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Original article

Uterine-derived progenitor cells are immunoprivileged and effectively improve cardiac regeneration when used for cell therapy

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ABSTRACT

Cell therapy to prevent cardiac dysfunction after myocardial infarction (MI) is less effective in aged patients because aged cells have decreased regenerative capacity. Allogeneic transplanted stem cells (SCs) from young donors are usually rejected. Maintaining transplanted SC immunoprivilege may dramatically improve regenerative outcomes. The uterus has distinct immune characteristics, and we showed that reparative uterine SCs home to the myocardium post-MI. Here, we identify immunoprivileged uterine SCs and assess their effects on cardiac regeneration after allogeneic transplantation. We found more than 20% of cells in the mouse uterus have undetectable MHC I expression by flow cytometry. Uterine MHC $I^{(neg)}$ and MHC $I^{(pos)}$ cells were separated by magnetic cell sorting. The MHC $I^{(neg)}$ population expressed the SC markers CD34, Sca-1 and CD90, but did not express MHC II or c-kit. *In vitro*, MHC $I^{(neg)}$ and $^{(pos)}$ SCs show colony formation and endothelial differentiation capacity. In mixed leukocyte co-culture, MHC $I^{(neg)}$ cells showed reduced cell death and leukocyte proliferation compared to MHC $I^{(pos)}$ cells. MHC $I^{(neg)}$ and $^{(pos)}$ cells had significantly greater angiogenic capacity than mesenchymal stem cells. The benefits of intramyocardial injection of allogeneic MHC $I^{(neg)}$ cells after MI were comparable to syngeneic bone marrow cell transplantation, with engraftment in cardiac tissue and limited recruitment of CD4 and CD8 cells up to 21 days post-MI. MHC $I^{(neg)}$ cells preserved cardiac function, decreased infarct size and improved regeneration post–MI. This new source of immunoprivileged cells can induce neovascularization and could be used as allogeneic cell therapy for regenerative medicine.

1. Introduction

In response to an ischemic injury to the heart, the recruitment of regenerative cells from both the bone marrow and the heart is essential for recovery [1,2]. Currently, bone marrow stem cells are used in the clinic as cell therapy for the treatment of heart disease [3,4]. However, recipients of autologous cells received only marginal benefits [5] in comparison to the extensive regeneration seen in pre-clinical animal studies [4]. The advanced age of the cardiac patients and age-related stem cell dysfunction have been suggested to play a major role in the difference between these preclinical and clinical findings [5,6]. To overcome this limitation, allogeneic cells from younger donors have been tested. Allogeneic mesenchymal stem cells (MSCs), embryonic stem cells and induced pluripotent stem (iPS) cells have promising regenerative properties, however their suitability as cell therapy agents remains an ongoing question because of the host immune response generated after transplantation [7,8]. Cell rejection remains a major clinical concern when using cell therapy. Strategies to maintain immunoprivilege in stem cell transplantation may directly improve the outcomes of cell therapy.

Research on immunoprivileged stem cells remains an ongoing area of investigation and new sources of allogeneic stem cells are being studied. Uterine stem cells are of interest for two reasons. The uterus is a unique organ with distinct immune characteristics allowing the presence of a (semi)allogeneic fetus within an exceptional tolerogenic environment [9]. Thus, regenerative uterine stem cells may also preserve some unique immunoprivileged properties. Additionally, the endometrium is a rare site of physiological angiogenesis in the post-development adult body, with regenerative cells that cyclically create and shed decidual tissue without scarring. In the context of heart regeneration after ischemic injury, angiogenesis is a crucial process to rescue cardiomyocyte death and restore blood flow. Therefore, uterine stem cells are an attractive candidate for testing.

Our group has previously demonstrated that the uterus is a source of potent progenitor cells which induce angiogenesis when injected into the infarcted heart [10]. Furthermore, females may have the advantage

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Table 1
Real-time PCR primer sets.

Gene	Forward	Reverse
MMP2	ACCAGAACACCATCGAGACC	CCATCAGCGTTCCCATACTT
MMP9	CGTCGTGATCCCCACTTACT	AGAGTACTGCTTGCCCAGGA
TIMP1	GCATCTGGCATCCTCTTGTT	CTCAGAGTACGCCAGGGAAC
TIMP2	AAGCAGTGAGCGAGA AGGAG	GGGTCCTCGATGTCAAGAAA
TIMP3	CCGAGGCTTCAGTAAGATGC	TTGCTGATGCTCTTGTCTGG
TIMP4	ACTTCTGCCACTCGGCTCTA	ACATGGCACTGCATAGCAAG
IL6	CCGGAGAGGAGACTTCACAG	GGAAATTGGGGTAGGAAGGA
IL10	CCAAGCCTTATCGGAAATGA	TTTTCACAGGGGAGAAATCG
TGFb2	TGGCTTCACCACAAAGACAG	TTCGATCTTGGGCGTATTTC
AKT1	GCAGGAAGAAGAGACGATGG	GTCGTGGGTCTGGAATGAGT
ANGPT1	CAGTGGCTGCAAAAACTTGA	TCTGCACAGTCTCGAAATGG
VEGFC	CAAGGCTTTTGAAGGCAAAG	TTAGCTGCCTGACACTGTGG
TNFα	CCCCAAAGGG ATGAGAAGTT	CTCCTCCACTTGGTGGTTTG
INFgamma	GCGTCATTGAATCACACCTG	TGAGCTCATTGAATGCTTGG
PIGF	TGCTGGTCATGAAGCTGTTC	ACCCCACACTTCGTTGAAAG

of a utero-cardiovascular axis in support of cardiac healing, as we have reported that hysterectomized rats without oorphorectomy showed progressive cardiac dilatation and heart failure following myocardial infarction (MI) which was comparable to males [11]. The transplantation of a green fluorescent protein (GFP^+) uterus complete with vascular anastomosis in a hysterectomized wild-type recipient animal resulted in GFP^+ cell mobilization to and engraftment into the heart, rescuing ventricular dysfunction after MI. GFP^+ cells were found around blood vessels supporting angiogenesis in these recipient animals [11]. Intravenous injection of uterine cells following hysterectomy and MI also enhanced tissue repair and prevented hysterectomy-related cardiac dysfunction [11]. These data support the notion that a functional uterus serves as a reservoir of highly regenerative cells that are mobilized in response to injury to function in cardiac regeneration.

These highly angiogenic uterine stem cells with distinct immunoprivileged characteristics could represent an excellent source of cells able to overcome the key challenges impairing the efficacy of currently used cell therapy for ischemic heart disease, such as dysfunctional aged cells and rejection. We identified a unique population of immunoprivileged, highly angiogenic uterine progenitor cells and have demonstrated their potential as an allogeneic cell therapy in regenerative medicine, particularly addressing cardiac regeneration post-ischemic injury.

2. Methods

For detailed methods, see the online Supplemental Information.

2.1. Experimental animals

For *in vitro* studies, C57BL/6N mice were used for uterine cell isolation and allogeneic leukocytes were isolated from FVB mice. For *in vivo* studies, female FVB mice of mouse major histocompatibility complex (MHC) haplotype 2, q variant ($H2^q$) were used as cell recipients, while female C57BL/6N mice of mouse MHC haplotype 2, b variant ($H2^b$) with green fluorescent protein (GFP) were used as cell donors. All animal procedures were approved by the Animal Care Committee of the University Health Network, and all animals received humane care in compliance with the *Guide for the Care and Use of Laboratory Animals* (8th edition, NIH, 2011).

2.2. Uterine cell isolation and characterization

Mice were sacrificed, their uteri dissected, and the uterine cells processed to create a single cell suspension. Uterine cells were sorted into MHC $I^{(pos)}$ and MHC $I^{(neg)}$ populations by allophycocyanin-targeted magnetic isolation and their purity analyzed by flow cytometry. Both uterine cell populations were characterized with respect to the expression of the following markers: Sca-1 and c-kit (considered hematopoietic lineage markers [12]), CD34 (identifies early hematopoietic and endothelial stem cells [13,14]), CD90 (a cell surface marker associated with differentiation potential in uterine stromal cells [15]), and MHC I and II.

2.3. In vitro assays

To quantify leukocyte-mediated cytotoxicity and cytotoxic T cell activation, mixed spleen leukocytes (5×10^5) from FVB mice were isolated and co-cultured with MSCs or MHC $I^{(neg)}$ and MHC $I^{(pos)}$ uterine cells. Leukocyte-mediated cytotoxicity was evaluated by lactate dehydrogenase (LDH) release from the damaged cells after 5 days of co-culture and by proliferation of cytotoxic leukocytes measured using 5-carboxyfluorescein diacetate succinimidyl ester (CFSE) staining.

Colony-forming unit (CFU) assays were performed on MHC $I^{(neg)}$ or MHC $I^{(pos)}$ uterine cells to assess their fibroblast (CFU-F) and hematopoietic (CFU-GM) progenitor potential. Endothelial differentiation potential was assessed by flow cytometry and immunostaining for expression of von Willebrand factor (vWF).

The angiogenic potential of MHC $I^{(neg)}$ or MHC $I^{(pos)}$ uterine cells was assayed *in vitro* by a scratch wound healing assay and by endothelial cord formation and compared to MSCs.

2.4. In vivo experiments

The angiogenic potential of the MHC $I^{(neg)}$ and MHC $I^{(pos)}$ uterine cells was compared with MSCs *in vivo* by an abdominal subcutaneous Matrigel implantation assay. Cells mixed with Matrigel were implanted into male mice. Seven days later, the nodules were excised. The vessel network in the nodule was photographed and assessed by hematoxylin and eosin (H&E) staining. Arteriole and capillary density was assessed and leukocyte recruitment was visualized by staining for CD45.

To assess the functional benefits of uterine cell implantation after ischemia, the left coronary artery of female FVB mice was permanently ligated and 0.5×10^6 allogeneic MSCs (at passage 4), or MHC $I^{(neg)}$ or MHC $I^{(pos)}$ uterine cells from C57BL/6 N GFP^+ mice were injected into the border zone. Uterine cells were freshly isolated on the day of transplantation. As each uterus contains 25–30% MHC $I^{(neg)}$ cells, approximately 1.5×10^6 MHC $I^{(neg)}$ cells could be isolated from each donor animal. 0.5×10^6 freshly isolated syngeneic GFP^+ bone marrow mononuclear cells (BMMCs) were injected into positive control animals and media injection alone was used as a negative control. Cardiac function was measured by echocardiography at baseline and at 7, 14 and 21 days post-MI to assess percent fractional shortening, left ventricular internal systolic dimension (LVIDS), left ventricular internal diastolic dimension (LVIDD), percent fractional change, left ventricular external systolic area (LVESA) and left ventricular external diastolic area (LVEDA).

Flow cytometry was used to quantify engraftment of the GFP^+ transplanted cells and infiltration of $CD4^+$ and $CD8^+$ T cells at day 1 (baseline), and at days 7 and 21. These markers were also assessed by immunohistochemistry on heart frozen sections.

