Hirosaki Med. J. 64(Suppl.) : S90—S98, 2013

ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS ATTENUATE FULMINANT HEPATITIS IN MICE INDUCED BY CONCANAVALIN A

Norihito Kubo[1, 3], Shunji Narumi[2], Kenichi Hakamada[1], and Akio Nakane[3]

Abstract Stem cell therapy is one of the remarkable treatments for fatal diseases. Mesenchymal stem cells (MSCs) are adult stem cells and detected in various tissues such as bone marrow, umbilical cord blood and adipose tissue. MSCs have some abilities to differentiate into multi-lineage mature cells, modify immune responses and play a role for tissue repairing. The purpose of the present study was to investigate the effect of adipose tissue-derived mesenchymal stem cells (ASCs) against fulminant hepatitis, a life threatening liver injury due to massive destruction of hepatocytes.

BALB/c mice were sacrificed and adipose tissues were harvested. ASCs were purified from the tissues and confirmed by characterization of cell surface markers and induction of differentiation into multi-lineage mature cells. Concanavalin A (ConA), a protein of jackbean, can induce fulminant hepatitis for mice. BALB/c mice were administered lethal or sublethal dose of ConA intravenously and treated by ASCs, phosphate buffered saline (PBS) or splenocytes (SPLCs). The survival rates, liver enzymes, cytokines, histopathological changes and localization of ASCs were investigated.

ASCs could increase the survival rates, inhibit elevation of liver enzymes and cytokines, and attenuate necroses of hepatocytes compared to PBS or SPLCs. Fluorescent stained ASCs were detected in inflammatory liver, but not in normal liver.

These findings suggest that ASCs have an ability to improve or attenuate fulminant hepatitis.

Hirosaki Med. J. **64, Supplement** : S90—S98, 2013

Key words: acute liver failure; autoimmune hepatitis; mesenchymal stem cell; cell therapy

Introduction

Fulminant hepatitis is a severe state of liver injury because of massive destruction of hepatocytes caused by excessive immune responses, viral eradication, or chemical reactions. It progresses rapidly toward death if patients are not provided with a quick and appropriate treatment[1, 2]. Although there are many medical treatments to recover liver function, liver transplantation is the only definitive way to improve the situation. However, there are many unsolved problems relating to liver transplantation such as lack of donors, operative damages, risk of rejection, and side effects of immunosuppressants[3]. Alternative effective treatments for fulminant hepatitis without relying on liver transplantation are expected.

Concanavalin A (ConA)-induced liver injury in mice is a well-known model of fulminant hepatitis due to excessive immune responses [4, 5]. It is believed that T cells and NKT cells play a crucial role for establishment of ConA-induced hepatitis[6], and cytokines including tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) produced by those cells are exacerbating factors.

[1] Department of Gastroenterological Surgery, Hirosaki University Graduate School of Medicine
[2] Department of Advanced Transplant and Regenerative Medicine, Hirosaki University Graduate School of Medicine
[3] Department of Microbiology and Immunology, Hirosaki University Graduate School of Medicine
Corresponding author: Norihito Kubo
FAX: +81 172 39 5080
E-mail: n_kubo@cc.hirosaki-u.ac.jp

Mesenchymal stem cells (MSCs) are one of the somatic stem cells residing in bone marrow and other various tissues[7, 8]. MSCs possess the abilities to differentiate into adipocytes, osteocytes, and chondrocytes[9-11] to regulate immune system[12], and to play a role for tissue repairing. MSCs can particularly affect various immune cells for suppressing their activation and proliferation[12-15].

Previous studies have shown the immunoregulatory effects of MSCs on T cells and NKT cells[16]. We therefore hypothesized that MSCs would be able to inhibit the activation of T cells and NKT cells in a mouse model of ConA-induced hepatitis leading to a reduction of liver injury and mortality.

MSCs also exist in adipose tissue[8, 17], and they have the similar abilities of differentiation and immunoregulation as MSCs residing in bone marrow[18, 19]. They are easy to obtain from adipose tissue and to establish.

In the present study, the therapeutic efficacy of adipose tissue-derived mesenchymal stem cells (ASCs) for ConA-induced hepatitis was demonstrated.

