side effects. Alternative or adjuvant therapies for treatment of liver dysfunction are urgently needed (Dahlke et al., 2003, Lee et al., 2004). In recent years cell transplantation strategies represent a promising approach for overcoming the need for solid organ Transplantation.

Hepatocyte transplantation has been proposed as an alternative to whole organ transplantation (Malhi et al., 2002). It does not only provide temporary liver function in patients awaiting liver transplantation but has also been shown to be curative in certain metabolic conditions (Guha et al., 2002).

Several reports have demonstrated the capacity of BMSc's to transdifferentiate into hepatocytes (Alison et al., 2002). These findings created enormous interest because they uncovered a new property of BMSc's and opened the possibility that these cells could be used in the treatment of liver injury and acute or chronic liver failure (Mitchell and Fausto, 2002, Wang et al., 2002, Dalakas et al., 2005, Lysy et al., 2008). Also there are reports have shown the capacity of BMSc's to differentiate into a variety of nonhematopoietic cell lineages, including lung, bone, cardiac muscle, endothelium, and neurons (Mangi et al., 2003). In a suitable microenvironmen, bone marrow-derived cells can differentiate into hepatocytes (Jang et al., 2004, Sato et al., 2005). Autologous bone marrow cell transplantation for the treatment of the diseased liver is becoming an increasingly promising strategy.

Clinically, chronic Schistosoma mansoni (S. mansoni) infection in humans presents as a spectrum of disease forms, ranging from the relatively mild hepato-intestinal form to the other end of the spectrum, the severe hepatic decompensation and failure (Ezzat et al., 1989, El-Garem, 1998). Murine schistosomiasis provides a model for the study of the pathogenetic mechanisms underlying both granulomogenesis and fibrogenesis of the infected liver.

The present study was designed using the murine model of schistosomiasis to highlight part of the potential capabilities of BMSc's and their role in cell based therapy in chronic hepatic disease.

MATERIALS AND METHODS

The Experimental Mice:

Forty (40) female Swiss albino mice; 8 weeks old weighing 20 +/- 5 gm were obtained, fed a standard commercial pelleted diet and maintained under conventional conditions at the Schistosome Biological Material Supply Center (SBSC) of the Theodor Bilharz Research Institute (TBRI), Giza, Egypt (www.tbri.sci.eg).

Infection of mice with Schistosoma mansoni:

Mice were infected with a single subcutaneous injection of 80±10 S. mansoni cercariae. All animal protocols were conducted in accordance with the valid international guidelines for animal experimentation and were approved by the TBRI's Ethics Research Committee.

Control mice:

Age and weight matching control female mice which were either non-infected non-transplanted, non-infected but transplanted, were subjected to the same procedures described below. There are donor healthy non-infected male mice group of age and weight matching. Accordingly mice were divided into 4 main groups in addition to the male donor mouse group.
Group 1: Female mice infected with *S. mansoni* and transplanted (Tx) with bone marrow cells (BMC's).

Group 2: Control female mice; non-infected and transplanted with BMC's.

Group 3: Control female mice non-infected non-transplanted.

Group 4: Control mice infected with *S. mansoni*.

**Donor male group.**

**Bone marrow cells separation and cell culture:**

Donor bone marrow cells (BMC's) were obtained from the male mice. Mice femurs were flushed with phosphate buffered saline (PBS) using a needle to force the marrow out of marrow niches. The collected cells were filtered, centrifuged, washed, and cultivated in Panserin 401 (PAN Biotec Gmbh, Aldenbach, Germany) with 10% FCS (PAA laboratories GMBH, Colbe, Germany) and penicillin/steptomycin (10.000U/ml/10.000μg/ml) for 3 weeks until the cells reached a final concentration of 3-5x10⁶/μl.

**Transplantation of cells:**

Transplant experiments involved a single intravenous injection in tail vein of mice of 0.5 x 10⁶ male non-fractionated BMC's into the non-ablated female schistosoma infected mice on their 16th week post schistosoma infection using an insulin needle. The same BMC's injection was given to the other non-infected female mice groups.

**Sacrifice of mice:**

The sacrifice schedule started 2 weeks after transplantation with a weekly sacrifice of female mice (of all 4 groups) until we reached 10 weeks post transplantation, which was the planned duration of this experiment. The Livers of sacrificed mice groups were cut and processed for paraffin sections and EM examination.

