Mobilization of CD34/CXCR4⁺, CD34/CD117⁺, c-met⁺ Stem Cells, and Mononuclear Cells Expressing Early Cardiac, Muscle, and Endothelial Markers Into Peripheral Blood in Patients With Acute Myocardial Infarction

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Background—Adult stem cells can contribute to myocardial regeneration after ischemic injury. Bone marrow and skeletal muscles contain a population of CXCR4⁺ cells expressing genes specific for muscle progenitor cells that can be mobilized into the peripheral blood. The aims of the study were (1) to confirm the presence of early tissue-committed cells expressing cardiac, muscle, and endothelial markers in populations of mononuclear cells in peripheral blood and (2) to assess the dynamics and magnitude of the mobilization of CD34⁺, CD117⁺, CXCR4⁺, c-met⁺, CD34/CD117⁺, and CD34/CXCR4⁺ stem cells into peripheral blood in relation to inflammatory and hematopoietic cytokines in patients with ST-segment–elevation acute myocardial infarction (STEMI).

Methods and Results—Fifty-six patients with STEMI (<12 hours), 39 with stable angina, and 20 healthy control subjects were enrolled. Real-time reverse transcription–polymerase chain reaction (RT-PCR) was used for detection of tissue-specific markers. The number of the cells was assessed by use of a flow cytometer on admission, after 24 hours, and after 7 days. RT-PCR revealed increased expression of mRNA (up to 3.5-fold increase) for specific cardiac (GATA4, MEF2C, Nkx2.5/Csx), muscle (Myf5, Myogenin, MyoD), and endothelial (VE-cadherin, von Willebrand factor) markers in peripheral blood mononuclear cells. The number of CD34/CXCR4⁺ and CD34/CD117⁺ and c-met⁺ stem cells in peripheral blood was significantly higher in STEMI patients than in stable angina and healthy subjects, peaking on admission, without further significant increase after 24 hours and 7 days.

Conclusions—The study demonstrates in the setting of STEMI a marked mobilization of mononuclear cells expressing specific cardiac, muscle, and endothelial markers as well as CD34/CXCR4⁺ and CD34/CD117⁺ and c-met⁺ stem cells and shows that stromal cell–derived factor-1 is an important factor influencing the mobilization. (Circulation. 2004; 110:3213-3220.)

Key Words: blood cells ■ myocardial infarction ■ inflammation ■ molecular biology ■ genetics

Genetics

It has been postulated that bone marrow–derived adult progenitor cells may have a potential to regenerate the myocardium after ST-segment–elevation acute myocardial infarction (STEMI). In experimental STEMI, both bone marrow– and peripheral blood–derived progenitor cells showed the ability to regenerate the myocardium by enhanced neovascularization and to a much lesser extent by transdifferentiation into functional cardiomyocytes.¹⁻⁴

See p 3158

Basically, 2 mechanisms are involved in the endogenous stem cell–associated myocardial regeneration. The concept of stem cell plasticity involves the mobilization of stem cells from the bone marrow and other putative “niches” (eg, skeletal muscles), cytokine-guided homing with subsequent engraftment into the ischemic area, and finally the transdifferentiation into functional cardiomyocytes.⁴ This concept has recently been challenged, and an alternative hypothesis was formulated, based on the observations that bone marrow and skeletal muscles contain a small population of cells positive for CXCR4 antigen and expressing genes specific for early muscle-committed stem/progenitor cells (myogenin, MyoD, Myf5).⁵,⁶ These tissue-committed stem cells (TCSCs) circulate in the peripheral blood at low numbers and can be mobilized by ischemia-related inflammatory and hematopoietic cytokines, such as granulocyte colony-stimulating factor (G-CSF) and stromal cell–derived factor-1 (SDF-1). The SDF-1/CXCR-4 axis seems particularly important in stem/muscle progenitor cell homing, chemotaxis,
engraftment, and retention in ischemic myocardium, as shown in experimental studies performed by our group. Moreover, we showed that SDF-1, a potent chemoattractant for CXCR4+ cells, is markedly upregulated in the myocardium under ischemia.5 The hypothesis of TCSCs “hiding out” in various niches and being mobilized in the setting of tissue damage is based primarily on experimental work in animal models, and more studies are needed to prove the concept.5,6 So far, the significant mobilization of endothelial progenitor cells (EPCs) in the setting of acute myocardial infarction (AMI) was demonstrated by Shintani et al.,7 but little is known about the stem/muscle progenitor cells positive for the CXCR4 receptor.

