Table 2. Baseline Pregnancy Patient Characteristics

Patient	Age at conception	LVEF in pregnancy (%)	NYHA class	No. ICD shocks	LVEF at delivery (%)	Anti-arrhythmic medications (mg/day)			
							1st trimester	2nd trimester	3rd trimester
1	26	61.1	2	0 (29 weeks ATP)	48.4	Metoprolol	40	160	200
2	27	47.7	2	0	44.2	Carvedilol/ Mexiletine/ Aprindine/ Digoxin	5/200/ 20/0.125	10/200/ 40/0.125	10/200/ 50/0.125
3	33	76.1	1	0	72.4	None			
4	29	61.8	1	0	68.8	Bisoprolol	2.5	5	5
5	25	54.8	1	0	51.3	Atenolol	50	50	50
6	28	56.2	1	0	57.3	Bisoprolol	5	5	5
Mean±SD	28±3	60±10			57±11				

ATP, anti-tachycardia pacing. Other abbreviations as in Table 1.

Table 3. Baseline Delivery Patient Characteristics

Patient	During delivery						After delivery		
	Weeks at delivery	ICD mode	Labor	Delivery mode	Indication for CS	Blood loss (ml)	Minimum LVEF (%)	No. ICD shocks	Follow-up period (months)
1	37	Off	Induced	Emergency CS	NRFS	1,190	42.1	1 (ATP 6)	12
2	37	Off	Induced	Emergency CS	NRFS	300	32.6	0	47
3	33	Off	None	CS	FGR	840	64.1	0	26
4	40	Off	Spontaneous	Emergency CS	NRFS	210	61.9	0	16
5	35	Off	Induced	Emergency CS	NRFS	340	59.3	0	12
6	38	On	Spontaneous	Emergency CS	NRFS	400	56.9	0	3
Mean±SD	37±2					547±384	53±13		19±15

Bood loss, total blood loss including amnion at cesarean section; CS, cesarean section; FGR, fetal growth restriction; NRFS, non-reassuring fetal status. Other abbreviations as in Tables 1,2.

Table 4. Baseline Fetus and Neonate Characteristics

Patient	Weeks at birth	Birth weight (g)	Apgar score (1 min)	Apgar score (5 min)	UmA pH	Fetal complications	Neonatal complications
1	37	2,684	7	9	7.312	NRFS	
2	37	2,622	8	9	7.283	NRFS	
3	33	1,240	8	9	7.332	FGR	Hypoglycemia, Hyperbilirubinemia
4	40	2,750	8	9	7.344	NRFS	
5	35	1,776	9	10	7.268	FGR, NRFS	Hypoglycemia, yperbilirubinemia, LQTS type1
6	38	2,188	8	10	6.963	FGR, NRFS	Metabolic acidosis, Hypoglycemia, LQTS type2
Mean±SD	37±2	2,210±603					

UmA, umbilical artery. Oher abbreviations as in Tables 1,3.

for maternal indication of increased non-sustained VT and reduction of cardiac function at 37 weeks' gestation, and 1 (patient 5) for fetal indication of fetal growth restriction and growth arrest at 35 weeks' gestation. All patients delivered by cesarean section under spinal and epidural anesthesia due to fetal indications. The ICD was turned off in patients 1–5 and turned on in patient 6 during labor and cesarean section. Electrocautery was not used during cesarean section. During delivery, there were no syncopal or hypotensive episodes and no patients received ICD discharges or shocks.

After Delivery

Baseline post-delivery patient characteristics are listed in Table 3. All but 2 women with DCM (patients 1, 2) breast-fed the neonate. Patient 1 had reduced LVEF before delivery and recovered within 1 month after delivery. She received an appropriate ICD shock after unsuccessful ATP for VT at 6 weeks after delivery. After an increase of β-blockers and construction of 2 more burst ATPs, there were no ICD shocks except for 6 ATP shocks for VT in 1 year after delivery. All ATP shocks were appropriate and successful. Patient 2 had reduced LVEF for 1 week and recovered within 1 month after delivery. In patient 4, PAF increased until 1 week after delivery. In the 2

women (patients 5, 6) with long QT syndrome, the corrected QT time was 505–510 ms and 460–490 ms, respectively; these were almost the same as before pregnancy, and there were no episodes of ventricular arrhythmia after delivery.

Fetus and Neonate Outcome

Baseline characteristics of fetuses and neonates are given in Table 4. Five neonates were born by emergency cesarean section due to non-reassuring fetal status. We observed persistent late decelerations in 3 fetuses and prolonged decelerations in 2 fetuses during labor on cardiotocogram. One neonate (patient 6) had metabolic acidosis that required infusion of bicarbonate. Two neonates (patients 3, 5) were born preterm and 3 (patients 3, 5, 6) were small for date. The 2 neonates of mothers with long QT syndrome (patients 5, 6) were also diagnosed with long QT syndrome on genetic testing. No major complications were observed in the observation period.

Discussion

To our knowledge, this is the largest single-center retrospective study to investigate the outcome of pregnancy in women with an ICD. According to the present 6 cases, pregnancy did not increase the risk of an ICD-related complication under appropriate management (eg, increase of β-blockers and change of the ICD setting), even though the number of ventricular arrhythmias increased after the end of the second trimester of pregnancy. Additional caution might be required in the postpartum period, as well as during pregnancy and labor.

Pregnancy and Ventricular Arrhythmia

Pregnancy is associated with reversible increases in blood volume, heart rate and cardiac output.[8,9] In some instances, these changes can trigger maternal cardiac deterioration during pregnancy.[10–13] Some studies have suggested that pregnancy may have an adverse effect on subsequent maternal cardiac outcome, perhaps as a result of the hemodynamic burden on ventricular structure and function during pregnancy.[14–17] Clearly, special caution is required for patients with an ICD with regard to cardiac function and arrhythmias. In this context, pregnancy can be thought of as a physiological stress test, and complications during pregnancy identify women at high risk for late events.[18] We monitored the ICD settings from before pregnancy to prevent inappropriate ICD discharges due to heart rate increases during pregnancy. In 1 case, β-blockers were introduced before pregnancy to avoid a recurrence of PAF during pregnancy. Although the number of tachyarrhythmias increased in all women after the end of the second trimester except in 2 with long QT syndrome, ICD discharges were not precipitated during pregnancy, when anti-arrhythmic medications were gradually increased and the setting of the ICD was changed.

Balint et al recommended that women at high cardiac risk should receive closer surveillance both during pregnancy and late after delivery.[19] Adverse events during pregnancy are associated with higher rates of late events, which makes it important to re-evaluate the cardiac status of women with pregnancy cardiac events more closely after pregnancy.[19] In the present study, 1 woman who had ATP at 27 weeks' gestation received her first ICD shock and several ATP events after delivery despite an increase of anti-arrhythmic medications and a change of the ICD setting. This suggests that additional caution may be required in the postpartum period, as well as during pregnancy and labor.

