Enhanced functional response of CD133+ circulating progenitor cells in patients early after acute myocardial infarction

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Aims Circulating progenitor cells (PC) may contribute to myocardial recovery following infarction. Growth factors including VEGF are produced during ischaemia and stimulate PC release and activation. In this study, we focused on the functional chemotactic response of PC to VEGF in subjects early after myocardial ischaemia.

Methods and results Number and phenotype of PC were characterized using flow-cytometry. CD133+PC were isolated from peripheral blood using positive MACS isolation. The chemotactic response towards members of the VEGF family (VEGF-A, PlGF-1, and VEGF-E) was analysed in three groups: (i) early period following acute myocardial infarction (days 2–4) treated with primary PCI (AMI) (n=35), (ii) stable coronary artery disease (CAD) (n=35), and (iii) controls (CTR) (n=20). CD133+PC number was 2-fold higher in AMI when compared with CAD and CTR (P=0.0001), whereas CAD was not different from CTR. The chemotactic response of CD133+PC to VEGF-A, PlGF-1, and VEGF-E was significantly enhanced (2-fold) in AMI when compared with CAD (P=0.0001). While the increase of the VEGFR-1-mediated/PlGF-triggered response was rapid (2 days following infarction), the VEGFR-2-mediated/VEGF-E-triggered response was maximally increased on day 4 post-AMI, thus correlating with the kinetics of maximal inflammatory activation reflected by increased CRP levels (P=0.019).

Conclusion The enhanced chemotactic response of CD133+PC following myocardial infarction represents a novel principle potentially involved in cardiovascular repair early after myocardial infarction. Acute inflammatory processes are closely associated with this increased cellular function.

Keywords Progenitor cells • CD133 • Myocardial infarction • Inflammation

Introduction

Accumulating evidence suggests that bone marrow-derived circulating progenitor cells (PC) may have a beneficial effect on the recovery of injured tissues including post-ischaemic myocardium.1–3 Thus, PC mobilization from bone marrow4–8 and recruitment into target tissues9,10 are important steps that may initiate myocardial healing. Chemotaxis, i.e. directed migration towards homing factors, is an important component of PC recruitment. PC chemotaxis towards a gradient of VEGF-A was suggested to be relevant in the recruitment of PC into tissues.11 As VEGF is a functionally important angiogenic growth factor rapidly expressed in ischaemic myocardium,12–14 it may be a good candidate for the recruitment of PC into the heart.9 It has been shown that VEGF, acting through its receptors VEGFR1 and/or VEGFR2, induces the activation of P38,11,15,16 The molecular mechanism of VEGF-A-induced activation of PC is still unknown. Different signalling molecules including phosphoinositide-3-kinase (PI3K), Akt,
and p38 mitogen-activated protein kinase (p38MAPK) are important for VEGF-induced function in adult cells but are probably involved in the activation of PC as well.

Among the multitude of PC subtypes, circulating CD133⁺PC have a high potential to integrate into ischaemic tissues and to contribute to healing by promoting local angiogenesis. CD133⁺PC constitute a heterogeneous population of progenitors which can further be subdivided according to the surface expression pattern of VEGFRs. VEGFR1⁺PC are likely to be early committed progenitors, mainly into the haematopoietic/myeloid-lineage. VEGFR2⁺PC are more immature PC as they are capable to differentiate into several lineages including the endothelial-lineage. However, both subpopulations are important for vascular growth: VEGFR1⁺PC participate in the initiation and stabilization of newly formed vessels, whereas VEGFR2⁺PC promote endothelialization.

Considering the potential of CD133⁺PC in the recovery of ischaemic tissues, a reduced number or dysfunction of these cells could negatively affect myocardial healing as it has been proposed for other circulating vasculogenic cells such as mononuclear cell-derived PC and monocytes.

In the present study, we tested the hypothesis whether CD133⁺PC supply (number) and/or function (VEGF-A-induced chemotaxis) are affected in acute or chronic coronary artery disease (CAD), namely within the early phase after acute myocardial infarction (AMI) or in stable CAD.

**Patients and methods**

The present study conforms to the principles outlined in the Declaration of Helsinki. The study protocol was approved by the Ethical Committee of the University Hospital of Maastricht (The Netherlands) and the one of Ulm University Medical Center (Germany).

