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ENHANCED EXPRESSION OF CELL-SPECIFIC SURFACE ANTIGENS ON ENDOTHELIAL MICROPARTICLES IN SEPSIS-INDUCED DISSEMINATED INTRAVASCULAR COAGULATION

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ABSTRACT—Sepsis-induced disseminated intravascular coagulation (DIC) is a major cause of death in patients admitted to intensive care units. Endothelial injury with microparticle production is reported in the pathogenesis of sepsis. Endothelial microparticles (EMPs) present several cell-specific surface antigens with different bioactivities, for example, tissue factor (TF), thrombomodulin (TM), and endothelial protein C receptor (EPCR). We investigated associations between these three different surface antigen–positive EMPs and sepsis-induced DIC. This cross-sectional study composed of 24 patients with sepsis and 23 healthy controls was conducted from November 2012 to September 2013. Blood samples were collected from patients within 24 h of diagnosis of severe sepsis and from healthy controls. Numbers of TF-positive EMPs (TF^+ EMPs), TM-positive EMPs (TM^+ EMPs), and EPCR-positive EMPs ($EPCR^+$ EMPs) were measured by flow cytometry immediately thereafter. Acute Physiology and Chronic Health Evaluation II and Sequential Organ Failure Assessment scores were assessed in the severe sepsis patients at enrollment. We assessed DIC with the International Society of Thrombosis and Haemostasis (ISTH) overt DIC diagnostic criteria algorithm. Numbers of antigen-positive EMPs were increased significantly in both severe sepsis patients and controls and with the increase in ISTH DIC score. Numbers of TF^+ EMPs and $EPCR^+$ EMPs correlated significantly with Sequential Organ Failure Assessment score, and numbers of $EPCR^+$ EMPs correlated significantly with Acute Physiology and Chronic Health Evaluation II score. Numbers of the three antigen-positive EMPs were increased significantly in severe sepsis patients versus those in healthy controls and with the increase of ISTH DIC score, suggesting that the specific bioactivity of each antigen-positive EMP may play a role in the progression of sepsis-induced DIC.

KEYWORDS—CD146 antigen, endothelial cell protein C receptor, flow cytometry, tissue factor, thrombomodulin

INTRODUCTION

Sepsis is one of the major causes of mortality in critically ill patients and is generally defined as systemic inflammatory response syndrome (SIRS) with infection (1). In this situation, various inflammatory mediators are activated, all of which can lead to endothelial injury (2). Disseminated intravascular coagulation (DIC) is always a secondary phenomenon triggered by specific disorders such as endothelial injury resulting from sepsis (3). Disseminated intravascular coagulation is one of the most common and clinically important acquired disorders of hemostasis and is associated with increased mortality during sepsis (4, 5). The pathogenesis of DIC is primarily caused by upregulation of tissue factor (TF) expression and downregulation of natural anticoagulant and fibrinolytic systems. Activation of coagulation, inhibition of fibrinolysis, and consumption of coagulation inhibitors lead to a procoagulant state, resulting in inadequate fibrin removal and fibrin deposition in microthrombi. Formation of these microthrombi aggravates microcirculatory failure and causes multiple organ failure (6).

Microparticles (MPs) are small shed membranous vesicles that are released from cells on activation. Microparticles are derived from various cell types such as platelets, erythrocytes, leukocytes, monocytes, and endothelial cells (7, 8). Expression of cell-specific surface antigens such as TF, thrombomodulin (TM), and endothelial protein C receptor (EPCR) on MPs has recently become a focus of both research and clinical investigations (9–12). Most recent evidence indicates that TF, TM, and EPCR on MPs have their own specific bioactivity. Tissue factor dependent on MPs shows significantly higher procoagulant activity than TF independent of MPs (13). Activated protein C (APC)–dependent anticoagulant activity was detected in MPs associated with TM (14). Also, APC binding MPs associated with EPCR activates PAR1, leading to cytoprotective and anti-inflammatory activities (15).

We previously reported that activated vascular endothelial cells and those with increased procoagulant activity enhance the production of EMPs, with increased binding to leukocytes in sepsis patients. Consequently, EMPs may be involved in the pathogenesis of endothelial injury, which includes sepsis-induced DIC (16). Also, Delabranche et al. (17) reported that concentrations of EMPs were both increased in, and associated with, septic shock–induced DIC during the first 24 h. There is presently no direct clinical evidence for an association between surface antigen–positive EMPs and sepsis-induced DIC.

