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.

Acknowledgments

We are indebted to the medical sonographers at the National Cerebral and Cardiovascular Center for their important contributions to the study.

Disclosure

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

Sources of Financial Support

Dr Miyoshi was supported by the Intramural Research Fund (24-6-7) for Cardiovascular Disease of the National Cerebral and Cardiovascular Center. Dr Shimizu was supported in part by a Research Grant for Cardiovascular Diseases (22-4-7, H24-033) from the Ministry of Health, Labour and Welfare, Japan, and a Grant-in-Aid for Scientific Research on Innovative Areas (22136011).

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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

This work was supported by a Research Grant for Cardiovascular Disease (18C-1) of Japan.
The authors declare no conflicts of interest.

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S.I. participated in research design, performance of the research, data analysis, and writing of the article. H.H. and T.I. participated in research design, performance of the research, and writing of the article. K.Y., K.M., K.I., M.F., M.M., and K.K. participated in the performance of the research and contributed new reagents or analytic tools. Y.A. and J.K. participated in the performance of the research and data analysis.
Supplemental digital content (SDC) is available for this article. Direct URL citations appear in the printed text, and links to the digital files are provided in the HTML text of this article on the journal's Web site (www.transplantjournal.com).
Received 13 November 2012. Revision requested 8 May 2013.
Accepted 6 June 2013.

ISSN: 0041-1337/13/9608-697
DOI: 10.1097/TP.0b013e31829f753d

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

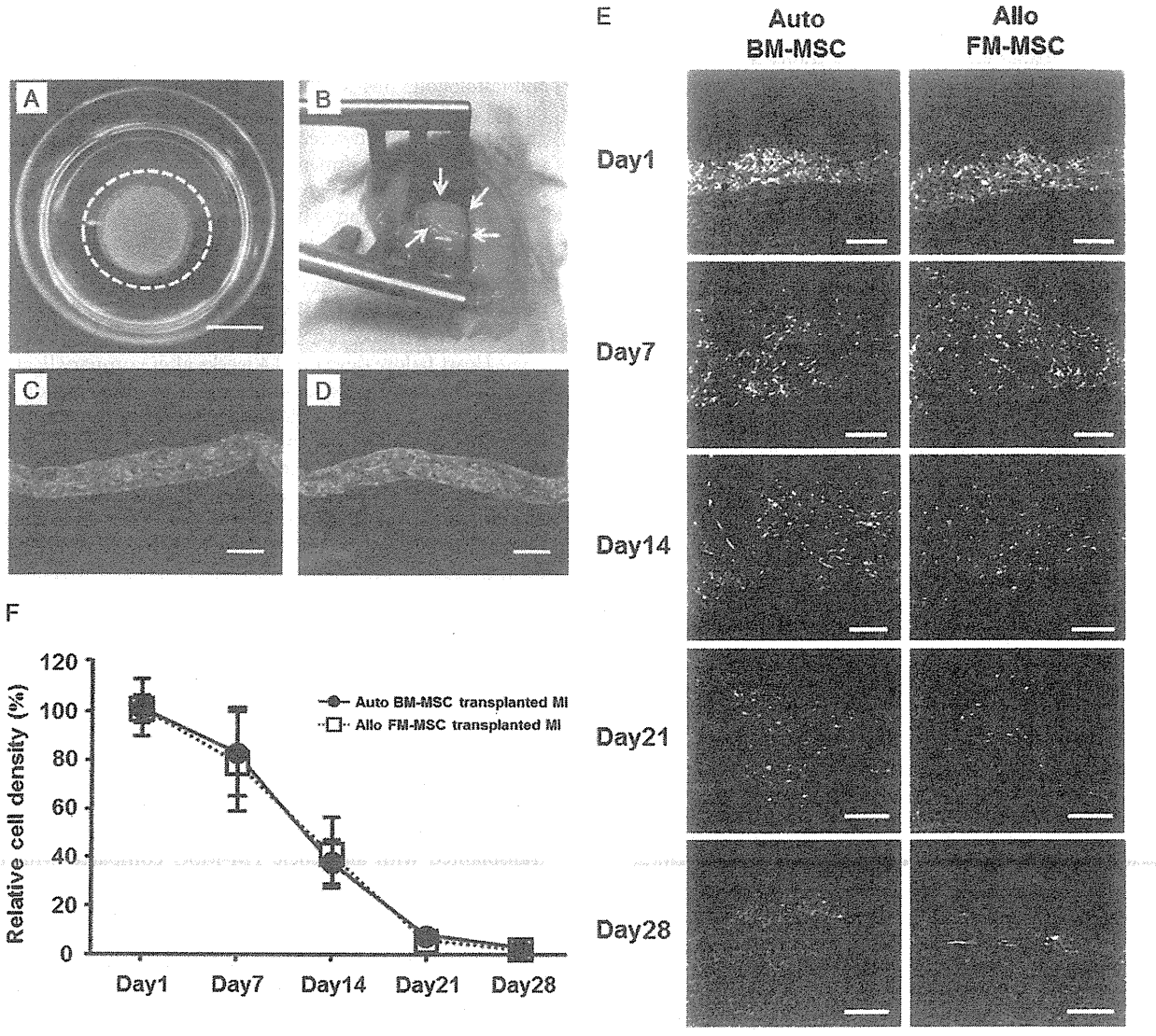

FIGURE 1. Stacked MSC sheets and their transplantation into infarcted hearts. A, two MSC sheets harvested from temperature-responsive culture surfaces were stacked successfully, producing a two-layer construct. Scale bar, 10 mm. B, two-layered MSC sheets were transplanted over the anterior wall of the 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