Therefore, we conducted this study to confirm the presence of CD34+, CD117+, CXCR4+, c-met+, CD34/CD117+, and CD34/CXCR4+, early TCSCs expressing cardiac, skeletal muscle, and endothelium-specific markers, and to assess the dynamics and magnitude of the mobilization of these cells into peripheral blood in relation to inflammatory and hematopoietic cytokines in the setting of STEMI.

**Methods**

**Study Population**

Fifty-six patients with STEMI admitted within 12 hours after the chest pain onset, 39 subjects with stable effort angina (SA), and 20 healthy control subjects were enrolled. All STEMI patients underwent urgent coronary angiography with subsequent primary percutaneous angioplasty. None of the patients had a history of systemic or cardiovascular inflammatory process, active infection, or febrile status. The institutional Ethics Committee approved the study protocol, and all patients gave written informed consent.

**Laboratory Measurements**

Peripheral blood samples (PBS) (2×5 mL) were drawn on admission, after 24 hours, and 7 days in STEMI patients, on admission in the SA group, and on an ambulatory basis in the control group. Plasma samples for the measurements of cytokine levels were frozen and stored at −40°C. EDTA-PBS samples for the assessment of stem cells were processed within 12 hours after drawing.

**Circulating Early Tissue-Committed Stem Cells**

The sample of whole blood (100 μL) was stained with phycoerythrin-conjugated (5 μL) anti-CD34, anti-CD117, anti-CXCR4 (Becton Dickinson), and anti-c-met (Sigma) monoclonal antibodies for 30 minutes at 4°C (Figure 1). The cells were also double-labeled for CD34/CXCR4- and CD34/CD117-positive staining. Cells were subsequently lysed for 15 minutes, centrifuged, washed twice, and resuspended in PBS and analyzed by use of a FACSCalibur flow cytometer (Becton Dickinson). Isotype-matched phycoerythrin-conjugated antibodies were used as controls (Becton Dickinson) (Figure 1). The number of early tissue-committed progenitor cells was expressed as the absolute number of cells per 1 μL of whole blood.
Real-Time RT-PCR

For analysis of mRNA levels for early myocardial (Nkx2.5/Csx, GATA-4, MEF2C), muscle (Myf5, MyoD, myogenin), and endothelial (VE-cadherin, von Willebrand factor) markers, total mRNA was isolated from peripheral blood mononuclear cells (PBMCs) by use of the RNeasy Mini Kit (Quiagen Inc). Subsequently, mRNA was reverse-transcribed by use of TaqMan Reverse Transcription Reagents (Applied Biosystems). Measurements of mRNA levels were performed by real-time reverse transcription–polymerase chain reaction (RT-PCR) by use of an ABI PRISM 7000 Sequence Detection System. A 25-µL reaction mixture containing 12.5 µL SYBR Green PCR Master Mix, 10 ng of cDNA template, and the following primers: 5'-ACCATGGATGGGGCAAAG-3' (sense), 5'-AAATCGATGCTGCAACTTGAG-3' (antisense) for Myf5; 5'-CCGGCCGCAGGACTCTAGC-3' (sense), 5'-GGGGGCGGG-GGGGAAACTC-3' (antisense) for MyoD; 5'-GGGCGGAAACTT-3' (sense), 5'-CTGGACATCTCGGTATG-3' (antisense) for GATA; 5'-CGTGGGAAACAAAA-3' (antisense) for Nkx2.5/Csx; 5'-GCTAGTGCAAGCTCCCAACTG-3' (sense) for MEF2C; and 5'-GGGCAATCTGGGTATGTCT-3' (sense), 5'-CCGACAGTTGTAGGCCCTGTT-3' (antisense) for VE-cadherin. Primers were designed with Primer Express software. The threshold cycle (Ct) was subsequently determined. Relative quantification of marker gene mRNA expression was calculated with the comparative Ct method. The Ct of endogenous control gene (HGF, HIF) was measured by use of commercially available high-sensitivity ELISA kits (Bender MedSystems, R&D). SDF-1 levels were measured by use of a commercially available high-sensitivity Enzyme-Linked Immunosorbent Assay kit (Bender MedSystems, R&D). The concentrations of SDF-1, G-CSF, vascular endothelial growth factor (VEGF), interleukin-6 (IL-6), and hepatocyte growth factor (HGF) were measured by use of commercially available high-sensitivity ELISA kits (Bender MedSystems, R&D). SDF-1 levels were assayed in platelet-depleted samples (centrifuged at 11 000g for 10 minutes).

