Cardiac Expression of Placental Growth Factor Predicts the Improvement of Chronic Phase Left Ventricular Function in Patients With Acute Myocardial Infarction

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OBJECTIVES
Our aim was to investigate cardiac expression of placental growth factor (PIGF) and its clinical significance in patients with acute myocardial infarction (AMI).

BACKGROUND
Placental growth factor is known to stimulate wound healing by activating mononuclear cells and inducing angiogenesis. The clinical significance of PIGF in AMI is not yet known.

METHODS
Fifty-five AMI patients and 43 control subjects participated in the study. Peripheral blood sampling was performed on days 1, 3, and 7 after AMI. Blood was also sampled from the coronary artery (CAos) and the coronary sinus (CS), before and after acute coronary recanalization. Cardiac expression of PIGF was analyzed in a mouse AMI model.

RESULTS
In AMI patients, peripheral plasma PIGF levels on day 3 were significantly higher than in control subjects. Plasma PIGF levels just after recanalization were significantly higher in the CS than the CAos, which indicates cardiac production and release of PIGF. Peripheral plasma levels of PIGF on day 3 were negatively correlated with the acute phase left ventricular ejection fraction (LVEF), positively correlated with both acute phase peak peripheral monocyte counts and chronic phase changes in LVEF. Placental growth factor messenger ribonucleic acid expression was 26.6-fold greater in a mouse AMI model than in sham-operated mice, and PIGF was expressed mainly in endothelial cells within the infarct region.

CONCLUSIONS
Placental growth factor is rapidly produced in infarct myocardium, especially by endothelial cells during the acute phase of myocardial infarction. Placental growth factor might be over-expressed to compensate the acute ischemic damage, and appears to then act to improve LVEF during the chronic phase. (J Am Coll Cardiol 2006;47:1559–67) © 2006 by the American College of Cardiology Foundation

After acute myocardial infarction (AMI), reducing the total amount of necrotic myocardium and minimizing ventricular remodeling are the most effective ways of preserving long-term left ventricular function and improving prognosis (1). In that regard, a variety of cytokines and circulating cells are known to participate in the wound healing processes that are ongoing in the injured myocardium of the post-myocardial infarction heart, and it has been suggested that appropriate manipulation of those molecules and/or cells could be used to exert a beneficial effect that promotes long-term improvement of cardiac function after AMI (2–4).

Recent progress in stem cell research has opened the possibility of regenerating lost cardiomyocytes and/or vascular tissue by recruiting autologous bone-marrow-derived stem cells to the heart after AMI. Among the candidate cytokines considered for this purpose, vascular endothelial growth factor (VEGF) has been extensively studied because of its established angiogenic capacity and its ability to mobilize endothelial progenitor cells (5). Vascular endothelial growth factor is known to be strongly expressed in the ischemic myocardium, and treatment with exogenous VEGF has been clearly demonstrated to enhance vessel formation in ischemic myocardium (6,7). Still, in vivo treatment with VEGF gene or recombinant VEGF protein does not always lead to improved ventricular function because of the development of non-functioning or unstable capillary vessels (8,9).

Placental growth factor (PIGF) is a member of the VEGF family that acts via VEGF receptor-1 (flt-1) (10,11). Like VEGF, PIGF has been shown to play a key role in vascular development under pathological conditions; for instance, by stimulating both angiogenesis and arteriogenesis, PIGF enhances not only capillary but also collateral formation in ischemic tissue (12). In addition, PIGF appears to promote mobilization of flt-1-positive hematopoietic stem cells that might be involved in regeneration of vessels and myocardium from bone marrow to the peripheral circulation (13,14).

With that as background, our aim in the present study was to assess cardiac expression of PIGF in both human AMI patients and a mouse model of AMI, and to determine the impact of PIGF expression on the clinical features of AMI in humans.

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The clinical parameters assessed included age, gender, and coronary risk factors (smoking, hypertension, diabetes mellitus, hyperlipidemia, and obesity). The diagnostic criteria for the coronary risk factors were as follows: hypertension, blood pressure more than 140/90 mm Hg, and/or a history of taking antihypertensive medication; diabetes mellitus, fasting plasma glucose more than 126 mg/dl, or casual plasma glucose more than 200 mg/dl, or a diabetic pattern in 75 g OGTT; hyperlipidemia, serum total cholesterol levels of >220 mg/dl or serum triglyceride levels of >150 mg/dl; obesity, body mass index of more than 25 kg/m². Infarct site, peak CK, peak CK-MB, LVEF, and coronary angiographical findings were selected for analysis as indicators of MI severity.

