MESENCHYMAL STEM CELLS ATTENUATE ACUTE LIVER FAILURE BY PROMOTING EXPANSION OF REGULATORY T CELLS IN AN INDOLEAMINE 2,3-DIOXYGENASE-DEPENDENT MANNER

Dragana Miloradovic[1], Dragica Miloradovic[1], Marina Gazdic Jankovic[2], Bojana Simovic Markovic[1], C. Randall Harrell[3], Crissy Fellabaum[3], Nebojsa Arsenijevic[1], Aleksandra Lukic[4] and Vladislav Volarevic[1]

[1]University of Kragujevac, Faculty of Medical Sciences, Center for Molecular Medicine and Stem Cell Research, Serbia

[2]Department of Genetics, Faculty of Medical Sciences, University of Kragujevac, Serbia

[3]Regenerative Processing Plant-RPP, LLC, 34176 US Highway 19 N Palm Harbor, Palm Harbor, Florida, United States of America

[4]University of Kragujevac, Faculty of Medical Sciences, Department of Dentistry, Serbia

MEZENHIMSKE MATIČNE ĆELIJE EKSPRIMIRAJU INDOLAMIN 2-3 DIOKSIGENAZU I PROMOVIŠU EKSPANZIJU REGULATORNIH ĆELIJA U JETRI UTIČUĆI NA SMANJENJE AKUTNOG HEPATITISA

Dragana Miloradović[1], Dragica Miloradović[1], Marina Gazdić Janković[2], Bojana Simović Marković[1], C. Randall Harrell[3], Crissy Fellabaum[3], Nebojša Arsenijević[1], Aleksandra Lukić[4] i Vladislav Volarević[1]

[1]Univerzitet u Kragujevcu, Fakultet medicinskih nauka, Centar za molekulsku medicinu i istraživanje matičnih ćelija, Kragujevac, Srbija

[2]Univerzitet u Kragujevcu, Fakultet medicinskih nauka, Katedra za genetiku, Kragujevac, Srbija

[3]Postrojenje za regenerativnu preradu-RPP, Palm Harbor, Florida, Sjedinjene Američke Države

[4]Univerzitet u Kragujevcu, Fakultet medicinskih nauka, Katedra za stomatologiju, Kragujevac, Srbija

Received / Primljen: 27. 09. 2018. Accepted / Prihvaćen: 01. 10. 2018.

ABSTRACT

The influence of mesenchymal stem cells (MSCs) on the phenotype and function of CD4+CD49b+FoxP3- regulatory cells has not been elucidated. We used Concanavalin A (ConA) - and α-galactosylceramide (α-GalCer)-induced acute liver injury to estimate the effects of MSCs on liver-infiltrating CD4+CD49b+FoxP3-regulatory cells. MSCs significantly reduced ConA- and α-GalCer-mediated liver injury in C57BL/6 mice, as demonstrated by biochemical tests, reduced influx of inflammatory CD4+ T cells, and increased presence of CD4+CD49b+FoxP3- regulatory cells in the injured livers. The number of CD4+CD49b+FoxP3-regulatory cells was also significantly increased in α-GalCer-treated mice that received MSC-derived conditioned medium (MSC-CM). The presence of 1-methyltryptophan, a specific inhibitor of indoleamine 2,3-dioxygenase (IDO), in MSC-CM completely abrogated the hepatoprotective effect of MSCs and significantly decreased the total number of liver-infiltrated CD4+CD49b+FoxP3- regulatory cells, indicating the crucial importance of MSC-derived IDO for the expansion of CD4+CD49b+FoxP3- regulatory cells and the consequent MSC-dependent attenuation of acute liver injury.

