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Mobilized Muse Cells After Acute Myocardial Infarction Predict Cardiac Function and Remodeling in the Chronic Phase

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Background: Multilineage differentiating stress-enduring (Muse) cells are SSEA3$^+$ and CD105$^+$ double-positive pluripotent-like stem cells. We aimed to examine the mobilization of Muse cells into peripheral blood after acute myocardial infarction (AMI) and their effects on left ventricular (LV) function and remodeling.

Methods and Results: In 79 patients with AMI, 44 patients with coronary artery disease (CAD), and 64 normal subjects (Control), we measured the number of Muse cells in the peripheral blood by fluorescence-activated cell sorting. Muse cells were measured on days 0, 1, 7, 14, and 21 after AMI. Plasma sphingosine-1-phosphate (S1P) levels were measured. Cardiac echocardiography was performed in the acute (within 7 days) and chronic (6 months) phases of AMI. Muse cell number on day 1 was significantly higher in the AMI (276±137 cells/100 μL) than in the CAD (167±89 cells/100 μL) and Control (164±125 cells/100 μL) groups. Muse cell number peaked on day 1, and had gradually decreased on day 21. Muse cell number positively correlated with plasma S1P levels. Patients with a higher increase in the number of Muse cells in the peripheral blood but not those with a lower increase in number of Muse cells in the acute phase showed improved LV function and remodeling in the chronic phase.

Conclusions: Endogenous Muse cells were mobilized into the peripheral blood after AMI. The number of Muse cells could be a predictor of prognosis in patients with AMI.

Key Words: Acute myocardial infarction; LV function; LV remodeling; Mobilization; Muse cells

Acute myocardial infarction (AMI) is a life-threatening event that causes the loss of cardiomyocytes and may lead to heart failure. Multilineage differentiating stress-enduring (Muse) cells were first discovered as stress-tolerant cells.[1] They can be isolated as pluripotent surface marker SSEA-3$^+$ and, more specifically, as cells double-positive for SSEA-3 and the mesenchymal stem cell (MSC) surface markers such as CD105 from the bone marrow (BM) (~0.03% of the mononucleated fraction), the peripheral blood, and the connective tissue of various tissues.[1–4] Muse cells are negative for CD34, CD31, von Willebrand factor, and c-Kit, and are thus distinct from hematopoietic stem cells (HSCs) and endothelial precursor cells (EPCs).[3] They can differentiate into endodermal-, ectodermal-, and mesodermal-lineage cells from a single cell, and are self-renewable in vitro,[1–3,5,6] which is inherent and not newly acquired by in vitro manipulation.[1] Their differentiation ability is also observed in vivo: intravenously or locally injected exogenous naïve Muse cells migrate to and accumulate in damaged tissue where they spontaneously differentiate into tissue-compatible cells according to the microenvironment of the site to which they home in and repair tissue.[1,7–10] Muse cells are also found in the peripheral blood, and their number increases in the acute phase of stroke.[4] Therefore, endogenous Muse cells may function as reparative stem cells, but their clinical relevance remains to be validated. We examined whether endogenous Muse cells mobilize after AMI and if mobilized Muse cells improve left ventricular (LV) function and attenuate LV remodeling in the chronic phase 6 months after AMI.

Methods

Study Protocol and Patients

This prospective study enrolled patients consecutively. Inclusion criteria were consecutive patients admitted to

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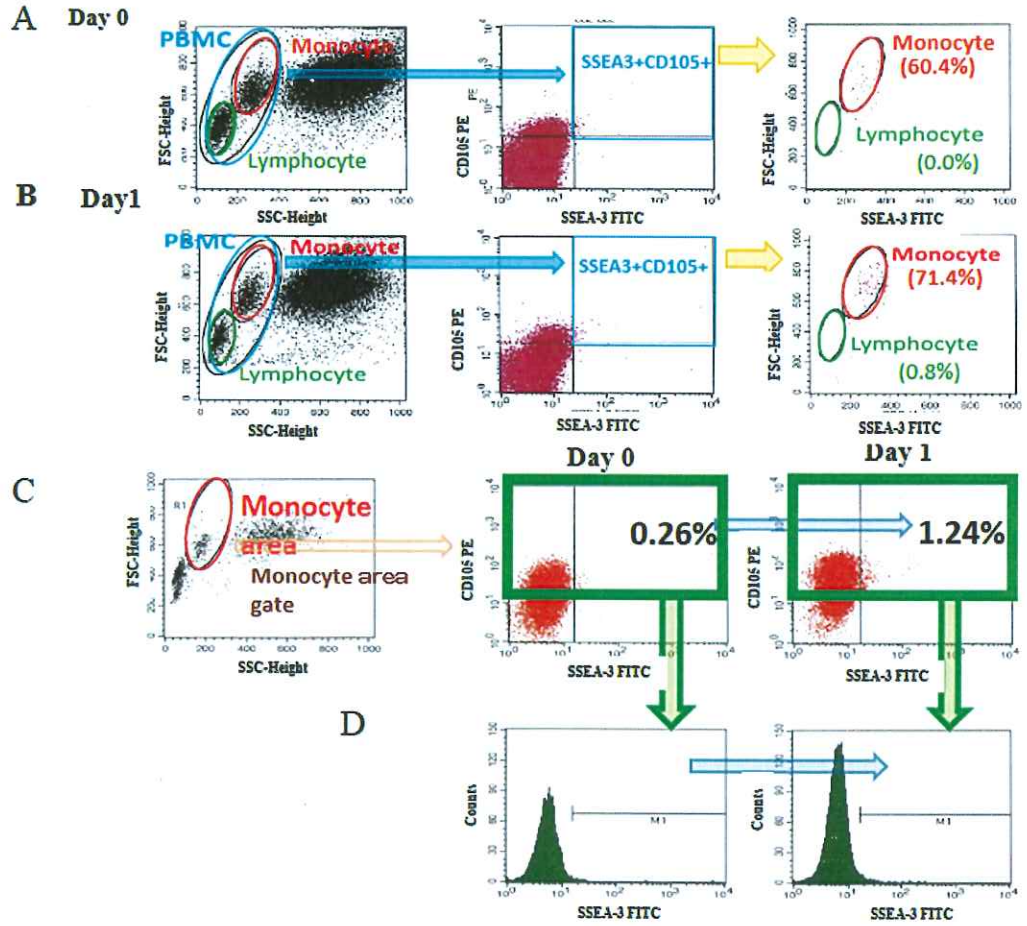

Figure 1. (**A**) Typical Control case. SSEA3+/CD105+ double-positive cells were measured in the monocyte area by FACS. (**B**) Typical case in AMI group on day 1. SSEA3+/CD105+ double-positive cells were measured in the monocyte area by FACS. (**C**) Typical case in AMI group on days 0 and 1 after the onset. FACS analysis of monocyte area. (**D**) Histogram presenting SSEA3+ cells within the gating area (monocyte and CD105+ areas) was drawn against cell count (Y-axis) and intensity of fluorescence (X-axis). AMI, acute myocardial infarction; FACS, fluorescence-activated cell sorting; PBMC, peripheral blood mononuclear cells.

