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Age-Related Impaired Efficacy of Bone Marrow Cell Therapy for Myocardial Infarction Reflects a Decrease in B Lymphocytes

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Abstract

Treatment of myocardial infarction (MI) with bone marrow cells (BMCs) improves post-MI cardiac function in rodents. However, clinical trials of BMC therapy have been less effective. While most rodent experiments use young healthy donors, patients undergoing autologous cell therapy are older and post-MI. We previously demonstrated that BMCs from aged and post-MI donor mice are therapeutically impaired, and that donor MI induces inflammatory changes in BMC composition including reduced levels of B lymphocytes. Here, we hypothesized that B cell alterations in bone marrow account for the reduced therapeutic potential of post-MI and aged donor BMCs. Injection of BMCs from increasingly aged donor mice resulted in progressively poorer cardiac function and larger infarct size. Flow cytometry revealed fewer B cells in aged donor bone marrow. Therapeutic efficacy of young, healthy donor BMCs was reduced by depletion of B cells. Implantation of intact or lysed B cells improved cardiac function, whereas intact or lysed T cells provided only minor benefit. We conclude that B cells play an important paracrine role in effective BMC therapy for MI. Reduction of bone marrow B cells due to age or MI may partially explain why clinical autologous cell therapy hasn't matched the success of rodent experiments.

KEYWORDS: Advancing age; B lymphocyte; bone marrow; ejection fraction; infarct size; myocardial infarction

Brief title: B cells for the treatment of myocardial infarction

Introduction

Cardiovascular disease including myocardial infarction (MI) has become the leading cause of mortality and morbidity in the world.[1, 2] Despite advances in revascularization for MI patients, post-MI remodeling still leads to a substantial heart failure burden, and cell therapy is viewed as a potential approach to regenerate, or at least to preserve, viable myocardium. Experimental cell therapy approaches have been based on a variety of cell types,[3] including bone marrow mononuclear cells, mesenchymal stem cells, resident cardiac stem or progenitor cells, cardiosphere-derived cells, stem cells from other tissues such as adipose tissue, embryonic stem cells, and myoblasts,[4-16] with and without genetic modification to increase functionality.[17-19] We and others reported that treatment with secreted products or cell lysates from various populations of bone marrow cells (BMCs) substantially improves post-MI cardiac function in animal models.[20-23] While considerable controversy has surrounded claims of bone marrow-derived regenerated myocardium,[24] therapeutic benefits in rodent models have been frequently observed regardless of whether endogenous myocardial regeneration is involved.[25]

However, attempts to translate this approach to the clinic have led to modest functional improvement in some trials and poor efficacy of cell therapy in others.[5, 8, 26-34] Notably, while laboratory experiments typically use young healthy mice as BMC donors, MI patients undergoing autologous cell therapy (i.e., they receive their own BMCs) are older and are post-MI. Recently, in two high-profile clinical trials from the NIH Cardiovascular Cell Therapy Research Network (CCTRN), acute MI patients were treated with their own BMCs 3 and 7 days post-MI

(TIME trial)[8] and 2-3 weeks post-MI (LateTIME trial).[32] These two clinical trials were based, in part, on observations from the REPAIR-AMI trial[29] that patients receiving their own BMCs 5-7 days post-MI fared better than those at 3-5 days post-MI, which was attributed chiefly to the state of the recipient heart rather than the BMCs. However, neither TIME nor LateTIME were successful.

We previously reported that BMCs from mice that are themselves post-MI or old, are less therapeutic than BMCs from healthy mice or young controls.[35, 36] We found that MI causes acute inflammation mediated by interleukin-1 leading to changes in BMC composition, including a decrease in B lymphocytes, and contributes to the poor efficacy of post-MI donor BMCs. Interestingly, BMCs became progressively less therapeutic over the first week post-MI, as the inflammatory response developed; and then became more therapeutic by 21 days post-MI as acute inflammation was subsiding. These results support the hypothesis that the 3-7 day post-MI BMCs in the TIME trial were still in the inflammatory state and problematic for therapy; while the 2-3 week post-MI BMCs in LateTIME may have actually regained some of their therapeutic capacity, but not enough to affect the outcome.

Intriguingly, B lymphocytes have been reported to be a therapeutic BMC subpopulation,[37] and B cell depletion and restoration have been shown to increase and reduce infarct size in a cerebral artery occlusion model.[38-40] In addition, the progressive influence of advancing age on the therapeutic efficacy of BMCs is relatively unexplored. Given the similar effects of donor age and MI on BMC therapeutic efficacy, we asked whether age also alters bone marrow B cell levels,

potentially accounting for the impaired therapeutic potential of BMCs from aged donors. We show here that increasing donor age drastically and progressively impairs donor BMC therapeutic efficacy, irrespective of the state of the recipient heart, and that advancing donor age impairs therapeutic potential of BMCs by B cell reduction. We also show that partial depletion of B cells from young healthy donor BMCs reduces the BMCs' therapeutic effect, mimicking the effects of donor age or MI. Furthermore, we demonstrate that injection of isolated bone marrow B cells or their lysate can reproduce the therapeutic effect of unfractionated BMC therapy for MI. Bone marrow B cell reduction by age or MI may explain why autologous BMC therapy in patients has been less successful in clinical trials than in rodent experiments.

Results

Progressive impairment of BMCs derived from progressively older mice. To confirm if donor advanced age impairs the therapeutic potential of BMCs for the treatment of MI, we implanted donor BMCs into groups of infarcted recipient mice, keeping the recipient conditions constant, but varying the donor conditions with increasing ages (10 weeks, 6 months, 1, 1.5, and 2 years; and Hank's balanced salt solution (HBSS)/BSA vehicle control). Injection of BMCs from donors of increasing ages resulted in progressively lower day 28 recipient left ventricular ejection fraction (LVEF) and progressively larger day 28 recipient end systolic volume and end diastolic volume (ESV and EDV; Figure 1A). Moreover, injection of BMCs from donors of increasing ages also led to progressively lower wall thickness (Figure 1B) and larger infarct size on day 28 (Figure 1C), despite the recipients being of constant age. Linear regression analysis

revealed a negative correlation between donor age and recipient EF change from day 2 to day 28 post-MI, as well as a positive correlation between donor age and recipient infarct size on day 28 (Figure 1D). Notably, BMCs from 2-year-old donor mice were completely devoid of therapeutic potential, comparable to the negative control vehicle injections, despite consistently good viability (96% assessed by Trypan blue staining) in all BMCs assessed before implantation.

Reduction of bone marrow CD19$^+$ B cells from old donors. To better understand the differences between BMC populations in young and old mice, BMCs harvested from donor mice at ages of 10 weeks, 1.5 years and 2 years were quantitatively analyzed by flow cytometry. Increasing age was associated with a significant decrease in the number and percentage of CD19$^+$ cells (B lymphocytes) and with significant increases in both the number and percentage of CD3$^+$ and CD11c$^+$ cells (T lymphocytes and potentially dendritic cells, respectively) (Figure 2). There was a trend toward increase with age of CD11b$^+$ cells that did not reach significance (P values are from 0.202 to 0.520). However, no significant differences were evident in other cell populations such as CD62L$^+$, Gr-1$^+$, and Ly6c$^+$ cells.

BMC therapeutic impairment by CD19$^+$ B cell depletion. Because we previously demonstrated that both donor age and donor MI decrease therapeutic efficacy of BMCs when implanted into post-MI hearts,[35, 36] we searched for any common differences in BMC composition resulting from age or MI in young donors. The only difference that we detected between young and old donors that we had also observed between healthy and post-MI mice was

the reduction in $CD19^+$ B cells (data not shown). Therefore, we depleted B cells from young healthy BMCs (10 weeks old) on the basis of $CD19^+$ expression to see if BMC therapeutic efficacy is impaired by B cell depletion. $CD19^+$ B cells were undetectable by FACS in the post-depletion BMCs (data not shown). Interestingly, young healthy donor BMCs became less therapeutic when manually depleted of B cells (Figure 3), as their implantation into young post-MI hearts led to significantly lower day 28 recipient EF ($P < 0.001$) and higher day 28 recipient ESV ($P < 0.05$) as compared to whole BMCs from donors at same age and condition.

Implantation of intact B cells reproduces the therapeutic effects of whole BMCs. Because (1) reduction in bone marrow B cells was associated with two distinct donor conditions (age and MI) that reduced therapeutic efficacy of BMCs, (2) direct depletion of B cells left the remaining BMCs in a less therapeutic state, and (3) B cells were reported to be a therapeutic subpopulation of BMCs when implanted immediately after MI in a rat model,[37] we tested the hypothesis that B cells account for the therapeutic efficacy of BMCs implanted 3 days post-MI. We isolated bone marrow B cells from young healthy donors by a subtractive selection strategy (see Methods) to avoid potential confounding effects of antibody binding to the cells, and implanted them into the recipient infarct border zone 3 days post-MI as in our previous experiments. Similarly prepared bone marrow T cells and vehicle (HBSS/BSA) were injected as negative controls. Because we have observed B cells to account for roughly 20% of the BMCs (Figure 2 and unpublished observations), B cells were suspended at 2×10^7 cells/ml, and 2×10^5 cells were injected split into two 5 μl injections as described above, with the goal of implanting a comparable number of B

cells to those in the original whole BMC implantations. T cells as a negative control were implanted at the same concentration and cell number as the B cells. As before, recipient mice were always young (10-12 weeks). Injection of B cells significantly improved recipient day 28 EF, and reduced day 28 ESV and EDV; in contrast, injection of T cells improved LV dimensions but provided only a modest benefit for EF relative to injection of HBSS (Figure 4A). The effect on EF, ESV, and EDV by isolated B cells was comparable to that of the whole BMCs in our earlier experiments (see Figure 1).