**Antibodies needed for the used techniques (FISH) and IF:**

Goat anti mouse chromodomain-Y-like (CDYL) protein antibodies were obtained from Abcam Limited, Cambridge, UK (www.abcam.com). For its detection; FITC labelled anti goat antibodies were used as a secondary antibody.

Rabbit anti mouse albumin antibodies were obtained from Accurate Chemical & Scientific Corporation, Westbury, NY. For its detection Alexa Fluor 594 labelled goats anti rabbit antibodies were used (Molecular Biologische Biotechnologie. Göttingen, Germany).

The FITC labeled murine specific anti Y chromosome painting probe used for the fluorescent in situ hybridization technique (FISH) was obtained kindly from Prof. Weier, University of California, and Berkeley.

**Histopathology study:**

Liver specimens from all recipient mice, were fixed in 10 % buffered formalin, processed to paraffin blocks and sectioned at 4-mm thickness. Sections were stained with Hematoxylin and eosin stain and Masson trichrome stain to study the hepatic morphological changes and hepatic schistosomal fibrosis in the sacrificed mice. Liver sections were studied by light microscope regarding the exerted hepatic schistosomal infection and portal fibrosis in the different weeks post infection. This study included the extent of fibrocellular granulomas in the portal areas and the background fibrosis. Histopathological studies also focused on tracing the homing of the transplanted BMSC's and their differentiation into newly formed hepatocytes as well as the detection of apoptotic hepatocytes and newly formed bile ductules in the vicinity of the schistosomal granulomas.

**Electron Microscopy:**

Tiny pieces of the liver specimens were minced into about 1mm³ in size, each, and immediately fixed in 4% glutaraldehyde solution buffered by 0.2M sodium cacodylate, for 1 hour at 4°C. The fixed specimens were then washed twice in equal volumes of sodium cacodylate 0.2M and sucrose 0.4M at 4°C, post fixed in 2% osmium tetroxide for 1 hour and washed in distilled water. Dehydration in ascending grades of ethyl alcohol was then performed and the specimens were embedded in Epon and polymerized at 60°C for 48 hours. Semithin sections were cut, stained with methylene blue azur II and examined by light microscope to choose the proper site for ultrathin sectioning. Then ultrathin sections were prepared using an Ultract R ultramicrotome, double stained with uranyl acetate and lead citrate and examined by a Philips EM 208 S electron microscope.
**Immunofluorescent study (IF):**

The Chromodomain-Y-linked (CDYL) protein, is encoded by a group of related genes, the Y linked genes. CDYL protein is expressed in somatic cells and is over expressed during spermatogenesis. Subcellularly; it is located in the mitochondrial matrix (Lahn et al., 2002), EMBL Bioinformatics Harvester). In this study, we used the anti CDYL antibody to trace the male donor derived cells in liver sections of female recipient mice.

Formalin-fixed liver specimen were processed to paraffin-embedded blocks and were cut into 4-μm sections and deposited on Super Frost Plus glass slides (Menzel-Glaser, Germany). Slides were dried completely, deparaffinized, rehydrated in xylene and grades of alcohol and then were ready for epitope retrieval of the Chromodomain-Y-linked (CDYL) protein, washed with Tris buffer solution (TBS), then incubated overnight with diluted anti CDYL antibody (Ab) at 4°C (1:100). For labelling, samples were washed with TBS and incubated for 30 minutes at room temperature with fluorescein isothiocyanate FITC-labelled secondary antibody at room temperature in the dark. Similarly, the liver sections were treated with rabbit anti mouse albumin antibodies followed by red Alexa fluor 594 labelled goats anti- rabbit antibodies for the detection of albumin secreting cells.

In case of double labelling for Y chromosome protein and albumin; a cocktail of the two primary antibodies was used in the same way followed by the two labelled secondary antibodies.

For each analysis, elimination of non-specific staining was done by addition of an appropriate non immune serum. Negative controls were performed by removal of the primary antibody from the protocol. The livers of the recipient's female mice were checked for donor-derived cells in 50 microscopic fields of liver sections/animal, using Zeiss Fluorescent Microscope, power of magnification x650).

**Fluorescence in situ hybridization (FISH) to detect the cells carrying Y chromosome:***

Sections were processed for FISH to detect Y chromosome in female recipient liver sections. We used the murine Y chromosome specific painting probe generated by Weier et al., 1994 and followed the protocol recommended by (Nilsson et al., 1996).