ICD Mode During Delivery

It remains unclear whether an ICD should be on or off during delivery. In the present study, no arrhythmias or ICD discharges were precipitated during delivery, as also reported by Natale et al.[7] In this respect, the status of the ICD during delivery appears to have no effect on the overall outcome. Recurrence of VT, however, decreases placental perfusion due to maternal hypotension and could be dangerous for the fetus. In contrast, ICD shocks are a concern for the safety of the fetus, although the amount of energy transferred to the uterus is very small and the fetal heart has a high fibrillatory threshold.[7,20] Based on these considerations, we have recently changed our policy to leave the device turned on during vaginal delivery or cesarean section, with the proviso that electrocautery is not used. Because elevated heart rate during labor may cause inappropriate ICD shock, a multidisciplinary approach involving specialists in maternal fetal medicine, cardiology and anesthesiology is needed for total management during labor and delivery for pregnant woman with an ICD. This management needs to be designed specifically to meet these needs at each hospital.

Fetal and Neonatal Complications

Three of the present fetuses (50%) had fetal growth restriction. Gelson et al found a significant reduction in fetal growth rates associated with maternal heart disease, and concluded that the presence of maternal cyanosis and reduced cardiac output are the most significant predictors of this condition.[21] These findings, however, are not necessarily applicable to the present cases.

In the present study, 5 patients (83%) were given β-blockers, and 2 of these experienced fetal growth restriction. Beta-blockers are considered to be reasonably safe for use during pregnancy, but may rarely cause fetal growth restriction, bradycardia, apnea, hypoglycemia, and hyperbilirubinemia of neonates.[22–25] Five patients delivered by emergency cesarean section due to non-reassuring fetal status (ie, hypoxia of the fetus or severe cord compressions in the uterus, which also occurs during labor in those without an ICD). Beta-blockers are thought to have little effect in the unstressed fetus, but adverse effects may become apparent during fetal distress because these drugs impair fetal response to distress.[25] Although the number of cases is small, β-blockers may have been related to fetal and neonatal complications, but these drugs are clearly effective for preventing life-threatening arrhythmias and inappropriate ICD shocks.[26] We consider use of β-blockers permissible during pregnancy on the condition that efficacy surpasses complications. Furthermore, as few drugs as possible and the safest drugs at the lowest effective doses should be chosen for use in pregnancy.

Study Limitations

There are several limitations in the study, including its retrospective design and the relatively small sample size. First, the present 6 patients were relatively low risk: ICD shocks were delivered before pregnancy only in 3 of the 6 patients; clinically documented ventricular arrhythmias were heterogeneous (VT in 2 patients and VF in the other 4 patients); and LVEF was preserved in 4 of the 6 patients. Because risk of recurrence of ventricular arrhythmias would be strongly associated with the clinical and arrhythmia background of pregnant women, further investigation is needed, including in patients with high risk for VT and VF. Second, it may be safe to leave the device turned on during vaginal delivery or cesarean section, but the sample size may have been too small to prove this

point. There were no ICD shocks during pregnancy, and therefore we are unable to determine whether ICD shocks are safe for the fetus. Third, the follow-up period after delivery was insufficient to permit analysis of long-term morbidity and mortality, which prevented evaluation of potential long-term benefits and the risks of use of an ICD after delivery. The present study, however, is worthwhile as a report of a single-center experience of a rare condition that we were able to follow up in 5 patients (83%) more than 1 year after delivery.

Conclusions

In the present 6 patients with an ICD, pregnancy did not increase the risk of an ICD-related complication under appropriate management (ie, increase of β-blockers and changing of the ICD setting). Additional caution may be required in the postpartum period as well as during pregnancy and labor. Guidelines are required for pregnancy and delivery in patients with an ICD. Further large prospective studies are needed to establish the most appropriate treatment strategies.

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Disclosure

None of the authors have a conflict of interest to disclose.

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Allogeneic Transplantation of Fetal Membrane–Derived Mesenchymal Stem Cell Sheets Increases Neovascularization and Improves Cardiac Function after Myocardial Infarction in Rats

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Background. Mesenchymal stem cell (MSC) transplantation has been pursued as a new method to repair damaged myocardium. We focused on the fetal membrane (FM) as an alternative source to bone marrow (BM)–derived MSCs. In this study, we investigated whether transplantation of allogeneic FM-MSC sheets could attenuate myocardial dysfunction in a rat chronic myocardial infarction (MI) model.

Methods. Sheets of allogeneic FM-MSC or autologous BM-MSC were transplanted into the scarred myocardium 4 weeks after coronary ligation.

Results. Four weeks after transplantation, both allogeneic FM-MSC and autologous BM-MSC sheets had significantly improved cardiac function and reduced myocardial fibrosis compared with the untreated MI group. In both MSC sheet-transplanted groups, the peri-infarct regional capillary density was increased. Some engrafted MSCs formed vascular structures and were positive for lectin I and α-smooth muscle actin. The numbers of engrafted cells and differentiated cells were very low after both types of MSC sheet transplantation. $CD3^+$ T cells did not increase in the transplantation site, but $CD163^+$ M2 macrophages increased in the groups transplanted with allogeneic FM-MSC and autologous BM-MSC.

Conclusions. Transplantation of allogeneic FM-MSC or autologous BM-MSC sheets attenuated myocardial dysfunction in a rat MI model to a similar degree. The engraftment rate of transplanted cells and immune cell infiltration into the transplanted area did not differ between the two types of MSC transplants. M2 macrophage induction has possible involvement in the therapeutic effects of MSC transplantation. Allogeneic FM-MSC sheet transplantation might be a new therapeutic strategy after MI.

Keywords: Fetal membrane, Mesenchymal stem cells, Cell sheet, Myocardial infarction, Allogeneic transplantation. (*Transplantation* 2013;96: 697–706)

Myocardial infarction (MI) causes loss of cardiac tissue and impairment of left ventricular function. Recent reports suggest that mesenchymal stem cells (MSCs) are a

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valuable cell source for cell therapy after MI and that bone marrow (BM) represents a major source of MSCs. Several clinical trials of autologous BM-MSC transplantation for MI have reported therapeutic success (*1–3*).

BM harvest is a surgical procedure that requires general anesthesia or sedation, and both the proliferative potential and the differentiation capacity of MSCs seem to decrease in older donors (*4, 5*). In addition, BM procurement procedures in humans may yield low numbers of MSCs after cell processing. To address this issue, we focused on the fetal membrane (FM) of the placenta, which is generally discarded as medical waste after delivery, as an alternative source of autologous MSCs. Several studies have reported that the human FM contains multipotent cells similar to BM-MSCs and that these cells are easy to expand (*6, 7*). We demonstrated previously that the allogeneic transplantation of FM-MSCs did not elicit any lymphocyte proliferative response despite their allogeneic origin and induced therapeutic effects in a rat model of hind-limb ischemia and acute myocarditis (*8, 9*).