**Study design**

The following steps were pursued in the present study. (i) Subjects were enrolled in the outpatient clinic or at the coronary care unit. (ii) Heparinized blood samples (100 mL) were drawn from all subjects. Additional blood samples were taken for assessing clinical chemistry and haematological parameters. (iii) The number of circulating CD133⁺PC, CD133⁺VEGFR1⁺, and CD133⁺VEGFR2⁺ subpopulations was enumerated using flow-cytometry. (iv) Freshly isolated CD133⁺PC were characterized for purity, phenotype, and intrinsic cell cycle activity using flow-cytometry. Moreover, CD133⁺PC were subjected to a chemotaxis assay evaluating their ability to respond to angiogenic stimuli including VEGF-A.

**Study population**

The present study is a prospective pilot study including subjects with acute/chronic CAD and controls (CTR). We discontinuously recruited 35 patients hospitalized for AMI, whereby screening and inclusion was limited by the capacity of the labour-intensive migration assay. The diagnosis of AMI was established following guidelines. Only patients with medium-large infarctions were included, presenting peak creatinine kinase (CKmax) levels >5-fold the upper normal limit (<24 h of hospitalization). All patients were successfully treated with primary PCI within 4 h from onset of symptoms and followed a similar anticoagulation and medication (beta-blocker, ACE-inhibitor, and statin) regimen according to established guidelines. For the initial 15 patients, blood sampling was done on day 2 (AMI-d2) following onset of symptoms and primary PCI. The next 20 patients were recruited on day 4 (AMI-d4) following primary PCI.

In addition, we studied 35 patients with stable CAD meeting the following criteria: (i) history of transient episodes of typical chest pain on effort, stable for more than 3 months and absent in rest; (ii) documented exercise-induced myocardial ischaemia; and (iii) angiographically proven CAD. Patients were recruited during a regular visit at the outpatient clinic.

Ten CTR subjects were included at the outpatient clinic as well. They were seen for benign arrhythmias, but were without a history or signs of CAD.

Exclusion criteria were history of renal, hepatic, hematological, or coagulation disorders, AMI in the preceding year, other acute or chronic inflammatory diseases including diabetes mellitus, malignancies, recent trauma, recent surgery or recent (<6 months) major bleeding requiring blood transfusion. Clinical parameters of the study population including demographic data and prevalence of cardiovascular risk factors are presented in Table 1.

A preliminary analysis of the initial data of the study indicated differences between VEGF-A- and PlGF-1-induced chemotaxis of CD133⁺PC. Therefore, VEGF-E-induced chemotaxis was introduced for a more accurate characterization of the VEGF system. The CD133⁺PC subpopulations, VEGFR1/2 expression and cell cycle profile of CD133⁺PC were analysed prospectively in a limited number of subjects randomly selected from the AMI, CAD, and CTR groups.

**Flow-cytometric analysis**

The analysis was accomplished on a FACSCalibur flow-cytometer using the CellQuest software (Becton Dickinson). Antibodies used for staining are listed in Supplementary material online, Supplement-1. Cellular staining followed protocols previously described.

**Enumeration of CD133⁺PC from whole blood**

The flow-cytometric analysis of the number of CD133⁺PC was performed to a standardized protocol (see Supplementary material online, Supplement-2).

**Isolation of circulating CD133⁺PC from peripheral venous blood**

CD133⁺PC were isolated from peripheral blood by immunomagnetic selection (CD133-MACS, Miltenyi Biotec) following manufacturer’s instructions. Purity of isolation was between 85 and 90% as detected by flow-cytometry. Subpopulations of CD133⁺PC were evaluated by flow-cytometry as presented in Supplementary material online, Supplement-3.

**Cell cycle analysis**

Cell cycle analysis was performed according to an established protocol. Briefly, freshly isolated CD133⁺PC were incubated with propidium iodide for DNA staining and analysed using flow-cytometry.

**Chemotaxis analysis**

The chemotactic response of freshly isolated CD133⁺PC was evaluated upon stimulation with VEGF-A, PlGF-1, or VEGF-E. For analysis of different sensing pathways involved in CD133⁺PC chemotaxis, inhibitors of different signalling cascades (LY294002, Akt inhibitor,
and SB203580) were used in the chemotaxis assay (see Supplementary material online, Supplement-4).

