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Mobilization of Bone Marrow-Derived Oct-4⁺ SSEA-4⁺ Very Small Embryonic-Like Stem Cells in Patients With Acute Myocardial Infarction

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Objectives This study sought to assess of the mobilization of nonhematopoietic very small embryonic-like stem cells (VSELs) in acute myocardial infarction (MI).

Background Acute MI induces mobilization of bone marrow stem cells. Recently, a rare population of VSELs, expressing markers of embryonic pluripotent stem cells (PSCs), was identified in adult murine bone marrow and human umbilical cord blood.

Methods Thirty-one patients with acute MI and 30 healthy subjects were enrolled. Blood was sampled on admission, after 24 h, and 5 days later. Erythrocytes were lysed and lin−CD45−VSELs were isolated using a live cell sorting system (FACSAria, Beckton Dickinson, San Jose, California).

Results In healthy subjects the median number of circulating VSELs was very low (median 0.8 [range 0 to 1.3] cells/μL). In acute MI, VSELs were mobilized early (median 2.7 [range 0.2 to 3.9] cells/μL; p < 0.001) and remained elevated after 24 h and 5 days (median 4.7 [range 0.2 to 6.4] cells/μL; p < 0.003, and median 2.6 [range 0.3 to 3.6] cells/μL; p < 0.03, respectively). The mobilization of VSEL was significantly reduced in patients older than 50 years and with diabetes in comparison with younger and nondiabetic patients. Circulating VSELs were small (7 to 8 μm) and enriched in the messenger ribonucleic acid of PSC markers (Oct-4, Nanog), cardiac lineage (GATA-4, Nkx2.5/Csx, MEF2C), and endothelial (VE-cadherin) markers. The presence of PSC markers (Oct-4, SSEA-4) and the chemokine receptor CXCR4 in circulating VSELs was confirmed at the protein level by immunofluorescent staining and ImageStream system (Amnis Corporation, Seattle, Washington) analysis.

Conclusions Acute MI induced mobilization of VSELs expressing pluripotent markers, early cardiac and endothelial markers, and chemokine receptor CXCR4. (J Am Coll Cardiol 2009;53:1–9) © 2009 by the American College of Cardiology Foundation

Acute myocardial infarction (MI) induces a generalized inflammatory response reflected by increased plasma levels of chemoattractants leading to subsequent mobilization of stem and progenitor cells from the bone marrow (BM) (1–5). These circulating cells represent predominantly committed lineages such as granulocytes, lymphocytes, and monocytes, but also much smaller subpopulations of monopotent and multipotent cells, which may play a role in cardiac and endothelial repair. Earlier studies showed that these cells released into peripheral blood (PB) after the acute MI consist of hematopoietic stem cells (HSCs), endothelial progenitor cells (EPCs), and multipotent mesenchymal stromal cells (2,3,6). In patients with acute MI, we found significant up-regulation of early cardiac and endothelial lineage markers in circulating mononuclear cells (MNCs).
stem cells (VSELs). Murine VSELs can differentiate into cell lineages from all 3 germ layers, including mesoderm-derived cardiomyocytes. This population of primitive cells can be isolated from the BM and PB using a multiparameter live cell sorting technique (7–9). We hypothesized that VSELs are the progeny of epiblast-derived stem cells and form a population of quiescent PSCs deposited early in organogenesis in developing BM and other organs (brain, heart) (10). As mentioned earlier, experimental MI in mice is associated with the mobilization of VSELs into PB; however, there are no data confirming the presence of such primitive stem cells in patients with acute MI (11–13).

In the present study, we provide evidence for the first time in humans using several experimental strategies showing that acute MI induces mobilization of VSELs expressing PSC markers.

Methods

Patient population. We studied 31 patients with acute ST-segment elevation MI referred within 12 h after the symptom onset for primary percutaneous coronary intervention and 30 healthy age- and sex-matched subjects (Table 1).

Fluorescence-activated cell sorting (FACS) analysis and sorting of circulating VSELs. Figure 1A shows the protocol of VSEL isolation and analysis. The PB samples were drawn on admission, 24 h after percutaneous coronary intervention, and 5 days later. The VSELs (lin−/CXCR4+/CD45− cells that coexpress CD133 and CD34 antigens) were sorted using a multiparameter, live sterile cell sorting system (FACSAria, Beckton Dickinson, San Jose, California) (Fig. 1B) and used for real-time reverse-transcriptase polymerase chain reaction (RT-PCR) and immunofluorescence and ImageStream system (ISS) (Amnis Corporation, Seattle, Washington) analysis (2,7) (Online Appendix).

