Ultrastructural Study Of Hepatic Changes After Human Umbilical Cord Blood Stem Cell Transplantation In Chronic Murine Schistosomiasis

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Abstract: Background: The contribution of hematopoietic stem cells to liver therapy in different forms of liver injury remains debatable. In the last decade, the number of transplantations of hematopoietic cells derived from cord blood has increased, where numerous literature reports documented the feasibility and effectiveness of the transplantation of cord blood for the treatment of a broad range of disorders. **Aim of the work:** This xenogenic research is designed to highlight, by light and electron microscopic study, the possibility of engraftment of human umbilical cord blood derived stem cells in the livers of immune-competent mice infected with chronic schistosomiasis. **Materials and Methods:** This study was conducted on 20 Swiss Albino immune-competent mice. The mice were subdivided into four groups (5 mice each). (Group 1) was infected with *Schistosoma mansoni* cercariae for 20 weeks' duration, then intrahepatically transplanted with CD133+ human cord blood mononuclear cells, cultured on nutrient media, and isolated using the MACS Separation Unit from Miltenyi Biotec. (Group 2) was infected with *S.mansoni*, but not transplanted, (group 3) was normal and transplanted, and (group 4) was normal and non transplanted. All mice were sacrificed 3 weeks following the transplantation of groups 1 and 3. Engraftment of transplanted human cells was assessed by means of immunohistochemistry; using antibodies against human Hep Par1 and α-fetoprotein. Histological examination was performed using the Zeiss light microscope, and ultrastructural study was carried out by the Philips TEM 208 S electron microscope. **Results:** By light microscopic examination, livers of the infected transplanted group (group 1) and the infected non transplanted group (group 2) showed variable sized fibrocellular and fibrous schistosomal granulomas. (Group 1) exhibited as well more prominent bile duct proliferation than (group 2). Sections of (groups 1&3) showed small and large eosinophilic cells different from the surrounding murine hepatocytes. By immunohistochemistry, some cells in (groups1&3) sections showed positive cytoplasmic staining for the two anti human hepatocyte markers used; (Hep Par 1 and α-fetoprotein). Electron microscopic examination of (group 1) grids distinguished variable immature cells in the form of small progenitor cells, intermediate hepatocyte-like oval cells and larger premature hepatocytic cells. The transplanted healthy group (group3) showed similar cells. The previously noted cells were not seen in the remaining control groups, (groups 2&4). **Conclusion:** This research proved engraftment of the human umbilical cord blood hematopoietic stem cells after their intrahepatic transplantation into the livers of mice suffering from chronic hepatic schistosomiasis, and their attempt to give rise to premature forms of cells with hepatocytic lineage. Extensive studies are still needed to clarify the possible utility of these cells in resolving damaged organs and tissues.
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Key words: Umbilical cord, blood stem cell transplantation, Schistosomiasis, Hepatic changes.

1. Introduction

Treatment of liver disease has been greatly improved by the advent and evolution of liver transplantation. However as demand for donor organs continues to increase beyond their availability, the need for alternative liver therapies is clear ***(Jared and Sangeeta, 2008).***

Liver fibrosis occurs in the setting of chronic injury caused by different etiologies, constituting a serious worldwide public health problem. Schistosomiasis, hepatopathies due to viral hepatitis, drugs, alcohol, metabolic and autoimmune diseases, and congenital abnormalities are important causes of liver fibrosis ***(Oliveira et al., 2008).***

In Egypt, viral hepatitis along with infection with *Schistosoma mansoni* (*S.mansoni*) is one of the major causes of chronic liver disease ***(Halim, 1999).***Chronic infection by *Schistosoma mansoni* is considered a good experimental model of chronic hepatic disease and fibrosis. New therapeutic strategies aiming to minimize damages caused by hepatic fibrogenesis in chronic liver diseases are of great interest ***(Oliveira et al., 2008).***

Several studies performed on animal models have highlighted the potential of hematopoietic stem cells (HSC) to differentiate into hematopoietic cell lineages, and to transdifferentiate into non-hematopoietic cells including hepatocytes ***(Bunting and Hawely, 2002; Schwartz et al., 2002; Wang et***

al., 2003).Because of the lack of matched related donors and the risk of severe graft versus host disease (GVHD) that accompanies unrelated bone marrow or peripheral blood hematopoietic cell transplantation, alternative sources of hematopoietic stem cells have been considered ***(Lubin and Greene, 2008)***. Over the last few years, we have seen the growth in use of the common, often discarded stem cell source, umbilical cord blood. Cord blood stem cell transplantation successfully treated many diseases and debilitating conditions ***(Ballen, 2005; Newcomb et al.,2007).***

The frequency of umbilical cord blood (UCB) hematopoietic stem/progenitor cells equals or exceeds that of bone marrow (BM), and greatly surpasses that of adult peripheral blood ***(Mayani and Lansdorp, 1998; Sieff, 2008)***.Furthermore, UCB collection can be performed in a manner that entails less discomfort and morbidity for the donor than collection of bone marrow or peripheral blood stem cells. It is less expensive to store, and once banked, can be retrieved much more quickly than peripheral blood or bone marrow (***Lubin and Greene, 2008)***. Efforts to understand the plasticity of the stem cells found in cord blood may lead to advances in regenerative medicine ***(Sieff, 2008)***.***The aim of this work is*** designed to highlight, by light and electron microscopic study, the possibility of engraftment of human UCB derived stem cells in the livers of immune-competent mice with chronic schistosomiasis.

