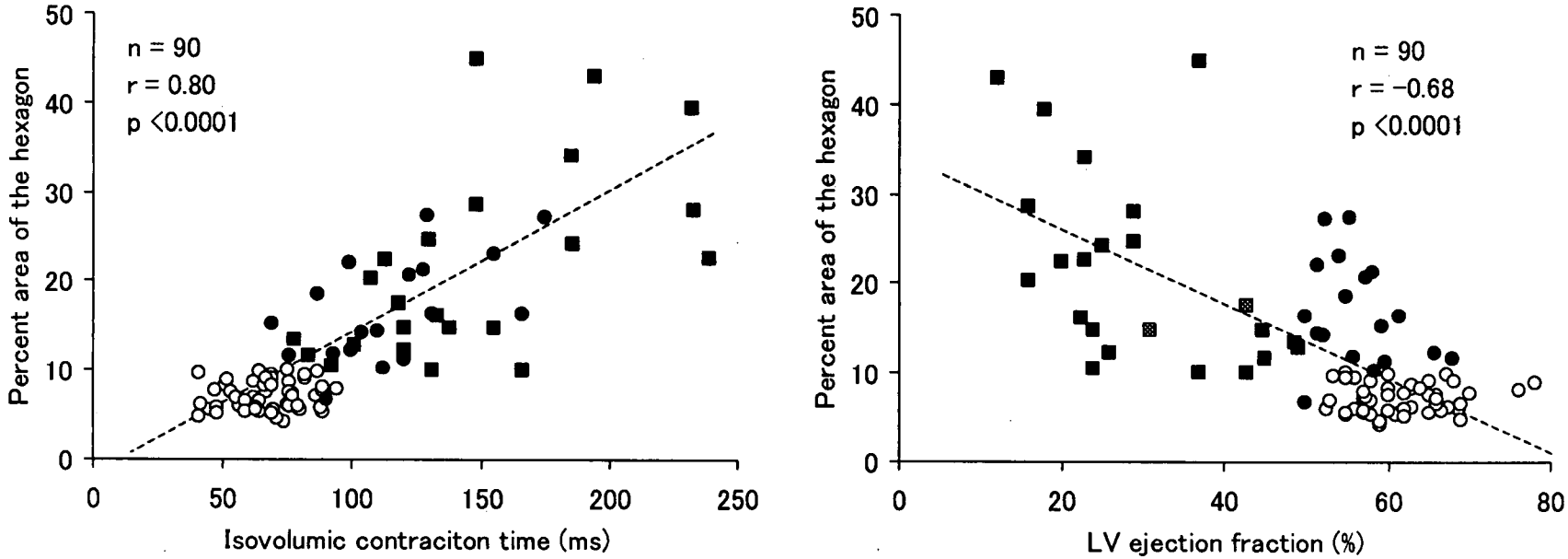

n = 90
r = 0.80
p <0.0001
Percent area of the hexagon
Isovolumic contraciton time (ms)
0
10
20
30
40
50
0
50
100
150
200
250
n = 90
r = −0.68
p <0.0001
Percent area of the hexagon
LV ejection fraction (%)
0
10
20
30
40
50
0
20
40
60
80

Figure 2

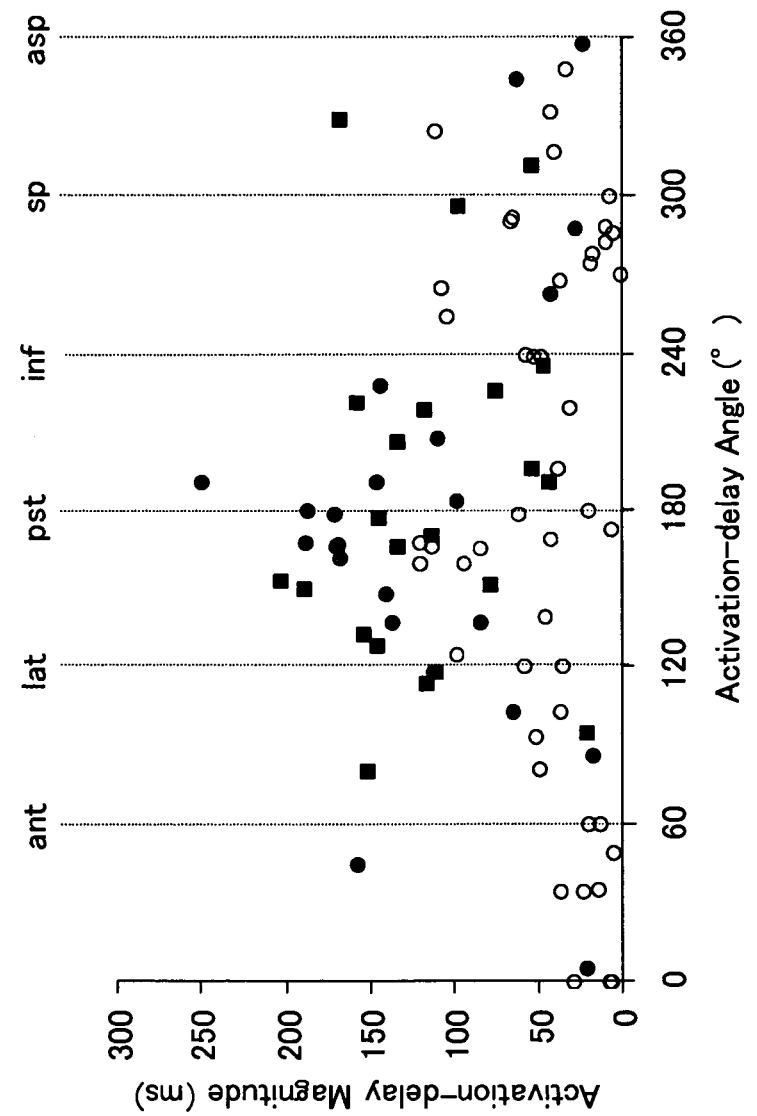

Activation–delay Magnitude (ms)
300
250
200
150
100
50
0
ant
lat
pst
inf
sp
asp
0
60
120
180
240
300
360
Activation–delay Angle (°)