Data Analysis

Data are expressed as median±range. Because the distribution of all parameters was skewed, the nonparametric tests (Mann-Whitney U and Wilcoxon tests) were used. The correlations between levels of cytokines and stem cells were assessed with linear regression analysis and Spearman rank correlation test. The logistic regression model was used for determination of the factor independently influencing the level of CD34+ cells. A probability value of P<0.05 was considered significant. Analyses were performed by use of Statistica 6.0 PL for Windows package.

Results

The characteristics of the study group are shown in Table 1. No significant differences between the AMI and SA groups regarding age, sex, smoking, diabetes, hypertension, and hypercholesterolemia were observed.

<table>
<thead>
<tr>
<th>TABLE 1. Characteristics of Patients</th>
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<tbody>
<tr>
<td>Control (n=20)</td>
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<tr>
<td>Age, y (mean±SD)</td>
</tr>
<tr>
<td>Men/women</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
</tr>
<tr>
<td>Hypercholesterolemia, n (%)</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
</tr>
<tr>
<td>Previous STEMI, n (%)</td>
</tr>
<tr>
<td>Family history of CAD, n (%)</td>
</tr>
<tr>
<td>Statins, n (%)</td>
</tr>
<tr>
<td>ACE inhibitors, n (%)</td>
</tr>
<tr>
<td>Aspirin, n (%)</td>
</tr>
<tr>
<td>Fibrinogen, mg/dL, n (%)</td>
</tr>
<tr>
<td>hsCRP, mg/dL, n (%)</td>
</tr>
<tr>
<td>Baseline CK-MB, U/L</td>
</tr>
<tr>
<td>Troponin I (24 h), ng/mL</td>
</tr>
<tr>
<td>Peak CK-MB, U/L</td>
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</table>

CD indicates coronary artery disease; CRP, C-reactive protein; and CK, creatine kinase. Values of CK-MB and troponin I are expressed as median and range.

*P<0.05 vs STEMI group.

Real-Time RT-PCR of Genes Expressed in Circulating Early Tissue-Committed Stem Cells

Real-time RT-PCR revealed markedly increased expression of early myocardial (Nkx2.5/Csx GATA-4 MEF2C), muscle (Myf5, MyoD, myogenin), and endothelial (VE-cadherin, von Willebrand factor) markers in circulating PBMCs. The levels of mRNA for myocardial markers increased 0.6 to 2.5 times, that for muscle markers increased 1.9 to 3.5 times, and that for endothelial VE-cadherin increased 1.8 times. The most pronounced increase in expression of cardiac, muscle, and endothelial markers was found in PBMCs of STEMI patients on admission, that is, at the same time at which the most significant increase in absolute number of cells occurred. The mRNA levels decreased but remained significantly higher compared with control values after 24 hours and 7 days for all muscle markers and for cardiac and endothelial VE-cadherin, but not for von Willebrand factor (Figure 2).