Because the B cells were isolated by depletion of non-B cells from the harvested BMCs, but the depletion antibodies used for this approach did not include those specific for markers of mesenchymal stem cells (MSCs), the possibility remained that a small number of MSCs contaminated the preparation, theoretically accounting for the therapeutic activity of the isolated cells. To confirm that this was not the case, we isolated the B cells from one additional donor using the same depletion approach and used flow cytometry to analyze the resulting population for MSCs defined as $CD105^{+}$ $CD29^{+}$ $Sca\text{-}1^{+}$ $CD45^{-}$ cells, based on the criteria used by R&D Systems in their mouse MSC identification panel FMC003 (see Methods). By these criteria, contaminating MSCs were undetectable in our B cell preparation (Supplementary Figure S1).

Therapeutic benefit of bone marrow B cells does not require the cells to be alive. We showed previously that the therapeutic effects of BMC implantation into post-MI hearts are mimicked by injection of cell-free extract from lysed BMCs,[22] and are thus paracrine in nature.

To determine if the therapeutic benefit of purified B cell implantation similarly was mediated by a paracrine mechanism, we repeated the implantation of B vs. T cells but used cell lysate rather than intact cells. Notably, B cell lysate, but not T cell lysate, also significantly increased recipient day 28 EF. B cell lysate decreased day 28 ESV and EDV comparably to the intact B cells and intact BMCs; whereas T cell lysate bestowed partial therapeutic benefit for day 28 ESV and led to improvement in EDV (Figure 4B). Infarct size was smaller in the B cells and B cell lysate treatment groups than in the HBSS control group (Figure 4A and B).

Discussion

Our results indicate that increasing donor age makes donor's BMCs progressively less able to prevent a decline in cardiac function when the aged BMCs are implanted into recipient hearts after MI. Advancing age lowers the number of bone marrow B cells, resulting in a less therapeutic BMC population, leading to lower recipient left ventricular EF and larger ESV and EDV on day 28 post-MI; along with larger infarct size and thinner anterior wall, independent of the recipient status. Implantation of intact and lysed B cells improved recipient post-MI cardiac function in EF, ESV, and EDV, as well as reduced infarct size; whereas implantation of BMCs with fewer B cells provided less therapeutic effect. Bone marrow B cells play a crucial role in BMC-based cell therapy via a paracrine mechanism involving intracellular mediators that can bestow benefit after a single intramyocardial administration.

It has long been understood that cells progressively lose function with advancing age, playing a large role in the loss of cardiac functionality.[41] Increasing age can also be correlated with reductions in various molecular or functional properties of cells in the bone marrow[42-49] and of circulating angiogenic cells from the bone marrow and peripheral blood.[50-52] Age-dependent reduction in plasticity of bone marrow-derived cells is a potential explanation for therapeutic functional impairment of those cells,[47] although our previous work[22] and the results reported here indicate that cellular plasticity is not required for the therapeutic effects that we have observed (i.e., the cells are still therapeutic when dead and lysed). We and others have shown that the age of the BMC donor can also reduce therapeutic properties of the BMCs[35, 53, 54] and intrinsic properties that may be mechanistically relevant.[47, 50] Thus, BMCs in general from old animals may suffer from a lifetime of progressive impairment, causing gradual functional declines. The results of our current study demonstrate this progressive impairment of donor BMCs over two years of aging, implying that age-related decrease in cellular therapeutic potential may limit efficacy of autologous BMC therapy. Additionally, we previously reported that inflammation resulting from donor MI impairs the therapeutic efficacy of the donor BMCs, leading to cellular alterations within the bone marrow compartment.[36] Together, these findings imply that one reason that clinical trials have been less effective than mouse experiments may be that the bone marrow used in human acute MI trials is impaired by both the age and post-infarct condition of the patients. Thus, regardless of the mechanisms that lead to functional improvement, the age and pathophysiological background from which the donor BMCs are derived may interfere with autologous cell therapy in patients.[55]

Notably, we showed previously that post-MI BMCs contain fewer $CD19^+$ B cells but more inflammatory cells such as $CD11b^+$ and $Gr1^+$ myeloid cells,[36] indicative of an inflammatory state. We now show that aged-donor BMCs also contain fewer $CD19^+$ B cells but more $CD3^+$ and $CD11c^+$ cells, and that removal of $CD19^+$ B cells from young healthy donor BMCs renders the BMCs less therapeutic. Interestingly, B cells have been reported as a therapeutic BMC subpopulation for MI treatment,[37] and the depletion and restoration of B cells have been shown to increase and reduce infarct size in a cerebral artery occlusion model.[38, 39] We show here that partial depletion of B cells from young healthy donor BMCs reduces the BMCs' therapeutic effect, mimicking the effects of donor age or MI. Therefore, despite presumed differences in the etiology of the reduction in B cells by age or MI, the therapeutic impairment that results from both conditions may result from the alteration of the bone marrow B cell compartment that they have in common.

Our results provide an insight that aged BMCs have impaired cellular efficacy and offer less B cells, which might be central to the therapeutic impairment. Thus, further investigation has also been addressed on bone marrow B cells' role in the therapeutic effect. As expected, injections of intact B cells or B cell lysate isolated from young healthy donor bone marrow significantly improved the post-MI cardiac function and reduced infarct size in the recipient MI hearts in mice. However, injections of bone marrow T cells or T cell lysate from young healthy donor bone marrow led to only a slight preservation of LVEF and to intermediate preservation of chamber

volumes. These data raise the possibility that B cells may also play an important role in human BMC therapeutic impairment as they did in mice, and that their lower numbers in BMCs from older animals may partially explain the poor outcomes of the clinical trials. Supporting this notion, CCTRN researchers observed that the level of $CD19^+$ B cells in bone marrow of the individual TIME and LateTIME participants correlated positively with clinical outcome.[56]

Importantly, our studies provide insights that cannot result from the study of only autologous human trials, because by delivering human or mouse BMCs to mice, we were able to vary the condition of the donor BMCs while keeping the recipient heart condition constant. For example, another CCTRN trial, FOCUS,[33] observed in post hoc subgroup analysis that patients under age 62 exhibited better LVEF than those over 62, but it is difficult to know whether this is related to the recipient heart or the donor BMCs in an autologous setting. This has been a powerful advantage of our approach using recipient mice that are constant while implanted BMCs are from variable conditions.

Many of the therapeutic effects of post-MI implantation of a range of cell types have ultimately proven to be paracrine in nature even if the particular cell type can differentiate into cardiomyocytes under appropriate culture conditions, and have been attributed to exosomes secreted by these cells.[21, 23, 57-59] The mechanistic basis for this observation remains unclear. We observed partial benefit from injection of T lymphocytes that did not reach the therapeutic effect of the B lymphocytes, so it is possible that the repertoire of exosomal microRNAs or other

factors within the cytoplasm from T cells includes a subset of the crucial factors in B cells. Still, it is notable that the reduction with age of BMC therapeutic efficacy correlated with a reduction in B cells and an increase in T cells, arguing against the T cells carrying the main therapeutic activity that is lost with age (Supplementary Figure S2). The observation that B cells play a major role in the therapeutic effect is intriguing, given that the post-MI heart experiences a robust multi-stage inflammatory response involving many bone marrow-derived cells that do not bestow this benefit naturally. Nonetheless, our results indicate that a key component of bone marrow-derived B cells, possibly the exosomes, is not present in this intrinsic response, at least not at sufficient levels to cause the therapeutic effect of direct B cell delivery to the tissue. The potential therapeutic use of exosomes from bone marrow B cells for post-MI therapy is the subject of ongoing studies.

We conclude that B lymphocytes account for at least a substantial portion of the therapeutic activity of unfractionated BMCs when administered post-MI. The therapeutic effect is mediated by intracellular or transmembrane factors that improve cardiac function and preserve viable myocardium. Our observations that therapeutic effect results from a single injection of whole BMC lysate[22] or B cell lysate indicate that the therapeutic benefits of these factors do not require active secretion from persisting living cells. The reduction of the number of B cells in the bone marrow is a consequence of both age and/or MI that limits therapeutic efficacy of autologous BMC therapy.

Materials and Methods

Donor and recipient mice. All animal experiments were approved by the Institutional Animal Care and Use Committee of the University of California, San Francisco. Male C57BL/6J mice were obtained from the Jackson Laboratory (Bar Harbor, ME). Donor mice were at various ages from young (10-12 weeks) to old (2 years). The donor mice at ages of 6 months, 1, 1.5 and 2 years were purchased from the National Institute of Aging. Recipient mice were always at 10-12 weeks old. Recipient group size was 15 or greater; BMCs from one donor mouse were used per five recipient mice.