2. Materials and Methods:

Twenty female Swiss albino mice, 8 weeks old, weighing 20 ± 5gm were obtained, fed a standard commercial pelleted diet, and maintained under conventional conditions at the Schistosome Biological Supply Center (SBSC) of Theodor Bilharz Research Institute (TBRI). All animal protocols were conducted in accordance with the valid international guidelines for animal experimentation and were approved by the TBRI's animal research committee.

Ten (10) Mice were inoculated with *Schistosoma mansoni* cercariae. Eighty cercariae, in a single dose, were administered subcutaneously to each mouse. The remaining 10 mice were not inoculated with the cercariae. All 20 mice used in the study were subsequently kept at the SBSC for a further period of 20 weeks.

After taking the informed consent of twelve pregnant mothers, and following delivery of the full term infant, the umbilical cord was clamped and cut in the usual manner. Prior to separation and delivery of the placenta, a four to eight inch of the cord was cleansed with an antiseptic solution .A gauge needle, connected to a sterile cord blood collection bag containing an anticoagulant solution (citrate-phosphate dextrose and adenine) was inserted into the umbilical vein at the cleansed site, and the blood was allowed to drain into the bag by gravity. A range of (60-120ml) of blood was collected from each umbilical cord. UCB samples were diluted 1:3 in phosphate buffer saline (PBS). Low-density mononuclear cells were separated over Ficoll–Hypaque (density 1.077 g/dl; Pharmacia, Uppsala, Sweden).

CD133+ve HSC were isolated from UCB mononuclear cell samples by the positive selection method using the magnetic activated cell sorting columns and microbead-conjugated antibodies (MACS® and the CD 133 positive selection Kit, Miltenyi Biotec Gmbh, Bergisch Gladbach, Germany), as described by the manufacturer, in order to reach an optimum enrichment of the stem cells with depletion of the mature cells so as to escape the rejecting effect of the mature T cell according to ***(Schwartz et al., 2002 & Wang et al., 2003)***. Purity of sorted cells was assessed by fluorescence-activated cell sorting (FACS) analysis using anti-CD133 antibodies labeled with phycoerythrin (PE) from Miltenyi Biotec Gmbh.

Cells were plated (5-6 x 10^6 cells/ml) in 9 well culture plates with Dulbecco's modified eagle's media, supplemented with 20% fetal calf serum (FCS), Penicillin/Streptomycin (100U/ml, 0.1mg/ml), L-glutamine (2mM), all from Stem Cell Technologies Inc, Vancouver, Canada together with the following human recombinant growth factors and cytokines: Stem Cell Factor (SCF 100ng/ml), Flt3/Flk2-ligand (100ng/ml), Il 3 (20ng/ml) and Il 6 20ng/ml ,all from R&D Systems, Inc. Cells were incubated at 37̊ C in 5 % CO_2 in a humidified atmosphere for one week. The positivity and concentration of CD 133+ve cells was confirmed in each step with flowcytometry using anti-CD133 antibodies labeled with phycoerythrin (PE). The cells were expanded in this culture media for a period of 3 weeks.

In the 20th week post schistosomal infection, 10 non ablated mice were transplanted with 0.3 x10^6 umbilical cord blood stem cells each, through an intrahepatic injection using an insulin needle. 5 of the transplanted mice were infected, and the other 5 were selected from the normal (non infected) group of mice. The studied immune-competent mice (n=20) were divided into four groups, consisting of 5 mice each.

- Group (1): Mice infected with *Schistosoma mansoni* and transplanted with human umbilical cord blood stem cells (HUCBSCs).
- Group (2): Mice infected with *Schistosoma mansoni* and not transplanted with HUCBSCs.

- Group (3): Normal mice not infected with *Schistosoma mansoni* and transplanted with HUCBSCs.
- Group (4): Normal mice not infected with *Schistosoma mansoni* and left out without stem cell transplantation.

(Groups 1&3) mice were sacrificed 3 weeks after their transplantation with stem cells and their livers were subsequently processed for further histological and immunohistochemical examination. (Groups 2&4) mice were accordingly also sacrificed at the same time.

Livers from all mouse groups were fixed in 10% phosphate-buffered formalin for 24 hours and processed to paraffin blocks. Four-micrometer thick paraffin sections were then rehydrated and stained with hematoxylin and eosin (H&E). 15 liver sections were prepared per mouse.

The standard avidin-biotin immunoperoxidase technique was used ***(Hsu et al., 1981)***. From each mouse 10 paraffin sections were prepared and stained with the primary antibodies against human Hep Par1 (Dako, Denmark), and the other 5sections were alternatively incubated with the primary antibodies against α- fetoprotein (Santa Cruz Biotechnology, USA). Liver sections with voluntary omission of the primary antibody and its replacement by PBS served as negative control.