Materials and Methods

Mice

BALB/c mice (Clea Japan, Tokyo, Japan), 8-12-week-old females, were housed in plastic cages with controlled light and dark cycle and fed a standard diet with water *ad libitum*. All the animal experiments were performed in accordance with Guidelines for Animal Experimentation of Hirosaki University..

Isolation and expansion of ASCs

We used a method for isolation and expansion of ASCs as previously described with minor modification[10, 17]. Subcutaneous and visceral adipose tissues were taken from BALB/c mice. Collected tissues were placed in Hank's balanced salt solution (HBSS). Tissues were minced finely and incubated in 3 ml of Iscove's modified Dulbecco's media (IMDM, Invitrogen, NY, USA) containing 0.2% of collagenase (type II, Sigma, St. Louis, USA) and 1% bovine serum albumin (BSA, Sigma) at 37°C for 40 min. Cells were collected from digested tissue through 70-μm mesh. After centrifuging at 800 x *g* for 10 min and discarding supernatant, 3 ml of 0.83% NH_4Cl was added to hemolyze, and the cells were washed twice with HBSS and once with IMDM. Cells were seeded in IMDM containing 9% fetal bovine serum (FBS), 9% horse serum, 100 U/ml penicillin and 100 μg/ml streptomycin[9]. The medium was changed every 3 or 4 days. Confluent cells were treated with 1 mM EDTA containing 0.25% trypsin for 2 min at 37°C and non-adherent cells were collected. Furthermore, the medium was changed every 3 or 4 days for 1 to 2 weeks. The cultured cells were used for the examinations.

Differentiation of ASCs

To ensure that the cultured cells were ASCs, an ability to differentiate to multi-lineage cells was investigated. For differentiation to osteocytes, adipocytes, and chondrocytes as mesodermal lineages, StemXVivo™ (R&D Systems, Minneapolis, USA) medium and supplements were used according to the manufacturer's instructions. Two to 4 weeks later, each cell population was stained by Oil red O staining, von Kossa staining, and Alcian blue staining for adipocytes, osteocytes, and chondrocytes, respectively.

Flow cytometric analysis for cell surface markers

Cultured cells were detached from cultured flask by 0.25% trypsin with 1 mM EDTA for 2 min at 37°C and re-suspended in phosphate buffered saline (PBS) containing 2% FBS (2% FBS-PBS). The cells were counted and 1.0 x 10^6 cells were collected by centrifugation at 800 x *g*

for 5 min. After discarding supernatants, 1 μl of anti-CD16 plus anti-CD32 antibodies (Abcam, Cambridge, UK) was added and incubated at 4°C for 30 min. The cells were labeled with phycoerythrin (PE)-labeled anti-CD34 antibody (GeneTex, Irvine, CA, USA), PE-labeled anti-CD45 antibody (R&D Systems), PE-labeled anti-CD117 (c-kit) antibody (R&D Systems), PE-labeled anti-CD90.2 antibody (Birmingham, AL, USA), fluorescein isothiocyanate (FITC) -labeled anti-CD106 antibody (Funakoshi, Tokyo, Japan), and PE/Cy5.5-labeled anti-Sca-1 antibody (GenWay Biotech, San Diego, CA, USA). The suspensions were incubated at 4°C for 30 min and washed with 2% FBS-PBS. Then the cells were analyzed by FACScalibur (Becton Dickinson, Franklin Lakes, NJ, USA).

Isolation of splenocytes

Mice were sacrificed by cervical dislocation. The spleen was harvested and minced by scissors, and then the cells were filtered through 70-μm mesh. After centrifuging at 800 x *g* for 10 min and discarding supernatant, 3 ml of 0.83% NH_4Cl was added, and the cells were washed twice with RPMI1640 medium containing 10% FBS. The cells were then resuspended in 2% FBS-PBS, counted and prepared at the concentration of 2.0 x 10^6 cells/ml before injection.