Gifu University Hospital because of chest pain or discomfort and who underwent coronary angiography (CAG), and consecutive patients with AMI who underwent CAG. Exclusion criteria were patients who were interpreted by the doctor in charge as inappropriate for this study. We did not establish any other exclusion criteria because this was the first study to examine the mobilization of Muse cells into peripheral blood after AMI and their effects on LV function and remodeling. Patients with ≥75% coronary artery stenosis were enrolled into the CAD group, whereas patients with <75% coronary artery stenosis were enrolled into the Control group. Diagnosis of AMI was defined as the presence of prolonged anterior chest pain, ST-segment elevation on ECG, and an occluded coronary artery on CAG. AMI patients were treated with percutaneous coronary intervention followed by standard pharmacological treatment. The Ethics Committee of Gifu University Graduate School of Medicine (Approval no. 23-97) approved this study. All patients provided written informed consent before the study commenced. The investigation conformed with the principles outlined in the Declaration of Helsinki (*BMJ* 1964; **ii:** 177). Public registry and trial registry number: R000022572.

SSEA3+/CD105+ Double-Positive Muse Cell Number in Peripheral Blood

Muse cells are double-positive for pluripotent marker SSEA-3 and mesenchymal markers such as CD105, CD90 and CD29.[11] In the present study, Muse cell number in the peripheral blood was measured as SSEA3+/CD105+ double-positive cells.[11] In the AMI group, Muse cells were measured on days 0 (admission), 1, 7, 14, and 21 after AMI. The highest and lowest Muse cell number measured on days 0–21 were defined as the maximum and minimum Muse cell number, respectively. An increase (maximum-minimum Muse cell number) in the Muse cell number was defined as ΔMuse.

Blood samples were obtained from the antecubital vein and collected into sterile tubes, immediately placed on ice, and the number of SSEA3+/CD105+ double-positive cells was measured by fluorescence-activated cell sorting (FACS). Briefly, a 250-μL aliquot of heparinized whole blood was

Table 1. Patients' Characteristics and Drugs Used

	CTRL (n=64)	CAD (n=44)	AMI (n=79) (on admission)	P value
Characteristics				
Age (years)	67.6±9.1	70.3±8.8	67.9±9.7	0.30
Sex (M/F)	39/25	38/6*	64/15*	0.003
HTN, n (%)	32 (50.0)	34 (77.3)*	55 (69.6)*	0.007
HL, n (%)	24 (37.5)	21 (47.7)	53 (67.1)*,+	0.002
DM, n (%)	17 (26.6)	21 (47.7)*	31 (39.2)	0.069
Smoking, n (%)	11 (17.7)	10 (22.7)	32 (40.5)*,+	0.006
CKD, n (%)	18 (28.1)	16 (36.4)	37 (45.6)	0.070
CVD, n (%)	9 (14.1)	6 (13.6)	4 (5.06)	0.14
PAD, n (%)	6 (9.4)	8 (18.2)	3 (3.8)+	0.029
TC	181±36.4	189±35.8	192±37.8	0.22
LDL-C	106±22.9	117±33.5	116±29.0	0.34
HDL-C	55.2±16.8	47.2±10.8*	43.8±11.6*	<0.001
TG	97 (64–128)	138 (100–198)*	109 (63–178)	0.017
HbA1C	5.9 (5.6–6.2)	6.2 (5.8–7.1)	6.0 (5.7–6.9)*,+	0.020
S-CRE	0.76 (0.62–0.91)	0.82 (0.72–1.15)	0.89 (0.71–1.10)*	0.006
BNP	37.0 (13.5–128.2)	50.8 (31.8–163.7)	74.5 (23.3–182.5)	0.066
EPO	20.1 (14.4–30.5)	16.6 (13.1–28.5)	20.0 (13.2–29.2)	0.77
CRP	0.06 (0.02–0.27)	0.13 (0.06–0.26)*	0.14 (0.07–0.42)	0.018
Drugs used				
ACEI, n (%)	5 (7.81)	1 (2.27)	3 (3.80)	0.36
ARB, n (%)	27 (42.2)	23 (52.3)	33 (41.8)	0.48
CCB, n (%)	26 (40.6)	18 (40.9)	30 (38.0)	0.79
BB, n (%)	10 (15.6)	7 (15.9)	7 (8.9)	0.38
Diuretics, n (%)	12 (18.8)	11 (25.0)	9 (1.4)	0.081
NTG, n (%)	10 (15.6)	5 (11.4)	7 (8.9)	0.46
Nicorandil, n (%)	3 (4.69)	4 (9.10)	3 (3.80)	0.44
Statins, n (%)	16 (25.0)	15 (34.1)	29 (36.7)	0.31
EPA, n (%)	1 (1.56)	2 (4.55)	2 (2.53)	0.64
Aspirin, n (%)	6 (9.38)	17 (38.6)*	11 (13.9)+	<0.001
Clopidogrel, n (%)	4 (6.25)	13 (29.5)*	6 (7.59)+	<0.001
Insulin, n (%)	4 (6.25)	3 (6.82)	4 (5.06)	0.91
Metformin, n (%)	7 (10.9)	5 (11.4)	5 (6.33)	0.53
Pioglitazone, n (%)	3 (4.69)	0 (0)	2 (2.53)	0.33
DPP-4 inhibitors, n (%)	9 (14.1)	9 (20.5)	6 (7.59)	0.12

