Video Article

Ultrasound-guided Intracardiac Injection of Human Mesenchymal Stem Cells to Increase Homing to the Intestine for Use in Murine Models of Experimental Inflammatory Bowel Diseases

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URL: https://www.jove.com/video/55367
DOI: doi:10.3791/55367

Keywords: Medicine, Issue 127, Mesenchymal stem cells (MSC), Crohn's disease, SAMP1/YitFc, ultrasound-guided intracardiac injection, hMSC transduction, bioluminescence imaging

Date Published: 9/1/2017

Citation: Dave, M., Menghini, P., Sugi, K., Somoza, R.A., Lee, Z., Jain, M., Caplan, A., Cominelli, F. Ultrasound-guided Intracardiac Injection of Human Mesenchymal Stem Cells to Increase Homing to the Intestine for Use in Murine Models of Experimental Inflammatory Bowel Diseases. *J. Vis. Exp.* (127), e55367, doi:10.3791/55367 (2017).

Abstract

Crohn's disease (CD) is a common chronic inflammatory disease of the small and large intestines. Murine and human mesenchymal stem cells (MSCs) have immunosuppressive potential and have been shown to suppress inflammation in mouse models of intestinal inflammation, even though the route of administration can limit their homing and effectiveness [1,3,4,5]. Local application of MSCs to colonic injury models has shown greater efficacy at ameliorating inflammation in the colon. However, there is paucity of data on techniques to enhance the localization of human bone marrow-derived MSCs (hMSCs) to the small intestine, the site of inflammation in the SAMP-1/YitFc (SAMP) model of experimental Crohn's disease. This work describes a novel technique for the ultrasound-guided intracardiac injection of hMSCs in SAMP mice, a well-characterized spontaneous model of chronic intestinal inflammation. Sex- and age-matched, inflammation-free AKR/J (AKR) mice were used as controls. To analyze the biodistribution and the localization, hMSCs were transduced with a lentivirus containing a triple reporter. The triple reporter consisted of firefly luciferase (fl), for bioluminescent imaging; monomeric red fluorescent protein (mrfp), for cell sorting; and truncated herpes simplex virus thymidine kinase (ttk), for positron emission tomography (PET) imaging. The results of this study show that 24 h after the intracardiac administration, hMSCs localize in the small intestine of SAMP mice as opposed to inflammation-free AKR mice. This novel, ultrasound-guided injection of hMSCs in the left ventricle of SAMP mice ensures a high success rate of cell delivery, allowing for the rapid recovery of mice with minimal morbidity and mortality. This technique could be a useful method for the enhanced localization of MSCs in other models of small-intestinal inflammation, such as TNFΔRE[6]. Future studies will determine if the increased localization of hMSCs by intra-arterial delivery can lead to increased therapeutic efficacy.

Video Link

The video component of this article can be found at https://www.jove.com/video/55367/

Introduction

Crohn's disease (CD) is a common chronic inflammatory disease of the small and large intestines and is thought to result from an inappropriate response of the host immune system to intestinal microbes[7,8]. Recent studies have shown that both murine and human mesenchymal stem cells (MSCs) can suppress inflammation in mouse models of intestinal inflammation[1,3,4,5]. There are multiple ongoing clinical trials that use human MSCs derived from bone marrow or adipose tissue to treat patients with inflammatory bowel disease (IBD), which includes CD[9]. Two routes for MSC therapy have been used in these clinical trials: one involves the systemic infusion (*i.e.,* intravenously) of MSCs for luminal IBD (including CD), and the other involves the localized application/injection of stem cells in the fistula tract of patients with perianal CD. In a recent meta-analysis of MSC therapy for IBD, systemic (*i.e.,* intravenous) MSC therapy for luminal IBD (including CD) was efficacious in up to 40% (95% CI: 7 - 79%) of patients, whereas the efficacy was much higher, at 61% (95% CI: 36 - 85%), when the MSCs were injected locally into the diseased CD fistula[9]. A recent phase III multicenter randomized placebo controlled trial of allogeneic adipose stem cells injected directly into the perianal fistula of CD patients showed statistically significant clinical and radiological evidence of perianal fistulae healing, corroborating the findings of the meta-analysis[10]. The reasons for the low efficacy of MSC therapy given intravenously for luminal CD has been inadequately investigated, but one reason may be the inadequate homing of MSCs to the site of inflammation. Murine studies in models of colonic inflammation have demonstrated that only a small percentage of MSCs (1 - 5%) injected intravenously reach the inflamed colon; the remaining MSCs are filtered by the lungs (first-pass effect)[1,2,5,11,12]. Multiple murine research studies have therefore used the intraperitoneal route (i.p.) for MSC administration in animal models of colitis[4]. However, they have also demonstrated that only a small fraction of cells reach and engraft the colon and that