In some types of MSC transplantation, dissociated MSCs are injected into the myocardium to induce cardiac regeneration. However, it is difficult to reconstruct sufficient cardiac mass in the thinned scar area after MI. Imanishi et al. (*10*) reported that approximately 90% of cells injected into the myocardium are lost within 1 day. Okano et al. recently developed cell sheets using temperature-responsive culture dishes (*11–14*). These cell sheets allow for cell-to-cell connections and maintenance of adhesion proteins. In a rat MI model, the engraftment rate of transplanted cells was higher after transplantation of cell sheets compared with intramyocardial transplantation of dissociated cells (*15, 16*). These results suggest that transplantation of allogeneic FM-MSC sheets may be a new strategy for the treatment of heart failure.

In this study, we designed a set of experiments with the following aims: (i) to compare the therapeutic effects of transplantation of allogeneic FM-MSC sheets and autologous BM-MSC sheets in a rat chronic MI model, (ii) to investigate the engraftment and differentiation of transplanted MSCs, and (iii) to investigate whether transplanted allogeneic FM-MSC sheets evade immune rejection.

RESULTS

Preparation and Transplantation of Two-Layered MSC Sheets

FM-MSCs derived from green fluorescent protein (GFP)-transgenic Sprague–Dawley rats (3.3×10^6 cells) or BM-MSCs derived from GFP-transgenic Lewis rats (3.3×10^6 cells) were cultured in temperature-responsive 35-mm dishes for 1 day. When the culture temperature was decreased from 37°C to 20°C, both types of MSC sheets detached spontaneously and floated into the culture medium as a monolayer MSC sheet that could be stacked into two-layer constructs (Fig. 1A, C, and D). We transplanted two-layered FM-MSC sheets or BM-MSC sheets over the anterior wall of the heart, including the infarcted area, and then attached them to the heart surface (Fig. 1B).

Engraftment of Transplanted Allogeneic FM-MSC and Autologous BM-MSC Sheets in Infarcted Hearts

One day and 1 and 2 weeks after transplantation, GFP-positive allogeneic FM-MSCs and autologous BM-MSCs were present as sheets on the infarcted area of the anterior wall (n=3 in each group). GFP-positive allogeneic FM-MSCs and autologous BM-MSCs were observed in the anterior infarcted area 3 and 4 weeks after transplantation (n=4 in each group) (Fig. 1E). However, semiquantitative analysis demonstrated that the engraftment rate decreased with time in both MSC sheet-transplanted groups (Fig. 1F). The engraftment rate did not differ significantly between the MI groups transplanted with allogeneic FM-MSC and autologous BM-MSC.

Improvement in Cardiac Function after Transplantation of Allogeneic FM-MSC and Autologous BM-MSC Sheets

Heart failure developed 4 weeks after coronary ligation, as indicated by deterioration of left ventricular function and thinning of the infarct wall. The ejection fraction, fractional shortening, anterior wall thickness, posterior wall thickness, left ventricular diastolic dimension, and left ventricular systolic dimension measurements at baseline did not differ significantly between the three MI groups. One of the 10 rats in the untreated MI group died on day 53 after coronary ligation; no rats died in the MI groups transplanted with allogeneic FM-MSC or autologous BM-MSC.

Hemodynamic analysis revealed significant improvements in the MI group transplanted with allogeneic FM-MSC compared with the untreated MI group for the left ventricular systolic pressure, maximum dP/dt, and minimum dP/dt ($P<0.05$; n=10 in each group) (Fig. 2B,C; see **Table S1, SDC,** http://links.lww.com/TP/A849).

Echocardiographic analysis revealed significant improvements in ejection fraction, fractional shortening, and left ventricular systolic dimension ($P<0.05$ for each) in the MI group transplanted with allogeneic FM-MSC compared with the untreated MI group. Anterior wall thickness was also significantly greater in the MI group transplanted with allogeneic FM-MSC than in the untreated MI group (Fig. 2D,E; see **Table S2, SDC,** http://links.lww.com/TP/A849).

The hemodynamic and echocardiographic parameters did not differ significantly between the MI groups transplanted with allogeneic FM-MSC and autologous BM-MSC.

Reduction in Myocardial Fibrosis after Transplantation of Allogeneic FM-MSC and Autologous BM-MSC Sheets

Eight weeks after coronary ligation, Masson's trichrome staining of the myocardium from the untreated MI group demonstrated prominent and diffuse interstitial fibrosis in the anterior scar area. This was attenuated markedly in the MI groups transplanted with allogeneic FM-MSC and autologous BM-MSC (Fig. 3A). Quantitative assessment of myocardial fibrosis of the left heart showed that the fraction of Masson's trichrome-stained collagen volume was significantly smaller in the MI groups transplanted with allogeneic FM-MSC or autologous BM-MSC than in the untreated MI group ($P<0.05$; n=10 in each group) (Fig. 3B).

Angiogenesis and Differentiation of Transplanted Allogeneic FM-MSC and Autologous BM-MSC Sheets in Infarcted Hearts

Four weeks after transplantation, vascularization was assessed by lectin I staining and was observed in the allogeneic FM-MSC–transplanted MI group and the autologous

