Intracoronary Transplantation of Non-Expanded Peripheral Blood-Derived Mononuclear Cells Promotes Improvement of Cardiac Function in Patients With Acute Myocardial Infarction

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Background   Transplantation of non-expanded peripheral blood mononuclear cells (PBMNCs) enhances neovessel formation in ischemic myocardium and limbs by releasing angiogenic factors. This study was designed to examine whether intracoronary transplantation of PBMNCs improves cardiac function after acute myocardial infarction (AMI).

Methods and Results   After successful percutaneous coronary intervention (PCI) for a ST-elevation AMI with occlusion of proximal left anterior descending coronary artery within 24 h, patients were assigned to either a control group or the PBMNC group that received intracoronary infusion of PBMNCs within 5 days after PCI. PBMNCs were obtained from patients by COBE spectra-apheresis and concentrated to 10 ml, 3.3 ml of which was infused via over-the-wire catheter. The primary endpoint was the global left ventricular ejection fraction (LVEF) change from baseline to 6 months' follow-up. The data showed that the absolute increase in LVEF was 7.4% in the control group and 13.4% (p=0.037 vs control) in the PBMNC group. Cell therapy resulted in a greater tendency of ΔRegional ejection fraction (EF) or significant improvement in the wall motion score index and Tc-99m-tetrofosmin perfusion defect score associated with the infarct area, compared with controls. Moreover, intracoronary administration of PBMNCs did not exacerbate either left ventricular (LV) end-diastolic and end-systolic volume expansion or high-risk arrhythmia, without any adverse clinical events.

Conclusion   Intracoronary infusion of non-expanded PBMNCs promotes improvement of LV systolic function. This less invasive and more feasible approach to collecting endothelial progenitor cells may provide a novel therapeutic option for improving cardiac function after AMI.   (*Circ J* 2007; **71:** 1199–1207)

Key Words: Acute myocardial infarction; Angiogenesis; Cardiac function; Peripheral blood-derived mononuclear cells

Differentiation of mesodermal cells to angioblasts and subsequent endothelial differentiation was believed to exclusively occur in embryonic development,[1] but this dogma was overturned when human adult peripheral blood mononuclear cells (PBMNCs) were demonstrated to differentiate into the endothelial lineage.[2] These cells named "endothelial progenitor cells" (EPCs) expressed endothelial markers, and were incorporated into the sites of ischemia.[3,4] We have recently demonstrated that bone marrow mononuclear cells (BMMNCs) contain EPCs in the CD34+ cell fraction and various proangiogenic factors, such as basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), and angiopoietin 1 in the CD34– cell fraction, and that implantation of BMMNCs into the site of ischemia enhances angiogenesis via harmonic supply of EPCs and angiogenic factors.[5,6] This technique has been used clinically and developed as a useful therapeutic option for human critical limb ischemia.[7]

The concept of the heart as an organ composed of terminally differentiated myocytes incapable of regeneration is also being challenged.[8–10] Although attempts to replace necrotic tissue by transplanting other cells (eg, fetal cardiac myocytes or skeletal myoblasts) succeeded in reconstituting heart muscle, these cells failed to completely integrate structurally and to display characteristic physiological function.[11–13] In contrast, bone marrow cells (BMCs) have the ability to differentiate into various tissue and are likely to

regenerate myocardium by inducing myogenesis and angiogenesis, as shown by improved cardiac function and myocardial perfusion in recent accumulating evidence from animals and humans![14–17] In particular, cardiac transfer of BMC-derived stem/progenitor cells can have a favorable impact in patients with acute myocardial infarction (AMI)![18,19] Clinical efficacy of intracoronary transplantation of BMCs after AMI has been the focus of recent large scale, randomized, and controlled trials. The potential benefit of intracoronary injection of BMCs for left ventricular (LV) function was reported in the randomized Bone marrow transfer to enhance ST-elevation infarct regeneration (BOOST) trial[20] and in the Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction (REPAIR-AMI) trial[21] In contrast, intracoronary injection of BMCs after AMI did not significantly improve LV function in the Autologous Stem-Cell Transplantation in Acute Myocardial Infarction (ASTAMI) trial[22] or in the trial reported by Janssens et al[23] Thus, the latest randomized clinical studies for transplantation of BMCs against AMI retain discrepancies that must be resolved in future trials.

We have previously reported that NOGA-catheter based implantation of non-expanded PBMNCs alone can significantly improve systolic function in ischemic hibernating myocardium of pigs[24] and that intramuscular injection of human PBMNCs markedly increases regional blood flow in hindlimb ischemia by releasing potent angiogenic factors such as VEGF and bFGF[25] Moreover, it has been demonstrated that EPCs are indeed mobilized in patients with AMI, peak at 7 days after the onset[26] and that stromal-cell-derived factor-1 (SDF-1), an important stem cell homing factor, is expressed in the myocardium immediately after AMI[27] Because the invasiveness of BMC collection in the acute phase of AMI limits its clinical application, we hypothesized that transplantation of non-expanded PBMNCs would even improve the cardiac function in patients with AMI. In this context, we started a clinical trial named the "Japan Trial for Therapeutic Angiogenesis by Cell Transplantation of Peripheral Blood-derived Mononuclear Cells for Acute Myocadial Infarction (TACT-PB-AMI)" in 2004. The primary aim of our study was to examine whether intracoronary injection of non-expanded PBMNCs results in an improvement in LV function, as measured by LV ejection fraction (LVEF), after AMI. Additional objectives were to test the feasibility and safety of this treatment, as well as to assess the effectiveness on regional wall motion, cardiac volumes, and arrhythmias.