Liver tissue specimens of the different groups of mice were minced into tiny pieces about $1mm^3$ 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 one hour and washed in distilled water. Dehydration in ascending grades of ethyl alcohol (50, 70, 90 and 100% ethanol) at 5 min gap was then performed. The specimens were immersed in Epon for 3 successive days, and polymerized at 60 ˚C for 48 hours. Semithin sections were cut, stained with methylene blue azur Π, and examined by light microscopy to choose the proper site for ultrathin sectioning. Then ultrathin sections were prepared using a Leica ultracut R ultramicrotome, and mounted on copper grids then double stained with uranyl acetate and lead citrate. Sections were examined under TEM (Philips EM 208S) electron microscopy.

3. Results:

Liver sections of Schistosoma infected and umbilical cord blood stem cell transplanted mice (group 1), and infected non transplanted mice (group 2) showed partial apparent disturbance of the hepatic lobular architecture due to the presence of many scattered different sizes rounded and ovoid schistosomal granulomas of mostly fibrocellular granulomas representing the characteristic picture of the chronic stage of schistosomal infection. Fibrocellular granulomas were formed of central schistosomal ova surrounded by concentrically arranged chronic inflammatory cells, mainly lymphocytes, macrophages, epithelioid cells and by bundles of fibrous tissue. The rest were fibrous granulomas formed of fibrous bundles and reticular fibers. The granulomas may showed central calcified ova, or degenerated or absent ova. The portal tracts were enlarged by the presence of granulomas, increased deposition of collagen fibers around the portal blood vessels and the bile ductules, and the proliferative reaction of bile ductules.

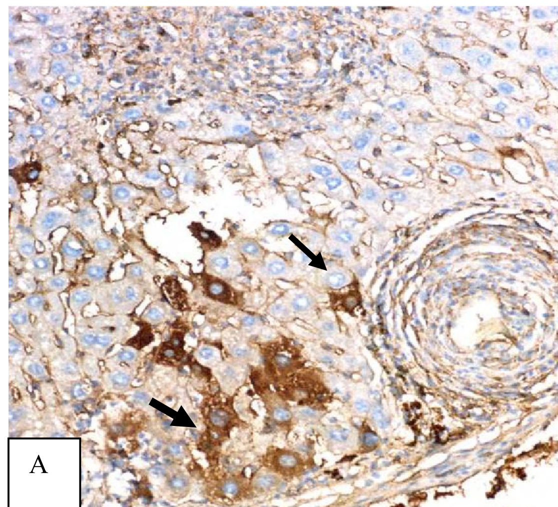
Besides the characteristic picture of schistosomal granulomas, sections of the infected transplanted livers of (group 1) mice revealed small and large cells with an intensely stained eosinophilic cytoplasm inside the hepatic lobule, which could be easily differentiated from the surrounding murine hepatocytic cells. Larger cells were more frequently seen than the smaller ones. These cells showed either central or eccentric basophilic nuclei, and were present either singly scattered throughout the hepatic lobule, or in groups. They were also present at the periphery of the granulomas, in periportal areas, and inside and around central veins This group exhibited as well more prominent bile duct proliferation as compared to the infected only group (group 2), where bile duct proliferation in both groups was represented by groups of primitive bile ductules lined by cubical and some flattened epithelial cells with centrally located basophilic nuclei occupying most of the cytoplasm. In this infected transplanted group, some of the previously mentioned eosinophilic cells could be identified in the vicinity of and inside some of the bile ductules and vessels. By examining the liver sections of the normal transplanted mice of (group 3), they equally revealed the presence of similar eosinophilic cells between the normal murine hepatocytes and encircling the central veins. The smaller-sized cells were the dominant type in this group, some of which were ovoid in shape and some had angulated borders. The normal histology of murine liver was illustrated in Hematoxylin and Eosin stained liver sections of the normal non transplanted group (group 4).

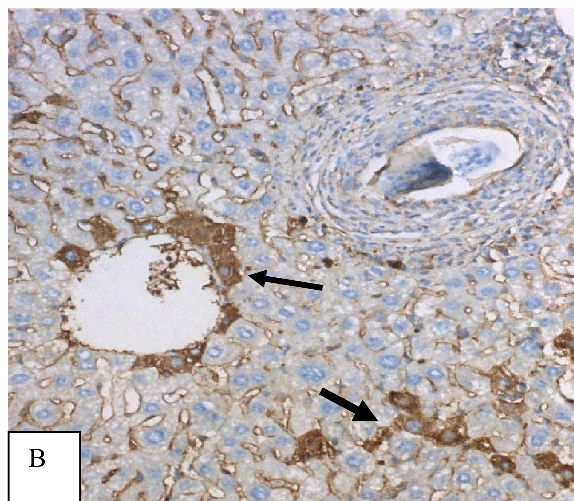
Immunohistochemically, By applying the two anti-human hepatocyte markers; Hep Par 1and α-fetoprotein to the four groups of mice, by light microscopic examination revealed that :

-Liver sections of the infected transplanted (group 1) mice showed positive cytoplasmic staining of some cells intercalated between mouse hepatocytes,

adjacent to blood vessels, around central veins, at the periphery of portal tracts and around granulomas, either present singly or in clusters.

