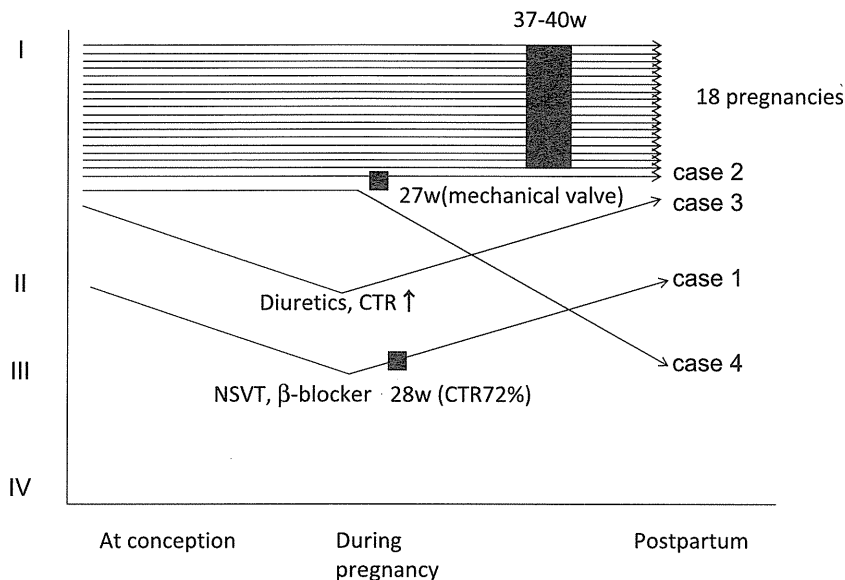


**FIGURE 3**  
**Changes of NYHA classification in the course of pregnancy**



CTR, cardiothoracic ratio; NSVT, nonsustained ventricular tachycardia; NYHA, New York Heart Association.

Katsuragi. Ebstein anomaly in pregnancy. *Am J Obstet Gynecol* 2013.

severity. However, patients with Ebstein anomaly are also susceptible to supraventricular tachycardia, and WPW syndrome is present in 20-50% of these patients.<sup>19</sup> However, arrhythmias in patients with Ebstein anomaly are not a contraindication to become pregnant. During pregnancy, supraventricular tachycardia can be treated with adenosine triphosphate or verapamil without adverse effects on the fetus, or direct current cardioversion can be used to safely terminate these arrhythmias. However, in such cases, we suggest that catheter ablation should be performed before conception for a subsequent pregnancy.<sup>20</sup>

If the patients are symptomatic, including exercise intolerance and cyanotic or progressive cardioomegaly up to CTR of 65%, TVR should be considered.<sup>21</sup> However, TVR with a mechanical valve is best avoided in patients who want to become pregnant, in case an anticoagulant is required during pregnancy. Both heparin and warfarin can be used during pregnancy, but use of warfarin may cause fetal brain hemorrhage and carries a risk of fetal bone anomaly, whereas heparin may cause maternal hemorrhage, as in one of our cases. A hypercoagulable state

may occur during pregnancy and a higher anticoagulant dose is needed as the glomerular filtration rate is increased.

Ebstein disease accounts for only 0.5% of congenital heart disease. Therefore, patients with Ebstein disease in pregnancy are relatively rare, and we were able to include only 13 such patients in our study. The small number of subjects prevented correction of the results for the effects of potential confounding factors such as hypertension and obstetric history through performance of multifactorial analysis. No newborn had congenital heart disease, but this needs to be reevaluated in a larger cohort. Also, few patients had undergone tricuspid valve repair, and this does not allow a discussion of this issue. In future work, we plan to investigate a larger cohort of patients to clarify the risk factors for cardiac dysfunction during pregnancy in patients with Ebstein anomaly.

In summary, pregnancy in Ebstein anomaly can be complicated with tachyarrhythmia or cardiac failure, and cardiac dysfunction and arrhythmias are a particular concern in post-TVR cases. However, maternal and fetal outcomes

are generally good without cardiac failure in patients with Ebstein anomaly with NYHA class I. Maternal outcome in these cases can be predicted by the preconceptional cardiac condition and NYHA functional class, which allows the evaluation of the risk for patients with Ebstein anomaly who want to become pregnant.

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## PERINATAL/NEONATAL CASE PRESENTATION

# Acute aortic dissection (Stanford type B) during pregnancy

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We report a case of acute aortic dissection (Stanford type B) that occurred in pregnant woman at 34-week gestation. She had no systemic characteristics of Marfan syndrome, however she exhibited a mutation of *FBN1*, Arg 545 Cys, which has been found to correlate with ectopia lentis but not with aortic dissection.

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**Keywords:** aortic dissection; gene analysis; pregnancy

### INTRODUCTION

Pregnancy is strongly associated with life-threatening problems in Marfan patients. Herein, we report a case of aortic dissection during pregnancy with a rare combination of the clinical manifestation and *FBN1* mutation.

