ORIGINAL ARTICLE

G-CSF Augments Small Vessel and Cell Density in Canine Myocardial Infarciton

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Abstract: We recently reported that granulocyte-colony stimulating factor (G-CSF) prevented cardiac remodeling by mobilization and differentiation of bone marrow-derived cells in murine experimental myocardial infarction (MI). Little is known, however, whether these findings can be reproduced in large animals. The aim of this study is to investigate the effect of G-CSF after MI in canine model. MI was generated in twenty-six beagle dogs by ligation of left anterior descending artery. They were divided into two groups: G-CSF group which received subcutaneous injection of G-CSF (10 μg/kg/day) for 10 days, and the control group with saline injection. After six weeks, they were subjected to echocardiography and catheterization to measure hemodynamic parameters, and histological analysis was performed. No dogs died during the period. No hemodynamic changes were observed between these two groups probably due to the smaller size of the MI than we expected. We found significant increase in wall thickness and higher cell density in G-CSF group. Immunohistochemical staining against α-smooth muscle actin and CD31 revealed increased vessel density mainly in the epicardium in G-CSF group. The number of survived cardiomyocytes in G-CSF group was slightly greater than that in the control group, although it was not statistically significant. These findings suggested G-CSF prevented cardiac remodeling in canine model not by increasing the cardiomyocytes but by increasing the vessel density and cell numbers in the infarcted area. (Keio J Med 57 (3) : 139–149, september 2008)

Key words: G-CSF, myocardial infarction, bone marrow derived cell, cardiac remodeling

Introduction

Recent studies have reported that granulocyte-colony stimulating factor (G-CSF) administration can improve cardiac function and survival after myocardial infarction (MI).[1–3] G-CSF can mobilize bone marrow (BM) stem cells into the systemic circulation, however it remained unknown whether mobilized-BM cells contribute to the healing process or prevention of cardiac remodeling after MI. Experimental and clinical studies have recently suggested that BM cells contribute to the repair of an infarcted heart, although the precise mechanism underlying this clinically promising effect remains to be determined.[4–6] A number of studies have suggested that BM cells can contribute to regeneration processes in various tissues.[7,8] Cardiomyocytes derived from BM cells have been observed both in mice and human,[1,9] and BM-derived cells mobilized by cytokines were capable of regenerating the myocardial tissue, leading to an improvement in survival and cardiac function after MI.[1] BM contains both hematopoietic and non-hematopoietic cells, and the origin of the BM cells with the ability to

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repair damaged myocardial tissue remained unknown. The identification of specific cell types involved in myocardial repair is a crucial step towards the development of effective stem cell-based therapies for MI. The most likely candidates for the BM-derived stem cells with the ability to regenerate myocardial tissue are hematopoietic stem cells[1,10,11] and mesenchymal stem cells.[12–14] Alvarez-Dolado *et al*. demonstrated that BM-derived cardiomyocytes sporadically detected in non-infarcted mice were exclusively generated by fusion with donor $CD45^+$ cells, possibly hematopoietic cells,[15] suggesting that the so-called phenomenon of hematopoietic stem cells 'plasticity' might result from the fusion of hematopoietic stem cells with cells residing in the target tissue. In addition, two recent studies also confirmed that hematopoietic stem cells can not transdifferentiate into cardiomyocytes *in vivo*.[16,17] Another candidates for the regeneration of cardiomyocytes are mesenchymal stem cells; we previously reported that BM mesenchymal stem cells could differentiate into spontaneously beating cardiomyocytes *in vitro*,[12,13] and other groups have repaired the myocardium using mesenchymal stem cells transplantation *in vivo*.[18,19] We recently examined two independent clonal studies to determine the origin of BM-derived cardiomyocytes, and concluded that mesenchymal stem cells had been mobilized and differentiated into cardiomyocytes, but hematopoietic stem cells could not. However, since the number of regenerated cardiomyocytes was only restricted, it is difficult to explain the effect of G-CSF by the cardiomyocyte regeneration.[20]

To further understand the mechanism of beneficial effect of G-CSF, this study is designed to investigate the changes in cellular density and cellular component (endothelial cells, smooth muscle cells, and cardiomyocytes) in the infarcted area of dogs treated with G-CSF administration. We demonstrate that G-CSF treatment increased the number of endothelial cells and myofibroblasts in the infarcted area, which might reinforce the infarcted ventricular wall, and be essential for the improved cardiac function and survival.

Materials and Methods

Animal experiment protocol

All experimental procedures and protocols were reviewed and approved by the Animal Care and Use Committees of the Keio University and conformed to the NIH Guide for the Care and Use of Laboratory Animals. Twenty-three beagle dogs (10 male and 13 female) were anesthetized with pentobarbital (30-35 mg/kg) intravenously, intubated, and the respiration was controlled by a Harvard respirator with a tidal volume of 20 ml/kg/stroke, the respiratory rate of 10-15 /min, and the room air mixed with 0.5-1 l/min oxygen. The left thoracotomy was performed at the 4^{th} intercostal space, and the pericardial cradle was prepared. MI was created by ligation of the left anterior descending coronary artery after the first diagonal branch. After ligation, the chest was closed, and the dogs were carefully observed in the recovery room. One day after MI, the dogs were randomly divided into two groups: a control group (n=10) and a G-CSF treated group (n=13). Dogs of G-CSF group were injected subcutaneously with recombinant human G-CSF (10 μg/kg/day), and control group received saline injection subcutaneously for 10 days after MI. The peripheral blood cell counts were examined on the 10^{th} day. Hemodynamic measurement and histological analysis were performed at 6 weeks after MI.