-Liver sections of the normal transplanted (group 3) mice showed positive staining of a number of cells for both markers. Negative staining for both markers was clearly demonstrated in the infected non transplanted group of mice, (group 2) and the normal non transplanted group of mice, (group 4).





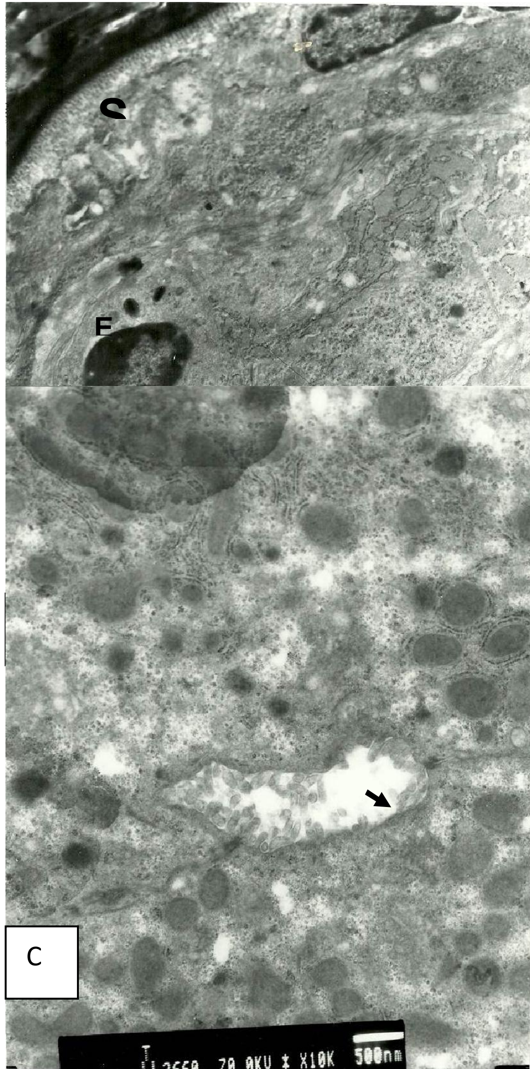
Fig(1) (A:)A liver section from infected transplanted mouse showing cells with positive cytoplasmic staining of anti-human (Hep par1)seen nearby a granuloma (thin arrow)and interspersed between the rest of negatively stained hepatocytes(thick arrow) x200.

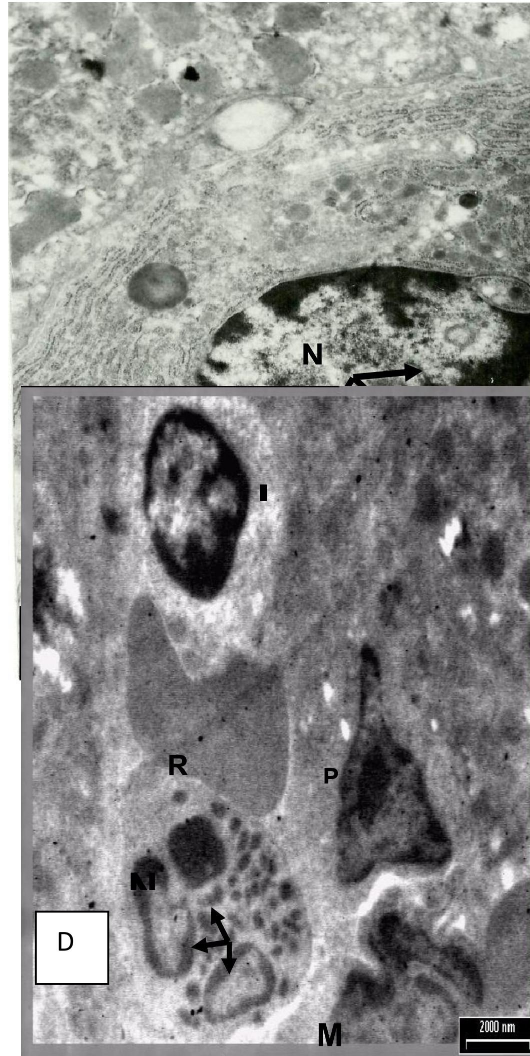
(B): A liver section from infected transplanted mouse showing cells with positive cytoplasmic staining for anti human(alpha fetoprotein).These cells are present around a central vein(thin arrow) and interspersed in between the rest of negatively stained hepatocytes(thick arrow) x200

Electron microscopic examination of the ultrathin sections of livers of the infected transplanted (group 1) mice revealed the presence of fibrocellular and fibrotic granulomas, which also represented the main constituent of ultrathin sections of the infected non transplanted mice of (group 2). Some damage attributed to schistosomal infection was seen in the bile canaliculi of the infected transplanted and infected non transplanted groups with partial loss of their microvilli Moreover, the infected groups of mice, (groups 1&2) showed congested sinusoidal spaces impacted with RBCs, and hypertrophied kupffer cells usually located at the junction of two sinusoids, having plumpy irregular nuclei, loaded with schistosomal pigment, and having primary and secondary lysosomes. Signs of hepatocyte regeneration were more clearly seen in (group 1) with proliferated rough endoplasmic reticulum encircling the mitochondria, binucleation, and irregularity of the nuclear membrane.