Induction of ConA hepatitis and survival analysis

Mice were injected intravenously with 30 to 50 mg/kg (body weight) of ConA (Sigma) diluted with PBS. Most of the mice injected with more than 40 mg/kg of ConA died within 72 h. In contrast, all of the mice administered with less than 35 mg/kg of ConA survived (data not shown). Based on this result, 40 mg/kg of ConA was used as the lethal dose in this study.

For survival analysis, mortality of mice was recorded every 3 h up to 72 h or until all mice died.

Treatment

ASCs and SPLCs were suspended in 2% FBS-PBS at the concentration of 2.0 x 10^6 cells/ml. At 30 min after ConA administration, mice were injected intravenously with 1.0 x 10^6 ASCs, 0.5 ml 2% FBS-PBS, or 1.0 x 10^6 SPLC for ASC group, control group, or SPLC group, respectively.

Measurement of liver enzymes and cytokines

Mice were anesthetized by diethyl ether and whole blood was obtained by cardiac puncture using with 23G needles. Blood samples were centrifuged at 2300 x *g* for 30 min, and sera were collected.

For measurement of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels, mice were injected with 20 mg/kg of ConA and treated as described previously. Twenty-four hours after hepatitis induction, sera were collected for AST and ALT measurement by SPOTCHEM EZ® (ARKLEY, Kyoto, Japan).

For cytokines analysis, mice were injected 40 mg/kg of ConA and treated. Sera were collected at 2 h, 6 h and 12 h after hepatitis induction, and IFN-γ and TNF-α were measured by enzyme-linked immunosorbent assays (ELISAs). Determination of IFN-γ was carried out as described previously[22], TNF-α was measured by using CytoSet™ ELISA kits (Invitrogen) according to the manufacturer's instructions.

Histological analysis

Mice were sacrificed by cervical dislocation at 24 h after injection of 20 mg/kg of ConA, and livers were harvested and fixed with 10% formaldehyde, and thereafter cut horizontally into two pieces. After embedding in paraffin, livers were sectioned at 5-μm in thickness and stained with hematoxylin and eosin. The pictures of whole livers were taken and all necrotic areas were painted in black and the percentages of necrotic areas were analyzed by ImageJ

software.

Detection of localization of ASCs

Fluorescent staining was used to trace injected ASC in mice. ASCs were stained with NEO-STEM® (Biterials, Seoul, Korea) according to manufacturer's instructions. Mice were divided in two experimental groups; hepatitis group and control group. Hepatitis group was injected 20 mg/kg of ConA and control group was 0.2 ml of PBS. At 30 min after injection, each group was administered 1 x 10^6 ASCs stained with NEO-STEM®. Twenty-four hours after the first injection, mice were sacrificed and the lungs, the liver, the spleen and the mesenteric lymph nodes were harvested. Those organs were fixed with 10% formaldehyde and embedded in paraffin, and then sectioned at 5-μm in thickness to be observed under fluorescent microscope (IX71, OLYMPUS).

Statistics

Statistical analysis was performed using Student's *t*-test. In case of a survival curve, the data were analyzed by the log-rank test. Significant difference between experimental and control group was considered by p value of <0.05. All data were given as a mean ± standard deviations.

Results

Characteristics of isolated ASCs

Multi-lineage differentiation into adipocytes, osteocytes and chondrocytes were induced, and each population was stained by Oil red O stain, von Kossa stain and Alcian blue stain, respectively. Differentiated cells were detected by each respective staining procedure (data not shown). These results indicate that ASCs can differentiate into multi-lineage cells.

Cell surface markers were also detected from expanded cells by flow cytometry. ASCs were negative for CD34, CD45 and CD117 (c-kit) and positive for CD90.2, CD106 and Sca-1 (data not shown).

Survival rate was significantly improved in ASC-injected mice, but the effect was observed only in high dose and early administration group

The survival rate of ASC-injected mice was significantly improved compared to control group and SPLC group. There was no difference of survival rates between control group and SPLC group (Figure 1A).

Next, dose dependency of ASCs was investigated. Survival rates were not improved in the groups injected with 1 x 10^5 or 5 x 10^5 ASCs (p = 0.45 and 0.73, respectively), but were significantly improved in 1 x 10^6 ASC-injected mice (Figure 1B).

All the mice survived 72 h could recover from severe condition and live over a month.