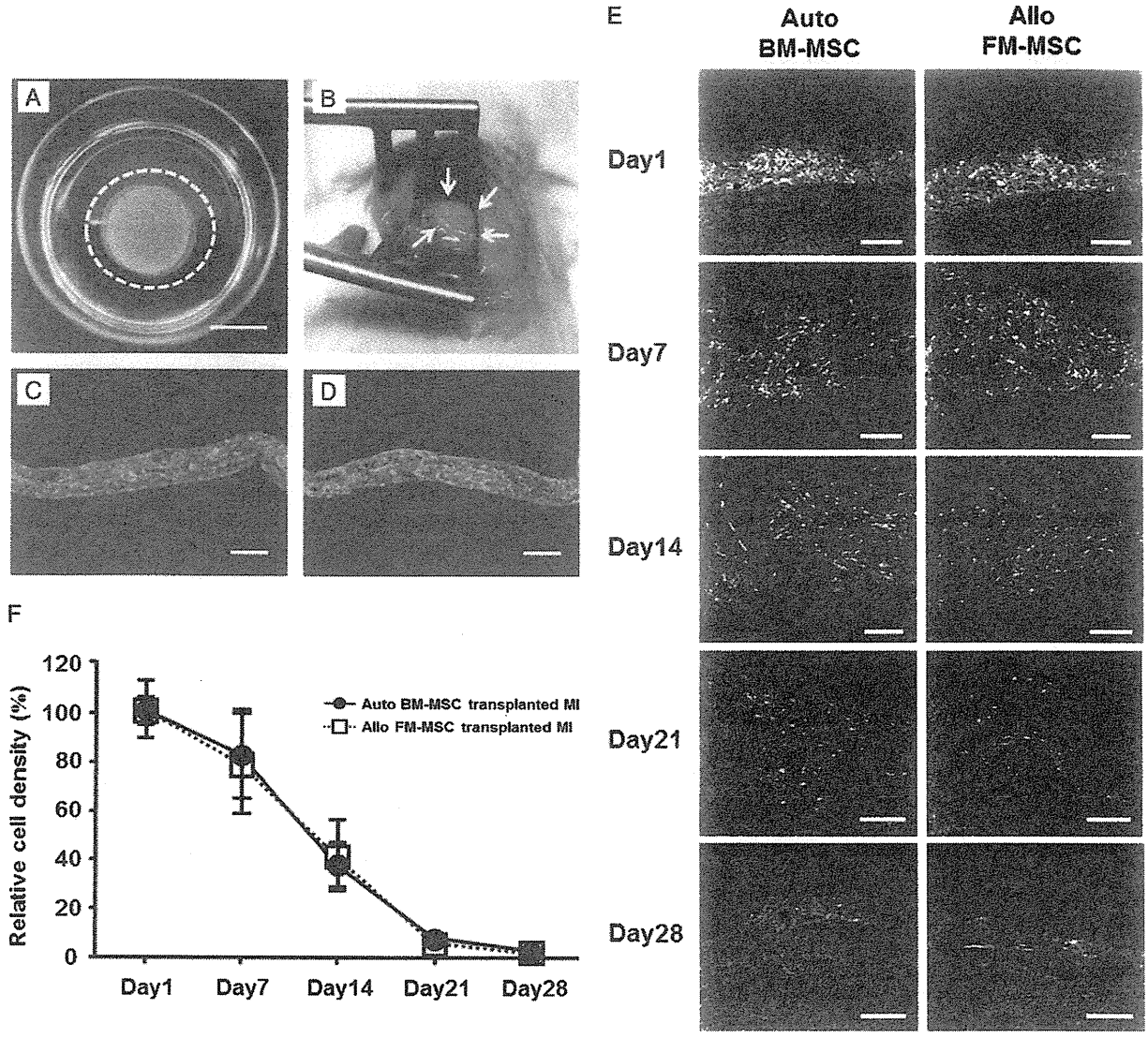

FIGURE 1. Stacked MSC sheets and their transplantation into infarcted hearts. A, two MSC sheets harvested from temperature-responsive culture surfaces were stacked successfully, producing a two-layer construct. Scale bar, 10 mm. B, two-layered MSC sheets were transplanted over the anterior wall of the infarcted heart and formed a stable attachment to the heart surface (arrows). C, cross-sectional staining of a GFP-expressing two-layered FM-MSC sheet. Scale bar, 100 μm. D, cross-sectional staining of a GFP-expressing two-layered BM-MSC sheet. Scale bar, 100 μm. E, allogeneic FM-MSCs and autologous BM-MSCs were present over the area surrounding the scar on days 1, 7, 14, 21, and 28. Scale bar, 100 μm. F, semiquantitative analysis showed that the engraftment rate of cells decreased with time in both groups transplanted with MSC sheets (days 1, 7, and 14, n=3 in each group; days 21 and 28, n=4 in each group). Data are expressed as mean±SE.

BM-MSC–transplanted MI group (Fig. 4B). Quantitative analysis showed increased capillary density in the infarcted area in both MSC-transplanted groups compared with the untreated MI group (n=6 in each group) (Fig. 4C). The capillary density in the peri-infarct area was similar in the MI groups transplanted with allogeneic FM-MSC and autologous BM-MSC; both values were significantly higher than in the untreated MI group ($P<0.05$; n=6 in each group) (Fig. 4D).

GFP-positive FM-MSCs and BM-MSCs were observed in the peri-infarct area of the anterior wall, but GFP–lectin I/α-smooth muscle actin (αSMA) double-positive cells were not observed 1 day or 1 week after transplantation (data not shown). Two weeks after transplantation, engrafted GFP-positive FM-MSCs and BM-MSCs formed vascular structures and were positive for lectin I and αSMA (Fig. 4E,F). The GFP–lectin I/αSMA double-positive cells comprised less than 1% of the engrafted cells.

Immune Responses to Transplanted Allogeneic FM-MSCs and Autologous BM-MSCs in Infarcted Hearts

To compare the host immune responses to transplanted allogeneic FM-MSCs and autologous BM-MSCs, we performed immunohistochemical staining for CD3 (T cells) and CD68