the efficacy is related to the secretion of soluble paracrine factors, like tumor necrosis factor-inducible gene 6 protein (TSG-6)[2]. The MSC mechanism of immunosuppression and healing involves a multipronged approach that involves paracrine; cell proximity-independent factors, like TSG-6; and cell proximity-dependent factors, like programmed death-ligand 1 (PD-L1); or Jagged 1. Therefore, MSC localization to the site of inflammation may result in increased efficacy[9,13]. In fact, a recent study showed that MSCs directly implanted at the site of colonic injury resulted in healing by secreting angiogenesis-promoting vascular endothelial growth factor (VEGF). On the other hand, a minimal healing effect was noted after intravenous injection[5]. To increase their localization to the site of inflammation (*i.e.,* the small intestine in SAMP mice), this ultrasound-guided intracardiac injection technique for MSC administration in the left ventricle was developed. Image-guided injection ensures an accurate injection, which leads to a higher rate of success and to decreased morbidity and mortality rates. Moreover, the injection of MSCs into the left ventricle delivers them to arterial circulation, where they can reach the inflamed small intestine before becoming trapped in the lungs.

In this study, human bone marrow-derived MSCs (hMSCs) were used for injection in the SAMP-1/YitFc (SAMP) murine model of CD[14]. SAMP is a well-characterized spontaneous murine model of chronic inflammation that develops small-intestinal inflammation with nearly 100% penetrance[14]. The inflammation develops in response to microflora in the absence of any chemical, immunological, or genetic manipulation and closely resembles human CD[11]. Sex- and age-matched inflammation-free AKR/J (AKR) mice, the parental control mice of SAMP, were used in this study.

The hMSCs were isolated and expanded in the laboratory from bone marrow (BM) samples obtained from normal, unidentified donors after informed consent using validated and previously published protocols[15,16]. After isolation and expansion, the MSC ability in osteogenic, adipogenic, and chondrogenic differentiation was evaluated in the laboratory by multiple assays[15]. The osteogenic functional assay was performed by implanting ceramic cubes of hydroxyapatite/tricalcium phosphate matrix containing hMSCs subcutaneously in immunocompromised CB17-Prkdc SCID mice[17]. The cube assay demonstrates osteogenesis and chondrogenesis potential and is considered the ultimate test for evaluating individual MSC preparations[17]. To visualize hMSCs *in vivo* after injection, lentivirus was used to transduce hMSCs with triple reporter gene construct that consists of firefly luciferase (fl), monomeric red fluorescent protein (mRFP), and herpes simplex viral thymidine kinase (ttk), driven by a modified myeloproliferative sarcoma virus (mnd) promoter[18]. The firefly luciferase in the triple reporter is an enzyme that coverts injected luciferin to oxyluciferin in hMSCs and produces photons/white light. This is detected by the sensitive charge-coupled device (CCD) camera (bioluminescence) in an *in vivo* optical imaging system, enabling the visualization of live hMSCs in mice. Bioluminescence imaging (BLI) is a sensitive technique that can be used serially for tracking cells and for *ex vivo* analysis. The use of a strong mnd promoter drives the continuous expression of the triple fusion reporter gene construct and allows for the imaging of injected hMSCs for more than 16 weeks[19]. The hMSCs are difficult to transduce and have a low transduction efficiency. Using an optimized protocol, the hMSCs transduction efficiency was improved and the transgene expression was enhanced[18]. Using mRFP expression (one of the triple reporter genes) on flow cytometry, the ability to transduce hMSCs with a high efficiency of up to 83% was demonstrated. Differentiation assays and *in vivo* cube assays demonstrated the ability of the transduced hMSCs to differentiate into chondrocytes, adipocytes, and osteocytes[17].

Protocol

All mice experiments and procedures in the study were approved by Case Western Reserve University's Institutional Animal Care and Use Committee. The procedures were conducted in Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) accredited facility. The BM was aspirated under a University Hospitals Institutional Review Board-approved protocol at the stem cell core facility at Case Western Reserve University after informed consent.