Myocardial infarction. MI was surgically induced as described previously.[60] Mice underwent the MI surgery under anesthetization with 2% isoflurane and received analgesics (buprenorphine 0.1 mg/kg, subcutaneous injection) at time of surgery. The heart was exposed via a parasternotomy and the left anterior descending coronary artery was permanently ligated ~3 mm below the tip of the left atrium. Recipient mice undergoing the parasternotomy MI were all consistent from experiment to experiment. The mortalities during and after MI surgery were 4% and 2%, respectively.

BMC harvest and injection. The protocol for BMC harvest and injection has been previously described.[22, 60] Briefly, mouse femurs were harvested, and bone marrow was flushed with cold HBSS with 0.5% BSA. The cell suspension was strained through a 70 μm filter and washed twice with HBSS/BSA. After centrifugation, the unfractionated whole BMCs were harvested for

injection. The cell concentration was adjusted to 10^8 viable cells/ml. 10^6 cells were injected into myocardium at the infarct border zone split into two 5 μl injections under ultrasound visualization using a Vevo660 micro-ultrasound system (VisualSonics Inc., Toronto).[60] Each donor mouse provided BMCs for 5 recipient mice in order to have multiple donors per group. BMCs were always implanted into recipient hearts on day 3 post-MI. Injection of HBSS with 0.5% BSA served as a negative control. Optimal intramyocardial injections were judged as 98% of visible changes in localized ultrasound signal resulting from the presence of cell suspension.[22, 36]

Flow cytometry. The fluorescent antibodies for cell-surface markers used for flow cytometry analysis were listed as follows: anti-panNKFITC, anti-Gr-1-PE, anti-MHCII-PE, anti-CD3-PerCPCy5.5, anti-CD25-PerCPCy5.5, anti-CD45R-PerCPCy5.5, anti-CD11b-PerCPCy5.5, anti-CD11c-PECy7, anti-CD69-PECy7, anti-CD11b-Pacific Blue, anti-CD44-Pacific Blue, anti-CD80-APC, anti-CD19-Alexa647, anti-CD4-APC750, and anti-Gr-1-APC750 (eBiosciences); anti-Ly6C-FITC and anti-CD19-APCCy7 (BD Biosciences); anti-CD86-Pacific Blue (Biolegend); anti-CD62LAlexa647 (UCSF Hybridoma Core); and anti-CD8-Pacific Orange (Invitrogen). Anti-CD11b-Pacific Blue, anti-CD11b-PerCPCy5.5, anti-Gr-1-PE, anti-Gr-1-APC750, anti-Ly6CFITC, and anti-CD11c-PE were used for identifying monocytes, macrophages, and neutrophils (Supplementary Table S1). Bone marrow was harvested from two femurs and BMCs were isolated as indicated above. Following red blood cell lysis, cells were resuspended in phosphate-buffered saline with 5% BSA, Fc-receptors blocked, stained and processed on a LSR II flow cytometer and analyzed using FlowJo v8.8.6. Absolute

numbers of cells were calculated based on the percentages of total live cell numbers (cell viability accessed by Trypan blue staining).

Bone marrow $CD19^+$ B cell depletion. Mouse $CD19^+$ MicroBeads (Miltenyi Biotec Inc., Auburn, CA) were used for the positive depletion of mouse B cells from bone marrow. Briefly, whole BMCs were harvested as described above from donor mice at age 10 weeks, the total cell number was determined, and the BMC suspension was incubated with $CD19^+$ MicroBeads for 15 min at 4°C. The mixture was loaded onto a MACS LD column, which was placed in a magnetic separator (QuadroMACS) to remove the magnetically labeled $CD19^+$ cells. 10^6 cells from the B cell-depleted fraction were injected into myocardium at the infarct border zone as described above.

Isolation and injection into myocardium of B cells and T cells. Whole BMCs were harvested from the 10-week-old donor mouse and were treated with red blood cell lysis buffer (eBioscience), and the total cell number was determined. B cells were isolated indirectly by MACS using a mouse Pan B cell isolation kit II (Miltenyi Biotec Inc.) to deplete non-B cells from the BMC suspension. The antibodies used to remove non-B cells were as follows: FITC anti-mouse $CD3\varepsilon^+$, FITC anti-mouse CD4, FITC anti-mouse CD8a, FITC anti-human/mouse CD11b, FITC anti-mouse Ly-6G, and FITC anti-mouse TER-119 (Tonbo Biosciences); and FITC anti-mouse CD49b (BioLegend). The remaining unlabeled fraction enriched in B cells was incubated with mixed antibodies listed above at a dilution of 1:400 for subsequent

fluorescence-activated cell sorting using an Avalon cell sorter, and the un-labelled cells that were highly enriched for B cells were collected for cell injection. T cells were prepared by a similar indirect isolation approach using a mouse Pan T cell isolation kit II (Miltenyi Biotec Inc.). Antibodies used for T cell isolation were as follows: FITC anti-mouse $CD19^+$, FITC anti-mouse CD4, FITC anti-mouse CD8a, FITC anti-human/mouse CD11b, FITC anti-mouse Ly-6G, and FITC anti-mouse TER-119 (Tonbo Biosciences); and FITC anti-mouse CD49b (BioLegend). For implantation of B or T cells into the myocardium at the infarct border zone, cells were suspended at 2×10^7 cells/ml, and 2×10^5 cells were injected split into two 5 µl injections as described above. Cell viability by Trypan blue staining was assessed in all cells before implantation. B cells isolated from two donor mice were pooled and used per five recipient mice; while T cells isolated from one donor mouse were used per five recipient mice.

Assessment of potential MSC contamination of B cell preparations. B cells were isolated from one more donor using the depletion approach described above. The resulting B cell preparation (1.2×10^6 cells) was divided and one portion was stained with fluorescent antibodies in a panel based on R&D Systems mouse identification kit (Catalogue #FMC003) but assembled independently. The panel antibodies used for flow cytometry analysis were the following: Alexa Fluor 647 anti-$CD105^+$, PE anti-$CD29^+$, BV421 anti-$Sca\text{-}1^+$, and FITC anti-$CD45^-$ (all from BD Biosciences). The remaining portion of the preparation was stained separately with anti-$CD19^+$ in Alexa Fluor 647, PE, BV421 and FITC (BD Biosciences) as positive controls for flow cytometry analysis.

Preparation of cell-free extracts from B or T cell lysates. Isolated B or T cells were diluted with HBSS with 0.5% BSA at a concentration of 2×10^7 cells/ml. This diluted cell suspension was subjected to four freeze–thaw cycles using an ethanol/dry ice bath followed by micro-centrifugation at 14,000 rpm (one minute) to remove insoluble material. Soluble cell-free lysate was collected and injected into myocardium at the infarct border zone as two 5 μl injections as described above.

Echocardiography. Echocardiography of recipient mice under anesthesia with 1.25% isoflurane was performed at baseline, and 2 and 28 days post-MI, using a Vevo660 micro-ultrasound system as described previously.[22, 36] Echocardiograms were obtained at long-axis view to measure the left ventricular end-systolic volume (ESV) and end-diastolic volume (EDV). Left ventricular ejection fraction (EF) was calculated by the following formula: EF (%) = [(EDV - ESV)/EDV] × 100. Wall thickness was measured at the apical-segment (infarct) and mid-segment (infarct border zone) of the anterior wall. Echocardiographic parameters were measured by two blinded investigators.

Measurement of infarct size. Mouse hearts were arrested in diastole with saturated KCl injected into the left ventricular chamber and removed. Frozen heart sections were stained with Masson trichrome for infarct size measurement by a midline arc length method.[61] The sections were read blind and scored for the extent of fibrosis.

Statistical analysis. Power calculation based on standard deviations from within-group comparisons in several of our previous MI experiments determined that n=10/group was sufficient to detect changes in cardiac function at a power of 0.8 and significance level of 0.05. For comparisons involving multiple groups and times, we fit a 2-factor (treatment condition and time) repeated-measures analysis of variance to all the data at once using a mixed model estimated with restricted maximum likelihood estimation with an unstructured covariance matrix of residuals, then tested for differences over time and across treatment condition using contrasts and pairwise comparisons, adjusted for multiple comparisons using the Sidak method. Calculations were done with Stata 13.1. All values are presented as mean ± SD.

Author Contributions

S.A. conducted the aging experiments; X.W. helped conceive of and performed the B and T cell experiments and data analysis with participation of M.A.R., H.J.R., D.S.K., M.V., E.L., and R.D.; S.C.K., M.L.H., and M.L.S. conceived of the project; M.L.H. and S.V.S. provided collaborative experimental support and ideas for the project. X.W. and M.L.S. wrote the manuscript. The authors declare that they have no competing interests.