Echocardiographic and hemodynamic analyses

The dogs were similarly anesthetized, mechanically ventilated, and left thoracotomy was performed at the 5^{th} intercostals space. Under direct observation, echocardiography was performed using a Power Vision SSA-380A (Toshiba Medical) equipped with a 2.5 MHz transducer (PSK-25AT, Toshiba Medical). Left ventricular end-diastolic diameter (LVEDD), left ventricular end-systolic diameter (LVESD) and heart rate (HR) were measured by M-mode echocardiography. Fractional shortening (FS) and ejection fraction (EF) were calculated from the following formula: FS=(LVEDD-LVESD)/LVEDD and EF=($LVEDD^3$-$LVESD^3$) /$LVEDD^3$. The measurement was performed three times and the mean value was used.

Thereafter, 7 Fr. double micro-tip catheter transducer (model SPC-771, Millar) was inserted from the left femoral artery to the left ventricle (LV), and LV systolic pressure (LVSP), LV end diastolic pressure (LVEDP), LVdP/dt, and -LVdP/dt were measured. Systolic arterial blood pressure (SBP), diastolic arterial blood pressure (DBP) and heart rate (HR) were also measured. Swan-Ganz thermo-dilution catheter was inserted from right femoral vein to pulmonary artery, and pulmonary artery pressure (PAP) and cardiac output (CO) were measured by thermo-dilution method. All the hemodynamic data was measured at three times and the mean value was used.

Histological analysis

After hemodynamic measurements, dogs were sacrificed by rapid intravenous injection of KCl. The hearts were quickly removed. Right ventricle was eliminated, and left ventricle was cut into five transverse slices. LV diameter, infarcted wall thickness were measured at the level of papillary muscles. The percentage of infracted area was calculated by following formula: infracted area (%)=(∑[(outer arc length of infarcted myocardium + in-

ner arc length of infracted myocardium)/(outer circle length of whole LV slice + inner circle length of whole LC slice)x(observed LV slice weight)]/(whole LV weight). The infarcted myocardium were sampled from the slice of papillary muscle level and fixed with periodate-lysine-paraformaldehyde (PLP) fixative. The samples were embedded in OCT compound or paraffin. Frozen sections (5 μm thickness) were cut by cryostat, and used for immunostaining. The sections were incubated with primary mouse monoclonal antibodies against α-actinin (SIGMA, 1:800), α-smooth muscle actin (αSM A (SIGMA, 1:5000), and CD31 (DAKO, 1:20) for overnight at 4℃. After rinsing with PBS, secondary antibodies labeled with Alexa488 were incubated for 30 min at room temperature. The samples were observed with confocal laser scanning microscopy (LSM510 ver3.0, Karl Zeiss, Germany). Azan staining was performed on paraffin-embedded sections.

Quantiative analysis of the histological samples

Cellular number of the infarcted area was measured by Azan staining or TOTO-3 staining. Red-stained area in the Azan staining was processed by Photoshop Ver7.0, and quantitated by NIH image Ver5.8. The nuclei in the samples were visualized by TOTO-3 staining, and their number was counted by NIH image Ver5.8.

Statistical Analysis

Values are shown as mean±SEM. The significance of differences was evaluated by Welch's t test. The probability level accepted for significance was $p<0.05$.

Results

No dogs died and were dropped out during experimental period in both G-CSF and control groups. There found no apparent adverse effect of G-CSF administration. Both groups of animals' behavior was normal during experiment.

Peripheral white blood cell counts

To check the bioactivity of human G-CSF in dogs, we measured peripheral blood cell counts (Fig. 1). At 10 days after MI, the WBC counts were significantly elevated to $5.7x10^4$/μl in the G-CSF group, whereas $1.3x10^4$/μl in the control group. There were no differences between the two groups in the RBC counts, but platelets were significantly decreased to 2.1 x 10^5/μl in G-CSF group than in control group (3.3 x 10^5/μl), probably due to well-known side effect of G-CSF.

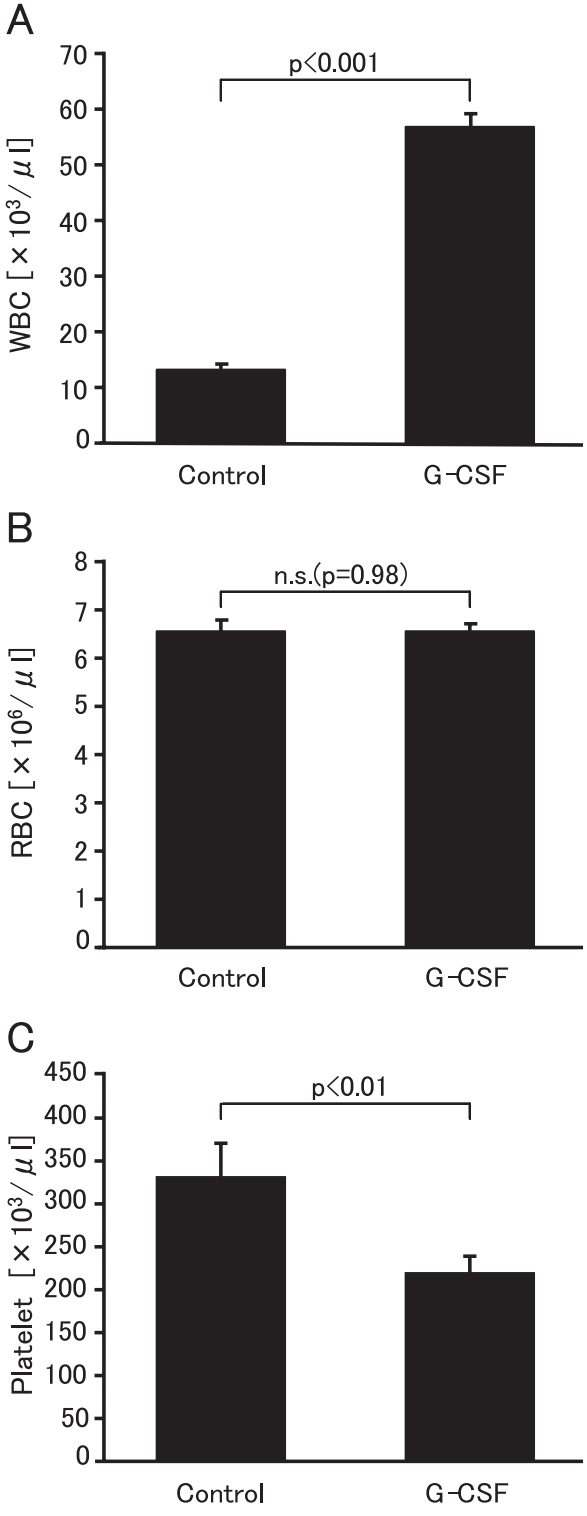


Fig. 1 Effect of G-CSF administration on blood cell counts at 10days.