Dynamics of Circulating Early Tissue-Committed Stem Cells

The absolute number of TCSCs was significantly higher in STEMI patients than in both SA and healthy subjects (Table 2). Figure 3 shows the time course of CXCR4+ stem cells in PBS in STEMI patients compared with SA patients. We observed significantly higher absolute numbers of CD34+ and CD117+ cells in STEMI patients at all time points compared with the SA group, whereas the number of CD117+ cells was significantly higher on admission only. The number of CD34+ and CD117+ cells was significantly higher in the STEMI group than in the SA group and healthy controls.
in STEMI patients peaked on admission, without further significant increases after 24 hours and 7 days. There was a trend toward further increase in c-met\(^+\) cell numbers after 24 hours compared with baseline, but the change did not reach statistical significance. The number of c-met\(^+\) cells was \( \approx 8 \) times higher compared with control, whereas the differences in number of CD34\(^+\), CD117\(^+\), and CXCR4\(^+\) cells ranged from 30\% to 200\%. The double staining revealed a significant increase of the CD34/CXCR4\(^+\) and CD34/CD117\(^+\) cells in STEMI subjects compared with healthy control subjects, who had no detectable numbers of CD34/CXCR4\(^+\) cells and only small numbers of CD34/CD117\(^+\) cells (Figure 4).

### Plasma Concentrations of Inflammatory and Hematopoietic Cytokines

The changes in levels of cytokines (G-CSF, IL-6, VEGF, HGF, and SDF-1) in STEMI patients compared with the control and SA groups are shown in Table 3. The levels of IL-6, G-CSF, VEGF, and HGF were significantly higher, whereas SDF-1 was lower, in STEMI patients than in control subjects and SA patients. The levels of G-CSF, IL-6, and HGF increased significantly in STEMI patients, peaking after 24 hours and lowering to baseline on day 7, whereas SDF-1 and VEGF concentrations peaked on day 7 and remained significantly higher compared with baseline. SDF-1 levels increased significantly throughout the observation but still remained below the levels observed in healthy subjects and SA patients.

### Correlations Between Cytokines and Tissue-Committed Stem Cells

In linear regression analysis, the number of CD34\(^+\) cells in STEMI patients correlated positively with the levels of IL-6 (\( R = 0.65, P = 0.002 \)), VEGF (\( R = 0.4, P = 0.01 \)), and SDF-1 (\( R = 0.34, P = 0.03 \)) and negatively with G-CSF levels on day 7 (\( R = -0.3, P < 0.03 \)). The number of CD117\(^+\) cells showed a positive correlation with VEGF (\( R = 0.37, P < 0.02 \)) and HGF (\( R = 0.39, P = 0.02 \)), and CXCR4\(^+\) cells with G-CSF levels (\( R = 0.37, P = 0.01 \)) and c-met correlated strongly negatively with IL-6 (\( R = -0.78, P = 0.02 \)). In logistic regression, only the significant increase of SDF-1 level (defined as SDF-1 concentration >1000 pg/mL at 24 hours after admission) was an independent predictor of significant increase (>100 cells/\( \mu \)L [median]) of CD34\(^+\) cell numbers (OR [95\% CI], 5.6 [1.4 to 23], \( P = 0.01 \)) (Figure 5).

### Discussion

In this study, we demonstrated for the first time a significant increase of mononuclear tissue-committed cells expressing early cardiac, muscle, and endothelial markers as well as stem cells expressing CD34, CXCR4, CD117 (c-kit), and c-met in peripheral blood in the setting of STEMI. The absolute number of TSCC cells in peripheral blood significantly increased in patients with STEMI compared with SA patients and healthy subjects. Moreover, a small but consistently detectable number of circulating stem cells (CD34, CXCR4, CD117, c-met, and CD34/CD117 double-positive cells, but not CD34/CXCR4 cells) was found in patients with SA and healthy subjects, suggesting that some baseline steady state exists. The time course of the mobilization showed a marked

### Table 2. Absolute Number of Early Stem/Muscle Progenitor Cells Expressing Surface Antigens CD34, CD117, CXCR4, and c-met in Peripheral Blood in Healthy Controls, Stable Angina, and STEMI

