Published in final edited form as:

J Surg Res. 2015 December ; 199(2): 615–621. doi:10.1016/j.jss.2015.06.023.

Mesenchymal Stem Cells Reverse T/HS Induced Bone Marrow Dysfunction

Amy V. Gore, MD, Letitia E. Bible, MD, David H. Livingston, MD, Alicia M. Mohr, MD, and Ziad C. Sifri, MD

Department of Surgery, Division of Trauma, Rutgers – New Jersey Medical School

Abstract

Intro—Lung contusion (LC) followed by hemorrhagic shock (HS) causes persistent bone marrow (BM) dysfunction lasting up to seven days after injury. Mesenchymal stem cells (MSC) are multipotent cells that can hasten healing as well as exert protective immunomodulatory effects. We hypothesize that MSC can attenuate BM dysfunction following combined LCHS.

Materials and Methods—Male Sprague-Dawley (SD) rats (n=5-6/group) underwent LC+45 minutes of HS (MAP of 30-35). Allogeneic MSCs (5×10^6 cells) were injected IV following resuscitation. At seven days, BM was analyzed for cellularity and growth of hematopoietic progenitor cell (HPC) colonies (CFU-E, BFU-E, CFU-GEMM). Flow cytometry measured %HPCs in peripheral blood (PB); plasma G-CSF levels were measured via ELISA. Data was analyzed by one-way ANOVA followed by Tukey's multiple comparison test.

Results—As previously shown, at seven days, LCHS resulted in 22, 30, and 24% decreases in CFU-GEMM, BFU-E and CFU-E colony growth respectively vs. naïve. Treatment with MSCs returned all BM parameters to naïve levels. There was no difference in %HPCs in PB between groups, however, G-CSF remained elevated up to seven days following LCHS. MSCs returned G-CSF to naïve levels. Plasma from animals receiving MSCs was not suppressive to the BM.

Conclusion—One week following injury, the persistent BM dysfunction seen in animals undergoing LCHS is reversed by treatment with MSCs with an associated return of plasma G-CSF levels to normal. Plasma from animals undergoing LCHS+MSCs was not suppressive to BM cells *in vitro*. Treatment with MSCs following injury and shock reverses BM suppression and returns plasma G-CSF levels to normal.

Address correspondence to: Ziad C. Sifri, MD, Rutgers New Jersey Medical School, Department of Surgery, 150 Bergen Street M232, Newark, NJ 07101, Tel: (973) 972-3808, FAX: (973) 972-7441, sifrizi@njms.rutgers.edu.

(goreav@njms.rutgers.edu)
(biblele@njms.rutgers.edu)
(livingst@njms.rutgers.edu)
(Alicia.mohr@surgery.ufl.edu)

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

No further disclosures to report.

Authorship Statement: The role of each author is as described below. Amy Gore was involved in experimental design, data acquisition, analysis and interpretation of data, and manuscript preparation. Letitia Bible in data acquisition and analysis. David Livingston, Alicia Mohr, and Ziad Sifri in design, data analysis and interpretation, and critical revision.

Keywords

Mesenchymal Stem Cells; Trauma; Hemorrhagic Shock; Hematopoietic progenitor cells; G-CSF

INTRODUCTION

Persistent injury-associated anemia and increased susceptibility to infection [1,2] are manifestations of the bone marrow (BM) dysfunction seen following severe injury with significant clinical ramifications. In a rodent model of lung contusion alone (LC), this dysfunction is manifested as a reduction of BM cellularity and growth of hematopoietic progenitor cells (HPCs) within the BM itself as well as mobilization of HPCs to the periphery with an associated rise in plasma G-CSF early after injury, however rats have recovered within seven days [3,4]. When animals undergo hemorrhagic shock following contusion (LCHS), BM dysfunction is both more acutely marked and persists for greater than one week [3,4].

Mesenchymal stem cells (MSC) are a type of multipotent cell with multiple immunomodulatory and paracrine functions that are essential to maintaining the BM microenvironment and supporting hematopoiesis [5-7]. They have been investigated in several disease states, such as graft-versus-host disease, aplastic anemia, and myocardial infarction, as a potential cellular therapy to expedite healing and restore homeostasis [8-10].