Figure 3

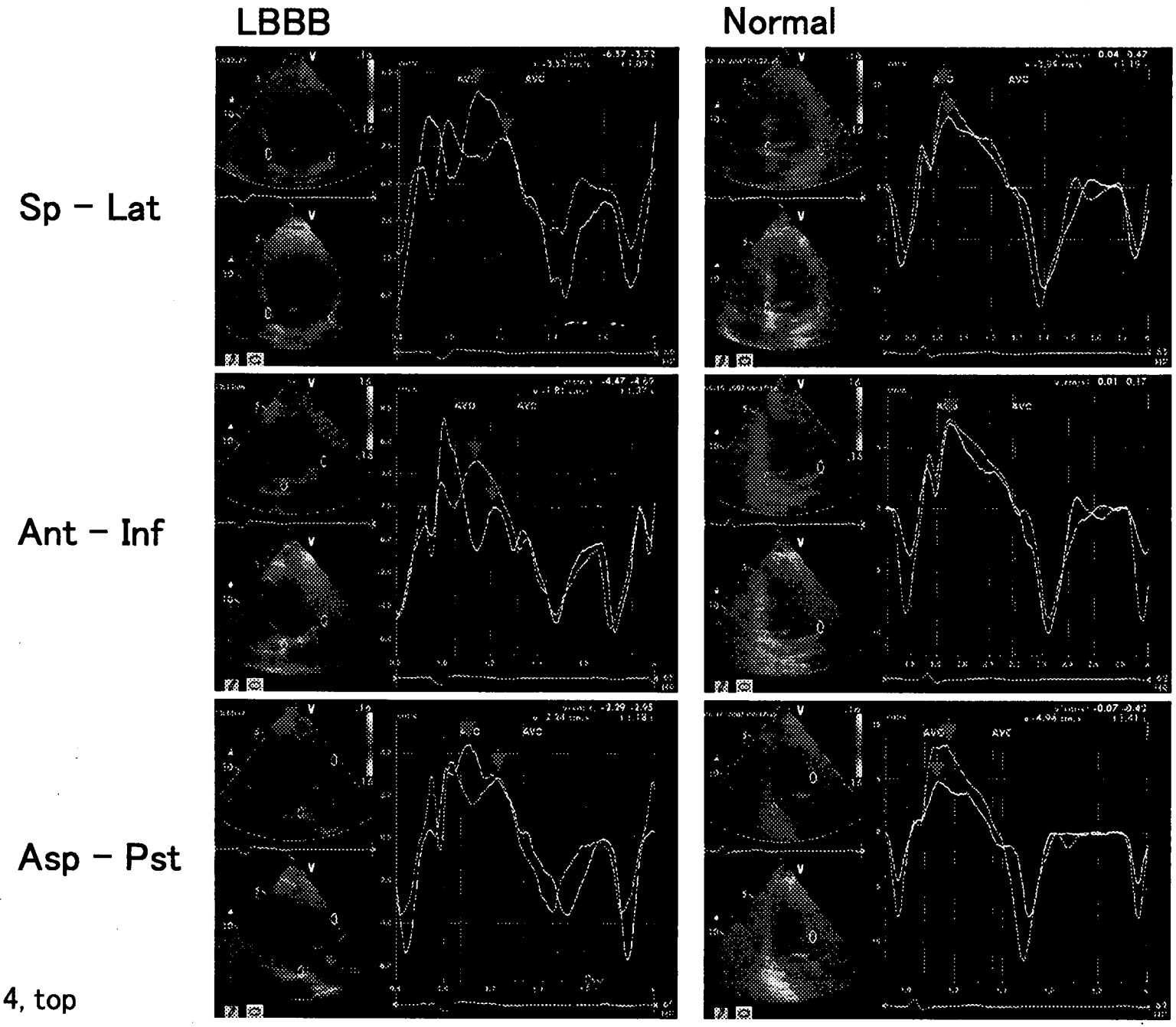
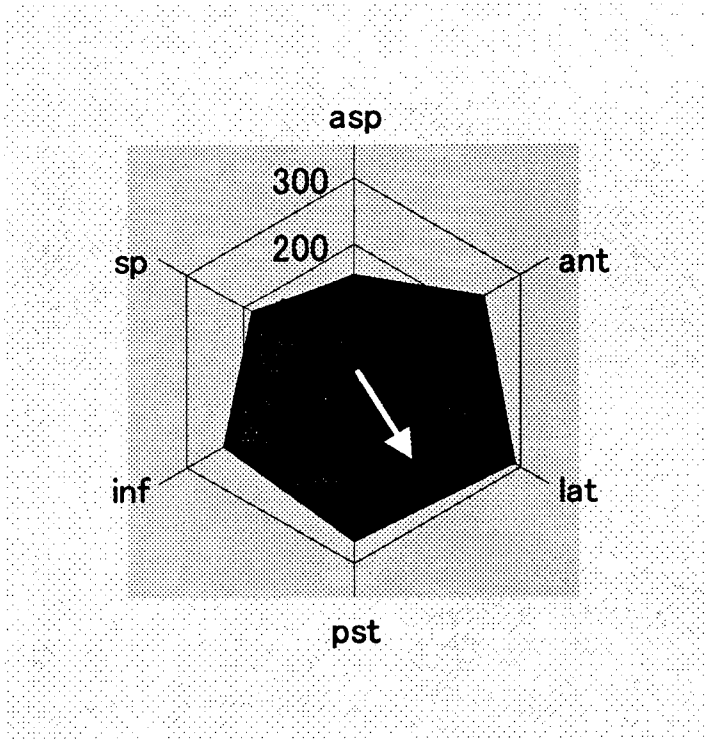