*vs. Control, +vs. CAD. ACEI, angiotensin-converting enzyme inhibitor; AMI, acute myocardial infarction; ARB, angiotensin II receptor blocker; BB, β-blocker; BNP, B-type natriuretic peptide; CAD, coronary artery disease; CCB, calcium-channel blocker; CKD, chronic kidney disease; CRP, C-reactive protein; DM, diabetes mellitus; EPA, eicosapentaenoic acid; EPO, erythropoietin; HDL-C, high-density lipoprotein-cholesterol; HL, hyperlipidemia; HTN, hypertension; LDL-C, low-density lipoprotein-cholesterol; NTG, nitroglycerine; PAD, peripheral artery disease; S-CRE, serum creatine; TC, total cholesterol; TG, triglycerides; WBC, white blood cells.

incubated with fluorescein isothiocyanate-anti-SSEA-3 (rat-SSEA3-anti-human antibody, Becton Dickinson, San Jose, CA, USA) and phycoerythrin-anti-CD105 (mouse-anti-human antibody, Becton Dickinson) at 18°C for 30 min.[3] Another aliquot was stained with mouse IgG2a to define the negative fluorescence range as a control. Red blood cells were lysed by adding lysing solution [154 mmol/L ammonium chloride, 0.9 mmol/L EDTA-2K (Dojindo), 7.5 mmol/L potassium bicarbonate]. After washing with phosphate-buffered saline, the cells were analyzed using FACS (FACS Calibur, Becton Dickinson). Muse cells are present in the mononuclear cell fraction,[3] so we gated the monocyte and lymphocyte areas (**Figure 1A,B**) because monocytes and lymphocytes are mononuclear cells (forward scatter (FSC) and side scatter (SSC) of flow cytometer were focused on mononuclear cells). Next, the $SSEA3^+/CD105^+$ double-positive cells (Muse cells) in the first selected gate were determined, and the distribution of Muse cells in the monocyte and lymphocyte areas and in other areas within the gated area was determined. Few Muse cells if any were detected in the lymphocyte area and the majority of Muse cells were detected in the monocyte area (**Figure 1A,B**). Therefore, we counted the Muse cells in the monocyte area but not in the lymphocyte area. **Figure 1C** shows a typical measurement of $SSEA3^+/CD105^+$ double-positive cells in the blood on days 0 and 1 in a patient with AMI. The histogram of counted $SSEA3^+$ cells within the gating area (monocyte and $CD105^+$ areas) was drawn against cell count (Y-axis) and intensity of fluorescence (X-axis) (**Figure 1D**: M1 shows the $SSEA3^+/CD105^+$ double-positive Muse cells). The number of Muse cells was expressed as cells per 100 μL of blood, as follows:

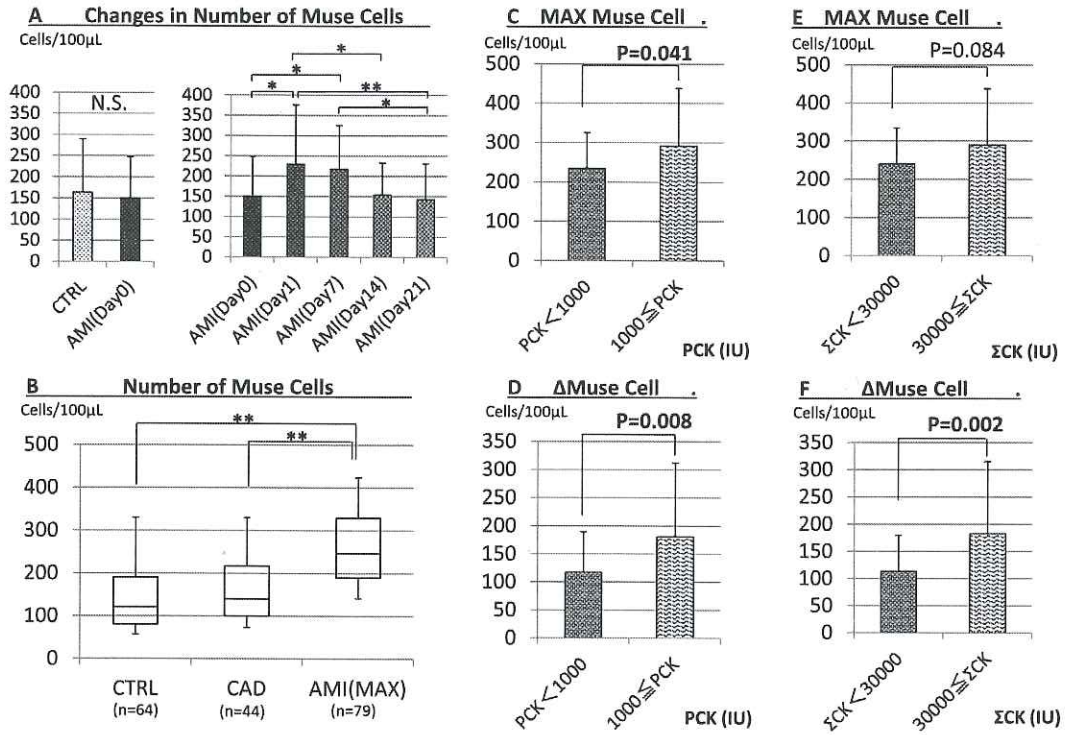

Figure 2. Muse cell mobilization into the peripheral blood. (**A**) Changes in the Muse cell number in peripheral circulating blood after AMI. (**B**) Muse cell number in the peripheral circulating blood. Number of Muse cells in the AMI group is the maximum Muse cell number on days 0, 1, 7, 14 and 21 after AMI. (**C**) Maximum Muse cell number in AMI patients with peak CK (PCK) <1,000 IU and those with PCK ≥1,000 IU. (**D**) ΔMuse cells between AMI patients with PCK <1,000 IU and those with PCK ≥1,000 IU. (**E**) Maximum Muse cell number in AMI patients with ΣCK <30,000 IU and those with peak ΣCK ≥30,000 IU. (**F**) ΔMuse in AMI patients with ΣCK <30,000 IU and those with peak ΣCK ≥30,000 IU. *P<0.05, **P<0.01. AMI, acute myocardial infarction; CK, creatinine kinase; CTRL, control; Muse, multilineage differentiating stress-enduring (cells).

absolute number of Muse cells (/100 μL)=white blood cells (/100 μL)×monocytes (%)×SSEA3$^+$/CD105$^+$ double-positive cells (%).