We have previously investigated the role of these cells in lung healing [11]; however, have not examined their role in treating the BM dysfunction that ensues after injury. We hypothesize that treatment of rats undergoing combined LCHS with MSCs given early following resuscitation can reverse injury-associated BM dysfunction. Furthermore, we hypothesize that this protection occurs via a systemic effect by altering the plasma in shocked animals such that it is no longer suppressive to BM cells *in vitro*.

MATERIALS AND METHODS

Experimental Groups

Male Rats (n=5-6/group) were assigned to experimental groups as follows: Lung contusion (LC) alone, lung contusion plus mesenchymal stem cells (LC+MSC), lung contusion followed by hemorrhagic shock (LCHS), and lung contusion followed by hemorrhagic shock plus mesenchymal stem cells (LCHS+MSC). A control naïve group of uninjured animals undergoing daily handling was also used. On day seven, rats were sacrificed and bone marrow and blood collected.

Animals

Male Sprague-Dawley (SD) rats weighing between 250 and 350g (Charles River, Wilmington, MA) were maintained according to the recommendations of the Guide for the Care and Use of Laboratory Animals and experiments were approved by the Rutgers New Jersey Medical School Animal Care and Use Committee. Rats were housed in a barrier-

sustained animal facility at 25° C with 12-hour light/dark cycle. Animals had free access to water and chow (Teklad 22/5 Rodent Diet W-8640; Harlen, Madison WI).

Lung Contusion Model

After intraperitoneal administration of sodium pentobarbital (50mg/kg), a 12 mm metal plate was secured to the rat's right axilla and a percussive nail gun (Craftsman 968514 Stapler, Sears Brands, Chicago IL) was used to induce a unilateral lung contusion.

Hemorrhagic Shock Model

As previously described [12], immediately following LC, rats in the LCHS and LCHS+MSC groups underwent cannulation of the right internal jugular vein and right femoral artery with polyethylene (PE-50; Becton Dickinson and Co., Sparks, MD) and Silastic (Dow Corning Corp., Midland, MI) tubing, respectively. To prevent clotting, all tubing was flushed with 10 units/ml of heparinized saline. The femoral artery catheter was then connected to a continuous blood pressure monitoring device for measurement of mean arterial pressure (MAP) and heart rate. Animals were bled to a MAP of 30-35mmHg for 45 minutes; additional blood was withdrawn or re-infused as needed in order to maintain MAP within range. Temperature was maintained at approximately 37 °C with the use of an electric heating pad placed under the surgical platform. Following the completion of the shock period, shed blood was re-infused at a rate of 1mL/min.

Mesenchymal Stem Cell Culture

As previously described [13], SD rat mesenchymal stem cell (MSC) cultures (Cyagen Biosciences, Santa Clara, CA) were established and expanded. Briefly, cells were thawed and transferred into 15mL OriCell MSC Growth Medium. Cells were washed and re-suspended 2-3 mL fresh growth medium. Cells were incubated in a 37°C humidified 5% CO₂ incubator after seeding into T25 flasks containing additional growth medium. Medium was changed at 24 hour and then every 72 hours. Cells were dissociated with Trypsin-EDTA when 80-90% confluent and re-seeded at $3 \times 10^3/\text{cm}^2$. This continued until adequate cell counts were available for harvest. On the day of injection, 5×10^6 cells MSCs were quantified and aliquoted into individual vials. Cells were washed with IMDM and resuspended in 1mL IMDM prior to injection.

Mesenchymal Stem Cell Injection

A previously prepared suspension of 5×10^6 MSC in one mL IMDM was infused via the previously cannulated right internal jugular vein over five minutes, beginning within ten minutes of injury in the LC and LC+MSC groups or within ten minutes of return of shed blood in the groups undergoing HS.

Bone Marrow Cellularity

BM cells were harvested from the right femur to determine cellularity and establish cell culture. An 18-gauge needle was inserted into the femur and BM was aspirated into 1mL IMDM. Cells were suspended, stained with 0.4% Trypan blue, and hemocytometer was used to determine total viable cell count.