White blood cell counts (WBC) increased by 4.4-fold of the control, while platelet counts decreased by 0.6-fold. The red cell counts was unchanged.

Table 1 Effects of treatment with G-CSF for 10 days on physiological parameters at 6 weeks after MI in anesthetized dogs

	Sham	Myocardial infarction		P value
		Control	GCSF	
n	4	10	13	
BW (kg)	11.7±0.1	10.1±0.2*	10.6±0.2	0.0875
SBP (mmHg)	118±7	113±5	110±3	0.6634
DBP (mmHg)	95±4	91±4	90±3	0.9047
HR (bpm)	161±12	135±7	146±4	0.1752
LVSP (mmHg)	124±3	108±4*	107±3	0.8894
LVEDP (mmH)	5±1	7±1	5±1	0.2798
LVdP/dt (mmHg/s)	3017±281	2367±237	2329±108	0.8887
-LVdP/dt (mmHg/s)	2996±184	2517±130	2700±134	0.3382
CO (L/min)	1.8±0.1	1.4±0.1*	1.6±0.1	0.3195
PAP (mmHg)	19±1	20±1	18±0	0.1853
pH	7.407±0.010	7.405±0.007	7.394±0.006	0.2334
pCO_2 (mmHg)	35.2±1.6	34.2±0.7	34.9±0.8	0.5053
pO_2 (mmHg)	123.1±4.7	116.4±2.0	115.1±2.2	0.6479
BT (℃)	35.9±0.1	36.5±0.2	36.5±0.1	0.9831

Values are expressed as mean ±SEM. BW: body weight, SBP: systolic arterial blood pressure, DBP: diastolic arterial blood pressure, HR: heart rate, LVSP: left ventricular (LV) systolic pressure, LVEDP: LV end diastolic pressure, LVdP/dt(+) and (-) : maximal and minimal first derivative of LV pressure, CO: cardac output, PAP: mean pulmonary arterial blood pressure, BT: body temperature.

Table 2 Effects of treatment with G-CSF for 10 days on infarct size, cardiac weight, and echocardiographic parameters at 6weeks after MI in anesthetized dogs

	Sham	Myocardial infarction		P value
		Control	G-CSF	
n	4	10	13	
BW (kg)	11.7±0.1	10.1±0.2	10.6±0.2	0.0875
Infarct size	0.012±0.012	0.132±0.011*	0.133±0.009	0.7963
LVW (g)	63.8±3.0	52.6±1.5*	54.5±1.7	0.3988
RVW (g)	21.3±0.6	19.3±0.7	19.6±0.8	0.7965
LVEDD (mm)	21.7±1.9	32.2±0.9*	33.5±0.8	0.2831
LVESD (mm)	12.9±3.1	26.0±0.8*	28.1±0.7	0.0793
HR (bpm)	157±13	138±7	147±4	0.1752
FS	0.42±0.10	0.19±0.02*	0.16±0.02	0.2241
EF	0.71±0.11	0.40±0.03*	0.34±0.03	0.1592

Values are expressed as mean ± SEM. BW: body weight, LVW: left ventricle weight, RVW: right ventricle weight; LVEDD: left ventricular end diastolic dimension, LVESD: left ventricular end systolic dimension, HR: heart rate, FS: fractional shortening, EF: ejection fraction.

Hemodynamic measurements and echocardiogram

Hemodynamic analysis performed at 6 weeks was shown in Table 1. Cardiac output was not significantly different between the control and G-CSF groups. LVEDP seemed to have a tendency to decrease in G-CSF group, but not significant. There was no significant difference between the two groups in other hemodynamic parameters such as body weight, systolic and diastolic blood pressure, LV end-diastolic pressure, pulmonary artery pressure, pH, pCO_2, pO_2, and body temperature.

Echocardiographic measurements were shown in Table 2. There was no significant difference in LV end-diastolic dimension, LV end-systolic dimension, heart rate, fractional shortening and ejection fraction between the two groups. These findings indicated that G-CSF administration did not improve or aggravate the cardiac function after MI.

Macroscopic findings of LV

Anatomical measurement had revealed that there was no difference in LV weight, RV weight and infarcted size between the two groups. Infarct size did not differ between the two groups (Table 2). Fig. 2 revealed the representative photograph of the LV slices at papillary muscle level. Using these LV slices, we investigated LV dimension and infracted wall thickness. There were no significant differences between the groups in LV dimension. However, the infracted wall thickness was significantly greater in G-CSF group, implying that G-SCF might affect the cardiac remodeling after myocardial infarction.

Histological analysis

Since G-CSF can mobilize bone marrow derived cells into peripheral blood, we next investigated the cellular density of the infarcted area. Fig. 3 A-D showed the representative photograph of the azan staining of the infarcted area. Red area indicated the cellular component and blue indicated the interstitial tissue. In both endocardium and epicardium, the red area in the G-CSF treated group seemed to be greater than that of the control group. Quantitative analysis of the red-stained area in both groups was shown in Fig. 3 E,F. The red-stained area of the epicardial layer in G-CSF group was significantly bigger than that of the control group. The red-stained area of the endocardial layer in G-CSF group had a tendency to be bigger than the control group, but it was not statistically significant.