Besides the previously detected pathological changes, while screening the grids obtained from the infected transplanted mice three types of immature cells were identified; small oval-shaped cells of average diameter (5μ), (progenitor cells), oval-shaped cells with an average diameter of (7μ) showing large oval nuclei, a high nuclear cytoplasmic ratio and marginated chromatin, (intermediate_hepatocyte-like cells), and lastly cells with an average diameter of (14μ) having few mitochondria and endoplasmic reticulum, looking larger and more mature than the former cells and resembling mature hepatocytes, but at the same time being smaller and less mature than the normal hepatocytes, (premature hepatocytes).The intermediate hepatocyte-like cells and the larger premature hepatocytes were more commonly seen in grids screened from this group. Such cells were seen in between inflammatory cells at the periphery of granulomas, at the sinusoidal poles of hepatocytes, and also in periportal areas. Similar cells sharing the same morphological criteria of the previously mentioned immature cells were also seen in liver sections from (group 3), the normal transplanted group of mice. In this group however, they were mainly composed of the first category of small oval-shaped cells (the progenitor cells). Apart from the above noted pathological changes, most of the surrounding hepatocytes of the hepatic lobule in (groups 1, 2 &3) revealed the normal ultrastructure of murine liver as presented in the normal non

transplanted control group of mice(group 4). Hepatocytes measured (20-30μ) across with their prominent nuclei having an average diameter of (10μ), showing finely dispersed chromatin condensed slightly around the nuclear envelope, and containing a large nucleolus. Binucleation was seen in some of the hepatocytes. The cytoplasm of hepatocytes was packed with organelles, including lots of mitochondria, rough and smooth endoplasmic reticulum, and Golgi complexes, which implies the active indulgement of the hepatocyte in protein synthesis. The sinusoids were clearly identified as wide vascular channels lined by endothelial cells.