Bone Marrow Hematopoietic Progenitor Cell Cultures

Growth of hematopoietic progenitor cells from the BM was assessed by culturing colony-forming unit-granulocyte-erythrocyte-, monocyte-, megakaryocyte (CFU-GEMM), burst-forming unit-erythroid (BFU-E), and colony-forming unit-erythroid (CFU-E). A stock solution of BM cells was prepared according to the cellularity to reach a concentration of 1×10^6 cells/mL of IMDM. A solution of IMDM supplemented with 30% FBS, 2% BSA, rat growth factor, 1% methylcellulose, 2×10^{-4} mol/L 2-mercaptoethanol, penicillin/streptomycin, and glutamine was prepared, and 1.5×10^5 cells added to it. All cultures were plated in duplicate. Those plates growing BFU-E/CFU-E were further supplemented with 1.3 U/mL rhEpo and 6 U/mL rhIL-3. Plates growing CFU-GEMM were supplemented with 3 U/mL rhGM-CSF. Cultures were incubated at 37°C in 5% CO₂. CFU-E colonies were counted on day 7, BFU-E on day 14, and CFU-GEMM on day 17 by an observer blinded to sample origin.

Cultures with Experimental Plasma

Bone marrow was collected from naïve rats and cellularity and stock solutions prepared as described above. Again, cells were plated in duplicate. While all BM cells came from naïve rats, cells were plated with the addition of 5% volume per volume of plasma from rats in one of the following experimental groups: unmanipulated control (UC), LCHS, and LCHS +MSC. An additional control group consisting of naïve BM cells incubated with media alone was used for comparison (naïve). Plasma was obtained following sacrifice on day 7 after injury. As above, cultures were counted following the appropriate number of days of incubation according to colony type by a blinded observer.

Flow Cytometry for Hematopoietic Progenitor Cells

At the time of sacrifice, blood was obtained via direct cardiac puncture using a 10-mL heparinized syringe. A well established, single-platform enumeration method was used to determine the frequency of CD71+, CD117+ hematopoietic progenitor cells within unfractionated blood samples. Briefly, 100 µl whole blood was labeled with 10 µL of BD Pharmingen™ mouse anti-rat CD71 antibody conjugated with fluorescein isothiocyanate (FITC) and 10 µL of BD Pharmingen™ rat anti-mouse CD117 (c-Kit) antibody conjugated to phycoerythrin (PE) (BD Biosciences, Franklin Lakes, NJ) for 30 minutes. Erythrocytes were then lysed by ammonium chloride in a 37°C in 5% CO₂ incubator for 10 minutes. Following lysis, cells were centrifuged at $300 \times g$ for five minutes and supernatant was discarded prior to washing three times. After fixation with BD Cytfix solution (BD), cells were analyzed using BD FACSCalibur flow cytometer (BD) equipped with CellQuest software (BD) with an event count of 50,000 each run.

Plasma G-CSF Levels

A commercial ELISA kit (R&D Systems, Minneapolis, MN) run in duplicate was used to determine plasma G-CSF levels. Briefly, 50 µL of assay diluent RD1-54 and 50 µL of standard, control, or sample was added to wells pre-coated with polyclonal anti-mouse G-CSF. Plate was covered and following two hour incubation at room temperature, wells were aspirated and washed a total of five times. 100 µL Mouse G-CSF conjugate was then added

to each well prior to another two hour covered incubation. Plate was again washed a total of five times before adding 100 μ L substrate solution to each well and a 30 minute dark incubation. 100 μ L stop solution was then added to each well, mixed gently, and plate was read using 450-540nm wavelength correction.

Reagents

Bovine serum albumin (BSA), and 2-mercaptoethanol were purchased from Sigma-Aldrich (St. Louis, MO). Methylcellulose was purchased from Stemcell Technologies (Vancouver, Canada). Fetal bovine serum (FBS), Iscove's Modified Dulbecco's Medium (IMDM), glutamine, penicillin/streptomycin, and trypan blue were obtained from Invitrogen (Carlsbad, CA). All cytokines rhEpo, rhIL-3, rhGM-CSF were purchased from R&D Systems (Minneapolis, MN). Sodium pentobarbital was purchased from B&B Pharmacy (Bellflower, CA) and heparin was obtained from Hospira Inc. (Lakefront, IL).