Keywords: *mesenchymal stem cells, regulatory T cells, acute liver failure.*

SAŽETAK

Uticaj mezenhimskih matičnih ćelija (engl. Mesenchymal Stem Cells, MSCs) na fenotip i funkciju CD4+CD49b+FoxP3-regulatornih ćelija nije razjašnjen. Da bismo procenili uticaj MSCs na CD4+CD49b+FoxP3- regulatorne ćelije, izazvali smo oštećenje jetre konkanavalinom A (engl. ConcanavalinA, ConA) i alfa-galaktozilceramidom (engl. α-Galactosylceramide, α-GalCer).MSCs, koje su aplikovane nakon indukcije hepatičnog oštećenja, značajno su smanjile oštećenje jetre C57BL/6 miševa, što je pokazano biohemijskim analizama, uz smanjenu infiltraciju inflamacijskih (IFN-γ-, TNF-α- i IL-4 produkujućih CD4+ T ćelija) i povećale ukupan broj CD4+CD49b+FoxP3-regulatornih ćelija u jetri. Broj CD4+CD49b+FoxP3- ćelija je značajno povećan u jetri miševa koji su primili kondicionirani medijum, dobijen od MSCs (engl. MesenchymalStem-Cell-ConditionedMedium, MSC-CM). Nakon dodavanja 1-metil-DL-triptofana, specifičnog inhibitora indoleamin 2,3-dioksigenaze (eng. indoleamine 2,3-dioxygenase, IDO) u MSC-CM, koji je injektiran u miševe tretirane GalCer-om, hepatoprotektivni efekat MSCs je neutralisan dok je broj CD4+-CD49b+FoxP3- regulatornih ćelija značajno smanjen, što ukazuje da MSCs smanjuju akutno oštećenje jetre povećavajući broj CD4+CD49b+FoxP3- regulatornih ćelija posredstvom IDO-a.

Ključne reči: *mezenhimalne matične ćelije, regulatorneT ćelije, akutno oštećenje jetre.*

sciendo
UDK: 616.32-08:602.9
Ser J Exp Clin Res 2020; 21 (3): 257-262
DOI: 10.2478/sjecr-2018-0043

Corresponding author:
Prof. Dr. Vladislav Volarevic
Postal address: 69 Svetozar Markovic Street, 34000 Kragujevac, Serbia
e-mail: drvolarevic@yahoo.com;
telephone number/fax number: +38134306800

INTRODUCTION

Autoimmune hepatitis, primary biliary cirrhosis, viral hepatitis, primary sclerosing cholangitis, and liver allograft rejection are induced by activated T lymphocytes, which infiltrate and destroy the liver parenchyma (1-2). Multipotent differentiation characteristics coupled to their capacity for self-renewal and capability of regulating immune responses point to mesenchymal stem cells (MSCs) as potentially new therapeutic agents for the treatment of various diseases (3). It is already known that MSCs suppress proliferation and effector functions of T lymphocytes; professional antigen presenting cells such as dendritic cells (DCs), macrophages, and B lymphocytes; and cytotoxicity of natural killer (NK) and natural killer T (NKT) cells (4-6). By suppressing immune responses in a juxtacrine or paracrine manner, MSCs attenuate liver inflammation and promote regeneration of hepatocytes. Thus, they represent promising therapeutic tools for acute liver injury (7). During recent decades, a variety of animal models have been used to study the mechanisms of MSC-based attenuation of acute hepatitis. Concanavalin A (ConA) and alpha-galactosylceramide (**α-GalCer)**-induced hepatitis are well-described experimental models of immune cell-mediated acute liver injury (8, 9). In ConA hepatitis, CD4+ T lymphocytes infiltrate the liver tissue and secrete large amounts of cytokines, such as tumour necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), interleukin (IL)-2, and granulocyte macrophage colony stimulating factor (GM-CSF) (10). CD8+ T cells, NK cells, NKT cells and macrophages can induce hepatocyte cell death by either cell-to-cell contact or in a paracrine manner, through the secretion of pro-inflammatory cytokines and reactive oxygen species (10, 11). In **α-GalCer**-induced hepatitis, liver injury is the result of interplay between DCs and NKT cells (12-13). By using these two murine models of immune-mediated acute liver failure, we have recently shown that injection of MSCs significantly reduced the hepatotoxicity of liver NKT cells and successfully increased the ratio between regulatory (NKTreg) and inflammatory IL-17 producing NKT (NKT17) cells in the injured livers (14). MSCs, through the production of indoleamine 2,3-dioxygenase (IDO), directly inhibited the secretion of inflammatory cytokines and the cytotoxic activity of NKT cells and induced increased production of IL-10 in Tregs, which in turn, in an IL-10 dependent manner, attenuated the hepatotoxicity of liver NKT cells (12,15).

Here, we describe another mechanism of MSC-based attenuation of acute liver failure relying on the interplay between MSCs and CD4+CD49b+FoxP3- regulatory cells. Our data strongly suggest that MSCs, in an IDO-dependent manner, induce expansion of hepatoprotective CD4+CD49b+FoxP3- regulatory cells that through the production of immunosuppressive IL-10 inhibit acute hepatitis.

MATERIALS AND METHODS

Cells

MSCs isolated from bone marrow of C57BL/6 mice were purchased from Gibco (catalogue no. S1502-100). These cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% heat-inactivated foetal calf serum (FCS), 100 IU/mL penicillin G and 100 μg/mL streptomycin (Sigma-Aldrich, Munich, Germany), at 37°C in a 5% CO_2 incubator. MSCs in passage 4 were used throughout the experiments.