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Therefore, the objective of this study was to investigate a possible association between these three different surface antigen–positive EMPs (TF, TM, and EPCR) and sepsis-induced DIC.

PATIENTS AND METHODS

Patients

This cross-sectional study was conducted at the Department of Traumatology and Acute Critical Medicine, Osaka University Graduate School of Medicine, during a period of 10 months from November 2012 to September 2013. Patients with *severe sepsis* defined as SIRS combined with an infectious episode and dysfunction of at least one organ based on the American College of Chest Physicians/Society of Critical Care Medicine consensus conference (18) and older than 18 years were included. Patients were excluded if they had surgery before consideration for inclusion. Healthy subjects with no previous history during the same period provided blood samples as controls. For comparison of the numbers of all three antigen-positive EMPs, we included two intensive care unit (ICU) control groups: 12 patients with trauma (Injury Severity Score >15) and SIRS criteria and six patients with cerebral hemorrhage and SIRS criteria.

The study, which followed the principles of the Declaration of Helsinki, was approved by the institutional review board of Osaka University. Written informed consent was obtained from all patients or their close relatives and the control subjects.

Blood samples

Blood samples were obtained through the arterial catheter from patients within 24 h after the diagnosis of severe sepsis to be used as initial biomarkers of sepsis-induced DIC. Similarly, blood samples were obtained within 24 h after trauma injury or after onset of cerebral hemorrhage in the patients with these conditions. Endothelial microparticles in the collection tubes containing trisodium citrate (Insepack II-ST; Kyokuto Pharmaceutical Industrial Co., Ltd., Tokyo, Japan) were measured by flow cytometry immediately after blood sampling. Serum/plasma samples for measuring concentrations of interleukin-6 (IL-6) and soluble TF (sTF) were separated immediately and stored at −40°C until the time of assay.

Analyses of EMPs

Endothelial microparticles in the blood were measured by flow cytometry (BD FACSCanto II; BD Biosciences, San Jose, Calif). Endothelial microparticles in the blood were counted by the method of Combes et al. (10) but with some modifications. For measurement of these variables, samples were centrifuged for 5 min at 1,800*g* to remove residual cells. Platelet-poor plasma was used for the assay as previously reported (19, 20). Subsequently, the cell-free supernatant was removed, and the platelet-poor plasma (platelet counts were less than measurement sensitivity; $5 \times 10^3/\mu L$) was carefully collected in a Trucount tube (BD Biosciences) in which 50 μL of platelet-poor plasma was mixed with 50 μL of a 5-fold dilution of Binding Buffer (BD Biosciences) and 20 μL of heparin (Novo-Heparin, 5,000 units/5 mL for Injection; Mochida Pharmaceutical Co., Tokyo, Japan). Then, 2.5 μL of annexin V, anti-CD146 antibody, and anti-CD142 antibody (clone HTF-1)/anti-CD141 antibody/anti-CD201 antibody (all from BD Biosciences) were added and gently vortexed at room temperature for 30 min in the dark. The antigen–antibody reactions were inhibited by adding 500 μL of a 10-fold dilution of Binding Buffer. Before the analysis, the flow cytometer was calibrated using BD Cytometer Setup and Tracking Beads (BD Biosciences). Measurement of MP diameter was calibrated with reference standard beads (2.00-μm-diameter Fluoresbrite YG carboxylate microspheres; Polysciences, Inc., Warrington, Pa). Measurements of EMPs were determined by flow cytometry (BD FACSCanto II) with an automated cell counter (XN9000; Sysmex Corporation, Kobe, Japan) for 5 min.

Endothelial microparticles were defined as events detected as annexin V^+/$CD146^+$ as previously described (9). Tissue factor–positive EMPs (TF^+ EMPs), TM-positive EMPs (TM^+ EMPs), and EPCR-positive EMPs ($EPCR^+$ EMPs) were defined as events detected by annexin $V^+/CD146^+/CD142^+$, annexin $V^+/CD146^+/CD141^+$, and annexin $V^+/CD146^+/CD201^+$, respectively. Endothelial microparticles were further defined as MPs having a calibrated diameter of 0.1 to approximately 2.0 μm on the basis of previous reports (9, 21). We excluded high-intensity signals caused by antibody aggregation from the flow cytometry analysis. The formation of MPs is expressed as the number of EMPs (Fig. 1).