Statistical Analysis

All data are expressed as mean $\pm$ SD. Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Tukey-Kramer's multiple comparison post-test or Student's t-test with GraphPad Prism (Version 4.0, San Diego, CA). Results were considered significant if * $p < 0.05$ vs. LC or ** $p < 0.05$ vs. LC/CS.

RESULTS

Effect of Mesenchymal Stem Cells on Bone Marrow Cellularity

There is no statistically significant difference in bone marrow cellularity in those animals undergoing either unilateral LC alone or LC+MSC as compared to naïve at seven days. Seven days following LCHS there is a 13% decrease in BM cellularity as compared to naïve (197 ± 19 vs 225 ± 6 ; $p < 0.05$). The addition of MSCs to animals undergoing LCHS resulted in a 9.7% increase in cellularity as compared to LCHS alone, returning cellularity to naïve levels (217 ± 4 vs 197 ± 19 ; $p < 0.05$) (Figure 1A).

Effect of Mesenchymal Stem Cells on Bone Marrow Hematopoietic Progenitor Cell Cultures

There is no significant change in growth of any of the hematopoietic progenitor cell types examined seven days following either LC alone or LC+MSCs. There is a 22% decrease in CFU-GEMM seven days following LCHS (28 ± 1 vs 35 ± 1 ; $p < 0.05$). The addition of MSCs at the time of resuscitation results in a 16% increase in CFU-GEMM growth, returning it to naïve levels (33 ± 1 vs 28 ± 1) (Figure 1B). At seven days following LCHS there are 30 and 24% decreases in BFU-E and CFU-E respectively (50 ± 3 vs 68 ± 3 and 60 ± 3 vs 77 ± 4 respectively; $p < 0.05$). When animals undergoing LCHS were given MSCs, there were 33 and 30% increases in BFU-E and CFU-E respectively, again returning colony growth to naïve levels (70 ± 1 vs 50 ± 3 and 81 ± 1 vs 60 ± 3 respectively, $p < 0.05$) (Figure 1C and 1D).

Effect of Mesenchymal Stem Cells on Mobilization of Hematopoietic Progenitor Cells and Plasma G-CSF

Seven days following either LC or LCHS there was no statistically significant difference in HPC mobilization (Figure 2A). Furthermore the addition of MSCs to LC or LCHS groups did not significantly alter the percentage of HPCs circulating in the peripheral blood. However, plasma G-CSF levels remained elevated in those animals undergoing LCHS (12.3 ± 3.6 vs 4.7 ± 2.1 ; $p < 0.05$). The addition of MSCs returned G-CSF to naïve levels (5.4 ± 1.7 vs 4.7 ± 2.1 ; $p > 0.05$) (Figure 2B).

Effect of Experimental Plasma on Naïve Bone Marrow Hematopoietic Progenitor Cell Growth *in vitro*

There was no difference in CFU-E colony counts in naïve BM incubated with media alone and naïve BM incubated with 5% volume/volume naïve plasma (UC). When naïve BM cells were incubated with 5% plasma from 7-day animals undergoing LCHS there was a 30% decrease in CFU-E colony growth as compared to naïve cells incubated with media alone (57.3 ± 2.6 vs 77.4 ± 4.0 $p < 0.001$). When naïve BM cells were incubated with plasma from animals undergoing LCHS with MSCs given following resuscitation, suppression was no longer seen (76.1 ± 2.3 vs 77.4 ± 4.0 ; $p > 0.05$) (Figure 3).

DISCUSSION

Bone marrow dysfunction has long been described following severe injury and hemorrhagic shock [1,2]. In human trauma patients, this dysfunction manifests as both disordered growth of hematopoietic progenitor cells within the bone marrow itself as well as increased mobilization of these cells to the periphery [2]. In critically injured patients, this dysfunction manifests as a persistent post-traumatic anemia [14]. In the current study we examine the effects of MSCs in a well-established rat model of combined LCHS resulting in BM dysfunction [3,4]. We demonstrate that treatment with MSCs immediately following resuscitation results in a return of HPC colony growth within the BM and plasma G-CSF levels to naïve within 7 days after injury. Furthermore, treatment with MSCs alters the composition of the plasma such that it is no longer suppressive to HPC colony growth in culture.