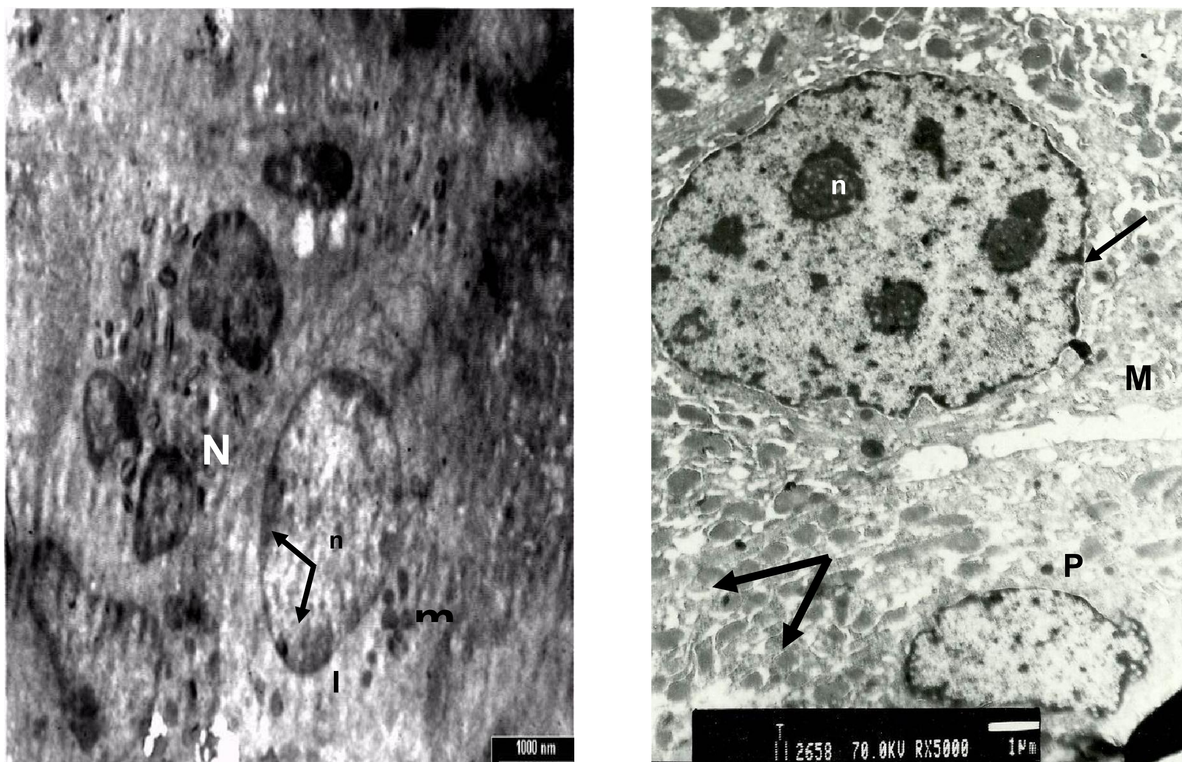


Figure (2).(A): Electron micrograph of a schistosomal egg shell(Sh) along with an eosinophil(E) showing the characteristic coffee-bean granules(arrows) and an adjacent fibroblast(F) as a part of fibrocellular granuloma. X6000**(B):** Electron micrograph showing part of fibrocellular granuloma. A plasma cell is identified by the characteristic cart-wheel appearance (arrow) of its nucleus (N) and Rer arranged in a circular configurations.x7500. **(C):** Electron micrograph displaying schistosoma infected transplanted mouse liver with damage to a bile canaliculus in the form of partial loss of its microvilli(arrow). There are adjacent hepatocytes showing cytoplasm full of mitochondria (Mt) x10000. **(D):** Electron micrograph showing small progenitor cell(Pr) allocated at the sinusoidal surface of a hepatocyte, and an intermediate oval hepatocyte like cells(Int) with an oval shaped nucleus and more condensed heterochromatin than euchromatin, along with inflammatory cells , Neutrophil (N) with trilobed nucleus(arrows) and macrophage(M).X2000. **(E):** Electron micrograph from infected transplanted group showing an intermediate hepatocyte like oval shaped cell (Int). The N/C ratio is high with the nucleus (N). Few mitochondria (mt) can be spotted in its cytoplasm. X4500.
(F): Electron micrograph showing premature hepatocytes(P) smaller in size than another adjacent large mature hepatocyte(M). The larger hepatocyte revelae signs of regeneration in the form of (irregularly nuclear membrane (thin arrow). Multiple nucleoli (n), and increased number of increased number of organelles e.g. mitochondria (thick arrow) x5000.

4. Discussion:

Hepatic fibrosis occurs in the setting of chronic liver diseases of different etiologies. Spontaneous regression of fibrosis occurs when the stimulus for hepatic liver damage is removed, and in some diseases the stimulus cannot be completely eradicated, in addition to being a slow process ***(Oliveira et al., 2008)***. Thus, it is of great interest to explore new effective therapeutic techniques in an attempt to ameliorate the damages caused by fibrogenesis in chronic liver diseases. ***Lorenzini et al. (2008)*** stated that in this scenario, stem cell therapy sounds particularly attractive for its potential to support tissue regeneration, requiring minimally invasive procedures with few complications.

BM hematopoietic stem cells for long have been clinically introduced in regenerating many diseased organs including the liver, but UCB hematopoietic and mesenchymal stem cells are now newly proposed in the field of regenerative medicine as a better and less invasive way to be used as a substitute for other types of stem cells ***(Lee et al., 2004)***.

This research intended to demonstrate the fate of human UCBHSCs after inoculation into murine diseased liver, in an attempt to set an experimental model of *in vivo* hepatocyte differentiation from stem

cells. In this case, chronic schistosomiasis was selected as an example of liver disease. Schistosomiasis has been studied extensively since its discovery by Theodore Bilharz in 1851 ***(Abdul-Ghani and Hassan, 2010)***. HCV infection along with schistosomiasis is two major prevalent chronic debilitating diseases that threaten the Egyptian population ***(Halim, 1999; El-Awady, 2006)***.

In the present study, histopathological examination of the livers of Schistosoma infected and UCB transplanted mice, and the infected non transplanted mice revealed the typical picture of chronic hepatic schistosomiasis, in the form of fibrocellular and fibrous granulomas of variable shapes and sizes. Bile duct proliferation was noticed in the liver sections as a consequence of schistosomal infection. It was though more prominent in the infected transplanted group, most probably due to the regenerative response induced by the inoculated stem cells.

In addition, light microscopic examination of the H&E stained liver sections of the infected transplanted group showed identifiable eosinophilic cells in between the murine hepatocytic cells, present singly and in groups. These cells were also allocated at the periphery of the granulomas , in periportal areas, and around central veins. Similar cells were present in livers of the normal transplanted mice as well. Such results demonstrate the presence of distinctive non murine hepatocytes in the hepatic lobule of the stem cell transplanted mice.

Using the anti human hepatocyte markers (Hep Par 1 and α-fetoprotein), the livers of the infected transplanted group and the normal transplanted group showed cytoplasmic positivity of a number of cells. Negative staining for both used markers was clearly noticed in the liver sections of the infected non transplanted group (group 2), and the normal non transplanted group (group 4). This provides evidence of successful transplantation of the injected UCBHSCs in the livers of the experimented mice of (groups 1 and 3), and enhances the fact that these detected cells are of human origin.

Though HepPar1 is considered an excellent marker of human hepatocyte lineage, it is known to be present, not only in fully differentiated human hepatocytes, but also in very early stages of liver development such as in human hepatoblasts ***(Turrini et al., 2004).*** Alpha fetoprotein is also considered to be an early hepatocytic marker ***(Synkers et al., 2006)***, thus expression of such markers in our experiment might help in the allocation of premature hepatocytic cells that appear in liver tissue of mice after their intrahepatic transplantation with stem cells.