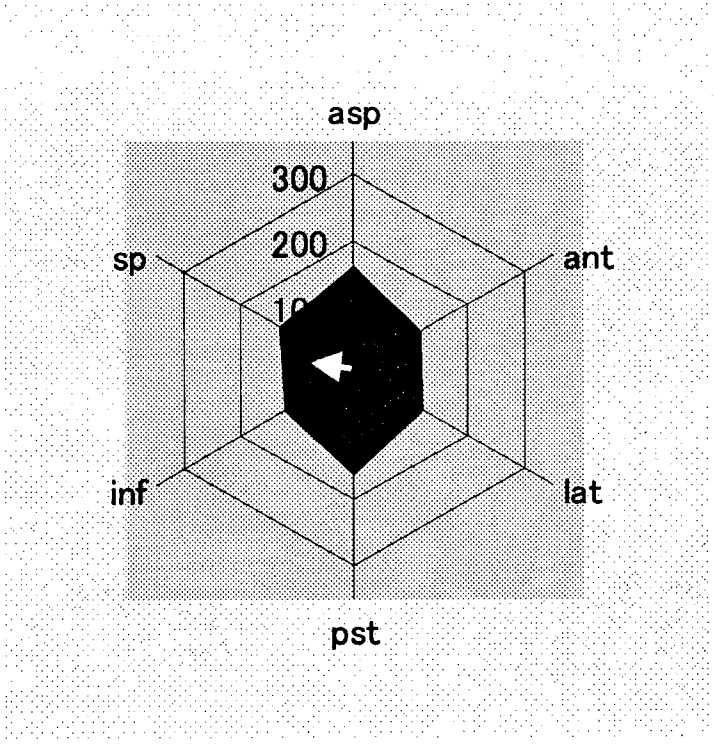
Figure 4, top

LBBB



%Area of Hexagon	20.3%
Activation–delay Magnitude	188 ms
Activation–delay Angle	150°
SD–6	51
CV–6	0.22
Dispersion of TPVs	135 ms

Normal



%Area of Hexagon	7.1%
Activation–delay Amplitude	17 ms
Activation–delay Angle	279°
SD–6	20
CV–6	0.14
Dispersion of TPVs	43 ms

Figure 4, bottom

Journal of the American College of Cardiology
© 2006 by the American College of Cardiology Foundation
Published by Elsevier Inc.

Vol. 47, No. 8, 2006
ISSN 0735-1097/06/$32.00
doi:10.1016/j.jacc.2005.11.064

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

Hajime Iwama, MD, Shiro Uemura, MD, Noriyuki Naya, BS, Kei-ichi Imagawa, PHD, Yasuhiro Takemoto, MD, Osamu Asai, MD, Kenji Onoue, MD, Satoshi Okayama, MD, Satoshi Somekawa, MD, Yoshitomi Kida, MD, Yukiji Takeda, MD, Kimihiko Nakatani, MD, Minoru Takaoka, MD, Hiroyuki Kawata, MD, Manabu Horii, MD, Tamio Nakajima, MD, Naofumi Doi, MD, Yoshihiko Saito, MD
Kashihara, Nara, Japan

OBJECTIVES Our aim was to investigate cardiac expression of placental growth factor (PlGF) 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 PlGF 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 PlGF was analyzed in a mouse AMI model.

RESULTS In AMI patients, peripheral plasma PlGF levels on day 3 were significantly higher than in control subjects. Plasma PlGF levels just after recanalization were significantly higher in the CS than the CAos, which indicates cardiac production and release of PlGF. Peripheral plasma levels of PlGF 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 PlGF 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 (PlGF) is a member of the VEGF family that acts via VEGF receptor-1 (flt-1) (10,11). Like VEGF, PlGF has been shown to play a key role in vascular development under pathological conditions; for instance, by stimulating both angiogenesis and arteriogenesis, PlGF enhances not only capillary but also collateral formation in ischemic tissue (12). In addition, PlGF 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 PlGF in both human AMI patients and a mouse model of AMI, and to determine the impact of PlGF expression on the clinical features of AMI in humans.

From the First Department of Medicine, Nara Medical University, Kashihara, Nara, Japan.

Manuscript received December 17, 2004; revised manuscript received October 26, 2005, accepted November 8, 2005.

JACC Vol. 47, No. 8, 2006
April 18, 2006:1559–67

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/m^2. 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 immunoadsorbent assay kits (DPG00, DVE00, respectively; R&D Systems, Minneapolis, Minnesota). This enzyme-linked immunoadsorbent assay kit also cross-reacts with human recombinant PlGF-2 isoform (50%) 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, PIGF cDNA was synthesized using Superscript II reverse transcriptase (Invitrogen Corp.). Relative PIGF mRNA levels were then determined using real-time polymerase chain reaction carried out using cDNA samples with Assay-on-Demand Product, Taqman

Probe and an ABI Prism 7700 Sequence Detection System (ABI, PE Biosystems, Foster City, California). Levels of PlGF mRNA were normalized to those of glyseraldehyde-3-phosphate dehydrogenase mRNA.

IMMUNOHISTOCHEMICAL AND IMMUNOFLUORESCENT STAINING OF PLGF 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 fixated in 4% paraformaldehyde for overnight. The sections were labeled with primary mouse anti-PlGF (AF465 1:100 dilution, R&D Systems) and then with secondary biotin-labeled anti-gout 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-PlGF (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) and/or 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, Inc., West Grove, Pennsylvania) for PlGF and/or anti-rabbit IgG (A21441, Molecular Probes Inc., Eugene, Oregon) for von Willebrand factor and/or Cy2-conjugated anti-biotin (016-220-084, Jackson Immunoresearch Laboratories Inc.) for CD11b. 4',6-Diamidino-2-phenylindole 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 thick of the left ventricle, including the infarct lesion, were labeled with mouse anti-alpha-smooth muscle actin (monocronal 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 PlGF 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 PlGF levels after AMI. When measured in peripheral plasma obtained on the 1st, 3rd, and 7th days after the onset of AMI, levels of both PlGF 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

Age (yrs)	66.7 ± 1.8
Gender (male/female)	43/12
Reperfusion time (h)	10.8 ± 1.8
Max CK (IU/l)	4,117 ± 502
Max CK-MB (IU/l)	274 ± 34
LVEF at the onset of MI (%)	57.1 ± 1.7
Peak WBC counts (cells/μl)	11,158 ± 494
Peak PBMC (cells/μl)	702 ± 40
Culprit lesion	
LAD	27
LCx	4
RCA	24
Coronary risk factor	
Diabetes	23
Hypertension	29
Hyperlipidemia	35
Smoking	25
Obesity	7

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.

