Bone Good to the Heart
Bone Marrow Cell Characteristics and Cardiac Repair After STEMI in the CCTRN TIME Cohort

Mario Picozza, Giulio Pompilio, Maurizio C. Capogrossi

In this issue of Circulation Research, Shutt et al1 explore the characteristics of resident bone marrow cells (BMC) in patients enrolled in the Timing In Myocardial infarction Evaluation (TIME) clinical trial, in search of cellular correlates of reduction of the infarcted area 6 months after acute myocardial infarction (MI). The central hypothesis is that endogenous BM properties could affect the clinical outcome. They found both phenotypic and biological BMC correlates of infarct size reduction, and the emerging picture is quite interesting.

Why should subset composition and functional status of BMC affect recovery after acute MI? One explanation relates to the fact that specific populations of BMC have been shown to mobilize from the BM into the peripheral blood (PB) after MI and to have prognostic and therapeutic values. The first clue of a cross talk between the human ischemic heart and the BM came more than a decade ago with the work of Shintani et al,2 which reported a peak in BM-derived, circulating CD34+ cells 7 days after MI, a trend that correlated with the rise in the number of putative endothelial cell clusters under in vitro angiogenic conditions (ie, in medium supplemented with endothelial cell growth factors). Mobilization of precursor cells after MI was confirmed by subsequent studies: Wojakowski et al3 showed that, immediately after admission, blood mononuclear cells of patients with ST-segment–elevation myocardial infarction (MI) had higher growth rates of colonies in the endothelial colony-forming unit assay,6 which uses CD45 expression to gate CD34+ hematopoietic stem cell cells in MI was confirmed by these previous studies on circulating BMC in acute MI, a detailed evaluation of resident BMC in humans with MI was missing. The work by Shutt et al1 analyzes the cells in the BM of patients with acute MI for their ability to give rise to endothelial, mesenchymal, and proangiogenic cell colonies, assessed in vitro by endothelial colony-forming cells, colony-forming units (CFU)-fibroblasts, and CFU-Hill assays as surrogate markers of the healing potential of regenerative cells. Furthermore, the authors evaluate BMC composition by the use of multiparametric flow cytometry. Two statistical methods are used to mine for parameters associated with infarct size reduction 6 months after ST-segment–elevation myocardial infarction. In the first, the entire cohort was dichotomized based on the reduction or the increase of infarct size. The data show that patients in whom there was a reduction in the infarcted area had higher growth rates of colonies in the endothelial colony-forming cell assay. However, this dichotomic categorization of patients produces a potential bias because of the higher chance of healing of larger infarcted areas. Authors address this issue by multiple regression analysis, which correlates BMC characteristics with infarct size as a continuous variable, taking as covariates factors presumably affecting the clinical outcome: baseline infarct size, smoking history, diabetes mellitus, BMC intracoronary injection assignment, and age. They found a significant correlation with reduction of infarct size in 3 parameters: (1) exponential constant of the colony growth curve in the endothelial colony-forming cell assay, (2) frequency of CD45+ CD31low small mononuclear leukocytes (ie, lymphocytes), and (3) CFU-Hill exponential curve constant. Importantly, no analysis of PB was performed, and the frequency of CD45+ CD31low cells in the systemic circulation is unknown.

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From the Laboratory of Vascular Pathology, Istituto Dermopatico dell’Immacolata-IRCCS, Rome, Italy (M.P., M.C.C.); Laboratory of Vascular Biology and Regenerative Medicine, Centro Cardiologico Monzino-IRCCS, Milan, Italy (G.P.); and Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy (G.P.).

Correspondence to Maurizio C. Capogrossi, MD, Laboratory of Vascular Pathology, Istituto Dermopatico dell’Immacolata-IRCCS, Via dei Monti di Creta 104, 00167 Rome, Italy. E-mail capogrossi@idi.it


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These findings require evaluation of the functional assays employed. Endothelial colony-forming cell are made up of true vascular wall cell precursors, clonally expandable and capable of functional integration into vascular networks in vivo, whereas the CFU-Hill assay gives a measure of hematopoietic cells likely predisposed to support the regeneration of vascular structures.\(^1\) The relationship between results obtained by counting CD31\(^+\) lymphocytes and the CFU-Hill assay is to some extent expected because these lymphocytes promote the formation of CFU-Hill cell clusters, are enriched in CFU-Hill colonies, and interact with macrophage-like myeloid cells.\(^2,3\) It is noteworthy that BM and circulating endothelial cells and monocytes expressing high levels of CD31 have been shown to have proangiogenic and vasculogenic activities\(^12,13\); however, in the study by Shutt et al,\(^1\) BM CD31\(^+\) cells that exhibit a positive correlation with the decrease in infarct size are lymphocytes (ie, CD45\(^+\) CD31\(^{hi}\)).