**BLOOD SAMPLING AND ANALYSIS.** Peripheral blood samples were collected from all subjects upon admission and then on the third and seventh hospital days. In 30 patients, moreover, blood was sampled during emergency cardiac catheterization from the coronary artery ostium (CAos) and coronary sinus (CS) using a 4-F coronary catheter (Goodman, Tokyo, Japan) and a 6-F CS catheter (Goodman, Tokyo, Japan) before and 30 to 45 min after emergency PCI. Plasma samples were collected in EDTA anticoagulant tubes and stored at −80°C until assayed.

White blood cell counts, profiles, and peripheral blood monocyte counts were obtained using Sysmex SE9000 (Sysmex, Kobe, Japan). Blood biochemistry data were obtained using commercially available assays.

Levels of PlGF and VEGF were measured using commercially available enzyme-linked immunosorbent assay kits (DPG00, DVE00, respectively; R&D Systems, Minneapolis, Minnesota). This enzyme-linked immunosorbent assay kit also cross-reacts with human recombinant PlGF-2 isofrom (5%) and also reacts with human recombinant VEGF/hrPlGF heterodimer (5%). For peripheral blood samples, maximum values on the day of admission and on the third and seventh hospital days were considered to be the peak levels for each cytokine at those times.

**ANALYSIS OF CORONARY ANGIOGRAPHY AND LEFT VENTRICULOGRAPHY.** Coronary angiograms and left ventriculograms were analyzed by two independent angiographers without knowledge of the patients’ background. Antegrade flow in the infarct-related coronary artery at the initial examination, after recanalization and during follow-up, was graded according to the Thrombolysis in Myocardial Infarction classification (15). Global LVEF was obtained from the right anterior oblique projection of contrast left ventriculography (QLV-CMS; MEDIS, Leiden, the Netherlands).

**Experimental study. Mouse model of AMI.** After anesthetizing 10- to 12-week-old male mice (C57BL/6, SLC, Hamamatsu, Japan) using isoflurane with artificial ventilation, thoracotomy was performed in the left intercostal space, and a proximal site of the left coronary artery was ligated as described previously (16). The duration of coronary artery ligation was 40 or 60 min in the ischemia-reperfusion (IR) model, and permanent ligation in the MI model. Mice were sacrificed under general anesthesia with pentobarbital, and the heart was excised on days 3 and 7 after surgery in the IR model and on days 1, 3, 7, and 28 after surgery in the MI model.

**QUANTITATIVE REAL-TIME POLYMERASE CHAIN REACTION.** Frozen infarct heart tissue, excised on day 3 after surgery, was homogenized in Trizol reagent (Invitrogen Corp., Carlsbad, California) using the standard protocol. After DNase processing, PlGF cDNA was synthesized using Superscript II reverse transcriptase (Invitrogen Corp.). Relative PlGF mRNA levels were then determined using real-time polymerase chain reaction carried out using cDNA samples with Assay-on-Demand Product, Taqman

### Abbreviations and Acronyms

- **AMI**: acute myocardial infarction
- **CAos**: coronary artery ostium
- **CK**: creatine kinase
- **CS**: coronary sinus
- **flt-1**: vascular endothelial growth factor receptor-1
- **IR**: ischemia-reperfusion
- **LVEF**: left ventricular ejection fraction
- **MI**: myocardial infarction
- **PCI**: percutaneous coronary intervention
- **PlGF**: placental growth factor
- **VEGF**: vascular endothelial growth factor

