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DIFFERENTIATION & HOMING OF BONE MARROW-DERIVED MESENCHYMAL STEM CELLS IN THE LIVER SCHISTOSOMA INFECTED MICE

By
AMIRA A. SALEH[1*], AYMAN A. SALEH[2], ENAS F. ABDEL HAMED[1], EMAN M. FAWZY[1] and MARWA A. SALAMA[1]
Department of Parasitology[1], Faculty of Medicine, and Department of Animal Wealth Development, Genetics & Genetic Engineering, Faculty of Veterinary Medicine[2], Zagazig University[1,2], Egypt (*Correspondence: amera.islam2019@gmail.com)

Abstract

Schistosomiasis is an endemic parasitic infective disease that remains widespread in several countries. This work aimed to evaluate the curative influence of bone marrow derived mesenchymal stem cells (BM-MSC) engraftment in liver of schistosomiasis chronic infected mice. 30 Swiss albino mice divided into 3 groups of 10 mice each. G1: normal healthy group, G2: infected with *Schistosoma mansoni* cercariae subcutaneously and G3: infected and transplanted with BM-MSC on their 8th week post infection by intravenous injection. All mice were sacrificed 4 weeks post-transplantation. Serum was collected for assessment of albumin, ALT & AST levels. Engraftment of BM-MSC was assessed by labeling with PKH26. Histopathological and ultrastructural studies were carried out. Expression of capase-3 & IL-1βas a marker of apoptosis and inflammation respectively were measured by quantitative RT-PCR. Homing of transplanted BM-MSC within the injured liver was confirmed by fluorescent microscopic examination. MSC labeled with PKH26 were recovered in liver. Histopathological and electron microscopic examinations of liver showed differentiation of transplanted BM-MSC into hepatocyte-like cells. Liver function was improved confirmed by elevation of serum albumin and decrease of ALT & AST in G3. Real-time PCR revealed that BM-MSC inhibited capase-3 and suppressinterleukin-1β mRNA expression in damaged liver tissues. MSC-treated group had the least apoptosis cells and inflammatory cells. Transplantation of BM-MSCs has therapeutic anti-apoptotic anti-inflammatory mechanisms effect in chronic liver disease.

Key words: Albino mice, *S. mansoni*, Liver, Mesenchymal stem cells.

Introduction

Schistosomiasis is an endemic parasitic infective disease prevalent in several countries. In spite of the major efforts to control schistosomiasis with considerable reduction in morbidity and mortality, it continue to spread to new areas (Patz *et al*, 2000). To elucidate mechanisms involved in fibrogenic process of *Schistosoma mansoni* chronic infection experimental models of hepatic fibrosis are used (Olivera *et al*, 2008). Along with schistosomiasis, hepatopathies caused by drugs, metabolic & autoimmune diseases, alcohol, viral hepatitis in addition to congenital anomalies considered as leading causes of hepatic fibrosis (Olivera *et al,* 2008). In Egypt, co-infection of viral hepatitis with *S. mansoni* considered as one of the leading causes of chronic hepatic disease (Halim *et al,* 1999). In chronic liver disease new therapeutic strategies are of great interest to minimize damage of hepatic fibrogenesis (Krause *et al,* 2001).

Mesenchymal stem cells (MSCs) were emerged as an effective advance in treatment of different diseases with minimal invasive procedures and few complications (Salama *et al,* 2010). MSCs are multipotents, capable of differentiating into functional hepatocytes. They are effective in liver structure regeneration together with recovery of liver functions (Ishii *et al,* 2008). Apoptosis of hepatocyte, an outcome of chronic hepatic disease, and one of the hall marks of hepatic fibrosis, can lead to a fibrous scar (Friedman, 2008). Caspases are cluster of enzymes participate in regulation of the apoptotic process with the subsequent classical features. Triggering of Capase-3 in response to variable apoptotic stimuli activate endonuclease and prompt DNA fragmentation (Luo *et al,* 2010). The damaged

hepatocytes alongside with the involved inflammatory cells release mediators as IL -6, IL -10, IL-13& IL-1β (Nasir *et al*, 2013). IL-1β, a potent inflammatory cytokine, considered as an important constituent of the innate immune response (Yang *et al*, 2003), that involved in various cellular functions such as proliferation, activation and differentiation (Dinarello, 1996).

The present study aimed to highlight homing & engraftment abilities of transplanted bone marrow derived MSCs (BM-MSCs) in murine schistosomiasis as a future anti-fibrotic anti-apoptotic therapeutic evolution. Engraftment of transplanted stem cells was assessed by labeling with PKH26 (Paul Karl Horan 26).