Animals

Male 6-8-week-old C57BL/6 mice were used. All animals received humane care, and all experiments were approved by and conducted in accordance with the Guidelines of the Animal Ethics Committee of the Faculty of Medical Sciences of the University of Kragujevac, Serbia. Mice were housed in a temperature-controlled environment with a 12-hour light-dark cycle and were administered standard laboratory chow and water *ad libitum.*

Con A-induced hepatitis

WT C57BL/6 mice were given a single intravenous injection of Con A (Sigma-Aldrich, St. Louis, MO) at 12 mg/kg body weight dissolved in 250 μL of saline (10). MSCs were intravenously injected (5 x 10^5 cells), via the tail vein, immediately after ConA administration (ConA+MSC-treated mice). Control animals received the appropriate amount of MSCs only or saline only. Serum levels of aspartate aminotransferase (AST) and alanine aminotransaminase (ALT) were measured 24 h after Con A administration, by a standard photometric method using the automated biochemistry analyser Olympus AU 400 (Olympus Diagnostica GMBH, Hamburg, Germany) and Olympus AU reagents, according to the manufacturer's instructions (11,16).

α-GalCer-induced hepatitis

WT C57BL/6 mice were given a single intravenous injection of α-GalCer (50 μg/kg) dissolved in 200 μL of saline (11). MSCs were intravenously injected (5 x 10^5 cells), via the tail vein, into C57BL/6 mice immediately after α-GalCer administration (α-GalCer+MSC-treated mice), while control animals received the appropriate amount of MSCs only or saline only. Serum levels of AST and ALT were measured 16 h after intravenous injection of α-GalCer (17).

Isolation of hepatic mononuclear cells and analysis with flow cytometry

The isolation of liver-infiltrating mononuclear cells was conducted as previously described (11). Hepatic mononuclear cells were screened for various cell surface and intracellular markers with flow cytometry 8 h after Con A and 2 h after α-GalCer injection. Briefly, MNC (1x10^6) were incubated with anti-mouse CD3, CD4, CD25, or CD49b mono-

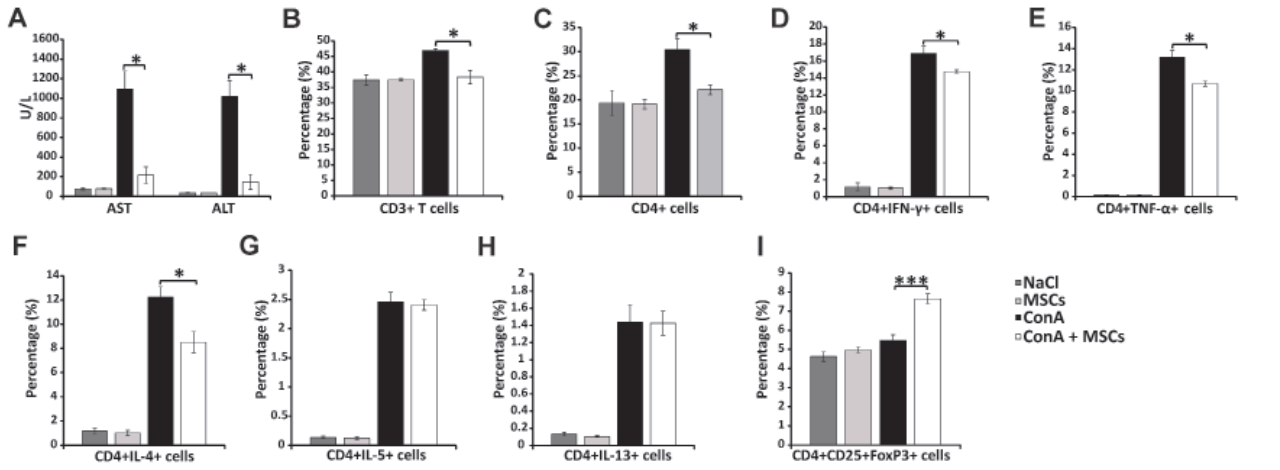

Figure 1. MSCs ameliorate Con A-induced hepatitis by reducing the total number of inflammatory CD4+ T cells and by increasing the presence of CD4+CD25+FoxP3+ T regulatory cells in the injured livers
(A) Serum levels of AST and ALT in Con A-treated mice. (B-I) Percentages of liver-infiltrating CD3+ T cells, CD4+ T cells, IFN gamma, TNFα, IL-4, IL-5, and IL-13-producing CD4+ T cells and CD4+CD25+FoxP3+ T regulatory cells. Data are presented as the mean ± SEM; n=10 mice per experimental group. **p<0.05, ***p<0.001.*