1. Culture and Transduction of Human Bone Marrow-derived Mesenchymal Stem Cells (hMSCs)

NOTE: The isolation of hMSCs is described in detail in previous publications[15,20].

1. Culture isolated hMSCs in Dulbecco's Modified Eagle Medium-low glucose (DMEM-LG) medium supplemented with 10% fetal bovine serum (FBS). Culture the cells at 37 °C, 95% humidity, and 5% CO_2 in T175 flasks with 25 mL of growth medium. Replace the medium every 3 - 4 days. On day 14, perform the primary passage of hMSCs.
    1. When the cells reach 80% confluence, completely remove the old medium and gently wash the cells with 5 mL of sterile phosphate-buffered saline (PBS; for 75-cm tissue culture flasks).
    2. Remove the PBS from the flask and add 4 mL of 0.25% trypsin-ethylenediaminetetraacetic acid (EDTA). Place the flask back in the incubator at 37 °C for 5 - 10 min. Keep the time of exposure as brief as possible.
    3. When the majority of cells have become well-rounded or have detached from the culture dish, stop the reaction by adding a volume of bovine calf serum equal to half the volume of trypsin.
    4. Transfer the cell suspension to an appropriate-size centrifuge tube. Centrifuge the cells at 400 x g for 5 min and then carefully remove the supernatant.
    5. Resuspend the cells in 5 mL of medium (DMEM-LG + 10% FBS) and count the cells with a hemocytometer.
    NOTE: At this point, the cells can be subcultured or transduced with triple reporter.

2. Perform the transduction of hMSCs with triple reporter.
    1. Seed 1.5 x 10^6 cells in one T175 flask. Culture the cells in 25 mL of growth medium at 37 °C, 95%, humidity and 5% CO_2. Wait 24 h for the cells to attach.
    2. Make a virus cocktail in 15-mL tubes, as described in reference [18].
        1. Thaw lentivirus at 37 °C in a water bath. Add 160 µL of a 5 mg/mL proteamine sulfate solution in Dulbecco's Modified Eagle Medium (DMEM) to 8 mL of culture medium (DMEM-LG + 10% FBS) for a final concentration of 100 µg/mL. Vortex for 3 s. Add a virus multiplicity of infection (MOI) of 5 (1st time thawed).

3. Remove the medium from the cell culture flask (step 1.2.1) and add the virus cocktail to the cells. Incubate for 10 h at 37 °C, 95% humidity, and 5% CO_2.

3. Add 4 mL of 100 µg/mL proteamine sulfate in 4 mL of DMEM-LG + 10% FBS and incubate for an additional 14 h.
4. Stop the transduction after 24 h by replacing with 25 mL of medium (DMEM-LG + 10% FBS).
5. Confirm transduction efficiency by evaluating mRFP expression by flow cytometry[18].
   NOTE: Here, hMSCs that were transduced with over 65% efficiency were used.
6. Expand the cells by replacing with 25 mL of fresh medium (DMEM-LG + 10% FBS) twice a week. Culture the cells at 37 °C, 95%, humidity and 5% CO_2. Passage cells after they reach 80% confluence.
7. Once the desired number of cells is reached, perform trypsinization per steps 1.1.2 - 1.1.4.
8. Count the cells and suspend them in sterile PBS (1x) in the final concentration of 1 x 10^6 cells/75 µL. Keep the cells on ice for transport to the ultrasound machine. For experiments, use hMSCs from passage numbers 2 - 5.

2. Ultrasound-guided Intracardiac Injection of hMSCs into the Left Ventricle

NOTE: Use SAMP1/YitFc with established small-intestinal inflammation and age- and gender-matched AKR/J mice for the experiments. Maintain the SAMP and AKR mice under specific pathogen-free conditions, feed them standard laboratory chow, and keep them on 12-h light/dark cycles. Here, the ultrasound-guided cardiac injections were performed in a dedicated pathogen-free cardiac ultrasound room located in the CWRU animal research facility that was sanitized with a disinfectant and rinsed with 70% alcohol. Laboratory research personnel must wear personal protective equipment, including gowns, face masks with eye shields, and sterile gloves during the intracardiac injections. The body heat of the mouse must be maintained for the duration of the procedure.

1. Set up the ultrasound imaging system before anesthetizing the mouse. For this protocol, use the ultrasound machine designed for mouse echocardiogram.
   1. Turn on the power and initialize the transducer (30 MHz) to set up the new study.