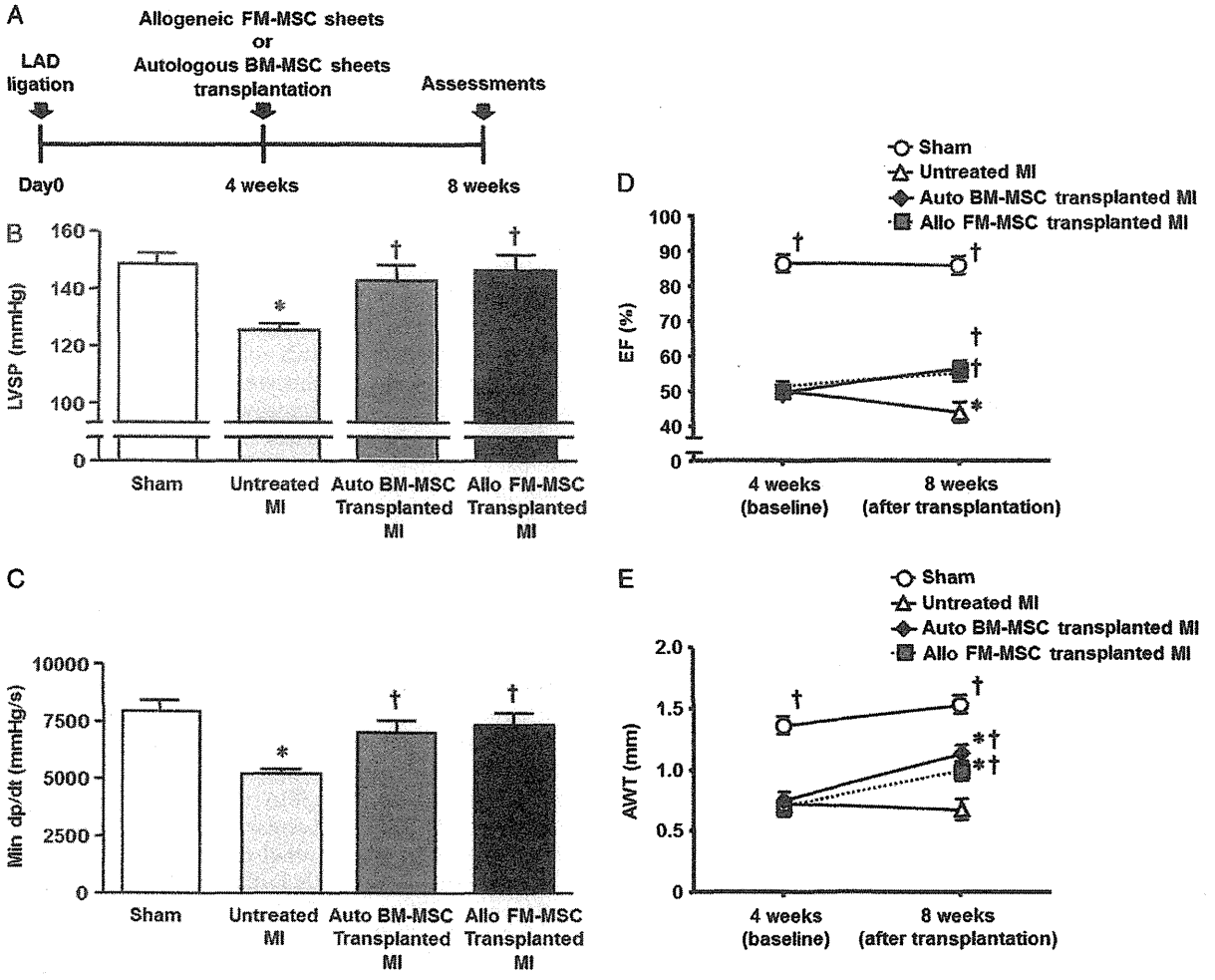

FIGURE 2. Effects of transplantation of allogeneic FM-MSC and autologous BM-MSC sheets on hemodynamic and echocardiographic parameters after MI. A, study flowchart. B and C, four weeks after transplantation, left ventricular systolic pressure and minimum d*P*/d*t* had improved significantly in the MI 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

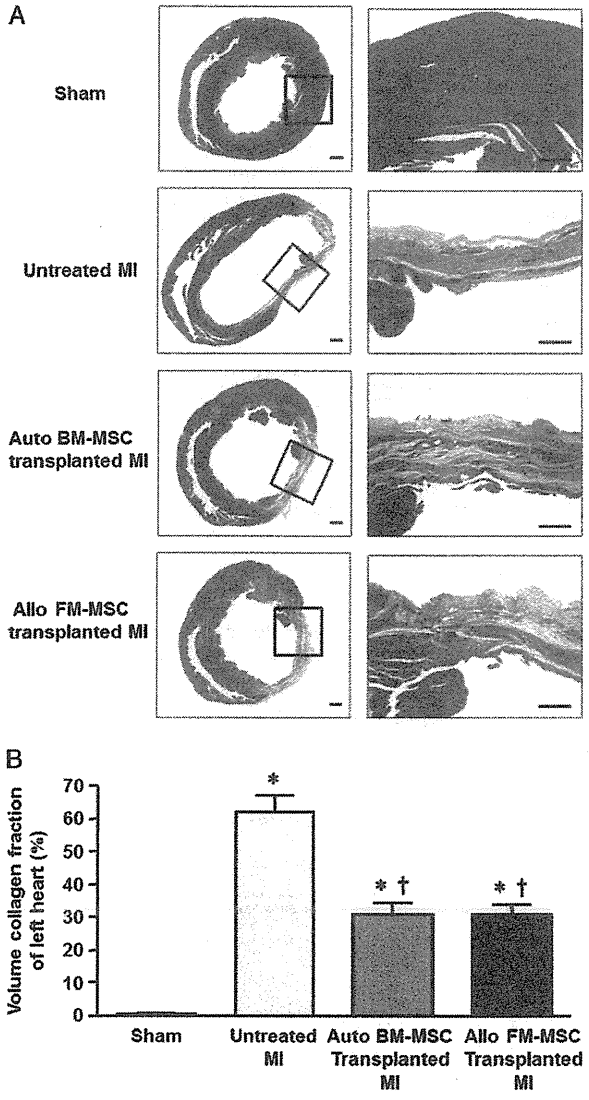

FIGURE 3. Masson's trichrome staining of heart cross-sections at the left ventricular papillary muscle level from MI rats transplanted with allogeneic FM-MSC and autologous BM-MSC sheets. A, four weeks after transplantation, the myocardial fibrosis area was smaller in the MI groups transplanted with 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.