JACC Vol. 47, No. 8, 2006
April 18, 2006:1559–67

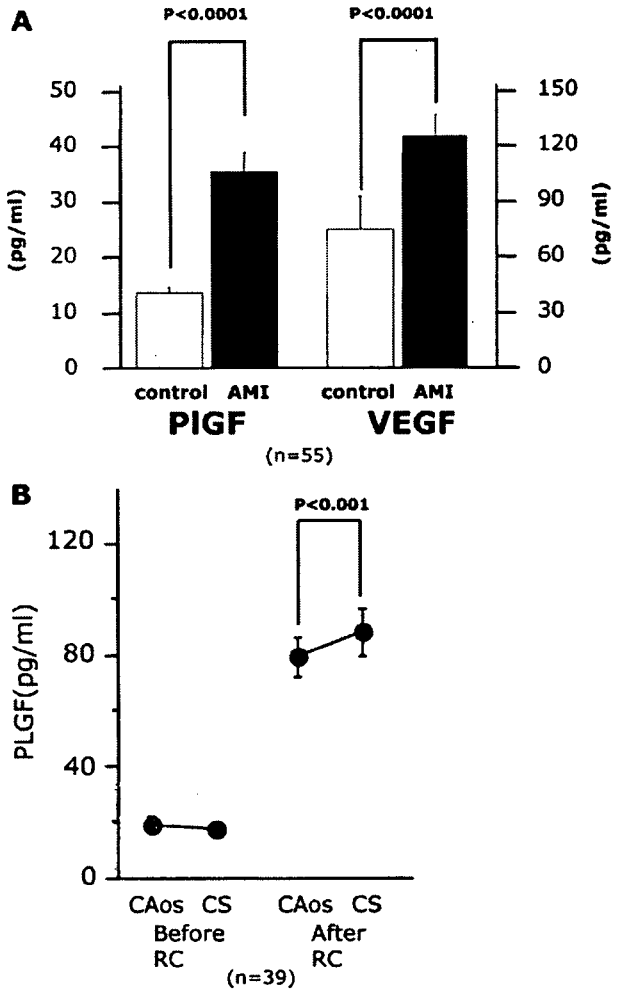


Figure 1. (A) Peripheral blood plasma cytokine levels in patients with acute myocardial infarction (AMI). Placental growth factor (PlGF) and vascular endothelial growth factor (VEGF) were significantly higher than control in blood samples from AMI patients. **Bars** represent means ± SE. **(B)** Placental growth factor levels in plasma samples taken from the coronary artery ostium (CAos) and coronary sinus (CS) before and just after percutaneous coronary intervention. Before recanalization (RC) of an infarct-related artery, plasma PlGF levels were similarly low in the CAos and CS. After RC, PlGF levels were significantly increased in both the CAos and CS, as compared to before RC, and levels in the CS were significantly higher than in the CAos. **Closed circles and bars** represent means ± SE.

Plasma PlGF levels in the coronary circulation during AMI. To determine whether the elevated PlGF levels in the peripheral blood plasma reflected release of the cytokine from the infarct heart, we measured PlGF levels in plasma samples collected from the CAos and CS at the time of emergency coronary intervention (Fig. 1B). Before reperfusion of the infarct-related artery, PlGF levels were similarly low in the CAos and CS (20.2 ± 3.1 pg/ml and 18.4 ± 2.7 pg/ml, respectively), and were comparable to the levels in healthy subjects. After reperfusion, however, PlGF levels in both the CAos and CS were significantly elevated, and the levels in the CS were significantly higher than those in the CAos (90.1 ± 8.8 pg/ml vs. 79.7 ± 7.6 pg/ml; $p < 0.01$), which is indicative of cardiac production and release of PlGF.

Clinical determinants of plasma PlGF levels. To identify the clinical determinants governing the plasma PlGF levels, we evaluated the relationship between plasma PlGF levels and the clinical characteristics of our patients. We found that plasma PlGF 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 PlGF levels (data not shown).

Clinical significance of PlGF during AMI. Plasma PlGF 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 PlGF 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 PlGF 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 PlGF 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 (ΔEF, $R = 0.38$, $p = 0.0196$; Fig. 3C), which reflects improvement in left ventricular function. The corresponding VEGF levels did not correlate with ΔEF (Fig. 3D). Multiple regression analysis revealed that the plasma PlGF 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 (ΔLVEF ≥0) ($n = 13$), as ΔLVEF >0, in the chronic phase had significantly higher plasma PlGF levels than patients without improvement in LVEF (ΔLVEF <0) ($n = 8$) (47.3 ± 22.6 pg/ml vs. 27.9 ± 12.6 pg/ml, respectively), supporting that peak PlGF 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 PlGF Levels on Third Day After AMI and the Coronary Risk Factors

Risk Factors	PlGF (pg/ml) (+)	PlGF (pg/ml) (−)	p Value
Diabetes	31.4 ± 21.2	37.8 ± 29.8	NS
Hypertension	35.9 ± 28.2	33.7 ± 23.7	NS
Hyperlipidemia	33.4 ± 23.2	37.0 ± 30.1	NS
Smoking	31.8 ± 20.3	37.8 ± 30.8	NS
Obesity	26.1 ± 8.7	36.4 ± 28.0	NS
	Male	**Female**	**p Value**
	31.1 ± 3.0	40.7 ± 11.4	NS

Data are expressed as mean value ± SE.
AMI = acute myocardial infarction; PlGF = placental growth factor.