Elevation of liver enzymes in sera was attenuated in ASC-injected mice

In order to evaluate the severity of liver injury by measuring liver enzymes, sera were harvested 24 h after ConA injection, and the level of AST and ALT was measured. In control and SPLC groups, AST and ALT was markedly elevated, but the level of these enzymes was significantly suppressed in ASC group (Figure 2A).

ASCs inhibited production of cytokines

Mice were injected with 40 mg/kg of ConA and treated as the same method described above. At 2 h, 6 h, and 12 h of hepatitis induction, sera were harvested and TNF-α and IFN-γ titers were measured by ELISAs. Levels of these cytokines in ASC group were significantly lower than those of control and SPLC groups at each time point. In contrast, there was a significant reduction of IFN-γ in SPLC group compared to control group only at 6 h after induction of

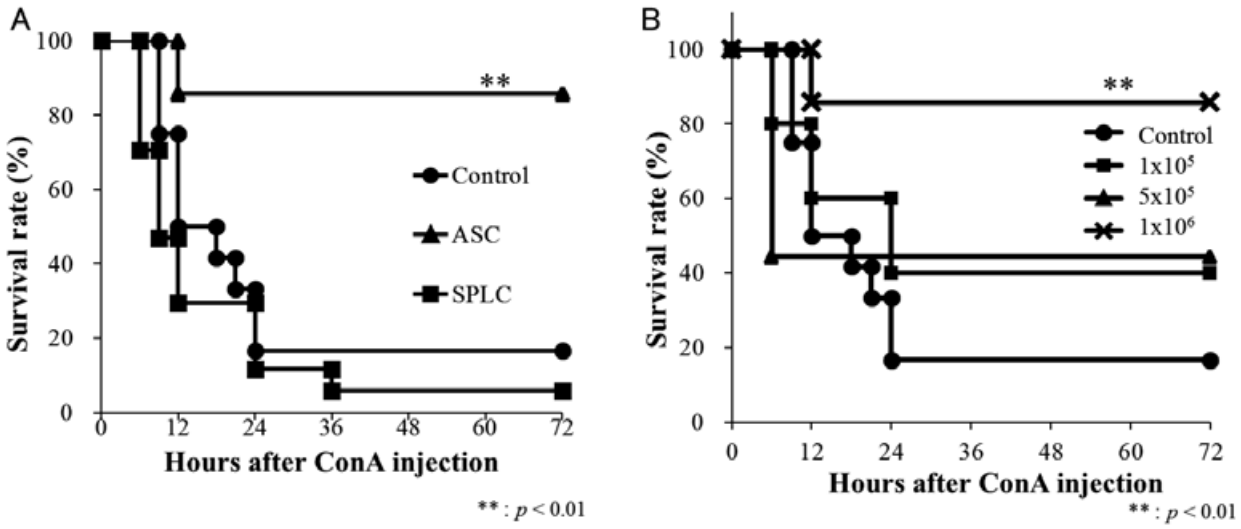

Figure 1. The survival rate was improved by ASC treatment in ConA-induced hepatitis.

Mice were injected with 40 mg/kg of ConA and survival rates were observed every 3 h until 72 h. A) ASC group (N=14) significantly survived compared to control (N=12), and SPLC (N=17) groups. B) 1 x 10^6 ASC- (N=14) treated groups could archived long term survival compared to control group, 1 x 10^5 ASC- (N=8), 5 x 10^5 ASC- (N=9)

**; $p < 0.01$ vs Control. Data are cumulative values of at least two independent experiments.

hepatitis (Figure 2B).

Liver injury was ameliorated microscopically in ASC-injected mice

At 24 h after hepatitis induction with 20 mg/kg ConA, histopathological changes of livers obtaining from ASC, control, and SPLC group were observed microscopically. Necrotic areas were mainly observed in the peripheral areas of each hepatic lobe (data not shown).

Percentages of necrotic areas were analysed by ImageJ software. The necrotic area of livers from ASC group was significantly lower than that of control and SPLC group (data not shown).