MSCs are multipotent cells that have been shown to have myriad paracrine and immunomodulatory functions [15]. These cells are considered to constitute an essential part of the BM microenvironment and are important in supporting hematopoietic homeostasis via the secretion of various cytokines as well as expressing adhesion molecules involved in hematopoietic stem cell homing [5-7]. While MSCs have not previously been examined in the treatment of post-traumatic BM dysfunction, their effects on the BM have been studied in the context of aplastic anemia, lupus-associated anemia, and in co-transplantation with MSCs as part of a BM transplant [16-18]. Wu et al treated a cohort of 21 patients with severe aplastic anemia with MSCs co-transplanted with haploidentical hematopoietic stem cells and demonstrated sustained hematopoietic engraftment without an increase in graft-versus-host-disease (GVHD) [19]. MSCs have also been shown to improve cell counts in patients with refractory cytopenia secondary to systemic lupus erythematosus [17]. While

these studies report dramatic results in severely ill patient populations, the exact mechanism of action of MSCs largely remains unknown. Our data demonstrates that a single dose of intravenous MSCs given immediately following resuscitation in LCHS animals resulted in increases of BM cellularity as well as hematopoietic progenitor cell growth to naïve levels by seven days after injury. The ability of MSCs to modulate and support the BM microenvironment may be protective against the dysfunction seen in the setting of combined LCHS.

Other manifestations of BM dysfunction following LCHS at seven days include HPCs returning from the periphery to the BM, despite modest elevation in plasma G-CSF levels. Elevations in plasma G-CSF have been shown in severely injured trauma patients and this elevation is associated with a significant increase in peripheral HPCs [20]. Petit et al reported that G-CSF within the BM modulates the release of HPC from their stromal attachments via elastase-dependent degradation, allowing mobilization of these cells to the periphery [21]. Clinically, G-CSF is often given in order to mobilize both hematopoietic and mesenchymal stem cells to the periphery in preparation for BM transplantation. The current data shows a relationship between exogenous MSC administration and an endogenous decrease in G-CSF. It appears that treatment with MSCs hastens the return of G-CSF to naïve levels, possibly indicating an earlier return of HPCs from the periphery and swifter recovery of BM homeostasis following injury.

While it is possible that MSCs have local effects within the BM niche, we hypothesized that their ability to protect the BM against post-traumatic dysfunction is due to their systemic effect following a global ischemia-reperfusion event. Plasma from trauma patients has been shown to inhibit HPC colony growth *in vitro* up to two weeks following injury [22]. Consistent with prior findings, we show that plasma from animals that had undergone LCHS significantly suppressed growth of CFU-E, whereas plasma from animals receiving MSCs did not. While this data indicates that MSCs modulate the plasma, reversing this *in vitro* suppressive effect, it is unknown what components of the plasma are altered. Nevertheless, this finding indicates that the effects of MSCs go beyond the local BM niche.

Our data establishes the protective role of MSCs in BM dysfunction seen following trauma and hemorrhagic shock. MSCs given immediately following resuscitation reverse BM dysfunction seen seven days after injury by returning BM cellularity and HPC colony growth to naïve levels. This protection is associated with a decrease in plasma G-CSF and the return of HPCs from the periphery. Furthermore, we demonstrate that MSCs modulate the suppressive effect of plasma on BM cells *in vitro*, indicating a systemic effect of these cells. Further studies are necessary to elucidate the mechanisms by which MSCs function on both a local level within the BM niche as well as systemically. Additionally, the optimal dosing of MSCs and therapeutic window for administration remains to be defined. The use of MSCs as a cellular therapy following severe trauma with hemorrhagic shock may provide great benefit in the treatment of BM dysfunction and its resultant anemia.

Acknowledgments

This research was supported by the National Institutes of Health Grant T32 GM069330.