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References

1. Mathers, CD, and Loncar, D (2006). Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med *3*: e442.
2. Sacco, RL, Roth, GA, Reddy, KS, Arnett, DK, Bonita, R, Gaziano, TA *et al.* (2016). The Heart of 25 by 25: Achieving the Goal of Reducing Global and Regional Premature Deaths From Cardiovascular Diseases and Stroke: A Modeling Study From the American Heart Association and World Heart Federation. Circulation *133*: e674-690.
3. Garry, GA, and Garry, DJ (2015). Somatic Cell Therapy for Chronic Heart Failure: In Search of Mechanistic Insights. J Card Fail *21*: 583-585.
4. Teerlink, JR, Metra, M, Filippatos, GS, Davison, BA, Bartunek, J, Terzic, A *et al.* (2017). Benefit of cardiopoietic mesenchymal stem cell therapy on left ventricular remodelling: results from the Congestive Heart Failure Cardiopoietic Regenerative Therapy (CHART-1) study. Eur J Heart Fail *19*: 1520-1529.
5. Rodrigo, SF, Mann, I, van Ramshorst, J, Beeres, SL, Zwaginga, JJ, Fibbe, WE *et al.* (2017). Safety and efficacy of percutaneous intramyocardial bone marrow cell injection for chronic myocardial ischemia: Long-term results. J Interv Cardiol *30*: 440-447.
6. Gallet, R, de Couto, G, Simsolo, E, Valle, J, Sun, B, Liu, W *et al.* (2016). Cardiosphere-derived cells reverse heart failure with preserved ejection fraction (HFpEF) in rats by decreasing fibrosis and inflammation. JACC Basic Transl Sci *1*: 14-28.
7. Assmus, B, Leistner, DM, Schachinger, V, Erbs, S, Elsasser, A, Haberbosch, W *et al.* (2014). Long-term clinical outcome after intracoronary application of bone

marrow-derived mononuclear cells for acute myocardial infarction: migratory capacity of administered cells determines event-free survival. Eur Heart J *35*: 1275-1283.

8. Traverse, JH, Henry, TD, Pepine, CJ, Willerson, JT, Zhao, DX, Ellis, SG *et al.* (2012). Effect of the use and timing of bone marrow mononuclear cell delivery on left ventricular function after acute myocardial infarction: the TIME randomized trial. JAMA *308*: 2380-2389.

9. Lopez, Y, Lutjemeier, B, Seshareddy, K, Trevino, EM, Hageman, KS, Musch, TI *et al.* (2013). Wharton's jelly or bone marrow mesenchymal stromal cells improve cardiac function following myocardial infarction for more than 32 weeks in a rat model: a preliminary report. Curr Stem Cell Res Ther *8*: 46-59.

10. Rasmussen, JG, Frobert, O, Holst-Hansen, C, Kastrup, J, Baandrup, U, Zachar, V *et al.* (2014). Comparison of human adipose-derived stem cells and bone marrow-derived stem cells in a myocardial infarction model. Cell Transplant *23*: 195-206.

11. Iso, Y, Yamaya, S, Sato, T, Poole, CN, Isoyama, K, Mimura, M *et al.* (2012). Distinct mobilization of circulating CD271+ mesenchymal progenitors from hematopoietic progenitors during aging and after myocardial infarction. Stem Cells Transl Med *1*: 462-468.

12. Skalicka, H, Horak, J, Kobylka, P, Palecek, T, Linhart, A, and Aschermann, M (2012). Intracoronary injection of autologous bone marrow-derived mononuclear cells in patients with large anterior acute myocardial infarction and left ventricular dysfunction: a 24-month follow up study. Bratisl Lek Listy *113*: 220-227.

13. Ye, J, Boyle, A, Shih, H, Sievers, RE, Zhang, Y, Prasad, M *et al.* (2012). Sca-1+ cardiosphere-derived cells are enriched for Isl1-expressing cardiac precursors and improve cardiac function after myocardial injury. PLoS One *7*: e30329.

14. Laflamme, MA, Chen, KY, Naumova, AV, Muskheli, V, Fugate, JA, Dupras, SK *et al.* (2007). Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. Nat Biotechnol *25*: 1015-1024.

15. Murry, CE, Wiseman, RW, Schwartz, SM, and Hauschka, SD (1996). Skeletal myoblast transplantation for repair of myocardial necrosis. J Clin Invest *98*: 2512-2523.

16. Lee, RJ, Springer, ML, Blanco-Bose, WE, Shaw, R, Ursell, PC, and Blau, HM (2000). VEGF gene delivery to myocardium: deleterious effects of unregulated expression. Circulation *102*: 898-901.

17. Mangi, AA, Noiseux, N, Kong, D, He, H, Rezvani, M, Ingwall, JS *et al.* (2003). Mesenchymal stem cells modified with Akt prevent remodeling and restore performance of infarcted hearts. Nat Med *9*: 1195-1201.

18. Kulandavelu, S, Karantalis, V, Fritsch, J, Hatzistergos, KE, Loescher, VY, McCall, F *et al.* (2016). Pim1 Kinase Overexpression Enhances ckit(+) Cardiac Stem Cell Cardiac Repair Following Myocardial Infarction in Swine. J Am Coll Cardiol *68*: 2454-2464.

19. Chen, Q, Varga, M, Wang, X, Haddad, DJ, An, S, Medzikovic, L *et al.* (2016). Overexpression of Nitric Oxide Synthase Restores Circulating Angiogenic Cell Function in Patients With Coronary Artery Disease: Implications for Autologous Cell Therapy for Myocardial Infarction. J Am Heart Assoc *5*.

20. Gnecchi, M, He, H, Liang, OD, Melo, LG, Morello, F, Mu, H *et al.* (2005). Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells. Nat Med *11*: 367-368.

21. Sahoo, S, and Losordo, DW (2014). Exosomes and cardiac repair after myocardial infarction. Circ Res *114*: 333-344.

22. Yeghiazarians, Y, Zhang, Y, Prasad, M, Shih, H, Saini, SA, Takagawa, J *et al.* (2009). Injection of bone marrow cell extract into infarcted hearts results in functional improvement comparable to intact cell therapy. Mol Ther *17*: 1250-1256.

23. Lai, RC, Arslan, F, Lee, MM, Sze, NS, Choo, A, Chen, TS *et al.* (2010). Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury. Stem Cell Res *4*: 214-222.

24. Orlic, D, Kajstura, J, Chimenti, S, Jakoniuk, I, Anderson, SM, Li, B *et al.* (2001). Bone marrow cells regenerate infarcted myocardium. Nature *410*: 701-705.

25. Eschenhagen, T, Bolli, R, Braun, T, Field, LJ, Fleischmann, BK, Frisen, J *et al.* (2017). Cardiomyocyte Regeneration: A Consensus Statement. Circulation *136*: 680-686.

26. Strauer, BE, Brehm, M, Zeus, T, Kostering, M, Hernandez, A, Sorg, RV *et al.* (2002). Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. Circulation *106*: 1913-1918.

27. Perin, EC, Dohmann, HF, Borojevic, R, Silva, SA, Sousa, AL, Mesquita, CT *et al.* (2003). Transendocardial, autologous bone marrow cell transplantation for severe, chronic ischemic heart failure. Circulation *107*: 2294-2302.

28. Wollert, KC, Meyer, GP, Lotz, J, Ringes-Lichtenberg, S, Lippolt, P, Breidenbach, C *et al.* (2004). Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial. Lancet *364*: 141-148.

29. Schachinger, V, Erbs, S, Elsasser, A, Haberbosch, W, Hambrecht, R, Holschermann, H *et al.* (2006). Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction. N Engl J Med *355*: 1210-1221.

30. Dill, T, Schachinger, V, Rolf, A, Mollmann, S, Thiele, H, Tillmanns, H *et al.* (2009). Intracoronary administration of bone marrow-derived progenitor cells improves left ventricular function in patients at risk for adverse remodeling after acute ST-segment elevation myocardial infarction: results of the Reinfusion of Enriched Progenitor cells And Infarct Remodeling in Acute Myocardial Infarction study (REPAIR-AMI) cardiac magnetic resonance imaging substudy. Am Heart J *157*: 541-547.

31. Bolli, R, Chugh, AR, D'Amario, D, Loughran, JH, Stoddard, MF, Ikram, S *et al.* (2011). Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): initial results of a randomised phase 1 trial. Lancet *378*: 1847-1857.

32. Traverse, JH, Henry, TD, Ellis, SG, Pepine, CJ, Willerson, JT, Zhao, DX *et al.* (2011). Effect of intracoronary delivery of autologous bone marrow mononuclear cells 2 to 3 weeks following acute myocardial infarction on left ventricular function: the LateTIME randomized trial. JAMA *306*: 2110-2119.

33. Perin, EC, Willerson, JT, Pepine, CJ, Henry, TD, Ellis, SG, Zhao, DX *et al.* (2012). Effect of transendocardial delivery of autologous bone marrow mononuclear cells on functional

capacity, left ventricular function, and perfusion in chronic heart failure: the FOCUS-CCTRN trial. JAMA *307*: 1717-1726.

34. Assmus, B, Walter, DH, Seeger, FH, Leistner, DM, Steiner, J, Ziegler, I *et al.* (2013). Effect of shock wave-facilitated intracoronary cell therapy on LVEF in patients with chronic heart failure: the CELLWAVE randomized clinical trial. JAMA *309*: 1622-1631.

35. Wang, X, Takagawa, J, Haddad, DJ, Pinnamaneni, K, Zhang, Y, Sievers, RE *et al.* (2011). Advanced Donor Age Impairs Bone Marrow Cell Therapeutic Efficacy for Cardiac Disease. J Tissue Sci Eng *S3*.