To determine other humoral effects of cell transplantation on ischemic hearts, mice were sacrificed and cardiac tissue was collected 5 days after MI. Total RNA was isolated from heart tissue (scar and border zone) of the MHC $I^{(neg)}$, MHC $I^{(pos)}$ and media control groups. RT-qPCR was performed to measure the level of gene expression of various angiogenic molecules, growth factors, proteases and matrix proteins, cytokines and genes involved in cell survival.

2.5. Staining and immnohistochemistry

Masson's trichrome staining was performed to depict scar thickness. Heart sections were immunolabeled with antibodies against GFP, Sca-1, α-smooth muscle actin (α-SMA), isolectin, vWF and CD45. Nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI). Cultured cells were

immunostained for vWF to identify endothelial cells and their nuclei were stained with DAPI.

3. Results

3.1. Characterization of uterine cells

When analyzing the uterus for markers associated with immune characteristics, the MHC I marker was identified as being of particular interest. Immunoprivileged sites in the body (such as the brain, used as control in this study, Fig. 1A) have lower or absent MHC I protein expression on the cell surface [16]. When comparing the uterus with other non-immunprivileged sites such as bone marrow and spleen (Fig. 1A), the uterus had the highest percentage of cells with undetectable levels of MHC I (MHC $I^{(neg)}$). When MHC $I^{(neg)}$ uterine cells were co-stained with stem cell markers (Fig. 1B), it was revealed that this fraction was enriched for $CD90^+$ (45%), $CD34^+$ (35%) and Sca-1^+ (25%) cells, but had negligible levels of c-Kit^+ (0.2%) and MHC II^+ (<1%) cells. Therefore, uterine MHC $I^{(neg)}$ cells represent a heterogeneous population that contains substantial endothelial, hematopoietic as well as stromal cell lineages. MHC $I^{(pos)}$ cells can be also considered a heterogeneous population due to their diverse cell marker expression patterns. This population contained a similar number of CD90 stromal lineage cells as the MHC $I^{(neg)}$ population, but the other markers examined were significantly different in expression. The number of $CD34^+$ cells was decreased, but the number of Sca-1^+, c-Kit^+ and MHC II^+ cells was significantly higher in the MHC $I^{(pos)}$ fraction than the MHC $I^{(neg)}$ population. Both populations were still heterogeneous, containing stromal, hematopoietic and endothelial potentials, but we hypothesized that the immunoprivileged MHC $I^{(neg)}$ population had less chance of rejection when used in allogeneic settings *in vitro* and *in vivo*.

3.2. Stemness of uterine cells

Comparing MHC $I^{(pos)}$ and MHC $I^{(neg)}$ uterine cells, we found that both fractions had stem and progenitor cell functions. Both populations had the same stromal/fibroblast colony formation capability (Fig. 2A), but the MHC $I^{(pos)}$ uterine cells had significantly more hematopoietic progenitors ($P < 0.01$, Fig. 2B). The capacity for differentiation towards the endothelial lineage was present in both fractions, however the MHC $I^{(neg)}$ population showed more endothelial progenitors than the MHC $I^{(pos)}$ ($P < 0.01$, Fig. 2C) as shown by vWF staining. Indeed, the differences in cell marker expression highlighted in Fig. 1B can give additional insights regarding the different endothelial progenitor capabilities of these two populations *in vitro*. Mouse hematopoietic stem cell populations with low CD34, and increased c-Kit and Sca-I antigen expression have a higher rank in the hematopoietic hierarchy [17], which might explain the greater hematopoietic potential in the MHC $I^{(pos)}$ population even though both populations had some hematopoietic potential. On the other hand, mouse endothelial progenitors are enriched for CD34 [14], which could explain the significantly higher endothelial differentiation potential of MHC $I^{(neg)}$ cells.

3.3. High angiogenic and regenerative potential of uterine cells

To evaluate the biological function of the uterine cell populations based on the presence or absence of MHC I surface levels, we performed cell migration and vessel formation assays. Both MHC $I^{(pos)}$ and MHC $I^{(neg)}$ uterine cells had the ability to close an *in vitro* wound by migration. The MHC $I^{(neg)}$ population was able to completely close the wound at 20 h while the MHC $I^{(pos)}$ cells took significantly longer ($P < 0.01$, Fig. 3A).

To investigate the angiogeneic potential of uterine cells, we performed an *in vitro* cord formation assay with MHC $I^{(pos)}$ and MHC $I^{(neg)}$ uterine cells and compared them to mouse MSCs currently in use in clinical practice for heart regeneration. As shown in Fig. 3B, all groups stimulated cord formation. MHC $I^{(neg)}$ uterine cells had significantly more cords than MHC $I^{(pos)}$ and MSCs ($P < 0.01$), and the pattern of web-like structures was also more organized and developed in the MHC $I^{(neg)}$ cell population.

Next, we used a Matrigel plug assay to evaluate the angiogenic capacity of these cells *in vivo*. The strong angiogenic potential of uterine

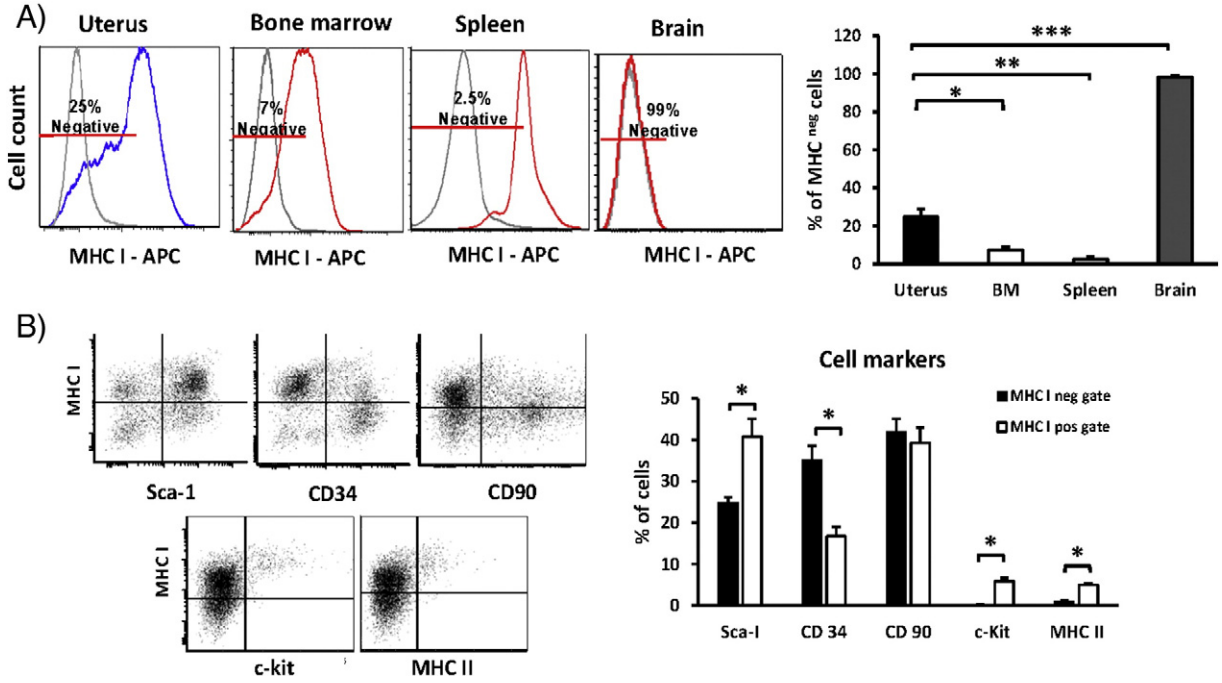

Fig. 1. MHC I expression and localization in tissues. (A) Representative flow cytometry charts of MHC I protein expression in uterus, bone marrow, spleen and brain (left panels). The uterus had a significantly higher proportion of cells with low or undetectable levels of MHC I compared to bone marrow and spleen (right panel, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$). Brain was used as negative control. (B) Flow cytometry charts of double staining for MHC I and common stem cell markers. The MHC $I^{(neg)}$ uterine cell population has a higher prevalence of $CD34^+$ cells, but lower expression of Sca-1, c-Kit and MHC II when compared to MHC $I^{(pos)}$ cells ($^* P < 0.05$). The number of $CD90^+$ cells was not significantly different between the groups.

cells could be visualized by color differences compared to controls when the plugs were dissected from the mouse (Fig. 3C). Further quantification of the angiogenic activity of uterine cells shown by the number of vessel-like structures/field visualized by H&E staining revealed a significantly higher density of vessels in uterine cell-seeded plugs compared to MSCs and the Matrigel only group ($P < 0.01$, Fig. 3C). Among uterine cell populations, MHC $I^{(pos)}$ had fewer vessels than MHC $I^{(neg)}$ cells ($P < 0.01$).

When analyzing the H&E stained nodules, we noticed more infiltrating cells when MHC $I^{(pos)}$ cells were used. In order to quantify this immune response, we labeled $CD45^+$ cells and found increased levels of infiltrating $CD45^+$ cells in the nodules implanted with MHC $I^{(pos)}$ cells ($P < 0.01$, Fig. 3D).

3.4. Immunoprivileged MHC $I^{(neg)}$ uterine cells

To evaluate the *in vitro* immune properties of MHC $I^{(pos)}$ and MHC $I^{(neg)}$ uterine cells, we established a co-culture system with uterine cells and allogeneic leukocytes generating a mixed leukocyte reaction (MLR). After 5 day co-culture, only 20% of leukocytes were proliferating in co-culture with MHC $I^{(neg)}$ uterine cells, while these proliferating cells increase to 60% when co-cultured with MHC $I^{(pos)}$ uterine cells (Fig. 4A). The level of cytotoxicity as assessed by LDH release in the culture media was significantly greater in MHC $I^{(pos)}$ compared to MHC $I^{(neg)}$ co-culture ($P < 0.001$, Fig. 4B). Fig. 4C shows bright-field images of the co-cultures, demonstrating a clear distinction between proliferating leukocyte patterns in the co-culture with MHC $I^{(pos)}$ and MHC $I^{(neg)}$ uterine cells, with increased proliferation in MHC $I^{(pos)}$ co-cultures. There was a 3-fold increase in $CD8^+$ cytotoxic T cells in the MHC $I^{(pos)}$ co-cultures when compared to MHC $I^{(neg)}$ uterine cells ($P < 0.001$, Fig. 4D), with $CD4^+$ cells proliferating to the same extent in both co-culture groups (data not shown). After the 5 days of co-culture, only 30% of MHC $I^{(neg)}$ cells started to express MHC I, while the MHC $I^{(pos)}$ population continued to express high levels of this molecule (Figs. 4 E–F).