RT-PCR. This study used RT-PCR for analysis of messenger ribonucleic acid (mRNA) levels for PSC (Oct-4, Nanog), early myocardial (Nkx2.5/Csx, GATA-4, MEF2C), and endothelial (VE-cadherin) markers (Online Appendix).

Immunofluorescent staining of VSELs. The VSELs were stained with anti-SSEA-4, Oct-4, and CXCR4 monoclonal antibodies (Online Appendix).

ISS analysis. After lysis of red blood cells and washing, the nucleated fraction of PB cells was fixed, permeabilized, and stained for Oct-4, CD45, lineage markers, and CXCR4. The PB-derived granulocytes and erythrocytes were stained for their specific markers. Samples were analyzed with ISS 100 (Online Appendix).

Results

Mobilization of Oct-4+ VSELs in acute MI. In healthy subjects, the number of circulating VSELs was very low (median 0.8 cells/μl, range 0 to 1.3 cells/μl). In contrast, the number of VSELs was higher in acute MI patients than in control subjects early (<12 h) after the onset of symptoms (median 2.7 cells/μl, range 0.2 to 3.9 cells/μl; p < 0.001). Further mobilization was observed after 24 h (median 4.7 cells/μl, range 0.2 to 7.4 cells/μl; p < 0.003) (Fig. 2) and 5 days (median 2.6 cells/μl, range 0.3 to 3.6 cells/μl; p < 0.0001) control versus MI group. *p < 0.03 control versus MI group. †p < 0.0001 control versus MI group.

ACEI = angiotensin-converting enzyme inhibitor; CAD = coronary artery disease; CK-MB = creatine kinase-MB; LVEF = left ventricular ejection fraction; MI = myocardial infarction.

Table 1. Demographic, Clinical, and Laboratory Characteristics of Patients With Acute MI and Control Group

<table>
<thead>
<tr>
<th></th>
<th>Acute MI (n = 31)</th>
<th>Control (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean(SD)</td>
<td>62.5 ± 12.1</td>
<td>40.4 ± 9.4*</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>20 (64)</td>
<td>19 (63)</td>
</tr>
<tr>
<td>History of CAD, n (%)</td>
<td>16 (51)</td>
<td>—</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>18 (58)</td>
<td>16 (53)</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>8 (25)</td>
<td>7 (23)</td>
</tr>
<tr>
<td>Family history of CAD, n (%)</td>
<td>7 (22)</td>
<td>6 (20)</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>11 (35)</td>
<td>10 (33)</td>
</tr>
<tr>
<td>Total cholesterol, mmol/l</td>
<td>4.9 (4.0–5.7)</td>
<td>4.8 (4.0–5.5)</td>
</tr>
<tr>
<td>Creatinine, μmol/l</td>
<td>112 (97–125)</td>
<td>109 (92–123)</td>
</tr>
<tr>
<td>Mean LVEF, %</td>
<td>45.5 (25–60)</td>
<td>56.7 (50–62)*</td>
</tr>
<tr>
<td>Prior statins, n (%)</td>
<td>10 (32)</td>
<td>9 (30)</td>
</tr>
<tr>
<td>Prior ACEI, n (%)</td>
<td>12 (38)</td>
<td>11 (36)</td>
</tr>
<tr>
<td>Maximum troponin I, ng/ml</td>
<td>6.4 (1.4–100)</td>
<td>—</td>
</tr>
<tr>
<td>Maximum CK-MB, U/l</td>
<td>124 (37–324)</td>
<td>—</td>
</tr>
<tr>
<td>12-h leukocytes, ×10^6 cells/μl (range)</td>
<td>11.2 (6.3–15)</td>
<td>5.9 (4.0–8.8)†</td>
</tr>
<tr>
<td>24-h leukocytes, ×10^6 cells/μl (range)</td>
<td>12.7 (7.4–16.2)</td>
<td>—</td>
</tr>
<tr>
<td>5-day leukocytes, ×10^6 cells/μl (range)</td>
<td>10.9 (5.1–14.2)</td>
<td>—</td>
</tr>
<tr>
<td>Lymphocytes, ×10^6 cells/μl (range)</td>
<td>2.28 (0.8–3.9)</td>
<td>2.1 (0.7–3.7)</td>
</tr>
<tr>
<td>Granulocytes, ×10^6 cells/μl (range)</td>
<td>7.6 (4.0–14.7)</td>
<td>2.7 (2.1–4.3)</td>
</tr>
<tr>
<td>Hemoglobin, g/dl (mean ± SD)</td>
<td>14.6 ± 1.2</td>
<td>14.7 ± 1.1</td>
</tr>
</tbody>
</table>

Values expressed as n (%) or median (range) unless otherwise specified. *p < 0.03 control versus MI group. †p < 0.0001 control versus MI group.
0.03). Populations of CD34⁺CD133⁺VEGFR2⁺ EPCs and CD34⁺CXCR4⁺ cells increased significantly in acute MI; however, the absolute numbers of cells were higher than VSELs (Table 2).