Immunohistochemical analysis

To further investigate the infarcted tissue, we performed immunostaining. Fig. 4 A-D indicated the representative photograph of the nuclear staining with Toto-3. Fig. 4 E,F showed the quantitative analysis of the cell density in the infarcted area. The cell density of both the epicardial and endocardial layer in G-CSF group was significantly higher than those of the control groups. Together with the results of Fig. 3, G-CSF significantly increased the number of cells in the infarcted area.

Fig. 5 A-D showed the representative immunofluorescent microscopy of the infarcted tissue in the control and G-CSF groups with anti-α-SMA antibody. The α-SMA was also expressed in the myofibroblasts in the infarcted area as well as smooth muscle cells. Quantitative analysis of the density of α-SMA-positive cells was shown in Fig. 5 E,F. G-CSF significantly increased the number of α-SMA-positive cells of the epicardial layer and had a tendency to increase that of the epicardial layer in G-CSF group than those in the control groups.

Fig. 6 A-D showed the representative immunofluorescent microscopy of the infarcted area stained with anti-CD31 (PECAM-1) antibody. Quantitative analysis of

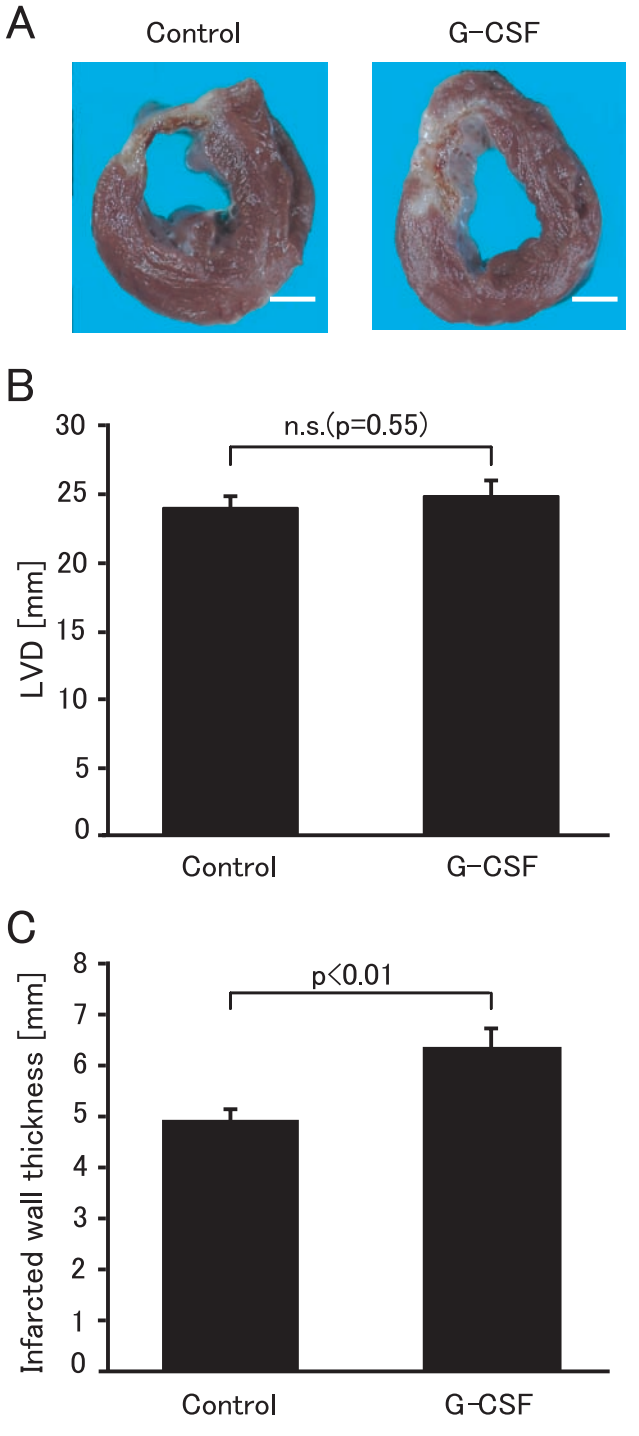


Fig. 2 Effect of G-CSF on left ventricular dimension (LVD) and infarcted wall thickness.

(A) Representative photograph of the slice of infarcted heart at the papillary muscle level in short axis view. Scale bars show 1 cm. (B) LVD of the control and the G-CSF groups was shown. Data were obtained from 10 (Control) and 13 (G-CSF) dogs, respectively. (C) Wall thickness of the infarcted area was shown. Data were obtained from 10 (Control) and 13 (G-CSF) dogs, respectively. Note that wall thickness in G-CSF group was significantly bigger than that of the control group.