Methods

Patients and Study Protocol

Patients between 18 and 80 years of age were eligible for inclusion in the study if they had a first acute ST-elevation myocardial infarction with occlusion of the proximal left anterior descending (LAD) coronary artery and a creatine kinase (CK) level >1,000 IU, which was successfully treated by percutaneous coronary intervention (PCI) within 24 h. According to previous observations[28,29] CK values were serially measured every 4 h for 24 h after the onset of AMI. Exclusion criteria were the presence of cardiogenic shock requiring intravenous pressors or intra-aortic balloon counterpulsation, pulmonary edema, advanced hepatic or renal dysfunction, evidence of malignant diseases, or unwillingness to participate. Because the patients were best suited for an evaluation of LV function by angiographic imaging,

we decided to include only patients with anterior wall infarction. This study protocol was approved by the Ethics Review Board of Kyoto Prefectural University School of Medicine, and written informed consent was given by each patient.

The study was designed as an open-label and non-randomized clinical trial. Briefly, after successful PCI (TIMI III), patients were assigned to either the control (PCI alone) group or non-expanded PBMNC group that received intracoronary infusion of PBMNCs within 5 days after PCI. Intracoronary cell transplantation was performed by over-the-wire balloon catheter. Neither collection of PBMNCs nor sham injection was performed in the control group. The primary endpoint was the global LVEF change from baseline to 6 months' follow-up.

Catheterization Procedure for Progenitor Cell Transplantation

A mean of 2.5±0.5 days after the AMI, an over-the wire balloon catheter was advanced into the infarct-related artery (eg, LAD). To allow for adhesion and transmigration of the infused cells through the endothelium, the balloon was inflated inside the stent previously implanted during the acute reperfusion procedure with low pressure to block blood flow for 3 min while 3.3 ml of the PBMNCs suspension was infused distally to the occluding balloon through the central port of the balloon catheter, as previously described![19] This maneuver was repeated 3 times to accommodate infusion of the total 10-ml cell suspension, interrupted by 3 min of reflow by deflating the balloon to minimize extensive ischemia. After completion of intracoronary cell transplantation, coronary angiography was repeated to ascertain vessel patency and unimpeded flow of contrast material.

Preparation of Progenitor Cells

A cell separator apheresis system with computer software (COBE Spectra, software version 6.1, Gambro BCT, Lakewood, Co, USA) was used to collect all PBMNC products via the standard MNC program. Acid citrate dextrose-A (ACD-A, Baxter Healthcare Corporation, Deerfield, IL, USA) was used as the anticoagulant at a whole body-to-ACD ratio of 20–25:1 in combination with 2,000 IU heparin sulfate. Apheresis was performed through central venous access from the femoral vein in all patients in the PBMNC group. With the aim of processing the largest amount of blood in the shortest possible time, apheresis procedures were performed with the highest possible but still tolerable blood flow rate, such as 55 ml/min. No more than 2.5-fold of the donor's blood volume was processed on a single day. We usually obtain PBMNCs ($\approx 5 \times 10^9$ cells) from patients by COBE spectra-apheresis and concentrate them to 10 ml by density gradient centrifugation (Kubota 9810, Japan).

After PBMNCs were harvested by COBE Spectra, 100μl of the cell suspension was diluted by PBS(–) containing 0.5% bovine serum albumin (Fraction V, Sigma, St Louis, MO, USA) used for flow cytometric analysis. The cells were stained with allophycocyanin-conjugated (APC)-anti-human CD34 (Becton Dickinson, San Jose, CA, USA) and phycoerythrin-conjugated (PE)-anti-human VEGFR2 (KDR) (R&D Systems, Minneapolis, MN, USA). Appropriate isotype controls were used for each staining procedure; 1×10^5 cells were gated within the lymphocyte region on forward-scatter vs side-scatter plots using a FACS Calibur (BD Bioscience). Next, the percentages of cells in

Table 1 Clinical Characteristics of the Study Population

	Control group (n=36)	PBMNC group (n=18)	p value
Age, years	60.4±11.3	61.8±8.7	0.65
Male sex, no. (%)	32 (88.9)	15 (83.3)	0.57
Hypertension, no. (%)	16 (44.4)	11 (61.1)	0.25
Hyperlipidemia, no. (%)	20 (55.6)	12 (66.7)	0.43
Diabetes, no. (%)	12 (33.3)	5 (27.8)	0.68
Smoking, no. (%)	20 (55.6)	12 (66.7)	0.43
PAD, no. (%)	3 (8.3)	1 (5.6)	0.71
Killip class on admission	1.20±0.48	1.22±0.43	0.66
Infarct segment	6.3±0.5	6.6±0.5	0.06
Vessel diameter, mm	3.24±0.38	3.22±0.31	0.89
Peak creatine kinase, IU/dl	3,764±2,506	4,255±1,615	0.47
Time to revascularization, h	6.1±5.7	5.2±2.4	0.49
Mean transplanted cells, no.	–	$4.92±2.82×10^9$	
Medication			
ACEI, no. (%)	26 (72.2)	15 (83.3)	0.37
ARB, no. (%)	11 (30.6)	5 (27.8)	0.83
-blocker, no. (%)	19 (52.8)	10 (55.6)	0.85
Diuretics, no. (%)	6 (16.7)	3 (16.7)	1.00
Statins, no. (%)	22 (61.1)	11 (61.1)	1.00

Values are expressed as mean±SD.
PBMNC, peripheral blood mononuclear cell; PAD, peripheral arterial disease; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin-receptor blocker.