**Statistical methods**

Statistical analysis was performed using SPSS software (SPSS Inc.). The frequency distribution of each variable was assessed for skewness to check whether the variable was approximately normally distributed. Results are expressed as mean ± standard deviation (SD). In addition, the medians (in the figures: median as line, 25th–75th percentiles as whiskers) are given in case of non-normally distributed results. Owing to the non-Gaussian distribution and relatively small number of subjects in some of the studied subgroups (CD133+PC subpopulations and AMI-d2 and AMI-d4 subgroups), non-parametric Mann–Whitney test was used to detect differences between groups and subgroups. Differences were considered significant when P < 0.05 (two-tailed) after Bonferroni correction. Due to differences in group sizes, power calculation was performed for 3 inter-group comparisons (CTR, CAD, and AMI) based on the overlap rate of the collected data (α = 0.0167 due to Bonferroni correction). For correlation analyses, we used Spearman’s rho.

**Results**

**Patient characteristics**

The present study was designed to analyse the number and function of immature CD133+PC early after AMI and in stable CAD. Both groups displayed comparable demographic characteristics and displayed comparable cardiovascular risk profiles. When compared with AMI-patients, significantly fewer patients with CAD took one or more cardiovascular drug (P < 0.05) (Table 1). No difference in infarct size (CKmax) was observed between the AMI subpopulations, AMI-d2 and AMI-d4, indicating an unbiased

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**Table 1 Clinical baseline characteristics of the study population**

<table>
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<th>CTR, n = 10</th>
<th>CAD, n = 35</th>
<th>AMI, n = 35</th>
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<td><strong>Demographic characteristics</strong></td>
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<tr>
<td>Age, years</td>
<td>59.8 ± 7.2</td>
<td>62.5 ± 9.5</td>
<td>59.9 ± 11.7</td>
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<td>Gender (male), n (%)</td>
<td>8 (80.0)</td>
<td>31 (88.6)</td>
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<td><strong>Cardiovascular risk factors</strong></td>
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<tr>
<td>Family history of CAD, n (%)</td>
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<td>21 (60.0)</td>
<td>27 (77.1)</td>
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<td>Hypertension, n (%)</td>
<td>5 (50.0)</td>
<td>18 (51.4)</td>
<td>12 (34.3)</td>
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<td>Hypercholesterolaemia, n (%)</td>
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<td>27 (77.1)</td>
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<td>6 (17.1)</td>
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<td>Diabetes mellitus, n (%)</td>
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<td><strong>Number of cardiovascular risk factors</strong></td>
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<td>0, n (%)</td>
<td>1 (10.0)</td>
<td>2 (5.7)</td>
<td>4 (11.4)</td>
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<td>1, n (%)</td>
<td>4 (40.0)</td>
<td>7 (20.0)</td>
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<td>&gt;1, n (%)</td>
<td>5 (50.0)</td>
<td>26 (74.3)</td>
<td>23 (65.7)</td>
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<td><strong>Laboratory parameters</strong></td>
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<td>Cholesterol (total), mmol/L</td>
<td>6 ± 3.8</td>
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<td>6 ± 1.8</td>
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<td>HDL-cholesterol, mmol/L</td>
<td>1.3 ± 0.9</td>
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<td>Triglycerides, mmol/L</td>
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<td>CK/CKmax, IU/L</td>
<td>n.d.</td>
<td>122 ± 70.9</td>
<td>2561 ± 1595.7</td>
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<td>Troponin L, ng/mL</td>
<td>n.d.</td>
<td>n.d.</td>
<td>11.8 ± 42.5</td>
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<td>WBC, ×10^9/L</td>
<td>7 ± 1.3</td>
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<td>12 ± 3.5</td>
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<td>CRP², mg/L</td>
<td>2.8 ± 5.4</td>
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<td><strong>Medication at admission in the present study</strong></td>
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<td>5 (50.0)</td>
<td>23 (65.7)</td>
<td>35 (100)²</td>
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<td>Beta-blockers, n (%)</td>
<td>4 (40.0)</td>
<td>18 (51.4)</td>
<td>35 (100)²</td>
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<td>19 (54.3)</td>
<td>35 (100)²</td>
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<td>Statins, n (%)</td>
<td>3 (30.0)</td>
<td>23 (65.7)</td>
<td>35 (100)²</td>
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<td>History of prior AMI, n (%)</td>
<td>—</td>
<td>17 (48.6)</td>
<td>0 (0)</td>
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<td>Time from prior AMI to present study, years</td>
<td>range 1–25</td>
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Data are presented as number (percentage) or as mean ± SD.

n.d., not detected.