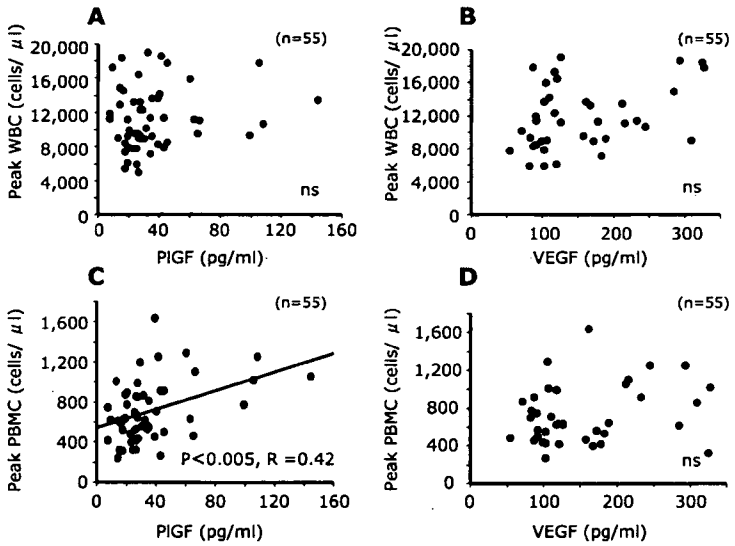


Figure 2. Relationship between plasma placental growth factor (PlGF) levels on day 3 post-myocardial infarction and peripheral white blood cell (WBC) counts. Neither PlGF **(A)** nor vascular endothelial growth factor (VEGF) **(B)** correlated with total peripheral WBC counts. Placental growth factor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

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).

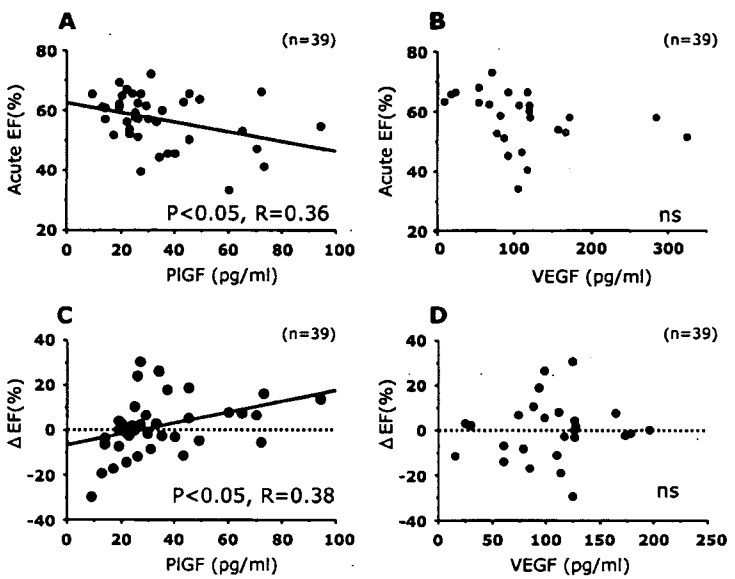


Figure 3. Relationship between plasma placental growth factor (PlGF) levels on day 3 post-myocardial infarction and left ventricular ejection fraction (EF) during the acute phase. Whereas plasma PlGF levels negatively correlated with left ventricular EF at the onset of acute myocardial infarction **(A)**, 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)**.

Table 3. Multiple Regression Analysis for Chronic Phase Left Ventricular Function

Clinical Parameters	p Value
PlGF	<0.05
Age	NS
Max CK	NS
Max CK-MB	NS
Reperfusion time	NS

Data are expressed as mean value ± SD.
CK = creatine kinase; Max = maximum; PlGF = placental growth factor.

Next, we evaluated the effect of severity of ischemia on the PlGF mRNA expression and the angiogenesis in ischemic myocardium. Placental growth factor mRNA expression on day 3 after surgery did not increase in mice with 40-min ischemia, but significantly increased in the 60-min ischemia model (1.9-fold increase), as well as in the MI model (26.6-fold increase) compared with sham-operated mice. Furthermore, PlGF mRNA expression levels were significantly higher in MI than in IR. Blood vessel densities in the marginal area of infarct myocardium were also significantly increased in both IR and MI models compared with sham, and blood vessel density of the MI model was significantly higher (2.5-fold increase) compared with the IR model (Fig. 7B).

DISCUSSION

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 infarct 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 infarct 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 infarct 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 overexpressed to compensate the ischemic damage during AMI. Like VEGF, PlGF is known to have powerful angiogenic properties, especially under pathological circumstances, such

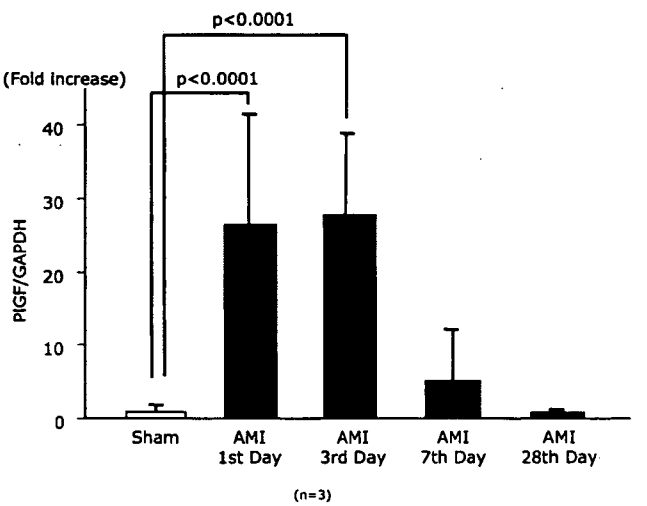


Figure 4. Expression of placental growth factor (PlGF) mRNA in the hearts of mice subjected to myocardial infarction induced by coronary artery ligation. Quantitative real-time polymerase chain reaction revealed that expression of PlGF mRNA was significantly up-regulated in infarct hearts, as compared 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.