Materials and Methods

Experimental animals: Thirty female Swiss Albino mice, 7 weeks old weighing about 25 gm were maintained at appropriate environmental conditions in the Schistosome Biological Material Supply Center (SBSC), Theodor Bilhariz Research Institute (TBRI), Giza. Animal protocols were followed in agreement with the international guidelines for animal research and were permitted by the TBRI's animal research team.

Animals infection: Each mice was infected by subcutaneous injection of 80±10 cercairae. Mice were classified into 3 main groups of 10 mice each. GI: healthy mice (control group). GII: *S. mansoni* infected mice injected with intravenous phosphate buffered saline (PBS). GIII: infected mice with *S. mansoni* cercariae and received MSCs. After twelve weeks post- infection, blood was obtained from the retro-orbital vein, then all mice groups were sacrificed and livers were processed for paraffin sections and electron microscopic (EM) examination (Philips TEM 208s).

BM-derived MSC Preparation: BM-MSCs were obtained from Department of Biochemistry, Kasr Al-Ainy Medical Faculty.

Donor marrow was obtained by flushing of femur & tibiae of 6-week-old male mice with Dulbecco's Modified Eagle's Medium (DMEM) fortified with 10% fetal bovine serum. Nucleated cells were separated according to a density gradient, and then suspended in complete culture medium after adding 1% penicillin-streptomycin to it. Incubation of cells were in 5% humidified CO_2 for 12-14 days at 37°C till development of enormous colonies (80-90% confluence). Phosphate-buffered saline (PBS) was used for culture wash then the culture released with trypsin (0.25%) in 1mL ethylene diamine-tetra acetic acid (EDTA for 5 min at 37°C). Finally, centrifugation was done and the cells were suspended with the serum-supplemented medium and incubated in $50cm^2$ culture flask (Falcon) (Al Hadlaq and Mao, 2004). Characterization of MSCs in the culture was recognized by the fusiform shape and their adhesiveness (Rochefort *et al*, 2005)

Concerning labeling, MSCs were collected throughout the 2^{nd} passage and marked with PKH-26 dye (Haas *et al*, 2000). After centrifugation, cells washed two times in a serum free medium, after that they pelleted and resuspended in dye solution.

Cells transplantation: Was done by injection of male non-fractionated BM-MSCs intravenously in infected female mice on 8^{th} week after-*Schistosoma* infection in the dose of $1x10^6$ cells/mouse suspended in 0.3ml (PBS).

Histopathological and ultrastructural studies were carried out. Liver function tests were assayed. Expression of capase-3 and IL-1βas markers of apoptosis and inflammation were measured by quantitative RT-PCR.

Liver function parameters: Level of Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and albumin were determined spectrophometrically in the serum by an automatic analyzer using conventional laboratory methods.

Liver histopathology: After fixation in buffered formalin (10%), collected tissue were dehydrated then processed for paraffin sections. After that, the samples were deparaffin-

ised then stained by hematoxylin & eosin (H & E). Hepatic inflammation graded (Knodell *et al*, 1981).

Transmission electron microscope (TEM): Small liver specimens ($1mm^3$) were fixed in 2.5% gluteraldehyde solution followed by 1%. Osmium tetroxide, dried up then placed in epoxy resin. Collected ultrathin sections on copper grids were stained with uranyl acetate & lead citrate. Lastly, they examined and photographed under electron microscope (Philips TEM 208s).

Quantitative RT-PCR analysis for mice caspase-3 & IL-1β*:* Liver of mice, total RNA was isolated by RNeasy Mini Kit including DNase I digestion according to manufacturer's guidelines (Qiagen, #74104). Quantiscript reverse transcriptase was used to reverse transcribe RNA. After reverse transcription a Rotor-Gene (Q2plex, Qiagen) was used to achieve quantitative real-time PCR analysis using SYBR green master mix (Roche Diagnostics). Primer 3 web-based tool used to design Primers by (http://www-genome.wi.mit.edu/cgibin/primer/ primer3_www.cgi) depending on the published mice sequence. The subsequent primers were used for the study: mice *caspase-3*, 5'-GACCAT ACATGGGAGCAAGT-3' and 5'-CCTTCA TCACCATGGCTTAGA3'; mice*IL-1β,* 5'-G GAGAACCAAAGCAACGACAAAAATA -3' & 5'-TGGGGAACTCTGCAGACTCAA AC-3'; mice *GAP D H*, Gen-Bank Accession No. M32599; 5'-TGTGTCCGTCGTGGAT CTGA -3'; and 5'- CCTGCTTCACCACCTT CTTGA -3'. Mice gene expression was standardized to mice *GAPDH*. Thermal cycling consist of a primary step at 95°C for 10min then 40 cycles of amplification (denaturation step at 95°C for 15s, annealing at 60°C for 1min) in accordance with the manufacturer's guide. The critical thresholds (Ct) of the targeted gene were standardized with *GAPDH* quantities (Ct) by $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). Calculation of $2^{-\Delta\Delta Ct}$ determined the fold change in gene expression in the treated groups with respect to controls.