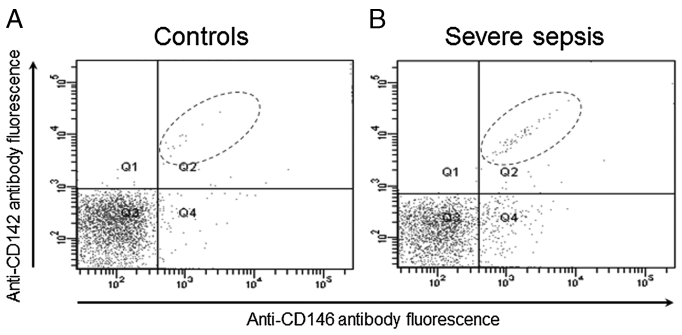

FIG. 1. **Representative flow cytometry dot plots of TF^+ EMPs in a healthy control subject (A) and a patient with severe sepsis (B)**. The TF^+ EMPs are distinctly immunostained positive for annexin V antibody, anti-CD146 antibody, and anti-CD142 antibody, shown in area Q2 indicated by the ellipse. The counts of TF^+ EMPs in the blood are higher in the patient with severe sepsis than in the healthy control subject.

Analyses of serum levels of platelets and IL-6 and plasma levels of sTF, fibrinogen degradation products, and D-dimer

Total platelet counts were determined with an automated cell counter (XN9000). The serum concentrations of IL-6 and plasma concentrations of sTF were measured with an enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, Minn). Plasma concentrations of fibrin/fibrinogen degradation products (FDPs) and D-dimer were measured with latex immunoassay (CS5100; Sysmex Corporation). Frozen samples were thawed and subsequently processed according to the manufacturer's instructions. Absorbance was measured with a microplate reader (SH-9000Lab; Corona Electric Co., Ltd., Ibaraki, Japan). The minimum detectable dose of IL-6 was less than 0.70 pg/mL and that for sTF was 0.16 pg/mL.

Definitions and DIC diagnosis

The Acute Physiology and Chronic Health Evaluation II (APACHE II) score and Sequential Organ Failure Assessment (SOFA) score were assessed in the patients at the time of enrollment. The APACHE II score assesses the severity of illness for critical patients based on routine physiologic measurements, age, and previous health status and is used to predict the outcome of the critical illness (22). The SOFA score allows calculation of the dysfunction of six organ systems, composed of the respiratory, coagulation, hepatic, cardiovascular, renal, and neurologic systems, as well as the severity of the dysfunction (23).

Disseminated intravascular coagulation is a life-threatening condition that is defined by the International Society of Thrombosis and Haemostasis (ISTH) as "an acquired syndrome characterized by the intravascular activation of coagulation with loss of localization arising from different causes. It can originate from and cause damage to the microvasculature, which, if sufficiently severe, can produce organ dysfunction" (24). We used the ISTH overt DIC diagnostic algorithm because it has high specificity to ensure an accurate diagnosis of DIC (25). The level of FDP was used for fibrin-related markers in the ISTH overt DIC criteria. *No increase*, *moderate increase*, and *strong increase* were defined as FDP levels of 0 to 9, 10 to 24, and more than 25 mg/L, respectively (26).

Statistical analysis

Continuous variables are expressed as the group median with interquartile range. The nonparametric Mann-Whitney *U* test was used to assess differences between the patients with severe sepsis and healthy control subjects. Correlations between EMPs and other markers were investigated by means of scatter plots and Spearman rank-correlation coefficients. A value of $P < 0.05$ was considered to indicate statistical significance. Statistical analyses were performed with JMP 9.0.2 for Windows (SAS Institute Inc., Cary, NC).