Plasma Sphingosine-1-Phosphate (S1P) Concentration

In 71 subjects from the Control, CAD, and AMI groups, blood samples were obtained from the antecubital vein. Blood was centrifuged (10,000 g for 15 min), and plasma was collected and frozen at −80°C until analysis of plasma S1P concentrations by liquid chromatography tandem mass spectroscopy (API 4,000, AB/MDS Sciex, Framingham, MA, USA) at the Toray Research Centre (Kamakura, Japan).

Blood Biochemical Analysis

Blood samples underwent complete blood cell counts and biochemical analysis including creatinine kinase (CK), aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, creatinine (CRE), blood urea nitrogen, C-reactive protein, hemoglobin A1c (HbA1c), total cholesterol, low-density lipoprotein-cholesterol, high-density lipoprotein-cholesterol (HDL-C), triglycerides (TG), and B-type natriuretic peptide (BNP).

Drugs and Complications

Drugs used by the patients before hospitalization and complications were examined.

Measurement of Cardiac Function by Echocardiography

Cardiac echocardiography (iE33, PHILIPS, Tokyo) was performed during the acute (within 7 days) and chronic (6 months) phases of AMI. LV ejection fraction (EF), LV end-systolic dimension (LVDs) and LV end-diastolic dimension (LVDd) were measured. We used the modified Simpson method, which is regarded as a reliable method of estimating the LVEF.

Statistical Analysis

Data are shown as mean±standard deviation of the mean. Non-parametric data are expressed as the median (interquartile range). Categorical data are summarized as percentages and compared with a chi-square test or Fisher's exact test as appropriate. The normality of the data distributions was tested using the Kolmogorov-Smirnov test. The significance of the differences between groups for variables that were normally distributed was determined by unpaired Student's t-test. Otherwise, a Mann-Whitney U test was used to compare the differences between groups. The significance of the differences among the Control, CAD and AMI groups for variables that were normally distributed was determined by analysis of variance (ANOVA) followed by the Tukey-Kramer method. Otherwise, a

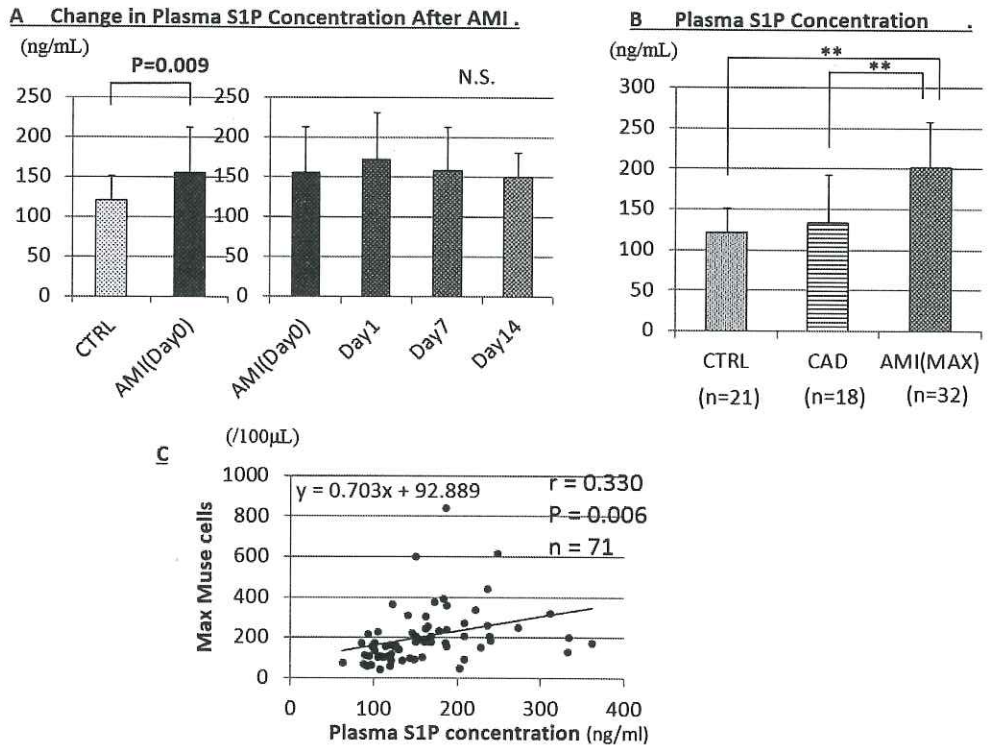

Figure 3. Plasma S1P concentrations and Muse cells. (**A**) Changes in the S1P concentrations after AMI. (**B**) Plasma S1P concentrations in the Control, CAD, and AMI groups. The plasma S1P concentration in the AMI group is the maximum plasma S1P concentration on days 0, 1, 7, and 14 after AMI. (**C**) Relationship between the plasma S1P concentration and maximum Muse cell number in the peripheral blood. **P<0.01. CAD, coronary artery disease; S1P, sphingosine-1-phosphate. Other abbreviations as in Figure 2.

Kruskal-Wallis test was used to compare the differences among groups. To evaluate the AMI follow-up data, ANOVA with repeated measure was used because data were normally distributed. Correlation coefficients between 2 variables were obtained by linear regression analysis. We performed multivariate analysis using clinical parameters with a P value <0.01 in the univariate analysis to adjust ΔMuse by confounding factors. A P value <0.05 was considered significant. All statistical analyses were performed using StatView version 5.0 (SAS Institute Inc., Cary, NC, USA).[7]

Results

Patients' Characteristics and Medications

Under the diagnosis based on CAG, 83 patients with AMI, 44 patients with CAD, and 64 patients with normal coronary arteries were included in the present study; 1 patient with AMI who was interpreted by the doctor in charge to be inappropriate for the study because baseline Muse cell number was greater than the average by 3 standard deviations and 3 patients with AMI who died during their admission were also excluded. Of the 79 remaining patients with AMI, 55 patients were followed up for 6 months after the onset. The incidence of hyperlipidemia (HL), prevalence of smokers and levels of HbA1c were higher in the AMI group than in the CAD/Control group (**Table 1**). Both the prevalence of male patients and incidence of hypertension (HTN) was higher and the HDL-C levels lower in the AMI/CAD groups than in the Control group. The levels of serum CRE were higher in the AMI group than in the Control group. Peripheral arterial disease (PAD) was lower in the AMI group than in the CAD group. The usage of aspirin and clopidogrel was higher in the CAD group than in the Control/AMI groups.