36. Wang, X, Takagawa, J, Lam, VC, Haddad, DJ, Tobler, DL, Mok, PY *et al.* (2011). Donor myocardial infarction impairs the therapeutic potential of bone marrow cells by an interleukin-1-mediated inflammatory response. Sci Transl Med *3*: 100ra90.

37. Goodchild, TT, Robinson, KA, Pang, W, Tondato, F, Cui, J, Arrington, J *et al.* (2009). Bone marrow-derived B cells preserve ventricular function after acute myocardial infarction. JACC Cardiovasc Interv *2*: 1005-1016.

38. Ren, X, Akiyoshi, K, Dziennis, S, Vandenbark, AA, Herson, PS, Hurn, PD *et al.* (2011). Regulatory B cells limit CNS inflammation and neurologic deficits in murine experimental stroke. J Neurosci *31*: 8556-8563.

39. Chen, Y, Bodhankar, S, Murphy, SJ, Vandenbark, AA, Alkayed, NJ, and Offner, H (2012). Intrastriatal B-cell administration limits infarct size after stroke in B-cell deficient mice. Metab Brain Dis *27*: 487-493.

40. Bodhankar, S, Chen, Y, Vandenbark, AA, Murphy, SJ, and Offner, H (2013).

IL-10-producing B-cells limit CNS inflammation and infarct volume in experimental stroke. Metab Brain Dis *28*: 375-386.

41. Hariharan, N, and Sussman, MA (2015). Cardiac aging - Getting to the stem of the problem. J Mol Cell Cardiol *83*: 32-36.

42. Mozid, AM, Jones, D, Arnous, S, Saunders, N, Wragg, A, Martin, J *et al.* (2013). The effects of age, disease state, and granulocyte colony-stimulating factor on progenitor cell count and function in patients undergoing cell therapy for cardiac disease. Stem Cells Dev *22*: 216-223.

43. Weksler, ME, and Szabo, P (2000). The effect of age on the B-cell repertoire. J Clin Immunol *20*: 240-249.

44. Edelberg, JM, Tang, L, Hattori, K, Lyden, D, and Rafii, S (2002). Young adult bone marrow-derived endothelial precursor cells restore aging-impaired cardiac angiogenic function. Circ Res *90*: E89-93.

45. Collado, M, Blasco, MA, and Serrano, M (2007). Cellular senescence in cancer and aging. Cell *130*: 223-233.

46. Kissel, CK, Lehmann, R, Assmus, B, Aicher, A, Honold, J, Fischer-Rasokat, U *et al.* (2007). Selective functional exhaustion of hematopoietic progenitor cells in the bone marrow of patients with postinfarction heart failure. J Am Coll Cardiol *49*: 2341-2349.

47. Pallante, BA, Duignan, I, Okin, D, Chin, A, Bressan, MC, Mikawa, T *et al.* (2007). Bone marrow Oct3/4+ cells differentiate Into cardiac myocytes via age-dependent paracrine mechanisms. Circ Res *100*: e1-11.

48. Rossi, DJ, Bryder, D, Seita, J, Nussenzweig, A, Hoeijmakers, J, and Weissman, IL (2007). Deficiencies in DNA damage repair limit the function of haematopoietic stem cells with age. Nature *447*: 725-729.

49. Sharpless, NE, and DePinho, RA (2007). How stem cells age and why this makes us grow old. Nat Rev Mol Cell Biol *8*: 703-713.

50. Rauscher, FM, Goldschmidt-Clermont, PJ, Davis, BH, Wang, T, Gregg, D, Ramaswami, P *et al.* (2003). Aging, progenitor cell exhaustion, and atherosclerosis. Circulation *108*: 457-463.

51. Heeschen, C, Lehmann, R, Honold, J, Assmus, B, Aicher, A, Walter, DH *et al.* (2004). Profoundly reduced neovascularization capacity of bone marrow mononuclear cells derived from patients with chronic ischemic heart disease. Circulation *109*: 1615-1622.

52. Heiss, C, Keymel, S, Niesler, U, Ziemann, J, Kelm, M, and Kalka, C (2005). Impaired progenitor cell activity in age-related endothelial dysfunction. J Am Coll Cardiol *45*: 1441-1448.

53. Ayala-Lugo, A, Tavares, AM, Paz, AH, Alegretti, A, Miquelito, L, Bock, H *et al.* (2011). Age-dependent availability and functionality of bone marrow stem cells in an experimental model of acute and chronic myocardial infarction. Cell Transplant *20*: 407-419.

54. Zhang, H, Fazel, S, Tian, H, Mickle, DA, Weisel, RD, Fujii, T *et al.* (2005). Increasing donor age adversely impacts beneficial effects of bone marrow but not smooth muscle myocardial cell therapy. Am J Physiol Heart Circ Physiol *289*: H2089-2096.

55. Dimmeler, S, and Leri, A (2008). Aging and disease as modifiers of efficacy of cell therapy. Circ Res *102*: 1319-1330.

56. Schutt, RC, Trachtenberg, BH, Cooke, JP, Traverse, JH, Henry, TD, Pepine, CJ *et al.* (2015). Bone marrow characteristics associated with changes in infarct size after STEMI: a biorepository evaluation from the CCTRN TIME trial. Circ Res *116*: 99-107.

57. Gallet, R, Dawkins, J, Valle, J, Simsolo, E, de Couto, G, Middleton, R *et al.* (2017). Exosomes secreted by cardiosphere-derived cells reduce scarring, attenuate adverse remodelling, and improve function in acute and chronic porcine myocardial infarction. Eur Heart J *38*: 201-211.

58. Grigorian-Shamagian, L, Fereydooni, S, Liu, W, Echavez, A, and Marban, E (2017). Harnessing the heart's resistance to malignant tumors: cardiac-derived extracellular vesicles decrease fibrosarcoma growth and leukemia-related mortality in rodents. Oncotarget *8*: 99624-99636.

59. Sahoo, S, Mathiyalagan, P, and Hajjar, RJ (2017). Pericardial Fluid Exosomes: A New Material to Treat Cardiovascular Disease. Mol Ther *25*: 568-569.

60. Springer, ML, Sievers, RE, Viswanathan, MN, Yee, MS, Foster, E, Grossman, W *et al.* (2005). Closed-chest cell injections into mouse myocardium guided by high-resolution echocardiography. Am J Physiol Heart Circ Physiol *289*: H1307-1314.

61. Takagawa, J, Zhang, Y, Wong, ML, Sievers, RE, Kapasi, NK, Wang, Y *et al.* (2007). Myocardial infarct size measurement in the mouse chronic infarction model: comparison of area- and length-based approaches. J Appl Physiol (1985) *102*: 2104-2111.