RESULTS

Patient characteristics

During the study period, 24 patients with severe sepsis (median age, 69.5 [59.8–78.0] years), 12 SIRS patients with trauma (median age, 62.0 [51.0–69.3] years), six SIRS patients with cerebral hemorrhage (median age, 54.0 [44.3–79.8] years), and 23 healthy control subjects (median age, 65.0 [36.0–73.0] years) were included (Table 1). Of the patients with severe sepsis, 15 were men and nine were women. The APACHE II, SOFA, and ISTH DIC scores of the patients with

TABLE 1. **Patient characteristics**

Characteristics	Severe sepsis (n = 24)	Trauma (n = 12)	Cerebral hemorrhage (n = 6)	Controls (n = 23)
Age, years	69.5 (59.8–78.0)	62.0 (51.0–69.3)	54.0 (44.3–79.8)	65.0 (36.0–73.0)
Sex, male/female	15/9	10/2	2/4	14/9
APACHE II score (0–71)	20.0 (15.0–23.5)	13.0 (15.0–23.5)	10.0 (5.8–13.0)	
SOFA score (0–24)	6.5 (3.0–10.8)	4.0 (3.0–6.5)	1.0 (0.75–4.3)	
ISTH DIC score (0–8)	2.5 (2.0–4.0)			
Site of sepsis				
Chest	11			
Abdomen	3			
Soft tissue	3			
Urinary	6			
Others	1			
ISS (0–75)		25 (22.8–39.8)		
Injury mechanism				
Motor vehicle collision		6		
Fall		6		
Location of injury				
Head		3		
Face		1		
Neck		1		
Thorax		5		
Abdomen		3		
Extremities		1		
Location of cerebral hemorrhage				
Cerebrum				5
Cerebellum				1

Reference ranges of severity scores are expressed as minimum and maximum.
Data are expressed as group number or median (interquartile range).
APACHE, Acute Physiology and Chronic Health Evaluation; SOFA, Sequential Organ Failure Assessment; ISTH, The International Society of Thrombosis and Hemostasis; ISS, Injury Severity Score.

severe sepsis were 20.0 (15.0–23.5), 6.5 (3.0–10.8), and 2.5 (2.0–4.0), respectively. The overall ICU mortality of the patients with severe sepsis was 12.5%. The sources of severe sepsis were chest (n = 11), abdomen (n = 3), soft tissue (n = 3), urinary (n = 6), and others (n = 1). The patients with trauma included 10 men and two women. The Injury Severity Score and APACHE II and SOFA scores of the patients with trauma were 25.0 (22.8–39.8), 13.0 (15.0–23.5), and 4.0 (3.0–6.5), respectively. The causes of trauma were motor vehicle collision (n = 6) and falls (n = 6). The locations of injury were the head (n = 3), face (n = 1), neck (n = 1), thorax (n = 5), abdomen (n = 3), and extremities (n = 1). Of the patients with cerebral

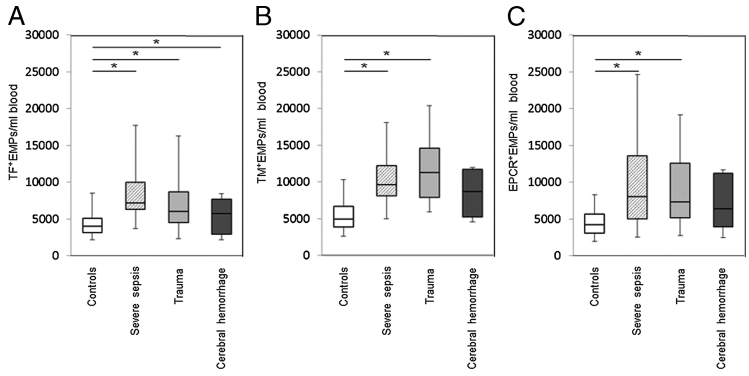

FIG. 2. **The numbers of TF$^+$ EMPs (A), TM$^+$ EMPs (B), and EPCR$^+$ EMPs (C) in the patients with severe sepsis, trauma, and cerebral hemorrhage.** The boxes indicate the lower and upper quartiles, the central dark line is the median, and the ends of the whiskers indicate the maximum and minimum values. Asterisks indicate a statistically significant ($P < 0.05$) difference between groups.