LV Angiography

LV angiograms were obtained according to standard acquisition guidelines immediately after PCI and at 6 months' follow-up. LVEF and LV volumes were calculated by the area–length method, and regional wall motion was determined with the use of the centerline chord method.

Measurement of Other Parameters

For the assessment of regional LV wall motion, echocardiography was carried out before cell transplantation and at 6 months' follow-up. Two-dimensional resting echocardiography was performed in the 4 standard views (parasternal long-axis and short-axis views and apical 4- and 2-chamber views) and regional LV wall motion analysis was performed as described by the Committee on the Standards of the American Society of Echocardiography, dividing the left ventricle into 16 segments and scoring wall motion as 1=normal, 2=hypokinesis, 3=akinesis, 4=dyskinesis for each segment. The wall motion score index (WMSI) was calculated as the sum of the scores of the segments divided by the number of the segments evaluated at the day of cell transplantation and 6 months' follow-up.

We performed resting Tc-99m (^{99m}Tc)-tetrofosmin gated single photon emission computed tomography (SPECT) before hospital discharge and at 6 months' follow-up. In all patients, 592 MBq of ^{99m}Tc-tetrofosmin was intravenously injected at rest. Immediately after the injection, each patient drank a glass of milk to accelerate tracer clearance from the hepatobiliary system. Data acquisition for SPECT imaging was performed at 30 min after ^{99m}Tc-tetrofosmin injection, using a rotating digital gamma camera (Picker PRISM IRIX) equipped with a low energy, high resolution, and parallel-hole collimator. Reconstructed transaxial images were reoriented in the vertical long-axis and short-axis of the LV. The basal and midventricular segments on short-axis views of the LV myocardium were divided into 8 segments each, and 16 segments were taken. An apical region

on the vertical long axis was also taken, and a total of 17 segments were analyzed. The ^{99m}Tc-tetrofosmin perfusion defects were visually evaluated by 2 experienced observers, who had no knowledge of the patient's clinical information, with a 5-point grading system (0=normal, 1=mildly decreased uptake, 2=moderately decreased uptake, 3=severely decreased uptake, 4=defect). The grading was decided on by consensus between the 2 observers, and the sum of the scores for all segments was used as the defect score.

To assess whether intracoronary cell transplantation was associated with proarrhythmic effects, we performed 24-h Holter recording for all patients before hospital discharge and at 6 months' follow-up, and estimated the Holter Lawn class by calculating premature ventricular complexes and ventricular tachycardias.

Follow-up Examinations

Six months after progenitor cell therapy, cardiac catheterization was repeated; left ventriculography was performed with identical projections and adequate contrast opacification for quantitative analysis according to standard guidelines and coronary angiograms were analyzed for the presence of restenosis in the infarct-related artery. Echocardiography, Holter ECG, and perfusion scintigraphy were also repeated after 6 months.

Statistical Analysis

Continuous variables are presented as means±SD. Control and cell therapy groups were compared using the chi-square test for discrete variables and unpaired Student's t-test for continuous variables according to standard statistical methods. Statistical significance was assumed at a value of p<0.05. All statistical analysis was performed with SPSS (Version 9.0, SPSS Inc, Chicago, IL, USA).

Results

Baseline Characteristics and Procedural Results of Cell Infusion

The clinical characteristics of the study population are

each population described below were calculated using CELLQuest software (BD Bioscience).

Table 2 Clinical Characteristics of the Patients' Measurements of Hemodynamics at the Time of AMI (Baseline) and 6-Months' Follow-up

	Control group (n=36)	PBMNC group (n=18)	p value
LVSP (mmHg)			
Baseline	121.7±13.0	120.3±13.9	0.73
6 months	125.0±19.2	126.9±8.7	0.67
p value (baseline vs 6 months)	0.47	0.10	
LVDP (mmHg)			
Baseline	3.9±4.8	4.6±5.1	0.67
6 months	3.1±2.4	4.1±3.5	0.27
p value (baseline vs 6 months)	0.4	0.73	
LVEDP (mmHg)			
Baseline	20.1±6.1	19.8±4.8	0.89
6 months	13.6±3.7	14.3±5.3	0.57
p value (baseline vs 6 months)	<0.0001	<0.0001	
AOMP (mmHg)			
Baseline	90.3±13.4	88.4±9.7	0.61
6 months	89.5±14.2	90.2±9.7	0.85
p value (baseline vs 6 months)	0.84	0.58	
HR (beats/min)			
Baseline	88.3±7.7	88.0±10.9	0.91
6 months	65.8±6.8	65.0±7.2	0.69
p value (baseline vs 6 months)	<0.0001	<0.0001	

Values are mean±SD.
AMI, acute myocardial infarction; LVSP, left ventricular systolic pressure; LVDP, left ventricular diastolic pressure; LVEDP, left ventricular end-diastolic pressure; AOMP, aortic mean pressure; HR, heart rate. Other abbreviation see in Table 1.