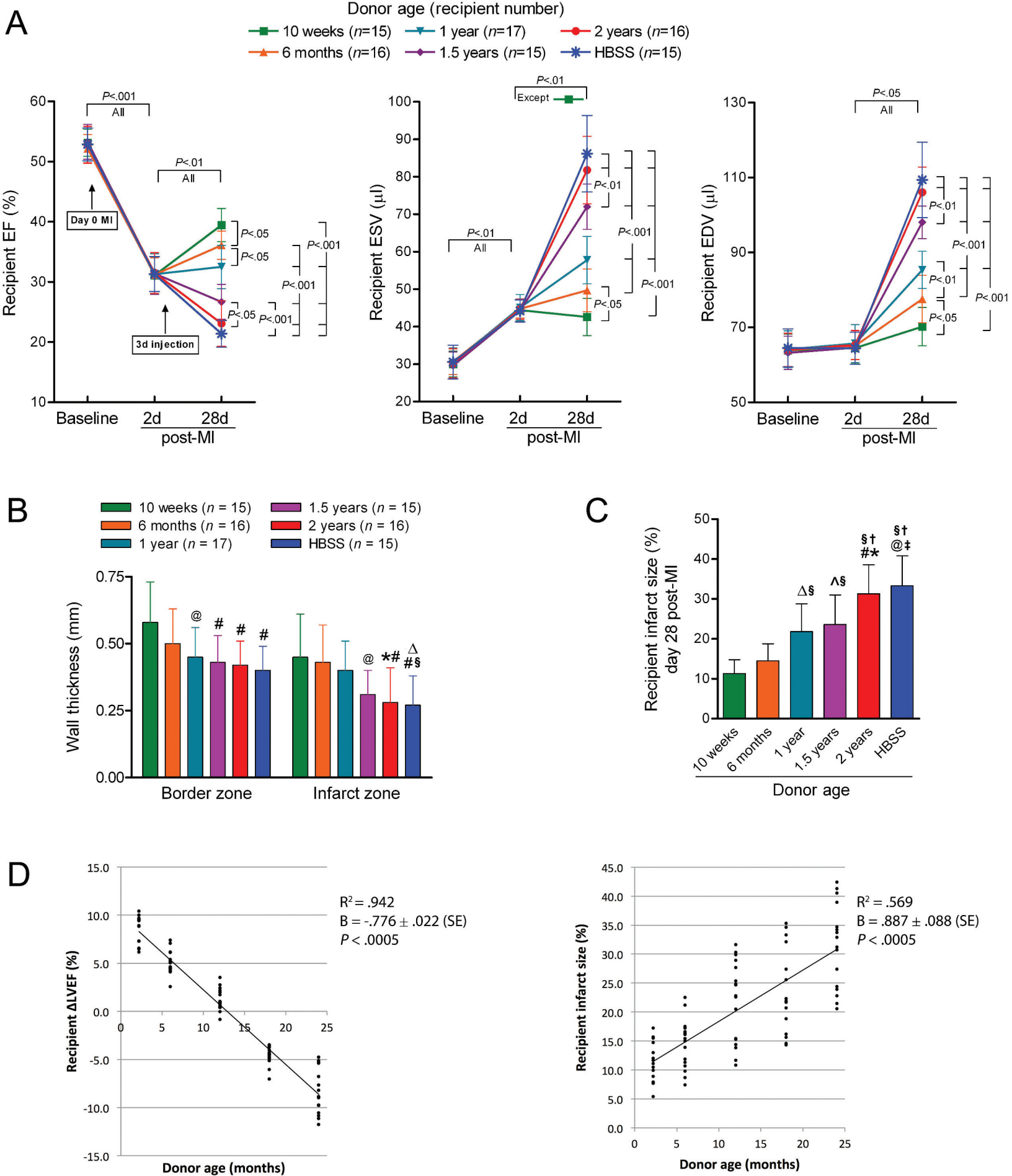
Figure Legends

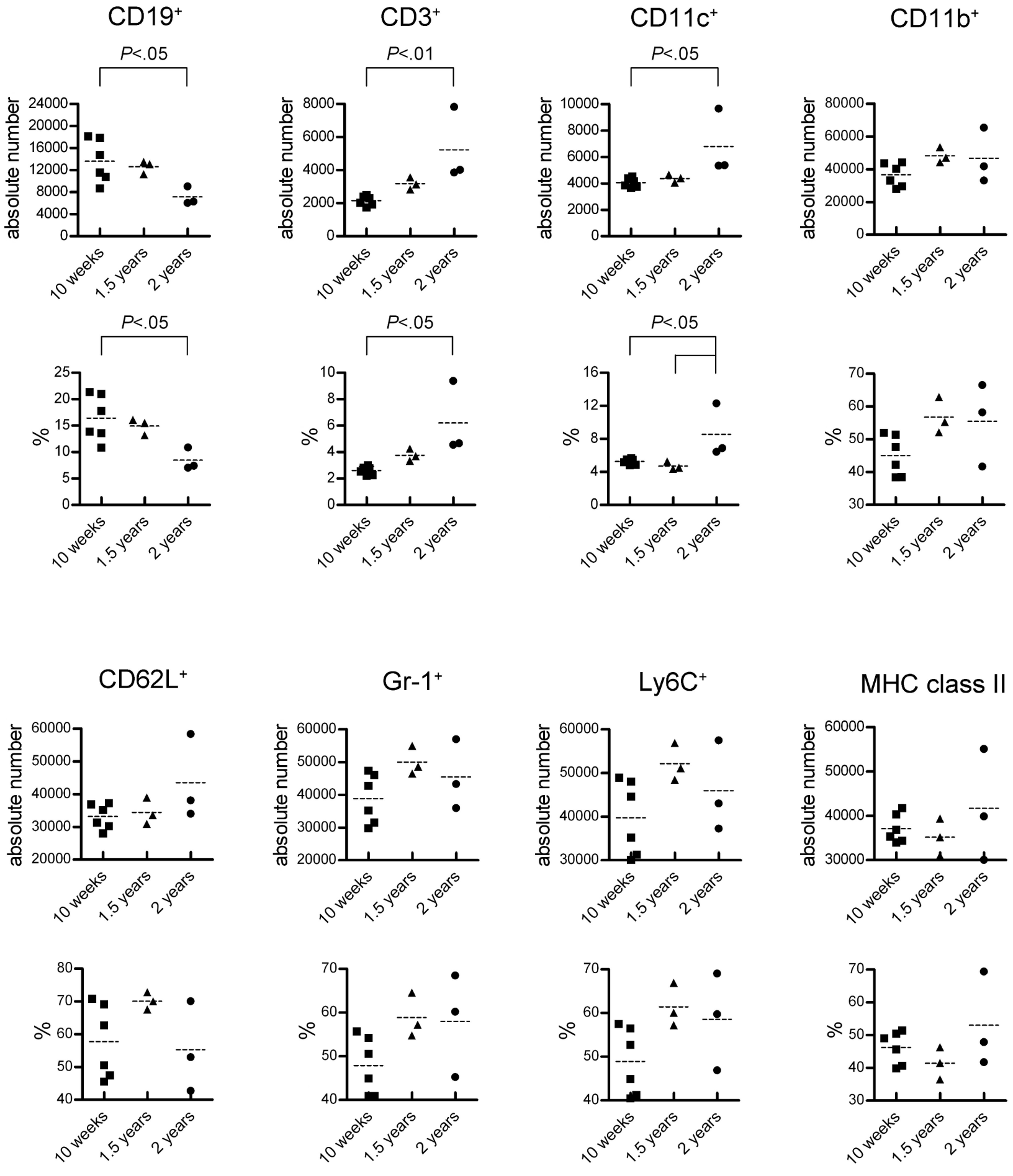
Figure 1. Progressive impairment of BMCs derived from progressively older mice. Donor BMCs were harvested from mice with increasing ages (from 10 weeks, 6 months, 1, 1.5, to 2 years). Increasing donor age led to progressively (A) lower recipient EF and larger ESV and EDV on day 28 post-MI, (B) reduced anterior wall thickness, and (C) increased infarct size on day 28. Linear regression analysis (D) for correlations between donor age and both recipient LVEF change from day 2 to day 28 and infarct size indicated that increase in donor age predicted decrease in recipient LVEF change from day 2 to day 28 post-MI ($P < 0.0005$), and increase in day 28 recipient infarct size ($P < 0.0005$). All data are means ± SD. (B) @ $P < 0.05$ vs 10 weeks, * $P < 0.05$ vs 6 months, Δ $P < 0.05$ vs 1 year, # $P < 0.01$ vs 10 weeks, and § $P < 0.01$ vs 6 months; (C) Δ $P < 0.05$ vs 6 months, * $P < 0.05$ vs 1.5 years, ^ $P < 0.01$ vs 6 months, # $P < 0.01$ vs 1 year, @ $P < 0.01$ vs 1.5 years, § $P < 0.001$ vs 10 weeks, † $P < 0.001$ vs 6 months, and ‡ $P < 0.001$ vs 1 year. Data are summarized numerically in Supplementary Table S2.

Figure 2. Old donor BMC composition analysis by flow cytometry. BMCs were harvested from donor mice at ages of 10 weeks, 1.5 years and 2 years for quantitative analysis by flow cytometry. The key differences observed were a significant decrease in $CD19^+$ B cells of total live cells and significant increases in $CD3^+$ T cells and $CD11c^+$ cells in the 2-year-old donor bone marrow. Cell amount is represented as absolute number of live cells per bone marrow as well as percentage of cells per total cellular population (%).

Figure 3. BMC therapeutic impairment by B cell depletion. Young healthy donor BMCs (10-week old) were harvested and depleted of B cells on the basis of $CD19^{+}$ expression. Implantation of the B cell-depleted donor BMCs led to less effective therapeutic outcomes in recipient day 28 EF and ESV as compared to whole BMCs from donors at same age and condition. Data are summarized numerically in Supplementary Table S3.

Figure 4. Therapeutic effects of B cells and B cell lysate on MI. Injection of purified bone marrow B cells (A) or B cell lysate (B) at day 3 post-MI significantly improved recipient day 28 EF, decreased day 28 ESV and EDV, and reduced infarct size. In contrast, bone marrow T cells (A) or T cell lysate (B) provided varying degrees of benefit to ESV and EDV relative to HBSS, but little or no benefit in EF. Data are summarized numerically in Supplementary Table S4.