Muse Cell Number in the Peripheral Blood

On day 0, the number of Muse cells in the peripheral blood was not significantly different between the AMI group (149±98 cells/100 μL) and Control group (164±125 cells/100 μL; **Figure 2A**). The number of Muse cells in AMI patients then increased on day 1 (229±147 cells/100 μL), peaked, and became significantly greater than on day 0 and in the Control group, and then decreased on day 7 (217±109 cells/100 μL), and returned to basal levels by days 14 and 21 (**Figure 2A**).

The Muse cell dynamics described above from days 0–21 in the AMI group (**Figure 2A**) were typical of most of the 79 patients, although some patients exhibited a peak on other days (i.e., day 0 or day 7). Therefore, we determined the maximum Muse cell number (max. Muse) for each patient regardless of the day after AMI onset, and compared it with Muse cell numbers in the Control and CAD groups. The max. Muse in the AMI group was significantly

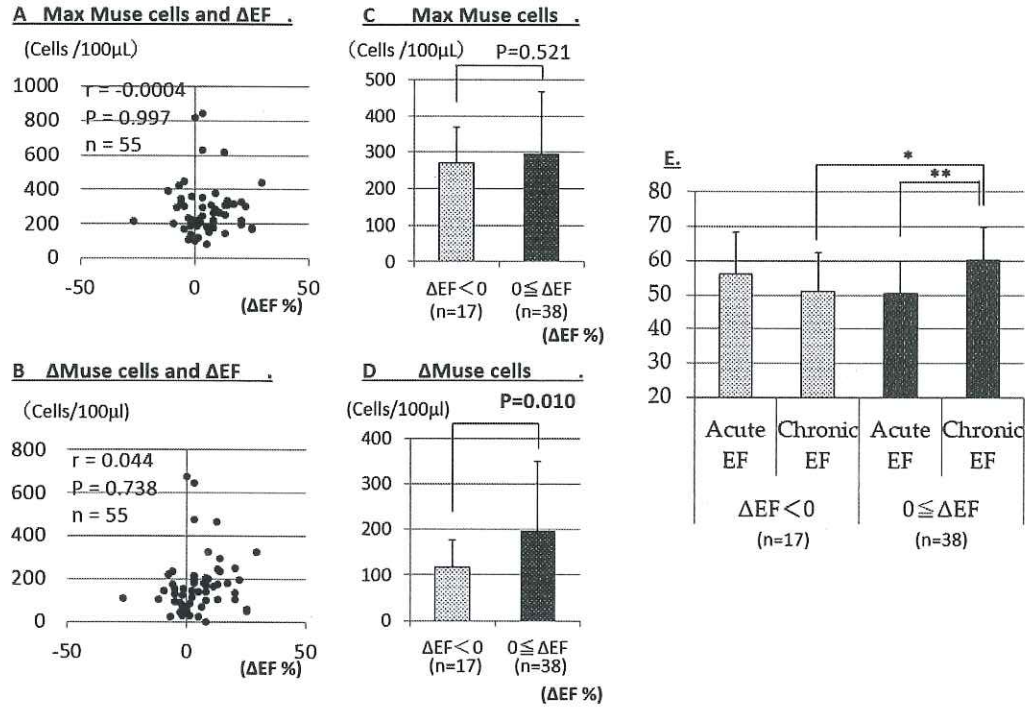

Figure 4. Muse cell mobilization and left ventricular (LV) function. (**A**) Relationship between maximum Muse cell number and ΔEF. (**B**) Relationship between ΔMuse cells and ΔEF. (**C**) Comparison of the maximum Muse cell number between patients with positive ΔEF and those with negative ΔEF. (**D**) Comparison of ΔMuse cells between patients with positive ΔEF and those with negative ΔEF. (**E**) EF in the acute and chronic phases in the negative ΔEF group with lower ΔMuse and in the positive ΔEF group with higher ΔMuse. *P<0.05, **P<0.01. EF, ejection fraction. Other abbreviations as in Figure 2.

greater than the Muse cell numbers in the Control and CAD groups (**Figure 2B**). When the data shown in **Figure 2B** were adjusted by the confounding factors in **Table 1**, the result was the same.

Peak CK and the sum of CK (ΣCK) are indicators of infarct size.[12] We measured CK at 8 points during days 0–2. When AMI patients were divided into 2 groups (peak CK <1,000 IU and peak CK ≥1,000 IU), the max. Muse was significantly higher in the group with peak CK ≥1,000 IU than in the group with CK <1,000 IU (P=0.041; **Figure 2C**). The ΔMuse (max. Muse−min. Muse number) was significantly greater in the group with peak CK ≥1,000 IU than in the group with CK <1,000 IU (P=0.008; **Figure 2D**). On the other hand, when the AMI patients were divided into 2 groups according to ΣCK (ΣCK <30,000 IU and ΣCK ≥30,000 IU), max. Muse was not significantly different between the 2 groups (P=0.084, **Figure 2E**). However, ΔMuse was significantly higher in the group with ΣCK ≥30,000 IU than in the group with ΣCK <30,000 IU (P=0.002, **Figure 2F**).

Other factors such as time between onset to balloon time or culprit coronary artery, medical treatment after AMI, smoking or drinking habits did not affect the max. Muse cell number or Muse cell number (data not shown).

Plasma S1P Concentration and Muse Cell Number

In the AMI group, the plasma S1P concentration began to increase significantly on day 0, and there were no differences among days 0–14, which was still significantly greater than that in the Control group, then significantly decreased on day14 (**Figure 3A**). Plasma S1P levels were significantly higher in the AMI group (maximum S1P level on day0, 1, 7 and 14 after onset) compared with the Control and CAD groups (**Figure 3B**). When the data shown in **Figure 3B** were adjusted by the confounding factors in **Table 1**, the result was the same. The max. Muse cell number positively correlated with plasma S1P level (P=0.006, **Figure 3C**).