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Stable Angina</th>
<th>( P ) vs Control</th>
<th>STEMI Baseline</th>
<th>( P ) vs Baseline</th>
<th>STEMI 24 h</th>
<th>( P ) vs Baseline</th>
<th>STEMI 7 days</th>
<th>( P ) vs Baseline</th>
<th>STEMI Day 7</th>
<th>( P ) vs Baseline</th>
<th>STEMI 7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD34(^+)</td>
<td>380 (56–402)</td>
<td>450 (0–790)</td>
<td>NS</td>
<td>752 (0–1368)</td>
<td>0.0005</td>
<td>0.00001/</td>
<td>1018 (0–1924)</td>
<td>0.0003</td>
<td>NS</td>
<td>882 (0–1472)</td>
<td>&lt;0.003</td>
<td>NS</td>
</tr>
<tr>
<td>CD117(^+)</td>
<td>289 (58–1879)</td>
<td>325 (124–3564)</td>
<td>NS</td>
<td>860 (174–1981)</td>
<td>0.04</td>
<td>0.001/</td>
<td>584 (0–1633)</td>
<td>NS</td>
<td>NS</td>
<td>730 (197–3322)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>CXCR4(^+)</td>
<td>1231 (487–4029)</td>
<td>1205 (556–4327)</td>
<td>NS</td>
<td>2655 (418–5078)</td>
<td>&lt;0.003</td>
<td>&lt;0.05/</td>
<td>2628 (127–6062)</td>
<td>0.03</td>
<td>NS</td>
<td>1810 (497–4864)</td>
<td>&lt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>c-met(^+)</td>
<td>213 (7–521)</td>
<td>303 (23–865)</td>
<td>NS</td>
<td>1623 (71–6011)</td>
<td>0.00008</td>
<td>0.000001/</td>
<td>1389 (103–9349)</td>
<td>0.00006</td>
<td>NS</td>
<td>924 (63–1193)</td>
<td>0.003</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are expressed as the number of cells per \( \mu \)L of blood (median±range). SA indicates stable angina.
early increase of TCSC numbers, with a subsequent decrease through the 7-day follow-up with concomitant changes in the levels of cytokines involved in the inflammatory response and stem cell recruitment. Interestingly, the most pronounced increase in expression of cardiac muscle and endothelial markers was found in PBMCs isolated in STEMI patients at admission, that is, at the same time at which the most significant increases of CD34+/H11001, CXCR4+/H11001, CD117+/H11001, c-met+/H11001, CD34/CXCR4+/H11001, and CD34/CD117+/H11001 stem cell numbers occurred. The enrichment of TCSCs in mRNA for these markers remained significantly higher after 24 hours and 7 days. The double FACS staining revealed the significant difference between the populations of mobilized cells, because in healthy subjects, only a small number of CD34/CD117+/H11001 cells but no detectable number of CD34/CDCXCR4+/H11001 cells was found, whereas in STEMI patients, a significant increase of both populations was detected.

Ratajczak et al. found that PBMCs expressing markers of early muscle (myogenin, myoD) and neural (GFAP) cells can be isolated from human peripheral blood and that the numbers of these circulating TCSCs increase after G-CSF mobilization. Furthermore, the CXCR4+ TCSCs expressing markers for muscle, liver, and neural tissue are significantly chemoattracted by SDF-1. An increased number of hematopoietic progenitor cells expressing CD34 and CD117 was shown in patients with persistent atrial fibrillation and in marathon runners. Another possible explanation is a release of mature cardiomyocytes and endothelial cells from the infarct zone and coronary vessels, but it would not explain an increased expression of muscle marker genes. The issue to be proved is whether the cells expressing tissue-specific markers are the same as the cells identified by FACS.

So far, in the setting of STEMI, only the mobilization of EPCs but not muscle/stem progenitor cells was investigated. Shintani et al. found a significant increase of CD34− mononuclear cells positive for lineage markers (KDR, VE-cadherin, CD31), function (Dil-acLDL uptake), and morphology specific for endothelial cells peaking 7 days after STEMI, with a subsequent decrease after 1 month.