Table 3 Clinical Characteristics of Patients' Baseline Cardiac Function

	Control group (n=36)	PBMNC group (n=18)	p value
LVEF (%)	48.8±11.3	43.8±12.5	0.20
EDVI (ml/m²)	60.9±14.8	70.0±15.0	0.07
ESVI (ml/m²)	32.0±13.1	39.9±14.9	0.054
Regional EF (%)			
Segment #2	11.7±6.1	12.3±7.0	0.77
Segment #3	8.4±6.5	5.5±5.8	0.11

LVEF, left ventricular ejection fraction; EDVI, end-diastolic volume index; ESVI, end-systolic volume index; EF, ejection fraction. Other abbreviation see in Table 1.

shown in Table 1. The PBMNC group and control group were well matched with respect to baseline characteristics and procedural characteristics, such as age, sex, and coronary risk factors, Killip class, infarct segment, and vessel diameter. Although particularly important factors influencing cardiac function are considered to be peak CK and time to revascularization, there were no significant differences in these factors between the 2 groups. All patients were treated with aspirin (100 mg/day), ticlopidine (200 mg/day for at least 4 weeks after PCI) or cilostazol (200 mg/day at least 4 weeks after PCI), statin, -blocker, and angiotensin-converting enzyme inhibitor (ACEI) or angiotensin-receptor blocker (ARB) during the hospitalization for AMI and continued until the 6-months' follow-up examination, unless these agents were contraindicated. There were no significant differences between the control and PBMNC groups in the administration of ACEIs, ARBs, -blockers or statins (Table 1).

No patient had either bleeding complications through the central venous access from the femoral vein or systemic blood pressure fall during the apheresis procedures. Although a transient further ST elevation associated with balloon occlusion was seen in the infarct-related ECG leads in most of the patients receiving PBMNCs, there were no serious symptomatic complaints or circulatory disturbances during or after cell transplantation. Neither intracoronary infusion nor the stop-flow procedure was performed in the

control group. There were no fatal cardiac events during the follow-up period, and no patient in either group had any clinical manifestation of heart failure.

Endothelial Progenitors in AMI Patients

FACS analysis showed that the percentage of CD34+ (0.12±0.2) or CD34/KDR+ (0.05±0.1) cells in the PBMNCs from AMI patients tended to be higher (2–10-fold, but not statistically significant), compared with healthy volunteers (CD34+; 0.06±0.1, CD34/KDR+; 0.003±0.001), and that these cells also possess the characteristics of EPCs, as demonstrated by DiI-acetylated LDL uptake and lectin binding.

Hemodynamics and LV Function by Angiography

Table 2 shows the hemodynamic measurements in the control and PBMNC groups at the time of AMI (baseline) and at 6-months' follow-up. Although LV end-diastolic pressure (LVEDP) and heart rate (HR) were significantly reduced from baseline to 6-months' follow-up in both groups, there were no significant differences in LV systolic pressure (LVSP), LV diastolic pressure (LVDP), or aortic mean pressure (AOMP). Moreover, there were no statistically significant differences in LVSP, LVDP, LVEDP, AOMP, or HR between groups at either baseline or 6-months' follow-up.

Table 3 shows the clinical characteristics of baseline LV cardiac function. There were no significant differences in
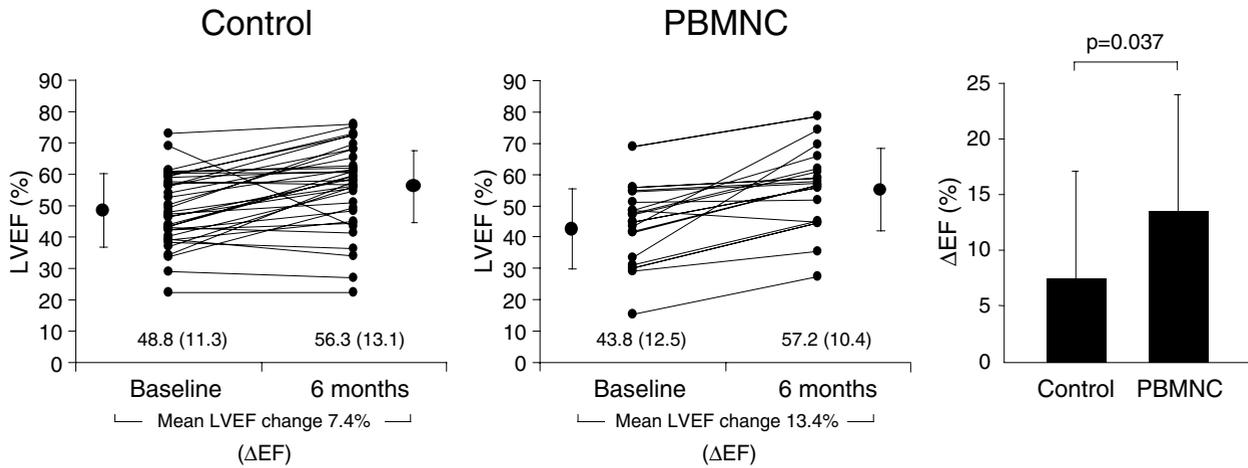
Control