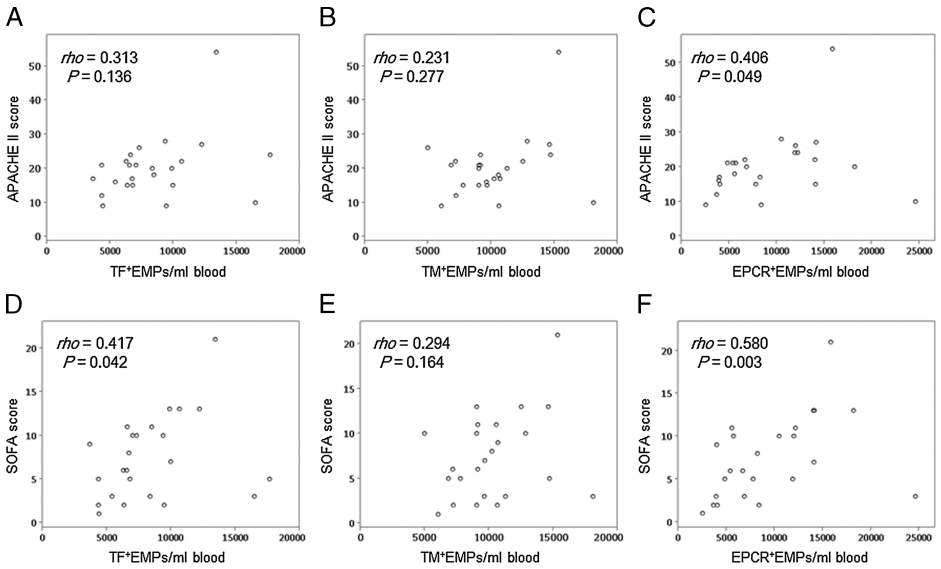

FIG. 3. **Correlations between EMPs and APACHE II and SOFA scores in the patients with severe sepsis**. (A) TF^+ EMPs and APACHE II score, (B) TM^+ EMPs and APACHE II score, (C) $EPCR^+$ EMPs and APACHE II score, (D) TF^+ EMPs and SOFA score, (E) TM^+ EMPs and SOFA score, and (F) $EPCR^+$ EMPs and SOFA score.

hemorrhage, two were men and four were women. The APACHE II and SOFA scores of the patients with cerebral hemorrhage were 10.0 (5.8–13.0) and 1.0 (0.75–4.3), respectively. The sources of cerebral hemorrhage were the cerebrum (n = 5) and the cerebellum (n = 1). The patients with severe sepsis, trauma, and cerebral hemorrhage and the control subjects were all similar with respect to age and sex.

Numbers of TF^+ EMPs, TM^+ EMPs, and $EPCR^+$ EMPs

Overall, in the patients with severe sepsis and trauma, the numbers of TF^+ EMPs, TM^+ EMPs, and $EPCR^+$ EMPs were significantly increased compared with those in the healthy control subjects. In the patients with cerebral hemorrhage, however, only the numbers of TF^+ EMPs were significantly increased compared with those in the healthy control subjects (Fig. 2, A–C).

Correlations between EMPs and severity of illness in the patients with severe sepsis

We assessed the correlation between the number of EMPs and the disease severity scoring systems (APACHE II and SOFA scores) (Fig. 3). A significant correlation was found between the number of $EPCR^+$ EMPs and the APACHE II score (Fig. 3C). There were no statistically significant correlations between the numbers of TF^+ EMPs and TM^+ EMPs and the APACHE II score (Fig. 3, A and B). Significant correlations were found between the numbers of TF^+ EMPs and $EPCR^+$ EMPs and the SOFA score (Fig. 3, D and F). There was no statistically significant correlation between the numbers of TM^+ EMPs and the SOFA score (Fig. 3E).

Correlations between EMPs and ISTH DIC score in the patients with severe sepsis

We investigated several variables associated with coagulant activity (TF^+ EMPs), anticoagulant activity (TM^+ EMPs), and cytoprotective role ($EPCR^+$ MPs) in the patients with severe sepsis. In these patients, the numbers of all three antigen-positive EMPs increased significantly with the increase in the ISTH DIC score (Fig. 4, A–C).