REFERENCES

1. Livingston DH, Gentile PS, Malangoni MA. Bone marrow failure after hemorrhagic shock. *Circ Shock*. 1990; 30:255–263. [PubMed: 2178799]
2. Livingston DH, Anjaria D, Wu J, Hauser CJ, Chang V, Deitch EA, Rameshwar P. Bone marrow failure following severe injury in humans. *Ann Surg*. 2003; 238:748–753. [PubMed: 14578739]
3. Baranski GM, Offin MD, Sifri ZC, Elhassan IO, Hannoush EJ, Alzate WD, Rameshwar P, Livingston DH, Mohr AM. Beta-blockade protection of bone marrow following trauma: the role of G-CSF. *J Surg Res*. 2011; 170:325–331. [PubMed: 21571320]
4. Elhassan IO, Hannoush EJ, Sifri ZC, Jones E, Alzate WD, Rameshwar P, Livingston DH, Mohr AM. Beta-Blockade prevents hematopoietic progenitor cell suppression after hemorrhagic shock. *Sur Infect*. 2011; 12:273–278.
5. Bueno C, Roldan M, Anguita E, Romero-Moya D, Martin-Antonio B, Rosu-Myles M, del Cañizo C, Campos R, García R, Gómez-Casares M, Fuster JL, Jurado M, Delgado M, Menendez P. Bone marrow mesenchymal stem cells from patients with aplastic anemia maintain functional and immune properties and do not contribute to the pathogenesis of disease. *Haematologica*. 2014; 99(7):1168–1175. [PubMed: 24727813]
6. Majumdar MK, Thiede MA, Mosca JD, Moorman M, Gerson SL. Phenotypic and functional comparison of cultures of marrow-derived mesenchymal stem cells(MSCs) and stromal cells. *J Cell Physiol*. 1998; 176:57–66. [PubMed: 9618145]
7. Haynesworth SE, Baber MA, Caplan AI. Cell surface antigens on human marrow-derived mesenchymal cells are detected by monoclonal antibodies. *Bone*. 1992; 13:69–80. [PubMed: 1316137]
8. Miyahara Y, Nagaya N, Kataoka M, Yanagawa B, Tanaka K, Hao H, Ishino K, Ishida H, Shimizu T, Kangawa K, Sano S, Okano T, Kitamura S, Mori H. Monolayered mesenchymal stem cells repair scarred myocardium after myocardial infarction. *Nat Med*. 2006; 12:459–465. [PubMed: 16582917]
9. Walker PA, Bedi SS, Shah SK, Jimenez F, Xue J, Hamilton JA, Smith P, Thomas CP, Mays RW, Pati S, Cox CS Jr. Intravenous multipotent adult progenitor cell therapy after traumatic brain injury: modulation of the resident microglia population. *J Neurol*. 2012; 9:228–240.
10. Le Blanc K, Frasson F, Ball L, Locatelli F, Roelofs H, Lewis I, Lanino E, Sundberg B, Bernardo ME, Remberger M, Dini G, Egeler RM, Bacigalupo A, Fibbe W, Ringden O. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. *Lancet*. 2008; 371(9624):1579–1586. [PubMed: 18468541]
11. Hannoush EJ, Elhassan I, Sifri ZC, Mohr AM, Alzate WD, Livingston DH, et al. Role of bone marrow and mesenchymal stem cells in healing after traumatic injury. *Surgery*. 2013; 153(1):44–51. [PubMed: 22862904]
12. Pasupuleti LV, Cook KM, Sifri ZC, Kotamarti S, Calderon GM, Alzate WD, Livingston DH, Mohr AM. Does selective beta-1 blockade provide bone marrow protection after trauma/hemorrhagic shock? *Surgery*. 2012; 152(3):322–30. [PubMed: 22938894]
13. Gore AV, Bible LE, Livingston DH, Mohr AM, Sifri ZC. Can Mesenchymal Stem Cells Reverse Chronic Stress-Induced Impairment of Wound Healing following Traumatic Injury? *J Trauma and Acute Care Surg*. In press.
14. Corwin HL, Surgenor SD, Gettinger A. Transfusion practice in the critically ill. *Crit Care Med*. 2003; 31:S668–S671. [PubMed: 14724464]
15. Caplan, AI. Why are MSCs therapeutic? New data: new insight. *J Pathol*. 2009; 271:318–324. [PubMed: 19023885]
16. Xiao Y, Jiang ZJ, Pang Y, Li L, Gao Y, Xiao HW, Li YH, Zhang H, Liu Q. Efficacy and safety of mesenchymal stromal cell treatment from related donors for patients with refractory aplastic anemia. *Cryotherapy*. 2013; 15(9):1118–25.
17. Li X, Wang D, Liang J, Zhang H, Sun L. Mesenchymal SCT ameliorates refractory cytopenia in patients with systemic lupus erythematosus. *Bone Marrow Transplant*. 2013; 48(4):544–550. [PubMed: 23064040]
18. Kim DH, Yoo KH, Yim YS, Choi J, Lee SH, Jung HL, Sung KW, Yang SE, Oh WI, Yang YS, Kim SH, Choi SY, Koo HH. Cotransplanted bone marrow derived mesenchymal stem cells (MSC)