Injected ASCs were detected in the liver of ConA injected mice

Under fluorescent microscopic observation, NEO-STEM®-labeled ASCs were detected in the lungs of hepatitis group or control group, and there was no significant difference between each group. However, significantly high amount of NEO-STEM®-labeled cells were observed in the livers of hepatitis group compared to control group. Fluorescent positive cells mostly existed around Glisson's sheaths (data not shown).

Discussion

ConA-induced hepatitis is well known as a murine model of fulminant hepatitis due to excess immune responses, such as autoimmune hepatitis[4, 5]. ASCs are noticed as a kind of cells showing immunoregulatory functions, and their therapeutic abilities for liver injury were recently reported[23-26]. However, the relationship between ASCs and fulminant hepatitis by ConA has not been well elucidated. In the present study, we have investigated the effect of ASCs for ConA-induced hepatitis.

ASC treatment improved the survival rate in ConA-induced hepatitis compared to control group. In SPLC group, even after receiving

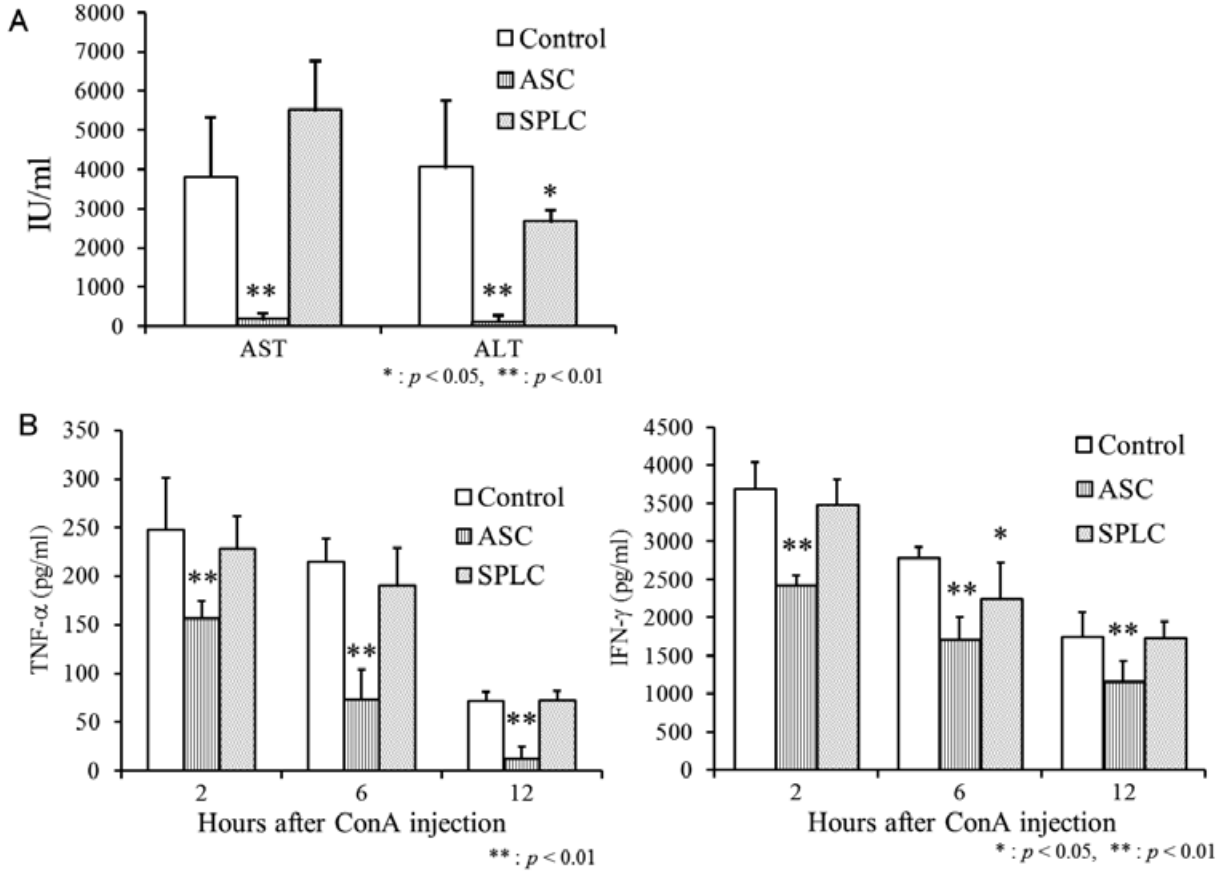

Figure 2. The levels of serum AST and ALT were normalized and serum TNF-α and IFN-γ were suppressed by ASC treatment in ConA-induced hepatitis.