2. Anesthetize the mouse in an induction chamber using 3 - 4% isoflurane in 100% oxygen at a rate of 0.2 - 0.5 L/min. Maintain the animal for the whole procedure with 2% isoflurane in 100% oxygen via a nose cone. Confirm proper anesthetization prior to performing imaging by pinching the toe, rolling the mouse, and observing the absence of movement.
   NOTE: Ophthalmic ointment should be applied to the eyes following the induction of anesthesia to prevent corneal drying. Transfer the anesthetized mice to the imaging machines (*i.e.,* bioluminescence imaging system and ultrasound), where they will receive a maintenance dose of anesthetic isoflurane. Determine the anesthesia start time and the depth of anesthesia by observing a lack of reaction to pinches on the tail and foot pad every 10 min for the entire duration of anesthesia.
3. Set the temperature of the imaging table and ultrasound gel to 37 °C.
4. Completely remove the fur over the thorax area of the anesthetized mouse using hair removal cream.
5. Place the mouse on the imaging table in supine position and secure both the upper and lower limbs with adhesive tape to avoid body movement during the procedure. Use an electrocardiogram monitor during the procedure for all mice.
6. Clean the skin of the thorax area using a 10% povidone/iodine swab followed by a 70% ethanol swab. Apply a thick layer of gel to the thorax area of the mouse.
7. Mount the transducer in the holder and adjust its position until the left ventricle is clearly visible on the field of view.
8. Load 150 µL of transduced hMSC cell suspension (2 x 10^6 hMSCs in 150 µL of sterile PBS) into a 1-mL syringe with a 28-G needle and secure the syringe to the appropriate holder. To visualize pulsating blood, keep a small air column in the syringe. To avoid clumping, suspend the cells before the injection.
9. Advance the syringe towards the mouse thorax, adjust the needle trajectory using the ultrasound guidance, and enter the left ventricle.
10. Following the guidance of the ultrasound imaging, penetrate the syringe needle through the intercostal space and into the left ventricle of the mouse.
    NOTE: The syringe needle tip should be clearly visible in the left ventricle on the ultrasonographic image. Proper placement should be further confirmed by fresh arterial blood outflow into the syringe. These are signs of a successful insertion.
11. Inject the hMSC cell suspension very slowly over 2 min, applying gentle pressure.
12. Following the injection of suspended cells, gently withdraw the needle, clean off the ultrasound gel, and release the mouse from the tape restraints.
13. Place the animal in a new, clean cage with a preheated pad.
    NOTE: After the completion of the procedure, a warm environment should be provided to the mice until recovery. Over-heating should be avoided, since peripheral vasodilation compromises the recovery. The mice should be monitored closely and kept isolated from other mice until their full recovery, indicated by their ability to maintain sternal recumbency.
14. Monitor the animal until they achieve complete recovery from the anesthesia and daily until the end of the experiment.

3. Bioluminescence (BLI) Imaging of hMSCs

1. Image the mice 24 h after the intracardiac injection in the bioluminescence imaging system. Start the *in vivo* imaging software. Initialize the imaging system and open a new study.
   1. In the control panel, set the imaging parameters by clicking "sequence setup." In the imaging mode, select "luminescence" and "photograph." Set the exposure times from 0.5 s to 10 min. Set the binning to "medium" and the F/stop to "1." Set the excitation filter to "block" and the emission filter to "open." Set the field of view to "C" for two mice. Add the sequence setup to the image wizard by clicking on the acquisition control panel.

2. Switch on the oxygen pump and set the isoflurane to 2.5% in both the induction chamber and the nose cone inside the machine.
3. Inject the mouse i.p. with 300 µL of D-Luciferin (12.5 mg/mL).
4. Place the mouse in the anesthesia induction chamber.

1. After 2 - 3 min, transfer the mouse into the *in vivo* imaging chamber, with its head in the nose cone on the anesthesia manifold. Apply optical ointment to protect the eyes during imaging. To image multiple mice, use black light baffle to prevent the reflection of light onto adjacent mice.

5. Click the "acquire" button to start the image acquisition 10 min after D-Luciferin injection.
6. Repeat steps 3.3 - 3.5 to image more mice.
7. After the *in vivo* image acquisition, euthanize the mice by CO_2 inhalation followed by cervical dislocation, to confirm death. Perform the *ex vivo* analysis.
    1. If the *ex vivo* analysis is performed after 20 min, repeat the luciferin injection (step 3.3) before sacrificing the mice; the BLI signal may decrease 20 min after the luciferin injection.