LV Function and Muse Cell Number

Of the 79 AMI patients subjected to the acute-phase analysis (days 0–7 after onset), 55 were followed up until the chronic phase (~6 months) for measuring LVEF and LVDd. Analysis of the difference between EF in the acute and chronic phases (ΔEF) revealed no correlation between ΔEF and acute-phase max. Muse (P=0.997) or ΔMuse (P=0.738; **Figure 4A,B**). When patients were divided into 2 groups based on ΔEF, positive ΔEF (EF increased in the chronic phase or remained unchanged from the acute phase, ΔEF ≥0; n=38) and negative ΔEF (EF decreased in the chronic phase compared with acute phase, ΔEF <0; n=17), max. Muse did not differ between the 2 groups (P=0.521; **Figure 4C**). However, ΔMuse was significantly higher in patients with positive ΔEF (196±153 cells/100μL) than in those with negative ΔEF (118±59 cells/100μL, P=0.010; **Figure 4D**). As shown in **Figure 4E**, the EF of the positive ΔEF group increased significantly from 51.3±9.3% (acute

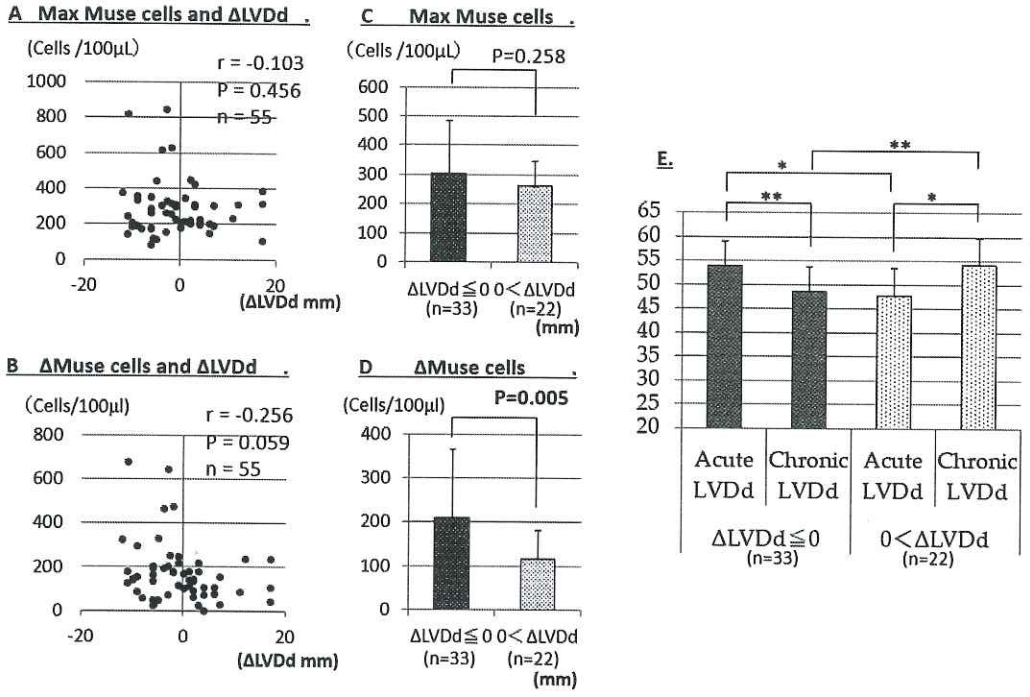

Figure 5. Muse cell mobilization and LV remodeling. (**A**) Relationship between the maximum Muse cell number and ΔLVDd. (**B**) Relationship between ΔMuse cells and ΔLVDd. (**C**) Comparison of the maximum Muse cells between patients with positive ΔLVDd and those with negative ΔLVDd. (**D**) Comparison of ΔMuse cells between patients with positive ΔLVDd and those with negative ΔLVDd. (**E**) LVDd at acute and chronic phases in the negative ΔLVDd group with higher ΔMuse and in the positive ΔLVDd group with lower ΔMuse. *P<0.05, **P<0.01. LVDd, LV end-diastolic dimension. Other abbreviations as in Figures 2,4.

phase) to 61.1±8.3% (chronic phase) (P<0.01), while EF decreased from 56.8±12.3% (acute phase) to 50.6±11.8% (chronic phase) in the negative ΔEF group (**Figure 4E**).

LV Remodeling and Muse Cell Number

LV remodeling, which indicates heart failure, is generally assessed by an increase in LVDd between the acute and chronic phases, namely a positive ΔLVDd. No relation was observed between acute-phase max. Muse and ΔLVDd (P=0.456; **Figure 5A**). ΔMuse at the acute phase, however, tended to be inversely correlated with ΔLVDd, but the difference was not significant (P=0.059, **Figure 5B**). We then divided the patients into a positive ΔLVDd group (LVDd increased in the chronic phase, ΔLVDd >0, suggesting progression to LV remodeling) and a negative ΔLVDd group (LVDd decreased in the chronic phase or unchanged from the acute phase, ΔLVDd ≤0, suggesting prevention of LV remodeling). The max. Muse did not differ between the 2 groups (P=0.258; **Figure 5C**), although ΔMuse cells was significantly higher in the negative ΔLVDd group (210±157 cells/100 μL) than in the positive ΔLVDd group (117±65 cells/100 μL, P=0.005; **Figure 5D**). As shown in **Figure 5E**, LVDd decreased significantly from 53.5±5.6mm (acute phase) to 48.0±5.6 mm (chronic phase) (P<0.01) in the negative ΔLVDd group, while, LVDd increased significantly (P<0.01) from 48.6±5.9mm (acute phase) to 54.6±5.4mm (chronic phase) in the positive ΔLVDd group (**Figure 5E**).

Factors Affecting EF and LV Remodeling

In the univariate analysis, many of the factors related to LV remodeling were compared between the negative and positive ΔEF groups (**Table 2**), and between the negative and positive ΔLVDd groups (**Table 3**). Among these factors, ΔMuse was significantly higher in the positive ΔEF group than in the negative ΔEF group (P=0.010; **Table 2**). Diabetes mellitus, chronic kidney disease, high HbA1c and high serum CRE levels correlated with a negative ΔEF (**Table 2**). In the univariate analysis, there were significant differences in serum CRE and HbA1c between the 2 groups (P<0.01). Therefore, we performed multivariate analysis including these 2 factors. ΔMuse was still significantly different between the 2 groups (P=0.049) even after adjustment for serum CRE and HbA1c.