Statistical analysis: One-way analysis of variance (ANOVA) was used to assess the influence of mscson serum levels followed by least significant difference (lsd) test as post hoc test. Analysis was performed via statistical package for social sciences version 22.0 (Ibm Corp., Armonk, NY, USA). Results were reported in means ± SD. *p* value < *0.05* was used to indicate significance

Results

Confirmation of homing of BM-MSCs: Fluorescent microscope was used to distinguish PKH-26 labeled cells appeared as bright dots in liver tissue in MSC treated group (Fig.1A).

Liver histopathology: H & E stained liver sections of GIII showed marked regenerative changes as well as decrease in apoptotic changes in hepatocytes and inflammatory cellular infiltrates compared to the infected control group. Histopathological studies were focused on the homing of transplanted BM-MSCs inside the liver of recipient females that exhibited presence of small dispersed cells resembling hepatocyte, with small rounded central nuclei (Fig.1B), a marked proliferation of primitive and incomplete small groups of bile ducts was observed as well as an increase of recently formed tiny blood vessels in liver parenchyma (Fig.1C). Regarding inflammatory reactions and apoptosis in GIII, there was marked inflammatory cellular infiltrate (grade IV) and mild induced apoptosis within hepatocytes (Fig.1D).

Electron microscopic examination of liver sections in GIII confirmed the regenerative histopathological findings. The peripheral parts of the granuloma revealed the presence of newly formed hepatocytes, in cells opposite the sinusoids as well as the periportal area. These cells were distinguished into: a rather small immature cells with big ovoid nuclei, increased nuclear cytoplasmic ratio with marginal chromatin plus moderate sized cells. Also, there were mature cells with the endoplasmic reticulum and mitochondria upcoming the morphological structures of

liver cells (Fig. 2A). These cells were not detected in the control infected and healthy groups. Mature hepatocytes with features of regeneration were observed as well. Cells with noticeable nucleoli, irregular nuclear membrane and proliferated rough endoplasmic reticulum encircling mitochondria also were detected (Fig. 2B). A newly formed bile ductile and newly formed blood vessels were in infected and transplanted group (Fig. 2C).

Liver function parameters: There was a significant increase in serum albumin in group III in comparison to infected controls (P<0.0001) (Tab. 1). Liver enzymes (ALT &AST) were significantly reduced in GIII comparable to the infected control group (P<0.0001, 0.0004) respectively. A high statistically significant difference in LSD between healthy control versus control infected and the control infected comparable to treated group at all liver functions investigations, But, without significant difference amongst healthy control and treated group at all of those parameters.

Real-time PCR: There was a significant low expression of apoptosis and inflammation related genes, IL-1β as well as caspase-3 in group III when compared to control infected group (Tab. 2).

Table 1: Mean values of ALT, AST and albumin in serum level of groups.

Groups	Albumin (gm/dl)	ALT (U/L)	AST (U/L)
Control healthy	3.90±0.29[a]	30.2±4.59[b]	60.2±12.68[b]
Infected control	2.65±0.71[b]	77.2±32.15[a]	156.3±88.76[a]
Infected + MSCs	3.4±0.59[a]	36±10.14[b]	65.7±11.98[b]
F- value	12.77	17.02	10.68
P-value	0.0001	<0.0001	0.0004
LSD	0.51	18.02	47.93

[a, b]=significant difference.*P<0.05, F = Test for ANOVA.

Table 2: Effect of BM-MSCs transplantation on apoptosis and inflammation related genes expression.