8. Dissect the mice to remove the whole gastrointestinal tract (*i.e.,* from the stomach to the rectum), mesenteric lymph node (MLN), lungs, spleen, and liver. Place them in petri dishes using forceps and scissors[2,4].
9. Place the petri dish containing the explanted organs in the imaging chamber and acquire BLI images per steps 3.1 - 3.6.
10. After the image acquisition, transfer the explanted organs to specimen molds containing optimal cutting temperature (OCT) compound. Cryofreeze the molds at -80 °C using dry ice. Perform immunohistochemistry using anti-luciferase antibody on frozen sections to confirm the presence of hMSCs[16].
11. For the quantification of light intensity, use the region of interest (ROI) tool in the tool palette[16,19]. Select the ROI frame and move it to region of interest on the image. Click "ROI measurement" and save the data in SEQ file format. Export the data as a .txt file and use statistical software to perform basic statistical tests for quantification[16].

Representative Results

Figure 1 shows that hMSCs can be transduced with the triple reporter at a high efficiency, preserving their stem cell properties. Transduced hMSCs can be visualized in real time by BLI (**Figure 1C**, **Figure 3**). This novel, ultrasound-guided injection of hMSCs into the left ventricle of SAMP mice ensures a very high success rate of injection, allowing for the rapid recovery of the mice with minimal morbidity and mortality (**Figure 2**). The average time for injecting one mouse with hMSCs is approximately 10 min, and less than 8 - 9% morbidity and mortality were observed with this technique. The major reason for mortality is stroke and cardiac tamponade from hemorrhagic pericardial effusion. The *ex vivo* analysis performed 24 h after the intracardiac (intra-arterial) administration of hMSC confirms that this route of administration results in the homing of hMSCs to the inflamed small intestine of SAMP mice, as opposed to inflammation-free control mice (**Figure 3C** and **D**). Furthermore, hMSCs tend not to stay in the small intestine of inflammation-free AKR mice and start accumulating or getting trapped in the lungs (**Figure 3B**).