clonal antibodies conjugated with fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin chlorophyll protein (PerCP) or allophycocyanin (APC) (all from BD Biosciences, San Jose, CA, USA) following the manufacturer's instructions. MNCs were concomitantly stained for their intracellular content of TNF-α, IFN-γ, IL-4, IL-5, IL-10 and IL-13 by using the fixation/permeabilization kit and anti-mouse monoclonal antibodies conjugated with FITC, PE, PerCP and APC (BD Bioscience). For intracellular cytokine staining, cells were stimulated with 50 ng/mL phorbol-12-myristate-13-acetate (PMA) and 500 ng/mL ionomycin for 5 h, and GolgiStop (BD Biosciences) was added. Cells were fixed in Cytofix/Cytoperm, permeated with 0.1% saponin, and stained with fluorescent Abs. Flow cytometric analysis was conducted on a BD Biosciences FACSCalibur flow cytometer and analysed by using the Flowing software analysis program.

Generation of MSC-conditioned medium (MSC-CM)

MSCs were first cultured in serum-containing complete medium and incubated at 37°C in a humid atmosphere with 5% CO_2 in order to generate the MSC-CM. At 80% confluence, the cells were washed twice with 1X phosphate buffered saline (PBS, Invitrogen), and the medium was then changed to serum-free medium. The media were collected, 48 h later, centrifuged at 13 000×g at 4°C for 10 min and stored at -80°C until used (18).

Pharmacological inhibition of IDO

MSCs were cultured for 48 h in culture medium which contained 1 mM 1-methyltryptophan, (1-MT, Sigma-Aldrich, St-Louis, MO), a well-known inhibitor of IDO enzymatic activity (19).

Statistical analysis

Results were analysed using the Student's t test and SPSS 22.0 for Windows software (SPSS Inc., Chicago, IL). All data in this study were expressed as the mean ± standard error of the mean (SEM). Values of $p<0.05$ were considered statistically significant.

RESULTS

MSCs ameliorate acute hepatitis by reducing the capacity of liver-infiltrated CD4+T cells to produce inflammatory cytokines

MSCs efficiently attenuated Con A-induced acute liver injury as determined by liver enzyme tests (Figure 1A) and by flow cytometric analysis. Levels of serum AST and ALT were significantly lower ($p<0.05$) in Con A+MSC-treated mice compared to mice that received Con A only (Figure 1A). Flow cytometric analysis revealed a significant decrease in the percentage of CD3+ T cells ($p<0.05$), CD4+ T cells, IFN-γ-, TNF-α-, and IL-4 producing CD4+ T cells in the livers of Con A+MSC-treated mice ($p<0.05$, Fig. 1 B- F). Transplanted MSCs successfully and notably increased the presence of CD4+CD25+FoxP3+ T regulatory cells in the livers of Con A+MSC-treated mice (Fig. 1I). Since Tregs are able to attenuate acute liver failure (20,21), MSC-mediated increases of CD4+CD25+FoxP3+ T regulatory cells might be responsible for beneficial effects of MSCs. There was no significant difference in the percentage of IL-5 and IL-13 producing CD4+ cells ($p>0.05$; Fig. 1 G, H) between Con A+MSC- and Con A-only treated mice, indicating that MSCs did not induce polarization of naive T cells into effector Th2 cells.

MSCs significantly reduced acute liver injury by promoting expansion of IL-10-producing CD4+CD49b+FoxP3- regulatory cells

Similar to what we saw in Con A-induced hepatitis, the serum levels of AST and ALT were significantly reduced ($p<0.05$) in α-GalCer +MSC-treated mice compared to