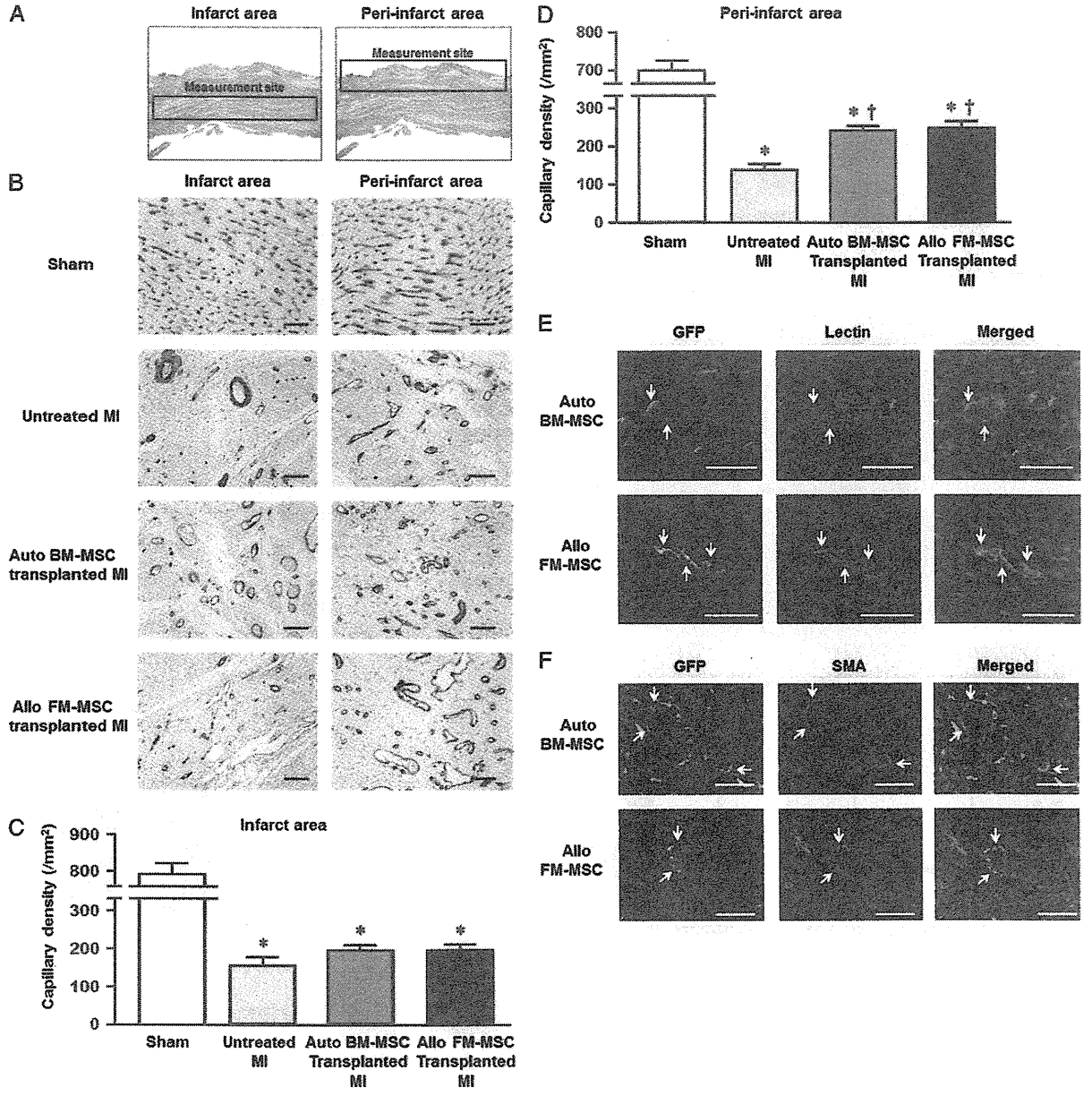

FIGURE 4. Vascularization and differentiation into vascular endothelial cells in the myocardial tissue grafted with allogeneic FM-MSC and autologous BM-MSC sheets. A, representative measurement section sites. B, four weeks after transplantation, the numbers of lectin I–positive capillaries were greater 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