3.5. MHC $I^{(neg)}$ transplanted uterine cells restore post-infarction cardiac function

To determine the effects of implanted allogeneic uterine cells on cardiac repair, we measured cardiac function by echocardiography at baseline (before MI), and 7, 14 and 21 days after MI and cell transplantation. Ejection fraction was similar in all groups at baseline (~60%). One week after MI, there was a significant decrease in fractional shortening and systolic fractional area change (%FAC) and an increase in left ventricular (LV) diameters in all animals. However, there was an improvement in these echocardiographic parameters in the animals receiving cells (syngeneic BMMCs, allogeneic MSCs, MHC $I^{(neg)}$ and MHC

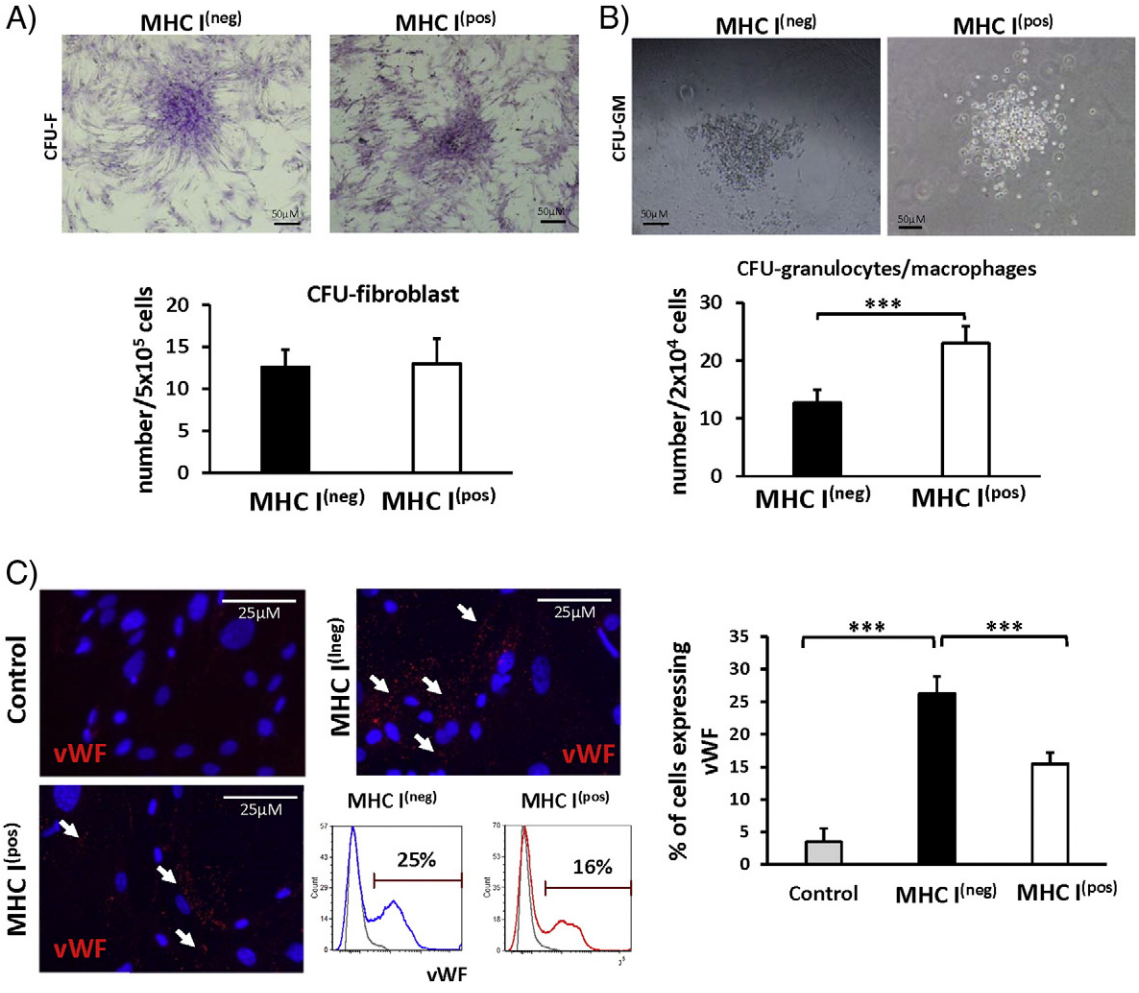

Fig. 2. MHC $I^{(neg)}$ or MHC $I^{(pos)}$ uterine cells have stromal/fibroblast, hematopoietic and endothelial progenitor capabilities. Freshly isolated uterine MHC $I^{(neg)}$ or MHC $I^{(pos)}$ were analyzed for progenitor cell characteristics (n = 4 done in duplicate/group). (A) Giemsa staining revealed fibroblast/stromal colonies in both uterine cells fractions 15 days after plating. CFU-F = fibroblast colony-forming unit. (B) Bright field images showing hematopoietic uterine colonies 15 days after plating. Quantification of colonies showed hematopoietic capacity in both cell fractions, however MHC $I^{(pos)}$ cells had significantly higher number of CFU-GM colonies. CFU-GM = granulocyte/macrophage colony-forming unit. (C) MHC $I^{(neg)}$ and MHC $I^{(pos)}$ plated in endothelial differentiation media expressed the endothelial marker vWF after 7 days in culture. Quantification of flow cytometry in MHC $I^{(neg)}$ cells revealed a significantly higher number of cells expressing vWF in this population when compared to MHC $I^{(pos)}$ or controls (cells not cultured in endothelial differentiation media, $^{***}P < 0.001$).

$I^{(pos)}$ uterine cells) when compared to media injection (Figs. 5A–G). The implantation of MHC $I^{(neg)}$ uterine cells resulted in significant preservation of cardiac function from the first week after cell implantation until the end of the study when compared to allogeneic MSCs and MHC $I^{(pos)}$ uterine cells. This preservation was comparable to the animals receiving syngeneic cells for all echocardiographic cardiac functional parameters ($P < 0.01$, Figs. 5A–G). At 21 days after MI, for example, there was significant improvement in fractional shortening in the MHC $I^{(neg)}$ group compared to the groups receiving either MSCs or MHC $I^{(pos)}$ cells. Therefore, a better survival of transplanted allogeneic MHC $I^{(neg)}$ uterine cells seems to have been correlated with the positive cardiac functional effects in this group. In order to address implanted cell survival and T cell response we compared only the MHC $I^{(pos)}$ and MHC $I^{(neg)}$ groups to the syngeneic BMMCs group which would not have the problem of cell rejection.

3.6. Allogeneic immunoprivileged MHC $I^{(neg)}$ uterine cell therapy after MI

To investigate survival of allogeneic uterine cells *in vivo*, allogeneic MHC $I^{(pos)}$ and MHC $I^{(neg)}$ uterine cells were transplanted into a female mouse model of MI. The immune response to the allogeneic cell

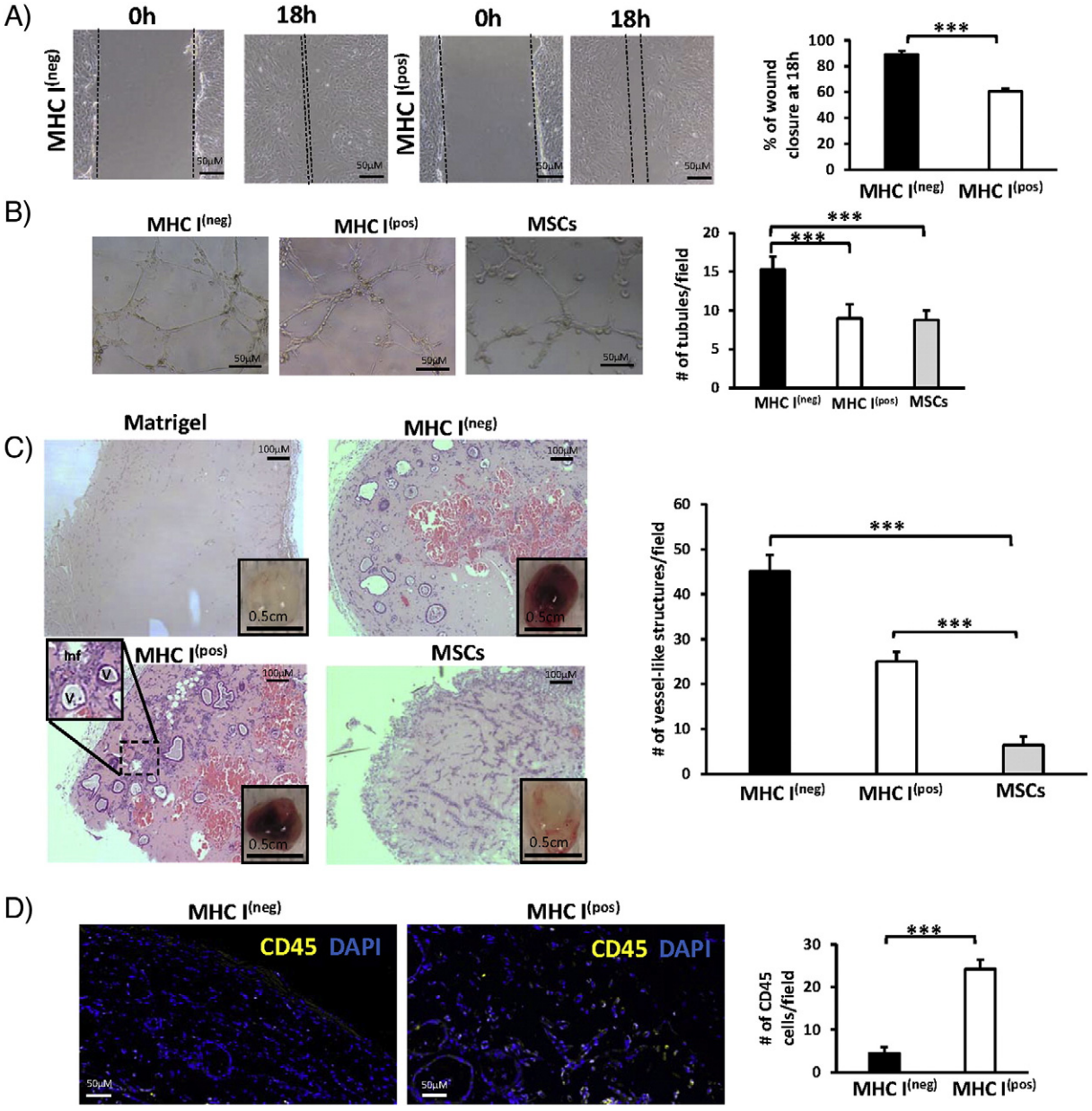

Fig. 3. Wound healing and angiogenic capacity of MHC $I^{(neg)}$ and MHC $I^{(pos)}$ uterine cells. (A) Both MHC $I^{(neg)}$ and MHC $I^{(pos)}$ uterine cells induced closure of the *in vitro* wound. Quantification of wound closure at 18 h showed MHC $I^{(neg)}$ cells migrated and proliferated faster than MHC $I^{(pos)}$ uterine cells (n = 4 done in duplicate/group). (B) Cord formation was induced in MHC $I^{(neg)}$ and MHC $I^{(pos)}$ uterine cells as well as mesenchymal stem cells (MSCs). All cells induced cord formation, however the MHC $I^{(neg)}$ group had more organized and defined cords. Quantification showed that MHC $I^{(neg)}$ cells had significantly higher tubule formation when compared to MHC $I^{(pos)}$ uterine cells and MSCs (n = 4 done in duplicate/group, $^{***}P < 0.001$). (C) Matrigel nodules with or without cells were implanted subcutaneously in mice and collected after 7 days (n = 4/group). Photographs of the nodules (insets) revealed visible vessels and blood in the nodules containing both MHC $I^{(neg)}$ and MHC $I^{(pos)}$ uterine cells. Hematoxylin and eosin staining was performed and quantification of the number of vessel-like structures showed significantly higher vessel density in the uterine cell groups when compared to MSCs ($^{***}P < 0.001$). MHC $I^{(pos)}$ nodules also presented inflammatory (inf) cells (lower left panel, upper inset) along with vessels (v). (D) CD45 was analyzed in MHC $I^{(neg)}$ and MHC $I^{(pos)}$ uterine cell nodules to measure the extent of infiltrating leukocytes. Quantification indicated a significantly higher density of inflammatory cells in MHC $I^{(pos)}$ uterine cell nodules.

transplantation was carefully evaluated over the course of the study, and correlated with implanted cell survival and cardiac function.