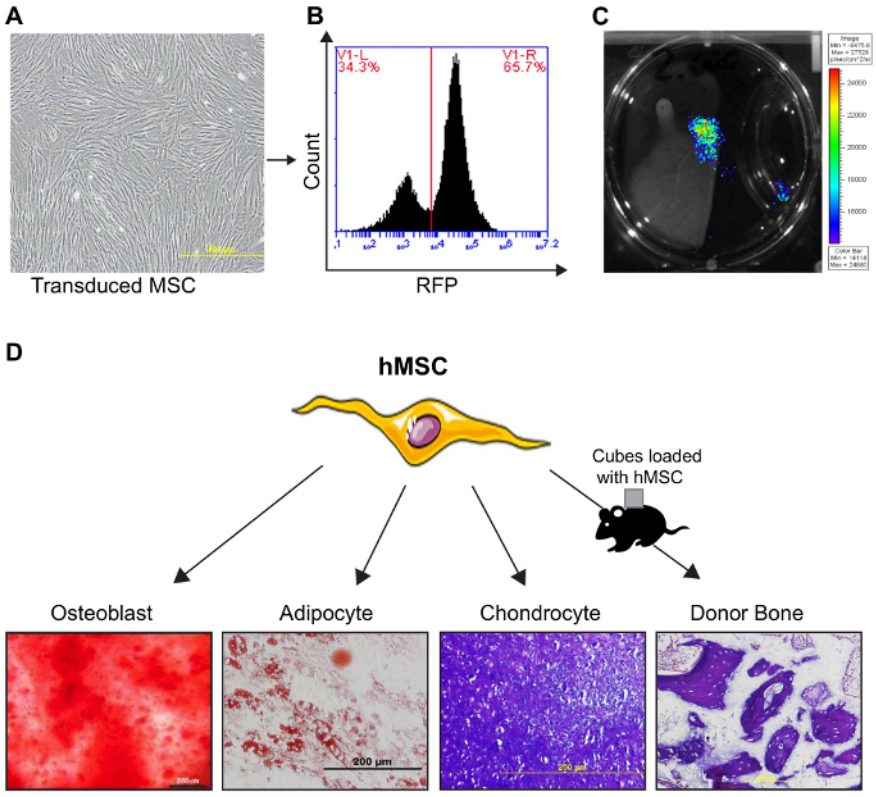

Figure 1. hMSCs can be Transduced with a High Efficiency for *In Vivo* Visualization and With the Retention of Stem Cell Properties. **(A)** Microscopic image of transduced hMSCs. Successful transduction of human MSCs, as determined by mRFP expression assessed with flow cytometry **(B)** and BLI **(C)**. Transduced hMSCs retain stem cell properties, as confirmed by differentiation assays that demonstrate the ability of transduced hMSCs to differentiate into chondrocytes, adipocytes, and osteoblasts. For a functional assay, the ceramic cubes of hydroxyapatite/tricalcium phosphate matrix were implanted subcutaneously in immunocompromised CB17-Prkdc SCID mice to demonstrate the ability of hMSCs to form ectopic donor bone **(D)**. Scale bars in A = 500 µm. Scale bars in C indicate units of BLI (10^3 photons/s/cm^2/steradian). Scale bars in D = 200 µm. Please click here to view a larger version of this figure.

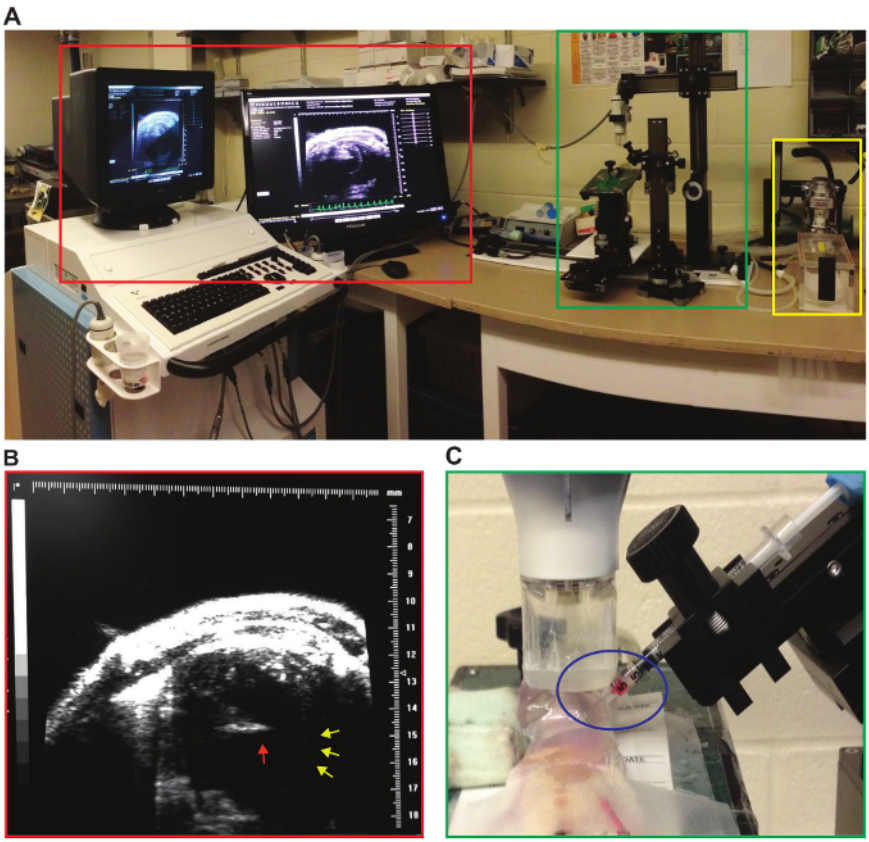

Figure 2: Instruments for Ultrasound-guided Injection. (A) A panoramic view of the instruments: cardiac ultrasound imaging system (red square), transducer mounted in its apposite holder, syringe holder and surgical table with nosecone for anesthesia (green square), and inhalation anesthesia machine with induction chamber (yellow square). **(B)** Ultrasonographic image of the syringe needle: the needle tip (red arrow) is clearly visible inside the left ventricle (yellow arrows). **(C)** Fresh arterial blood outflow into the syringe confirms the successful insertion of the syringe needle into the left ventricle prior to hMSC administration. Please click here to view a larger version of this figure.