¹Data presented also in Figure 3A and B; ²average levels of CRP between day 2 and day 4 post-AMI; ³medication on day 2 or day 4 post-AMI.
selection of the two patient groups (AMI-d2, 2478.7 ± 1428.4 vs. AMI-d4, 2346.4 ± 1295.7, \( P = 0.594 \)).

The number of circulating CD133\(^+\)PC increases following myocardial infarction

Although CD133\(^+\)PC number in CAD and in CTR was similar, a significant increase in CD133\(^+\)PC number was observed within days 2–4 post-AMI compared with both CAD and CTR (Figure 1A) (see Supplementary material online, Supplement-2B, Table-S2-1). In detail, the number of CD133\(^+\)PC remained constantly high between day 2 and day 4 (AMI-d2 vs. AMI-d4, \( P = 0.271 \); AMI-d2 vs. CAD, \( P = 0.001 \); AMI-d2 vs. CTR, \( P = 0.001 \); AMI-d4 vs. CAD, \( P = 0.004 \); AMI-d4 vs. CTR, \( P = 0.001 \)). This increase in CD133\(^+\)PC number was paralleled by the rise of total WBC early post-AMI. No significant differences in CD133\(^+\)PC percentages were observed between the groups (see Supplementary material online, Supplement-2B, Table-S2-1).

No correlation could be detected between the presence of risk factors and the number of CD133\(^+\)PC in CAD and AMI. Likewise, the number of CD133\(^+\)PC did not correlate with the left ventricular ejection fraction (LVEF) on day 7 post-AMI (data not shown).

We further analysed the absolute number of CD133\(^+\)VEGFR1\(^+\)PC and CD133\(^+\)VEGFR2\(^+\)PC. The number of both subpopulations increased significantly following AMI (Figure 1B and C) (see Supplementary material online, Supplement-2B, Table-S2-2). Their number increased already on day 2 post-AMI and remained constantly elevated to day 4 (AMI-d2 vs. AMI-d4, \( P = 0.120 \) and \( P = 0.647 \), respectively) (see Supplementary material online, Supplement-2B, Table-S2-2). By analysing the percentage of CD133\(^+\)VEGFR1\(^+\)PC and CD133\(^+\)VEGFR2\(^+\)PC within the total population of CD133\(^+\)PC, we observed for both subpopulations a tendency towards increase in the early post-AMI period (see Supplementary material online, Supplement-2B, Table-S2-2). This parallel increase of CD133\(^+\)VEGFR1\(^+\)PC and CD133\(^+\)VEGFR2\(^+\)PC cells may indicate a possible overlap between the expression of VEGFR1 and VEGFR2 and the presence of a double-positive CD133\(^+\)VEGFR1\(^+\)VEGFR2\(^+\) subpopulation following AMI. Similar levels of CD133\(^+\)VEGFR1\(^+\)PC and CD133\(^+\)VEGFR2\(^+\)PC subpopulations were observed in CAD and CTR (Figure 1B and C) (see Supplementary material online, Supplement-2B, Table-S2-2).

Cell cycle analysis of CD133\(^+\)PC

CD133\(^+\)PC were analysed for their proliferative state. Whereas the majority of freshly isolated CD133\(^+\)PC from CTR and CAD were in G0/G1-phase, the percentage of cells in G0/G1-phase tended to increase following AMI (median of 1.2% compared with 0.8% CTR and 0.7% CAD, respectively; \( n = 3 \) per group) (data not shown).

The chemotactic response of CD133\(^+\)PC to VEGF stimulation increases following myocardial infarction

First, we demonstrated that CD133\(^+\)PC from CTR show a consistent and strong chemotactic response to VEGF-A (Figure 2A) (See Supplementary material online, Supplement-4B). This chemotactic response involves the activation of downstream signalling
molecules including PI3K, Akt, and p38MAPK (see Supplementary material online, Supplement-4C).

In contrast, VEGF-A-induced chemotaxis of CD133⁺PC was influenced by pathological conditions such as CAD and AMI. CD133⁺PC from CAD showed a trend towards decreased chemotaxis compared with CTR. In contrast, VEGF-A-induced chemotaxis of CD133⁺PC was significantly enhanced early following AMI (P = 0.0001 AMI-d2 vs. CAD) and further increased between day 2 and day 4 post-AMI (Figure 2A).