Correlations between $EPCR^+$ EMPs/TF^+ EMPs and $EPCR^+$ EMPs/TM^+ EMPs ratios and ISTH DIC score in the patients with severe sepsis

We evaluated the ratios associated with the balance between coagulant activity and anticoagulant activity (TM^+ EMPs/TF^+ EMPs ratio) and the balance between coagulant activity and cytoprotective role ($EPCR^+$ EMPs/TF^+ EMPs ratio) in the patients with severe sepsis. A significant negative correlation was found between the TM^+ EMPs/TF^+ EMPs ratio and the ISTH DIC score, and a significant positive correlation was

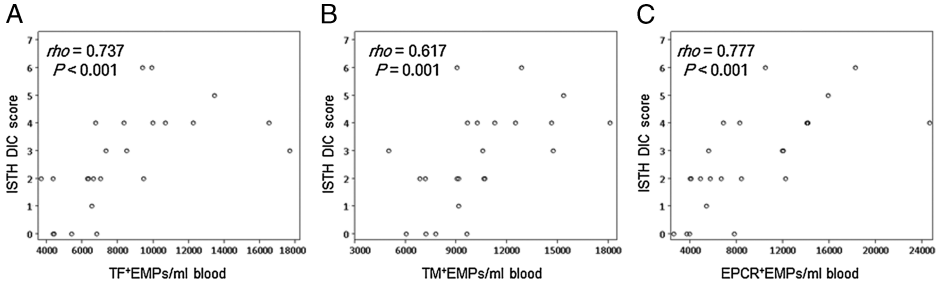

FIG. 4. **Correlations between TF^+ EMPs, TM^+ EMPs, and $EPCR^+$ EMPs and the ISTH DIC score in the patients with severe sepsis**. (A) TF^+ EMPs, (B) TM^+ EMPs, and (C) $EPCR^+$ EMPs.

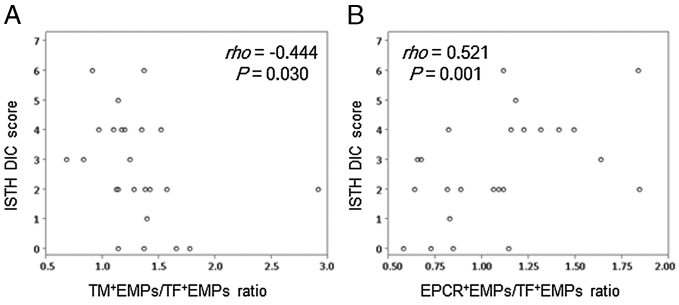

FIG. 5. **Correlations between the ratios of TM$^+$ EMPs/TF$^+$ EMPs and EPCR$^+$ EMPs/TF$^+$ EMPs and the ISTH DIC score in the patients with severe sepsis**. (A) TM$^+$ EMPs/TF$^+$ EMPs ratio and (B) EPCR$^+$ EMPs/TF$^+$ EMPs ratio.

found between the EPCR$^+$ EMPs/TF$^+$ EMPs ratio and the ISTH DIC score (Fig. 5, A and B).

Correlations between TF$^+$ EMPs and biochemical parameters in the patients with severe sepsis

To study the relation between the number of TF$^+$ EMPs and the markers of systemic proinflammation, the level of IL-6 was measured in each serum sample. Significant correlations were found between the number of TF$^+$ EMPs and IL-6 level (Fig. 6A). To determine the relation between the number of TF$^+$ EMPs and the markers of coagulative activity, levels of FDP and D-dimer were measured in each plasma sample (FDP median value, 14.6 [7.8–24.5] μg/dL; D-dimer median value, 6.1 [2.0–12.3] μg/mL). Significant correlations were also found between the number of TF$^+$ EMPs and FDP and D-dimer levels (Fig. 6, B and C). A significant negative correlation was found between the number of TF$^+$ EMPs and platelet count (Fig. 6D). A significant positive correlation was detected between the number of TF$^+$ EMPs and sTF levels (Fig. 6E). Correlations between the three EMP types and biochemical parameters are summarized in Table 2. Reference ranges are as follows: IL-6 (3.13–12.5 pg/mL), sTF (25.0–57.8 pg/mL), FDP (0–5.0 μg/dL), and D-dimer (0 – 0.5 μg/mL).