**METHODS**

### Clinical study. PATIENT POPULATION.

Fifty-five patients admitted to Nara Medical University Hospital with AMI were enrolled in this study. In addition, 43 age-matched healthy subjects (mean age 62.7 ± 1.5 years, 33 men, 10 women) served as control subjects. Acute MI was diagnosed when patients experienced chest pain within 24 h before admission and lasted more than 30 min and was not relieved by sublingual nitroglycerin, and exhibited ST-segment elevation and/or abnormal Q waves on an electrocardiogram and elevated serum creatine kinase (CK) levels. Exclusion criteria were AMI more than 24 h from the onset, a history of renal dysfunction requiring dialysis, evidence of malignant disease, or an unwillingness to participate. All patients received coronary angiography on admission. When patients had an occluded coronary artery suitable for percutaneous coronary intervention (PCI) using coronary stents, the patients underwent emergency PCI. After PCI, patients underwent left ventriculography to assess the left ventricular ejection fraction (LVEF) in acute phase. Then they were routinely treated with heparin, isosorbide dinitrate, nicorandil, ticlopidine, aspirin, and an angiotensin-converting enzyme inhibitor or angiotensin II receptor blocker.

The protocol was approved by our institutional ethics committee (#2002–009, Nara Medical University Ethics Committee), and written informed consent was obtained in all of cases from either the patient or his/her family members.

### CLINICAL PARAMETERS OF AMI PATIENTS.

The clinical parameters assessed included age, gender, and coronary risk factors (smoking, hypertension, diabetes mellitus, hyperlipidemia, and obesity). The diagnostic criteria for the coronary risk factors were as follows: hypertension, blood pressure more than 140/90 mm Hg, and/or a history of taking antihypertensive medication; diabetes mellitus, fasting plasma glucose more than 126 mg/dl, or casual plasma glucose more than 200 mg/dl, or a diabetic pattern in 75 g OGTT; hyperlipidemia, serum total cholesterol levels of >220 mg/dl or serum triglyceride levels of >150 mg/dl; obesity, body mass index of more than 25 kg/m². Infarct site, peak CK, peak CK-MB, LVEF, and coronary angiographical findings were selected for analysis as indicators of MI severity.
Probe and an ABI Prism 7700 Sequence Detection System (ABI, PE Biosystems, Foster City, California). Levels of PIGF mRNA were normalized to those of glyceraldehyde-3-phosphate dehydrogenase mRNA.

**IMMUNOHISTOCHEMICAL AND IMMUNOFLUORESCENT STAINING OF PIGF IN INFARCT MYOCARDIUM.** The left ventricular apex, including the infarct lesion, was perfused with 4% paraformaldehyde and harvested, then cut into 3-μm sections followed by immersion fixed in 4% paraformaldehyde for overnight. The sections were labeled with primary mouse anti–PIGF (AF465 1:100 dilution, R&D Systems) and then with secondary biotin–labeled anti–goat IgG (BA-9500 1:800 dilution; Vector Laboratories, Burlingame, California) antibodies, and processed using the ABC-DAB (3,3′-diaminobenzidine tetrahydrochloride) method (Vectastain Elite ABC Kit, Vector Laboratories). Hematoxylin was used for nuclear staining.

For immunofluorescent staining, acetone-fixed sections were incubated for 1 h at room temperature with a primary anti–PIGF (sc-1882, 1:100 dilution, Santa Cruz Biotechnology Inc., Santa Cruz, California) and/or fluorescein isothiocianate-conjugated anti–alpha-smooth muscle actin (F3777, 1:400 dilution, Sigma-Aldrich Inc., St. Louis, Missouri) and/or anti–von Willebrand factor (A0082, 1:200 dilution, DakoCytomation Corp., Carpinteria, California) antibodies, and processed using the biotin–labeled anti–mouse CD11b (13-0112, eBioscience, San Diego, California). After washing, the sections were incubated for 30 min at room temperature with Cy3-conjugated donkey anti–goat IgG (705-165-003, Jackson Immunoresearch Laboratories, West Grove, Pennsylvania) and/or Cy2-conjugated anti–PlGF (sc-1882, 1:100 dilution, Santa Cruz Biotechnology Inc.) antibodies, and processed using the Vectastain Elite ABC Kit, Vector Laboratories. Hematoxylin was used for nuclear staining.