PlGF-1-induced chemotaxis (VEGFR1⁺ cells) was weaker in CAD than in CTR. The chemotactic response increased early following AMI (P = 0.002 AMI-d2 vs. CAD) and did not further increase on day 4 post-AMI (Figure 2B).

In case of VEGF-E-induced chemotaxis (VEGFR2⁺ cells), no significant difference was noticed between CAD and CTR. As for VEGF-A, stimulation of CD133⁺PC with VEGF-E resulted in significantly enhanced chemotaxis, mainly on day 4 post-AMI (P = 0.0001 vs. CAD, P = 0.047 vs. AMI-d2), as AMI-d2 reached comparable levels to CAD (Figure 2C).

The presence of cardiovascular risk factors (Table 1) had no effect on CD133⁺PC response in CAD and AMI (data not shown).

To test whether differences in medication may influence CD133⁺PC migratory response, we analysed the potential influence of anticoagulants, beta-blockers, ACE-inhibitors, and statins as part of the standard therapy of CAD and AMI (Table 1). In case of CAD, no significant differences were observed between treated and non-treated subgroups with regard to the number or VEGF-induced chemotaxis of CD133⁺PC (data not shown). As all AMI-patients had a similar standardized medication, we could only analyse, whether the pre-AMI medication influences CD133⁺PC post-AMI. A fraction of 22.9% of AMI-patients had a history of CAD and was on standard therapy prior to the AMI event. CD133⁺PC reached similar numbers and chemotactic levels post-AMI irrespective of a history of CAD. No correlation between pre-medication and CD133⁺PC number or VEGF-A-induced chemotaxis was observed (data not shown).

In addition, a moderate correlation between CD133⁺PC chemotaxis and LVEF on day 7 post-AMI was observed (Figure 3A).

The enhanced number and chemotactic response of CD133⁺PC correlates with the activation of inflammation following acute myocardial infarction

To understand the activation of CD133⁺PC post-AMI, we have examined a possible relation between different AMI parameters (infarct size, level of acute inflammation) and CD133⁺PC function.

The CRP level, reflecting infarct size, was reached between 3–6 h following primary PCI (n = 8, 3 h, 3310.3 ± 940.6 U/L; 6 h, 2740.6 ± 4336.6 U/L; 24 h, 1659.4 ± 4242.2 U/L) (Figure 2A). Likewise, CRP levels significantly increased from admission and reached a maximum about day 4 (n = 8, 7.1 ± 3.0 vs. 127.0 ± 11.4 mg/L, P = 0.0001) (Figure 3B).

The number of CD133⁺PC was significantly higher in AMI-patients with a larger infarct size compared with those with smaller infarcts (CKₘₐₓ > median 2300 U/L, 8671.1 ± 1187.7 cells/mL blood; CKₘₐₓ < 2300 U/L, 6524.4 ± 1001.8, P = 0.017).

CRP levels correlated with enhanced CD133⁺PC number early after AMI (AMI-d2, R = 0.775, P = 0.002) (data not shown). No such correlation was detected in AMI-d4 patients.

The enhancement of CD133⁺PC response towards VEGF-A correlated with both CKₘₐₓ values and CRP levels on day 4 post-AMI (Figure 3C and D, left). PlGF-1-induced chemotaxis (VEGFR1⁺ cells) was lacking a significant correlation with CKₘₐₓ or CRP levels on day 4 post-AMI, suggesting an early (<4 days) activation of the corresponding CD133⁺VEGFR1⁺PC (Figure 3C and D, middle). In contrast, VEGF-E-induced chemotaxis (VEGFR2⁺ cells) correlated with the CKₘₐₓ or CRP levels on day 4 post-AMI (Figure 3C and D, right).

Subanalysis of the correlation of CD133⁺PC number and function (chemotaxis) revealed that AMI-patients with higher CRP levels (CRP>median of 78 mg/L) had both significantly elevated CD133⁺PC numbers as well as stronger chemotactic responses compared to those with lower CRP levels (9295.6 ± 5083.3 vs. 4894.7 ± 2623.9 cells/mL, P = 0.010; 190.7 ± 29.5% vs. 169.3 ± 21.6% migration, P = 0.008) (data not shown). This clearly points towards an influence of acute inflammation on PC number and function.