DISCUSSION

This is the first study, to our knowledge, that evaluates the association between three different surface antigen-positive EMPs (TF, TM, and EPCR) and sepsis-induced DIC. All three of the antigen-positive EMPs were significantly increased in patients with the three types of SIRS including severe sepsis, trauma, and cerebral hemorrhage compared with those in the healthy control subjects. In particular, the numbers of TF$^+$ EMPs and EPCR$^+$ EMPs were higher in the patients with severe sepsis than in the trauma and cerebral hemorrhage patients with SIRS. These data may reflect stronger systemic endothelial injury in the patients with severe sepsis compared with the other patients with SIRS.

Our results clearly showed a significant correlation between the numbers of TF$^+$ EMPs and the ISTH DIC score in the patients with severe sepsis. Disseminated intravascular coagulation is usually triggered by the release of blood-borne TF. Three forms of blood-borne TF have been described: cell-borne TF, an alternatively spliced form of TF that is soluble, and MP-borne TF (27). Nieuwland et al. (28) reported that plasma from patients with sepsis-induced DIC contained MPs that expressed TF, and these MPs demonstrated extreme thrombin generation *in vitro*. These data suggest that the procoagulant activity of TF$^+$ EMPs may play an essential role in the progression of sepsis-induced DIC and other blood-borne TFs, including cell-borne TF and the alternatively spliced form of TF.

In our study, the numbers of TM$^+$ EMPs correlated significantly with the ISTH DIC score in the patients with severe sepsis. Inflammation has been reported to downregulate TM expression in endothelial cells (29, 30), with the loss of anticomplement activity. Thrombomodulin also exists in a soluble form in plasma. Although this form is increased during inflammation, the soluble form of TM may be inadequate in either amount or quality to provide sufficient anticoagulant activity (31). By contrast, our results suggested that the anticoagulant activity of TM$^+$ EMPs increased with the progression of

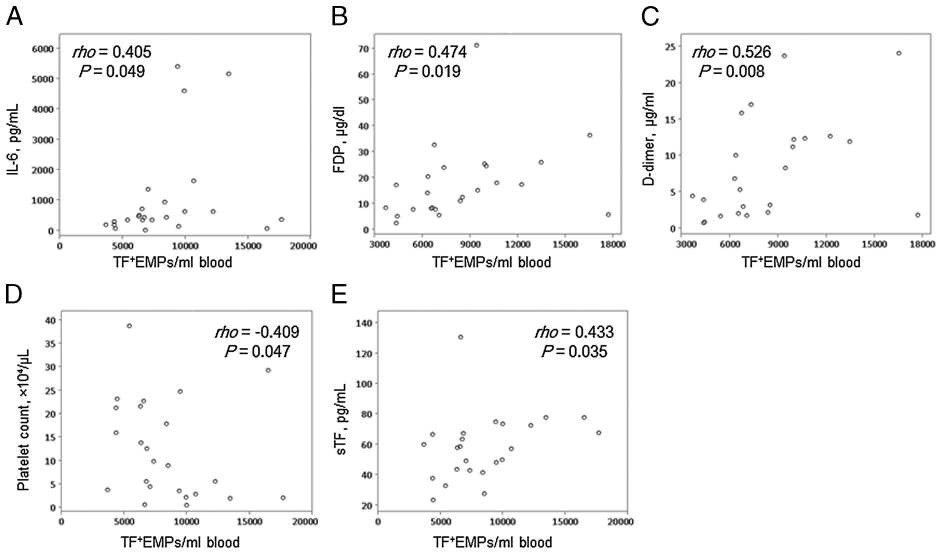

FIG. 6. **Correlations between TF$^+$ EMPs and biochemical parameters in the patients with severe sepsis**. A, IL-6. B, FDP. C, D-dimer. D, Platelet count. E, sTF.