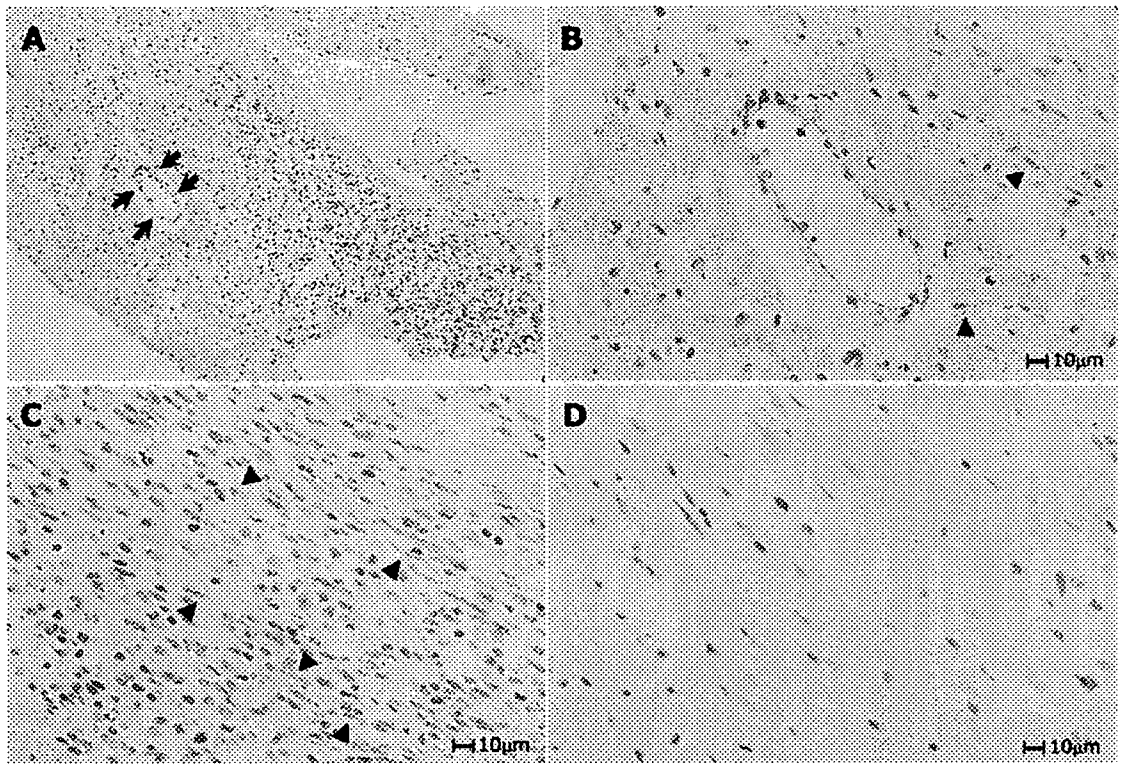


Figure 5. Photomicrographs showing immunohistochemical staining of placental growth factor in mouse heart on day 3 post-myocardial infarction. **(A)** Infarct myocardium (original magnification, 60×). **(B and C)** Higher magnification (240×) of the corresponding box **(A)**. The staining shows strong expression of placental growth factor protein in the endothelium within the infarct region **(B)**, as well as in interstitial cells **(C)**. The staining shows an absence of placental growth factor protein in the non-infarct area **(D)**. **Arrows** indicate vascular tissue, **arrowheads** interstitial cells.

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

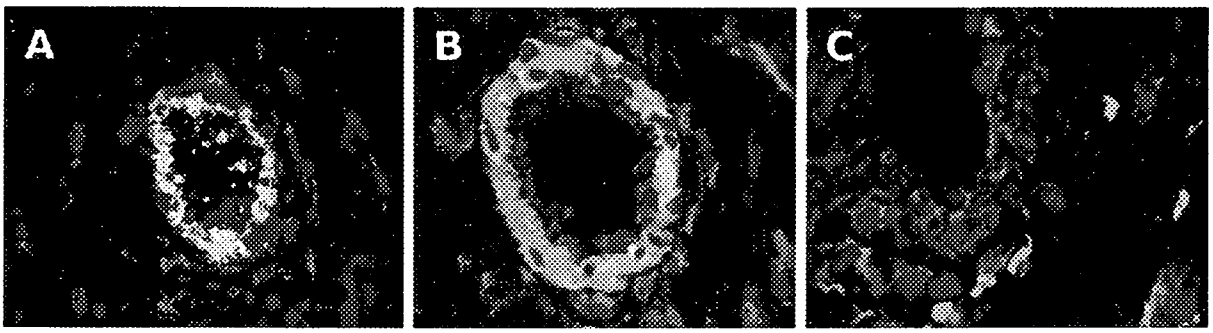


Figure 6. Photomicrographs showing immunofluorescent staining of a small artery and infiltrating cell within the infarct myocardium. **(A)** Combination staining of the target vessel with anti-placental growth factor **(red)** and anti-von Willebrand factor **(green)** antibodies. **Yellow** coloration indicates placental growth factor expression within vascular endothelial cells. **(B)** Combination staining with anti-placental growth factor **(red)** and anti-alpha-smooth muscle actin **(green)** antibodies. Note that placental growth factor expression is restricted to the intima of the target vessel. **(C)** Combination staining with anti-placental growth factor **(red)** and anti-CD11b **(green)** antibodies. Placental-growth-factor-expressing cell in the interstitium did not cross-react with CD11b-positive cell (macrophage). Original magnification, 800×. DAPI was used for nuclear staining **(blue)**.