In accordance with the above results, ***Newsome et al. (2003)*** demonstrated the expression of the HepPar1 human hepatocyte-specific antigen in livers of NOD/SCID mice after inoculation with HUCBSCs, and concluded that cells from human cord blood "become mature hepatocytes" in livers of such mice. Likewise, ***Kogler et al.(2004)*** transplanted adherently proliferating cells isolated from human cord blood into livers of fetal sheep. They observed the expression of albumin and human hepatocyte-specific antigen after transplantation, and concluded that the human cord blood cells differentiated into human parenchymal hepatic cells.

Ishikawa et al. (2003) detected human albumin and the HepPar1 antigen in livers of immunodeficient mice, postulating that the engrafted cells from human cord blood "functioned as hepatocytes." However, other scientists remained skeptical because they observed the expression of some, but not all hepatocyte markers that should be expressed by a real human hepatocyte ***(Brulport et al., 2007)***.

In a study done by ***Akl et al. (2009),*** transplantation with HUCBSCs in mice following their injury with CCL4, the inoculated undifferentiated cord blood cells proved homing into the livers with subsequent development of hepatocyte-like cells. In addition, a reduction in collagen deposition in livers of these mice was noticed.

By electron microscopy, ultrathin sections of the livers of mice of both the infected transplanted and the infected non transplanted groups in the current experiment showed many fibrocellular and fibrous schistosomal granulomas. Sinusoidal spaces were seen congested with RBCs, and showed scattered hypertrophied kupffer cells with schistosomal pigment and primary and secondary lysosomes. This was perceived as a reaction to the Schistosomal infection. Regenerating hepatocytes were also present, having proliferated rER encircling the mitochondria, irregular nuclear membranes, and showing binucleation. Regenerative signs were otherwise more prominent in the infected transplanted group than in the infected non transplanted group of mice.

Electron microscopic examination of the grids prepared from the transplanted groups of mice helped distinguish immature relatively small ovoid progenitor cells of average diameter (5μ), medium sized intermediate hepatocyte-like oval cells of average diameter (7μ) showing large oval nuclei, a high nuclear cytoplasmic ratio and marginated chromatin, and larger premature hepatocytic cells with an average diameter of (14μ). These large cells had few cytoplasmic organelles in the form of mitochondria and endoplasmic reticulum, approaching the morphological characteristics of mature hepatocytes. The above mentioned different forms of cells were situated mostly at the sinusoidal surface of hepatocytes.

In accordance with the present results, ***Sell (1997)*** previously described proliferation of small "null" intraportal cells that sequentially acquired markers of differentiation to hepatocytes after the acute form of allyl-alcohol liver injury. He noted that the progeny of these cells then extended across the necrotic zone, and acquired differentiation markers that reflected markers seen during maturation of the fetal liver. His examination of these cells by electron microscopy revealed three cell types similar to those found in chronic ductular reactions and focal nodular hyperplasia of human liver which he named: type I, small immature or progenitor cells; type II, bile duct-like progenitor cells; and type III, hepatocyte-like progenitor cells. Sell concluded that liver stem cells differentiate through stages of "restitutive" and "transitional" hepatocytes before developing the appearance of mature hepatocytes. ***Sell (2001)*** stated that the terms oval cell, transitional hepatocyte, atypical ductular cell, neocholangiole,etc., are used to describe the previous cells. He also reported the presence of redundant proliferating bile ducts in zones of liver injury which we previously mentioned in our study by light microscopic examination of the specimens of the infected transplanted group of mice.

The results of the present work suggest that the transplantation of cord blood stem cells in the livers of experimented mice has contributed in some way to the generation of premature forms of hepatocytic cells on their way to differentiate into mature hepatocytes. This proves that HUCBSCs can give rise to cells of hepatic lineage.

In agreement with the results obtained in this work, ***Sobaniec-Lotowska et al. (2007)*** detected two types of oval cells in liver biopsies from children with chronic hepatitis B; *hepatic progenitor cells* and *intermediate hepatic-like cells.*These cells were present in the parenchyma and were seen most commonly in areas of intense periportal fibrosis, and in the vicinity of the limiting plate of the lobule. There was a distinct relationship between the prevalence of these oval cells and fibrosis stage. Hepatic progenitor cells were small (usually not exceeding 5 microns) and oval or nearly oval in shape. Intermediate hepatocyte-like cells varied in size, and were twice as large as the hepatic progenitor cells, whereas their diameter did not exceed one-half of the diameter of the mature hepatocytes. The nuclei were less abundant in heterochromatin compared to the progenitor cell nuclei, and occasionally contained nucleoli. Frequently, these nuclei resembled the nuclei of mature hepatocytes. The intermediate hepatocyte-like cell cytoplasm contained better developed cell organelles, mainly mitochondria and elements of the endoplasmic reticulum.

In a study done by ***Xiong et al. (2001)***, investigating cases of chronic viral hepatitis, similar hepatic oval cells were allocated predominantly in the periportal region and fibrosis septa, characterized by an ovoid nucleus, small size, and scant cytoplasm.
These results showed that UCB and BM cells cultured with the combination of FGF-1, FGF-2, SCF, and HGF were capable of producing hepatocyte lineage cells in vitro. Thus human UCB as well as BM cells proved their ability to generate hepatoblasts in an in vitro culture system in a proportion noted to be about (13%) in UCB and (23%) in BM according to the CK-18 expression. They concluded that UCB cells as well as BM cells differentiated into mature hepatocytes via the hepatic progenitor cells in the primary culture system. For clinical application, it is necessary to refine the methods to expand and isolate the generated hepatoblasts as well as to determine the specific hepatoblast markers ***(Jung et al., 2008)***.