TABLE 2. **Correlations between EMPs and biochemical parameters in patients with severe sepsis**

	TF^+ EMPs		TM^+ EMPs		$EPCR^+$ EMPs	
	rho	*P*	rho	*P*	rho	*P*
IL-6, pg/mL	0.405	0.049	0.326	0.120	0.358	0.086
sTF, pg/mL	0.433	0.035	0.458	0.013	0.621	0.001
Platelet count ($\times 10^4/\mu L$)	−0.409	0.047	−0.294	0.163	−0.517	0.010
FDP, μg/dL	0.474	0.019	0.438	0.033	0.604	0.002
D-dimer, μg/mL	0.526	0.008	0.472	0.023	0.673	<0.001

EMPs, endothelial microparticles; TF^+, tissue factor–positive; TM^+, thrombomodulin-positive; $EPCR^+$, endothelial protein C receptor–positive; IL-6, interleukin-6; sTF, soluble tissue factor; FDP, fibrin/fibrinogen degradation products.

sepsis-induced DIC. In addition, we calculated the TM^+ EMPs/TF^+ EMPs ratio to evaluate the balance between TM and TF on EMPs. A negative correlation was found between the TM^+ EMPs/TF^+ EMPs ratio and the ISTH DIC score. This suggested that the anticoagulant activity of TM^+ EMPs decreased as compared with the procoagulant activity of TF^+ EMPs with the progression of sepsis-induced DIC.

Our study demonstrated significant correlations between $EPCR^+$ EMPs and the ISTH DIC score in the patients with severe sepsis. Inflammation has been reported to downregulate EPCR expression in endothelial cells (32). The EPCR also exists in a soluble form in plasma. Although soluble EPCR has been shown to bind to protein C and APC and to inhibit the anticoagulant properties of APC (33), our results suggested that the cytoprotective and anti-inflammatory activities of $EPCR^+$ EMPs increased with the progression of sepsis-induced DIC. In addition, we calculated the $EPCR^+$ EMPs/TF^+ EMPs ratio to evaluate the balance between EPCR and TF on EMPs. A positive correlation was found between the $EPCR^+$ EMPs/TF^+ EMPs ratio and the ISTH DIC score. This suggested that the cytoprotective and anti-inflammatory activities of $EPCR^+$ EMPs increased as compared with the procoagulant activity of TF^+ EMPs as sepsis-induced DIC progressed.

We previously reported that EMPs may play an important role in the pathogenesis of endothelial injury in sepsis (16). In the present study, the numbers of TF^+ EMPs correlated significantly with the levels of IL-6, platelets, FDP, and D-dimer in the patients with severe sepsis. The SOFA score, the scoring system used to assess organ dysfunction, was significantly associated with the numbers of TF^+ EMPs in the patients with severe sepsis. Thus, it is possible that the procoagulant activity of TF^+ EMPs reflects the progression of endothelial injury and resultant organ injury in severe sepsis. Previous reports demonstrated that endothelial injury plays a crucial role in the pathogenesis of sepsis (2, 34). Amabile et al. (35) showed that an increased level of EMPs in the plasma may be associated with endothelial dysfunction and is a robust independent predictor of severe cardiovascular disease outcome in end-stage renal failure patients. These data support our hypothesis that EMPs may be released depending on the progression of endothelial injury in sepsis-induced DIC.

We demonstrated a correlation between the numbers of TF^+ EMPs and the severity of sepsis as evaluated with the SOFA score, which is a scoring system for the assessment of organ dysfunction. Our finding suggested that the procoagulant activity of TF^+ EMPs might play a role in the progression of sepsis-induced DIC. Interestingly, platelet count, which is one of the parameters evaluated in ISTH DIC scoring, correlated negatively with the numbers of TF^+ EMPs. This suggested that hypercoagulability leads to the formation of microthrombi, which causes the consumption of platelets. In turn, these microthrombi might induce thrombogenesis that leads to multiple organ failure.

As limitations of this study, the numbers of patients and controls were relatively small and the data were collected at a single institution. Second, the change in EMPs was not thoroughly delineated in our study, the number of EMPs was measured at only one time point, and there was no follow-up period. Third, the specific bioactivity of TF, TM, and EPCR was not clarified in our study. Thus, further study is required to thoroughly assess the association between TF, TM, and EPCR and sepsis-induced DIC.

CONCLUSIONS

In conclusion, we demonstrated that the numbers of all three surface antigen–positive EMPs (TF, TM, and EPCR) in the patients with severe sepsis increased in comparison with those in the healthy control subjects. In addition, the numbers of these EMPs increased significantly with the increase in ISTH DIC score. Our findings suggest that the specific bioactivity of each of these antigen-positive EMPs may play a role in the progression of sepsis-induced DIC.

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