### CASE

A 31-year-old woman, 2 gravida 1 para, was referred to our hospital at 34-week gestation because of acute aortic dissection type B. She had no family history of aortic dissection, or sudden death. She was 167 cm tall and weighed 80 kg before pregnancy. She had experienced sudden severe pain in her chest and was transferred to a tertiary care center. She was conscious and alert. She did not show either thumb sign, wrist sign or hind foot deformity in physical examination. Her arm span/height ratio was 1.02 (<1.05). Her blood pressure (BP) was 110~140 mm Hg, heart rate 80 b.p.m., oxygen saturation SpO<sub>2</sub> 99~100%, cardiac ultrasonography revealed good left ventricular ejection fraction of 74%, left ventricle end-diastolic dimension/end-systolic dimension of 53/33 mm. She did not exhibit mitral valve prolapse or aortic valve regurgitation, or dilatation of ascending aorta. Tricuspid valve regurgitation was minimal, the diameter of the inferior vena cava was 16 mm, and there were no indications of pulmonary arterial hypertension. Electrocardiography showed no specific ST changes. Contrast computerized tomography showed aortic dissection from just after the branch of the left subclavian artery to the level of the atrium, but did not show dura ectasia. To control the BP, Nicardipine 3 ml per hour (0.5  $\gamma$ ) was started and the BP was maintained at around 110~120/mm Hg.

Fentanyl (0.5 mg per day) was administered as an analgesia. Three hours later, she felt another chest pain and the dissected area progressed to the level of the renal artery and she was transferred to our hospital. She was conscious and alert, with BP 124/60, heart rate 110 b.p.m. There was no sign of rales and the respiratory rate was 20 per minute. She had no clinical features suggestive of Marfan syndrome. The fetus was in position for cephalic presentation and the estimated fetal body weight was 2200 g. Cardiotocography reassured that the fetus was in no difficulty. As celiac, supra mesenteric and renal arterial blood flow

was adequate with no elevation of lactate level, there was no indication for the operation for the aortic dissection.

As the baby was mature enough for extra-uterine life and the mother required further intensive care, including BP control, pain control and follow-up CT scan examination, we decided to perform cesarean section. Cesarean section was performed under general anesthesia, and a 2350 g healthy baby was delivered. During the postpartum course, for BP control, Nicardipine was continued with 0.5  $\gamma$  and dose was increased to 2.5  $\gamma$  when she was extubated the next day and was maintained until 7 days postpartum. For the pain control Loxoprophen 120 mg per day was started on the day 1 postpartum, and maintained for 7 days.

The patient increased her activities incrementally each day after the surgery. Schellong test and 50-m walking test was performed on the day 3 and 7 postpartum, respectively, and there were no changes in her BP or heart rate. The patient was discharged from the hospital on the day 10 postpartum with no changes in the dissected areas. The genetic study was performed by direct sequencing of genomic DNA from peripheral blood leukocytes and complementary DNA from fibroblast RNA derived from intraoperative skin biopsy. Study results revealed a heterozygous missense mutation of c.1633C to T (p.Arg545Cys) in *FBN1* gene.

### DISCUSSION

This patient had a missense mutation of *FBN1* (p.Arg545Cys), which has been specifically observed in six patients with lens subluxation in previous reports.<sup>1–4</sup> The patient developed descending aortic dissection, but had no enlargement of the ascending aorta, which might not be typical of aortic lesions in Marfan syndrome, though Marfan patients with aortic dissection in the descending aorta were reported.<sup>5,6</sup> Furthermore, no physical findings specific to Marfan syndrome were observed in this patient. Although aortic dissection is a rare complication that can develop during pregnancy,<sup>7,8,12</sup> pregnancy is strongly associated with life-threatening problems particularly in Marfan patients.<sup>8,9</sup> Aortic dissection events in pregnancy were reviewed in the aspect of its prevalence, etiology and subtypes of type A and type B, previously.<sup>10,11</sup> The risk of aortic dilatation or dissection increases during and after pregnancy in patients with Marfan syndrome due to superimposition of the hyperdynamic and

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hypervolemic circulatory state of pregnancy on the preexisting weakness of the aortic media.<sup>9</sup> Marfan syndrome is an autosomal dominant connective tissue disorder, and is associated with a higher risk of aortic dissection.<sup>13–15</sup> The above-mentioned results suggests that we should explain the genetics of the disease and provide genetic diagnosis, such as *FBN1*, for aortic dissection patients during pregnancy in order to identify the possible risk of aortic dissection, even if they are not considered as having Marfan syndrome.

#### CONFLICT OF INTEREST

The authors declare no conflict of interest.

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## Safety and Efficacy of Implantable Cardioverter-Defibrillator During Pregnancy and After Delivery

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**Background:** There are few studies of pregnancy and delivery in patients with an implantable cardioverter-defibrillator (ICD). The purpose of this study was to investigate maternal and fetal outcome in these patients.

**Methods and Results:** Six pregnant women with an ICD were retrospectively reviewed. All women underwent implantation of an ICD before pregnancy and delivered at the National Cerebral and Cardiovascular Center. The mean age at pregnancy and the mean follow-up period after ICD implantation were  $28\pm 3$  years old and  $5\pm 3$  years, respectively. There was no device-related complication during pregnancy. In 4 women, the number of tachyarrhythmias such as non-sustained ventricular tachycardia increased after the end of the second trimester of pregnancy and anti-arrhythmic medications were gradually increased. No patient received discharges or shocks from the ICD during pregnancy, however, and only one required anti-tachycardia pacing at 27 weeks' gestation. Mean gestational age at delivery was  $37\pm 2$  weeks and all deliveries were by cesarean section, including 5 as emergency deliveries due to a fetal indication. After delivery, 2 mothers had reduced cardiac function and 1 received an ICD shock for the first time.