GENE	Caspase-3	IL1β
Control	1±0.18 [b]	1.1±0.16 [b]
Infected	4.23±0.23[a]	4.97±0.34 [a]
Infected + MSCs	1.61±0.11 [b]	1.94±0.27 [b]

Discussion

Liver fibrosis complicates chronic liver diseases of different etiologies. In Egypt, co-infection of viral hepatitis and *S. mansoni* considered as the most important cause of chronic hepatic illness (Halim *et al,* 1999). *S. mansoni* infection reflects a good experimental model of chronic hepatic disease and fibrosis. Regression of fibrosis occurs spontaneously as soon as the stimulus for liver damage is removed (Olivera *et al,* 2008). It was crucial to find novel effective therapeutic methods as a challenge aimed to improvement of the damage produced by fibrogenesis in chronic hepatic diseases. Stem cell therapy had supported tissue regeneration with minimal invasive procedures and few complications (Lorenzini *et al,* 2008). BM-MSCs are clever in homing to specific tissues with the ability to differentiate and stimulate local repair response (Fu and Li, 2009)

The current research determined the effect of murine BM-MSCs transplantation on diseased liver as an experimental *in vivo* trial of hepatocyte differentiation from BM-MSCs. After transplantation, the fluorescent microscope detected PKH-26 labeled MSCs incorporated in the liver tissues. Some authors reported the homing of inoculated MSCs in damaged liver (Anan *et al,* 2016; Fikry *et al,* 2016).

The MSC can protect against *S. mansoni*-induced liver fibrosis through alteration of liver microenvironment at engraftment site. Numerous cytokines and growth factors as hepatic growth factor could be secreted by MSC (Matsuda-Haashii *et al,* 2004) It exhibited anti-apoptotic effect on hepatocytes as well as having a crucial role in hepatic

regeneration (Ueki, 1999) as well as nerve growth factor (NGF) (Li *et al,* 2002). In the present study, there were newly formed hepatocytes at the edge of the granuloma in hepatocytes facing the sinusoids and similarly around periportal areas. The newly formed blood vessels and the newly formed bile ductules were seen in infected transplanted group. The oval cells count raised by 3-4 folds in liver of infected mice following MSCs transplantation denoting new hepatocytes formation (Oliveira *et al,* 2008). Similar findings were recorded by other authors (Elkhaff *et al,* 2010a, Shizhu *et al,* 2012; Yu *et al,* 2012). The origin from the injected MSCs or from the liver itself regenerated hepatocytes. The noted improvement after the MSCs inoculation; could attributed their soluble growth factors secretion more than their transdifferentiation to liver cells (Alison *et al,* 2009).

The effect of MSCs based on primary hepatic illness. It either substitutes the injured tissue or enhances hepatic regeneration via antiapoptotic, anti-inflammatory as well as their proliferative action (Christ and Stock, 2012). Histopathological data of newly formed blood vessels was confirmed by TEM (Elkhaff *et al,* 2010b). In the present study, liver functions were improved by significant elevation of serum albumin level and suppression of transaminase activity ALT, AST in mice transplanted with MSCs as compared with control infected group. Consistent with current results, infused BM-MSCs were confined to liver with subsequent improvement of liver functions (Abdel Aziz *et al,* 2012). BM-MSCs transplantation in mice with fibrosed liver induced by administration of the chemokine ligand 4 (CCL4), exhibited significant decrease in hepatic fibrosis evaluated by enhancement of liver functions, such as levels of albumin, bilirubin, as well as prothrombin time (Sakaida *et al,* 2004). Oyagi *et al.* (2006), Wu *et al.* (2005) and Luk *et al.* (2005) reported that autologous transplanted BM-MSCs could distinguish to liver cells, reestablish albumin level and inhibit transaminase action and hepatic fibrosis in experimentally injured liver. *S. mansoni* infection resulted in hepatocellular injury; enzymes were released from the affected liver cells into the blood stream (Oyagi *et al,* 2006). AST, ALT and albumin levels proved to be indicators to the schistosomiasis pathological changes. Aminotransferases are indicators for toxicity of the cells. Their elevation provided an evidence for *S. mansoni* cell toxicity. So, the current study revealed that the induced inflammatory reactions in the liver of infected mice were confirmed by hepatic dysfunctions shown by increased AST and ALT levels. This was attributed to egg deposition with liver cell injury. Therefore, levels of transaminase revealed a close association with cellular necrosis beside increased permeability to release the enzymes to the circulation (El-Rigal and Hetta, 2006). Reduced serum albumin level reflects an impairment of protein synthesis caused by destruction of parenchymal liver cells (Abdel-Rahium *et al,* 1990). There was a noted significant decrease in serum albumin caused by *S. mansoni* infection (Mahmoud *et al,* 2002). It was reported that the elevation of serum albumin and reduction in ALT &AST levels express a stabilization of the hepatic function induced by treatment with MSCs. So, the reversal of liver functions in fibrotic liver either caused by inhibition of parenchymal cells apoptosis or inhibition of proliferation and infiltration of inflammatory cells (Pulavendran *et al,* 2010)