Expression of PSC markers by RT-PCR and immunofluorescent staining. We found that the expression of PSC markers was significantly higher 12 h after MI (Oct-4: 17.9 ± 4.3-fold; Nanog: 18.2 ± 3.7-fold) than in control
subjects. The highest levels of mRNA were detected 24 h after acute MI, at the same time as when the most significant mobilization of VSELs occurred (Oct-4: 206 ± 32-fold; Nanog: 282 ± 41-fold). After 5 days, levels of mRNA were no different from control subjects (Oct-4: 2.3 ± 0.4-fold; Nanog: 2.3 ± 0.38-fold) (Fig. 3).

The presence of the PSC markers Oct-4, SSEA-4, and CXCR4 was subsequently confirmed at the protein level using immunofluorescence staining (Fig. 4A). We noticed that 54 ± 11.2% sorted VSELs express Oct-4 antigen. This corresponds to our previously published data on mobilization of VSELs in a murine model (8).

**Size and immunophenotype of VSELs.** The population of VSELs consists of nonhematopoietic cells negative for lineage markers and CD45 antigen (lin−CD45−) and positive for CXCR4, CD133, and CD34 antigens as shown by immunofluorescent staining and ISS. Expression of the PSC markers Oct-4 and SSEA-4 was confirmed in populations of small cells (approximately 7 to 8 µm). Direct ISS-based comparison of mobilized VSELs with other populations of circulating nucleated cells (monocytes, granulocytes) showed that VSELs are significantly smaller and have distinct morphology (Fig. 4B).

**Expression of early cardiac and endothelial markers in circulating VSELs by RT-PCR.** The highest relative expression level of early cardiac markers was detected 24 h after acute MI for GATA-4 (210 ± 34-fold), Nkx2.5/Csx (174 ± 28-fold), MEF2C (209 ± 32-fold), and VE-cadherin (3,884 ± 37-fold). After 5 days, relative expression levels of GATA-4 (1.7 ± 0.6-fold), Nkx2.5/Csx (2 ± 0.5-fold), and VE-cadherin (3.3 ± 0.9-fold) were comparable to control subjects, whereas expression of MEF2C remained higher (97.5 ± 19-fold) (Fig. 5).

**Plasma concentrations of chemokines, growth factors, cytokines, and inflammatory markers.** In acute MI, the up-regulation of plasma levels of chemoattractants (SDF-1, HGF, SCF, G-CSF, and VEGF) was found (Online Table). The number of circulating VSELs in MI patients positively correlated with levels of SDF-1. More information is available in the Online Appendix.

**Clinical and demographic correlates of VSELs mobilization.** The mobilization of VSELs was significantly higher in younger (age <50 years) compared with older patients (median 4.9 cells/µl; range 0 to 6.4 cells/µl vs. median 2.7 cells/µl; range 0.2 to 5.1 cells/µl; p < 0.05). In diabetic patients the number of circulating VSELs was significantly lower than in nondiabetic patients (median 1.9 cells/µl; range 0 to 2.3 cells/µl vs. median 4.8 cells/µl; range 0.2 to 6.2 cells/µl; p < 0.05). In addition, mobilization of VSELs was impaired in patients with a left ventricular ejection fraction (LVEF) <40% compared with an LVEF >40% (median 3.3 cells/µl; range 0 to 4.9 cells/µl vs. median 4.7 cells/µl; range 0.4 to 6.4 cells/µl; p < 0.05). The differences were significant after 12 and 24 h, but not after 5 days. The number of VSELs had a significantly negative correlation with maximum levels of troponin I on admission (R = −0.41; p = 0.03) and after 24 h (R = −0.34; p = 0.04), but not after 5 days (R = −0.31; p = 0.45). Similar negative correlations were found in relation to maximum activity of CK-MB (on admission: R = −0.45; p = 0.033; after 24 h: R = −0.39; p = 0.042; after 5 days R = −0.21; p = 0.23).