Liver sections (from the studied two transplanted groups) of 5 μm thick were cut with microtome on positively charged slides and mounted at 37°C overnight. The sections were deparaffinized in three changes of xylene, each for 10 min, and rehydrated in graded ethanol (twice at 100% for 10 min, 95%, 90%, 70% and double-distilled water each for 5 min). The sections were then rinsed in two changes of Phosphate buffer solution (PBS), each for 10 min, and placed in 0.1 M glycine in PBS for 5 min to inactivate the formaldehyde from the original fixative. The slides were washed in PBS containing 0.3% Triton X-100 for 15 min, then further rinsed in PBS for 5 min.

Because of the fixative used we identified the optimal permeability method to be enzymic digestion by 5 μg/ml proteinase K (Boehringer Mannheim; Mannheim, Germany) in 0.1 M Tris-HCl (pH 8.0) containing 50 mM EDTA,. Disodium salt for 40 min at 37°C before incubation with the slides. The proteinase K was pre-incubated at 50°C for 1 hr to remove any nucleases. The sections were then further fixed in freshly prepared 4% paraformaldehyde in PBS (pH 7.2) and washed in two changes of PBS for 2 min each. The slides were then placed in freshly prepared 0.25% acetic acid anhydride in 0.1 M Tris (pH 8.0) for 10 min. Sections were prehybridized for 1.5 hr at 45°C in 20 μl of prehybridization buffer. After prehybridization, a hybridization mix consisting of 1μ of FITC-labelled Y chromosome probe in 7 μl of MN 2.1, 1μl Salmon Sperm DNA and 1μ mouse Cot-1 DNA was applied. The sections were then placed in a humidified chamber, denatured at 60°C for 10 min, and incubated overnight at 45°C. Unbound probe was removed by stringent washing in four changes of 0.1 x SSC (15 mM NaCl and 1.5 mM sodium citrate, pH 6.4) each for 15 min at 45°C followed by a rinse in PBS for 2 min. To preserve fluorescent labelling, sections were mounted using Dabco antifade (Sigma, Missouri, USA). Each section was mounted in approximately 5 μl of mounting medium and the slide cover is sealed with entallan. Mounted slides were stored at 4°C protected from light.

**Microscopy and Photomicroscopy:**

Liver sections were evaluated using an Olympus BX 61 microscope equipped for fluorescence (100-W high pressure mercury lamp) (Germany). Auto fluorescent nonspecific signals were excluded by examining green emission (528 nm) and red emission (617 nm). Light microscopic and fluorescent photomicrographs were taken with a CCD camera connected to an Image Analysis Soft ware.
RESULTS AND DISCUSSION

Light Microscopy:
Hematoxyline and eosin stained liver sections of schistosoma infected and gender mismatched bone marrow cells transplanted female mice revealed focal appearance of small scattered hepatocyte like cells exhibiting small rounded centrally located nuclei as well as oval cells with relatively large nuclei facing the sinusoids and scattered in hepatic parenchyma (Figure 1a,b). A marked proliferation of primitive and incomplete small groups of bile ductules lined by epithelial cells with abundant effaced faint pink cytoplasm and central small nuclei at the perigranulomatous areas at the portal tracts were also observed (Figure 2a,b). A relative increase in the newly formed small blood vessels especially surrounding the periovular schistosomal granulomas was seen. These cells were only observed in the infected transplanted female mic group. They were neither observed in the non transplanted schistosoma infected control mice group, nor in the other control group which was transplanted with BMC’s without being infected. With increased post transplantation time; starting from 4th week, post transplantation, an increase in the number of these newly formed bile ductules and small hepatocyte like cells and blood vessels was noticed.

Fig. 1(a, b): Liver tissue sections from recipient female mice Tx with BMC’s, at 4th weeks post transplantation, showing newly formed hepatocyte like cells, scattered within the hepatic parenchyma exhibiting; small rounded centrally located nuclei (arrows)(1a). As well as oval cells with relatively large nuclei facing the sinusoids with rounded or elongated dark nuclei (arrows)(1b), (H&E, original magnification x200, x400 respectively).