The quantitative RT-PCR showed that there was a significant the low expression of caspase-3 and IL-1β (apoptosis & inflammation-related genes) in the BM-MSCs-treated group comparable with controls. These findings evidently demonstrate that BM-MSCs can improve hepatic dysfunction in vivo by reducing inflammation; apoptosis also, promoting proliferation as well as recovery (reinforced by ALT & AST normal levels). The results showed that MSCs were innovative cells that could repair liver damage as well

as dysfunction. However; the mechanisms of hepatocytes differentiation as well as hepatic function restoration are indistinct. It was found that BM-MSCs secrete hepatocyte growth factor (HGF) alongside with suppression of inflammation following transplantation to CCL4-injured mice (Oyagi *et al*, 2006). HGF has the capability to stimulate hepatic differentiation in addition to decrease the hepatocytes death (Matsuda *et al*, 1995).

Conclusion

The outcome results showed that the BM-MSCs have a potential therapeutic effect on *Schistosoma mansoni*-induced fibrotic liver model by capacity to differentiate into the hepatocytes, and, via anti-apoptotic and anti-inflammatory mechanisms. So, it could be used as a new therapeutic line in chronic liver disease.

Conflict of interest: The authors declared that they neither have conflict of interests nor received fund.

References

Abdel Aziz, MI, Atta, HM, Roshdy, NK, *et al*, 2012: Amelioration of murine *Schistosoma mansoni* induced liver fibrosis by mesenchymal stem cells. ISRM 8:28-34.

Abdel-Rahium, IM, Kaiser, C, Homeida, MA, *et al*, 1990: Enzyme activities and protein concentration in serum of patients with hepatosplenic schistosomiasis. Trop. Med. Parasitol. 41:262-4.

Alhadlaq, A, Mao, J, 2004: Mesenchymal stem cells: isolation and therapeutics. Stem Cell Dev. 13:436-48.

Alison, MR, Lim, S, Houghton, JM, 2009: Bone marrow-derived cells and epithelial tumors: More than just an inflammatory relationship. Curr. Opin. Oncol. 21, 1:77-82.

Anan, H, Zidan, R, Shaheen, M, *et al*, 2016: Theraputic efficacy of bone marrow derived mesenchymal stromal cells versus Losartan in adriamycin-induced renal cortical injury in adult albino rats. Cytotherapy 18, 8:970-84.

Christ, B, Stock, P, 2013: Mesenchymal stem cell-derived hepatocytes for functional liver replacement. Front Immunol. 3:168-71.

Dinarello, CA, 1996: Biologic basis for interleukin-1 in disease. Blood 87:2095-147.

Elkhafif, N, El-Baz, H, Hammam, O, *et al*, 2010: CD133 (+) human umbilical cord blood stem cells enhance angiogenesis in experimental chronic hepatic fibrosis. APMIS 119, 1:66-75.

Elkhafif, N, Voss, B, Hammam, O, *et al*, 2010: Homing of transplanted bone marrow cells in livers of *Schistosoma mansoni*-infected mice. APMIS 118, 4:277-87.

El-Rigal, NS, Hetta, MH, 2006: Effect of citrus reticulate on serum protein fractions of mice after *Schistosoma mansoni* infection. J. Appl. Sci. 6:1447-55.

Friedman, S, 2008: Hepatic fibrosis-Overview. Toxicology 254:120-9.

Fu, X, Li, H, 2009: Mesenchymal stem cells and skin wound repair and regeneration: Possibilities and questions. Cell tissue Res. 335:317-21.

Haas, SJ, Bauer, P, Ruffs, A, *et al*, 2000: Immunocytochemical characterization of *in-vivo* PK-H26-labelled and intracerebral transplanted neonatal cells. Acta Histochem. 102:273-80.

Halim, AB, Garry, RF, Dash, S, *et al*, 1999: Effect of schistosomiasis and hepatitis on liver disease. Amer. J. Trop. Med. Hyg. 6:915-20.

Ishii, K, Yoshida, Y, Akechi, Y, *et al*, 2008: Hepatic differentiation of human bone marrow-derived mesenchymal stem cells by tetracycline-regulated hepatocyte nuclear factor 3 beta. Hepatology 48:597-606.