- enhanced engraftment of hematopoietic stem cells in a MSC-dose dependent manner in NOD/SCID mice. *J Korean Med Sci.* 2006; 21(6):1000–4. [PubMed: 17179676]
19. Wu Y, Cao Y, Li X, Xu L, Wang Z, Liu P, Yan P, Liu Z, Wang J, Jiang S, Wu X, Gao C, Da W, Han Z. Cotransplantation of haploidentical hematopoietic and umbilical cord mesenchymal stem cells for severe aplastic anemia: successful engraftment and mild GVHD. *Stem Cell Research.* 2014; 12(1):132–138. [PubMed: 24185180]
20. Cook KM, Sifri ZC, Baranski GM, Mohr AM, Livingston DH. The role of plasma granulocyte colony stimulating factor and bone marrow dysfunction after severe trauma. *J Am Coll Surg.* 2013; 216(1):57–64. [PubMed: 23063381]
21. Petit I, Szyper-Kravitz M, Nagler A, Lahav M, Peled A, Habler L, Ponomaryov T, Taichman RS, Arenzana-Seisdedos F, Fugii N, Sandbank J, Zipori D, Lapidot T. G-CSF induces stem cell mobilization by decreasing bone marrow SDF-1 and up-regulating CXCR4. *Nat Immunol.* 2002; 3(7):687–787. [PubMed: 12068293]
22. Wu JC, Livingston DH, Hauser CJ, Deitch EA, Rameshwar P. Trauma inhibits erythroid burst-forming unit and granulocyte-monocyte colony-forming unit growth through the production of TGF-beta1 by bone marrow stroma. *Ann Surg.* 2001; 234(2):224–232. [PubMed: 11505069]

Figure 1. A-D: Effect of MSCs on BM seven days following injury and shock

A. The addition of MSCs to LCHS causes a significant increase in BM cellularity, returning cellularity to naïve levels. **B.** The addition of MSCs to LCHS causes a significant increase in BM CFU-GEMM colony growth, returning it to naïve levels. **C.** The addition of MSCs to LCHS causes a significant increase in BM BFU-E colony growth, returning growth to naïve levels. **D.** The addition of MSCs to LCHS causes a significant increase in BM CFU-E colony growth, returning growth to naïve levels. N=5-6/group (dotted line represents naïve; BM= Bone Marrow; LC = lung contusion; HS= hemorrhagic shock; MSC = mesenchymal stem cell). *p<0.05 vs naïve, **p<0.05 vs LCHS

A

B

Figure 2. A-B: Effect of MSCs on HPC mobilization and G-CSF seven days following injury and shock

A. There is no change in the percentage of HPCs found in the peripheral blood following LC or LCHS with or without MSCs. **B.** The addition of MSCs to LCHS causes a significant decrease in the plasma G-CSF level, returning plasma G-CSF to naïve levels. N=5-6/group (dotted line represents naïve; HPC= hematopoietic stem cell; LC = lung contusion; HS = hemorrhagic shock; MSC = mesenchymal stem cell). *p<0.05 vs naïve, **p<0.05 vs LCHS

Figure 3. Effect of experimental plasma on CFU-E Colony Growth

Naïve BM cells cultured with media plus the addition of 5% plasma from 7-day animals undergoing LCHS suppresses CFU-E colony growth. When incubated with plasma from animals receiving MSCs suppression is no longer seen. Dotted line represents naïve BM cells incubated with media alone. UC represents naïve BM cells incubated with 5% naïve plasma. (n=5-6/group; *p<0.001 vs Naïve, **p<0.001 vs LCHS)