A
Donor age (recipient number)
10 weeks (n=15)
1 year (n=17)
2 years (n=16)
6 months (n=16)
1.5 years (n=15)
HBSS (n=15)
Recipient EF (%)
P<.001
All
P<.01
All
Day 0 MI
3d injection
P<.05
P<.05
P<.001
P<.001
P<.05
Baseline
2d
28d
post-MI
Recipient ESV (µl)
P<.01
Except
P<.01
All
P<.01
P<.001
P<.001
P<.05
Recipient EDV (µl)
P<.05
All
P<.01
P<.001
P<.01
P<.05
P<.001
B
10 weeks (n = 15)
6 months (n = 16)
1 year (n = 17)
1.5 years (n = 15)
2 years (n = 16)
HBSS (n = 15)
Wall thickness (mm)
Border zone
Infarct zone
C
Recipient infarct size (%) day 28 post-MI
Donor age
10 weeks
6 months
1 year
1.5 years
2 years
HBSS
D
Recipient ΔLVEF (%)
R² = .942
B = -.776 ± .022 (SE)
P < .0005
Donor age (months)
Recipient infarct size (%)
R² = .569
B = .887 ± .088 (SE)
P < .0005
Donor age (months)


CD19+
P<.05
absolute number
24000
20000
16000
12000
8000
4000
0
10 weeks
1.5 years
2 years
P<.05
%
25
20
15
10
5
0
CD3+
P<.01
8000
6000
4000
2000
P<.05
10
8
6
4
2
CD11c+
P<.05
10000
16
12
CD11b+
80000
60000
40000
70
60
50
CD62L+
60000
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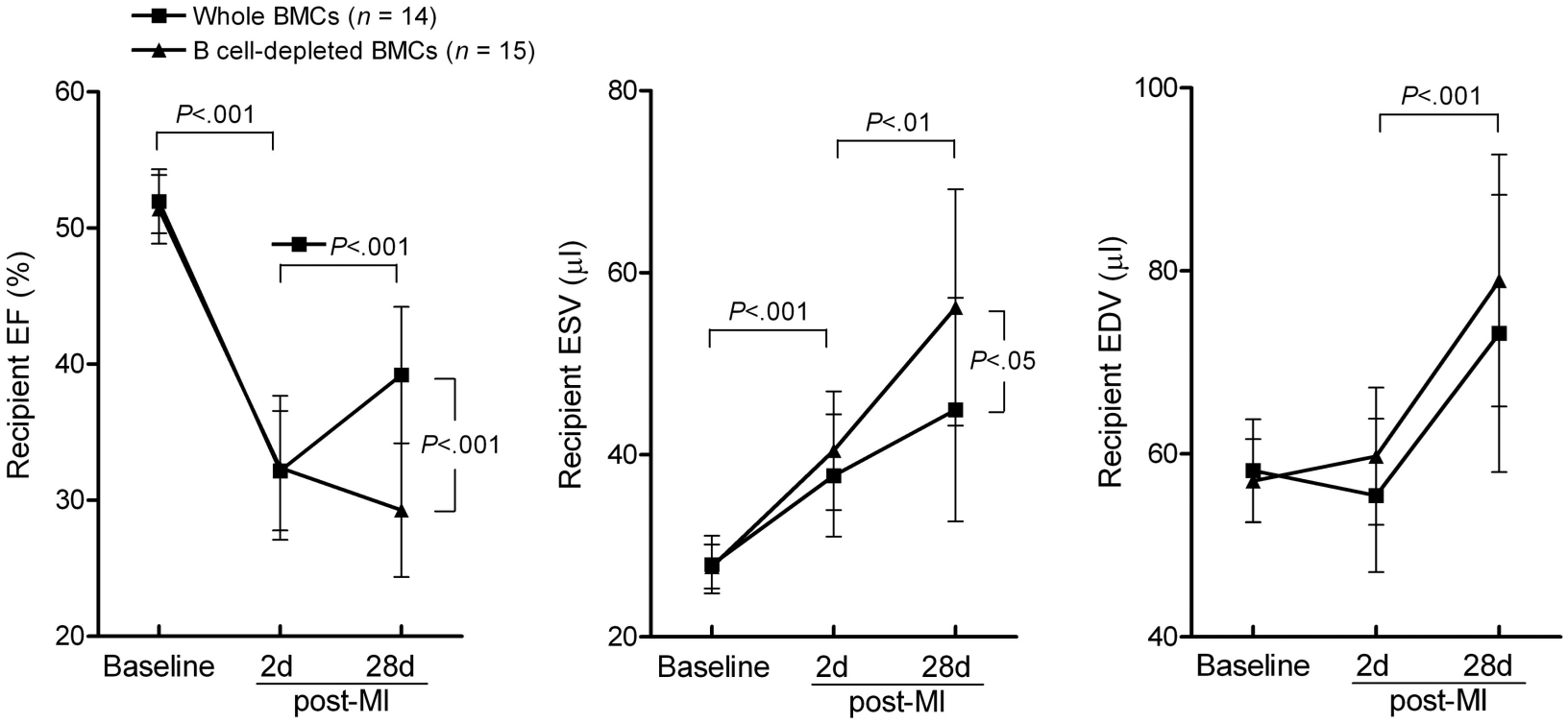
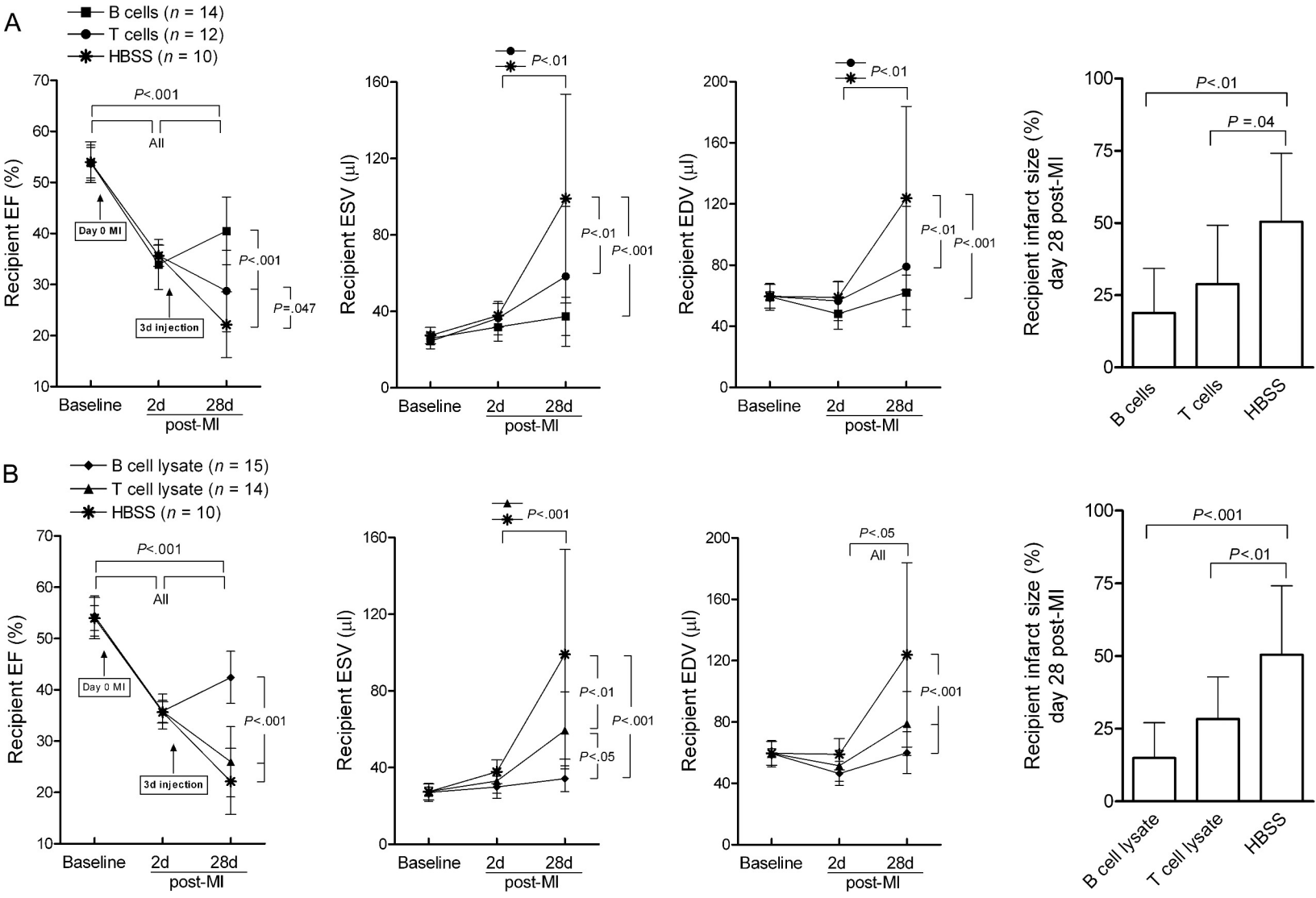
40000
30000
20000
80
70
Gr-1+
Ly6C+
MHC class II


Whole BMCs (n = 14)
B cell-depleted BMCs (n = 15)
Recipient EF (%)
60
50
40
30
20
P<.001
P<.001
P<.001
Baseline
2d
28d
post-MI
Recipient ESV (μl)
80
60
40
20
P<.01
P<.001
P<.05
Baseline
2d
28d
post-MI
Recipient EDV (μl)
100
80
60
40
P<.001
Baseline
2d
28d
post-MI


A
B cells (n = 14)
T cells (n = 12)
HBSS (n = 10)
Recipient EF (%)
P<.001
All
Day 0 MI
3d injection
P<.001
P=.047
Recipient ESV (μl)
P<.01
P<.01
P<.001
Recipient EDV (μl)
P<.01
P<.01
P<.001
Recipient infarct size (%) day 28 post-MI
P<.01
P =.04
B cells
T cells
HBSS
Baseline
2d
28d
post-MI
B
B cell lysate (n = 15)
T cell lysate (n = 14)
HBSS (n = 10)
Recipient EF (%)
P<.001
All
Day 0 MI
3d injection
P<.001
Recipient ESV (μl)
P<.001
P<.01
P<.001
P<.05
Recipient EDV (μl)
P<.05
All
P<.001
Recipient infarct size (%) day 28 post-MI
P<.001
P<.01
B cell lysate
T cell lysate
HBSS
Baseline
2d
28d
post-MI

Supplementary material

Supplementary Table S1. List of cell-surface markers used for identifying cell subpopulations

Cell types	Markers
B cells	$CD19^{+}$
T cells	$CD3^{+}$
NK cells	$CD69^{+}$
Monocytes	$Gr\text{-}1^{intermediate}$
Monocytes/macrophages	$Ly6C^{+}$
Inflammatory monocytes	$CD11b^{+}$, $Gr\text{-}1^{low}$, $Ly6C^{+}$
Circulating myeloid/monocytes	$CD11b^{+}$, $CD62L^{+}$
Myeloid cells	$CD11b^{+}$
Dendritic cells	$CD11c^{high}$
Tip dendritic cells	$CD11c^{+}$, $Ly6C^{+}$, $Gr\text{-}1^{-}$
Granulocytes	$Gr\text{-}1^{high}$
Neutrophils	$CD11b^{+}$, $Gr\text{-}1^{high}$, $Ly6C^{+}$
Lymphoid homing cells	$CD62L^{+}$
Mesenchymal stem cells	$CD105^{+}$, $CD29^{+}$, $Sca\text{-}1^{+}$, $CD45^{-}$

To detect disproportions between cell size versus cell signal, FSC-A x FSC-H was used to gate on singlets.