Knodell, RG, Ishak, KG, Black, WC, Chen, T S, Craig, R, *et al*, 1981: Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. Hepatology 1:431-5.

Krause, DS, Theise, ND, Collector MI, *et al*, 2001: Multi-organ multi-lineage engraftment by a single bone marrow-derived stem cell. Cell 105:369-77.

Li, Y, Chen, J, Chen, XG, *et al*, 2002: Human marrow stromal cell therapy for stroke in rat: Neurotrphins and functional recovery. Neurology 59:514-23.

Livak, KJ, Schmittgen, TD, 2001: Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C}{}_{T}$ Method. Methods 25, 4:402-8.

Lorenzini, S, Gitto, S, Grandini, E, *et al*, 2008: Stem cells for end stage liver disease: How far have we got? World J. Gastroenterology 14, 29:4593-9.

Luk, JM, Wang, PP, Lee, CK, *et al*, 2005: Hepatic potential of bone marrow stromal cells: Development of in vitro co-culture and intra-

portal transplantation models. J. Immunol. Methods 305, 1:39-47.

Luo, M, Lu, Z, Sun, H, *et al*, 2010: Nuclear entry of active caspase-3 is facilitated by its P3-recognition-based specific cleavage activity. Cell Res. 20, 2:211-22.

Mahmoud, MR, El-Abhar, HS, Saleh, SA, 2002: The effect of Nigella sativa oil against the liver damage induced by *Schistosoma mansoni* infection in mice. J. Ethno-pharmacol. 79:1-11.

Matsuda, Y, Matsumoto, K, Ichida, T, *et al*, 1995: Hepatocyte growth factor suppresses the onset of liver cirrhosis and abrogates lethal hepatic dysfunction in rats. J. Biochem. 118:643-9.

Matsuda-Haashii, Y, Takai, K, Ohta, H, et al, 2004: Hepatocyte growth factor plays roles in the induction and auotcrine maintenance of bone marrow stromal cell IL-11 SDF-1 alpha, and stem cell factor. Exp. Hematol. 32:955-61.

Nasir, A, Mohsin, S, Shams, S, Ali, G, Khan, S, *et al*, 2013: Mesenchymal stem cells and Interleukin-6attenuate liver fibrosis in mice. J. Translational Med. 11:78-80.

Olivera, SA, Souza, BS, Guimaraes-Ferreira, CA, *et al*, 2008: Therapy with bone marrow cells reduces liver alterations in mice chronically infected by *Schistosoma mansoni*. World J. Gastroenterol. 14, 38:5842-50.

Oyagi, S, Hirose, M, Kojima, M, *et al*, 2006: Therapeutic effect of transplanting HGF-treated bone marrow mesenchymal cells into CCL4-injured rats. J. Hepatol. 44, 4:742-84.

Patz, JA, Graczyk, TK, Geller, N, *et al*, 2000: Effects of environmental change on emerging parasitic diseases. Inter. J. Parasitol. 30:1395-405.

Pulavendran, S, Vignesh, J, Rose, C, 2010: Differential anti-inflammatory and anti-fibrotic activity of transplanted mesenchymal vs. hematopoietic stem cells in carbon tetrachloride-induced liver injury in mice. Inter. Immunopharma. 10:513-9.

Rochefort, G, Vaudin, P, Bonnet, N, *et al*, 2005: Influence of hypoxia on the domiciliation of mesencymal stem cells after infusion into rats, possibilities of targeting pulmonary artery remodeling via cells therapies. Inter. J. Stem Cells 6, 3:74-88.

Sakaida, I, Terai, S, Yamamoto, N, *et al*, 2004: Transplantation of bone marrow cells reduced CCL4 induced liver fibrosis in mice. Hepatology 40:1304-11.

Salama, H, Zekri, AR, Bahnassy, AA, *et al*, 2010: Autologous CD34+ & CD133+ stem cells transplantation in patients with end stage liver disease. World J. Gasteroenterol. 16:5297-305.

Shizu, J, Xiangwei, M, Sun, S, *et al*, 2012: Bone marrow mononuclear cell transplant therapy in mice with CCL4-induced acute liver failure. Turk. J. Gastroenterol. 23, 4:344-52.

Ueki, T, Kaneda, Y, Tsurtsui, H, *et al*, 1999: Hepatocyte growth factor gene therapy of liver cirrhosis in rats. Nat. Med. 5:226-30.

Wu, X, Zhao, L, Xu, Q, *et al*, 2005: Differentiation of bone marrow mesenchymal stem cells into hepatocytes in hepatectomized mouse. Tang H Sheng Wu Yi Xue Gong Cheng Xue Za Zhi 22, 6:1234-7.