Allograft rejection is associated with recruitment of T cells [18]. To investigate whether allogeneic MHC I$^{(neg)}$ uterine cells can escape the adaptive immune response after implantation, we compared the

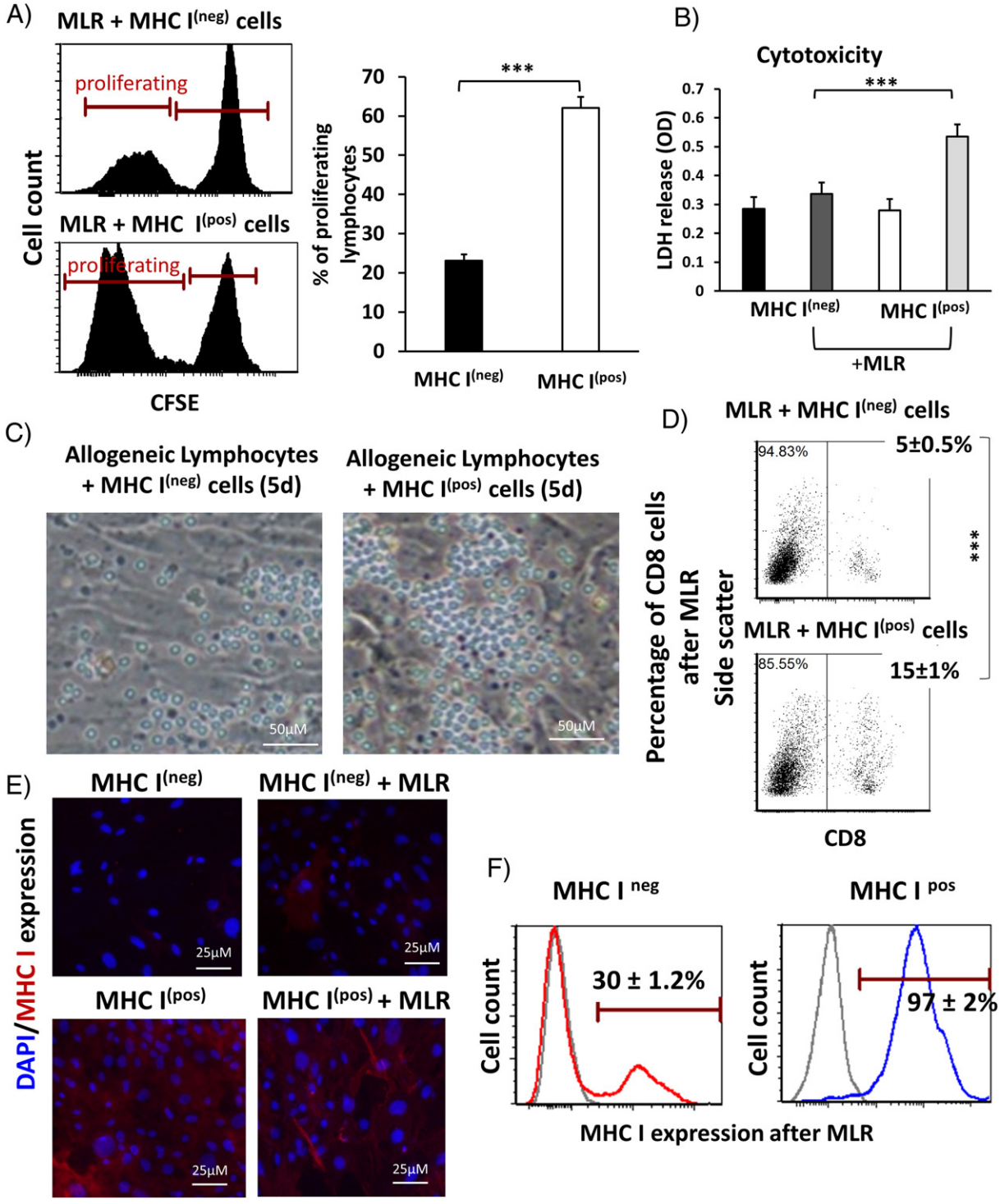

Fig. 4. MHC I$^{(neg)}$ uterine cells show reduced allogeneic lymphocyte-mediated cytotoxicity in a mixed lymphocyte co-culture system compared to MHC I$^{(pos)}$ uterine cells. Mouse splenic allogeneic lymphocytes were co-cultured for 5 days with either MHC I$^{(neg)}$ or MHC I$^{(pos)}$ uterine cells. MLR = mixed lymphocyte reaction. (A) Flow cytometry was used to quantify the proliferating fraction of carboxyfluorescein succinimidyl ester (CFSE)-labeled lymphocytes. Significantly higher lymphocyte proliferation is seen when they are co-cultured with MHC I$^{(pos)}$ compared to MHC I$^{(neg)}$ uterine cells. (B) Significantly higher cytotoxicity (measured by the level of released lactate dehydrogenase; LDH) was observed only in co-cultures containing MHC I$^{(pos)}$ uterine cells (***$P < 0.001$). (C) Representative micrographs (20× magnification) of MHC I$^{(neg)}$ (left) and MHC I$^{(pos)}$ (right) uterine cells co-cultured with allogeneic lymphocytes for 5 days (5d). (D) After 5 days in co-culture, the lymphocytes were removed and stained for CD8 (marking cytotoxic lymphocytes). Flow cytometry showed co-cultures of lymphocytes with MHC I$^{(pos)}$ cells had a 3-fold increase in CD8^{+} cells compared to MHC I$^{(neg)}$ co-cultures. (E) Immunostaining demonstrated that after 5 days co-culture, only a few MHC I$^{(neg)}$ cells started to express MHC I on their surface while all MHC I$^{(pos)}$ cells continued to express high levels of MHC marker. Blue is 4′,6-diamidino-2-phenylindole- (DAPI-) stained nuclei, red is MHC I. (F) Flow cytometry revealed that, after co-culture, only 30% of MHC I$^{(neg)}$ cells acquired MHC I expression while 97% of MHC I$^{(pos)}$ cells remained positive for MHC I.

recruitment of T cells ($CD4^+$ and $CD8^+$) at 1, 7 and 21 days after MI. We also followed GFP^+ cell survival and cardiac function at the same time points. From 5×10^5 cells implanted, cell retention on day 1 was 10% (5×10^4) in all groups. The group receiving syngeneic BMMCs had the highest cell survival (41% of day 1 totals) at 7 days as expected, but MHC $I^{(neg)}$ uterine cells also had a high percentage of implanted cell survival (35% of day 1 totals) which was significantly higher ($P < 0.001$) than the MHC $I^{(pos)}$ group (data not shown). At 21 days after MI, GFP^+ cells were still found in the hearts of the MHC $I^{(neg)}$ group. GFP immunohistochemistry in heart sections showed that these cells were usually round and small and were observed in the peri-infarct region (Fig. 6A, one GFP^+ cell is shown in detail). Flow cytometry analysis of single cell suspensions from the whole heart demonstrated that the number of GFP^+ cells in the MHC $I^{(neg)}$ group at 21 days was comparable to the BMMC syngeneic group (around 0.19% of the whole heart and 20% of the day 1 total). However GFP^+ cells were nearly completely depleted from the MHC $I^{(pos)}$-implanted hearts (Fig. 6A) and difficult to find in heart sections.

The levels of $CD8^+$ and $CD4^+$ T cells (Figs. 6B–C) in the MHC $I^{(neg)}$ group were low throughout the 21 days after MI and was comparable to the syngeneic group, while the MHC $I^{(pos)}$ group had a more robust T cell response which correlated to the decrease in transplanted cell survival. $CD8^+$ and $CD4^+$ cells were significantly higher in the MHC $I^{(pos)}$ group, particularly at 21 days (Figs. 6B–C). These T cells were mainly localized in the scar region (Fig. 6D). These data suggested that the greater survival rate of implanted MHC $I^{(neg)}$ uterine cells in the recipients' hearts was due to reduced immunogenicity.

3.7. MHC $I^{(neg)}$ transplanted uterine cells reduce scar formation, improve angiogenesis and express Sca-1 when engrafted in cardiac tissue after MI

GFP^+ cells were shown to have survived more in the animals receiving MHC $I^{(neg)}$ *vs* MHC $I^{(pos)}$ uterine cells. We determined the possible mechanisms that might account for the functional benefits seen in this immunoprivileged group. The infarct size at 21 days was significantly smaller in the MHC $I^{(neg)}$ group compared to MHC $I^{(pos)}$ (Fig. 7A), and the scar was also thinner in the MHC $I^{(pos)}$ group, with less muscle in between the collagen fibers (Fig. 7B). This suggests prevention of adverse cardiac remodeling in the MHC $I^{(neg)}$ group. At 7 days post-MI, we studied angiogenesis by analyzing histological heart sections and staining for α-SMA to evaluate mature blood vessels, vWF to detect endothelial cells and isolectin for capillaries. Fig. 7C shows that the injected uterine MHC $I^{(neg)}$ cells significantly increased the number of blood vessels in and around the infarcted myocardium at this earlier time point. Sca-1 is a marker of progenitor cells, and Sca-1^+ cells in