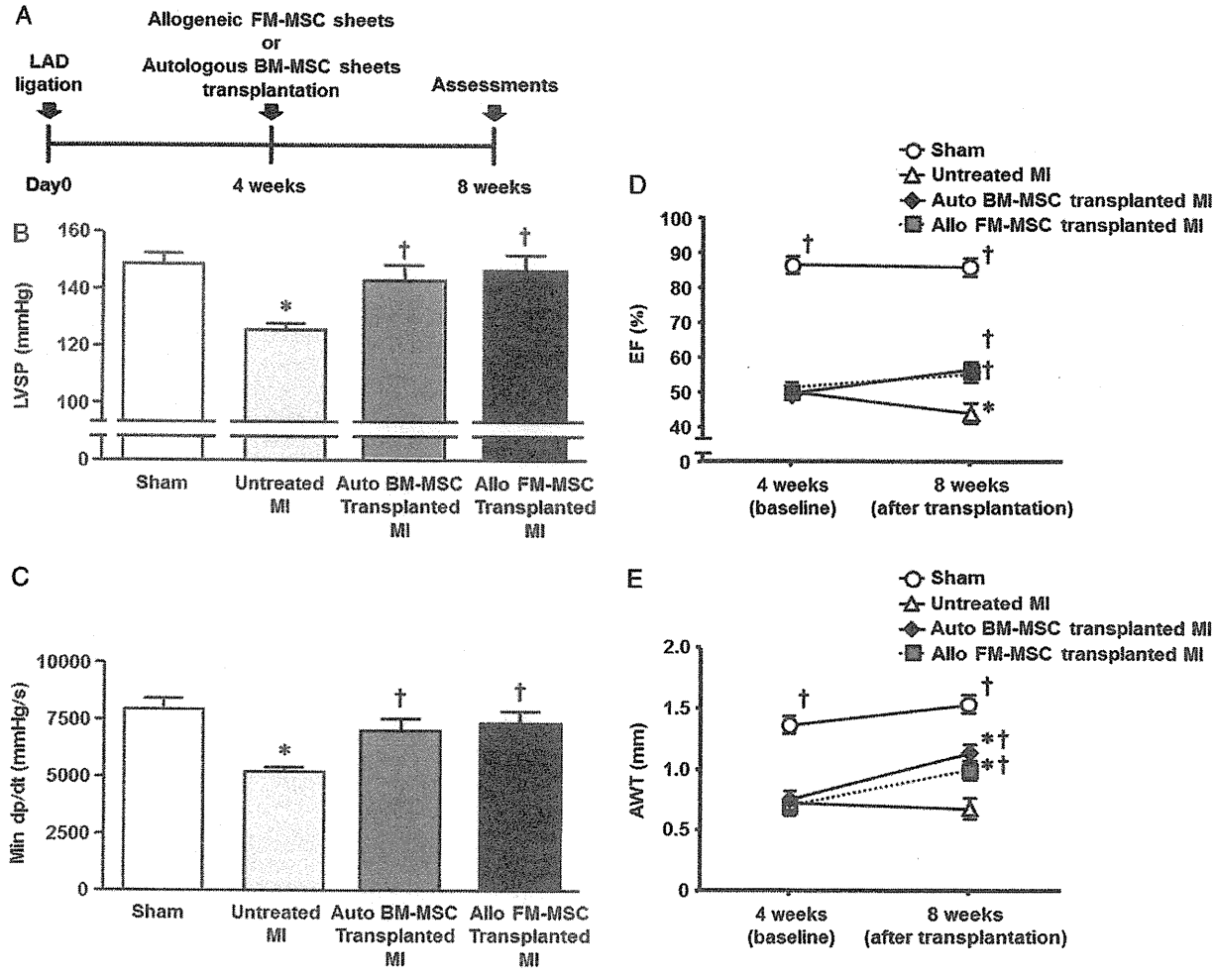

FIGURE 2. Effects of transplantation of allogeneic FM-MSC and autologous BM-MSC sheets on hemodynamic and echocardiographic parameters after MI. A, study flowchart. B and C, four weeks after transplantation, left ventricular systolic pressure and minimum d*P*/d*t* had improved significantly in the MI groups transplanted with allogeneic FM-MSC and autologous BM-MSC compared with the untreated MI group (n=10 in each group). Data are expressed as mean±SE. **P*<0.05 vs. sham group; †*P*<0.05 vs. untreated MI group. D and E, four weeks after transplantation, the ejection fraction and anterior wall thickness in the MI groups transplanted with allogeneic FM-MSC and autologous BM-MSC had improved significantly compared with the untreated MI group (n=10 in each group). Data are expressed as mean±SE. **P*<0.05 vs. baseline; †*P*<0.05 vs. time-matched untreated MI group. AWT, anterior wall thickness; EF, ejection fraction; LVSP, left ventricular systolic pressure.

(monocytes and macrophages) in sections of MSC-transplanted infarcted hearts 4 weeks after transplantation. Compared with the sham group, the numbers of $CD3^+$ and $CD68^+$ cells in the infarcted and peri-infarct areas were increased in the untreated MI group and in the MI groups transplanted with allogeneic FM-MSC or autologous BM-MSC (Fig. 5A,D). Quantitative analysis demonstrated no significant differences in $CD3^+$ cell infiltration between the MI groups that were untreated or transplanted with allogeneic FM-MSC and autologous BM-MSC (n=8 in each group) (Fig. 5C).

In the infarcted areas, there were no differences in the number of $CD68^+$ cells between the three MI groups, untreated or transplanted with allogeneic FM-MSC or autologous BM-MSC (n=8 in each group). By contrast, the number of $CD68^+$ cells in the peri-infarct area was significantly higher in both MSC-transplanted MI groups than in the untreated MI group (*P*<0.05 vs. untreated MI group; n=8 in each group) (Fig. 5E,F). The intensity of CD3 and CD68 staining did not differ between the two MSC-transplanted groups. $CD163^+$ cells were observed in the serial sections of the sites infiltrated by $CD68^+$ cells from all three MI groups (see **Figure S2b, SDC**, http://links.lww.com/TP/A849).

DISCUSSION

In the present study, we have demonstrated five points. First, transplantation of allogeneic FM-MSC sheets and autologous BM-MSC sheets improved cardiac function and prevented ventricular remodeling in a rat model of MI to a similar degree. Second, massive angiogenesis was observed in the areas transplanted with allogeneic FM-MSC sheets and autologous BM-MSC sheets but was not observed in the area

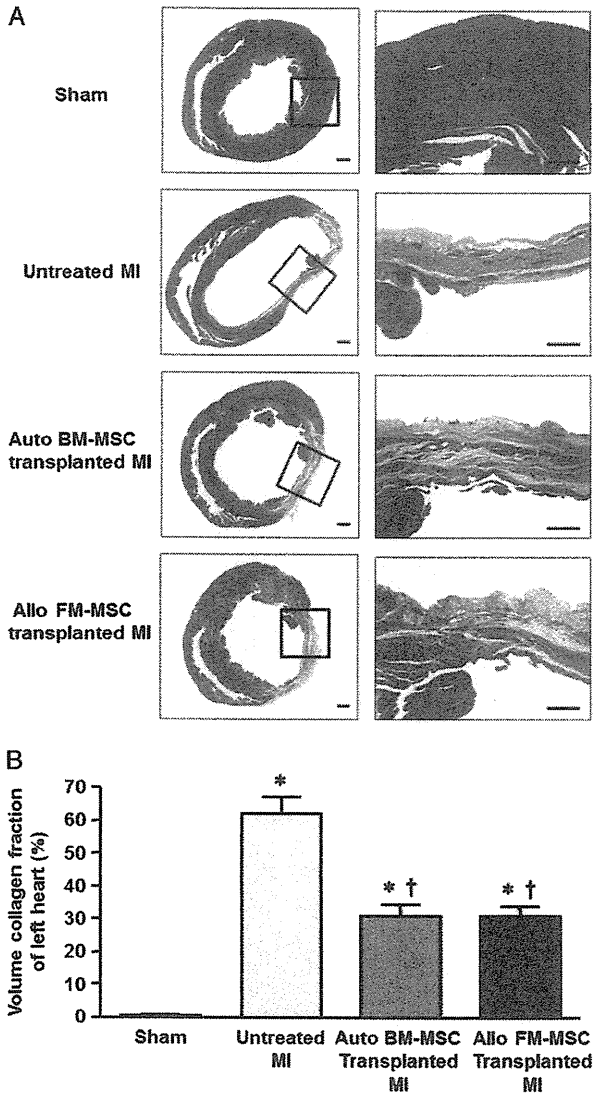

FIGURE 3. Masson's trichrome staining of heart cross-sections at the left ventricular papillary muscle level from MI rats transplanted with allogeneic FM-MSC and autologous BM-MSC sheets. A, four weeks after transplantation, the myocardial fibrosis area was smaller in the MI groups transplanted with FM-MSC and autologous BM-MSC than in the untreated MI group. Right row shows a higher resolution of the image in the black box in the respective left row. Scale bar, 1 mm (left row) and 500 μm (right row). B, quantitative analysis demonstrated that the fibrosis area was significantly smaller in the MI groups transplanted with FM-MSC and autologous BM-MSC compared with the untreated MI group (n=10 in each group). Data are expressed as mean±SE. *P<0.05 vs. sham group; †P<0.05 vs. untreated MI group.

of the infarcted myocardium. Third, transplanted allogeneic FM-MSCs engrafted in the infarcted myocardium from 1 day to 4 weeks after transplantation, but the number of engrafted cells decreased markedly with time. Fourth, some of the engrafted FM-MSCs were positive for lectin I or αSMA, but these cells comprised less than 1% of the engrafted cells. Fifth, the engraftment rate and host immune cell responses did not differ between groups transplanted with allogeneic FM-MSC and autologous BM-MSC.