Fig. 2(a,b): Liver tissue sections from recipient female mice Tx with BMC's, at 4th weeks post transplantation showing newly formed bile ductules in portal areas close to the schistosomaal granulomas (arrows) (2a). A marked proliferation of the bile ductules lined by epithelial cells with abundant effaced faint pink cytoplasm and central small nuclei at the perigranulomatous areas at portal tracts(arrows) (2b),(H&E, original magnification x400).

Electron microscopic examination:
Electron microscopic examination revealed the presence of oval cells with large nuclei in between the inflammatory cells in the periphery of the granuloma, in between the hepatocytes facing the sinusoids and also in the periportal areas. These cells could be distinguished into: small relatively immature cells with large oval nuclei, high nuclear cytoplasmatic ratio and margnated chromatin and medium sized, more mature cells with mitochondria and endoplasmic reticulum approaching the morphological characteristics of hepatocytes (Figure. 3, Figure 4, Figure 5). These cells were neither seen in the control mice, which were schistosoma infected but did not have bone marrow transplantation nor in the other control group which was transplanted with BMC's without being infected. Mature hepatocytic cells with signs of regeneration were also detected in the studied group of mice. Hepatocytes with irregular nuclear membrane, prominent nucleoli, proliferated rough endoplasmic reticulum encircling the mitochondria were discerned. These signs of regeneration were less evident in the two control groups.
Indirect immunofluorescence (IF) staining:

The liver sections of infected female mice transplanted with male BMC's, stained by IF for anti CDYL protein, showed positive strong signals for CDYL protein in the cytoplasm of scattered hepatocytic cells (Figure 6). These CDYL-positive cells were located related to the sinusoids, and appear to us distinguishable from mature hepatocytes that are large polyhedral cells with round nuclei, a total of 10-15 isolated hepatocytes were positive for CDYL proteins per liver section. The initial number of the donor derived cells was small but appeared to increase starting from 4th week post-Tx on. With increasing post transplantation time few positive donor derived cells also appeared in the hepatic parenchymal tissue. In case of double IF, using the cocktail of the two primary antibodies anti-CDYL and anti-albumin antibodies followed by the two labelled secondary antibodies, only few cells showed double positivity denoting the scarcity of functioning hepatocytes of donor origin (Fig. 7 a, b, c).
**Fig. 6:** Immunofluorescence of CDYL protein, in liver sections of infected female mice Tx with BMC's after 23 weeks post infection (5 weeks post Tx), CDYL expression in the cytoplasm of hepatocyte like cells (IF, original magnification x 625).

**Fig. 7a,b,c:** Immunofluorescence of both CDYL protein and albumin, in liver sections of infected female mice Tx with BMC's after 23 weeks post infection (5 weeks post Tx) a. CDYL expression localized in the cytoplasm of hepatocyte like cells, b. Albumin expression localized in the cytoplasm of hepatocyte like cells, c. Double Immunofluorescence for AFP expression and CDYL protein in the same cell (IF, original magnification x 625).

**Fluorescent in Situ Hybridization (FISH):**

FISH analysis was performed to detect Y-chromosome in the recipient female mice livers. In the group of transplanted animals we found many Y-chromosome-positive nuclei in the perigranulomatous area (Figure 8) and only few positive nuclei with typical hepatocyte morphology in the hepatic parenchymal tissue of the analyzed sections of recipient female livers (7 cells per 10 sections) (Figure 9). Female controls infected with schistosoma but not transplanted showed complete absence of Y-chromosome fluorescein isothiocyanate signals.

**Fig. 8:** Liver sections of infected female mice Tx with BMC's after 26 weeks post infection (8 weeks post Tx) showing some hepatocytes like cells around the schistosomal granuloma showed green signals in the nuclei stained with DAPI. (FISH, original magnification x 1000).
Fig. 9: Female recipient infected liver after male BMC's Tx , 26 weeks post infection (8 weeks post Tx) revealed by FISH, positive Y chromosome signals appear as green dots in the hepatocyte like nuclei stained with DAPI(FISH, original magnification ×1000).

**Discussion:**
Advantages of stem cells for tissue regenerative medicine are multiple: ease of harvest, proliferation capacity, efficiency of *in vitro* transfection and potential use of autologous cells. Different types of stem cells are eligible for liver cell therapy according to their hepatic potential, for instance mesenchymal stem cells (MSCs), hematopoietic stem cells (HSCs) and adult liver stem/progenitor cells. Several studies showed the capability of bone marrow derived stem cells to transdifferentiate to hepatocytes (Alison *et al.*, 2002, Dalakas *et al.*, 2002, Petersen *et al.*, 1999, Lagasse *et al.*, 2000, Mallet *et al.*, 2002).