Given that T cells are the major lymphocyte subset in BMC, and that CD31\(^+\) T lymphocytes decrease while CD31\(^+\) increase during life,\(^1\) the results by Shutt et al\(^1\) of diminished myocardial healing in individuals with low frequency of CD31\(^+\) lymphocytes among BMC strengthen the hypothesis that aging of the immune system plays an important role in the lack of myocardial regeneration. There is a subset of young naïve T cells called recent thymic emigrants, which express CD31 and whose number strikingly declines with the age because of thymic involution and antigen triggering.\(^4\) Recent thymic emigrant cell frequency can influence clinical outcome of MI because recent thymic emigrants are preferential precursors of induced Treg cells\(^5\) and reduced recent thymic emigrant Treg in patients with non–ST-segment–elevation myocardial infarction is accompanied by elevated levels of the proinflammatory cytokine tumor necrosis factor-\(\alpha\).\(^6\) Furthermore, Treg cells inhibit leukocyte-mediated myocardial damage and improve healing after MI through activation of M2-like myocardial macrophages and myofibroblast induction.\(^7\) In addition, age-dependent decrease of CD31\(^+\) T lymphocytes is associated with a progressive increase of CD31\(^+\) T lymphocytes and a subset of those cells, that is, CD31\(^+\) CD57\(^+\) CD28\(^{−}\) terminally differentiated senescent T cells, has been shown to have a detrimental effect on cardiac healing after MI because of enhanced production of inflammatory cytokines, such as interferon-\(\gamma\) and tumor necrosis factor-\(\alpha\).\(^8,9\) The number of B cells, which express CD31, is also known to decline with age,\(^10\) and, interestingly, Shutt et al\(^1\) also found a statistically significant decrease in B lymphocytes (CD19\(^+\) lymphocytes) in patients who exhibit an increase in infarct size when treatment with BMC coronary infusion was taken into consideration. Aging of the immune system progressively raises the levels of basal activation, promoting a chronic, deleterious low-grade inflammatory state called inflamming that may contribute to impaired healing of the infarcted heart. Taken together, immunosenescence-related pathways offer a credible framework to explain the results of the work by Shutt et al\(^1\) (Figure).

It is noteworthy that Circulation Research has recently published another report from the Cardiovascular Cell Therapy Research Network (CCTRN) comparing composition and functionality of BMC collected from patients with ST-segment–elevation myocardial infarction at different times after MI (in TIME and LateTIME trials) and from patients with chronic ischemic heart failure (in the Effectiveness of Stem Cell Treatment for Adults With Ischemic Cardiomyopathy, the FOCUS Study).\(^11\) Interestingly, in that study, the same authors of the present work showed a positive correlation of BM CD34\(^+\) precursors and a negative correlation of CD11b\(^+\) cells with recovery of cardiac function as assessed by left ventricular ejection fraction. The discrepancy between those findings and data presented in this issue can be attributed, at least in part, to the distinct end point chosen and to the extended cohort used to generate the former. However, taken together, these reports highlight the need to look at different aspects of the response to cardiac damage, from hematologic/regenerative to immunologic ones in which the BM plays a fundamental role.

Contemporary high-throughput technologies offer the opportunity to in-depth analyze the plethora of BM populations and to correlate phenotypic patterns to clinical parameters. Recent introduction of mass cytometry, for example, makes feasible to analyze >30 parameters at the single-cell

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**Figure.** Schematic representation of age-dependent relevant cell dynamics among bone marrow (BM), thymus and systemic circulation and secondary lymphoid tissues. 

**A.** Human BM produces mature myeloid subsets (eosinophils [Eos], neutrophils [Neu], and monocytes [Mono]) through granulocyte macrophage progenitor (GMP), and mature B cells through common lymphoid progenitor (CLP). Progenitors of T cells migrate from the BM to the thymus where selection and expansion take place. When naïve T cells (T naïve) exit the thymus under the name of recent thymic emigrant (RTE), they express CD31. Homeostatic proliferation, aimed at maintaining the T naïve pool causes the loss of CD31. RTE are preferential precursors of peripherally induced Treg (iTreg). Antigens drive the expansion and differentiation of T naïve cells into memory T cells (Tmem) clones. Continuative and life-long antigenic triggering causes accumulation of terminally differentiated Tmem (tdTmem). **B.** Aging of the immune system is accompanied by the progressive loss of CD31\(^+\) cells (mainly RTE and B cells) and expansion of CD31\(^−\) lymphocytes. HSC indicates hematopoietic stem cells; nTreg, natural regulatory T cells; pre-B, B-cell precursor; and T-prog, progenitor of T cells.
level simultaneously, discriminating an extremely large number of cell populations.\textsuperscript{23} In the near future, if genome-wide expression analysis will be combined with polychromatic (>8 parameters) fluorescence-assisted cell-sorting, the exact profiling of single, highly pure, relevant populations in heart diseases will give critical information on regenerative mechanisms and how to exploit them for heart recovery. The works by the CCTRN, by performing standardized multiparametric cytometry on BMC from large cohorts, represent a reference point for such studies.

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Disclosures

None.

References


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