JACC Vol. 47, No. 8, 2006
April 18, 2006:1559–67

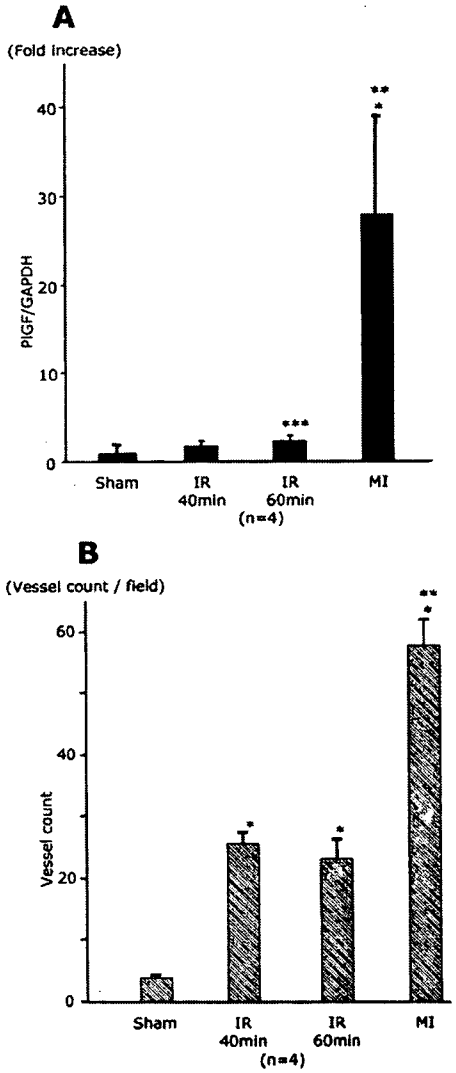


Figure 7. Placental growth factor (PlGF) gene expression and angiogenesis in ischemic myocardium. **(A)** Relation between the duration of ischemia and the PlGF gene expression three days after surgery in mouse models of myocardial infarction (MI, as permanent occlusion) and ischemia-reperfusion (IR). **Bars** represent PlGF mRNA levels of infarct heart 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.

al. (26) indicate that, in animal ischemic limb models, PlGF administration significantly enhances collateral vessel formation by activating and recruiting mononuclear cells to the ischemic region in an flt-1–dependent fashion. We speculate that same mechanisms might work in MI. It has also been reported that monocytes infiltrating into the injured tissue directly stimulate collateral vessel growth by releasing various cytokines, including basic fibroblast growth factor and monocyte chemoattractant protein-1 (27), which is consistent with the idea that, in addition to its direct angiogenic activity, PlGF facilitates wound healing by helping home circulating mononuclear and bone marrow-derived stem cells to the injured myocardium by inducing the expression of adhesion molecules on the surfaces of both endothelial and mononuclear cells (28). Furthermore, macrophage is also known to express flt-1, so elevated PlGF might enhance wound healing by activating and recruiting macrophages that have important roles in removing necrotic tissue from an injured area (27,29).

A crucial consideration when interpreting the results of the present study is to determine whether plasma PlGF levels in the range of 30 to 120 pg/ml, which were the highest concentrations observed in our AMI patients, are sufficient to exert a biological effect, such as angiogenesis or mobilization of mononuclear cells from bone marrow. It is noteworthy in that regard that an earlier report showed that PlGF at a concentration of 100 pg/ml stimulates DNA synthesis in flt-1–expressing endothelial cells (30), which suggests that the plasma PlGF levels we observed in our patients were indeed sufficient to be biologically active.

Differences between PlGF and VEGF. We found that, like PlGF, plasma VEGF levels were elevated in patients' blood, but they did not correlate with either monocyte counts or improvement in LVEF. One possible explanation for the difference between these two angiogenic cytokines is that PlGF only binds to flt-1, whereas VEGF binds to both flt-1 and VEGF receptor 2 (31). Another possible explanation is that flt-1–mediated intracellular signaling is ligand-specific. For example, PlGF-mediated stimulation of flt-1 induces MAP kinase activation in porcine aortic endothelial cells, which, in turn, leads to enhanced DNA synthesis (30). By contrast, VEGF-mediated stimulation of flt-1 does not activate the MAP kinase pathway (30).

Clinical implications. Blood vessel growth is a principle component of the wound healing process. Recently, administration of bone marrow-derived or peripheral blood-derived mononuclear cells into infarct-related coronary arteries was shown to improve left ventricular function by enhancing angiogenesis and regeneration of the myocardium (32–34). Similarly, Hojo et al. (35) reported that patients showing improved left ventricular systolic function after MI exhibited significantly higher monocyte counts than patients without improvement. We, therefore, suggest that an efficient and safe method for mobilizing bone marrow mononuclear cells may be a useful approach to treating AMI. Our finding that local expression of PlGF is associated with increased monocyte counts and improved LVEF after AMI suggests administration of human PlGF might be a possible means of improving left ventricular function in the chronic phase. On the other hand, several studies have reported a negative relationship between monocyte counts and long-term prognosis after AMI (22,36,37), which suggests peripheral monocytosis may also have adverse effects. Additional studies analyzing the relation be-

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.

Reprint requests and correspondence: Dr. Shiro Uemura, 840 Shijo-cho, Kashihara, Nara, Japan 634-8522. E-mail: suemura@naramed-u.ac.jp.