Stimulation of the bone marrow to release progenitor cells in the setting of tissue injury is related to the increased levels

### TABLE 3. Plasma Concentrations of Cytokines in Healthy Controls, Stable Angina, and STEMI.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Stable Angina</th>
<th>P vs Control</th>
<th>P vs Baseline</th>
<th>P vs SA</th>
<th>P vs Baseline</th>
<th>P vs Control</th>
<th>P vs SA</th>
<th>P vs Baseline</th>
<th>P vs Control</th>
<th>P vs SA</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-CSF, pg/mL</td>
<td>47 (22–85)</td>
<td>58 (24–103)</td>
<td>NS</td>
<td>&lt;0.05</td>
<td>&lt;0.03</td>
<td>84 (12–165)</td>
<td>&lt;0.00001</td>
<td>0.0006</td>
<td>67 (23–210)</td>
<td>0.01</td>
<td>NS</td>
</tr>
<tr>
<td>IL-6, pg/mL</td>
<td>2.12 (0.25–6.87)</td>
<td>3.6 (0.23–12.2)</td>
<td>&lt;0.05</td>
<td>6.4 (1.2–144.4)</td>
<td>0.001</td>
<td>19.5 (3.8–90.6)</td>
<td>&lt;0.00001</td>
<td>0.01</td>
<td>7 (1.8–97.6)</td>
<td>0.001</td>
<td>NS</td>
</tr>
<tr>
<td>HGF, pg/mL</td>
<td>106 (71–234)</td>
<td>213 (79–403)</td>
<td>&lt;0.05</td>
<td>271 (98–465)</td>
<td>0.001</td>
<td>379 (188–763)</td>
<td>&lt;0.00001</td>
<td>&lt;0.00001</td>
<td>220 (103–453)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>VEGF, pg/mL</td>
<td>155.25 (51–238.5)</td>
<td>173 (78–451)</td>
<td>NS</td>
<td>246 (76–471)</td>
<td>0.0002</td>
<td>&lt;0.00003</td>
<td>241 (100–446)</td>
<td>0.0002</td>
<td>NS</td>
<td>401 (123–717)</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>SDF-1, pg/mL</td>
<td>3789 (1022–4710)</td>
<td>2123 (778–4012)</td>
<td>&lt;0.03</td>
<td>987 (246–2245)</td>
<td>&lt;0.00001</td>
<td>&lt;0.00001</td>
<td>1045 (386–3481)</td>
<td>&lt;0.00001</td>
<td>0.05</td>
<td>1769 (557–4204)</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Data are expressed as median range. SDF-1 indicates stromal cell–derived factor-1; G-CSF, granulocyte colony-stimulating factor; VEGF, vascular endothelial growth factor; IL-6, interleukin-6; HGF, hepatocyte growth factor; and SA, stable angina.
of hematopoietic and inflammatory cytokines. Ischemic myocardium can produce angiogenic and hematopoietic cytokines, such as VEGF and SDF-1, which may play a role in bone marrow stimulation.10,11

We observed lower baseline levels of SDF-1 in STEMI patients than in healthy control subjects and patients with SA. In STEMI patients, the differences were noted already on admission, with subsequent increases throughout the observation period but still remaining lower than in SA. Damas et al10 also reported significantly decreased concentrations of SDF-1α in patients with SA and particularly low levels in unstable angina compared with healthy control subjects. SDF-1 is an important signaling factor in embryonic development, particularly in cardiogenesis, vasculogenesis, and hematopoiesis.10–13 SDF-1 interacts with a single specific receptor, CXCR4, thus forming an SDF-1/CXCR4 axis, which is crucial to mobilization, homing, and survival of stem cells (CD34+), EPCs, and mononuclear cells in AMI patients on admission and throughout the follow-up period. The altered function of the SDF-1/CXCR4 axis was described by Damas et al10 in patients with SA and particularly low levels in unstable angina compared with healthy control subjects.