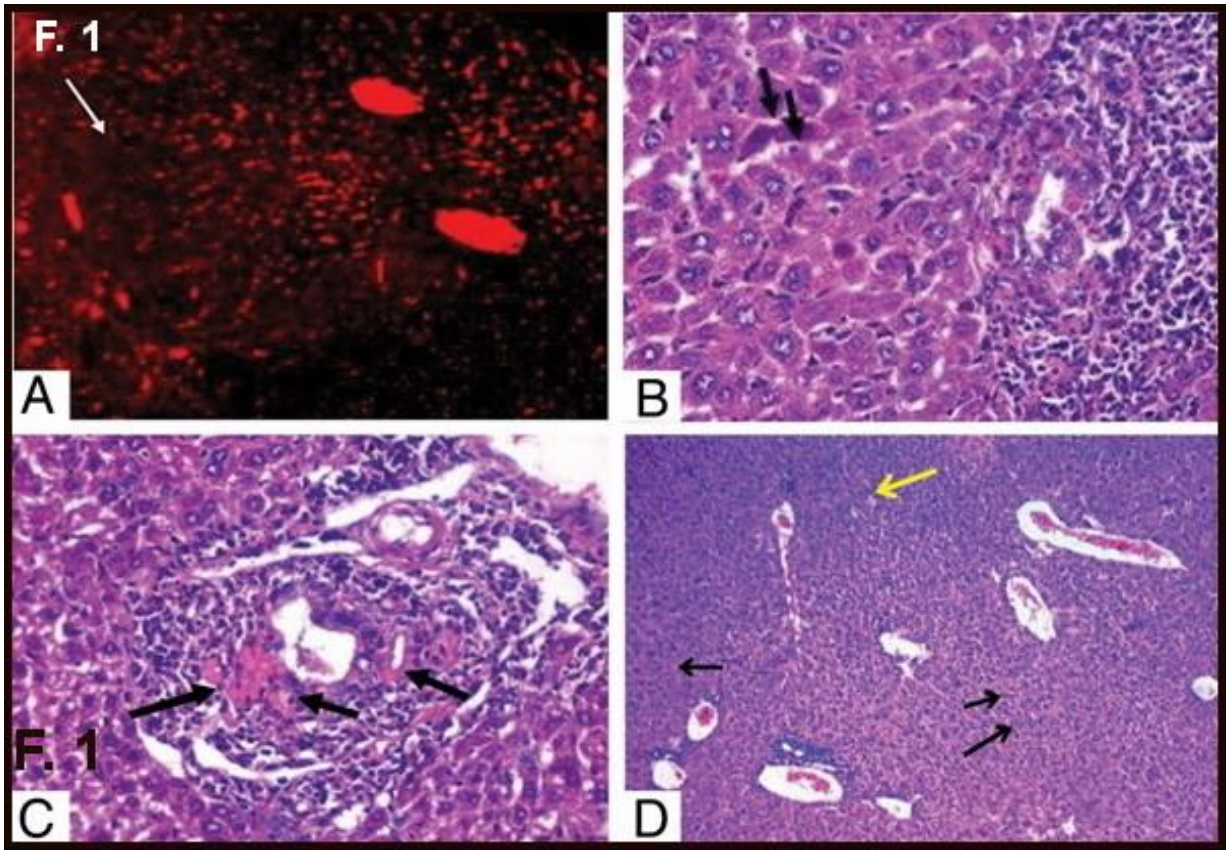
Yang, JW, Hooper, DJ, Phillips, DF, *et al*, 2003: Interleukin-1 beta responses to Mycoplasma pneumonia infection are cell-type specific. MicrobPathol2003;34: 17-25.

Yu, JY, Zhu, SH, Zou, DH, 2012: The study of osteogenic differentiation in canine bone marrow stromal cell induced by point mutant type of HIF-1α Shanghai Kou Qiang Yi Xue 21, 5:481-7.

Explanation of figures

Fig.1: Liver section from infected transplanted group. A- PKH26 marked cells seem like bright dots in hepatic tissue (Fluorescent Microscope, X 200). B-new hepatocytes like (black arrow; H&E, x400) C-proliferating bile ducts and congested blood vessels (black arrow; H&E,x400) D-mild inflammatory infiltrate (yellow arrow) and mild apoptosis (black arrow; H&E X100).