Supplementary Table S2. Recipient echocardiographic parameters before (2d post-MI) and after (28d post-MI) the implantation of BMCs from healthy donors of varying ages shown in Figure 1

Donor age (recipient number)	EF (%)	ESV (µl)	EDV (µl)	AWTd (mm) (border zone)	AWTd (mm) (infarct zone)
10 weeks (n=15)					
Baseline	53.13 ± 2.25	30.04 ± 3.45	63.91 ± 4.34		
2d post-MI	31.10 ± 3.10	44.40 ± 2.89	64.50 ± 4.20		
28d post-MI	39.47 ± 2.75	42.61 ± 4.98	70.20 ± 5.12	0.58 ± 0.15	0.45 ± 0.16
6 months (n=16)					
Baseline	52.17 ± 2.34	30.68 ± 3.44	63.96 ± 4.44		
2d post-MI	31.20 ± 2.80	44.80 ± 2.48	65.10 ± 3.60		
28d post-MI	36.09 ± 2.36	49.72 ± 5.70	77.59 ± 6.31	0.50 ± 0.13	0.43 ± 0.14
1 year (n=17)					
Baseline	52.86 ± 2.76	30.36 ± 3.99	64.16 ± 4.92		
2d post-MI	31.30 ± 3.40	45.10 ± 3.48	65.70 ± 5.07		
28d post-MI	32.51 ± 3.60	57.78 ± 6.34	85.35 ± 4.99	0.45 ± 0.11	0.40 ± 0.11
1.5 years (n=15)					
Baseline	53.28 ± 2.89	29.64 ± 3.64	63.22 ± 4.45		
2d post-MI	31.40 ± 3.50	44.30 ± 3.08	64.60 ± 4.49		
28d post-MI	26.69 ± 2.90	72.01 ± 6.04	98.07 ± 4.37	0.43 ± 0.10	0.31 ± 0.09
2 years (n=16)					
Baseline	52.81 ± 3.07	30.29 ± 3.89	64.03 ± 4.44		
2d post-MI	31.50 ± 3.40	44.70 ± 2.66	65.30 ± 3.89		
28d post-MI	23.10 ± 3.77	81.80 ± 9.01	106.08± 6.75	0.42 ± 0.09	0.28 ± 0.13
HBSS (n=15)					
Baseline	52.87 ± 2.71	30.58 ± 4.45	64.51 ± 5.08		
2d post-MI	31.30 ± 2.90	44.20 ± 2.93	64.40 ± 4.27		
28d post-MI	21.39 ± 2.20	86.18 ± 10.20	109.38 ± 10.05	0.40 ± 0.09	0.27 ± 0.11

AWTd, anterior wall thickness in diastole; BMCs, bone marrow cells; EDV, end-diastolic volume; EF, ejection fraction; ESV, end-systolic volume; HBSS, Hank's buffered saline solution; MI, myocardial infarction. Statistically significant differences between all groups were shown in Figure 1.

Supplementary Table S3. Recipient echocardiographic parameters before (2d post-MI) and after (28d post-MI) BMC implantations shown in Figure 3

Donor condition (recipient number)	EF (%)	ESV (µl)	EDV (µl)
Whole BMCs (n=14)			
Baseline	51.97 ± 2.35	27.96 ± 3.16	58.17 ± 5.61
2d post-MI	32.17 ± 4.39	37.73 ± 6.71	55.46 ± 8.38
28d post-MI	39.20 ± 5.02	44.96 ± 12.28	73.18 ± 15.15
B cell-depleted BMCs (n=15)			
Baseline	51.38 ± 2.52	27.72 ± 2.41	57.06 ± 4.54
2d post-MI	32.39 ± 5.30	40.47 ± 6.51	59.74 ± 7.50
28d post-MI	29.28 ± 4.92	56.19 ± 12.98	78.94 ± 13.76

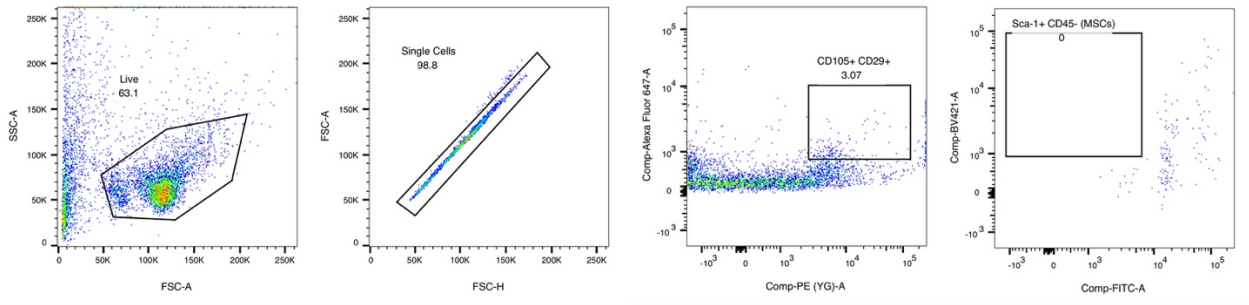
BMCs, bone marrow cells; EDV, end-diastolic volume; EF, ejection fraction; ESV, end-systolic volume; MI, myocardial infarction. Statistically significant differences between two groups were shown in Figure 3.

Supplementary Table S4. Recipient echocardiographic parameters before (2d post-MI) and after (28d post-MI) implantations of bone marrow B or T cells as well as B cell or T cell lysate shown in Figure 4

Donor condition (recipient number)	EF (%)	ESV (µl)	EDV (µl)
B cells (n=14)			
Baseline	53.88 ± 3.45	25.95 ± 3.32	56.35 ± 6.58
2d post-MI	33.95 ± 4.90	31.88 ± 7.49	48.13 ± 9.99
28d post-MI	40.52 ± 6.60	37.37 ± 10.05	62.13 ± 11.35
T cells (n=12)			
Baseline	53.88 ± 2.99	24.44 ± 4.02	52.86 ± 6.88
2d post-MI	35.69 ± 2.10	36.45 ± 8.67	56.63 ± 12.91
28d post-MI	28.76 ± 7.97	58.35 ± 36.64	79.03 ± 39.44
B cell lysate (n=15)			
Baseline	54.40 ± 3.91	27.09 ± 4.76	59.34 ± 8.70
2d post-MI	35.84 ± 2.16	29.89 ± 5.73	46.43 ± 7.84
28d post-MI	42.42 ± 5.08	34.25 ± 6.73	59.99 ± 13.58
T cell lysate (n=14)			
Baseline	53.97 ± 2.41	27.48 ± 2.35	59.65 ± 2.94
2d post-MI	35.78 ± 3.39	33.00 ± 6.35	51.49 ± 10.20
28d post-MI	25.97 ± 6.84	59.43 ± 20.00	78.99 ± 20.85
HBSS (n=10)			
Baseline	53.97 ± 4.01	27.39 ± 4.22	59.58 ± 7.78
2d post-MI	35.64 ± 2.09	37.84 ± 6.16	58.92 ± 10.23
28d post-MI	22.14 ± 6.43	99.07 ± 54.65	123.80 ± 60.03

EDV, end-diastolic volume; EF, ejection fraction; ESV, end-systolic volume; MI, myocardial infarction. Statistically significant differences between groups were shown in Figure 4.

Supplementary Figure S1. Confirmation of absence of contaminating MSCs in negatively selected B cells. MSCs as defined as $CD105^+$ $CD29^+$ $Sca\text{-}1^+$ $CD45^-$ cells were undetectable. 3% of live cells in the preparation were $CD105^+$ $CD29^+$, none of which were also $Sca\text{-}1^+$ $CD45^-$.



Supplementary Figure S2. Relationship between donor age, donor bone marrow B and T cell proportions, and therapeutic efficacy as evidenced by recipient LVEF at day 28 LVEF post-MI (day 25 post-implantation)