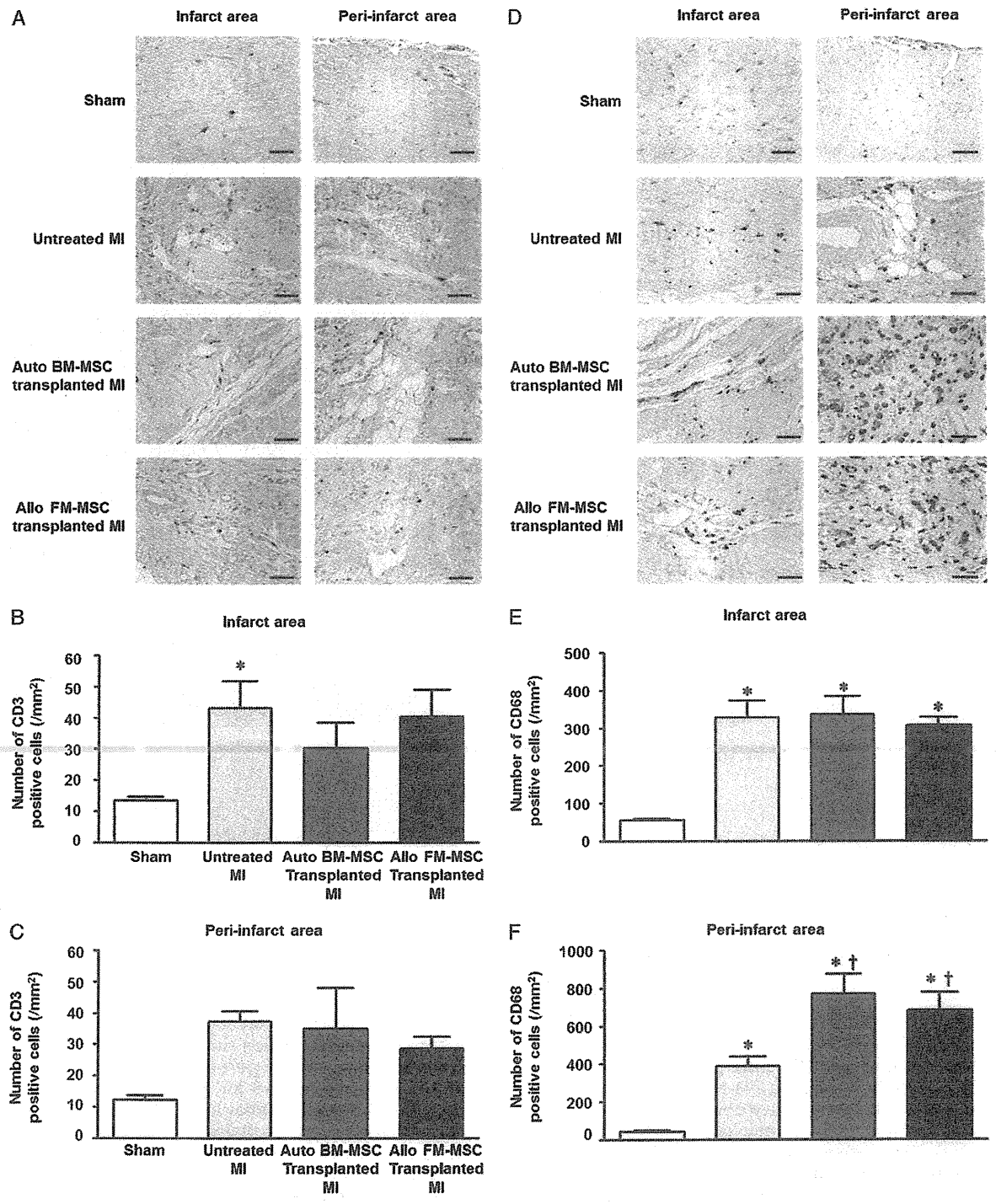

FIGURE 5. Immune responses in the myocardium transplanted with MSC sheets. A, in the infarcted and peri-infarct areas, the numbers of infiltrating $CD3^+$ cells did not differ between the untreated MI group and MI groups transplanted with allogeneic FM-MSC and autologous BM-MSC (yellow arrows: $CD3^+$ 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 $P<0.05$.

ACKNOWLEDGMENTS

The authors are grateful to the National BioResource Project for the Rat in Japan (http://www.anim.med.kyoto-u.ac.jp/NBR/) for providing rat strain LEW-TgN(CAG-EGFP)1Ys.

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C1 Esterase Inhibitor Activity in Amniotic Fluid Embolism

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AQ3 **Introduction:** Amniotic fluid embolism exhibits activation of the complement system and the kallikrein-kinin and coagulofibrinolytic systems. C1 esterase inhibitor is a major inhibitor of C1 esterase and can inhibit plasma kallikrein and also factors XIIa and XIa. Its activity has been shown to be significantly lower in pregnancy and labor than in the nonpregnant state. The purpose of this study was to determine C1 esterase inhibitor activity levels in amniotic fluid embolism.

Methods: This study was retrospectively conducted on 194 singleton pregnant women. One hundred six cases of amniotic fluid embolism had applied to the Japan amniotic fluid embolism registration center in Hamamatsu University School of Medicine between January 2010 and December 2011. In amniotic fluid embolism cases, 85 cases were nonfatal and 21 cases were fatal. Eighty-eight women who delivered without amniotic fluid embolism were regarded as a control. C1 esterase inhibitor activity levels at the onset of amniotic fluid embolism in amniotic fluid embolism cases and at the completion of labor in control cases were measured and compared using the Mann-Whitney *U* test.

Results: C1 esterase inhibitor activity levels were significantly lower in amniotic fluid embolism patients (30.0% ± 1.8%) than in control women (62.0% ± 2.0%) ($p < 0.0001$). C1 esterase inhibitor activity levels in fatal amniotic fluid embolism cases (22.5% ± 3.4%) were significantly lower than those in nonfatal amniotic fluid embolism cases (32.0% ± 2.1%) ($p < 0.05$).

Conclusions: These results demonstrated that low C1 esterase inhibitor activity levels were closely associated with the pathogenesis of amniotic fluid embolism suggesting that C1 esterase inhibitor activity levels have potential as a prognosis factor of amniotic fluid embolism. (*Crit Care Med* 2014; XX:00–00)

Key Words: amniotic fluid embolism; C1 esterase inhibitor; disseminated intravascular coagulopathy; kallikrein; postpartum hemorrhage; serpin

Amniotic fluid embolism (AFE) is one of the most serious complications of obstetrics, anesthetics, and critical care. Despite earlier recognition and intensive critical care, the mortality of AFE remains high and has been estimated at between 5% and 15% of all maternal deaths (1). Maternal mortality rates due to AFE have been estimated at between 37% and 80% (2, 3). Maternal death has been decreasing year by year in Japan; however, the prevalence of maternal death due to AFE has remained unchanged. The maternal mortality rate due to AFE has increased to 24.3% in Japan (4). AQ4

AFE is recognized as a kind of syndrome characterized by the abrupt onset of hypoxia, hypotension, and disseminated intravascular coagulopathy (DIC) (5). Benson et al (6) reported that maternal complement levels were significantly decreased in AFE. These findings suggested a disorder in the coagulofibrinolytic system as well as the complement system that may play important roles in the pathogenesis of AFE.

We developed the Japan AFE registration system in 2003 and collected clinical data, maternal serum, and uterine tissue from nearly all cases of fatal AFE in Japan (4, 7). Under the system, maternal serum has been applied to determine mainly the levels of specific amniotic fluid complements such as Sialyl Tn and zinc coproporphyrin 1 (8, 9). These clinical and histopathological observations demonstrated that AFE was frequently associated with uterine atony due to angioedema (unpublished data). AQ5

C1 esterase inhibitor (C1INH), belonging to the serpin group/family, is a major inhibitor not only of C1 esterase but also of kallikrein and factors XIIa and XIa (10–12). Its deficiency has been known to be a direct cause of hereditary angioedema (HAE) as well as acquired angioedema (13). Since

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AQ2 Dr. Kanayama's institution received grant support from JSPS KAKENHI (grant number: 24390379). The remaining authors have disclosed that they do not have any potential conflicts of interest.