Fig.2: Electromicrograph of infected transplanted group. A- Newly formed small sized hepatocyte (h) with oval shaped nucleus (n) surrounded by developing cytoplasm. An adjacent regenerating hepatocyte with larger nucleus (N), well developed cytoplasm (C) with abundant mitochondria (M). Nucleus chromatin formed of euchromatin with prominent nucleolus. B- Signs of regeneration, increase in rough endoplasmic reticulum (rER) encircling proliferated mitochondria (M). Nuclear chromatin formed mainly of euchromatin with prominent paracentral nucleolus (n) and segregation of its constituents (arrow). C- Newly formed blood vessel (blue arrow) with luminal RBC,an adjacent progenitor cell (P) surrounded by few cytoplasmic organelles (C),macrophage (m) with cytoplasmic 1ry lysosomes (red arrow). D- Area of inflammatory cellular infiltrates represented by newly formed blood vessels (red arrows), progenitor cells (P), macrophage (m) with cytoplasmic lysosomes (blue arrow).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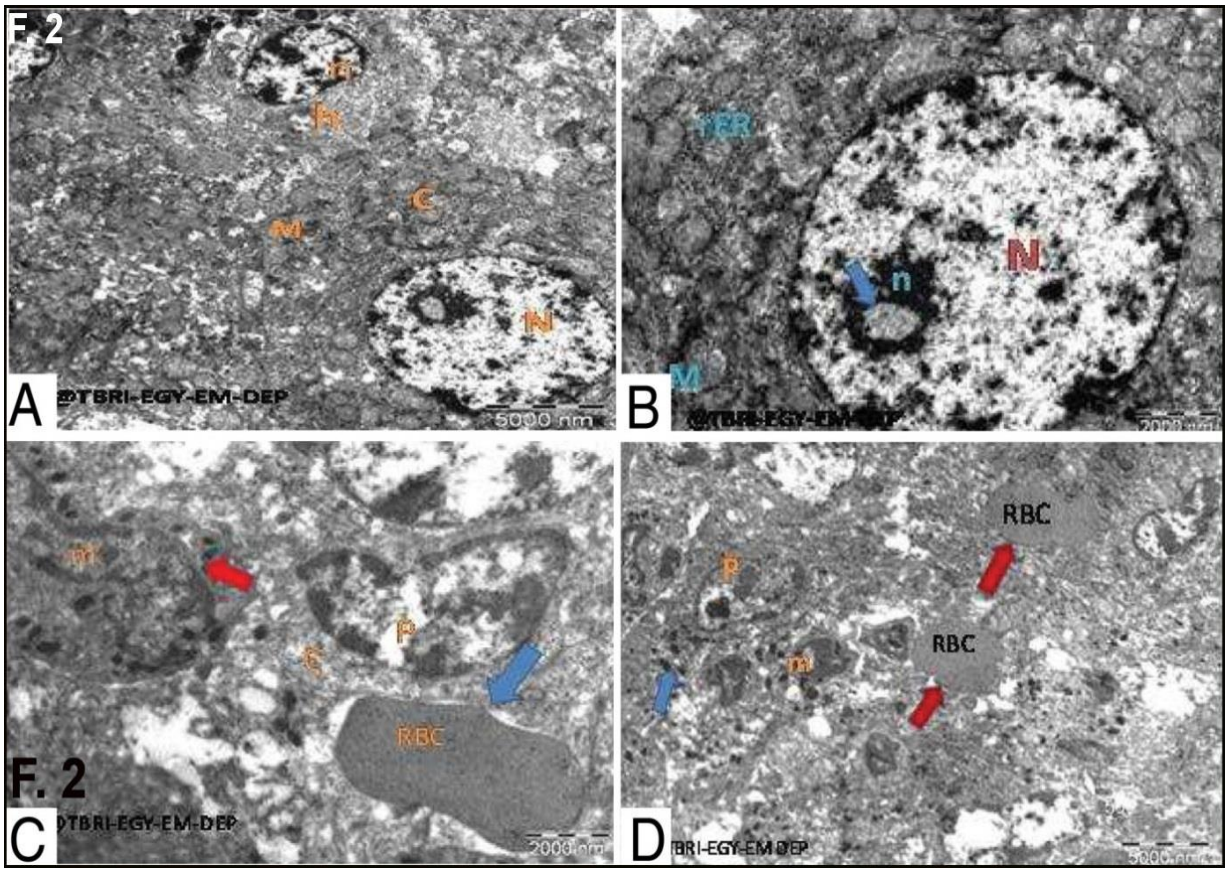
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