PBMNC

Fig 1.   Global left ventricular ejection fraction (LVEF) at baseline and 6 months' follow-up, and the absolute increase in LVEF (Δejection fraction (EF)) in the control and peripheral blood mononuclear cell (PBMNC) groups. Small dots show data for individual patients; large dots show mean values. Vertical bars show SD.
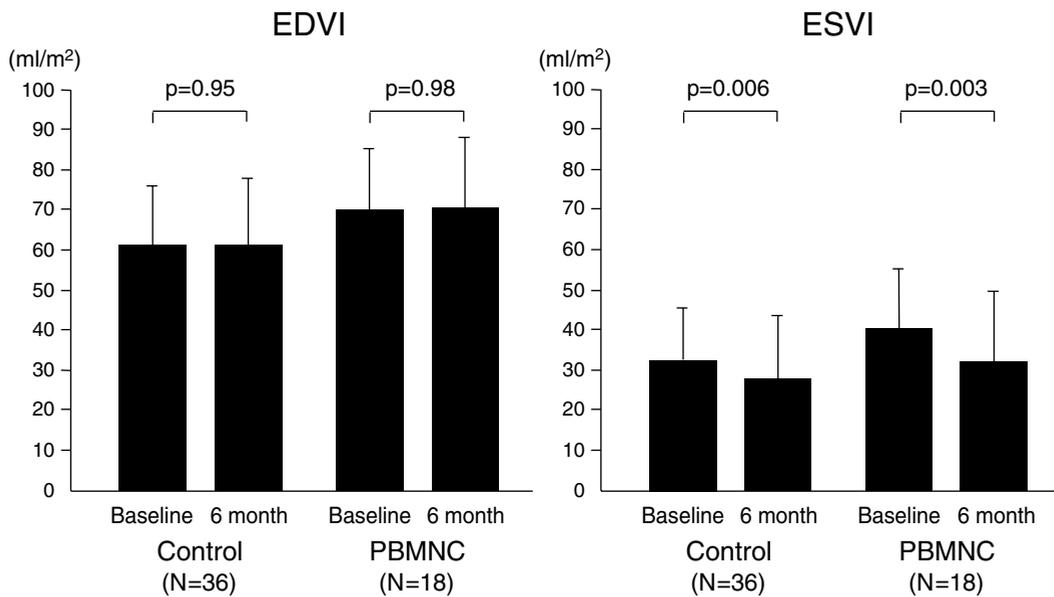
EDVI

ESVI

Fig 2.   End-diastolic volume index (EDVI) and end-systolic volume index (ESVI) at baseline and 6 months' follow-up. Data are mean ± SD. PBMNC, peripheral blood mononuclear cell.

LVEF, end-diastolic volume index (EDVI), end-systolic volume index (ESVI), or regional ejection fraction (EF) between the control and PBMNC groups. Fig 1 illustrates LV function as assessed by cineventriculography at baseline and 6-months' follow-up. In the control group, LVEF was 48.8% at baseline and gradually increased to 56.3% after 6 months. In contrast, LVEF was 43.8% at baseline and increased to 57.2% after 6 months in the PBMNC group. Although the baseline measurement of LVEF did not differ significantly between the 2 groups, the absolute increase in LVEF (ΔEF) was 7.4% in the control group and 13.4% in the PBMNC group. Our data therefore show that cell transplantation significantly improved LVEF, and there was a modest but significant increase in global LVEF, even in the patients with PCI alone (ie, controls). It is notable that the ΔEF value in thePBMNC group was significantly greater than that in the control group (PCI alone) (Fig 1).