In an attempt to explain the increased chemotactic response of CD133⁺PC to VEGF, we analysed possible differences in VEGF-receptor levels in AMI or CAD. However, we could not detect any changes in VEGFR1- or VEGFR2-expression between the study groups (see Supplementary material online, Supplement-3A).

Discussion

The release of PC from the bone marrow and their subsequent migration into the heart are two critical steps preceding the involvement of PC in myocardial healing and functional myocardial recovery. It is therefore important to elucidate whether these steps are affected under pathologic conditions associated with myocardial ischaemia. We have demonstrated that acute myocardial ischaemia leads to an increased number of circulating CD133⁺PC. Moreover, CD133⁺PC show a significantly increased chemotactic response towards VEGF-A in the early post-infarction period. Both CD133⁺PC number and chemotactic response to VEGF-A positively correlate with infarct size (CKₘₐₓ) and with the acute inflammatory response (CRP) post-AMI.

Under physiologic conditions, only low levels of PC are released in the peripheral blood, but acute myocardial ischaemia was shown to significantly enhance the mobilization of PC. Accordingly, we observed that the pool of circulating CD133⁺PC (CD133⁺CD45⁻CD34⁺) significantly increases in patients within the first days after AMI following successful reperfusion therapy. The release of PC after AMI is a rapid and prolonged process as the number of CD133⁺PC is increased already on day 2 and remains constantly elevated within the early period following AMI in accordance with previous reports on CD133⁺PC and CD34⁺PC.

The majority of the mobilized CD133⁺PC are in a non-proliferating, quiescent phase. However, we observed a tendency towards an increased fraction of CD133⁺PC in G2/S/M-phase, potentially indicating intense mobilization from bone marrow similar to the one observed under strong, i.e. multifactorial...
Figure 2 Chemotactic response of CD133⁺PC towards VEGF family members. CD133⁺PC were isolated from controls (CTR), stable coronary artery disease (CAD), and acute myocardial infarction (AMI) (samples from either day 2 or day 4, AMI d2–d4). Chemotactic response of CD133⁺PC towards VEGF-A (1 ng/mL) (A), PlGF-1 (1 ng/mL) (B), or VEGF-E (1 ng/mL) (C) was determined. Moreover, subanalysis of CD133⁺PC chemotactic response on day 2 (AMI-d2) vs. day 4 post-acute myocardial infarction (AMI-d4) is shown (right panels). Degree indicates outlier.
The physiologic relevance of PC mobilization after AMI is not well understood. Mobilized PC may just replenish the pool of inflammatory cells in circulation or, as demonstrated in experimental models, may promote the autoprotective mechanism of vessel growth. Neovessels arise around day 2 after AMI and have a positive impact on myocardial healing by supporting myocardial metabolism and by transporting inflammatory cells involved in healing. Since CD133⁺ PC are proved to be highly effective in in vitro capillarization, the fast rise of CD133⁺ PC post-AMI may be related to the early initiation of vascular growth in ischaemic conditions.

**Figure 3** Time course of CK and CRP after acute myocardial infarction. Correlation with chemotactic response of CD133⁺ PC towards VEGF family members. Serum levels of CK (A) and CRP (B) in the early post-acute myocardial infarction period. (C) The chemotactic response of CD133⁺ PC towards VEGF-A (1 ng/mL), PlGF-1 (1 ng/mL), or VEGF-E (1 ng/mL) was correlated with CKmax and the levels of CRP (day 4 post-acute myocardial infarction). *P < 0.05.
myocardium. Moreover, experimental and clinical data have shown that PC, once mobilized into circulation, incorporate rapidly into ischaemic tissues. The accumulation of PC in the heart relies on their directed-migration (chemotaxis) towards growth factors and cytokines locally expressed in ischaemic/infarcted myocardium.