Mice were injected with 20 mg/kg or 40 mg/kg of ConA and treated by PBS or 1 x 10^6 ASCs or 1 x 10^6 SPLCs. A) Sera were harvested at 24 h after hepatitis induction. AST and ALT were significantly low in ASC treated group (**; $p < 0.01$, *; $p < 0.05$ vs control). Data are shown as mean of three independent experiments (N=15). B) Sera were harvested at 2, 6 and 12 h later. TNF-α and IFN-γ titers were significantly low in ASC treated group (**; $p < 0.01$, *; $p < 0.05$ vs Control). Data are shown as mean of two independent experiments (N=10).

the same number of splenocytes as ASCs, the survival rate was not improved. This result suggests that an efficacy of ASCs on hepatitis is not due to the number of cells but some specific features of the ASCs. Furthermore, ASCs seem to act as a therapeutic effector by themselves, not a trigger for host's therapeutic reactions, since survival rates were not improved in groups with low doses of ASCs.

There was improvement from liver injury in ASC group as evidenced by survival rates, levels of serum liver enzymes and histopathology data. AST and ALT levels were supposed to be in tandem with small areas of necrotic hepatocytes detected in histopathology, and suppression of their appearance meant prevention of necrosis of hepatocytes and this correlated with improved survival.

Previous reports have shown that ConA-induced hepatitis is mainly due to activation of NKT cells and T cells[5, 6], and these cells produce TNF-α and IFN-γ. Suppression of TNF-α and IFN-γ production in these cell populations might be due to attenuation of liver injury in ASC-injected mice, therefore involvement of ASCs and NKT cells or T cells interaction is speculated.

Mechanisms of the immunoregulatory effect of ASCs are reported to be due to direct cell-

to-cell contact[29] and their products such as indoleamine 2,3-dioxygenase, prostaglandin E2 and nitric oxide[12, 29, 30]. Furthermore, it has been reported that ASCs can induce differentiation and activation of regulatory T cells (Tregs) [31, 32]. Since Tregs suppress immune response, it is possible that ASCs modulate innate immune responses through Tregs expansion and lead alleviation of liver injury induced by ConA. These are three possible mechanisms, namely a direct interaction by cell-to-cell contact, an indirect influence by products from ASCs, and an indirect effect by expanding Treg cells, in protection from ConA-induced hepatitis. In the present study, fluorescent stained ASCs were detected in inflammatory liver more than normal liver. These results suggest a possibility that ASCs migrate from systemic circulation into inflammatory lesion and suppress the activities of NKT and T cells stimulated by ConA. Therefore a mechanism including a direct interaction by cell-to-cell contact might be possible.

ASCs can be harvested easily and less invasively in humans, such as liposuction[20, 21], indicating that ASC is an attractive source of stem cells for clinical use. However, safety of ASCs administration still remains controversial. First, intravenous injection of a large amount of ASCs induces pulmonary embolism especially in mice[27, 33]. Secondly, ASCs can be a vector of malignant cells and help their growing[34-36]. Therefore, these problems should be solved before clinical use.

In conclusion, ASCs have various potentials for application as stem cell therapies. Additionally, ASCs harvesting procedure is easy and minimally invasive for humans. As our study demonstrated, ASC treatment for fulminant hepatitis is effective and has a potential to be an innovative therapy for severe hepatic failures, but the safety and its mechanisms have not been well elucidated. Further investigations are necessary.

References

1) Ichai P, Samuel D. Etiology and prognosis of fulminant hepatitis in adults. *Liver Transpl* 2008;**14** Suppl 2:S67-79.

2) Bernal W, Auzinger G, Dhawan A, Wendon J. Acute liver failure. *Lancet* 2010;**376**:190-201.