However, the real challenge for considering stem cells for therapy is the proof of their safety and functionality at a long term. Still, the research on the hepatic fate of stem cells is facing difficulties to demonstrate the acquisition of a fully mature hepatocyte phenotype, both in vitro and in vivo (Lysy *et al.*, 2008). Many authors are reevaluating the role of bone marrow derived cells as a source for hepatocyte regeneration (Cantz *et al.*, 2004). Other authors questioned the efficiency of their conversion into hepatocytes both in vivo and in vitro (Lian *et al.*, 2006, Thorgerisson and Grisham 2006, Yamaguchi *et al.*, 2006, Zhang *et al.*, 2003). Thus, the determination of the stem cell’s in vivo functionality in small and large animal models allowing objective screening of cell engraftment and functionality through detailed experimental studies remains to be a necessity. Our in vivo study is concerned with the potentials of transplanted bone marrow derived stem cells in murine schistosomiasis as an experimental model of a chronic liver disease.

In the current study we transplanted a relatively small dose (0.5 x 10⁶) of unfractionated male BMC’s into non irradiated gender mismatched female mice infected with schistosoma mansoni cercariae without immunosuppression therapy. The heterogenic cell population of BMC’s used may already contain different lines of stem cells capable of transformation into hepatocytes that can get lost during the complicated enrichment procedures. Similarly, in an extensive study done by Wang *et al.*, 2002, lethally irradiated fumarylacetoacetate hydrolase (FAH) mutant mice were transplanted with different doses of non fractionated BMC’s in comparison to highly purified hematopoietic stem cells (HSC). They stated that transplantation of enriched HSC does not enhance hepatocyte engraftment in comparison to unfractionated bone marrow. Also, Weiss *et al.*, 2003 transplanted porcine stem cells derived from umbilical cord mucous connective tissue into rat brains without immunosuppression. They demonstrated that these cells survived at 6 weeks without immunosuppression of the host animals after transplantation into either the brain or the peripheral blood.

In the present study histopathological examination of the infected female recipients liver Tx with BMC’s sections revealed focal appearance of small scattered hepatocyte like cells exhibiting small rounded centrally located nuclei as well as oval cells with relatively large nuclei facing the sinusoids and scattered in hepatic parenchyma. A marked proliferation of primitive and incomplete small groups of bile ductules lined by epithelial cells with abundant effaced faint pink cytoplasm and central small nuclei at the perigranulomatous areas at the portal tracts were also observed. A relative increase in the newly formed small blood vessels especially surrounding the periovular schistosomal granulomas was seen. These cells were only observed in the infected Tx female mouse group. They were neither observed in the non Tx schistosoma infected control mice group, nor in the other control group which was Tx with bone marrow cells without being infected. With increased post transplantation time; starting from 4th week, post transplantation, an increase in the number of these newly formed bile ductules and small hepatocyte like cells and blood vessels was noticed. These results were confirmed by electron microscopic examination; we were able to distinguish 2 different types of cells, small and medium sized oval cells, present in the periphery of the granuloma.
between the inflammatory cells, the hepatocytes facing the sinusoids and also in the periportal areas. The detected small cells had an oval shape, marginal chromatin and few cellular organelles resembling the type I primitive cells described by Sell. He studied the hepatic regeneration after liver injury with allyl alcohol and described oval cells with marginal chromatin, few cellular organelles, rare tonofilaments, that formed desmosomal junctions with adjacent liver cells.