Several studies have reported that transplantation of autologous BM-MSC improves cardiac function in ischemic heart disease (*17–20*). However, there are several limitations when using autologous BM-MSCs for clinical applications, including the invasiveness of the harvesting procedure, inadequate cell numbers, and donor site morbidity (*21*). We have reported that allogeneic FM-MSCs are an alternative to autologous BM-MSCs (*8, 9*). Although allogeneic, transplanted FM-MSCs exerted therapeutic effects in experimental rat models of hind-limb ischemia and acute myocarditis and did not elicit alloreactive lymphocyte proliferation. In this study, we showed a significant improvement in cardiac function and a reduction in myocardial fibrosis in rats with chronic MI that were transplanted with allogeneic FM-MSC sheets or with autologous BM-MSC sheets. The FM contains large quantities of MSCs, and their use is considered to present few ethical concerns; thus, FM-MSCs can provide a cell source for regenerative medicine (*22, 23*).

The mechanisms underlying the effectiveness of MSC therapy in treating ischemic heart failure may involve both the differentiation of transplanted MSCs into vascular cells and cardiomyocytes and the secretion of several growth factors by transplanted cells (paracrine effects). Two to 4 weeks after transplantation, some of the engrafted FM-MSCs and BM-MSCs stained positively for lectin I and participated in vessel formation. Staining for αSMA revealed that both types of MSCs differentiated into vascular smooth muscle cells, which play an important role in vessel maturation. A few engrafted MSCs may transdifferentiate in the vessel, but the number of such cells would be insufficient to be the main mechanism responsible for the therapeutic gain. We did not find desmin-positive or troponin T–positive engrafted allogeneic FM-MSCs or autologous BM-MSCs (data not shown). Earlier studies reported that transplanted MSCs differentiated into cardiomyocytes, vascular endothelial cells, and smooth muscle cells (*24–26*), but more recent studies have reported that transplanted MSCs appear to differentiate into these cells at a very low frequency (*27–30*).

After the discovery of the paracrine effect of MSCs, many studies have confirmed that the success of stem cell therapy for heart failure depends on this mechanism mainly by the promotion of angiogenesis, myocardial protection, and immune regulation (*31, 32*). In our previous study, transplanted FM-MSCs and BM-MSCs secreted angiogenic and cardioprotective cytokines, including vascular endothelial growth factor (VEGF) and hepatocyte growth factor, in the ischemic tissues (*8, 33*). These growth factors secreted from engrafted MSCs may help prevent ventricular remodeling. The response of the MSC sheets was similar, with large amounts of VEGF secreted into the culture media by FM-MSCs and BM-MSCs (see **Figure S1a, SDC,** http://links.lww.com/TP/A849). In both MI groups transplanted with MSC sheets, VEGF expression was up-regulated in the peri-infarct areas (see **Figure S1b, SDC,** http://links.lww.com/TP/A849). These results suggest that the therapeutic effects observed in our study may be attributable to the paracrine effects of transplanted FM-MSCs rather than to their differentiation into vascular endothelial cells and cardiomyocytes.