3) Lopez MM, Valenzuela JE, Alvarez FC, Lopez-Alvarez MR, Cecilia GS, Paricio PP. Long-term problems related to immunosuppression. *Transpl Immunol* 2006;**17**:31-5.

4) Lohse AW MM, Dienes HP, Meyer zum Büschenfelde KH, Cohen IR. Experimental autoimmune hepatitis: disease induction, time course and T-cell reactivity. *Hepatology* 1990;**11**:24-30.

5) Tiegs G HJ, Wendel A. A T cell-dependent experimental liver injury in mice inducible by concanavalin A. *J Clin Invest* 1992;**90**:196-203.

6) Takeda K, Hayakawa Y, Van Kaer L, Matsuda H, Yagita H, Okumura K. Critical contribution of liver natural killer T cells to a murine model of hepatitis. *Proc Natl Acad Sci U S A* 2000;**97**:5498-503.

7) Campagnoli C, Roberts IA, Kumar S, Bennett PR, Bellantuono I, Fisk NM. Identification of mesenchymal stem/progenitor cells in human first-trimester fetal blood, liver, and bone marrow. *Blood* 2001;**98**:2396-402.

8) Huss R. Isolation of primary and immortalized CD34-hematopoietic and mesenchymal stem cells from various sources. *Stem Cells* 2000;**18**:1-9.

9) Peister A, Mellad JA, Larson BL, Hall BM, Gibson LF, Prockop DJ. Adult stem cells from bone marrow (MSCs) isolated from different strains of inbred mice vary in surface epitopes, rates of proliferation, and differentiation potential. *Blood* 2004;**103**:1662-8.

10) Shimizu K, Sakai M, Ando M, Chiji H, Kawada T, Mineo H, et al. Newly developed primary culture of rat visceral adipocytes and their in vitro characteristics. *Cell Biol Int* 2006;**30**:381-8.

11) Delalat B, Pourfathollah AA, Soleimani M, Mozdarani H, Ghaemi SR, Movassaghpour AA, et al. Isolation and ex vivo expansion of human

umbilical cord blood-derived CD34+ stem cells and their cotransplantation with or without mesenchymal stem cells. *Hematology* 2009;**14**:125-32.

12) Siegel G, Schafer R, Dazzi F. The immunosuppressive properties of mesenchymal stem cells. *Transplantation* 2009;**87**:S45-9.

13) Di Nicola M, Carlo-Stella C, Magni M, Milanesi M, Longoni PD, Matteucci P, et al. Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. *Blood* 2002;**99**:3838-43.

14) Spaggiari GM, Capobianco A, Abdelrazik H, Becchetti F, Mingari MC, Moretta L. Mesenchymal stem cells inhibit natural killer-cell proliferation, cytotoxicity, and cytokine production: role of indoleamine 2,3-dioxygenase and prostaglandin E2. *Blood* 2008;**111**:1327-33.

15) Asari S, Itakura S, Ferreri K, Liu CP, Kuroda Y, Kandeel F, et al. Mesenchymal stem cells suppress B-cell terminal differentiation. *Exp Hematol* 2009;**37**:604-15.

16) Prigione I, Benvenuto F, Bocca P, Battistini L, Uccelli A, Pistoia V. Reciprocal interactions between human mesenchymal stem cells and gammadelta T cells or invariant natural killer T cells. *Stem Cells* 2009;**27**:693-702.

17) Bunnell BA, Flaat M, Gagliardi C, Patel B, Ripoll C. Adipose-derived stem cells: Isolation, expansion and differentiation. *Methods* 2008;**45**:115-20.

18) Yoo KH, Jang IK, Lee MW, Kim HE, Yang MS, Eom Y, et al. Comparison of immunomodulatory properties of mesenchymal stem cells derived from adult human tissues. *Cell Immuno*. 2009;**259**: 150-6.

19) van der Bogt KE, Schrepfer S, Yu J, Sheikh AY, Hoyt G, Govaert JA, et al. Comparison of transplantation of adipose tissue- and bone marrow-derived mesenchymal stem cells in the infarcted heart. *Transplantation* 2009;**87**:642-52.