In our study the medium sized cells constituted the dominant newly formed cells. They were hepatocyte-like, showed prominent mitochondria and rough endoplasmic reticulum, and some had lysosomes and a poorly developed Golgi apparatus similar to the type III progenitor cell described as hepatocyte-like (Sell). Sell stated that the terms oval cell, transitional hepatocyte, biliary hepatocyte, hepatocyte-like cell, atypical ductular cell, neocholangiole, etc., are used to describe these cells. In our infected and transplanted mice livers binucleated hepatocytic cells with signs of regeneration, irregular nuclear membrane, and proliferated rough endoplasmic reticulum encircling prominent mitochondria were also discerned with abundance relative to the control group of mice. It is explainable that the progenitor cells revealed in our study are mostly of the more differentiated type, since the mice in this experiment were sacrificed starting from 2 weeks after transplantation. Thus, the stem cells here, could be originated from the liver itself and from the transplanted cells, both had plenty of time to develop and differentiate, on the contrary to other studies where the sacrifice of mice was performed hours after the acute liver injury as seen in the ultrastructural study by (Sell, 1997). On the other hand histopathological and electron microscopic examination of the control group of mice which were schistosoma infected but were not transplanted showed regenerative signs; but to a lesser extent than the infected and transplanted study group. This hepatocyte like cells were not seen in control livers Tx with BMC’s and not infected with schistosomiasis. These control livers showed congested sinusoids, circulating inflammatory cells, and proliferating hepatocytes and hypertrophied Kupffer cells. These signs of congestion and proliferation may be a reaction to the transplanted cells.

In the current study the detection of the Y chromosome by FISH technique and of the CDYL protein by IF in infected recipient female mice liver sections; Tx with BMC’s liver sections, enabled the tracking of the transplanted cells until 10 weeks post transplantation. Moreover, these cells appeared to increase in number as time passed after the transplantation. It is noteworthy to mention here that donor derived cells were mainly localized in the hepatic parenchyma facing the sinusoids, in the periportal areas and also peri-granulomatous areas; the main sites of immune reaction characteristic of liver pathology in schistosoma infected livers. Furthermore, with increasing post transplantation time, few positive donor derived cells appeared in the hepatic parenchymal tissue and showed positivity for both albumin secretion and CDYL protein. Similarly, in another study done by Petersen et al., 1999, irradiated female rats received HSCs from male rats and their livers were subsequently injured by a treatment of carbon tetrachloride and 2-acetyl-aminofluorene, which prevented hepatocyte division and stimulated oval cell proliferation. The results obtained demonstrated that oval cells arose from hematopoietic precursors and gave rise to Y-chromosome-positive hepatocytes.

It is clear that a certain selective pressure is required to direct the donor cells towards liver repopulation and to reach an efficient conversion to hepatocytes. Mallet et al. highlighted the need for selection procedures to achieve significant hepatocyte replacement by HSC’s. They showed that bone marrow reconstitution and the presence of selective pressure are both necessary to obtain detectable levels of hepatocyte replacement.

(Kollet et al., 2003), stated that stress induced signals, such as increased expression of the chemokine stromal derived factor SDF-1, hepatocyte growth factor (HGF), and other proteolytic enzymes recruit human CD34+ progenitors with hematopoietic and/or hepatic-like potential to the liver of NOD/SCID mice and suggested this response as an important mechanism for tissue targeting and repair. The chemokine SDF, which attracts human and murine progenitors, is expressed by liver bile duct epithelium. SDF and HGF are increased following hepatic injury, inflammation or irradiation. Both mediate hepatic migration by CD34+ progenitors and synergize with stem cell factor. Accordingly, they suggest that an interplay between cytokines, chemokines and proteolytic enzymes regulates the migration of HSCs to the injured liver and their differentiation as part of liver repair.

A recent study of (Stadtfeld et al., 2005), demonstrated in a transgenic mouse model the minor role of the hematopoietic compartment in the adult liver ontogenetic development. They stated that hepatocyte differentiation from extrahepatic SC’s does not occur (or at very low level) in a non-injured model, and that the injury has to be strong enough and give advantage to the donor cells to seed and proliferate into the liver parenchyma. In our study the experimental murine schistosomiasis model showed the importance of liver injury as a selective pressure since we observed nearly no donor Y chromosome or CDYL positive cells in control liver sections of mice transplanted with BMC’s in absence of hepatic schistosomiasis. Also, most of the donor derived engrafted cells seemed to be trans-differentiated to inflammatory cells, bile ductules, blood vessels together with few hepatocyte like cells.
Conclusion:

Unfractionated BMC’s can repopulate the injured schistosomiasis liver without immuno suppression. The extent of liver injury, the timing of cell administration, the threshold number of transplanted cells and the interplay of cytokines in the liver pathology are all important variables in the process of engraftment and differentiation that still need to be further defined. However, the transformation of bone marrow derived stem cells into viable hepatocytes sustains the idea of in vivo cell transplantation to be a successful therapeutic approach.

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