**Conclusions:** Pregnancy did not increase the risk of an ICD-related complication under appropriate management. Additional caution might be required in the postpartum period as well as during pregnancy and labor. (*Circ J* 2013; **77**: 1166–1170)

**Key Words:** Beta-blocker; Delivery; Implantable cardioverter-defibrillator; Pregnancy; Ventricular tachycardia

Cardiac disease complicates approximately 1% of all pregnancies, and women with arrhythmias comprise only a small number of these cases.<sup>1</sup> Although arrhythmias are uncommon during pregnancy, they may jeopardize the health of both mother and fetus. Ventricular tachyarrhythmia may be triggered during pregnancy as a result of hemodynamic changes and autonomic nervous system modification.<sup>2,3</sup> Recurrence of malignant ventricular arrhythmias can be treated by defibrillation and anti-tachycardia pacing (ATP) to prevent sudden cardiac arrest.<sup>4</sup> An implantable cardioverter-defibrillator (ICD) improves survival in patients with life-threatening arrhythmias.<sup>5</sup> The number of women with congenital heart disease continues to increase and the use of an ICD has resulted in an increasing number of these women reaching a reproductive age.<sup>6</sup> Natale et al performed a multicenter retrospective analysis of 44 pregnant women with ICDs and found that the majority completed and tolerated pregnancy without serious

complications.<sup>7</sup> There are few studies, however, of pregnancy with an ICD managed at a single center and it remains unclear how to manage pregnant women with ICDs. The aim of this study was to investigate the maternal and fetal outcomes in these patients during pregnancy and after delivery.

### Editorial p1146

### Methods

#### Study Design

The subjects were all pregnant women with an implanted ICD who delivered at the National Cerebral and Cardiovascular Center. Data were retrospectively collected for age at the time of initial ICD implantation and delivery; heart disease and arrhythmia; New York Heart Association class; anti-arrhythmic medications and other anti-arrhythmic treatment; indication

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Table 1. Baseline Pre-Pregnancy Patient Characteristics

Patient	Heart disease	NYHA class	Age at ICD implantation (years)	LVEF at ICD implantation (%)	No. ICD shocks	Anti-arrhythmic medication	Other treatment
1	DCM, VT	2	25	37.5	0	Metoprolol	Catheter ablation
2	DCM, VF	2	23	21.3	2	Carvedilol, Mexiletine, Aprindine, Digoxin	
3	CHD <sup>†</sup> , VF	1	30	73.4	0	Mexiletine, Propranolol	
4	SSS, VT, PAF	1	26	62.7	16	Propranolol	PMI (DDD)
5	LQTS type 1	1	14	68.2	3	Atenolol	
6	LQTS type 2	1	26	56.5	0	Propranolol	
Mean±SD			25±6	53±20			

<sup>†</sup>Repair of coarctation of the aorta and patent ductus arteriosus, and aortic valve replacement for congenital bicuspid aortic valve. CHD, congenital heart disease; DCM, dilated cardiomyopathy; DDD, dual-chamber inhibits and triggers; ICD, implantable cardioverter-defibrillator; LQTS, long QT syndrome; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; PAF, paroxysmal atrial fibrillation; PMI, pacemaker implantation; SSS, sick sinus syndrome; VF, ventricular fibrillation; VT, ventricular tachycardia.

for ICD implantation; device information; device-related complications; number of ICD discharges and shocks; gestational age at delivery; mode of delivery; total blood loss at delivery; device status at time of delivery; and fetal and neonatal complications.

Data for maternal age, gestational age, left ventricular ejection fraction (LVEF), total blood loss during cesarean section, birth weight, and follow-up period are given as mean±SD.

### Device Implantation

All ICDs were implanted via transvenous placement of a ventricular lead for defibrillation and pacing using standard techniques under fluoroscopic guidance. Pacing, sensing and defibrillation thresholds were tested during implantation. The devices used were manufactured by Medtronic (Minneapolis, MN, USA), Guidant (St Paul, MN, USA), and Boston Scientific (Natick, MA, USA).

### Management of Pregnancy and Delivery

Fetal growth restriction was defined as an estimated fetal body weight <−1.5 SD of the Japanese standard value. Non-reassuring fetal status was diagnosed by cardiotocogram. Induction and augmentation of labor was performed according to obstetric or maternal indications using i.v. oxytocin following mechanical cervical dilation. Epidural anesthesia was electively used to minimize hemodynamic changes arising from pain or bearing down during labor and after cesarean section.

## Results

### Baseline Characteristics

Six Japanese women with an ICD who delivered between 2006 and 2012 were enrolled in the study. The mean follow-up after ICD implantation was 5±3 years (range, 2–9 years). The baseline pre-pregnancy characteristics of the 6 patients are given in Table 1. The indication for ICD implantation was secondary prevention in all women.