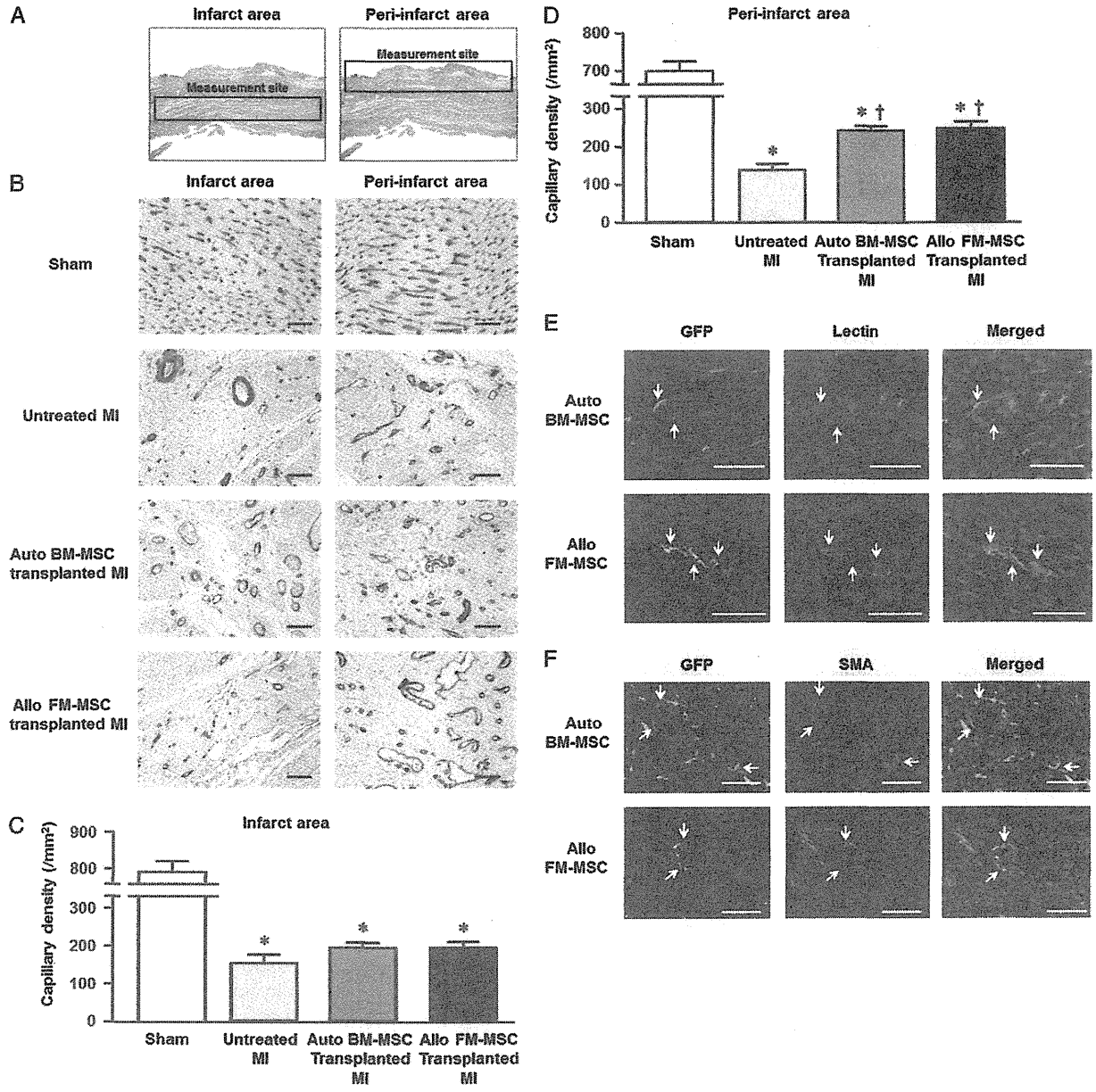

FIGURE 4. Vascularization and differentiation into vascular endothelial cells in the myocardial tissue grafted with allogeneic FM-MSC and autologous BM-MSC sheets. A, representative measurement section sites. B, four weeks after transplantation, the numbers of lectin I–positive capillaries were greater in the infarcted and peri-infarct areas in the MI groups transplanted with allogeneic FM-MSC and autologous BM-MSC than in the untreated MI group. Quantitative analysis demonstrated that the capillary densities in the transplanted area were significantly higher in the MI groups transplanted with allogeneic FM-MSC and autologous BM-MSC compared with the untreated MI group (infarcted area [C] and peri-infarct area [D]; n=10 in each group). E, two weeks after transplantation, GFP-expressing FM-MSCs and BM-MSCs were identified in a thick stratum on the epicardial side of the myocardium. Some allogeneic FM-MSCs and autologous BM-MSCs (green; white arrows) were positive for lectin I (red). F, some allogeneic FM-MSCs and autologous BM-MSCs (green; white arrows) were positive for αSMA (red). Nuclei are stained with TOPRO3 (blue). Scale bar, 50 μm. Data are expressed as mean±SE. *P<0.05 vs. sham group; †P<0.05 vs. untreated MI group.

MSCs are positive for major histocompatibility complex (MHC) I and negative for MHC II and costimulatory factors such as CD40, CD80, and CD86, so are considered to be nonimmunogenic (*34, 35*). We reported previously that FM-MSCs did not express MHC class II and did not induce alloreactive T lymphocyte proliferation (*8*). In this study, immunohistochemical staining showed few infiltrating $CD3^+$ T cells in the areas transplanted with allogeneic

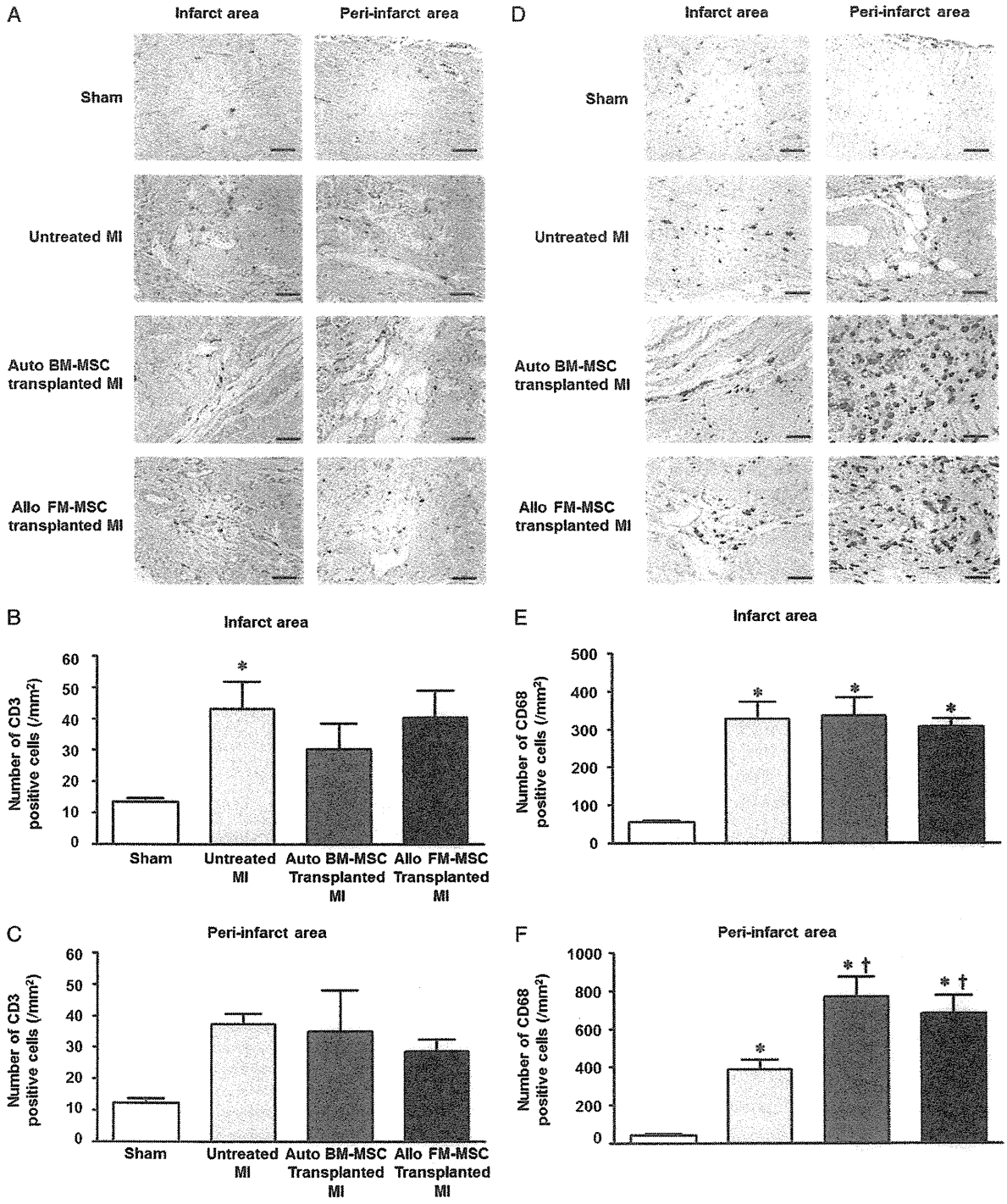

FIGURE 5. Immune responses in the myocardium transplanted with MSC sheets. A, in the infarcted and peri-infarct areas, the numbers of infiltrating $CD3^+$ cells did not differ between the untreated MI group and MI groups transplanted with allogeneic FM-MSC and autologous BM-MSC (yellow arrows: $CD3^+$ cells). B and C, quantitative analysis of $CD3^+$ cells in the infarcted and peri-infarct areas showed no significant differences between the three MI groups (n=8 in each group). D, in the infarcted area, the number of infiltrating $CD68^+$ cells did not differ between the three MI groups, but marked $CD68^+$ cell infiltration was found in the peri-infarct area in the MI groups transplanted with allogeneic FM-MSC and autologous BM-MSC. E and F, quantitative analysis of $CD68^+$ cells in the infarcted area showed no significant difference between the three MI groups. In the peri-infarct area, significantly more $CD68^+$ cells were found in the MI groups transplanted with allogeneic FM-MSC and autologous BM-MSC than in the untreated MI group (n=8 in each group). Scale bar, 50 μm. Data are expressed as mean±SE. *P<0.05 vs. sham group; †P<0.05 vs. untreated MI group.