**MEASURING THE BLOOD VESSEL DENSITY IN THE INFARCT MYOCARDIUM.** We quantified the blood vessel density in the MI and IR models in mice sacrificed on day 7 after surgery. Sliced sections with 3-μm thickness were stained with monoclonal antibodies to α-smooth muscle-alkaline phosphatase antibody produced in rabbit (F3777, 1:400 dilution, Sigma-Aldrich Inc., St. Louis, Missouri), and/or fluorescein isothiocianate-conjugated anti–alpha-smooth muscle actin (monoclonal anti–actin, alpha-smooth muscle-alkaline phosphatase antibody produced in mouse clone 1A4) (A5691, 1:200 dilution, Sigma-Aldrich Inc.) and processed using 5-bromo-4-chloro-3-indoxyl phosphate/nitro blue tetrazolium chloride substrate (K0598, DakoCytomation Corp.) for measuring the blood vessel density in the marginal area of infarct myocardium. Blood vessels were counted in a stained slide from at least 10 randomly selected fields under ×400 confocal microscopy (AX70, OLYMPUS, Tokyo, Japan) and expressed as count per high power field.

**Statistical analysis.** Continuous data are expressed as means ± SE. Comparisons between control and AMI groups were made using paired or unpaired t tests, as appropriate. To assess correlations between two parameters, simple linear regressions were calculated using the least squares method. Values of p < 0.05 were considered significant. The relationship between the improvement of LVEF and clinical baseline parameters including PIGF were analyzed using simple and multiple linear regression analysis. All statistics were calculated using Stat View for Windows, version 5.0 (SAS Institute Inc., Cary, North Carolina).

**RESULTS**

**Patient characteristics.** Among the 55 enrolled patients, 39 received follow-up cardiac catheterization six months after the onset of AMI. Of the 16 patients who did not receive follow-up cardiac catheterization, 3 died during hospitalization because of pump failure, 5 were transferred to other hospitals, and 8 refused a follow-up examination. 

**Table 1** shows the clinical characteristics of the AMI patients participating in this study. There were 43 men and 12 women with a mean age of 66.7 ± 1.8 years (range, 31 to 88 years). The mean interval from the onset of AMI to reperfusion was 10.8 ± 1.8 h. The mean value of the maximum CK level was 4,117 ± 502 IU/l, and mean LVEF during the acute phase was 57.1 ± 1.7%. The mean peak monocyte count was 702 ± 40 cells/μl, which was apparently unrelated to the severity of the AMI estimated from the LVEF and maximum CK levels. The mean interval from admission to the peak monocyte count was 3.2 ± 0.1 days.

**Changes in peripheral plasma PIGF levels after AMI.** When measured in peripheral plasma obtained on the 1st, 3rd, and 7th days after the onset of AMI, levels of both PIGF and VEGF were found to peak on day 3 after AMI in most cases, at which time they were significantly higher than in control subjects (Fig. 1A). They then tended to decline about one week after AMI (data not shown).

**Table 1. Clinical Characteristics of AMI Patients**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value (mean ± SE)</th>
</tr>
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<tbody>
<tr>
<td>Age (yrs)</td>
<td>66.7 ± 1.8</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>43/12</td>
</tr>
<tr>
<td>Reperfusion time (h)</td>
<td>10.8 ± 1.8</td>
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<tr>
<td>Max CK (IU/l)</td>
<td>4,117 ± 502</td>
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<tr>
<td>Max CK-MB (IU/l)</td>
<td>274 ± 34</td>
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<tr>
<td>LVEF at the onset of MI (%)</td>
<td>57.1 ± 1.7</td>
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<tr>
<td>Peak WBC counts (cells/μl)</td>
<td>11,158 ± 494</td>
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<tr>
<td>Peak PBMC (cells/μl)</td>
<td>702 ± 40</td>
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<tr>
<td>Culprit lesion</td>
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<td>LAD</td>
<td>27</td>
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<td>LCx</td>
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<tr>
<td>RCA</td>
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<td>Coronary risk factor</td>
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<tr>
<td>Hyperlipidemia</td>
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</tr>
<tr>
<td>Smoking</td>
<td>25</td>
</tr>
<tr>
<td>Obesity</td>
<td>7</td>
</tr>
</tbody>
</table>