Patient 1 had dilated cardiomyopathy (DCM) with spontaneous ventricular tachycardia (VT) causing hemodynamic instability, for which catheter ablation was not effective. Patient 2 had DCM with chronic heart failure and repeated ventricular fibrillation (VF) that required cardioversion. Patient 3 had congenital heart disease, including coarctation of the aorta and patent ductus arteriosus that had been repaired at 2 years old. Aortic regurgitation progressed gradually because of a congenital bicuspid aortic valve and the patient had cardiopulmonary

arrest caused by VF at 30 years of age. ICD implantation was performed following aortic valve replacement with a Carpentier-Edwards perimount valve. Patient 4 had sick sinus syndrome with repeated syncope and underwent permanent pacemaker implantation (dual-chamber inhibits and triggers) at 23 years old. This patient had wide QRS tachycardia, and ICD implantation was performed for spontaneous VT causing hemodynamic instability. This patient had experienced 16 ICD shocks in response to VF following paroxysmal atrial fibrillation (PAF) caused by acute pharyngitis. Patient 5 had repeated syncope once a year since 3 years of age and had been diagnosed with long QT syndrome type 1 on genetic testing at 10 years old. After introduction of atenolol at 18 years old, syncope reduced to once every 3 years. The severe long QT syndrome was linked to a double-point mutation in the potassium voltage-gated channel KQT-like subfamily, member 1 in re-testing at 25 years old. Her corrected QT time was 470–500ms. Patient 6 had experienced repeated syncope since 25 years of age and had been diagnosed with long QT syndrome type 2 on genetic testing at 26 years old. Her corrected QT time was 430–470ms.

Patients 1 and 4 had implanted dual-chamber ICDs with DDI pacing. The other 4 patients had implanted single-chamber ICDs with VVI pacing. All devices were programmed for the VF zone and 4 (patients 1–4) were also programmed for the VT zone with ATP such as burst and ramp pacing and cardioversion. Patient 2 had inappropriate ICD shocks due to sinus tachycardia, and the VT zone was used only for sensing before pregnancy. Patient 3 had no inappropriate ICD shocks due to discrimination of supraventricular tachycardia. Patient 4 received propranolol before pregnancy to avoid a recurrence of PAF during pregnancy.

### Pregnancy and Labor

Baseline pregnancy and labor patient characteristics are given in Tables 2,3. There were no device-related complications. In 4 women the number of arrhythmias (patients 1–3, non-sustained VT; patient 4, PAF) increased after the end of the second trimester and anti-arrhythmic medications were gradually increased. During pregnancy, no patient received discharges or shocks from the ICD, and only 1 (patient 1) received ATP at 27 weeks' gestation. After ATP in patient 1, the detection zone was changed from 2 zones (VT 180beats/min with 3 burst ATPs; VF 240beats/min) to 3 zones (VT-1 160beats/min with 3 burst and 3 ramp ATPs; VT-2 180beats/min with 3 burst ATPs; VF 220beats/min).

Labor was induced as planned in 3 cases: 2 (patients 1, 2)

Patient	Age at conception	LVEF in pregnancy (%)	NYHA class	No. ICD shocks	LVEF at delivery (%)	Anti-arrhythmic medications (mg/day)			
						1 <sup>st</sup> trimester	2 <sup>nd</sup> trimester	3 <sup>rd</sup> 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.

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

Blood 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.

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. Other 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  $\beta$ -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  $\beta$ -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.<sup>8,9</sup> In some instances, these changes can trigger maternal cardiac deterioration during pregnancy.<sup>10–13</sup> 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.<sup>14–17</sup> 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.<sup>18</sup> We monitored the ICD settings from before pregnancy to prevent inappropriate ICD discharges due to heart rate increases during pregnancy. In 1 case,  $\beta$ -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.<sup>19</sup> 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.<sup>19</sup> 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.<sup>7</sup> 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.<sup>7,20</sup> 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.<sup>21</sup> These findings, however, are not necessarily applicable to the present cases.

In the present study, 5 patients (83%) were given  $\beta$ -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.<sup>22–25</sup> 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.<sup>25</sup> Although the number of cases is small,  $\beta$ -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.<sup>26</sup> We consider use of  $\beta$ -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  $\beta$ -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  $\alpha$ -smooth muscle actin. The numbers of engrafted cells and differentiated cells were very low after both types of MSC sheet transplantation. CD3<sup>+</sup> T cells did not increase in the transplantation site, but CD163<sup>+</sup> 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)

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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 ( $3.3 \times 10^6$  cells) or BM-MSCs derived from GFP-transgenic Lewis rats ( $3.3 \times 10^6$  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>).