Similarly, only ΔMuse was significantly higher in the negative ΔLVDd group than in the positive ΔLVDd group (P=0.005) (**Table 3**). Therefore, among the many factors, the extent of Muse cell mobilization into the peripheral blood favorably correlated with both LV functional recovery and prevention of LV remodeling.

Discussion

In the present study, endogenous Muse cells in the peripheral blood were substantially increased 1 day after the onset of AMI and returned to basal levels by day 14 in patients with AMI. These findings, together with the absence of a significant difference in Muse cell numbers between the CAD

Table 2. Comparison of the Factors Affecting ΔEF

	ΔEF <0 (n=17)	ΔEF ≥0 (n=38)	P value
Age (years)	65.1±8.2	68.4±9.3	0.22
Sex (M/F)	15/2	28/10	0.39
HTN, n (%)	12 (70.6)	30 (78.9)	0.74
HL, n (%)	10 (58.8)	26 (68.4)	0.49
DM, n (%)	12 (70.6)	13 (34.2)	0.012
Smoking, n (%)	8 (47.1)	15 (39.5)	0.82
Drinker, n (%)	7 (41.2)	8 (21.1)	0.22
CKD, n (%)	12 (70.6)	13 (34.2)	0.012
CVD, n (%)	1 (5.9)	1 (2.6)	0.85
PAD, n (%)	1 (5.9)	2 (5.3)	0.58
TIMI grade			
Pre TIMI=0	10 (58.8)	26 (68.4)	0.49
Post TIMI=3	17 (100)	35 (92.1)	0.58
Target lesion			
RCA/LAD/LCX	6/9/2	11/23/4	0.88
Pre LVEF	56.8±12.3	51.3±9.3	0.12
Peak CK	1,230 (715–3,412)	1,831 (930–3,771)	0.28
Peak CK-MB	235±244	228±193	0.91
ΣCK	57,793±48,778	69,372±58,166	0.49
ΣCK-MB	5,157±5,103	4,453±3,229	0.62
Max. Muse	271.1±97.1	295.1±170.9	0.52
ΔMuse	118.2±58.8	196.7±153.4	0.010
WBC	10,240±5,938	9,309±2,835	0.56
Neutrophils	7,090±3,379	6,806±2,644	0.81
Lymphocytes	1,644±608	1,884±1,079	0.39
Eosinophils	208±197	135±132	0.28
Basophils	17.8±17.3	27.0±23.5	0.19
Monocytes	693±244	688±328	0.96
TC	188±46.2	191±29.4	0.84
LDL-C	110±37.8	119±25.9	0.49
HDL-C	38.2±10.6	46.1±11.9	0.029
TG	189±136	130±110	0.14
HbA1C	6.6 (6.2–7.6)	6.0 (5.7–6.2)	0.006
S-CRE	1.15±0.40	0.88±0.30	0.008
BNP	65.7 (17.3–153.7)	70.0 (24.2–210.8)	0.49
EPO	20.2 (14.3–28.9)	20.3 (12.8–31.4)	0.84
CRP	0.28 (0.08–0.76)	0.15 (0.05–0.41)	0.17
Drugs (6 months later)			
ACEI, n (%)	7 (41.2)	15 (39.5)	0.86
ARB, n (%)	8 (47.1)	18 (47.4)	0.79
CCB, n (%)	2 (11.8)	9 (23.7)	0.51
BB, n (%)	11 (64.7)	28 (73.7)	0.72
Diuretics, n (%)	12 (70.6)	25 (65.8)	0.97
Statins, n (%)	16 (94.1)	34 (89.5)	0.96
EPA, n (%)	1 (5.9)	1 (2.6)	0.85
Aspirin, n (%)	17 (100)	38 (100)	>0.99
Clopidogrel, n (%)	14 (82.4)	34 (89.5)	0.77
Insulin, n (%)	4 (23.5)	0 (0)	0.011
DPP-4 inhibitors, n (%)	5 (29.4)	8 (21.1)	0.74

CVD, cerebrovascular disease; LAD, left anterior descending; LCX, left circumflex artery; Muse, multilineage differentiating stress-enduring (cells); RCA, right coronary artery. Other abbreviations as in Table 1.

and Control groups, suggested that Muse cells are mobilized by signals caused by AMI, and not simply by coronary artery stenosis (≥75%).

ΔMuse, the extent of Muse cells mobilized into the peripheral blood after AMI, was higher in patients with greater peak CK and ΣCK, indicators of infarct size[12]