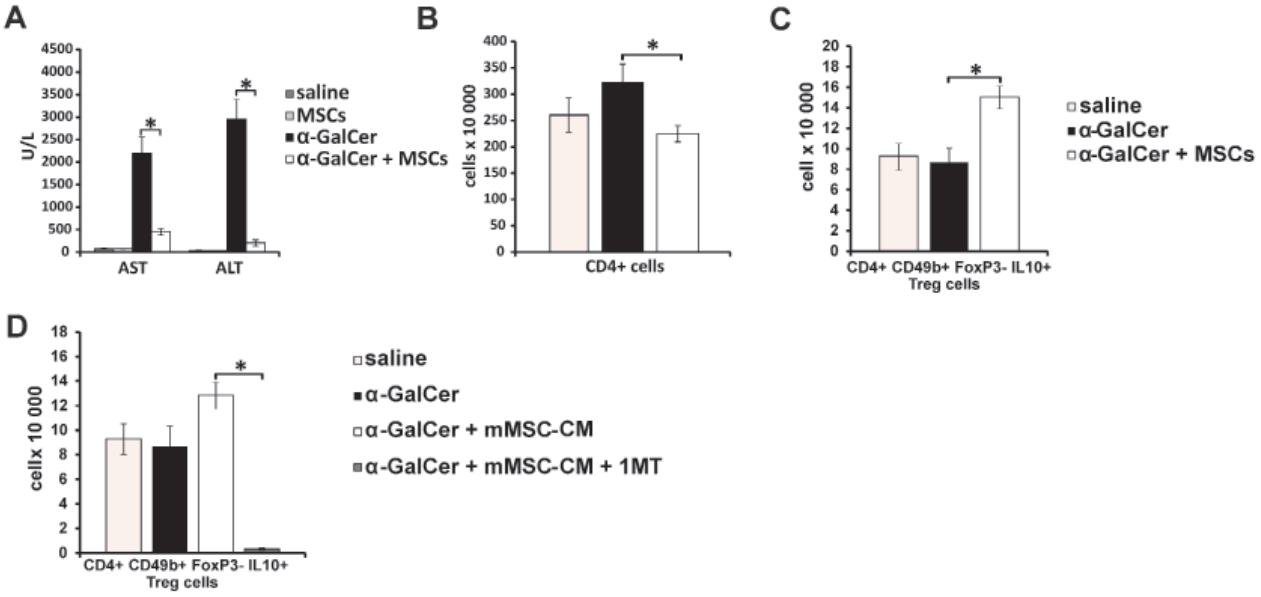

Figure 2. IDO is critically involved in MSC-based expansion of CD4+CD49b+FoxP3- T regulatory cells
(A) Serum levels of AST and ALT in α-GalCer-treated mice. (B) Total number of liver-infiltrating CD4+ T cells and (C) IL-10-producing CD4+CD49b+FoxP3- T regulatory cells in Con A and MSCs+α-GalCer-treated mice. (D) Total number of liver-infiltrating IL-10-producing CD4+CD49b+FoxP3- T regulatory cells in α-GalCer, MSC+CM+α-GalCer and 1-MT+MSC-CM+α-GalCer-treated mice. Data are presented as the mean ± SEM; n=10 mice per experimental group. *$p<0.05$.

mice that received α-GalCer only (Figure 2A). The total number of CD4+ T cells was significantly decreased in the livers of α-GalCer+MSC-treated mice compared to animals treated with α-GalCer only ($p<0.05$; Fig. 2B). Most importantly, intravenous injection of MSCs successfully significantly increased the number of CD4+CD49b+FoxP3-IL10+ cells in the livers of α-GalCer-treated mice ($p<0.05$; Fig. 2C), confirming that the beneficial effects of MSCs are at least partially reliant on MSC-dependent expansion of regulatory cells in the injured livers.

To investigate whether soluble factors were responsible for the immunomodulatory effects of MSCs and their capacity to induce expansion of regulatory cells in the livers, mice were intravenously injected with MSC-CM immediately after α-GalCer administration (α-GalCer+MSC-CM-treated mice). Similar to the effects observed after injection of MSCs, MSC-CM treatment significantly increased the total number of CD4+CD49b+FoxP3-IL10+T regulatory cells in the livers of α-GalCer treated mice ($p<0.05$; Fig. 2D), indicating that MSCs promoted the expansion of CD4+CD49b+FoxP3-IL10+T regulatory cells in a paracrine manner.

IDO is critically involved in MSC-based expansion of CD4+CD49b+FoxP3- T regulatory cells

Since we previously demonstrated the crucial importance of IDO for MSC-dependent expansion of IL-10-producing CD4+CD25+FoxP3+ Tregs in injured livers of α-GalCer-treated animals (15), we now examined the effects of IDO on MSC-dependent expansion of CD4+CD49b+FoxP3-IL10+Tregs. For this purpose, we used 1-MT, which inhibits mRNA expression of IDO in MSCs through p38 mitogen-activated protein kinase (MAPK) and c-Jun N-terminal kinase (JNK) signalling (22). Intracellular staining of liver MNCs revealed significantly lower total numbers of CD4+CD49b+FoxP3-IL10+Tregs in the livers of α-GalCer+MSC-CM+1-MT-treated mice compared to α-GalCer+MSC-CM-treated animals, confirming that MSC-mediated expansion of IL-10-producing CD4+CD49b+FoxP3-regulatory cells in injured livers was IDO dependent.