We have previously demonstrated that in murine models, the cells involved in myocardial regeneration and derived from muscle satellite cells express the functional CXCR4 receptor, which mediates chemotaxis to SDF-1, which can also be secreted by human myocardial, muscle, and bone marrow stromal cells. Moreover, both the myocardium and bone marrow chemoattract the CXCR4+ satellite cells and CD34+ hematopoietic/muscle progenitor cells. The importance of SDF-1/CXCR4 axis in chemotraction and homing of CXCR4+/CD34+ cells is evidenced by selective inhibition of CXCR4, which significantly reduces the chemotaxis of these cells. It also seems that other chemoattractants have to be involved, because the blockade of CXCR4 does not completely stop the chemotaxis.5 Thus, a possible role of CXCR4+ cells is to maintain the pool of progenitor cells, not only hematopoietic but also other tissue-specific cells, eg, myocardial, and in response to various forms of injury, to be mobilized by SDF-1 secreted by involved tissues. Because the gradient of SDF-1 concentration through the endothelium is an important signal for the stem/muscle progenitor cell homing, perhaps increased secretion of SDF-1 in the ischemic myocardium can direct the flux of cells into the myocardium, thus facilitating tissue repair.10

The levels of the inflammatory cytokines VEGF and G-CSF increase significantly in STEMI patients at all time points compared with the control and SA groups. In the present study, we also observed a significant increase in HGF levels, peaking in the first 48 hours after STEMI, and decreasing to baseline after 7 days. These findings are compatible with other studies showing a rapid increase of HGF and VEGF levels in the setting of STEMI.17,20 HGF has a stimulatory effect on hematopoietic progenitor cells and may play an important role in endothelial and myocardial regeneration.23 In animal models of STEMI, HGF was shown to be upregulated both at the level of transcription, and increased secretion leading to higher plasma levels and treatment with HGF improved the cardiac contractility and may be associated with development of collaterals in the myocardium.24,25 Other possible sources of VEGF, G-CSF, and HGF are EPCs, which were shown to express these cytokines in vitro.26 Cellular effects of HGF are mediated by c-met receptor expressed in endothelial and vascular smooth muscle cells and coupled with cytoplasmic tyrosine kinase.27 We observed a significant increase in the absolute number of the inflammatory cytokines (IL-1, TNF-α) downregulated the SDF-1/CXCR4 axis, but the data pertain to in vitro models (astrocytes, dermal wound healing model) and not to the circulating mononuclear cells.15,16 The use of acetylsalicylic acid (ASA) in patients with angina could interfere with the SDF-1/CXCR4 axis in the platelet-inhibitory mechanism. In this study, different proportions of SA and STEMI patients were treated with ASA, but previously described variations of SDF-1 levels after ASA administration were minor (<5%). Also, the use of statins may affect the mobilization of stem cells, as was shown in regard to atorvastatin. In our study, a significantly higher percentage of patients from the SA group were on statins compared with the STEMI group (60% versus 42%, respectively), so even if the effect exists, the differences between the groups would still be significant.10,12

We observed lower baseline levels of SDF-1 in STEMI patients than in healthy control subjects and patients with SA. In STEMI patients, the differences were noted already on admission, with subsequent increases throughout the observation period but still remaining lower than in SA. Damas et al10 also reported significantly decreased concentrations of SDF-1α in patients with SA and particularly low levels in unstable angina compared with healthy control subjects. SDF-1 is an important signaling factor in embryonic development, particularly in cardiogenesis, vasculogenesis, and hematopoiesis.10–13 SDF-1 interacts with a single specific receptor, CXCR4, thus forming an SDF-1/CXCR4 axis, which is crucial to mobilization, homing, and survival of stem cells (CD34+) and EPCs. In experiments on mice, it was shown that SDF-1–dependent migration of EPCs led to increased neovascularization and improved ischemic tissue perfusion.11,14 We found that of all cytokines, only the SDF-1 level was an independent predictor of a significant increase of CD34+ cells in peripheral blood. As to the receptor for SDF-1, we found a significant increase in the number of CXCR4+ cells in AMI patients on admission and throughout the follow-up period. The altered function of the SDF-1/CXCR4 axis was described by Damas et al10 in patients with SA and unstable angina, in whom low levels of SDF-1α coexisted with reduced cellular membrane expression of CXCR4 on PBMCNCS despite overexpression of the corresponding gene, as evidenced by high CXCR4 mRNA levels in these cells. We observed an increase in absolute number of CXCR4+ cells per unit of blood volume and not an increase of the fluorescence intensity, so perhaps our findings can be explained by increased CXCR4+ cell mobilization, which does not rule out the previously described decrease of the surface expression of this receptor. Other studies showed that
c-met+ cells in STEMI patients compared with both healthy subjects and stable ischemic heart disease patients. The peak mobilization of the c-met+ cells preceded the maximum increase of HGF levels by 24 hours. A possible explanation is either the release of c-met+ cells from the bone marrow and other niches or marked upregulation of its synthesis in the already circulating progenitor cells. Because there is a parallel significant increase in the number of cells positive for CD34 and CD117 antigens, the first mechanism seems more plausible. In an autopsy study, Sato et al28 reported that in the areas of the myocardium surrounding the infarction-related necrosis, the expression of c-met was significantly enhanced compared with control subjects without AMI. Similar findings were described by Ono et al29 who found an increase in HGF mRNA and c-met in first 12 hours, peaking at 24 hours after AMI in the rat. Increased mobilization of the CD34/c-met+ cells in the setting of STEMI may represent a myocardial repair mechanism, because there is a population of tissue-committed muscle/stem progenitor cells circulating in low numbers in peripheral blood and residing in the niches in other tissues that may be readily mobilized and chemotacted into an ischemic area.