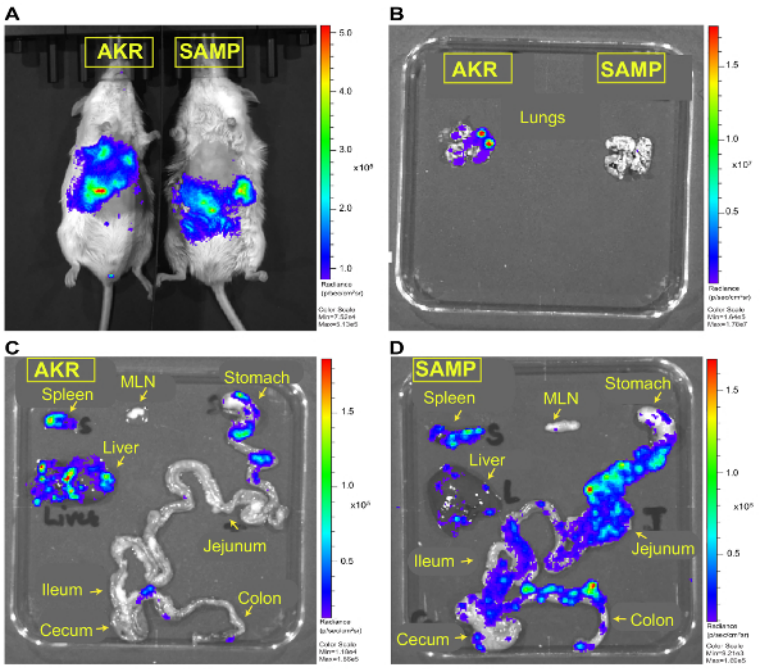

Figure 3. hMSCs Localize to the Small Intestine after Intracardiac/Intra-arterial Administration in SAMP Mice. Representative images of 3 - 4 mice/group, euthanized 24 h after injection and from three independent experiments are shown. *In vivo* bioluminescence imaging demonstrates the presence of hMSCs in SAMP and AKR mice 24 h after injection **(A)**. *Ex vivo* analysis in SAMP mice demonstrates that hMSCs preferentially localize to the small intestine (*i.e.,* the site of inflammation) **(D)**, whereas they tend to not stay in the small intestine of inflammation-free control AKR mice **(C)**. In fact, after the first arterial passage, hMSCs start accumulating more in the lungs of AKR mice, as opposed to SAMP mice **(B)**. A fraction of hMSCs can also be detected in the spleen, liver and lungs of SAMP and AKR mice (B,C,D). The scale bars indicate units of BLI (10^3 photons/s/cm^2/steradian). Please click here to view a larger version of this figure.

Discussion

This study describes a novel technique for the ultrasound-guided intracardiac injection of hMSCs in a small-intestinal mouse model of experimental CD. This technique has very high rate of survival and success, as the trajectory of the needle can be adjusted based on real-time, high-resolution images of the mouse left ventricle, provided by the ultrasound. The advantage of delivery to the left ventricle is that hMSCs are then distributed intra-arterially and bypass venous circulation, thus avoiding the aggregation of cells in the lungs. Previously, carotid artery catheterization for intra-arterial delivery was used to inject MSCs[16,21]. The carotid catheterization method involves surgery to expose the carotid artery in order to place a catheter inside the artery. The catheter is advanced towards the aortic arch for the transfusion of cells. The catheter is subsequently removed and the carotid artery ligated. This novel technique for the ultrasound-guided injection of MSCs in the left ventricle, as compared to carotid artery catheterization, is less invasive, is much faster (approximately 10 min/mouse versus 60 min/mouse), and has less morbidity and mortality (<10% *versus* 30%). The clinical translation of the mouse ultrasound-guided intracardiac injection of hMSCs to human clinical trials would be through the catheterization of the superior mesenteric artery (currently performed for the treatment of chronic mesenteric ischemia and intractable gastrointestinal bleeding) to deliver hMSCs to the diseased small intestine.

There are several considerations regarding modifications and troubleshooting of the ultrasound-guided intracardiac injection of hMSCs in the left ventricle of mice. A critical aspect of the procedure is mastering the use of the ultrasound to guide the injection needle into the left ventricle. Like any procedure, proper training, practice, and expert feedback are important components to successfully perform this procedure. Poor technique can lead to cardiac tamponade from hemorrhagic pericardial effusion and to death. To minimize cell clumping and the risk of embolization to brain (*i.e.,* stroke), it is essential to resuspend the hMSCs prior to each injection. Before implementing this procedure, it is important to consult the veterinarian in charge of the local animal research facility to receive expert advice. Ensure that guidelines for the safe and effective use of animals are followed to minimize inadvertent injury to the mice.