### Table 2

**Mobilization of CD34+ CD133+VEGFR2+ EPCs, CD34+CXCR4+ Cells, and lin−CD133−CD45− VSELs in Patients With Acute MI in Comparison With Healthy Control Subjects**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Acute MI Admission</th>
<th>p Value Versus Control</th>
<th>Acute MI 24 h</th>
<th>p Value Versus Control</th>
<th>Acute MI Day 5</th>
<th>p Value Versus Control</th>
<th>p Value Versus Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EPC, cells/µl, median (range)</strong></td>
<td>1.6 (0–2.1)</td>
<td>3.7 (0.1–5.6)</td>
<td>&lt;0.003</td>
<td>5.7 (0–8.1)</td>
<td>&lt;0.0001</td>
<td>&lt;0.003</td>
<td>5.1 (0.2–7.3)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>CD34+ CXCR4+ , cells/µl, median (range)</strong></td>
<td>1.9 (0–3.2)</td>
<td>5.4 (0.4–7.7)</td>
<td>&lt;0.0001</td>
<td>7.3 (0.3–9.7)</td>
<td>&lt;0.0001</td>
<td>&lt;0.003</td>
<td>6.8 (0.3–9.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>VSEL, cells/µl, median (range)</strong></td>
<td>0.8 (0–1.3)</td>
<td>2.7 (0.2–3.9)</td>
<td>&lt;0.001</td>
<td>4.7 (0.3–7.4)</td>
<td>&lt;0.001</td>
<td>&lt;0.003</td>
<td>2.6 (0.3–3.6)</td>
<td>&lt;0.03</td>
</tr>
</tbody>
</table>

EPC = endothelial progenitor cells; MI = myocardial infarction; VSEL = very small embryonic-like stem cell.
Discussion

Adult BM harbors various populations of cells that can potentially contribute to myocardial and endothelial repair (7,14–18). Data from the experimental model of acute MI in mice showed that VSELs are mobilized into PB in response to myocardial ischemia (11). Furthermore, intramyocardial injection of VSELs was more efficient than HSCs and placebo at improving global and regional left ventricular contractility, left ventricular end-systolic diameter, and systolic thickening, as well as reducing myocardial hypertrophy. This effect was obtained using a small total number of VSELs (10,000 cells), whereas a much higher number of HSCs (100,000 cells) was not effective (19).

**VSELs circulate in PB in adult humans.** Recently, VSELs were identified in human cord blood, but there were no data on their presence in adults (20). In the present work we showed for the first time that VSELs are present in the PB in healthy adult subjects and undergo significant mobilization in patients with acute MI. We used an FACS live cell sorting system to define the immunophenotype of human VSELs and to isolate these cells for subsequent analyses. We showed that the number of circulating VSELs increases 12 h after the onset of the symptoms, reaching a maximum after 24 h and remaining elevated after 5 days. The time course of the VSEL mobilization is similar to that for EPCs; however, the absolute cell number is significantly lower and decreases more rapidly after acute MI.

**Characteristics of murine and human VSELs.** In adult murine BM, VSELs comprise a rare population (approximately 0.01%) of MNCs (7,21). In mice, VSELs are nonhematopoietic Sca-1<sup>+</sup>/Lin<sub>−</sub>/CD45<sub>−</sub> cells expressing markers of PSCs Oct-4, Nanog, and SSEA-1 at the mRNA and protein levels. Data from electron microscopy and ISS showed that VSELs have a morphology consistent with embryonic stem cells. These cells are small (approximately 3.6 μm in diameter) and have a large nucleus surrounded by a narrow rim of cytoplasm with numerous mitochondria and open-type chromatin (7,21). Approximately 5% to 10% of purified VSELs form in coculture with C2C12 myoblast line spheres that resemble embryoid bodies. Cells derived from these spheres can be expanded if replated over a C2C12 feeder layer or differentiated after replating into tissue-specific differentiation media into cells representing all 3 germ layers including cardiac myocytes (7,9,21). In the present work, the expression of mRNA for early developmental markers was shown in circulating VSELs using RT-PCR, which confirmed their enrichment in mRNA for Oct-4 and Nanog. The highest relative expression level of mRNA for these genes was detected 24 h after acute MI, at the same time when the most significant mobilization of VSELs occurred. The presence of PSC markers Oct-4 and SSEA-4 was subsequently confirmed at the protein level using immunofluorescent staining and ISS analysis. The ISS is an imaging system that integrates the information obtained by FACS and immunofluorescence, allowing a combination of the data on cell morphometry and quantitative image analysis, for example, information on colocalization of multiple cellular markers (21). The VSELs were positive for nuclear transcription factor Oct-4 and cell surface antigen SSEA-4. These small (approximately 7 μm) Oct-4<sup>+</sup> and SSEA-4<sup>+</sup>–positive cells coexpressed CD34, CD133, and CXCR4 and were negative for lineage markers and the panhematopoietic marker CD45. The presence of CXCR4 on the surface of the cells was consistent with the previously described VSEL profile (7). Moreover, we no-
noticed that approximately 50% to 60% of circulating VSELs in PB in acute MI patients express Oct-4 at a protein level that corroborates with our previously published data on mobilization of VSELs in mice (8). Comparison of mobilized VSELs with other populations of circulating nucleated cells (monocytes, granulocytes) showed that not only are...
VSELs significantly smaller, but they also have a distinct morphology. We noticed that similar to mice, the size of human VSELs is slightly smaller than erythrocytes. Nevertheless, human circulating VSELs in adult patients are larger than those isolated from human neonatal umbilical cord blood (3 to 5 μm) as well as murine (3 to 4 μm) BM-derived VSELs (11,20).