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DOI: 10.1097/CCM.0000000000000217

<DOI:10.1097/CCM.0000000000000217>

C1INH has the potential to regulate the coagulofibrinolytic system, complement system, and kallikrein-kinin system, we have become greatly interested in C1INH activity levels in AFE in Japan.

MATERIALS AND METHODS

Definition of AFE

AFE was defined based on the Japan consensus criteria for the diagnosis of AFE based on the United States/United Kingdom criteria as shown in **Figure 1** (7, 14). A pathological diagnosis was determined when fetal debris was found in the maternal pulmonary arteries. The diagnosis of nonfatal AFE depended on clinical manifestations and was done when factors B1–B3 were all present, but more than one of the signs and symptoms listed in B1 needed to be present. Consumptive coagulopathy/ DIC due to evident etiologies such as abnormal placentation (placental abruption, etc.), trauma during labor and delivery, and severe preeclampsia/eclampsia should be excluded from the criteria.

Patients

The Japan AFE registration system was started in 2003 (7). This system has included the procedure of consent to apply and analyze their clinical data and blood samples. Consent was obtained from patients or patient's family, when physicians regarded their patients with significant symptoms as AFE based on the diagnostic criteria of AFE. Clinical data and serum from nearly all cases of AFE have accumulated in Hamamatsu University School of Medicine. Subjects of the present study were extracted from entry cases in the Japan AFE registration center in Hamamatsu University School of Medicine, Shizuoka, between January 2010 and December 2011. Women with multiple pregnancies, preeclampsia, thrombophilia, preterm labor, uterine disorder such as uterine myoma, and a history of systemic disease were excluded from this study. Cesarean section was carried out due to obstetrical indications, such as breech presentation, history of cesarean section, arrest of labor, and nonreassuring fetal status. Women, who delivered at Hamamatsu University Hospital between April 2011 and September 2011, without AFE and any medical intervention other than general birth and surgical assistances were analyzed as the control subjects. One hundred six cases of AFE and 88 cases of control were defined (**Table 1**). Among the AFE cases, 85 cases survived and 21 cases died due to AFE.

The Japan consensus criteria for the diagnosis of AFE

A. Pathological confirmation; A diagnosis is made on the basis of clinical presentation after excluding differential diagnosis and at autopsy in the event of death of the parturient. The diagnosis is confirmed by histochemical studies.

B. Clinical manifestation; The patients has the hallmark clinical manifestations of AFE following 1, 2, and 3:
1. Signs and symptoms: Cardiac arrest/ Respiratory arrest/ Consumptive coagulopathy
2. Onset of all of the signs and symptoms during pregnancy, labor, or cesarean section or within 12 hours of delivery
3. Absence of other illness that could explain the signs and symptoms described above

Figure 1. The Japan consensus criteria for the diagnosis of amniotic fluid embolism (AFE). A pathological diagnosis was determined when fetal debris was found in the maternal pulmonary arteries. The diagnosis of nonfatal AFE depended on clinical manifestations and was done when factors B1–B3 were all present, but more than one of the signs and symptoms listed in B1 needed to be present.

Blood Collection and Measurement of C1INH Activity

Blood samples from registered AFE patients were collected at the Japan AFE registration center in Hamamatsu, and serum and plasma samples were then kept at –30°C until use. Time points of blood samples obtained were at onset of and before interventions against AFE. Control blood samples were obtained at the completion of labor. The determination of C1INH activity was performed using the Berichrom C1 inhibitor kit (Siemens Healthcare Diagnostics) according to the manufacturer's instructions. The intra-assay coefficients of variation (CV) ranged between 1.8% and 7.9% and the interassay CV were between 3.2% and 6.6%. We analyzed all the samples at the same time under a blind fashion. In the present study, we demonstrated the measurement of C1INH activity in serum. Furthermore, C1INH activity was measurable in serum as well as plasma; there were no significant differences ($p < 0.0001$, $R^2 = 0.9881$) in the activity level between serum and plasma under the Berichrom C1 inhibitor kit (data not shown).

Approval

Written informed consent was obtained after full explanation of the study. The study was carried out under the approval of the Ethics Committee of Hamamatsu University School of Medicine (Number 24–130 and 25–107), which conforms to the provisions of the Declaration of Helsinki (as revised in Tokyo 2004).

Data Analysis

Values of C1INH activity (%) were presented as the median ± SE. Significant differences were assessed with the Mann-Whitney *U* test. A *p* value of less than 0.05 was considered significant.

RESULTS

As shown in **Figure 2**, C1INH activity levels in the controls and AFE cases were 62.0% ± 2.0% and 30.0% ± 1.8%, respectively. C1INH activity levels in the AFE cases were significantly lower than those in the controls ($p < 0.0001$). C1INH activity levels in fatal and nonfatal AFE cases were 22.5% ± 3.4% and 32.0% ± 2.1%, respectively. A significant difference was observed between the two groups ($p = 0.0121$).

Changes in C1INH activity levels in one survivor case and one case that died due to AFE are shown in **Figure 3**. Both cases were defined as AFE by the Japan consensus criteria for the diagnosis of AFE shown in Figure 1. C1INH activity levels were potentially very low before the onset of AFE. C1INH activity in the survivor case was at its lowest level at the onset 3 hours after the selective cesarean section due to history of cesarean section when AFE was defined due to the development of DIC. Immediate replacement therapy with FFP successfully increased the activity of C1INH. In the case that died,

AQ6 **TABLE 1. XXX**

AQ7

	Control	Total AFE	Nonfatal AFE	Fatal AFE
No. of subjects	88	106	85	21
Age (yr)	31.0±4.8	33.8±5.8	33.3±5.4	35.6±3.8
Gravida[a]	1.27±1.02	1.64±1.77	1.74±1.82	1.23±1.47
Parity[a]	0.72±0.63	0.83±1.06	0.89±1.12	0.57±0.72
Nulliparous (%)	28 (31.8)	52 (49.0)	41 (48.3)	11 (52.4)
Multiparous (%)	60 (68.2)	54 (51.0)	44 (51.7)	10 (47.6)
Gestational period (d)	273±12	268±19	267±20	270±17
Delivery methods				
Vaginal delivery (%)	60 (68.2)	52 (49.0)	44 (51.7)	10 (47.6)
Cesarean section (%)	28 (31.8)	54 (51.0)	41 (48.3)	11 (52.4)
Blood loss at delivery (mL)				
Vaginal delivery	395±170	4,864±3,039	5,038±3,111	4,097±2,569
Cesarean section	840±279	4,270±2,988	4,314±2,657	4,107±3,961

AFE = amniotic fluid embolism.
[a]Woman without previous history of pregnancy and delivery was determined as gravida 0 and parity 0, respectively.