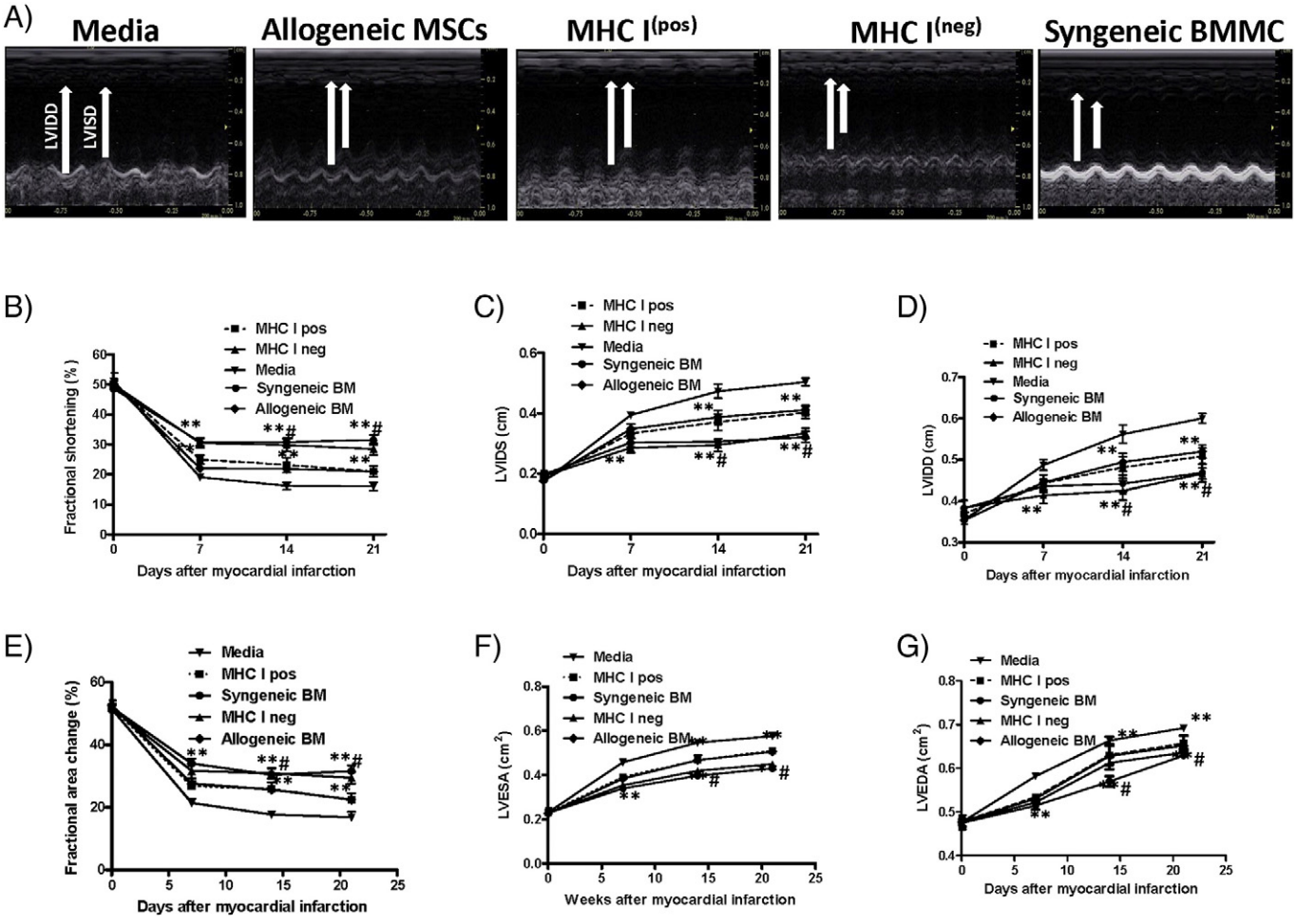

Fig. 5. Transplantation of allogeneic MHC $I^{(neg)}$ uterine cells restored cardiac function comparably to syngeneic bone marrow mononuclear cell (BMMC) transplantation. Allogeneic uterine cells, syngeneic bone marrow cells, and allogeneic mesenchymal stem cells (MSCs) (5×10^5 cells/mouse) were transplanted into the infarcted area of FVB mice hearts. Animals receiving only media injection (Media) were used as negative controls (n = 8/group). (A) Representative M-mode echocardiographic images. (B) Fractional shortening. (C) Left ventricular internal end systolic dimension (LVIDS). (D) Left ventricular internal end-diastolic dimension (LVIDD). (E) Percentage of fractional area change. (F) Left ventricular end systolic area (LVESA). (G) Left ventricular end diastolic area (LVEDA). Fractional shortening and the percentage of fractional area change were greater and the internal ventricular dimensions and areas were smaller when allogeneic MHC $I^{(neg)}$ uterine cells were implanted, and this was comparable to the bone marrow (BM) syngeneic group. $^{**}P < 0.01$ when compared to Media, $\#P < 0.01$ when compared to MHC $I^{(pos)}$ uterine cells.

the heart are associated with angiogenesis. Flow cytometry of heart cells 21 days post-MI showed that the group receiving MHC I$^{(neg)}$ cells had a 4% increase in the total number of Sca-1^{+} cells when compared to the MHC I$^{(pos)}$ group ($P < 0.001$, Fig. 7D). In addition, all GFP^{+} cells in the hearts of animals from the MHC I$^{(neg)}$ group co-stained with the Sca-1 marker, as shown by the double-positive cells analyzed by flow cytometry, as well as immunohistochemistry for GFP in heart sections of the MHC I$^{(neg)}$ group (Fig. 7E). This confirms that angiogenesis contributes to the improvement in cardiac function when allogeneic cells are not readily rejected from the heart, and Sca-1-expressing cells may be a key population that coordinates this process.

3.8. Expression of angiogenic molecules, growth factors, matrix proteins, cytokines and cell survival genes is involved in modulating improvement in heart function after transplantation of uterine cells

Although all the groups that received cells had improved cardiac function, cell engraftment was better in the allogeneic MHC I$^{(neg)}$ group when compared to the MHC I$^{(pos)}$ group. To determine other humoral effects of cell transplantation on ischemic hearts, mice were sacrificed and cardiac tissue was collected at 5 days post-MI. Although there was a trend showing increased vascular endothelial growth factor (VEGF) in uterine groups, this parameter did not reach statistical significance (Fig. 8A). The expression level of angiopoietin 1 was significantly increased in the groups that received uterine cells compared with media alone (Fig. 8B). Angiopoietin 2 was not different among groups (Fig. 8C). Matrix metalloproteinase (MMP)-2 as well as tissue inhibitor of metalloproteinases (TIMP)-2 were significantly increased in all uterine groups when compared to control (Figs. 8D and F), however TIMP1 was significantly decreased in MHC I$^{(neg)}$ animals when compared to the other two groups (Fig. 8E). TIMP3 and 4 did not show any statistical difference (data not shown). Expression of cell survival Ak strain transforming oncogene (AKT1) was significantly increased only in the MHC I$^{(neg)}$ group (Fig. 8G). Expression of the anti-inflammatory factors transforming growth factor β-2 (TGFβ2) (Fig. 8H) and interleukin-(IL-)10 (data not shown) as well as pro-inflammatory IL-6 (Fig. 8I),

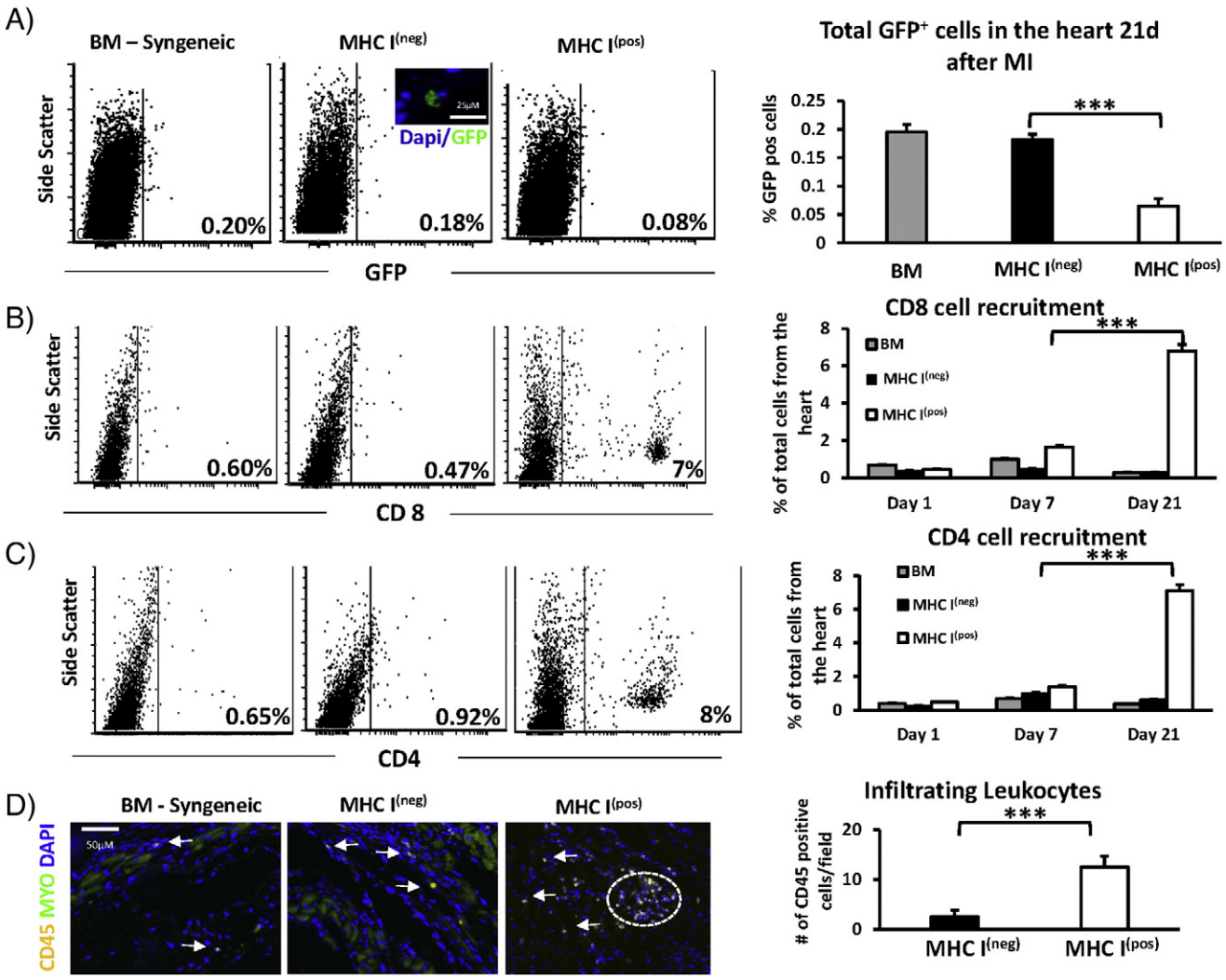

Fig. 6. After a myocardial infarction (MI), MHC I$^{(neg)}$ allogeneic uterine cells are comparable to syngeneic cells and superior to MHC I$^{(pos)}$ cells for post-implantation survival and reduced T cell recruitment. Animals underwent permanent ligation of the left anterior descending artery (LAD) and received either syngeneic bone marrow unfractionated mononuclear cells (BM) or allogeneic MHC I$^{(neg)}$ or MHC I$^{(pos)}$ uterine cells in the border zone of the infarcted area (n = 4/group). All implanted cells were GFP^{+}. GFP^{+} cell survival and T cell recruitment were analyzed at 1 (baseline), 7 and 21 days after MI. (A) Flow cytometry for the total number of GFP^{+} cells in the heart 21 days after MI showed more cells surviving in animals receiving syngeneic and MHC I$^{(neg)}$ uterine cells. MHC I$^{(neg)}$-implanted heart sections showed GFP^{+} cell staining but the MHC I$^{(pos)}$ group did not. A higher percentage of GFP^{+} cells survived in the syngeneic BM and the allogeneic MHC I$^{(neg)}$ uterine cell groups (***$P < 0.001$). (B) Flow cytometry of CD8^{+} and (C) CD4^{+} T cells recruited to the heart 21 days after MI. Animals receiving allogeneic MHC I$^{(pos)}$ uterine cells had significantly more infiltrating CD8^{+} and CD4^{+} T cell recruitment to the heart, particularly at 21 days post-MI (***$P < 0.001$). (D) CD45 expression in the allogeneic MHC I$^{(neg)}$ and MHC I$^{(pos)}$ groups showed the number and localization of infiltrating leukocytes. A significantly higher (***$P < 0.001$) density of inflammatory cells was seen in the scar region of the MHC I$^{(pos)}$ group. Yellow is CD45, green is autofluorescence from cardiomyocytes (MYO) and blue is DAPI-stained nuclei.

tumor necrosis factor-α and interferon-γ (data not shown) was not statistically different among groups at the time studied. These data indicate that direct cardiac injection of uterine cells augmented multiple biological factors associated with vascularization and matrix remodeling, and that the MHC I$^{(neg)}$ group was able to affect the AKT gene, which is involved in cell survival.