DISCUSSION

In several recently published papers, we and others demonstrated the therapeutic potential of MSCs in acute liver failure (8,9,23). In this study, we introduce the first evidence that MSCs are able to attenuate hepatitis by promoting expansion of "non-classical" populations of T regulatory cells (CD4+CD49b+FoxP3-cells) in a paracrine IDO-dependent manner.

MSCs significantly reduced Con A- and α-GalCer-mediated hepatitis in C57BL/6 mice by inducing the conversion of inflammatory T cells (Fig. 1B-F) into immunosuppressive regulatory T cells (Fig. 1I), which is in line with our previous findings (3, 15, 23). We recently described the molecular mechanisms involved in the crosstalk between MSCs and regulatory cells during acute liver failure (15). MSC-dependent attenuation of α-GalCer-induced acute liver injury in mice was accompanied by an increased presence of IL-10-producing CD4+ CD25+ FoxP3+ T regulato-

ry cells and IL10- and TGFβ-producing marginal zone-like B regulatory cells in the liver (15). Among a variety of immunosuppressive factors which were produced by MSCs, the interplay between nitric oxide (NO) and IDO was responsible for MSC-based expansion of liver-infiltrated CD4+ CD25+ FoxP3+ T regulatory cells (15).

In addition to conventional CD4+CD25+Foxp3+T regulatory cells (24), another "non-classical" type of T regulatory cells, named type 1 T regulatory cells (Tr1 cells) was identified several years ago (25). Tr1 cells are found in both humans and mice and are characterized by their copious secretion of IL-10 and their lack of Foxp3 expression. Human and mouse Tr1 clones co-express CD49b, the α2 subunit of the adhesion molecule very late antigen (VLA)-2, which specifically binds to collagens I, II, and X, and lymphocyte activation gene 3 (LAG-3), which differentiates Tr1 cells from other CD4+ T cells, including Th1, Th2, Th17, and Foxp3+ T regulatory cells (26). We found that administration of MSCs attenuated α-GalCer-induced liver injury by increasing the total number of CD4+CD49b+FoxP3- Tr1 cells (Figure 2 C). Additionally, MSCs as well as MSC-CM increased the capacity of Tr1 cells to produce immunosuppressive IL-10, leading to the attenuation of acute hepatitis (Figure 2D). It is well known that Tr1 cells suppress tissue inflammation, graft-versus-host disease, and autoimmunity by producing anti-inflammatory cytokine IL-10 in an antigen-specific manner (27,28). Tr1-derived IL-10 enhances the production of human leukocyte antigen (HLA)-G5 in MSCs and promotes MSC-dependent expansion of CD4+CD25+FoxP3+ T regulatory cells (29). CD4+CD49b+FoxP3- Tr1 cells exhibit a suppressive function in a hepatitis B virus (HBV)-carrier mouse model (29) and human hepatitis C virus (HCV) infection (30). Interestingly, their suppressive effect is even higher than that of the classical CD4+CD25+FoxP3+ T regulatory cells (31). It has been reported that naïve T cells can be induced to become Foxp3-Tr1 cells by IL-10, and these Foxp3-Tr1 cells secrete high levels of IL-10 and TGF-β to modulate the inflammatory microenvironment.

It has been elucidated that IDO promotes the degradation of tryptophan into kynurenine and toxic metabolites (quinolinic acid and 3-hydroxy-anthranillic acid), which suppress proliferation or induce apoptosis of T cells through activation of the stress response kinase GCN2 (32). Additionally, IDO triggers the production of immunosuppressive IL-10 in activated lymphocytes, while 1-MT significantly impairs the capacity of stimulated lymphocytes to secrete IL-10 (33). In our recently published paper we demonstrated that MSC-derived IDO is crucially important for the expansion of IL-10-producing CD4+CD25+FoxP3+ T regulatory cells and consequent attenuation of acute liver injury (12). In line with these findings, here we demonstrated that IDO inhibition completely abrogated the capacity of MSC-CM to induce expansion of IL-10-producing CD4+CD49b+FoxP3- Tr1 cells (Figure 2 D) and to attenuate acute hepatitis.