It was previously demonstrated that the chemotactic response of CD34\(^+\)PC towards SDF-1, a potent stem cell chemoattractant highly expressed in bone marrow, strongly correlated with in vivo repopulation and haematopoietic bone marrow recovery after autologous PC-transplantation.\(^2\) On this basis, we have analysed the chemotactic response of CD133\(^+\)PC as a surrogate ex vivo-parameter correlating with recruitment of CD133\(^+\)PC and contribution to myocardial healing post-AMI. In ischaemic myocardium, VEGF-A is a dominant stimulus for vessel growth, rapidly accumulating early after onset of AMI.\(^1\) Vessel growth following AMI closely follows the time course of VEGF-A expression,\(^3\) SDF-1, a potent chemoattractant for PC, was shown to presents only a transient expression post-AMI with increased expression in the first hours\(^6\) but baseline\(^13,14\) or even reduced expression after a few days.\(^26,27\) Intramyocardial injection of SDF-1 increases the recruitment of PC, but this effect seems VEGF-A-dependent since blocking local VEGF-A significantly affected PC recruitment.\(^28\) Likewise, IL-8-expression is significantly up-regulated in the peri-infarct area.\(^24\) However, CD133\(^+\)PC (own unpublished data) and other subpopulations of immature PC\(^29\) show no consistent migratory response towards IL-8.

We demonstrate here that CD133\(^+\)PC from AMI-patients exhibit a strong chemotactic response towards VEGF-A. The response of CD133\(^+\)PC is significantly enhanced already on day 2 and even increases further to day 4 post-AMI suggesting an active recruitment of CD133\(^+\)PC to infarcted myocardium. In contrast, CD133\(^+\)PC from CAD show only a poor chemotactic response to VEGF-A. This indicates that low-grade chronic inflammation in the absence of prolonged myocardial ischaemia is unable to a sustained PC activation as also seen in other chronic diseases.\(^30\) Likewise, acute inflammation was shown to increase PC levels even in the absence of myocardial necrosis (unstable angina or exercise-induced ischaemia) or of myocardial ischaemia (burns, pneumonia, or sepsis).\(^30\) Indeed, the mobilization of CD133\(^+\)PC from bone marrow is unlikely to represent a specific pro-healing response post-AMI. Our data indicate that the increased PC number is not due to increased percentage of CD133\(^+\)PC in the circulation, but is rather due to increased leukocyte mobilization following the general inflammatory stress response. Moreover, migration and consequent repopulation of bone marrow were more efficient when PC transplantation coincided with the maximum increase of inflammatory cytokines.\(^31\)

We demonstrate here that acute inflammation per se (elevated CRP levels) and infarction size (CK\(_{\max}\)) positively correlate with the number and chemotaxis of CD133\(^+\)PC in the early post-AMI period. Many inflammatory factors may be involved in PC activation including IL-6, IL-8, and G(M)-CSF.\(^30\) Of these, IL-6, an acute-phase-reaction cytokine, increases within hours post-AMI.\(^4\) Previous reports demonstrated a rapid mobilization of both CD34\(^+\)PC\(^6,7\) and CD133\(^+\)PC\(^32\) in \(<24\) h after onset of AMI. Therefore, a correlation between early IL-6 levels and PC mobilization may be incriminated.\(^4\) Moreover, IL-6-treated progenitors showed an increased recruitment into irradiated bone marrow.\(^31\) IL-8, another inflammatory factor, increases early post-AMI correlating with CD133\(^+\)PC number.\(^5\) However, owing the pleiotropic and overlapping action of many inflammatory factors, it is difficult to establish a strong relation between cytokine levels and PC number/function,\(^30\) reflecting the complex nature of this process.

Furthermore, our data show that particularly those AMI-patients with higher CRP levels presented with abundant CD133\(^+\)PC numbers and with a stronger chemotactic response. Acute inflammation may therefore be an essential component initiating myocardial healing by inducing CD133\(^+\)PC mobilization from bone marrow and recruitment to infarcted myocardium. However, despite a possible link between PC and vascular healing, our data showed only a weak correlation between CD133\(^+\)PC number/chemotaxis and improvement of myocardial function (LVEF) within the first week post-AMI. This time-point may be too early for assessment of myocardial function as suggested previously for CD34\(^+\)PC.\(^5\) On the other hand, our study was neither designed nor powered to establish such a causal relationship. Nevertheless, long-term follow-up studies have demonstrated that greater PC mobilization early post-AMI correlates with better improvement of myocardial function.\(^7,33\)