4. Discussion

Ischemic heart disease is an important clinical problem worldwide [19]. Cell therapy directed to preserve cardiac function is being explored and has the potential to greatly improve cardiac outcomes [20]. Ideal cells for therapy in this context should be able to improve cardiac

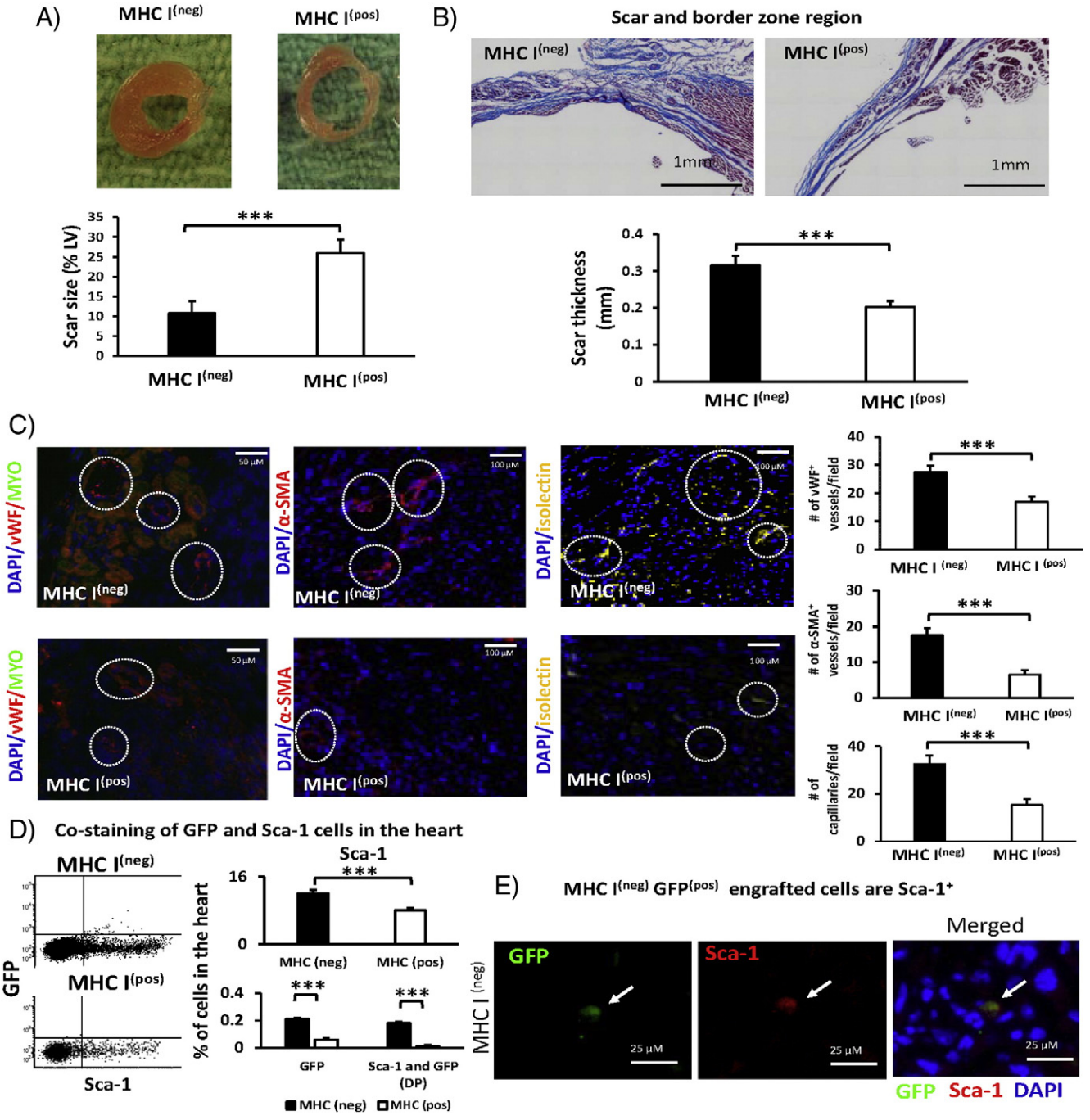

Fig. 7. Allogeneic immunoprivileged MHC I$^{(neg)}$ uterine cells are angiogenic and prevent adverse remodeling of the heart after myocardial infarction (MI). (A) Representative heart sections including the scar area of animals receiving MHC I$^{(neg)}$ or MHC I$^{(pos)}$ uterine cells 21 days post-MI. Infarct size (calculated by the percentage of scar area in the left ventricle) was significantly smaller in the MHC I$^{(neg)}$ group. (B) Scar areas (light blue) and muscle (red) were visualized with Masson's trichrome staining, and scar thickness was measured. MHC I$^{(neg)}$-injected hearts had significantly thicker scars compared to those injected with MHC I$^{(pos)}$ uterine cells. (C) Staining for von Willebrand factor (vWF, red), α-SMA (red) and isolectin (yellow) was used to visualize the extent of blood vessel density. The number of capillaries and mature blood vessels was significantly higher in the MHC I$^{(neg)}$ group in the peri-infarct and scar areas. Autofluorescence from cardiomyocytes (MYO) is shown in green on vWF-labeled images and healthy myocytes are more common in the infarcted region of MHC I$^{(neg)}$-injected hearts. (D) Flow cytometry showing co-staining of GFP and Sca-1 in cells from the heart 21 days post-MI. The MHC I$^{(neg)}$ group recruited more Sca-1 cells ($P < 0.001$), and the GFP cells were all Sca-1^{+}. (E) GFP and Sca-1 immunostaining and the merged image demonstrating co-localization of the two markers in heart sections of the MHC I$^{(neg)}$ group. Blue is DAPI-stained nuclei. n = 4/group for all assays, $^{***}P < 0.001$.

healing by successfully engrafting in the injured site to promote restoration of blood flow, impede cardiomyocytes from dying or replace already dead tissue with new muscle. However, most investigators agree that the optimal cells or ideal combination of cells for transplantation have not been identified. In this study, we were able to overcome the major obstacle of cell rejection as we identified and isolated immunoprivileged progenitor cells derived from the uterus with great potential for angiogenesis to be used for cell therapy after myocardial infarction to improve cardiac function and regeneration. Our data reveals the discovery of a unique cell population that can be effectively used as allogeneic cell therapy in regenerative medicine.

The most well known immunologically privileged sites in the body are the eye [21], the placenta and fetus [22], the testes [23] and the central nervous system [24]. In all these sites, expression of MHC class Ia and class II molecules are reduced or eliminated. Our data showed that the uterus contains a high percentage (>20%) of cells with low or undetectable expression of MHC I. Although the non-pregnant uterus is not an immunoprivileged organ as a whole, it may contain special sites with unique immune properties that may have contact with a semi-allogeneic fetus not normally rejected by the mother. Since the uterus is known for its unique regenerative properties, we divided uterine cells into two populations (MHC $I^{(neg)}$ and MHC $I^{(pos)}$) to test their effectiveness as cell therapy. The MHC $I^{(neg)}$ uterine cells represented a heterogeneous population with a common feature: immunoprivilege. The *in situ* reproductive functions of these MHC $I^{(neg)}$ cells remain unknown. Functional characterization was not the aim of this study and further work is necessary to better understand the role of these cells in tissue homeostasis and pathology. Lack of surface MHC I has been previously reported for stem cell populations [25–27]. In the bone marrow compartment, Quarta and colleagues reported two subpopulations of non-erythroid-derived stem cells, bone marrow-derived liver stem cells and the multipotent adult progenitors, which shared the characteristic of a lack of MHC I on the cell surface [26]. Although adult stem cells are rare, our findings revealed that MHC $I^{(neg)}$ uterine cell populations contain progenitor and stem cells capable of self-renewal and differentiation. Uterine stem cells in particular have also been used to rescue other diseases, suggesting a versatility in function [28,29]. In our study, the heterogeneity of the cell fraction may have contributed to multiple mechanisms of improvement in cardiac function: higher survival of immunoprivileged cells, angiogenesis counteracting ischemic injury, activation of extracellular matrix