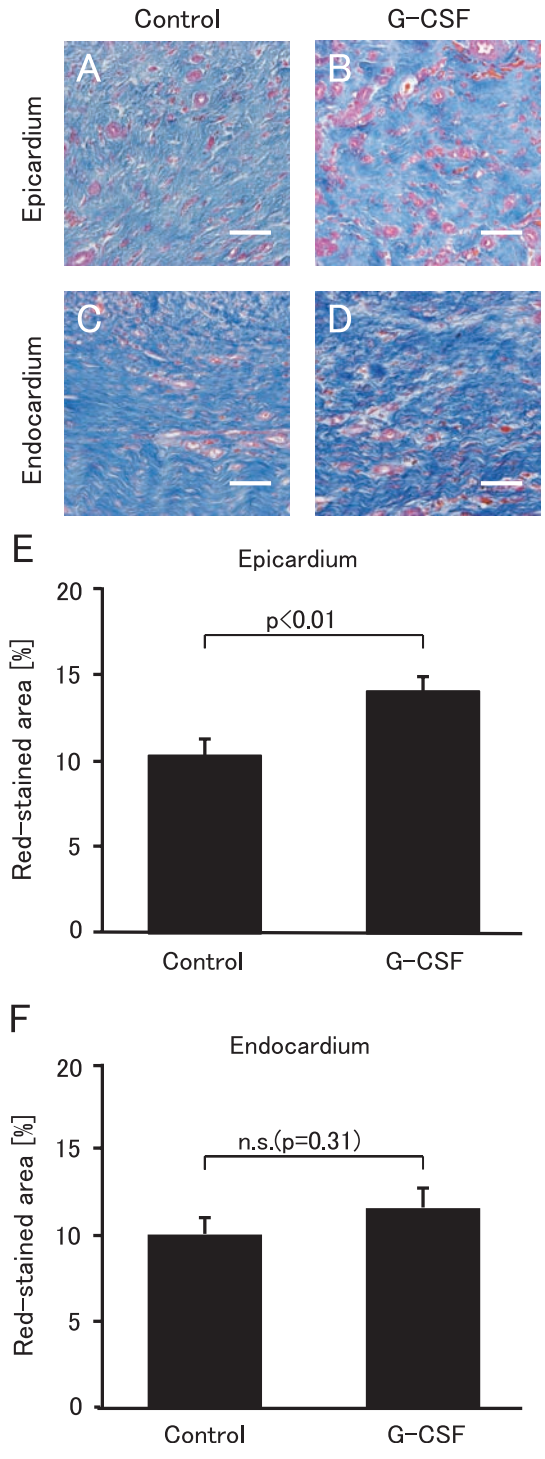


Fig. 3 G-CSF increased the cellular component in the infarcted area

(A-D) Representative microphotograph of the Azan staining of the infarcted area. Blue indicated extracellular matrix and red indicated cellular component. Note that red area was increased by G-CSF administration. Scale bars showed 200 μm. (E, F) Quantative analysis of the red stained area was shown. E and F showed epicardial and endocardial layer, respectively. G-CSF significantly increased the cellular component in the epicardial layer.

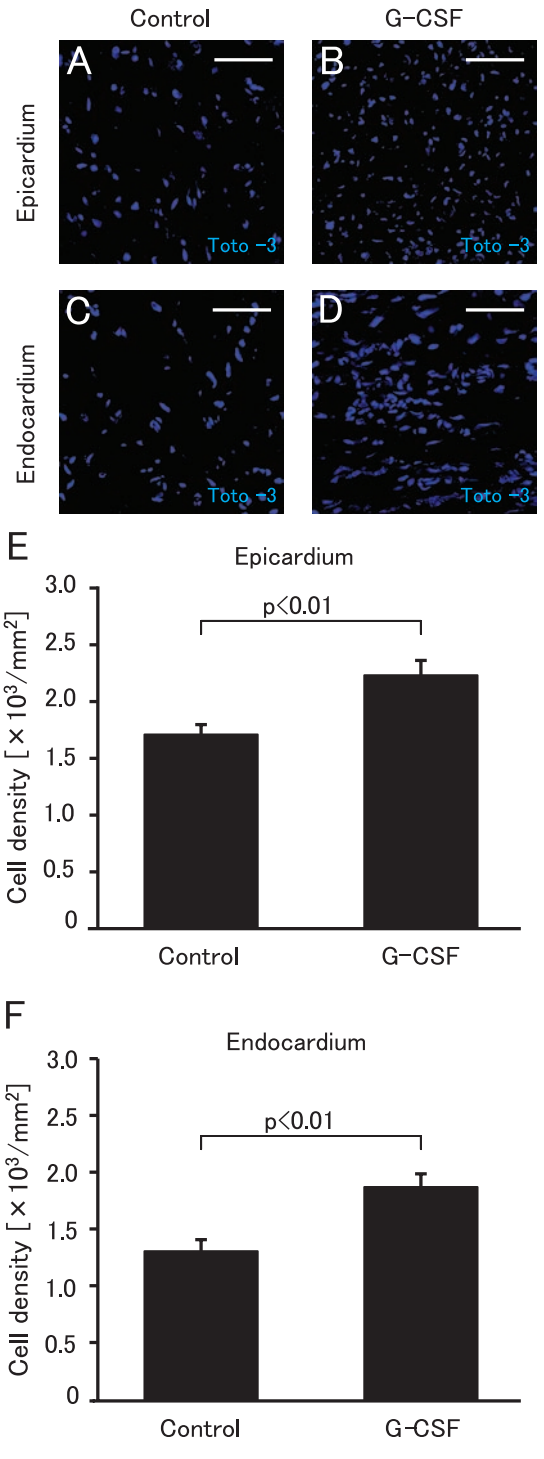


Fig. 4 G-CSF increased the cell number in the infarcted area.

(A-D) Representative microphotograph of the infarcted area by Toto-3, by which nucleus was specifically stained. Scale bars showed 50 μm. (E, F) The cell number in the infarcted area was quatitated. E and F showed epicardial and endocardial layer, respectively. G-CSF significantly increased the cell number in the infarcted area in both epicardial and endocardial layer.

