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Bone Marrow-Derived B Cells Preserve Ventricular Function After Acute Myocardial Infarction

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Objectives In view of evidence that mature cells play a role in modulating the stem cell niche and thereby stem cell potential and proliferation, we hypothesized that a mature bone marrow (BM) mononuclear cell (MNC) infusion subfraction may have particular potency in promoting hematopoietic or resident stem cell-induced cardiac repair post-infarction.

Background Treatment of acute myocardial infarction (MI) with BM MNC infusion has shown promise for improving patient outcomes. However, clinical data are conflicting, and demonstrate modest improvements. BM MNCs consist of different subpopulations including stem cells, progenitors, and differentiated leukocytes.

Methods Stem cells (c-kit+) and subsets of mature cells including myeloid lineage, B and T-cells were isolated from bone marrow harvested from isogeneic donor rats. Recipient rats had baseline echocardiography then coronary artery ligation; 1×10^6 cells (enriched subpopulations or combinations of subpopulations of BM MNC) or saline was injected into ischemic and ischemic border zones. Cell subpopulations were either injected fresh or after overnight culture. After 2 weeks, animals underwent follow-up echocardiography. Cardiac tissue was assayed for cardiomyocyte proliferation and apoptosis.

Results Fractional ventricular diameter shortening was significantly improved compared with saline ($38 \pm 3.2\%$) when B cells alone were injected fresh ($44 \pm 3.0\%$, $p = 0.035$), or after overnight culture ($51 \pm 2.9\%$, $p < 0.001$), or after culture with c-kit+ cells ($44 \pm 2.4\%$, $p = 0.062$). B cells reduced apoptosis at 48 h after injection compared with control cells ($5.7 \pm 1.2\%$ vs. $12.6 \pm 2.0\%$, $p = 0.005$).

Conclusions Intramyocardial injection of B cells into early post-ischemic myocardium preserved cardiac function by cardiomyocyte salvage. Other BM MNC subtypes were either ineffective or suppressed cardioprotection conferred by an enriched B cell population. (J Am Coll Cardiol Intv 2009; 2:1005–16) © 2009 by the American College of Cardiology Foundation

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Heart failure is the leading cause of morbidity and mortality in the Western world, affecting approximately 5 million people in the U.S. (1) and at least 10 million people in Western Europe (2). Heart transplantation remains the best available treatment option for end-stage heart disease; however, donor availability is limited. A potential alternative approach to the treatment of end stage heart disease is cell therapy in which bone marrow (BM) stem cells or mononuclear cells (MNCs) are injected into sites of cardiac injury. Currently, BM is the most frequent source of cells used for cardiac repair in clinical trials and has been shown to induce modest improvements in cardiac function in patients with acute myocardial infarction (MI) (3–5) and chronic ischemia (6–9).

While the mechanisms for improvement remain unclear, cardiac injection of autologous BM cells in recovery from an acute MI is an attractive therapeutic option. Potential mechanisms include transplanted cell differentiation into cardiac myocytes (10,11), fusion with resident myocytes (12–14), cytokine-supported recruitment of circulating progenitor cells (15,16), and secretion of beneficial paracrine factors (17–20). Clinical results from this novel approach have been mixed, and pre-clinical studies vary in terms of therapeutic cell types, administration methods, timing of cell delivery, and analytical methods. The optimal BM MNC population that induces maximal cardiac repair remains unclear.

Abbreviations and Acronyms

BM = bone marrow
BrdU = 5-bromo-2′-deoxyuridine pellets
HSC = hematopoietic stem cell
LAD = left anterior descending artery
LV = left ventricle/ventricular
MI = myocardial infarction
MNC = mononuclear cell
NHSC = nonhematopoietic stem cell

BM contains a complex assortment of progenitor cells, including hematopoietic stem cells (HSCs), mesenchymal stem cells, and multipotential adult progenitor cells, along with lineage-positive cells such as monocytes, basophils, eosinophils, and B and T lymphocytes. Furthermore, differentiated hematopoietic cells including T and B lymphocytes have been previously shown to contribute to tissue repair and regeneration, and T cells in particular have been shown to influence the HSC niche (20). The aim of this study was to examine the possibility that a unique mature subpopulation of BM cells that, either alone or in combination with HSC subpopulations, was primarily beneficial for repair of infarcted myocardium. The objective was to evaluate left ventricular (LV) function in rats that received intramyocardial injection of various cell subpopulations from the BM, administered at the time of left anterior descending coronary artery ligation. We hypothesized that by removing certain inhibitory or noncontributory mature leukocyte populations, the beneficial effects of cell therapy in the setting of acute MI could be potentiated. Our results demonstrate that a B cell-enriched subfraction induces maximal therapeutic effect during recovery from acute MI.

Methods

The Saint Joseph's Research Institute is accredited by the Association for Accreditation and Advancement of Laboratory Animal Care. Experimental animal use conformed to National Institutes of Health and American Heart Association guidelines and was approved by the Institutional Animal Care and Use Committee of the Saint Joseph's Research Institute, in accordance with the "Guide for the Care and Use of Laboratory Animals" (NIH publication no. 85-23, National Academy Press, Washington, DC, revised 1996).

BM harvest and cell preparation. Donor male Sprague-Dawley rats were anesthetized with ketamine 80 mg/kg with xylazine 2.0 mg/kg and euthanized via exsanguination. Femurs and tibias were harvested and flushed with Dulbecco's modified essential medium (Gibco, Grand Island, New York) supplemented with 2% fetal bovine serum (Gibco), penicillin 100 U/ml (Gibco), and streptomycin (100 μg/ml Gibco). The BM was collected and diluted 1:2 with Dulbecco's phosphate-buffered saline then layered onto a Ficoll density gradient (Histopaque-1077, Sigma, St. Louis, Missouri). The buffy coat was collected and washed with Dulbecco's phosphate-buffered saline then subjected to the Miltenyi Biotech (Auburn, California) cell separation procedure. BM MNCs were washed twice in cold buffer (phosphate-buffered saline, pH 7.2, 0.5% bovine serum albumin and 2 mmol/l ethylenediaminetetraacetic acid) then depleted of non-B cells by a negative selection procedure. BM cells were incubated with antibodies specific for mature lineage (Lin) markers for: T cells (CD3, clone G4.18, BD Pharmingen, San Jose, California), helper/inducer T cells (CD4, clone OX-35, BD Pharmingen), suppressor/cytotoxic T cells (CD8, clone X8, Antigenix America, Huntington Station, New York), monocytes (CD11b/c, clone OX-42, BD Pharmingen), and neutrophils (Granulocyte, clone RP-1, BD Pharmingen) and further incubation with phycoerythrin-labeled magnetic microbeads (Miltenyi Biotech). The unlabeled BM MNC fraction (Lin negative, Lin−) was then subjected to positive selection for B cells (CD45RA, clone OX-33, BD Pharmingen) or HSCs (Lin-c-kit+, HSC, c-kit, clone H-300, Santa Cruz Biotechnologies, Santa Cruz, California). While Lin-c-kit+ consist of very primitive cells including stem cells, for simplicity we will refer to this subset as HSC. Nonhematopoietic stem cells (NHSC) were isolated by the addition of CD45RA to the negative selection cocktail followed by positive selection with c-kit and considered to be Lin-CD45RA-c-kit+-selected cells.

For B cell characterization studies, CD45RA-positive cells (21) were stained with markers for lymphocytes