There were no significant difference in EDVI between

baseline and 6 months' follow-up in either control or PBMNC group (Fig 2) and also no significant difference in the absolute difference in EDVI (ΔEDVI) between the control and PBMNC groups (p=0.90). ESVI was significantly decreased from baseline to 6 months' follow-up in both groups, such that the absolute difference in ESVI (ΔESVI) was –4.3% in the control group and –8.2% in the PBMNC group. Thus, although there was no statistically significant difference in ΔESVI value between the 2 groups (p=0.09), the ΔESVI value tended to be lower in the PBMNC group, compared with controls. Selective analysis of the infarcted zone showed that baseline measurements of regional wall motion (regional EF), that is, segments #2 and #3 of the AHA classification, did not differ significantly between the control and PBMNC groups (Table 3). Although regional wall motion in the infarct area was significantly improved from baseline to 6 months' follow-up in both groups, the absolute value of regional EF (ΔRegional EF)
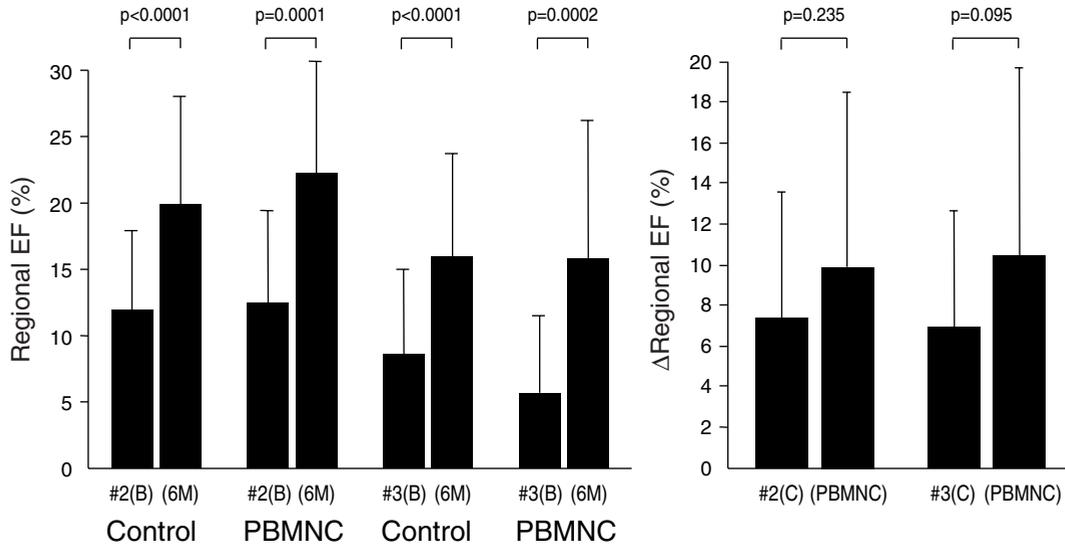
Fig 3.   Regional ejection fraction (EF) at baseline and 6 months' follow-up, and the absolute increase in regional EF (ΔRegional EF) in the control and peripheral blood mononuclear cell (PBMNC) groups. Regional EF in the infarct area (segments #2 and #3) was estimated as described in Methods. Data are mean±SD.
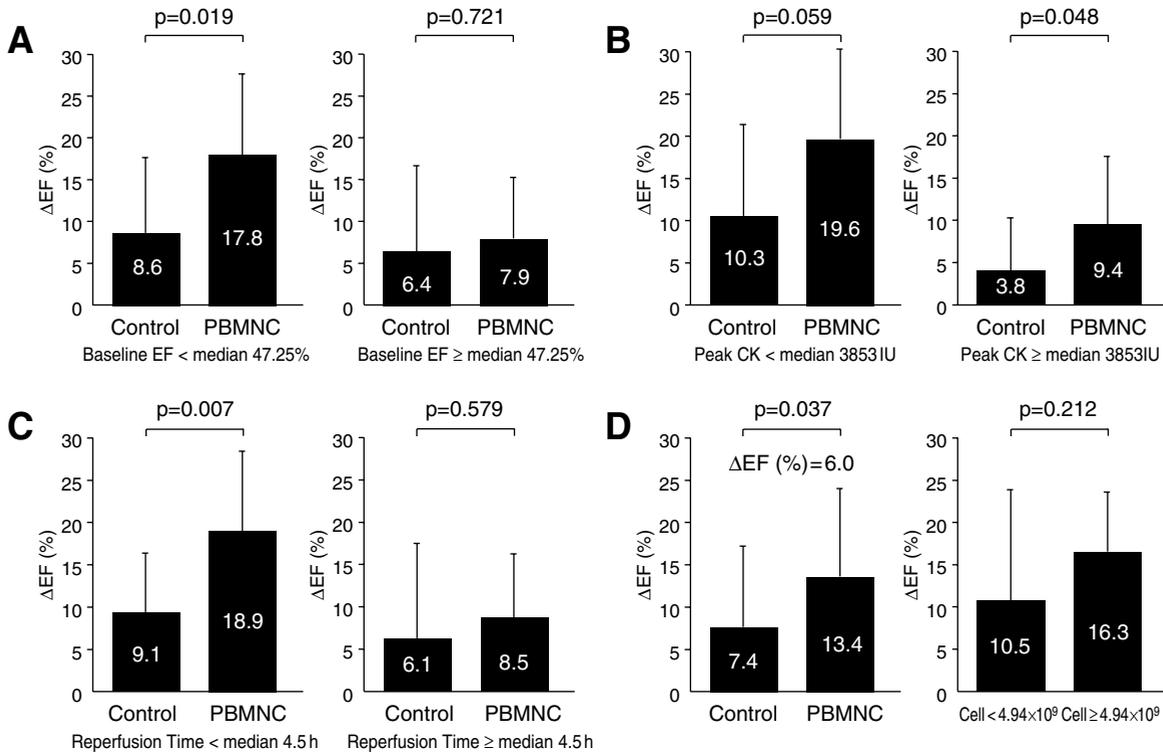

Fig 4.   Impact of baseline ejection fraction (EF), peak creatine kinase (CK), reperfusion time, and transplanted cell number on cardiac function. All patients were divided into 2 groups by median baseline EF (A), median CK (B), median reperfusion time (C), and median transplanted cell number (D), and the treatment effect of peripheral blood mononuclear cell (PBMNC) infusion on the absolute increase in left ventricular EF (ΔEF) was analyzed. Data are mean±SD.3

tended to be greater in PBMNC group, compared with control (Fig 3).