In support of the idea that PC contribute to vascular growth, we found an increase of two specific subpopulations of PC, which were shown to participate in the process of vascular growth: VEGFR1\(^+\)PC show a fast recruitment into ischaemic tissues, where they initiate the angiogenic process.\(^16\) Further, VEGFR2\(^+\)PC promote endothelialization of the growing vasculature,\(^16\) but might still require the presence of VEGFR1\(^+\)PC,\(^16\) which sustain the angiogenic process in a paracrine fashion.\(^3\) Of novelty, we tested the chemotactic response of both PC subpopulations. Since VEGF-A acts through both VEGFR1 and VEGFR2, we used the receptor-specific ligands PIGF-1 and VEGF-E to test receptor-specific chemotaxis. CD133\(^+\)VEGFR1\(^+\)PC showed a constantly higher chemotactic response within the first four days post-AMI, whereas chemotaxis of CD133\(^+\)VEGFR2\(^+\)PC increased between day 2 and 4 post-AMI, strongly correlating with increased CRP levels. This is compatible with the notion that CD133\(^+\)VEGFR1\(^+\)PC play a role in the initial period of vascular healing, while CD133\(^+\)VEGFR2\(^+\)PC reach their maximal functional responsiveness around day 4 post-AMI or even later.

As we did not detect any changes in the levels of VEGFR1- and VEGFR2-expression, the enhanced chemotactic response post-AMI appears to be localized downstream of the receptors. The signalling cascade responsible for CD133\(^+\)PC migration might be pre-activated in the cytokine-rich environment early post-AMI. As this explanation remains unproven so far, the activation of CD133\(^+\)PC in AMI warrants further investigation. This is even more interesting as the functional PC increase observed post-AMI seems not to be related to the pre-existence of CAD. CAD-patients present normal CD133\(^+\)PC or CD34\(^+\)PC numbers,\(^5\) but PC have a tendency towards a reduced chemotactic response similar to a reduced clonogeneity and differentiation as described previously.\(^18\) CD133\(^+\)VEGFR1\(^+\)PC in particular show a decreased responsiveness suggesting that not the endothelialization (CD133\(^+\)VEGFR2\(^+\)PC-dependent), but the initiation or stabilization of newly formed vessels (CD133\(^+\)VEGFR1\(^+\)PC-dependent) is inefficient in CAD.
As seen in other chronic conditions (heart failure, rheumatoid arthritis, chronic obstructive pulmonary disease), cardiovascular risk factors or a steady low-grade inflammation may decrease the function of CD133⁺PC. We are currently lacking a molecular explanation for the improved PC chemotaxis post-AMI, which overcomes PC dysfunction seen in CAD. However, it is tempting to hypothesize that the dysfunction of PC is reversible and that in vitro or even in vivo treatment of PC, mimicking acute inflammatory conditions, could upregulate their function and improve their recruitment into ischemic myocardium.

The novelty of the current study lies in the functional characterization of PC ex vivo, largely resembling the in vivo situation. We depicted PC based on the expression of the specific marker CD133. Other markers such as CD34⁺ are not PC-specific, as they are also present on adult endothelial cells released into the circulation. The in vivo characteristics of PC are closely reflected in our experimental setting, as we analysed migration of freshly isolated PC. Using this strategy, we are avoiding long-term in vitro differentiation and selection of PC, which could artificially affect both PC phenotype and function.

Study limitations

The major limitation of this study is the lack of mechanistic explanation on the enhanced CD133⁺PC mobilization and chemotaxis. Detailed analyses are limited by the low number of these cells in circulation. Due to the complexity of inflammatory reactions, the study was not designed and powered to find relevant correlations between specific cytokine levels and PC number/function. We did not determine the fate of mobilized CD133⁺ cells; therefore, the functional characterization of these cells in vivo remains to be done. This study was designed to identify CAD- and AMI-related changes in PC number and function, but it was not powered nor designed to assess the prognostic significance of these parameters. Patients with diabetes mellitus, an important cardiovascular risk factor, were excluded from the present study as diabetes itself influences many of the mechanisms which we analysed.

Conclusions

Taken together, our data indicate that CD133⁺PC are sensitive to the pathological conditions of AMI regarding their number and functional properties: the number and the migratory response of CD133⁺PC significantly increases early following AMI, a finding that correlated well with the elevated level of inflammation post-AMI. Therefore, distinct inflammatory stimuli may not only indicate adverse events in acute coronary syndromes but may also induce beneficial effects such as PC mobilization. Our observation may help to improve the understanding of cellular repair processes in cardiovascular pathology.

Supplementary material

Supplementary material is available at European Heart Journal online.

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