In conclusion, the capacity of MSCs to promote expansion of immunosuppressive CD4+CD49b+FoxP3- Tr1 cells in an IDO-dependent manner should be explored in future studies as new therapeutic approach for the treatment of liver diseases which are mediated by T cells.

ACKNOWLEDGEMENTS

This study was supported by the Macroproject of the Faculty of Medical Sciences University of Kragujevac (MP 01/18).

CONFLICT OF INTEREST

None.

REFERENCES:

1. Zhou Y, Dai W, Lin C, Wang F, He L, Shen M, Chen P, Wang C, Lu J, Xu L, XuX,Guo C. Protective effects of necrostatin-1 against concanavalin A-induced acute hepatic injury in mice. Mediators Inflamm. 2013;2013:706156.
2. Eggink HF, Houthoff HJ, Huitema S, Gips CH, Poppema S. Cellular and humoral immune reactions in chronic active liver disease. I. Lymphocyte subsets in liver biopsies of patients with untreated idiopathic autoimmune hepatitis, chronic active hepatitis B and primary biliary cirrhosis. ClinExpImmunol. 1982;50:17-24.
3. Volarevic V, Arsenijevic N, Lukic ML, Stojkovic M. Concise review: Mesenchymal stem cell treatment of the complications of diabetes mellitus. Stem Cells. 2011 (1):5-10.
4. Li N, Hua J. Interactions between mesenchymal stem cells and the immune system. Cell Mol Life Sci. 2017;74:2345-2360.
5. Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, Reyes M, Lenvik T, Lund T, Blackstad M, Du J, Aldrich S, Lisberg A, Low WC,Largaespada DA, Verfaillie CM. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature. 2002;418:41-9.
6. 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.
7. Nikolic A, SimovicMarkovic B, Gazdic M, Randall Harrell C, FellabaumC,Jovicic N, Djonov V, Arsenijevic N, L Lukic M, Stojkovic M, VolarevicV.Intraperitoneal administration of mesenchymal stem cells ameliorates acute dextran sulfate sodium-induced colitis by suppressing dendritic cells. Biomed Pharmacother. 2018;100:426-432.
8. Biburger M, Tiegs G. Alpha-galactosylceramide-induced liver injury in mice is mediated by TNF-alpha but independent of Kupffer cells. J Immunol. 2005;175:1540-50.