FM-MSCs and the infarcted areas at 4 weeks after transplantation, and the infiltrating T cells were almost all $CD8^+$ T cells (see **Figure S2a, SDC,** http://links.lww.com/TP/A849). However, there were no differences between the infarcted hearts transplanted with allogeneic FM-MSC sheets and untreated infarcted hearts in the number of infiltrating $CD3^+$ and $CD8^+$ T cells and the degree of $CD3^+$ T-cell infiltration, and the engraftment rate did not differ between allogeneic FM-MSC transplantation and autologous BM-MSC transplantation. Thus, this limited T-cell infiltration may have been caused by chronic myocardial inflammation. In addition, there were few infiltrating $CD45RA^+$ B cells in the allogeneic FM-MSC-transplanted areas (see **Figure S3, SDC,** http://links.lww.com/TP/A849). These results suggest that allogeneic FM-MSCs are unlikely to activate host immune responses. In contrast, massive $CD68^+$ macrophage/monocyte infiltration was observed in the areas transplanted with either type of MSCs. There are two conceivable reasons for this macrophage infiltration. First, the infiltrating macrophages may have phagocytosed apoptotic cells, because terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling–positive cells were observed in areas transplanted with both types of MSCs (see **Figure S4, SDC,** http://links.lww.com/TP/A849). Second, there is a possibility that the macrophage infiltration was induced by the MSCs. It was reported that MSCs increased macrophage infiltration via a paracrine mechanism during wound healing after MI (*36*). There are two types of macrophages/monocytes: the classically activated proinflammatory M1 type and the alternatively activated anti-inflammatory M2 type (*37*). In our present study, many of the infiltrating macrophages were $CD163^+$ M2 macrophages (see **Figure S2b, SDC,** http://links.lww.com/TP/A849). M2 macrophages secrete several angiogenic factors promoting neovascularization (*38, 39*). Freytes et al. (*40*) reported that M2 macrophages modulated the viability of MSCs, and MSCs were reported to mediate a switch of macrophages to an anti-inflammatory activation state, which may be associated with the enhancement of cardiac function (*36, 41, 42*). Although further studies are needed, the induction of M2 macrophages may be one of the therapeutic mechanisms of MSC transplantation in MI.

The mechanisms responsible for the therapeutic effects of transplantation of allogeneic FM-MSC sheets in chronic MI are still unclear, and poor long-term survival and low differentiation rates of both types of transplanted MSC sheets are limitations of our study. In our previous study using the MI model, monolayer adipose tissue–derived MSC sheets gradually grew and developed into a thick stratum (*12*). The different results obtained in this study may have been caused by the difference in the cell sources. Several studies indicate differences between adipose tissue–derived MSCs and BM-MSCs (*43, 44*). Some studies tried to increase the therapeutic effects of cell transplantation by, for example, gene transduction or using a combination of drugs (*45–48*). Xu et al. (*49*) reported that lovastatin protected BM-MSCs from hypoxia-induced apoptosis, and Yang et al. (*50*) demonstrated that simvastatin improved the therapeutic efficacy of BM-MSC transplantation in an acute MI model by promoting cell survival and cardiovascular differentiation. These drug treatments may improve cell viability and increase the therapeutic effects of transplantation of MSC sheets in heart failure.

In conclusion, transplantation of allogeneic FM-MSC sheets improved cardiac function in a rat model of MI possibly by inducing angiogenesis and inhibiting myocardial fibrosis. The therapeutic effects were similar to those of transplanting autologous BM-MSC sheets and might be caused by the paracrine effects and the M2 macrophage induction. FM-MSC could be considered a new cell source, allowing wider clinical applications of MSC transplantation therapy. Although further experiments are needed to apply the current results to human cardiomyoplasty, transplantation of allogeneic FM-MSC sheets may provide a new therapeutic strategy for the treatment of MI.

MATERIALS AND METHODS

Animals

Male 8-week-old Lewis rats (Japan SLC, Hamamatsu, Japan) were used in this MI model. Male GFP-transgenic Lewis rats (Institute of Laboratory Animals, Kyoto University, Japan) and female GFP-transgenic Sprague–Dawley rats (Japan SLC) were also used for the harvest of transplanted cells. The experimental protocols were approved by the Animal Care Committee of the National Cerebral and Cardiovascular Center Research Institute (Osaka, Japan).

Expansion of FM-MSCs and BM-MSCs

The isolation and expansion of FM-MSCs and BM-MSCs were performed as described previously (see details in the **Materials and Methods, SDC,** http://links.lww.com/TP/A849) (*8*). In all experiments, FM-MSCs and BM-MSCs were used at passages 4 to 8.

Preparation of MSC Sheets

To prepare MSC sheets, we used 35-mm temperature-responsive dish (UpCell, CellSeed, Tokyo, Japan). Preparation of MSC sheets was performed as described previously (see details in the **Materials and Methods, SDC,** http://links.lww.com/TP/A849) (*12*).

Model of MI

To create an MI model, male Lewis rats (220–250 g) were anesthetized, and the left coronary artery was ligated, as described previously (see details in the **Materials and Methods, SDC,** http://links.lww.com/TP/A849) (*12*). The sham group underwent thoracotomy and cardiac exposure without coronary ligation.

We randomly assigned the rats to four groups: (a) rats with chronic heart failure that underwent transplantation with allogeneic FM-MSC sheets (allo-FM-MSC–transplanted MI group; n=10), (b) rats with chronic heart failure that underwent transplantation with autologous BM-MSC sheets (auto-BM-MSC–transplanted MI group; n=10), (c) rats with chronic heart failure without transplantation (untreated MI group; n=10), and (d) sham-operated rats without transplantation (sham group; n=10). Four weeks after coronary ligation, the allo-FM-MSC–transplanted MI group and auto-BM-MSC–transplanted MI group underwent transplantation with the respective two-layered cell sheets. The sheets were placed on the anterior wall, including the scar area, and then covered with oxidized regenerated cellulose (INTERCEED [TC7], Johnson & Johnson Medical, Tokyo, Japan). The other two groups underwent the same operative procedures without transplantation.

Hemodynamic Studies

Hemodynamic studies were performed 8 weeks after coronary ligation (4 weeks after transplantation (see details in the **Materials and Methods, SDC,** http://links.lww.com/TP/A849).

Echocardiographic Studies

Echocardiography was performed 4 weeks (before transplantation) and 8 weeks (4 weeks after transplantation) after coronary ligation (see details in the **Materials and Methods, SDC,** http://links.lww.com/TP/A849).

Immunohistochemical Studies

Immunohistochemical details (see **Materials and Methods, SDC,** http://links.lww.com/TP/A849).

Statistical Analysis

Data are expressed as mean±SE. Analysis of variance was used to compare each variable between groups, and the post hoc Tukey's test was used to locate significant differences. Differences were considered significant at $P<0.05$.

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