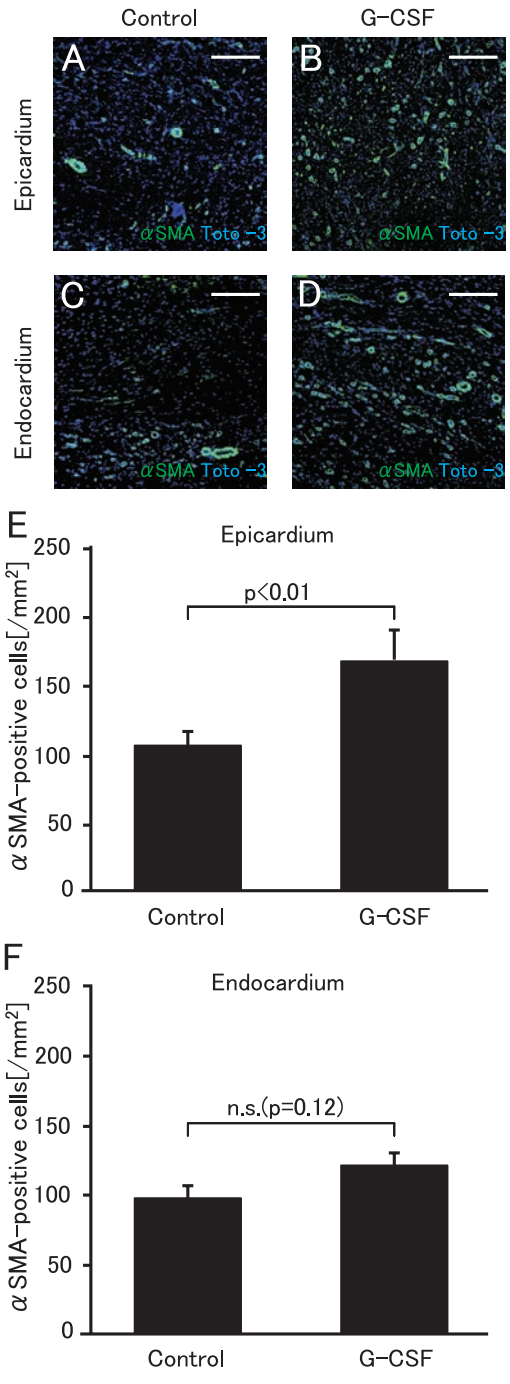


Fig. 5 G-CSF increased the number of α-smooth muscle actin(+) cells in the infarcted area.

(A-D) Epicardial and endocardial layer of the infarced area was separately shown in the control and G-CSF treated groups. Green and blue signals indicated α-smooth muscle actin (α-SMA) (+) and nuclei, respectively. Scale bars show 200μm. (E, F) The number of α-SMA(+) cells of the infarcted area was obtained with immunofluorescent staining, quantiated by NIH image and shown in E and F. Vessel density of the epicardial layer in G-CSF group was significantly higher than that of the control. The number of α-SMA(+) cells of the endocardial layer in G-CSF group has a tendency to be higher than that the control group, but was not statistically significant.

the CD31-positive cell density of the infarcted area was shown in Fig. 6 E,F. G-CSF significantly increased the number of CD31-positive cells of the epicardial layer and had a tendency to increase those of the endocardial layer in G-CSF than those of the control groups. These findings indicated that G-CSF increased the number of endothelial cells, smooth muscle cells and fibroblasts.

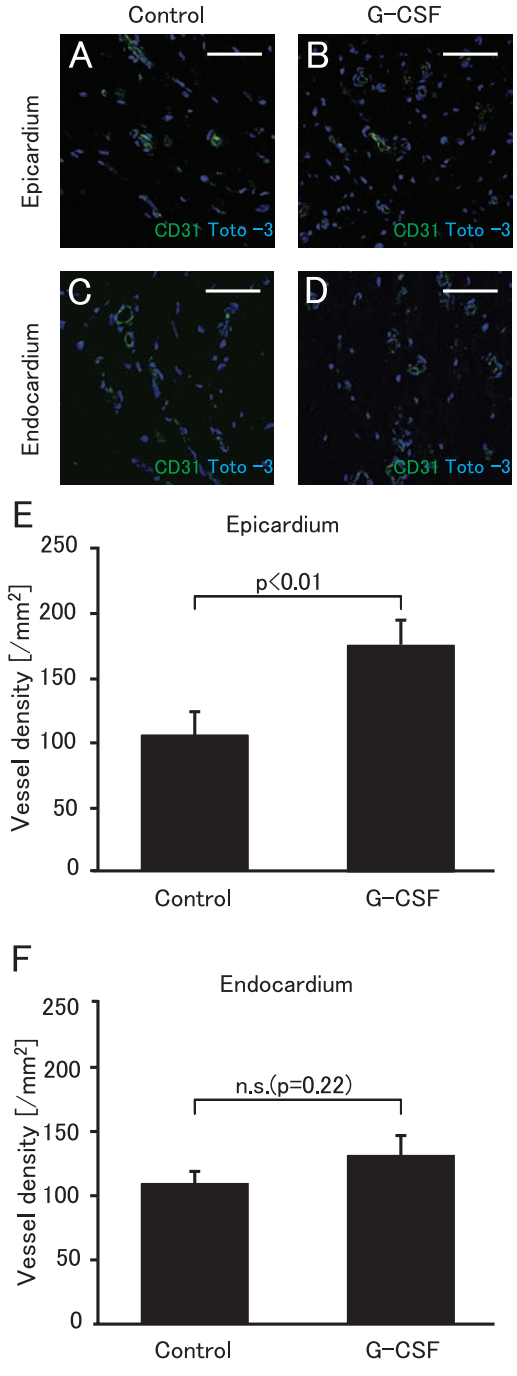
Fig. 7 A-D indicated the representative photograph of the infarcted area stained with anti-actinin antibody, and Fig. 7 E,F showed the quantitative analysis. As it is well known, the cardiomyocytes was spared at the subendocardial and subepicardial layer. G-CSF did not significantly affect the survived cardiomyocyte at the epicardial and endocardial layers. G-CSF did not significantly increase the number of cardiomyocytes in the infarcted area. These findings indicated that the survived or regenerated cardiomyocytes were not increased by the administration of G-CSF at the statistically significant level.

Fig. 8 A-D indicated the representative photograph of the infarcted area stained with vimentin antibodies, and Fig. 8 E,F showed the quantitative analysis. G-CSF administration slightly increased the number of vimentin positive cells in the epicardium, although it is not significant. Taken together, these findings indicated that G-CSF administration slightly increased the density of endothelial cells, smooth muscle cells, and fibroblasts in the infarcted area, and its tendency was observed especially at the epicardium.

Discussion

We previously showed that mesenchymal stem cells, not hematopoietic stem cells, could differentiate into cardiomyocytes *in vivo* after MI.[20] We also reported that bone marrow derived cells can both transdifferentiate into and fuse with cardiomyocytes in pressure overloaded heart using murine pulmonary hypertention and transaortic constriction model.[21] However, the significance of this phenomenon with respect to the G-CSF-induced improvement of cardiac function and survival after MI was not determined. This is due to the fact that the number of regenerated cardiomyocytes was very few even G-CSF was administered, and that it is difficult to explain the beneficial effect of G-CSF to be regenerated cardiomyocytes. Since the fundamental effect of G-CSF was to mobilize the granulocytes and monocytes into peripheral circulation, these cells might infiltrate into the infarcted area.