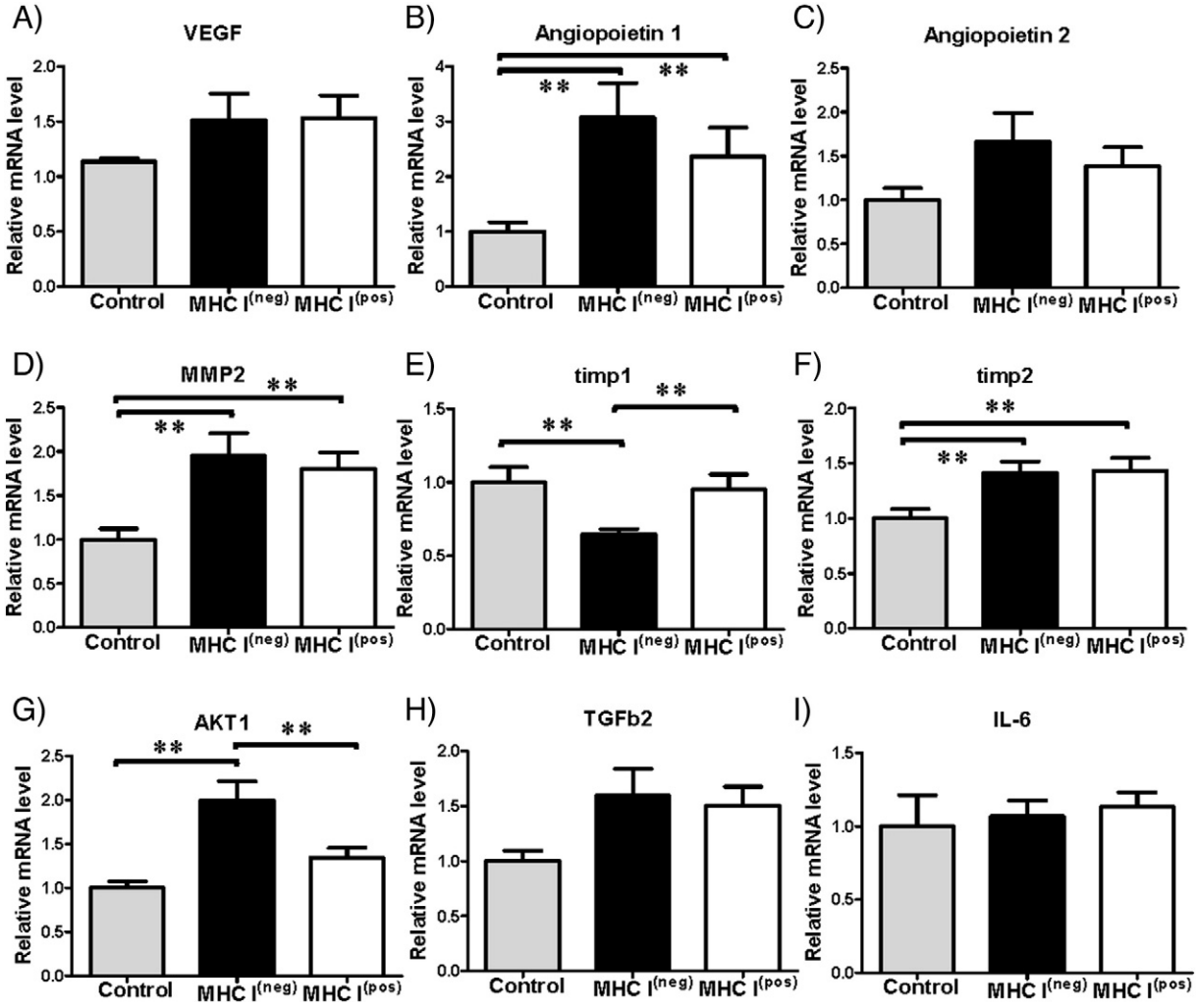

Fig. 8. Angiogenic, matrix remodeling and cell survival genes are involved in rescuing the ischemic heart after myocardial infarction (MI) when using uterine cell therapy. RT-qPCR was performed to measure the level of gene expression in heart tissue of animals receiving MHC $I^{(neg)}$ or MHC $I^{(pos)}$ uterine cells and media control 5 days post-MI. Various angiogenic (A–C), matrix remodeling (D–F), cell survival (G) anti-inflammatory (H), and pro-inflammatory (I) genes were analyzed. n = 4/group for all assays, $^{**}P < 0.001$. Individual values were normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH). VEGF = vascular endothelial growth factor; MMP2 = matrix metalloproteinase-2; timp = tissue inhibitor of metalloproteinases; AKT1 = Ak strain transforming oncogene; TGFb2 = transforming growth factor β-2; IL = interleukin.

mechanisms, smaller scar size and modulation of ventricular remodeling. Although it would be ideal to characterize one single cell population responsible for the reparative effect, multiple cells types are needed to potentiate cardiac regeneration. In this regard, the stem cell field for cardiac repair is also moving towards a broader approach, using a combination of different cell types which allows for a synergistic beneficial effect [30].

Recognition of MHC I molecules by cytotoxic T lymphocytes can stimulate the killing of allogeneic MHC I-presenting cells. CD4 and CD8 cytotoxic T cells have been shown to be the major cause of allogeneic graft rejection [31]. *In vitro* and *in vivo*, the MHC $I^{(pos)}$ population elicited proliferation of cytotoxic $CD8^+$ cells which was associated with decreased MHC $I^{(pos)}$ cell survival and rejection. $CD4^+$ and $CD8^+$ cells were already reported to be recruited to the heart within the first week post-MI in murine models [32]. In our study, the number of $CD4^+$ and $CD8^+$ cells started to increase in the heart at 7 days post-MI in the MHC $I^{(pos)}$-injected hearts and continued to increase up to 21 days. At these time points, we noticed a further decrease in cardiac function and survival of cells in the animals receiving MHC $I^{(pos)}$ cells. MHC $I^{(neg)}$ cells did not trigger this T cell response to the same extent, and were similar to syngeneic BM cell therapy, indicating their *in vivo* immunoprivilege. From the present data, we conclude that MHC $I^{(neg)}$ cells implanted into the infarcted myocardium prevented decreased cardiac function as effectively as syngeneic BM cells. Although the number of surviving cells after 21 days was relatively low, both in the syngeneic as well as the allogeneic MHC $I^{(neg)}$ groups, the decrease in cell numbers appeared not to be due to cell rejection and induction of an immune response that could prevent engraftment across histocompatibility antigens. Development of new delivery devices with enhanced biologic homing factors or using a combination of cells with biomaterials could further enhance cell retention and requires more investigation. We identified cells expressing low or undetectable levels of MHC I which are suitable for allogeneic cell transplantation, and we propose this cell population can be developed as a new cell treatment in regenerative medicine.

Moreover, although we injected a heterogeneous population of cells, our *in vivo* as well as *in vitro* findings revealed that these cells have high angiogenic and regenerative potential and all might have contributed to the beneficial effects seen in the heart. The relatively few MHC $I^{(neg)}$ cells that survived in cardiac tissue after 21 days also expressed the Sca-1^+ marker. The group that received MHC $I^{(neg)}$ cells seems to have recruited more endogenous Sca-1^+ cells compared to the MHC $I^{(pos)}$ group as we noticed a significant 4% increase in the total number of these cells (from 8 to 12% of the total heart). While GFP^- Sca-1^+ cells were found distributed through the heart tissue, the few GFP^+ Sca-1^+ cells were found only in MHC $I^{(neg)}$-implanted heart sections and were usually found adjacent to the scar. Progenitor cells in the adult heart were previously reported to express Sca-1 [33–35]. Although the role of progenitor cells in heart structure and function is poorly understood, different subsets of cells have been shown to be committed to form cells of cardiac lineage such as cardiomyocytes, smooth muscle cells, and endothelial cells in support of tissue repair under ideal conditions [33,36,37]. Unpublished data from our laboratory identified a Sca-1^+ $CD34^-$ population from the uterus as a pro-angiogenic cell subtype. Angiogenesis, with restoration of blood flow to the injury site, has been identified as a key factor in the preservation of cardiac function [26]. In our study, the hearts of the animals that received immunoprivileged uterine cells had highly organized blood vessels as well as improved capillary density which was able to restore blood flow, meaning this cell treatment has the potential to avoid the need for intralumenal or surgical interventions. Although more studies are necessary, our results further support the use of uterine regenerative cells as effective cell therapy agents.

Additionally, when we compared all uterine cells to MSCs in Matrigel assays for angiogenic function, uterine cells were superior to MSCs. Indeed, the endometrium is a rare site of non-pathological angiogenesis, repeatedly recreating decidual tissue in the post-development adult body and is a unique niche for regenerative cells [38]. Our functional data also showed allogeneic MHC $I^{(neg)}$ cells to have better improvement in cardiac function than allogeneic MSCs. We have previously shown that, upon differentiation, allogeneic MSCs rapidly lose their privileged status and are rejected [39,40]. This accounts for the fact that allogeneic MSCs improve heart function in the short term post-implantation, but then are rejected and heart function declines at later time points [39]. In our study, the high angiogenic capacity of uterine cells, coupled with the distinct immunoprivilege of the selected uterine population, led to a more suitable cell type for treating ischemic injury. This brings new hope for the use of cell therapy in the context of ischemic diseases. The main focus of this study was to show that the immunoprivilege capability of the implanted cells increased the time available to promote the repair mechanisms necessary to induce cardiac regeneration. Angiogenesis alone is unlikely to explain this greater improvement in cardiac function. After uterine cell treatment, especially in animals that received MHC $I^{(neg)}$ cells, multiple biological factors that have been previously demonstrated to enhance angiogenesis, cell survival, and tissue repair were significantly activated in the heart at 5 days post-MI. At this early time point, greater matrix remodeling and pro-survival activities were found in the group receiving MHC $I^{(neg)}$ cells which may have worked synergistically with improved angiogenesis to promote and sustain neovascularization and preservation of viable myocytes to support tissue regeneration *in vivo*. At 21 days post-MI, the lack of T cell recruitment with immunoprivileged cells may have been pivotal to avoid implanted cell rejection and the deleterious effects of the recruited immune cells in secreting cytokines to further increase tissue damage.

In addition, according to our previous studies regarding the uterus and uterine stem cells [11,41], this fully functional organ may contribute as an independent factor in support of cardioprotection in women, as it is well known that pre-menopausal women fare better in both cardiovascular disease incidence and outcomes [42]. Our group has already shown that uterine cells traffic to the heart in response to injury and collaborate in cardiac regeneration [11], and in the present study we translated this previous knowledge and used intramyocardial transplantation of allogeneic uterine cells, which proved to be an effective therapy providing increased angiogenesis and preserving cardiac function. Increased understanding about the “uterine stem cell reservoir” may lead to better cardiovascular outcomes in post-menopausal women as well as men, populations at the highest risk for adverse cardiovascular outcomes. In the current study, we did not include a group of male animals receiving immunoprivileged female cells. This will be investigated in the future studies.

The isolation of immunoprivileged reparative cells may have the potential to restore this reservoir in the specific patient populations with the highest need for a healthy and young cell source. This cell therapy may have great impact in regenerative medicine, contributing to the treatment of other ischemia-related diseases such as ischemia-induced renal injury, lower-limb ischemia, and stroke. In our study, we used freshly isolated cells in order not to lose the benefits of non-adherent (hematopoietic) cells in the population of interest, as well as to assure culture conditions and storage would not change their immunoprivilege. As we deepen our understanding about MHC $I^{(neg)}$ cells, it will be important to know whether these cells can be stored, cultured and passaged to generate a cell product which is readily available and functionally consistent for clinical use. Although we demonstrated some mechanisms that may account for the beneficial effects of these cells, further studies should specifically clarify which pathways are activated and the cytokines or other proteins involved. We also did not study cell rejection past 21 days post-MI, however, the cells survived long enough in the target tissue to effectively counteract adverse cardiac remodeling either by direct cell response or mobilization of host-associated repair mechanisms. Further studies are needed to provide the specific mechanism of action of these cells, along with

optimization of cells for clinical trials to determine whether our findings can be translated to future therapy.

5. Conclusion

We identified uterine progenitor angiogenic cells with distinct immunoprivilege abilities that can be used as an effective allogeneic cell therapy for cardiac regeneration. Uterine MHC $I^{(neg)}$ cells, like their MHC $I^{(pos)}$ counterparts, show angiogenic and progenitor properties *in vitro*. The MHC $I^{(neg)}$ cells, however, demonstrate improved immunoprivilege compared to uterine cells that express the MHC I antigen and show improved engraftment when injected after MI. This persistent engraftment allows them to significantly improve cardiac function. The angiogenic and immunoprivileged nature of these uterine cells suggests that they should be further explored as an allogeneic cell therapy for aged patients with cardiac ischemia.