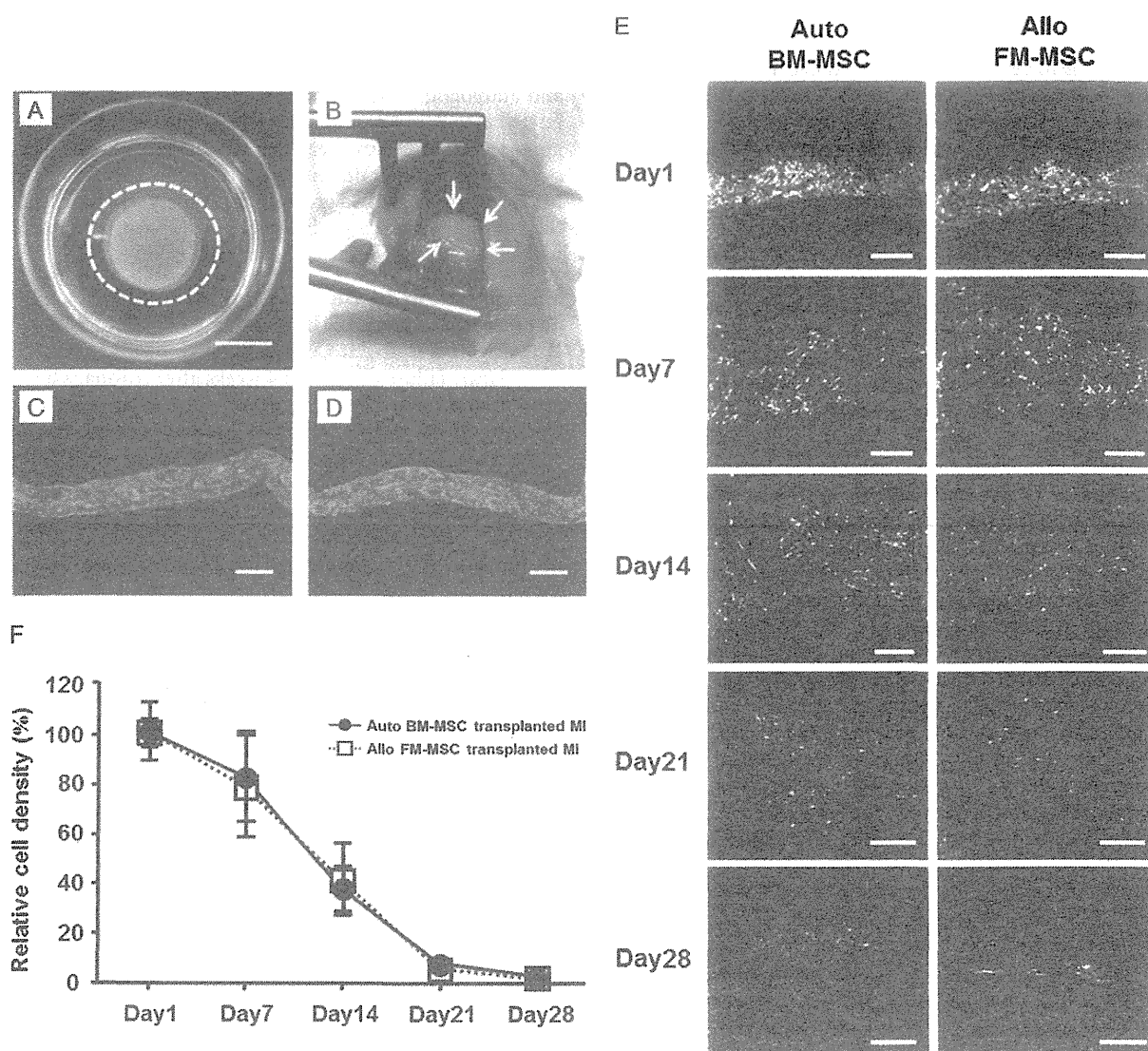
The hemodynamic and echocardiographic parameters did not differ significantly between the MI groups transplanted with allogeneic FM-MSC and autologous BM-MSC.

### 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  $\mu$ m. D, cross-sectional staining of a GFP-expressing two-layered BM-MSC sheet. Scale bar, 100  $\mu$ m. 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  $\mu$ m. 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 $\pm$ SE.