We recently performed BM transplantation using GFP-transgenic mice as a donor, and investigated the contribution of hematopoietic stem cells -derived cells to the healing process after MI in mice.[22] We found that many hematopoietic stem cell-derived cells, which have a fibroblast-like elongated morphology, are retained at the infarcted myocardium at 7 days post-MI. These cells



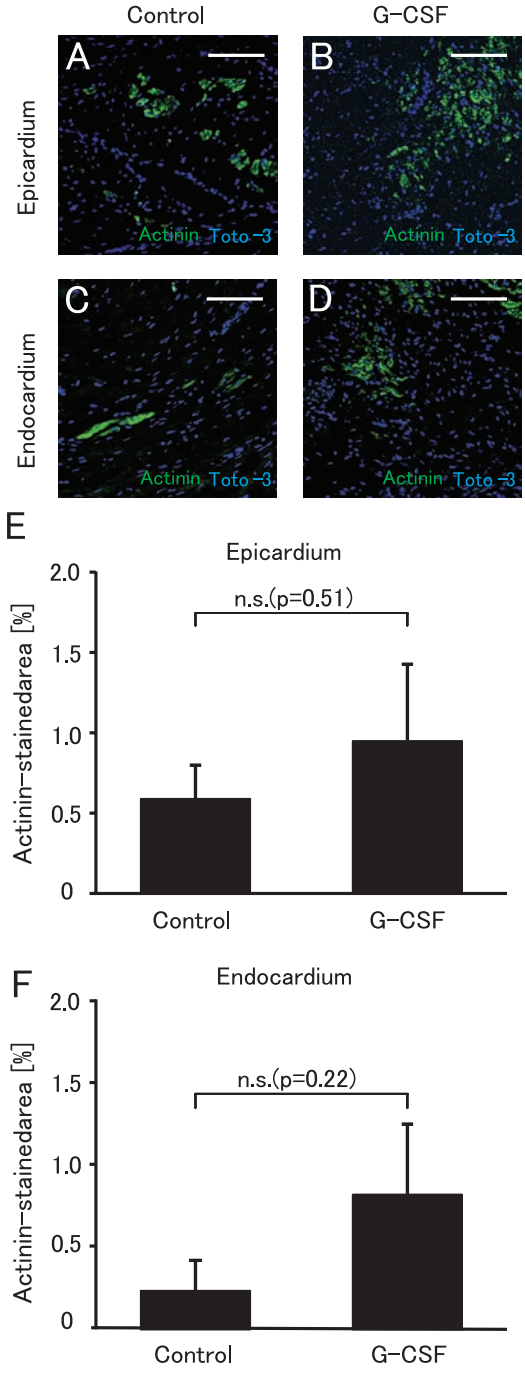


Fig. 6 G-CSF increased the number of CD31(+) cells in the infarcted area.

(A-D) Epicardial and endocardial layer of the infarced area was separately shown in the control and G-CSF treated groups. Green and blue signals indicated CD31 and nuclei, respectively. Scale bars show 50μm. (E, F) The number of CD31(+) cells of the infarcted area was obtained with immunofluorescent staining, quantiated by NIH image and shown in E and F. CD31(+) cell density of the epicardial layer in G-CSF group was significantly higher than that of the control. CD31(+) cell density of the endocardial layer in G-CSF group has a tendency to be higher than that the control group, but was not statistically significant.

Fig. 7 Immunofluorescent staining for actinin on the infarcted area

(A)~(D) Representative immunofluorescent microphotograph of the infarcted area using cardiac actinin antibody. (A, B) Comparison of the endocardium between control (A) and G-CSF group (B). (C, D) Comparison of the epicardium between control (C) and G-CSF group (D). Blue = Nuclei stained by TOTO-3. Scale bars show 50μm. (E, F) The actinin-positive cell density in the endocardium (E) and the epicardium (F). The actinin-positive cell density of G-CSF group had a tendency to be greater than that of the control group in both endocardium and epicardium, although it was not statistically significant.