The results of the current research showed that the intermediate- sized oval cells and premature hepatocytes constituted the dominant forms of newly formed cells observed in the grids of the infected transplanted group. It is explainable that the immature cells allocated here are mostly of the more differentiated type, since the mice in this experiment were sacrificed 3 weeks after transplantation. Thus, the stem cells here had plenty of time to develop and differentiate, in contrary to other reported studies where the sacrifice of mice was performed hours after the acute liver injury ***(Sell, 1997; Sell, 2001)***. In the normal transplanted group however, light and electron microscopic examination highlighted that the relatively small ovoid progenitor hepatocytic cells were present more obviously than the medium sized intermediate oval cells and the larger premature hepatocytes. An explanation could be that the inoculated stem cells have successfully engrafted into the murine hepatic lobule, but there was no triggering infectious stimulus to motivate these cells to differentiate into the larger more mature forms, leaving the small progenitor cells as the mainly recognized type of cells.

Transplantation of UCBHSCs into the livers of non irradiated mice infected with *S.mansoni* cercariae was carried out in this study, without prior immunosuppression therapy. Non-ablated immune-competent Swiss Albino mice were sacrificed 3 weeks after their intrahepatic infusion with the stem cells, and demonstration of the actual transplantation of UCBHSCs followed by the study of their influence on the liver was carried out by utilizing both light and electron microscopy. This study demonstrated that the human-derived stem cells successfully engrafted into the mouse livers that were not previously subjected to immunosuppression.

It was previously reported by ***Nilsson et al. (1999)*** as well as ***Weiss et al. (2003)*** that stem cells could survive till 6 weeks after transplantation into either the brain or the peripheral blood without the need for immunosuppression of the host. Similarly, ***Weiss et al. (2003)*** transplanted porcine stem cells derived from umbilical cord mucous connective tissue into rat brains without immunosuppression ***(Elkhafif et al., 2006)***.

This fact that hematopoietic stem cells were able to overcome host immunosurveillance, thus avoiding problems of immune rejection , could be an important advantage of the stem cell based therapy in comparison with whole organ transplantation ***(Elkhafif et al., 2006)***.

Induction of tolerance, as another alternative to immunosuppression, has been suggested and explored by other authors. It is generally well established that auto-reactive T cell clones are completely deleted from the immune repertoire during fetal development. Indeed rats, intrafetally tolerated against human liver antigens, accept xenotransplantation with hepatocytes coming from the same donor ***(Turrini et al., 2004)***.

In conclusion, the transplanted UCBHSCs in this current study proved engraftment into the livers of mice. However, to what extents can these cells repopulate a damaged liver is still under investigation. The stage of schistosomiasis, and the time interval between their infection, their transplantation with stem cells, and their sacrifice might be important variables affecting different experimental models used by different researchers.

Establishment of a suitable model to study the extent of adult extrahepatic stem cell contribution to hepatic regeneration was felt mandatory. Mouse infected with schistosomiasis was chosen and was considered a suitable option for this study. Schistosomiasis was selected, being a disease that causes less complex architectural changes compared to known forms of liver cirrhosis ***(Coelho et al., 2008 a,b).***

There are several potential advantages of using adult rather than embryonic stem cells to regenerate tissues including fewer ethical concerns, better known biological behaviour, easier accessibility and, therefore, lower costs. Cord blood contains multiple populations of embryonic-like and other pluripotent stem cells capable of originating hematopoietic, epithelial, endothelial, and neural tissues both in vitro and in vivo. The isolation of HSCs and MSCs from cord blood is a relatively new procedure and only few studies have been published ***(Lorenzini et al., 2008)***.

It would be helpful to use immuno- electron microscopy techniques in such studies. This helps to obtain a more precise evaluation of the source of the identified immature hepatocytic cells. Such technique might clarify whether the immature hepatocyte-like cells that appeared on electron microscopic examination were the product of the transplanted human umbilical cord blood hematopoietic stem cells, or the mobilized bone marrow hematopoietic stem cells from the mouse itself. Although anti human hepatocyte (Hep Par 1and α- fetoprotein) marker positivity claims the actual transplantation of human cord blood stem cell in this study, this positivity could be quantitatively and more objectively traced more accurately by immuno-electron microscopy.

A broader understanding of the biological properties and use of extra-hepatic adult stem cells is required before proceeding into clinical applications. The possible carcinogenic potential of former types of stem cells should be investigated. We concluded that the engraftment of the human umbilical cord blood hematopoietic stem cells after their intrahepatic transplantation into the livers of mice suffering from chronic hepatic schistosomiasis , and their attempt to give rise to premature forms of cells with hepatocytic lineage. Extensive studies are still needed to clarify the possible utility of these cells in resolving damaged organs and tissues.

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