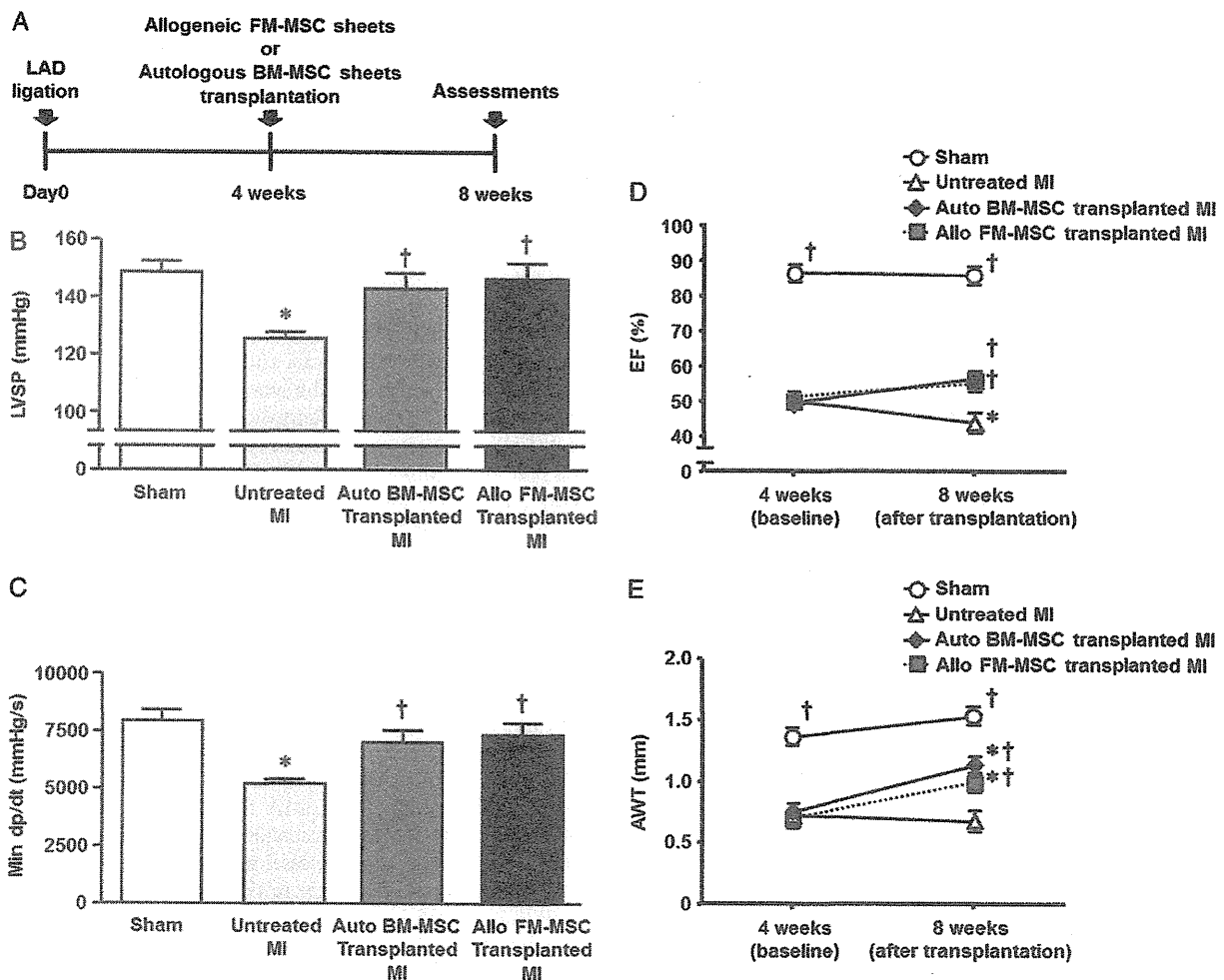
BM-MSC-transplanted MI group (Fig. 4B). Quantitative analysis showed increased capillary density in the infarcted area in both MSC-transplanted groups compared with the untreated MI group ( $n=6$  in each group) (Fig. 4C). The capillary density in the peri-infarct area was similar in the MI groups transplanted 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/ $\alpha$ -smooth muscle actin ( $\alpha$ SMA) 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  $\alpha$ SMA (Fig. 4E,F). The GFP-lectin I/ $\alpha$ SMA 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  $dp/dt$  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 $\pm$ 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 $\pm$ 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.

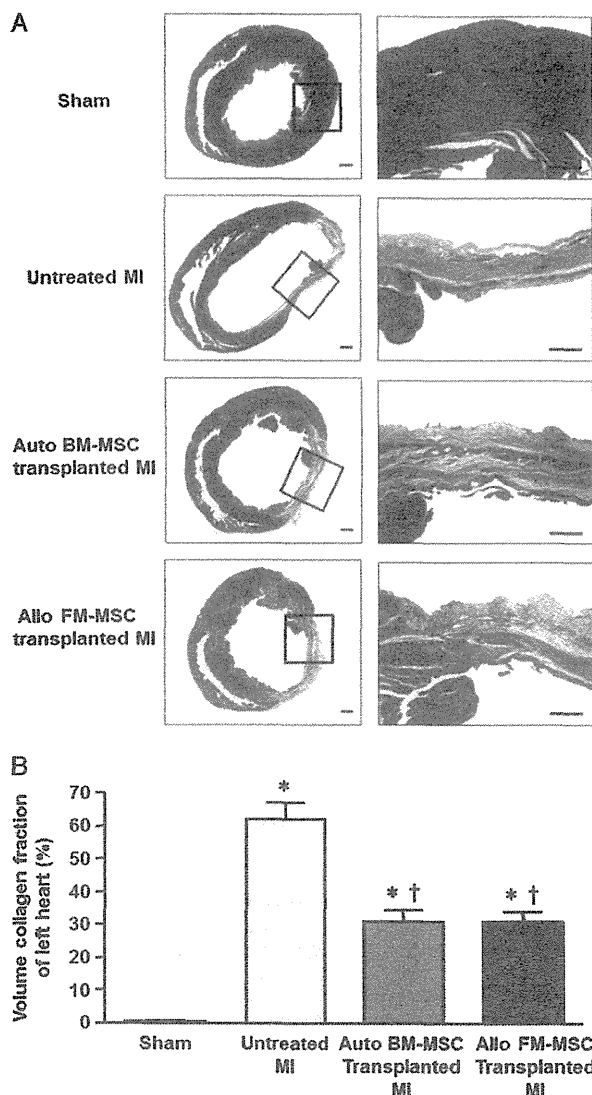
(monocytes and macrophages) in sections of MSC-transplanted infarcted hearts 4 weeks after transplantation. Compared with the sham group, the numbers of CD3<sup>+</sup> and CD68<sup>+</sup> cells in the infarcted and peri-infarct areas were increased in the untreated MI group and in the MI groups transplanted with allogeneic FM-MSC or autologous BM-MSC (Fig. 5A,D). Quantitative analysis demonstrated no significant differences in CD3<sup>+</sup> 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<sup>+</sup> 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<sup>+</sup> 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<sup>+</sup> cells were observed in the serial sections of the sites infiltrated by CD68<sup>+</sup> 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  $\mu$ m (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 $\pm$ SE. \* $P<0.05$  vs. sham group; † $P<0.05$  vs. untreated MI group.