Effects of Cell Transplantation on Other Parameters
    Resting echocardiography indicated that cell transplantation significantly decreased the WMSI from baseline (1.67±0.18) to 6 months' follow-up (1.50±0.34) and this im-

provement in regional wall motion was especially seen in the infarct-related area of 15 of 18 patients. Resting [99mTc]-tetrofosmin gated SPECT also showed that cell transplantation significantly decreased the perfusion defect score from baseline (20.4±9.0) to 6 months' follow-up (14.1±9.4), and this improvement in regional myocardial perfusion was seen in the infarct-related area of 14 of 18 patients. In con-

trast, cell transplantation did not significantly exacerbate but rather tend to decrease the Holter Lawn Class (data not shown).

Impact of Baseline Parameters and on Cardiac Function

We observed that cell transplantation significantly improved the ΔEF value, compared with controls (Fig 1). We further analyzed the effect of baseline parameters on the absolute increase in LVEF. In order to avoid our "arbitrary decision" on subgroup analysis, the total patient population was dichotomized according to the "median values" of baseline EF, peak CK, reperfusion time, and transplanted cell number at baseline, as previously reported[21] We then reanalyzed the data for addressing the clinical relevance of PBMNC administration.

We first examined the impact of baseline EF on cardiac function. When we divided all the patients into 2 groups by median baseline EF 47.25%, there was a significant interaction between the treatment effect of PBMNC infusion and the baseline EF. Among patients with a baseline EF below the median value, patients in the PBMNC group had an absolute increase in LVEF (ΔEF value) that was 2-fold that of the control group (Fig 4A) (absolute difference, 9.2%; 95% confidence interval (CI), 5.3 to 13.1). In contrast, among patients with a baseline EF at or above the median, the absolute difference between the 2 groups was only 1.5% (absolute difference, 1.5%; 95% CI, −2.1 to 5.1), suggesting that cell transplantation preferentially improved LV function in patients with relatively depressed contractility.

We next examined the impact of peak CK on cardiac function. When we divided all the patients into 2 groups by median peak CK 3,853 IU/dl, there was again a significant interaction between the treatment effect of PBMNC infusion and the peak CK. Among patients with a baseline peak CK at or above the median value, those in the PBMNC group had an absolute ΔEF value that was more than 2-fold the value for the control group (Fig 4B) (absolute difference, 5.6%; 95% CI, 2.8 to 8.4). Among patients with a baseline peak CK below the median, those in the PBMNC group also had an absolute ΔEF value that was ≈2-fold that in the control group, although there was not a significant difference in the ΔEF value between the 2 groups. The data suggest that cell transplantation preferentially improved LV function irrespective of infarct size.

We also examined the impact of reperfusion time on cardiac function. When we divided all the patients into 2 groups by a median reperfusion time of 4.5 h, there was again a significant interaction between the treatment effect of PBMNC infusion and the reperfusion time. Among patients with a baseline reperfusion time below the median value, those in the PBMNC group had an absolute ΔEF value that was almost 2-fold that in the control group (Fig 4C) (absolute difference, 9.8%; 95% CI, 6.1 to 13.5). In contrast, among patients with a baseline reperfusion time at or above the median, the absolute difference between the 2 groups was only 2.4% (absolute difference, 2.4%; 95% CI, −1.6 to 13.8), suggesting that cell transplantation preferentially improved LV function in patients with relatively early reperfusion.

We further examined the impact of transplanted cell number on cardiac function. When we divided the patients receiving cell therapy into 2 groups by a median cell number of 4.94×10^9, there was no significant interaction between the treatment effect of PBMNC infusion and the

number of transplanted cells, suggesting that cell number did not significantly affect LV function, at least in our study (Fig 4D).

Clinical Manifestations and Adverse Effects

The occurrence of individual major adverse cardiac events of death, recurrence of myocardial infarction, or rehospitalization for heart failure did not differ significantly between the control and PBMNC groups. The rate of in-stent restenosis at the culprit lesion in patients who received PBMNC transplantation was 22.2%, which was not significantly different from that in the control patients (p=0.21).

Discussion

The major finding of the present study is that the intracoronary administration of non-expanded PBMNCs significantly enhanced the recovery of LV contractile function in patients optimally treated for AMI. After 6 months, the absolute increase in LVEF (ΔEF) was significantly higher in the PBMNC group than in controls. The enhanced recovery of LV contractile function after the administration of PBMNCs appeared to be related to a reduction in regional LV dysfunction within the territory of the infarct, because cell therapy resulted in a greater tendency of ΔRegional EF or significant improvement of WMSI and ^{99m}Tc-tetrofosmin perfusion defect score associated with the infarct area, compared with controls. Moreover, intracoronary administration of PBMNCs did not exacerbate LV expansion or high-risk arrhythmia after the infarction. Taken together, our findings indicate that when combined with optimal reperfusion therapy and standard medical treatment, intracoronary administration of PBMNCs is able to enhance the recovery of global and regional LV function after AMI.

Our results of subgroup analysis also provide some meaningful suggestions in the choice of patients for cell therapy against AMI; cell transplantation preferentially improved LV function in patients with relatively depressed contractility, irrespective of infarct size, and with relatively early reperfusion. Thus, patients with relatively early reperfusion and depressed LV contractile function had better improvement in contractile function after the intracoronary administration of PBMNCs. Our data therefore suggest that PBMNC transplantation may rescue dying myocytes that were severely stunned in the infarct border zone, irrespective of the infarct size.