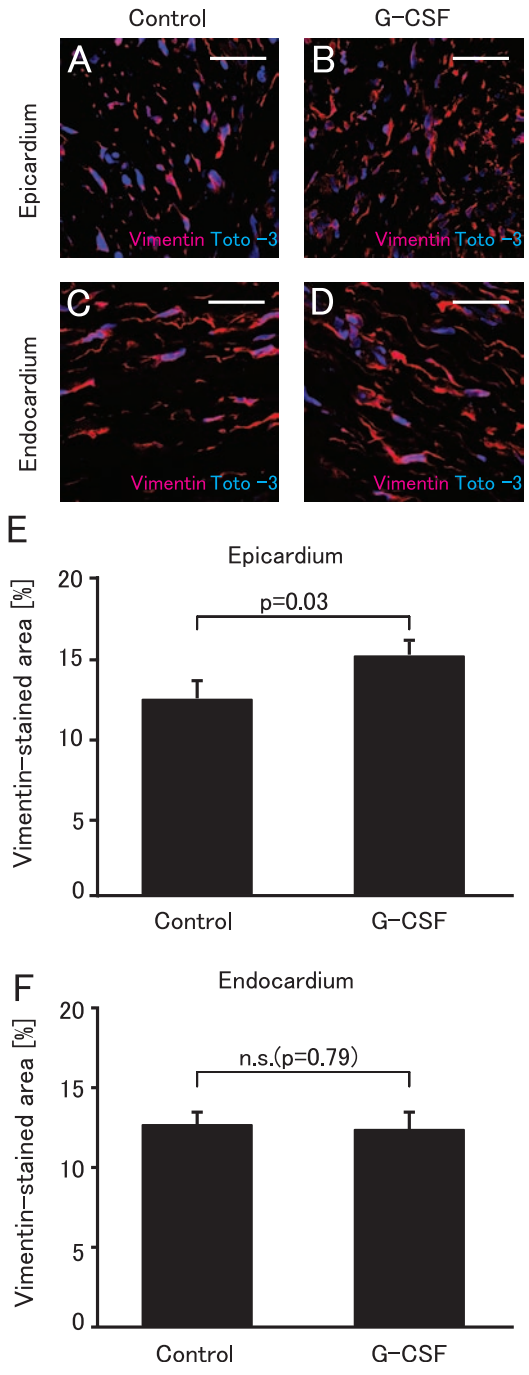


Fig. 8 Immunofluorescent staining for vimentin on the infarcted area

(A)~(D) Representative immunofluorescent microphotograph of the infarcted area using anti-vimentin antibody. (A, B) Comparison of the endocardium between control (A) and G-CSF group (B). (C, D) Comparison of the epicardium between control (C) and G-CSF group (D). Blue = Nuclei stained by TOTO-3. Scale bars show 50μm. (E, F) The vimentin-positive cell density in the endocardium (E) and the epicardium (F). The vimentin-positive cell density of G-CSF group had a tendency to be greater than that of the control group in epicardium, although it was not statistically significant.

clearly expressed α-SMA and vimentin, indicating that they were myofibroblasts. Some also weakly expressed CD45. In the chronic phase of cardiac remodeling, GFP $^{+}$ fibroblast-like cells were still present in the scar tissue of the heart. These cells were positive for vimentin, but most of them were negative for α-SMA. These results indicated that hematopoietic stem cell-derived cells migrated into the infarcted area, and differentiated into myofibroblasts in the acute phase, and became mature fibroblasts by the chronic phase. G-CSF treatment was associated with an increased number of infiltrating myofibroblasts in both groups, as well as a decrease in mortality and an improvement cardiac function in the chronic phase. Moreover, we found that hematopoietic stem cell-derived cells (possibly monocytes) contributed the accumulation of myofibroblasts at the perivascular fibrosis area in pressure overload-induced cardiac hypertrophy model.[21] These results implicate the infiltration of myofibroblasts as critical in the prevention of cardiac remodeling.

The present study showed that G-CSF significantly increased the thickness of the infarcted wall. Moreover, we found that G-CSF significantly increased the cell density of the endothelial cells, α-SMA positive cells and vimentin positive cells but not the cardiomyocytes. However, both α-SMA and vimentin is not a specific marker for special cell types. The α-SMA was expressed in various cell types such as smooth muscle cells, cardiac myofibroblasts, and juvenile cardiomyocytes. Vimentin was mainly expressed in myofibroblasts, but was expressed in other cell types. From the finding of the cell morphology, we supposed that some of α-SMA positive cells might be vascular smooth muscle cells. We also think that most of the cells positive for α-SMA and vimentin might be myofibroblasts, the origin of which might be the mobilized monocytes by G-CSF administration. Taken together, the present findings indicated that G-CSF increased the BM-derived cells in the infarcted area, which may play an important role in modulating post-MI remodeling. This phenomenon was basically in accordance with our previous observation obtained from BM transplanted mice. A number of cell transplantation experiments revealed that fibroblasts, smooth muscle cells, cardiomyocytes and even skeletal muscle cells improved cardiac remodeling and function when they were transplanted into the infarcted area after MI.[6,23–28] The increased cellular density might be critically involved in the healing process of myocardial infarction by secreting extracellular matrix, angiogenic growth factors and cytokines, which may accelerate healing process. Previous papers reported the molecular mechanism of the action of G-CSF.

Recently, Harada reported that G-CSF directly binds to G-CSF receptors on cardiomyocytes, activating the JAK/STAT pathway, and preventing apoptosis in the late

phase after MI. This effect was proposed to improve cardiac remodeling.[29] An anti-apoptotic effect on cardiomyocytes might also play a role in the improvement of cardiac remodeling mediated by G-CSF in our study. In addition, endothelial progenitor cells might also play a role in the regeneration of the infarcted myocardium in the chronic phase. Cardiac progenitor cells have also been shown to be resident in the heart,[30] and we supposed that they also participate in the healing process after MI. These findings indicated that these mechanisms may collaboratively work and improved the cardiac function. A great deal of clinical research up until 2005 involved harvesting mononuclear cells from BM or peripheral blood and infusing them through a catheter into a coronary artery to treat acute myocardial infarction. Strauer and colleagues first reported transplantation of BM mononuclear cells 4.8-13.5 days after a myocardial infarction in 10 acute myocardial infarction patients, which resulted in a slight decrease in left-ventricular end-systolic dimension and infarct region, and an increase in the left-ventricular ejection fraction and regional function.[6] The TOPCARE-AMI trial allocated 20 patients with reperfused acute myocardial infarction to receive intracoronary infusion of either BM-derived or circulating blood-derived progenitor cells into the infarct artery at 4.3±1.5 days. The results from this trial indicated a significant increase in global left ventricular ejection fraction, improved regional wall motion, and reduced end-systolic left ventricular volumes at the 4-month follow-up investigation. At the one year follow-up investigation the transplanted group revealed an increased EF, reduced infarct size, and absence of reactive hypertrophy without significant complication, suggesting functional regeneration of the infarcted ventricles[31–33] (41-43). The BOOST trial, and Fernandes-Aviles and colleagues also transplanted BM mononuclear cells 4.8-13.5 days after a myocardial infarction in acute myocardial infarction patients. In most of the studies, transplantation resulted in similar results.[34,35] The IACT Study transplanted BM mononuclear cells via catheter in patients with chronic MI, and reported that functional and metabolic regeneration of infarcted and chronically avital tissue could be realized.[36]

In conclusion, the precise investigation of molecular mechanisms and the double-blind clinical trial will clarify the usefulness of this cytokine therapy for the prevention of cardiac remodeling after myocardial infarction.

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