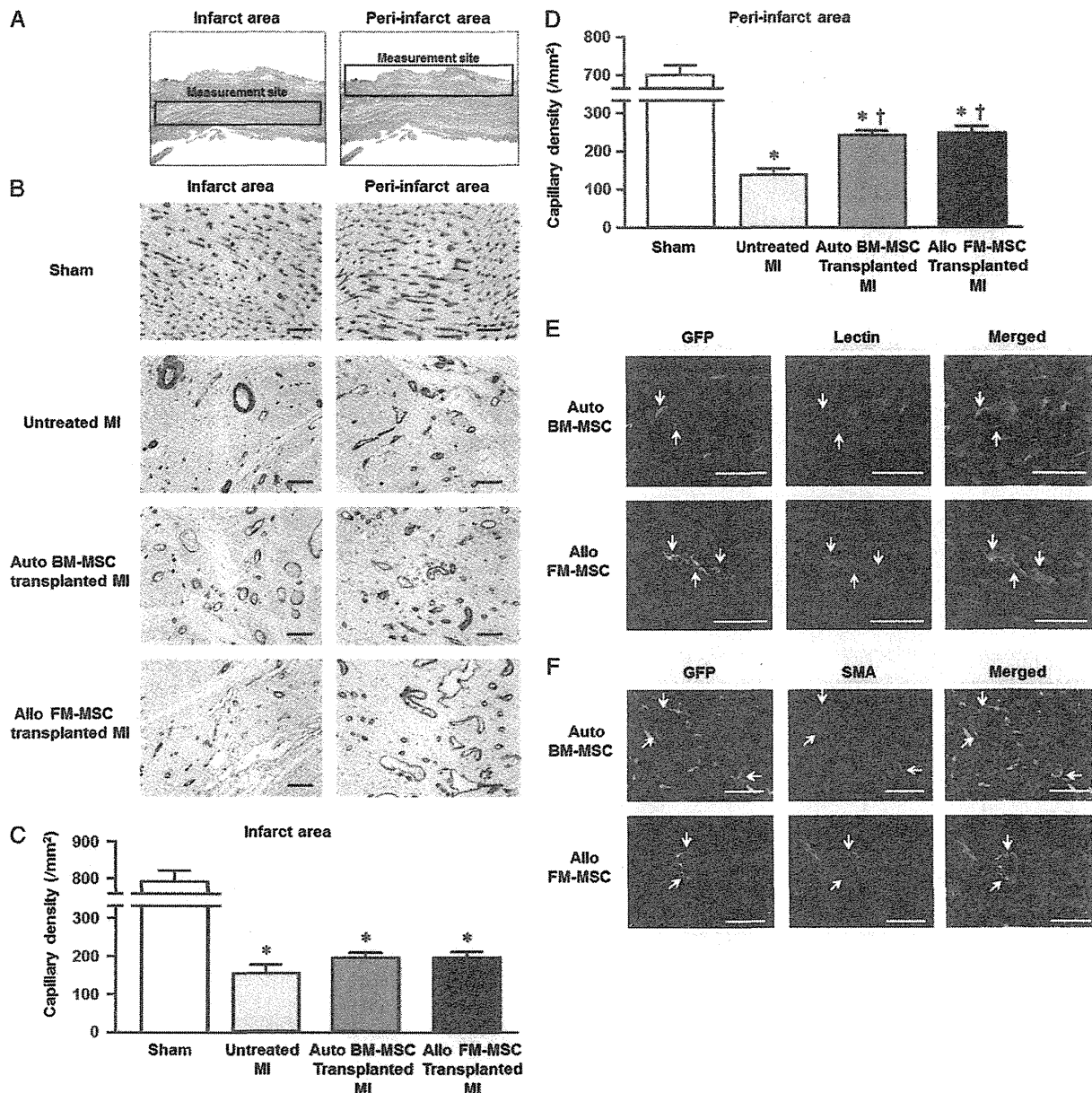
of the infarcted myocardium. Third, transplanted allogeneic FM-MSCs engrafted in the infarcted myocardium from 1 day to 4 weeks after transplantation, but the number of engrafted cells decreased markedly with time. Fourth, some of the engrafted FM-MSCs were positive for lectin I or  $\alpha$ SMA, but 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  $\alpha$ SMA 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).