Data are expressed as mean value ± SE.
AMI = acute myocardial infarction; CK = creatine kinase; LAD = left anterior descending artery; LCx = left circumflex artery; LVEF = left ventricular ejection fraction; Max = maximum; MI = myocardial infarction; PBMC = peripheral blood; RCA = right coronary artery; WBC = white blood cell.
Clinical determinants of plasma PI GF levels. To identify the clinical determinants governing the plasma PI GF levels, we evaluated the relationship between plasma PI GF levels and the clinical characteristics of our patients. We found that plasma PI GF levels were unaffected by gender or by the presence or absence of any of the coronary risk factors (Table 2). Likewise, age, the extent of coronary atherosclerosis, site of the culprit lesion, reperfusion time, and maximum CK did not correlate with plasma PI GF levels (data not shown).

Clinical significance of PI GF during AMI. Plasma PI GF levels on day 3 post-MI positively correlated with peak monocyte counts ($r = 0.415$, $p = 0.0016$), but did not correlate with total peripheral white blood cell counts, suggesting PI GF may be involved in the peripheral mobilization of mononuclear cells during AMI. By contrast, plasma VEGF levels on day 3 post-MI did not correlate with either the total white blood cell counts or the monocyte counts (Fig. 2). At the same time, plasma PI GF levels negatively correlated with acute phase LVEF ($r = 0.434$, $p = 0.021$), though plasma VEGF levels did not (Figs. 3A and 3B). In addition, plasma PI GF levels on day 3 post-MI were positively correlated with the subsequent changes in LVEF observed during the chronic phase 6 months after the onset of AMI ($\Delta$EF, $R = 0.38$, $p = 0.0196$; Fig. 3C), which reflects improvement in left ventricular function. The corresponding VEGF levels did not correlate with $\Delta$EF (Fig. 3D). Multiple regression analysis revealed that the plasma PI GF level was the strongest independent predictor of improvement of LVEF during the chronic phase ($p = 0.0098$) (Table 3). Furthermore, when we studied the subgroup of patients with substantially impaired left ventricular function (LVEF <60%), we found that patients who showed improvement in LVEF ($\Delta$LVEF ≥0) ($n = 13$), as $\Delta$LVEF >0, in the chronic phase had significantly higher plasma PI GF levels than patients without improvement in LVEF ($\Delta$LVEF <0) ($n = 8$) ($47.3 \pm 22.6$ pg/ml vs. $27.9 \pm 12.6$ pg/ml, respectively), supporting that peak PI GF levels in the acute phase, independent from baseline LVEF, is the determinant factor for the improvement of LVEF in the chronic phase. Placental growth factor might be over-expressed to compensate for ischemic damage during AMI.

**Table 2.** Plasma PI GF Levels on Third Day After AMI and the Coronary Risk Factors

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>PI GF (pg/ml)</th>
<th>(+)</th>
<th>(−)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes</td>
<td>31.4 ± 21.2</td>
<td>37.8 ± 29.8</td>
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<tr>
<td>Hypertension</td>
<td>35.9 ± 28.2</td>
<td>33.7 ± 23.7</td>
<td>NS</td>
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<tr>
<td>Hyperlipidemia</td>
<td>33.4 ± 23.2</td>
<td>37.0 ± 30.1</td>
<td>NS</td>
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<tr>
<td>Smoking</td>
<td>31.8 ± 20.3</td>
<td>37.8 ± 30.8</td>
<td>NS</td>
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<tr>
<td>Obesity</td>
<td>26.1 ± 8.7</td>
<td>36.4 ± 28.0</td>
<td>NS</td>
<td></td>
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</tbody>
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<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
<th>p Value</th>
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<tbody>
<tr>
<td>PI GF (pg/ml)</td>
<td>31.1 ± 3.0</td>
<td>40.7 ± 11.4</td>
<td>NS</td>
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</tbody>
</table>

Data are expressed as mean value ± SE.
AMI = acute myocardial infarction; PI GF = placental growth factor.
Expression of PlGF in a mouse AMI model. To confirm the augmented production of PlGF in infarct hearts, we assessed the expression of PlGF mRNA in a mouse model of MI. We found that levels of PlGF transcript were increased 26.6-fold (p < 0.0001) in infarct hearts of MI models, as compared to sham-operated hearts, on days 1 and 3 post-MI, and that transcript levels had returned to control levels on days 7 and 28 post-MI (Fig. 4).