The study by Shintani et al,7 which revealed the increase in number of EPCs and CD34+ mononuclear cells (CD34+ MNC) in unstable angina, also showed that among multiple cytokines, only plasma VEGF levels correlate positively with CD34+ MNC number. In our study, the number of CD34+ cells correlated positively with levels of IL-6, VEGF, and SDF-1 and negatively with G-CSF in simple regression. However, in multiple logistic regression, only SDF-1 levels >1000 pg/mL were independent predictors of a significant increase of CD34 cells.

This study has certain limitations. The most important one is the lack of evidence that the cells expressing early cardiac, muscle, and endothelial markers are the same cells as identified in FACS analysis, because the real-time RT-PCR performed in the entire population of mononuclear cells isolated from peripheral blood. Further studies are needed to show the expression of tissue-specific markers in isolated populations of CD34/CXCR4 and CD34/CD117+ cells. Also, we did not establish whether the increased number of progenitor cells results in their homing and migration into the ischemic zone of the myocardium, their actual role in myocardial regeneration. Second, all STEMI patients underwent cardiac catheterization with subsequent percutaneous coronary angioplasty, which is known to evoke an inflammatory reaction and might have influenced the levels of cytokines and progenitor cells. However, the major increase in cell counts was observed at admission, which suggests that percutaneous coronary intervention had no significant effect.

This experiment aimed to test the hypothesis of TCSCs “hiding” in various niches and being mobilized in the setting of tissue damage, which was based on our experimental work in animal models, because it seems a novel and plausible approach to cardiac regeneration research; however, more studies are needed to prove the concept, and its accuracy does not exclude other explanations of myocardial regeneration. Also, the source, or “hide-out,” of the cells has to be established, because this study only showed their increased number in peripheral blood but did not document their place of origin.5,6

Our study demonstrates for the first time the mobilization of mononuclear cells expressing early cardiac, muscle, and endothelial markers in peripheral blood in the setting of STEMI. The principal finding of the study is the presence of a pool of progenitor cells (CD34+, CD117+, CXCR4+, c-met+, and CD34/CD117+) circulating in low numbers in peripheral blood of both healthy subjects and coronary artery disease patients and their rapid mobilization in STEMI. Mobilization of the cells coexists with an increase of CD34/CXCR4+ cells, which were not detected in healthy subjects, and a simultaneous increase in expression of the early cardiac, muscle, and endothelial markers in a population of circulating mononuclear cells that may be a pool of early tissue-committed stem cells.

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References


Mobilization of CD34/CXCR4⁺, CD34/CD117⁺, c-met⁺ Stem Cells, and Mononuclear Cells Expressing Early Cardiac, Muscle, and Endothelial Markers Into Peripheral Blood in Patients With Acute Myocardial Infarction

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