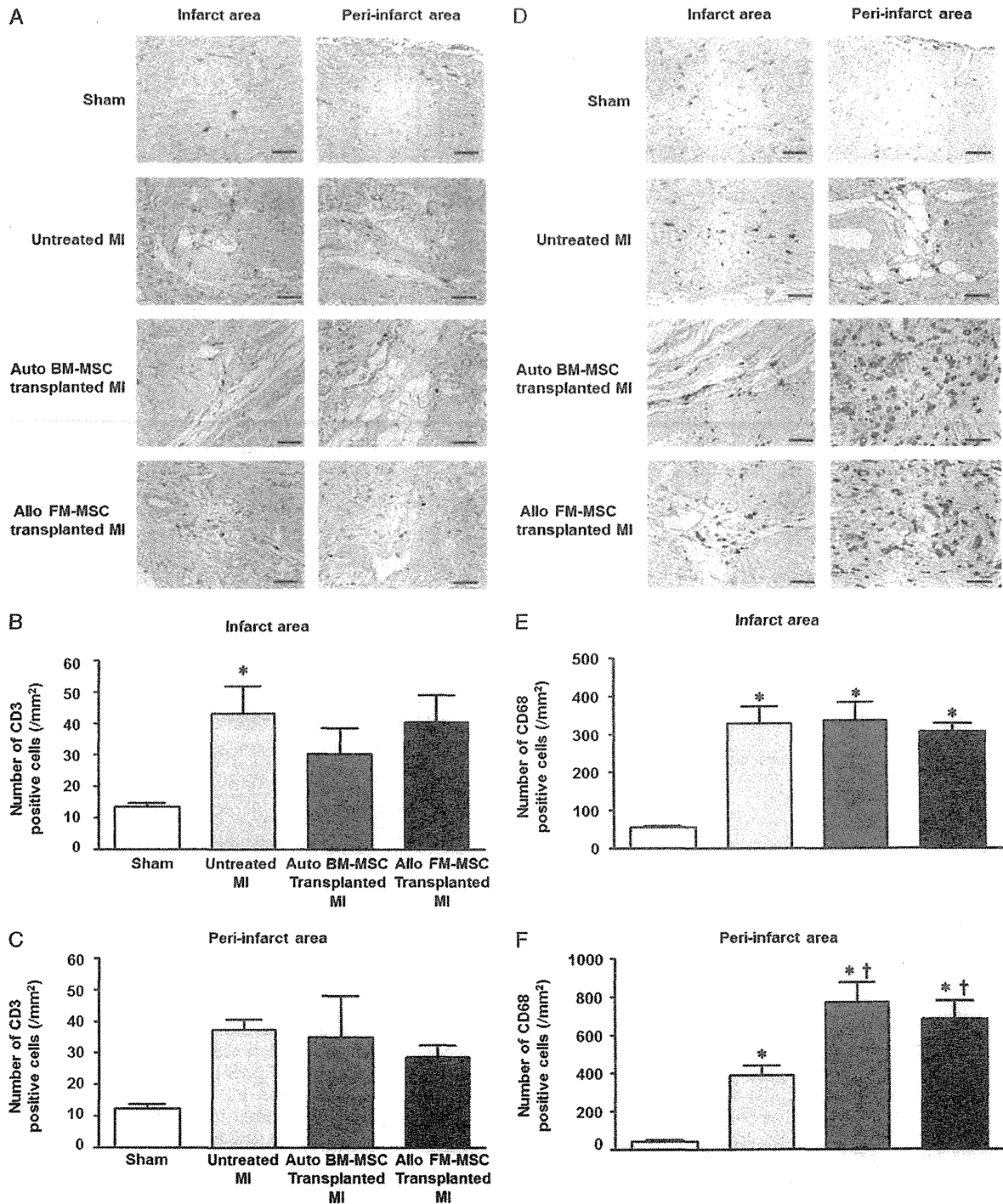
After the discovery of the paracrine effect of MSCs, many studies have confirmed that the success of stem cell therapy for heart failure depends on this mechanism mainly by the promotion of angiogenesis, myocardial protection, and immune regulation (31, 32). In our previous study, transplanted FM-MSCs 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  $\alpha$ SMA (red). Nuclei are stained with TOPRO3 (blue). Scale bar, 50  $\mu$ m. Data are expressed as mean $\pm$ 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<sup>+</sup> 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<sup>+</sup> cells did not differ between the untreated MI group and MI groups transplanted with allogeneic FM-MSC and autologous BM-MSC (yellow arrows: CD3<sup>+</sup> cells). B and C, quantitative analysis of CD3<sup>+</sup> 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<sup>+</sup> cells did not differ between the three MI groups, but marked CD68<sup>+</sup> 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<sup>+</sup> cells in the infarcted area showed no significant difference between the three MI groups. In the peri-infarct area, significantly more CD68<sup>+</sup> 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<sup>+</sup> 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<sup>+</sup> and CD8<sup>+</sup> T cells and the degree of CD3<sup>+</sup> 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<sup>+</sup> 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<sup>+</sup> 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<sup>+</sup> 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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