REFERENCES

1. Pfeffer MA, Braunwald E. Ventricular remodeling after myocardial infarction. Experimental observations and clinical implications. Circulation 1990;81:1161–72.
2. Deten A, Volz HC, Briest W, Zimmer HG. Cardiac cytokine expression is upregulated in the acute phase after myocardial infarction. Experimental studies in rats. Cardiovasc Res 2002;55:329–40.
3. de Lemos JA, Morrow DA, Sabatine MS, et al. Association between plasma levels of monocyte chemoattractant protein-1 and long-term clinical outcomes in patients with acute coronary syndromes. Circulation 2003;107:690–5.
4. Birdsall HH, Green DM, Trial J, et al. Complement C5a, TGF-beta 1, and MCP-1, in sequence, induce migration of monocytes into ischemic canine myocardium within the first one to five hours after reperfusion. Circulation 1997;95:684–92.
5. Asahara T, Masuda H, Takahashi T, et al. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. Circ Res 1999;85: 221–8.
6. Hashimoto E, Ogita T, Nakaoka T, Matsuoka R, Takao A, Kira Y. Rapid induction of vascular endothelial growth factor expression by transient ischemia in rat heart. Am J Physiol 1994;267:H1948–54.
7. Schwarz ER, Speakman MT, Patterson M, et al. Evaluation of the effects of intramyocardial injection of DNA expressing vascular endothelial growth factor (VEGF) in a myocardial infarction model in the rat—angiogenesis and angioma formation. J Am Coll Cardiol 2000; 35:1323–30.
8. Hedman M, Hartikainen J, Syvanne M, et al. Safety and feasibility of catheter-based local intracoronary vascular endothelial growth factor gene transfer in the prevention of postangioplasty and in-stent restenosis and in the treatment of chronic myocardial ischemia: phase II results of the Kuopio Angiogenesis Trial (KAT). Circulation 2003; 107:2677–83.
9. Henry TD, Annex BH, McKendall GR, et al. The VIVA trial: Vascular endothelial growth factor in Ischemia for Vascular Angiogenesis. Circulation 2003;107:1359–65.
10. Mattei MG, Borg JP, Rosnet O, Marme D, Birnbaum D. Assignment of vascular endothelial growth factor (VEGF) and placenta growth factor (PLGF) genes to human chromosome 6p12-p21 and 14q24-q31 regions, respectively. Genomics 1996;32:168–9.
11. Autiero M, Luttun A, Tjwa M, Carmeliet P. Placental growth factor and its receptor, vascular endothelial growth factor receptor-1: novel targets for stimulation of ischemic tissue revascularization and inhibition of angiogenic and inflammatory disorders. J Thromb Haemost 2003;1:1356–70.
12. Carmeliet P, Moons L, Luttun A, et al. Synergism between vascular endothelial growth factor and placental growth factor contributes to angiogenesis and plasma extravasation in pathological conditions. Nat Med 2001;7:575–83.
13. Hattori K, Heissig B, Wu Y, et al. Placental growth factor reconstitutes hematopoiesis by recruiting VEGFR1(+) stem cells from bone-marrow microenvironment. Nat Med 2002;8:841–9.
14. Rafii S, Avecilla S, Shmelkov S, et al. Angiogenic factors reconstitute hematopoiesis by recruiting stem cells from bone marrow microenvironment. Ann N Y Acad Sci 2003;996:49–60.
15. TIMI Study Group. The Thrombolysis In Myocardial Infarction (TIMI) trial. Phase I findings. N Engl J Med 1985;312:932–6.
16. Izumi T, Saito Y, Kishimoto I, et al. Blockade of the natriuretic peptide receptor guanylyl cyclase-A inhibits NF-kappaB activation and alleviates myocardial ischemia/reperfusion injury. J Clin Invest 2001;108:203–13.
17. Hauser S, Weich HA. A heparin-binding form of placenta growth factor (PlGF-2) is expressed in human umbilical vein endothelial cells and in placenta. Growth Factors 1993;9:259–68.
18. Persico MG, Vincenti V, DiPalma T. Structure, expression and receptor-binding properties of placenta growth factor (PlGF). Curr Top Microbiol Immunol 1999;237:31–40.
19. Khaliq A, Li XF, Shams M, et al. Localisation of placenta growth factor (PlGF) in human term placenta. Growth Factors 1996;13:243–50.
20. Green CJ, Lichtlen P, Huynh NT, et al. Placenta growth factor gene expression is induced by hypoxia in fibroblasts: a central role for metal transcription factor-1. Cancer Res 2001;61:2696–703.
21. Perelman N, Selvaraj SK, Batra S, et al. Placenta growth factor activates monocytes and correlates with sickle cell disease severity. Blood 2003;102:1506–14.
22. Heeschen C, Dimmeler S, Fichtlscherer S, et al. Prognostic value of placental growth factor in patients with acute chest pain. JAMA 2004;291:435–41.
23. Luttun A, Tjwa M, Moons L, et al. Revascularization of ischemic tissues by PlGF treatment, and inhibition of tumor angiogenesis, arthritis and atherosclerosis by anti-flt1. Nat Med 2002;8:831–40.
24. Adini A, Kornaga T, Firoozbakht F, Benjamin LE. Placental growth factor is a survival factor for tumor endothelial cells and macrophages. Cancer Res 2002;62:2749–52.
25. Sawano A, Iwai S, Sakurai Y, et al. Flt-1, vascular endothelial growth factor receptor 1, is a novel cell surface marker for the lineage of monocyte-macrophages in humans. Blood 2001;97:785–91.
26. Pipp F, Heil M, Issbrucker K, et al. VEGFR-1-selective VEGF homologue PlGF is arteriogenic: evidence for a monocyte-mediated mechanism. Circ Res 2003;92:378–85.
27. Heil M, Ziegelhoeffer T, Pipp F, et al. Blood monocyte concentration is critical for enhancement of collateral artery growth. Am J Physiol Heart Circ Physiol 2002;283:H2411–9.
28. Selvaraj SK, Giri RK, Perelman N, Johnson C, Malik P, Kalra VK. Mechanism of monocyte activation and expression of proinflammatory cytochemokines by placenta growth factor. Blood 2003;102:1515–24.
29. Semenza GL. Regulation of hypoxia-induced angiogenesis: a chaperone escorts VEGF to the dance. J Clin Invest 2001;108:39–40.
30. Landgren E, Schiller P, Cao Y, Claesson-Welsh L. Placenta growth factor stimulates MAP kinase and mitogenicity but not phospholipase C-gamma and migration of endothelial cells expressing Flt 1. Oncogene 1998;16:359–67.
31. Autiero M, Waltenberger J, Communi D, et al. Role of PlGF in the intra- and intermolecular cross talk between the VEGF receptors Flt1 and Flk1. Nat Med 2003;9:936–43.
32. Assmus B, Schachinger V, Teupe C, et al. Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI). Circulation 2002;106:3009–17.
33. Britten MB, Abolmaali ND, Assmus B, et al. Infarct remodeling after intracoronary progenitor cell treatment in patients with acute myocardial infarction (TOPCARE-AMI): mechanistic insights from serial contrast-enhanced magnetic resonance imaging. Circulation 2003;108: 2212–8.
34. Stamm C, Westphal B, Kleine HD, et al. Autologous bone-marrow stem-cell transplantation for myocardial regeneration. Lancet 2003; 361:45–6.
35. Hojo Y, Ikeda U, Zhu Y, et al. Expression of vascular endothelial growth factor in patients with acute myocardial infarction. J Am Coll Cardiol 2000;35:968–73.
36. Jugdutt BI. Monocytosis and adverse left ventricular remodeling after reperfused myocardial infarction. J Am Coll Cardiol 2002;39: 247–50.
37. Maekawa Y, Anzai T, Yoshikawa T, et al. Prognostic significance of peripheral monocytosis after reperfused acute myocardial infarction: a possible role for left ventricular remodeling. J Am Coll Cardiol 2002;39:241–6.