Several lines of evidence suggest that the level of circulating CD34+ EPCs is predictive of future cardiovascular events[30] and that bone marrow-derived CD34+ cells could be important for cardiovascular repair[31] In the present study, we used a mean of 4.92×10^9 PBMNCs containing ≈6×10^6 CD34+ cells for intracoronary injection and obtained an increase of 6% in ΔEF value. In the BOOST and REPAIR-AMI trials, ≈2.5×10^9 unfractionated BMCs and ≈2.4×10^8 Ficoll-separated BMCs (≈$2-3 \times 10^6$ CD34+ cells) were transplanted, with increases of 6% and 2.5% in ΔEF values, respectively. In contrast, in Janssens's report and the ASTAMI trial, ≈3×10^8 Ficoll-separated BMCs (≈2.8×10^6 CD34+ cells) and ≈7×10^7 Ficoll-separated BMCs (≈0.7×10^6 CD34+ cells), respectively, were used, and there was no significant increase in ΔEF value. These data therefore indicate that the total number of injected cells or CD34+ cells does not always correlate with the improvement in cardiac performance after cell transplantation, although trans-

planted cell numbers appear to have been relatively low in the ASTAMI trial. Indeed, cell number did not significantly affect LV function in our study (Fig 4). Importantly, our study results also suggests that PBMNCs, which were even not culture-expanded, show great capability as a comparable cell source to BMCs.

In view of BMCs homing into the heart, the microenvironment (eg, niche) within the infarct tissue and the timing of cell delivery may be important for the incorporation of BMCs. Recent observations indicate that after intracoronary transfer only 1.3–2.6% of ^{18}F-FDG-labelled unselected BMMNCs were detected in the infarcted heart, whereas most cells homed into the liver and spleen within ≈1 h after intracoronary delivery[32] Therefore, the findings do not support the likelihood that progenitor cells home into jeopardized myocardium and transdifferentiate into cardiac myocytes capable of generating active force development in scar tissue, but rather suggest other potential mechanisms through angiogenesis and reduced apoptosis. Indeed, recent articles have shed light on the potential of BMCs to differentiate into hematopoietic and endothelial lineages able to secrete proangiogenic factors,[33] rather than transdifferentiation into other cell lineages such as cardiac myocytes.[34,35] These subsets of mature hematopoietic cells, either derived from bone marrow or peripheral blood, may cooperate with transplanted or resident cardiac and endothelial stem/progenitor cells to enhance their capacity for tissue repair through angiogenesis, anti-apoptosis, and myocyte proliferation after ischemic injury.[36,37]

The most potential advantage of our method is its feasibility and safety in collecting PBMNCs from patients with depressed cardiac performance and bleeding tendency, by administration of enough antiplatelet agents, such as aspirin and ticlopidine. Although previous studies emphasize the feasibility and safety of BMCs aspiration, this maneuver is always accompanied by the risk of s serious bleeding accident from bone in patients receiving antiplatelet therapy. There are also a few other reports indicating a benefit of intracoronary infusion of granulocyte colony-stimulating factor (G-CSF) mobilized-PBMNCs for AMI[38–40] However, this procedure still involves several possible adverse effects of G-CSF, including serious thrombosis, bone pain, fever, and aggravation of in-stent restenosis[38–40] In contrast to those previous studies, we could easily collect ≈5×10^9 cells PBMNCs, avoiding contamination with neutrophils, within 2 h without any hemodynamic or bleeding problems. We could concentrate the collected PBMNCs to 10 ml by density gradient centrifugation aseptically through bag to bag, instead of by Ficoll gradient sedimentation methods. Our present data, therefore, show for the first time that intracoronary infusion of non-expanded PBMNCs alone can promote improvement of LV function without any bleeding accident or G-CSF-related serious adverse effects. Because we can easily obtain levels of ≈6×10^6 CD34$^+$ cells, which is higher than in either the BOOST or REPAIR-AMI trials, we never need another laboratory to expand the PBMNCs, giving substantial merit that this protocol can be easily accepted in any hospital worldwide.

A major limitation of our study is that evaluation of the present regeneration therapy was not randomized, double-blind, and controlled. Moreover, cardiac function was not assessed with state-of-the-art imaging modalities, such as magnetic resonance imaging (MRI), and LV angiography was used exclusively for the serial assessment of LV function. Although angiography is well suited to delineate

regional contractile function for AMI by LAD occlusion, the use of MRI to assess global LV function would have more precisely depicted changes in the distorted geometry of the infarcted hearts. Although we choose contemporary controls, the control group does not reproduce the exact conditions of the cell therapy group to which the cells were transferred, including PBMNC collection and a placebo intracoronary injection. Therefore, the true benefit of cell transfer can not be fully appreciated and further research is needed to address these issues.

In conclusion, intracoronary infusion of PBMNCs in patients with AMI is associated with improved global LV contractile function; cell therapy preferentially improves LV function in patients with early reperfusion, but relatively depressed contractility after AMI, prevents end-diastolic and end-systolic LV volume expansion, and has not increased any adverse clinical events so far. Transplantation of PBMNCs might be an effective and novel therapeutic option for AMI, if cell transfer occurs expeditiously and in appropriate subjects. This less invasive and more feasible approach to collecting EPCs may be a novel therapeutic option for improving cardiac function after AMI.

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