**Mobilization of VSELs as a hypothetical reparatory mechanism in acute MI.** It is hypothesized that stem cells residing in BM and other tissues to reduce the cardiac ischemic injury must undergo mobilization and subsequently home and engraft into the myocardium (22–26). Because BM is also a potential source of cardiac stem cells, the mobilization of cardiac stem cells from the BM may be an additional physiological pathway to replenish these stem cells (27). Shintani et al. (5) described for the first time a rapid increase of EPC number after acute MI. Massa et al. (2) documented mobilization of both HSCs and EPCs in patients with acute MI. The number of circulating cells reached a maximum early, within a few hours after the onset of symptoms, decreased after 1 week, and returned to a level comparable to that of healthy subjects within 2 months. In addition to EPCs and HSCs, other less well defined subpopulations (c-met+, c-kit+, mesenchymal stromal cells) were also mobilized in acute MI (28).

**Circulating human VSELs are enriched in mRNA for early cardiac and endothelial markers.** We found that circulating human VSELs are significantly enriched for mRNA for cardiac lineage (GATA-4, Nkx2.5/Csx, MEF2C) and endothelial (VE-cadherin) markers. The highest relative expression level of mRNA for these genes was detected 24 h after acute MI. The decrease in mRNA expression probably could be related to “back-homing” to the BM of mobilized cells not incorporated in the myocardium. Other possible mechanisms could also be involved, such as: 1) an increase in expression of cardiac committed genes in VSELs by signals released from damaged myocardium; or 2) some point-selective mobilization of VSELs already enriched in cardiac markers (committed to myocardial lineage). Our previous data from patients with acute MI showed significant mobilization of CD34+CXCR4+ cells early in acute MI. Parallel to the release of CD34+CXCR4+ cells, we found a significant increase of the mRNA for early myocardial, muscle, and endothelial markers in the PB MNCs, but we were not able to show which population of circulating MNCs was responsible for the enrichment in the tissue-specific markers (3). In a murine model of MI, it was consistently shown that stem cells are mobilized into PB and the relative increase in expression levels of cardiac and endothelial markers correlates with stem cell mobilization (1).

The presence of Oct-4+ cells in adult murine BM raises an important question about their ability to differentiate into cardiomyocytes. Pallante et al. (29) showed that the murine BM contains cells capable of cardiogenic differentiation that give rise to the population of spontaneously contracting cardiomyocytes expressing the cardiac structural proteins, beta-adrenergic receptors and connexins. These cells were identified as rare (0.05% of BM MNCs) Oct3/4+ c-kit+ CXCR4+Sca-1−CD34−CD45− cells localized...
adjacent to the osteoblastic niche. Thus, this study somehow supports our results showing that the population of mobile nonhematopoietic Oct-4+/CXCR4+/CD133+ cells is enriched for the cardiac markers and capable of cardiac differentiation (1,7).

The number of circulating VSELs correlates with plasma SDF-1 levels. We found that although the number of circulating VSELs in acute MI positively correlated with levels of SDF-1, there was no significant correlation with other cytokines, growth factors, chemokines, or inflammatory markers. This correlation between SDF-1 level and the circulating VSEL expressing SDF-1-binding receptor CXCR4 seems to be biologically important. Murine BM-derived VSELs express the CXCR4 as well as other receptors for cytokines and growth factors (c-met and leukemia inhibitory factor receptor). We have previously reported that the population of murine BM-derived cells expressing cardiac markers migrated to the homogenates prepared from infarcted myocardium in a SDF-1/CXCR4, leukemia inhibitory factor/leukemia inhibitory factor receptor, and HGF/c-met dependent manner (1,4,7).