Table 3. Comparison of the Factors Affecting LVDd

	ΔLVDd ≤0 (n=33)	ΔLVDd >0 (n=22)	P value
Age (years)	66.5±8.4	68.5±10.0	0.45
Sex (M/F)	24/9	19/3	0.39
HTN, n (%)	24 (72.7)	18 (81.8)	0.70
HL, n (%)	22 (66.7)	14 (63.6)	0.82
DM, n (%)	15 (45.5)	10 (45.5)	>0.99
Smoking, n (%)	14 (42.4)	9 (41.0)	0.91
Drinker, n (%)	8 (24.2)	7 (31.8)	0.76
CKD, n (%)	14 (42.4)	11 (50.0)	0.58
CVD, n (%)	2 (6.06)	0 (0)	0.66
PAD, n (%)	1 (3.03)	2 (9.09)	0.72
TIMI grade			
Pre TIMI=0	25 (75.8)	11 (50.0)	0.049
Post TIMI=3	30 (90.9)	22 (100)	0.40
Target lesion			
RCA/LAD/LCX	11/18/4	6/14/2	0.80
Pre LVEF	52.6±10.9	53.5±10.1	0.78
Peak CK	1,606 (841–2,980)	1,761 (894–4,800)	0.60
Peak CK-MB	214±198	254±226	0.50
ΣCK	65,194±58,745	66,693±50,755	0.92
ΣCK-MB	4,213±3,259	5,358±4,656	0.33
Max. Muse	304.7±181.8	262.1±85.8	0.26
ΔMuse	209.8±157.4	116.6±64.9	0.005
WBC	9,970±4,900	9,037±2,281	0.35
Neutrophils	7,248±3,333	6,328±1,843	0.28
Lymphocytes	1,684±630	2017±1,316	0.37
Eosinophils	129±131	198±183	0.22
Basophils	26.9±24.1	20.1±18.2	0.33
Monocytes	690±354	687±220	0.97
TC	196±39.5	181±28.4	0.13
LDL-C	119±33.4	112±24.7	0.44
HDL-C	44.9±13.1	41.9±10.0	0.37
TG	143±120	156±124	0.72
HbA1C	6.43±1.47	6.47±1.17	0.42
S-CRE	0.95±0.36	0.91±0.35	0.69
BNP	79.9 (24.0–201.9)	56.8 (19.9–150.1)	0.49
EPO	22.2 (14.4–34.6)	19.3 (9.4–24.4)	0.27
CRP	0.18 (0.07–0.62)	0.12 (0.05–0.44)	0.30
Drugs (6 months later)			
ACEI, n (%)	13 (39.4)	9 (40.9)	0.91
ARB, n (%)	15 (45.5)	11 (50.0)	0.74
CCB, n (%)	6 (18.2)	5 (22.7)	0.95
BB, n (%)	23 (69.7)	16 (72.7)	0.95
Diuretics, n (%)	24 (72.7)	13 (59.1)	0.45
Statins, n (%)	29 (87.9)	21 (95.5)	0.63
EPA, n (%)	1 (3.0)	1 (4.5)	0.66
Aspirin, n (%)	33 (100)	22 (100)	>0.99
Clopidogrel, n (%)	28 (84.8)	20 (90.9)	0.80
Insulin, n (%)	1 (3.0)	3 (13.6)	0.34
DPP-4 inhibitors, n (%)	8 (24.2)	5 (22.7)	0.85

Abbreviations as in Tables 1,2.

(**Figure 2D,F**). When the infarct is extensive, the damage to the heart causes LV functional deterioration and LV remodeling, leading to heart failure. It is reasonable to consider that endogenous Muse cells are mobilized into the peripheral blood to repair the infarcted myocardium and prevent the cascade of deterioration of LV function and remodeling. The larger the infarct, the greater the extent of Muse cell mobilization might be as a consequence of the

body's protective reaction to tissue damage.

S1P mobilizes CD34+ cells and MSCs from the BM and was produced by ischemic myocardium in an animal model.[13,14] S1P may be one of the factors that trigger Muse cell mobilization into the peripheral blood after AMI as shown in **Figure 3C**. The SDF-1-CXCR4 axis may also be a candidate factor that triggers Muse cell mobilization. However, it has been already reported that CXCR4 antagonist AMD3100 in animal serum could only partially suppress Muse cell migration towards the damaged liver, and its suppression effect did not differ significantly between Muse and non-Muse cells,[15] suggesting that the effect of SDF-1 on mobilizing Muse cells is smaller than that of S1P.

The organ that supplies Muse cells to the peripheral blood has not yet been clearly identified. Muse cells localize in clusters in the BM and are sparsely present in the connective tissue of various organs, including adipose tissue, dermis, trachea, and spleen.[1,4,16] Among these candidate sources, the BM microcirculation is directly connected to the peripheral blood, and thus the BM most likely supplies Muse cells to the peripheral blood.

In the present study, patients with a higher number of endogenous Muse cells mobilized into the peripheral blood in the acute phase exhibited significantly greater improvement in LV function and greater attenuation of LV remodeling. In contrast, patients with a lower number of Muse cells showed deteriorated LV function and accelerated LV remodeling in the chronic phase (6 months) (**Figures 4D,E,5D,E**). As shown in **Table 2** and **Table 3**, statistical analysis showed that, among many factors, the extent of Muse cells mobilization into the peripheral blood favorably correlated with both improvement of LV function and prevention of LV remodeling. It is suggested that DM and CKD negatively interfered with the improvement in LV function despite the presence of Muse cells (**Table 2**). In AMI patients, several types of endogenous stem/progenitor cells, such as CD34+ mononuclear cells, HSCs, and EPCs, as well as MSCs, are mobilized to the peripheral blood from the BM.[17–20] CD34+ mononuclear cells are vascular stem/progenitor cells and might contribute to tissue repair through neovascularization.[21–23] A previous study of AMI patients demonstrated that the number of CD34+CXCR4+ cells mobilized into the peripheral blood after AMI was significantly higher in patients with improved LV function and attenuated LV remodeling at the 1-year follow-up compared with patients without functional improvement.[18] Another study of AMI patients reported that the number of mobilized EPCs in the peripheral blood and the potential of those EPCs to differentiate into endothelial cells correlated with greater myocardial salvage, decreased end-systolic volume, and increased EF recovery 6 months after AMI.[24] Based on these reports, the main contribution of CD34+ cells and EPCs to AMI is considered neovascularization.[17–19] In animal models other than AMI, Muse cells have been shown to home into damaged tissue and differentiate spontaneously into cells compatible with the tissue they targeted and repair damaged tissue.[1,7–10] If the same mechanism is applied, it is suggested they repair AMI tissue more directly through replenishing new cardiomyocytes, and in fact, we have reported this in a rabbit model of AMI.[25]

Measurements of the increase in peripheral blood Muse cells after AMI in the acute phase might allow physicians to predict the prognosis of LV function and LV remodeling in the chronic phase. Endogenous Muse cells may not be sufficient to deliver robust recovery in some patients. In such cases, administering exogenous Muse cells could be a practical strategy for treating AMI. In addition, the development of new drugs to enhance the mobilization of endogenous Muse cells could be a novel therapeutic target for AMI.

Study Limitations

There were several limitations to note. First, it is difficult to demonstrate in the clinical studies that circulating Muse cells home into the AMI heart and differentiate into cardiomyocytes. Second, circulating progenitors such as EPCs, MSCs and HSCs, which are considered to improve outcomes for AMI patients, were not evaluated in this study. Finally, the number of AMI patients with 6 months' follow-up was relatively small. A clinical study with a larger number of AMI patients is required.

Author Contributions

Shinya M. designed the experiment, T.T., KN, Shingo M., T.N., Y.Y., H.K., A.M., H.U. and M.K. obtained the data, and M.D., T.T. and Shinya M. wrote the manuscript.

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Disclosures

There is no conflict of interest and authors have nothing to disclose.

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