C1INH activity was also low before the manifestation of AFE symptoms. In this case, amniotic fluid, fetal substance, and gram-positive coccus were observed and autopsy diagnosis was AFE and bacteremia.

DISCUSSION

AFE is an unpredictable and serious disorder of pregnancy characterized by hypotension, hypoxia, and coagulopathy (5). In most pregnant women, the entry of small amounts of amniotic fluid into the maternal circulation may be innocuous; however, such exposure is associated with a fatal outcome in other women. Anaphylactic reactions have been suggested as a concept of AFE to explain such an individual difference in the response to amniotic fluid (2, 15). Benson (6, 16) reported that serum tryptase and urinary histamine increased and complement levels decreased in AFE patients, suggesting that contact and maternal immune activation played important roles in the pathophysiology of AFE.

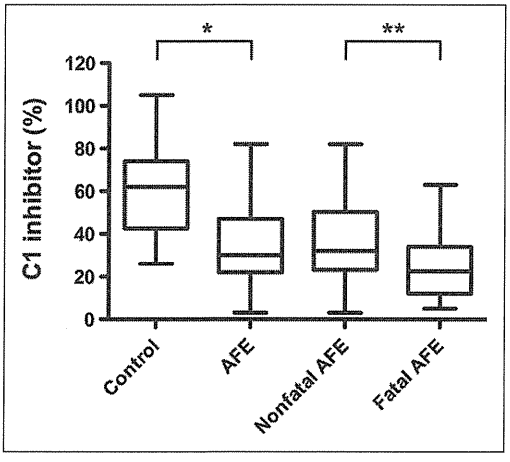

Figure 2. C1 esterase inhibitor (C1INH) activity levels in control, amniotic fluid embolism (AFE), nonfatal AFE, and fatal AFE cases. Columns indicate the medians and whiskers represent the minimum and maximum values. Significant differences were $^{*}p < 0.0001$ and $^{**}P = 0.0121$, respectively.

Clinically, DIC-type postpartum hemorrhage accompanying uterine atony is one of the recognized symptoms of AFE (4, 17). To explain this, coagulation factor XII (FXII) may be responsible for the pathological condition as it is activated by contact with various artificial or biological negatively charged surfaces, resulting not only in blood coagulation but also in the activation of the complement system and kallikrein-kinin system to produce bradykinin (18). We demonstrated that FXII inactivated plasminogen activator inhibitor 1 and enhanced fibrinolysis (19). Interestingly, bradykinin has strong vasodilation effects, a hypotensive effect, and causes an increase in vascular permeability resulting in a hypotonic uterus (20, 21). These findings suggest that FXII activation by contact triggers the subsequent catastrophic chain of AFE. We are continuing to investigate the possible role of FXII in AFE.

C1INH, which is mainly synthesized in hepatocytes and endothelial cells and belongs to serpin family, is a major inhibitor of not only C1 esterase but also FXIIa and kallikrein (11, 12). Its deficiency is known to be a specific cause of HAE (13). Since C1INH is capable of not only inhibiting the complement system but also modulating the coagulofibrinolytic and kallikrein-kinin systems (22, 23), we hypothesized that C1INH was key in the pathophysiology of AFE.

Halbmayer et al (24) reported that basal C1INH activity levels decreased markedly with pregnancy up to labor. Although the mechanism remains unclear, estradiol (E2) was shown to suppress the potential activity of C1INH (25, 26).

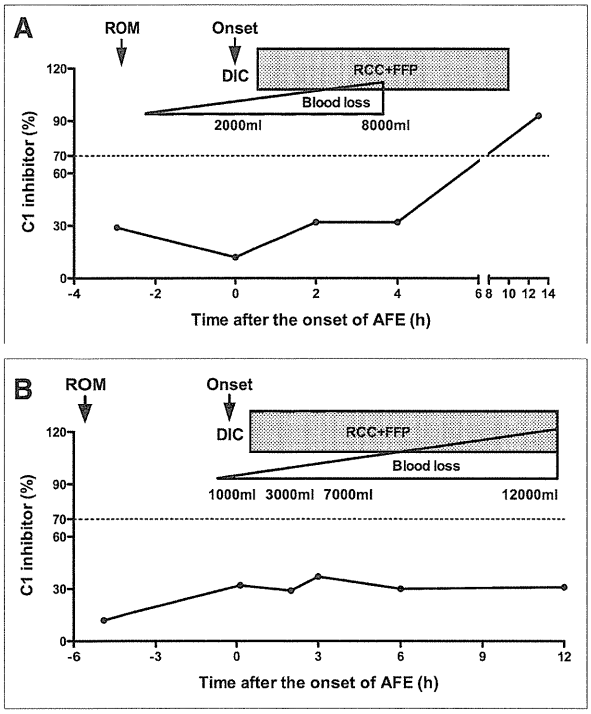

Figure 3. Chronological changes in C1 esterase inhibitor (C1INH) activity levels in amniotic fluid embolism (AFE) cases. A survivor of AFE (**A**) and a case that died due to the coexistence of AFE with bacteremia (**B**). Potential C1INH activity levels were low before the onset of AFE. In case A, the sudden onset of AFE presented disseminated intravascular coagulopathy (DIC) 3 hr after rupture of membrane (ROM) under a selective cesarean section. When AFE was recognized as abnormal coagulopathy, blood replacement therapy was immediately administrated resulting in an increase in C1INH activity levels. In case B, despite intensive care including adequate blood transfusions, C1INH activity levels did not recover. A *dotted line* indicates 70% of C1INH activity. Normal C1INH activity is more than 70%. RCC = , FFP = .