Mobilization of VSELs is decreased in older patients with diabetes and reduced LVEF. Several factors, including age, presence of diabetes, and the use of statins, can influence the mobilization of cells into the PB. In murine models, age seems to be the most important determinant of mobilized VSELs and level of expression of PSC markers in these cells (7). We report that the mobilization of VSELs was significantly more efficient in younger patients than in older patients. This finding is consistent with our previous data suggesting that the number of circulating CD34+ CXCR4+ cells is significantly reduced in patients older than 50 years (12). To support this clinically relevant observation, we previously reported that in 1-year-old mice the number of Sca-1+lin-CD45− VSELs in the BM is significantly decreased compared with young 1-month-old animals. Data from animal models showed that both younger and older mice had a similar time course and number of circulating VSELs after the acute MI; however, the level of expression of PSC markers in circulating VSEL was significantly lower in older animals (11). In diabetic patients, the number of mobilized pluripotent VSELs is lower than in nondiabetics. It is worth mentioning that so far no study has investigated the influence of diabetes on tissue distribution and mobilization of PSCs. However, diabetes is associated with reduced mobilization, functional capacity, and survival of EPCs as well as promoting the senescence of cardiac stem cells (30,31).

Mobilization of VSELs was also impaired in our study in patients with reduced LVEF. In addition, the number of VSELs showed significant negative correlations with cardiac necrosis markers, which suggests that reduced mobilization of VSELs is associated with larger infarct size and a higher degree of impairment of the left ventricular contractility. This could be potentially explained by the fact that proteases are probably more abundantly released from the larger necrotic areas of myocardium and may negatively affect stability of certain stem cell–mobilizing chemoattractants. To support this, SDF-1 is a very-well-known substrate for several tissue proteases (32). Current findings are consistent with our previous data suggesting that the numbers of circulating CD34+CXCR4+ cells correlate positively with LVEF and negatively with markers of myocardial necrosis and N-terminal pro-B-type natriuretic peptide in patients with acute MI (13). In the prospective 1-year follow-up of 55 patients with acute MI, we showed that the mobilization of CD34+CXCR4+ cells in the acute phase positively correlated with LVEF after 1 year (12). In 15 patients followed up using cardiac magnetic resonance imaging, we showed that mobilization of CD34+CXCR4+ cells in acute MI inversely correlated with the parameters of left ventricular end-systolic and end-diastolic remodeling, infarct area (delayed enhancement), and number of segments showing impaired perfusion after infarction of adenosine (3,12). The association between the mobilization of stem and progenitor cells was confirmed by Leone et al. (33), who showed that a higher number of CD34+ cells assessed 1 year after the MI is associated with better improvement of LVEF. Numaguchi et al. (34) showed that in patients with acute MI the capability of mobilized EPCs to differentiate into mature endothelial cells may be associated with improved recovery of LVEF, lower end-systolic volume, and decreased infarct size. However, these findings need confirmation in larger populations of patients before use of the stem cell number as the cardiac recovery marker can be recommended.

The search for the most suitable cell for cardiac repair continues, and cells showing the features of PSCs (e.g., VSELs) are good candidates for further studies in this area. The VSELs can be easily isolated from the BM using a live cell sorting system. In addition, murine VSELs can be expanded and differentiated into cardiomyocytes. We are aware that the obvious limitation of our current study is the lack of direct proof that the human PB-derived VSELs can differentiate into the cardiac myocytes similar to their murine counterparts. Indeed, this issue needs further investigation (8). In conclusion, we believe that human VSELs might be potentially useful in future clinical studies in patients with ischemic cardiomyopathy, providing that the technical issues with their large-scale isolation, expansion, and differentiation are resolved.

This study supports the hypothesis that a rare population of VSELs expressing markers of PSCs (Oct-4, Nanog, SSEA-4) as well as early cardiac and endothelial markers is mobilized to the PB in acute MI. Mobilization of VSELs is reduced in older patients with high levels of cardiac necrosis markers, reduced LVEF, and diabetes.
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Key Words: very small embryonic-like stem cells, pluripotent stem cells, acute myocardial infarction, mobilization.
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