20) Locke M, Windsor J, Dunbar PR. Human adipose-derived stem cells: isolation, characterization and applications in surgery. *ANZ J Surg* 2009;**79**:235-44.

21) Meyerrose TE, De Ugarte DA, Hofling AA, Herrbrich PE, Cordonnier TD, Shultz LD, et al. In vivo distribution of human adipose-derived mesenchymal stem cells in novel xenotransplantation models. *Stem Cells* 2007;**25**:220-7.

22) Mizuki M, Nakane A, Sekikawa K, Tagawa YI, Iwakura Y. Comparison of host resistance to primary and secondary *Listeria monocytogenes* infections in mice by intranasal and intravenous routes. *Infect Immun* 2002;**70**:4805-11.

23) Banas A, Teratani T, Yamamoto Y, Tokuhara M, Takeshita F, Osaki M, et al. Rapid hepatic fate specification of adipose-derived stem cells and their therapeutic potential for liver failure. *J Gastroen Hepatol* 2009;**24**:70-7.

24) Kuo TK, Hung SP, Chuang CH, Chen CT, Shih YR, Fang SC, et al. Stem cell therapy for liver disease: parameters governing the success of using bone marrow mesenchymal stem cells. *Gastroenterology* 2008;**134**:2111-21, 21 e1-3.

25) Zhao Q, Ren H, Zhu D, Han Z. Stem/progenitor cells in liver injury repair and regeneration. *Biol Cell* 2009;**101**:557-71.

26) Banas A, Teratani T, Yamamoto Y, Tokuhara M, Takeshita F, Osaki M, et al. IFATS collection: in vivo therapeutic potential of human adipose tissue mesenchymal stem cells after transplantation into mice with liver injury. *Stem Cells* 2008;**26**:2705-12.

27) Yukawa H, Noguchi H, Oishi K, Takagi S, Hamaguchi M, Hamajima N, et al. Cell transplantation of adipose tissue-derived stem cells in combination with heparin attenuated acute liver failure in mice. *Cell Transplant* 2009;**18**:611-8.

28) Aurich H, Sgodda M, Kaltwasser P, Vetter M, Weise A, Liehr T, et al. Hepatocyte differentiation of mesenchymal stem cells from human adipose tissue in vitro promotes hepatic integration in vivo. *Gut* 2009;**58**:570-81.

29) Nemeth K, Leelahavanichkul A, Yuen PS, Mayer B, Parmelee A, Doi K, et al. Bone marrow stromal cells attenuate sepsis via prostaglandin E(2) -dependent reprogramming of host macrophages to increase their interleukin-10 production. *Nat Med* 2009;**15**:42-9.

30) Ghannam S, Bouffi C, Djouad F, Jorgensen C, Noel D. Immunosuppression by mesenchymal stem

cells: mechanisms and clinical applications. *Stem Cell Res Ther* 2010;**1**:2.

31) Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood* 2005;**105**:1815-22.

32) Augello A, Tasso R, Negrini SM, Cancedda R, Pennesi G. Cell therapy using allogeneic bone marrow mesenchymal stem cells prevents tissue damage in collagen-induced arthritis. *Arthritis Rheum* 2007;**56**:1175-86.

33) Furlani D, Ugurlucan M, Ong L, Bieback K, Pittermann E, Westien I, et al. Is the intravascular administration of mesenchymal stem cells safe? Mesenchymal stem cells and intravital microscopy. *Microvasc Res* 2009;**77**:370-6.

34) Bergfeld SA, DeClerck YA. Bone marrow-derived mesenchymal stem cells and the tumor microenvironment. *Cancer Metastasis Rev* 2010;**29**: 249-61.

35) Corcoran KE, Trzaska KA, Fernandes H, Bryan M, Taborga M, Srinivas V, et al. Mesenchymal stem cells in early entry of breast cancer into bone marrow. *PLoS One* 2008;**3**:e2563.

36) Kuhn NZ, Tuan RS. Regulation of stemness and stem cell niche of mesenchymal stem cells: implications in tumorigenesis and metastasis. *J Cell Physiol* 2010;**222**:268-77.