9. Tiegs G, Hentschel J, Wendel A. A T cell-dependent experimental liver injury in mice inducible by concanavalin A. J Clin Invest. 1992;90:196-203.
10. Volarevic V, Misirkic M, Vucicevic L, Paunovic V, SimovicMarkovicB,Stojanovic M, Milovanovic M, Jakovljevic V, Micic D, Arsenijevic N, Trajkovic V, Lukic ML. Metformin aggravates immune-mediated liver injury in mice. Arch Toxicol. 2015;89:437-50.
11. Volarevic V, Mitrovic M, Milovanovic M, Zelen I, Nikolic I, MitrovicS,Pejnovic N, Arsenijevic N, Lukic ML. Protective role of IL-33/ST2 axis in Con A-induced hepatitis. J Hepatol. 2012;56:26-33.
12. Gazdic M, SimovicMarkovic B, Vucicevic L, Nikolic T, Djonov V, ArsenijevicN,Trajkovic V, Lukic ML, Volarevic V. Mesenchymal stem cells protect from acute liver injury by attenuating hepatotoxicity of liver natural killer T cells in an inducible nitric oxide synthase- and indoleamine 2,3-dioxygenase-dependent manner. J Tissue EngRegen Med. 2018;12:e1173-e1185.
13. Biburger M, Tiegs G. Alpha-galactosylceramide-induced liver injury in mice is mediated by TNF-alpha but independent of Kupffer cells. J Immunol. 2005;175:1540-50.
14. Milosavljevic N, Gazdic M, SimovicMarkovic B, Arsenijevic A, NurkovicJ,Dolicanin Z, Djonov V, Lukic ML, Volarevic V. Mesenchymal stem cells attenuate acute liver injury by altering ratio between interleukin 17 producing and regulatory natural killer T cells. Liver Transpl. 2017;23:1040-1050.
15. Gazdic M, Markovic BS, Arsenijevic A, Jovicic N, Acovic A, Harrell CR,Fellabaum C, Djonov V, Arsenijevic N, Lukic ML, Volarevic V. Crosstalk between mesenchymal stem cells and T regulatory cells is crucially important for the attenuation of acute liver injury. Liver Transpl. 2018;24:687-702.
16. Volarevic V, Milovanovic M, Ljujic B, Pejnovic N, Arsenijevic N, Nilsson U, Leffler H, Lukic ML. Galectin-3 deficiency prevents concanavalin A-induced hepatitis in mice. Hepatology. 2012;55:1954-64.
17. Volarevic V, Markovic BS, Bojic S, Stojanovic M, Nilsson U, Leffler H, Besra GS, Arsenijevic N, Paunovic V, Trajkovic V, Lukic ML. Gal-3 regulates the capacity of dendritic cells to promote NKT-cell-induced liver injury. Eur J Immunol. 2015;45:531-43.
18. Linero I, Chaparro O. Paracrine effect of mesenchymal stem cells derived from human adipose tissue in bone regeneration. PLoS One. 2014;9:e107001.
19. Yang SH, Park MJ, Yoon IH, Kim SY, Hong SH, Shin JY, Nam HY, Kim YH, Kim B, Park CG. Soluble mediators from mesenchymal stem cells suppress T cell proliferation by inducing IL-10. ExpMol Med. 2009;41:315-24.
20. Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood. 2005;105:1815-22.
21. Milosavljevic N, Gazdic M, SimovicMarkovic B, Arsenijevic A, NurkovicJ,Dolicanin Z, Jovicic N, Jeftic I, Djonov V, Arsenijevic N, Lukic ML, Volarevic V. Mesenchymal stem cells attenuate liver fibrosis by suppressing Th17 cells – an experimental study. Transpl Int. 2018;31:102-115
22. Lim JY, Im KI, Lee ES, Kim N, Nam YS, Jeon YW, Cho SG. Enhanced immunoregulation of mesenchymal stem cells by IL-10-producing type 1 regulatory T cells in collagen-induced arthritis. Sci Rep. 2016;6:26851.
23. Volarevic V, Nurkovic J, Arsenijevic N, Stojkovic M. Concise review: Therapeutic potential of mesenchymal stem cells for the treatment of acute liver failure and cirrhosis. Stem Cells. 2014;32:2818-23.
24. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. Science. 2003;299:1057-61.
25. Groux H, O'Garra A, Bigler M, Rouleau M, Antonenko S, de Vries JE, Roncarolo MG. A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. Nature. 1997;389:737-42.
26. Gagliani N, Magnani CF, Huber S, Gianolini ME, Pala M, Licona-Limon P, et al. Coexpression of CD49b and LAG-3 identifies human and mouse T regulatory type 1 cells. Nature medicine. 2013; 19:739–46.
27. Ahangarani RR, Janssens W, VanderElst L, Carlier V, VandenDriessche T, Chuah M, Weynand B, Vanoirbeek JA, Jacquemin M, Saint-Remy JM. In vivo induction of type 1-like regulatory T cells using genetically modified B cells confers long-term IL-10-dependent antigen-specific unresponsiveness. J Immunol. 2009;183:8232-43.
28. Xu L, Yin W, Sun R, Wei H, Tian Z. Liver type I regulatory T cells suppress germinal center formation in HBV-tolerant mice. ProcNatlAcadSci U S A. 2013;110:16993-8.
29. Nasef A, Mazurier C, Bouchet S, François S, Chapel A, Thierry D, Gorin NC, Fouillard L. Leukemia inhibitory factor: Role in human mesenchymal stem cells mediated immunosuppression. Cell Immunol. 2008;253:16-22.
30. Carpentier A, Conti F, Stenard F, Aoudjehane L, Miroux C, Podevin P, Morales O, Chouzenoux S, Scatton O, Groux H, Auriault C, Calmus Y, Pancre V, DelhemN. Increased expression of regulatory Tr1 cells in recurrent hepatitis C after liver transplantation. Am J Transplant. 2009;9:2102-12.
31. Wildbaum G, Netzer N, Karin N. Tr1 cell-dependent active tolerance blunts the pathogenic effects of determinant spreading. J Clin Invest. 2002;110:701-10
32. Munn DH, Sharma MD, Baban B, Harding HP, Zhang Y, Ron D, Mellor AL. GCN2 kinase in T cells mediates proliferative arrest and anergy induction in response to indoleamine 2,3-dioxygenase. Immunity. 2005;22:633-42.
33. Eleftheriadis T, Pissas G, Karioti A, Antoniadi G, Liakopoulos V, Dafopoulou K, Pournaras S, Koukoulis G, Stefanidis I. The indoleamine 2,3-dioxygenase inhibitor 1-methyl-tryptophan suppresses mitochondrial function, induces aerobic glycolysis and decreases interleukin-10 production in human lymphocytes. Immunol Invest. 2012;41:507-20.