Immunohistochemical analysis with confocal microscopy (AX70, OLYMPUS) showed that PlGF protein was strongly expressed in vascular tissues, especially vascular endothelial cells of both capillaries and small vessels in the infarct myocardium (Fig. 5), within the infarct myocardium, but was scarcely detected in non-infarct regions, and was not detected at all in sham-operated hearts (data not shown). Although we cannot totally deny the PlGF expression in vascular smooth muscle cell in the same vessel, results from double immunofluorescent staining indicate that endothelial cells of vascular tissue in infarct myocardium is the major site of PlGF production (Fig. 6). We also found a small number of PlGF-positive cells in interstitial space in infarct myocardium. However, PlGF-positive cells did not cross-react with known macrophage antibody (anti-CD11b) (Fig. 6).

Figure 2. Relationship between plasma placental growth factor (PlGF) levels on day 3 post-myocardial infarction and peripheral white blood cell (WBC) counts. Neither PlGF (A) nor vascular endothelial growth factor (VEGF) (B) correlated with total peripheral WBC counts. Placental growth factor levels positively correlated with peak monocyte counts (C), though VEGF levels did not (D). PBMC = peripheral blood.

Figure 3. Relationship between plasma placental growth factor (PlGF) levels on day 3 post-myocardial infarction and left ventricular ejection fraction (EF) during the acute phase. Whereas plasma PlGF levels negatively correlated with left ventricular EF at the onset of acute myocardial infarction (A), plasma vascular endothelial growth factor (VEGF) levels did not (B). Plasma PlGF levels on day 3 post-myocardial infarction were positively correlated with the changes of left ventricular EF (ΔEF) that occurred between the acute and chronic phases (C); VEGF levels were not (D).
The present study demonstrates for the first time that: 1) cardiac production of PlGF is increased after AMI; 2) the major site of augmented expression of PlGF is the endothelium of vessels within the infarcted region; 3) PlGF production after AMI is determined, in part, by the amount of injured myocardium; and 4) plasma PlGF levels on day 3 post-MI are positively correlated with both the circulating monocyte counts during the acute phase and the degree of improvement of LVEF during the chronic phase of MI. Taken together, these findings may suggest that cardiac expression of PlGF has a beneficial effect on wound healing processes after AMI, possibly by inducing peripheral mobilization of mononuclear cells and enhancing angiogenesis.

Cardiac expression of PlGF during AMI. Earlier studies showed that PlGF is expressed in placental tissue, trophoblasts, leukocytes, and endothelial cells under normal physiological conditions (17–19). The present findings indicate that, during AMI, vascular tissue, especially the endothelium within the infarcted myocardium, is also a major site of PlGF production. Clinically, we found that LVEF during the acute phase is negatively correlated with plasma PlGF levels on day 3 post-MI, making it likely that the degree of injury to the vascular endothelium within the infarcted myocardium is a key determinant of cardiac PlGF production after AMI. Furthermore, our observation that induction of PlGF begins very early (within 24 h) after the onset of cardiac ischemia and is sustained for about a week thereafter suggests that tissue PlGF is importantly involved in the early phase of tissue healing after ischemic injury (e.g., angiogenesis). Although the signaling pathway leading to up-regulation of PlGF expression in the ischemic myocardium is not yet clear, expression level of PlGF mRNA levels was regulated by the degree and duration of myocardial ischemia in this study. Supporting our observations, one in vitro study reported that PlGF is induced by hypoxia (20). In that regard, the promoter region of the PlGF gene does not contain a hypoxia-inducible, factor-responsive element, but it does contain other hypoxia-responsive elements, such as those for metal transcription factor-1 and Sp-1 (20,21). It is, thus, plausible that hypoxia is an important stimulus of PlGF expression within the infarcted myocardium.

Although their study population and the study design are not the same with the present study, Heeschen et al. (22) have reported that plasma levels of PlGF in patients with non–ST-segment elevated MI and refractory unstable angina pectoris were significantly elevated compared with healthy control subjects, supporting that prolonged and/or severe ischemia induces the cardiac expression of PlGF.