To examine the biodistribution of hMSCs after the injection in mice, hMSCs were successfully transduced with triple reporter, with high efficiency. Differentiation assays performed demonstrated the ability of transduced hMSCs to differentiate into chondrocytes, adipocytes, and osteocytes[18]. For the osteogenic functional assay, the ceramic cubes of hydroxyapatite/tricalcium phosphate matrix were implanted subcutaneously in immunocompromised SCID mice and demonstrated the ability of hMSCs to form ectopic donor bone[17,18]. These differentiation and functional assays demonstrate that transduced hMSCs retain their stem cell properties. The results of this study show that transduced hMSCs used for intracardiac injection into SAMP mice localize to the small intestine, which is the site of inflammation in the SAMP mice. In the intestinal inflammation-free AKR mice, only a minority of hMSCs localized to the intestines 24 h after injection, implying that, in the absence of inflammation, hMSCs do not remain in the intestine. This data is in concordance with multiple other studies that have shown the ability of MSCs to migrate and engraft at the site of injury, such as the heart in acute myocardial infarction[22] and the injured BM in a radiation-injured leg[16]. Like in other diseases, the local application of MSCs to colonic injury models has shown efficacy at ameliorating inflammation[23]. In a

2,4,6-trinitrobonzene sulfonic acid (TNBS) model of colitis in rats, Hayashi *et al.* injected BM-derived MSCs into the submucosa of the rat colon, surrounding the area exposed to TNBS, and demonstrated accelerated healing of the colitis[23].

A recent study by Manieri *et al.* used a novel technique of endoscope-assisted injection of colonic MSCs in the distal colon, the site of colonic injury, in prostaglandin-deficient mice and showed their effectiveness in preventing the formation of penetrating ulcers[5]. SAMP is a model of small-intestinal inflammation, and these mice do not develop spontaneous inflammation in their colon. Our laboratory has developed and validated an endoscopic method for the assessment and quantification of colonic inflammation and tumor development in mice. However, endoscopic devices are not able to reach the small intestine in a live mouse[24]. The scoring system can only be used as terminal method for the assessment of inflammation in the SAMP mice after euthanasia. Therefore, the endoscopic method is not suitable for the local application of MSCs in the SAMP model. By using this novel technique of injection, enhanced localization of MSCs to the small intestine can be achieved. Whether the increased localization to the inflamed small intestine will translate into increased efficacy is not known and will be the focus of future studies. In addition to the SAMP model, the ultrasound-guided injection of MSCs would be a useful method for the enhanced localization of hMSCs in other models of small intestinal inflammation, such as the TNFΔRE[6] murine model of experimental CD.

BLI is a sensitive and convenient technique that can be used serially to track hMSCs *in vivo*, but it does not allow for the precise quantification of hMSC homing. An advantage of using the triple reporter-transduced hMSCs in mice is that the enzyme ttk in the triple reporter allows for more quantitative positron emission tomography (PET) imaging[19]. Combining highly quantitative PET imaging (with a radiotracer probe) with a computed tomography (CT) scan (providing localization) allows for a quantitative estimate of the number of engrafted hMSCs at the diseased site, as the gamma counts are proportional to the number of the viable hMSCs labeled with the ttk gene. Exact quantification of hMSC localization can help to determine the percentage of hMSCs homing and therapeutic efficacy and will be the focus of future studies.

This technique, despite its many advantages, has some limitations: i) the requirement for an expensive cardiac ultrasound imaging system for mice, ii) the mortality and morbidity associated with intracardiac injection, and iii) the inability to be used for the slow infusion (*i.e.,* over hours) of cells and other molecules. Despite these limitations, it has many benefits over the current technique of carotid artery catheterization for the intra-arterial injection of hMSCs, as highlighted above.

In conclusion, the results of this study demonstrate the effectiveness and convenience of the ultrasound-guided injection technique to improve the localization of hMSCs to the site of inflammation in the SAMP model of experimental CD. Future studies will determine if the increased homing of hMSCs by intra-arterial delivery can lead to increased therapeutic efficacy in models of small-intestinal inflammation.

Disclosures

The authors have nothing to disclose.

Acknowledgements

Maneesh Dave is supported by Department of Defense grant PR141774 and the Crohn's and Colitis Foundation of America Career Development Award. The laboratory of Fabio Cominelli is supported by NIH grants DK042191 (F.C.), DK055812 (F.C.), DK091222 (F.C.), and DK07948 (F.C.). The laboratory of Arnold Caplan is supported in part by the L. David and E. Virginia Baldwin Foundation. The funding agencies had no role in the study analysis or writing of the manuscript. Its contents are solely the responsibility of the authors.

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