The increase in E2 levels during pregnancy may be associated with the decrease in C1INH activity levels in pregnant women. They also observed that C1INH activity levels were significantly lower in preeclampsia patients than in normal pregnant women (24). Increases in C3a and C5a have been reported not only in AFE patients but also in patients with preeclampsia and eclampsia (27), which suggests that the consumption of C1INH is due to activation of the complement system. This may explain the high risk of AFE associated with preeclampsia and eclampsia as risk factors of AFE (28, 29).

The present study demonstrated that C1INH activity levels in AFE cases were significantly lower than those of controls. Furthermore, when we compared fatal cases to nonfatal cases using Pearson chi-square test for C1INH activity less than 25% as a cutoff value almost comparable to "attack of angioedema," there was a significant difference with p equal to 0.026 (degree of freedom 1 and chi-square value 4.956). In addition, the chronological assessment of C1INH activity levels in two AFE patients indicated that their basal C1INH activity levels before delivery and onsets of AFE were also lower at 29% and 12% than that of healthy pregnant controls at 74.3% ± 15.5% during the third trimester (24). These results suggest that low C1INH activity levels before onset of AFE could be a predictive factor as well as low levels at onset and the persisting low levels of C1INH activity could be a prognostic factor of AFE.

It has been reported that the levels of C1INH may be increased during infection as an acute phase protein, then cleaved and inactivated by neutrophil elastase and bacterial proteases under developing inflammatory conditions due to bacteremia and sepsis resulting in a functional C1INH deficiency (30–32). As demonstrated in the fatal case in **Figure 3B**, we should note here that not only C1INH consumption under AFE condition but also C1INH inactivation under inflammatory conditions due to bacteremia may be involved in the persistent low levels of C1INH activity (32).

As a treatment for DIC with AFE, the rapid administration of FFP or cryoprecipitate was sufficient to extricate the patient from a critical situation. FFP contains several essential proteins such as ATIII and fibrinogen. One hundred units of C1INH are contained in FFP derived from 200 mL blood. Our chronological assessment of C1INH activities in the AFE patient shown in **Figure 3A** demonstrated that a suited blood transfusion including FFP was able to improve C1INH activity. Clinically, the use of 500–1,500 U of human plasma–derived C1INH concentrates can revert HAE in C1INH-deficient patients (33–35). Since AFE patients certainly have significant lower levels of C1INH activity, similar to a C1INH deficiency, the clinical application of human plasma–derived C1INH concentrates may become one of the promising candidates for the treatment of AFE.

In summary, we reported here that C1INH activity levels were significantly lower in AFE cases, particularly in fatal cases. These results indicate that C1INH activity levels reflect the severity of AFE and can be a prognostic factor of AFE. We speculate that the clinical application of C1INH concentrates will be effective for the treatment of AFE. Although the chronological measurement of C1INH activity was small, our results suggest that pregnant women with potentially low C1INH activity levels may be at a high risk of the onset of AFE. Further clinical studies are required to elucidate the etiological role of C1INH in AFE and determine whether C1INH activity may be a predictive factor of AFE.

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特集　事例から学ぶ妊産婦死亡の予防対策

日本産婦人科医会による妊産婦死亡報告事業の運用状況

石渡　勇　　関沢明彦

はじめに

周産期医療の特徴は，①同時に母児双方の健康管理が要求されること，②突然の急変が起こりやすいリスクの高い医療であること，③紛争・訴訟が起こりやすい医療であること，等である。

日本産婦人科医会（以下，医会）は平成16年より産婦人科偶発事例報告事業（いわゆる医療事故の届け出制度）を医会会員の協力の下に開始した。その目的は，原因分析と再発防止，医療安全の資料作成と啓発，さらに早期の紛争解決のためである。当初は約半数の報告にとどまっていたが，平成22年1月より，会員の支援を目的に，妊産褥婦死亡症例届け出システムを構築した（妊産婦死亡報告事業）。この事業は，羊水塞栓症の血清検査事業（浜松医科大学産婦人科 金山教授）および妊産婦死亡の調査と評価に関するモデル事業（厚生労働省科学研究 池田班）とも連携し，高い評価を得ている。本稿においては報告事業の運用状況についてまとめてみたい。

妊産婦死亡にかかわる届け出

1. 世界の状況

世界保健機関（WHO）や国連児童基金（ユニセフ）の報告によると，2008年の妊産婦死亡率は推定で出生10万人当たり約260人で，年間約35万8,000人が妊娠や出産に絡み死亡している。地域別ではサハラ以南のアフリカ諸国が最悪で，死亡率は10万人当たり約640人。開発途上国では極端に高い妊産婦死亡で，アフリカの女性が出産時に亡くなる割合は先進国の175倍である[1]。世界各国の報告は数，質ともに十分とはいえない状況である。一方，イギリスではすべての妊産婦死亡の報告を行うことが政府により義務づけられている。Centre for Maternal and Child Enquiries（CMACE）から2011年までに8回のreviewがなされ，recommendationとして発表されている[2]。

2. 厚生労働省人口動態統計

厚生労働省統計局は，毎年，妊産褥婦の死亡統計[3]を報告しているが，母子保健統計は死亡診断書（死亡届は2週間以内）に基づいた統計であり，死因の詳細な検討がなされる前の段階で作成されたものである。したがって，妊産婦の正確な死因の把握は不十分といわざるを得ない。

3. 日本産婦人科医会妊産婦死亡症例届け出システム

医会がシステムを構築した目的は，速やかな会員への支援，医学的な原因究明，事例の再発防止に向けた問題点の抽出，さらに再発防止および医療安全に向けた提言の発信である。その手順については**図1**[4]に示す。妊産婦死亡に遭遇した時，①当該医療機関（医師）は連絡票（**図2**）を医会ホームページからダウンロードするか，偶発事例報告事業・妊産婦死亡報告事業（報告事業概要平成23年版）をコピーして，記載の上，医会および都道府県産婦人科医会にFAX等で連絡する。②医会から当該機関へ調査票（**図3**，**表1**）を送付する。③当該機関は医会に調査票を記述し提出する。④原因分析などで必要な場合には追跡調査を行うこと