Disclosure of potential conflicts of interest

There are no conflicts of interest associated with this work.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.yjmcc.2015.04.019.

References

[1] Smith MA, Pallister CJ, Smith JG. Stem cell factor: biology and relevance to clinical practice. Acta Haematol 2001;105:143–50 [doi:46556].
[2] Ayach BB, Yoshimitsu M, Dawood F, Sun M, Arab S, Chen M, et al. Stem cell factor receptor induces progenitor and natural killer cell-mediated cardiac survival and repair after myocardial infarction. Proc Natl Acad Sci U S A 2006;103:2304–9. http://dx.doi.org/10.1073/pnas.0510997103.
[3] Boyle AJ, Schulman SP, Hare JM. Stem cell therapy for cardiac repair: ready for the next step. Circulation 2006;114:339–52.
[4] Sanganalmath SK, Bolli R. Cell therapy for heart failure: a comprehensive overview of experimental and clinical studies, current challenges, and future directions. Circ Res 2013;113:810–34. http://dx.doi.org/10.1161/CIRCRESAHA.113.300219.
[5] Zhang H, Fazel S, Tian H, Mickle DA, Weisel RD, Fujii T, et al. Increasing donor age adversely impacts beneficial effects of bone marrow but not smooth muscle myocardial cell therapy. Am J Physiol Heart Circ Physiol 2005;289:H2089–96. http://dx.doi.org/10.1152/ajpheart.00019.2005.
[6] Dimmeler S, Leri A. Aging and disease as modifiers of efficacy of cell therapy. Circ Res 2008;102:1319–30. http://dx.doi.org/10.1161/CIRCRESAHA.108.175943.
[7] Pearl JI, Kean LS, Davis MM, Wu JC. Pluripotent stem cells: immune to the immune system? Sci Transl Med 2012;4(164 ps):25. http://dx.doi.org/10.1126/scitranslmed.3005090.
[8] Dhingra S, Huang XP, Li RK. Challenges in allogeneic mesenchymal stem cell-mediated cardiac repair. Trends Cardiovasc Med 2010;20:263–8. http://dx.doi.org/10.1016/j.tcm.2011.12.002.
[9] Arck P, Solano ME, Walecki M, Meinhardt A. The immune privilege of testis and gravid uterus: same difference? Mol Cell Endocrinol 2014;382:509–20. http://dx.doi.org/10.1016/j.mce.2013.09.022.
[10] Huang ML, Tian H, Wu J, Matsubayashi K, Weisel RD, Li RK. Myometrial cells induce angiogenesis and salvage damaged myocardium. Am J Physiol Heart Circ Physiol 2006;291:H2057–66. http://dx.doi.org/10.1152/ajpheart.00494.2006.
[11] Xaymardan M, Sun Z, Hatta K, Tsukashita M, Konecny F, Weisel RD, et al. Uterine cells are recruited to the infarcted heart and improve cardiac outcomes in female rats. J Mol Cell Cardiol 2012;52:1265–73. http://dx.doi.org/10.1016/j.yjmcc.2012.03.002.
[12] Yang L, Bryder D, Adolfsson J, Nygren J, Månsson R, Sigvardsson M, et al. Identification of $Lin^{-}Sca1^{+}kit^{+}CD34^{+}Flt3^{-}$ short-term hematopoietic stem cells capable of rapidly reconstituting and rescuing myeloablated transplant recipients. Blood 2005;105:2717–23. http://dx.doi.org/10.1182/blood-2004-06-2159.
[13] Krause DS, Ito T, Fackler MJ, Smith OM, Collector MI, Sharkis SJ, et al. Characterization of murine CD34, a marker for hematopoietic progenitor and stem cells. Blood 1994;84:691–701.
[14] Yang J, Ii M, Kamei N, Alev C, Kwon S-M, Kawamoto A, et al. $CD34^{+}$ cells represent highly functional endothelial progenitor cells in murine bone marrow. PLoS One 2011;6. http://dx.doi.org/10.1371/journal.pone.0020219.
[15] Schwab KE, Chan RW, Gargett CE. Putative stem cell activity of human endometrial epithelial and stromal cells during the menstrual cycle. Fertil Steril 2005;84(Suppl. 2):1124–30. http://dx.doi.org/10.1016/j.fertnstert.2005.02.056.
[16] Ito T, Meyer KC, Ito N, Paus R. Immune privilege and the skin. Curr Dir Autoimmun 2008;10:27–52. http://dx.doi.org/10.1159/000131412.
[17] Nakauchi H, Takano H, Ema H, Osawa M. Further characterization of CD34-low/negative mouse hematopoietic stem cells. Ann N Y Acad Sci 1999;872:57–66 [discussion 66–70].
[18] Wood KJ, Bushell A, Hester J. Regulatory immune cells in transplantation. Nat Rev Immunol 2012;12:417–30. http://dx.doi.org/10.1038/nri3227.
[19] Massie BM, Shah NB. Evolving trends in the epidemiologic factors of heart failure: rationale for preventive strategies and comprehensive disease management. Am Heart J 1997;133:703–12. http://dx.doi.org/10.1016/S0002-8703(97)70173-X.
[20] Fazel S, Tang GHL, Angoulvant D, Cimini M, Weisel RD, Li R-K, et al. Current status of cellular therapy for ischemic heart disease. Ann Thorac Surg 2005;79:S2238–47. http://dx.doi.org/10.1016/j.athoracsur.2005.02.085.
[21] Streilein JW. Ocular immune privilege: the eye takes a dim but practical view of immunity and inflammation. J Leukoc Biol 2003;74:179–85.
[22] Zenclussen AC, Schumacher A, Zenclussen ML, Wafula P, Volk H-D. Immunology of pregnancy: cellular mechanisms allowing fetal survival within the maternal uterus. Expert Rev Mol Med 2007;9:1–14. http://dx.doi.org/10.1017/S1462399407000294.
[23] Fijak M, Meinhardt A. The testis in immune privilege. Immunol Rev 2006;213:66–81. http://dx.doi.org/10.1111/j.1600-065X.2006.00438.x.
[24] Carson MJ, Doose JM, Melchior B, Schmid CD, Ploix CC. CNS immune privilege: hiding in plain sight. Immunol Rev 2006;213:48–65. http://dx.doi.org/10.1111/j.1600-065X.2006.00441.x.
[25] Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, et al. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 2002;418:41–9. http://dx.doi.org/10.1038/nature00870.
[26] Quarta M, Stroka D, Keogh A, Sidler D, Avital I, Gloor B, et al. Multiparameter flow cytometry characterization of MHC class I negative mouse bone marrow cells. Med Sci Monit Inter Med J Exp Clin Res 2008;14:BR286–93.
[27] Beerheide W, von Mach MA, Ringel M, Fleckenstein C, Schumann S, Renzing N, et al. Downregulation of beta2-microglobulin in human cord blood somatic stem cells after transplantation into livers of SCID-mice: an escape mechanism of stem cells? Biochem Biophys Res Commun 2002;294:1052–63. http://dx.doi.org/10.1016/S0006-291X(02)00596-X.
[28] Santamaria X, Massasa EE, Feng Y, Wolff E, Taylor HS. Derivation of insulin producing cells from human endometrial stromal stem cells and use in the treatment of murine diabetes. Mol Ther 2011;19:2065–71. http://dx.doi.org/10.1038/mt.2011.173.
[29] Deane JA, Gualano RC, Gargett CE. Regenerating endometrium from stem/progenitor cells: is it abnormal in endometriosis, Asherman's syndrome and infertility? Curr Opin Obstet Gynecol 2013:25.
[30] Williams AR, Hatzistergos KE, Addicott B, McCall F, Carvalho D, Suncion V, et al. Enhanced effect of combining human cardiac stem cells and bone marrow mesenchymal stem cells to reduce infarct size and to restore cardiac function after myocardial infarction. Circulation 2013;127:213–23. http://dx.doi.org/10.1161/CIRCULATIONAHA.112.131110.
[31] Mason D. The roles of T cell subpopulations in allograft rejection. Transplant Proc 1988;20:239–42.
[32] Yan X, Anzai A, Katsumata Y, Matsuhashi T, Ito K, Endo J, et al. Temporal dynamics of cardiac immune cell accumulation following acute myocardial infarction. J Mol Cell Cardiol 2013;62:24–35. http://dx.doi.org/10.1016/j.yjmcc.2013.04.023.
[33] Oh H, Bradfute SB, Gallardo TD, Nakamura T, Gaussin V, Mishina Y, et al. Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. Proc Natl Acad Sci U S A 2003;100:12313–8. http://dx.doi.org/10.1073/pnas.2132126100.
[34] Matsuura K, Nagai T, Nishigaki N, Oyama T, Nishi J, Wada H, et al. Adult cardiac Sca-1-positive cells differentiate into beating cardiomyocytes. J Biol Chem 2004;279:11384–91. http://dx.doi.org/10.1074/jbc.M310822200.
[35] Nagai T, Matsuura K, Komuro I. Cardiac side population cells and Sca-1-positive cells. Methods Mol Biol Clifton NJ 2013;1036:63–74. http://dx.doi.org/10.1007/978-1-62703-511-8_5.
[36] Beltrami AP, Barlucchi L, Torella D, Baker M, Limana F, Chimenti S, et al. Adult cardiac stem cells are multipotent and support myocardial regeneration. Cell 2003;114:763–76.
[37] Linke A, Müller P, Nurzynska D, Casarsa C, Torella D, Nascimbene A, et al. Stem cells in the dog heart are self-renewing, clonogenic, and multipotent and regenerate infarcted myocardium, improving cardiac function. Proc Natl Acad Sci U S A 2005;102:8966–71. http://dx.doi.org/10.1073/pnas.0502678102.

[38] Demir R, Yaba A, Huppertz B. Vasculogenesis and angiogenesis in the endometrium during menstrual cycle and implantation. Acta Histochem 2010;112:203–14. http://dx.doi.org/10.1016/j.acthis.2009.04.004.
[39] Huang XP, Sun Z, Miyagi Y, McDonald KH, Zhang L, Weisel RD, et al. Differentiation of allogeneic mesenchymal stem cells induces immunogenicity and limits their long-term benefits for myocardial repair. Circulation 2010;122:2419–29. http://dx.doi.org/10.1161/CIRCULATIONAHA.110.955971.
[40] Li P, Li S-H, Wu J, Zang W-F, Dhingra S, Sun L, et al. Interleukin-6 downregulation with mesenchymal stem cell differentiation results in loss of immunoprivilege. J Cell Mol Med 2013;17:1136–45. http://dx.doi.org/10.1111/jcmm.12092.
[41] Sun Z, Zhang Y, Brunt KR, Wu J, Li SH, Fazel S, et al. An adult uterine hemangioblast: evidence for extramedullary self-renewal and clonal bilineage potential. Blood 2010;116:2932–41. http://dx.doi.org/10.1182/blood-2010-01-266882.
[42] Kalin MF, Zumoff B. Sex hormones and coronary disease: a review of the clinical studies. Steroids 1990;55:330–52.