Clinical significance of PlGF during healing after AMI. The function of PlGF after AMI has not been systematically studied. We noticed, however, that patients with higher plasma PlGF levels on day 3 post-MI showed greater improvement in LVEF during the chronic phase (6 months post-MI) than patients with lower plasma PlGF levels, and also that patients with improvement in LVEF in the chronic phase had significantly higher plasma levels of PlGF in the acute phase compared with patients without improvement, again suggesting the involvement of PlGF in the healing of the injured myocardium. Thus, PlGF might be over-expressed to compensate the ischemic damage during AMI. Like VEGF, PlGF is known to have powerful angiogenic properties, especially under pathological circumstances, such

<table>
<thead>
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<th>Clinical Parameters</th>
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<td>PlGF</td>
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<tr>
<td>Age</td>
<td>NS</td>
</tr>
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<td>Max CK</td>
<td>NS</td>
</tr>
<tr>
<td>Max CK-MB</td>
<td>NS</td>
</tr>
<tr>
<td>Reperfusion time</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are expressed as mean value ± SD.

CK = creatine kinase; Max = maximum; PlGF = placental growth factor.

Figure 4. Expression of placental growth factor (PlGF) mRNA in the hearts of mice subjected to myocardial infarction induced by coronary artery ligation. Quantitative real-time polymerase chain reaction revealed that expression of PlGF mRNA was significantly up-regulated in infarct hearts, as compared to sham-operated hearts on days 1 and 3 post-myocardial infarction, but had returned to levels that did not differ from control by days 7 and 28. AMI = acute myocardial infarction.
as in cancer and limb ischemia (23,24). This suggests that, in cases of AMI, locally expressed PlGF might directly stimulate angiogenesis in both the infarct and infarct-border regions. In mouse experiments of our study, we observed that both temporal and permanent occlusion of coronary artery promoted PlGF gene expression, as well as angiogenesis in ischemic myocardium. As shown in Figure 7, the increment of vessel density and PlGF expression in the permanent occlusion model was substantially higher than that in temporal ischemia. Accordingly, cardiac PlGF expression seems to be involved in the angiogenesis under the situation of severe myocardial ischemia. Moreover, we observed that plasma PlGF levels on day 3 post-MI were significantly correlated with peak monocyte counts 3.2 ± 0.1 days post-MI, suggesting that PlGF released from the infarct heart exerts a stimulatory effect on monocyte mobilization. Consistent with that idea, Hattori et al. (13) observed an increase in circulating stem cells after adenovirus-mediated gene transfer of PlGF to BALB/c mice (14). They suggested that PlGF induces stem cell mobilization via flt-1, which is expressed on monocyte, CD34+ cells, and hematopoietic stem cells (25). Furthermore, recent observations by Pipp et
Placental growth factor (PIGF) gene expression and angiogenesis in ischemic myocardium. (A) Relation between the duration of ischemia and the PIGF gene expression three days after surgery in mouse models of myocardial infarction (MI, as permanent occlusion) and ischemia-reperfusion (IR). Bars represent PIGF mRNA levels of infarct tissue. Placental growth factor mRNA expression levels were significantly increased in both in 60 min. Ischemia-reperfusion and MI models compared with sham operated mice. (B) Relation between the duration of ischemia and the blood vessel density in the marginal area of infarct myocardium in mouse models of MI and IR. Heart tissue was sampled seven days after surgery. Bars represent alpha-smooth muscle actin-positive blood vessel counts in infarct lesion per field under ×400 microscopy. Blood vessel densities in both MI and IR are significantly increased compared with sham, and blood vessel density in MI is significantly higher (2.5×) compared with IR. *p < 0.001 for MI and IR vs. sham; **p < 0.001 for MI vs. IR; ***p < 0.01 for IR 60 min vs. sham.
tween PlGF levels and monocyte counts during the acute phase of MI and subsequent left ventricular function and long-term prognosis in MI patients are needed to address this issue.

Conclusions. Placental growth factor is rapidly produced in infarct myocardial tissue, especially in the endothelium of vessels within the infarct myocardium during the acute phase of MI. The resultant elevation in plasma PlGF levels appears to contribute to the improved LVEF seen during the chronic phase, probably by activating monocytes and enhancing angiogenesis.

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REFERENCES