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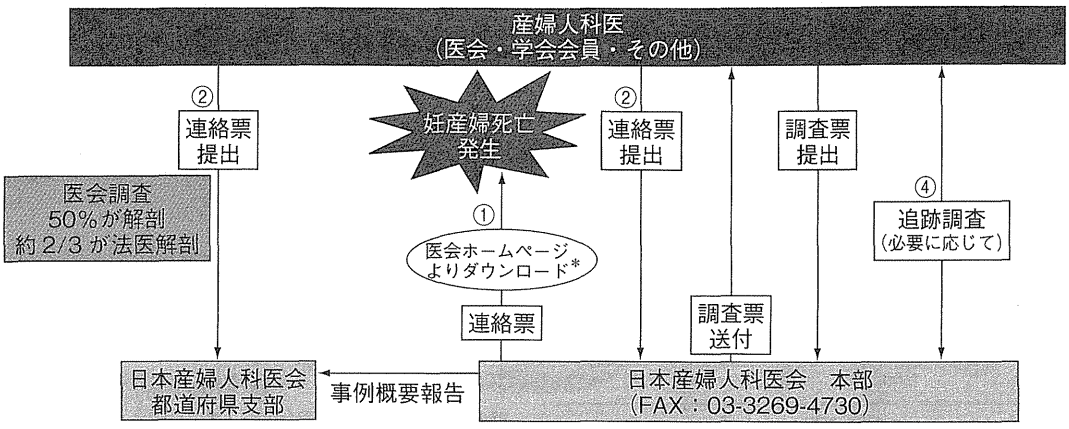

手順：①医会ホームページ（http://www.jaog.or.jp/）から連絡票をダウンロード*，②連絡票を日本産婦人科医会・都道府県産婦人科医会の２カ所にFAXまたは郵送，③医会より送付される調査票に記入して郵送，④必要に応じて送付される追跡調査票に記入して医会に郵送
（注）妊産婦死亡以外の偶発事例報告は，従来通り都道府県産婦人科医会宛に提出してください。

*連絡票は日本産婦人科医会に電話（03-3269-4739）で請求いただければFAXいたします。

図１　妊産婦死亡の届け出の手順（石渡ら，2011）[4]

様式３－１

妊産婦死亡 連絡票

社団法人日本産婦人科医会　殿

妊産婦死亡がありましたので報告いたします。

- 記載された住所に後日 妊産婦死亡調査票を送付いたします。
- 妊産婦死亡があった場合は速やかに本票を日本産婦人科医会および都道府県産婦人科医会までご提出ください。

施設名				
住　所	〒			
電話番号				
E-mail				
担当者 もしくは代表者				
報告日	西暦　　年　　月　　日			
死亡日	西暦　　年　　月　　日			
患者氏名 イニシャル	氏	名	患者年齢	

提出先：FAX：03-3269-4730
郵送先：〒162-0844　新宿区市谷八幡町14　市ヶ谷中央ビル４階

22

図２　妊産婦死亡連絡票

様式３－２－１a

妊産婦死亡 調査票（偶発的妊産婦死亡用）

施設名		事例登録番号	
都道府県		報告担当者・責任者氏名	
妊産婦死亡日	西暦　　年　　月　　日	妊産褥婦イニシャル	（氏）　　（名）
患者年齢	歳	経産回数(妊娠回数)	（　）経妊（　）経産
妊娠時期	（発症時）妊娠中（妊娠　　週）・分娩中（第　　期）・帝王切開中・ 産褥（　月　日　時間）・その他（　　）		
	（死亡時）妊娠　　週　　産褥　　日（妊娠　　週分娩）		
初発症状	初発症状発症場所：施設外・助産施設・有床診療所・産科病院・総合病院・その他（　　） 初回心停止発生場所：施設外・助産施設・有床診療所・産科病院・総合病院・その他（　　） 初発症状：　　初発症状から心停止までの時間：　　時間　　分		
分　娩	分娩施設：施設外・助産施設・有床診療所・産科病院・総合病院・その他（　　） 分娩様式：未分娩・経腟分娩・クリステレル・吸引・鉗子分娩・帝王切開		
死　亡	確認施設：有床診療所・産科病院・総合病院・その他（　　） 確認診療科：救急医学科・脳神経外科・循環器内科・整形外科・その他（　　）		
基礎疾患	なし・あり（　　）		
剖　検	なし・病理解剖・司法解剖・行政解剖・その他（　　）		
臨床診断			

複数の施設で妊産褥婦・新生児の管理が行われた場合にその施設名を記入してください

健診施設	施設名:	所属: □産婦人科　□その他（　　）	
	住所:	担当者名:	
	□助産施設　□無床診療所　□有床診療所　□産科病院　□総合病院（周産期センター）		
分娩施設	施設名:	所属: □産婦人科　□その他（　　）	
	住所:	担当者名:	
	□助産施設　□無床診療所　□有床診療所　□産科病院　□総合病院（周産期センター）		
搬送先施設	施設名:	所属: □産婦人科　□その他（　　）	
	住所:	担当者名:	
	□産科病院　□総合病院（周産期センター）		
新生児搬送先施設	施設名:	所属: □小児科　□その他（　　）	
	住所:	担当者名:	

（妊産婦死亡担当医師 → 日本産婦人科医会 FAX 03-3269-4730）

1　　2012.11 作成

様式３－２－２a

Ⅶ.具体的な臨床経過　※ 経過サマリー・事例検討会資料などがある場合は添付して提出してください

（原因分析に活用します。既往歴,妊娠中合併症,初発症状,バイタルサインの変化,処置なども含めて記載してください）

妊産婦死亡報告事業は、「妊娠・分娩中および分娩後１年未満の女性の死亡事故を報告する」日本産婦人科医会が学会の協力を得て行う事業で、厚労省の母子保健統計とほぼ同数の事例が報告されています。
妊産婦死亡の実態把握と再発予防策の策定・発信を目的としています。
妊産婦死亡が発生した場合に、本書式に記載の上、ご報告をお願いいたします。

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2　　2012.11 作成

図３　妊産婦死亡調査票（偶発的妊産婦死亡用）