의학박사 학위논문

동물 및 인간 패혈증 모델에서 혈청 Developmental endothelial locus-1 (Del-1)과 중증도 및 예후와의 상관성

Serum developmental endothelial locus-1 (Del-1) is associated with severity of sepsis in animals and humans

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이 논문을 의학박사학위 논문으로 제출함

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ABSTRACT

Objectives: Disruption of the endothelial glycocalyx has a prominent role in the pathophysiology of sepsis. Developmental endothelial locus-1 (Del-1) is an endothelial-derived anti-inflammatory factor. The study hypothesis was that degradation of the endothelial glycocalyx during sepsis may increase serum Del-1. In this study, the relationship between the serum Del-1 level and the severity and prognosis of sepsis was investigated.

Materials and Methods: Sepsis was induced in mice using the cecal ligation and puncture (CLP; non-pulmonary sepsis) and lipopolysaccharide (LPS)-induced pneumonia (pulmonary sepsis) models. Glycocalyx and Del-1 were immunolocalized in both septic and control mice. Del-1 expression in various septic mouse tissues was quantified and compared with the control mice. Serum Del-1 and other biomarkers in the CLP and LPS mice were measured. Serum Del-1 levels were also measured in 84 patients with sepsis and septic shock and in 20 control subjects.

Results: After 24 h of CLP, the endothelial glycocalyx was nearly completely degraded, with less formation of Del-1 in the endothelium and extracellular matrix than in control mice. Del-1 expression in various tissues in septic mice was decreased upon CLP. Serum Del-1 levels were significantly increased in the CLP mice with increasing severity of sepsis. LPS-induced serum levels of Del-1, syndecan-1, intercellular adhesion molecule-1, receptor for advanced glycation end products, interleukin-17, interleukin-6, and tumor necrosis factor-α rapidly increased after 1h but had returned to the basal level by 48 h after administration. Meanwhile, there was a steady increase in these biomarkers in the course of CLP-induced sepsis. The median serum Del-1 level in patients with sepsis was significantly higher than that in healthy controls (174.0 μg/ml vs. 88.2 μg/ml; $P < 0.001$). The high Del-1 group (serum Del-1 ≥ 375.96 μg/ml) had higher illness severity scores and contained more patients with organ dysfunction than the low Del-1 group (serum Del-1 < 375.96 μg/ml). The 90-day mortality rate was 74% in the high Del-1 group and 40% in the low Del-1 group ($P = 0.004$). Multivariate analysis indicated a tendency for a high serum Del-1 level to be associated with a higher mortality risk

(adjusted odds ratio, 1.87; 95% confidence interval, 0.995–3.50; $P = 0.052$).

Conclusions: In sepsis, serum Del-1 level is increased and associated with disease severity, organ dysfunction, and mortality. Endothelial glycocalyx degradation may be more prominent in non-pulmonary sepsis. Del-1 may be a promising approach for the diagnosis of sepsis and may require further studies.

Keywords: Del-1; endothelial cells; glycocalyx; inflammation; sepsis; serum

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INTRODUCTION

Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection.[1)] The global burden of sepsis is substantial with an estimated 15 to 19 million cases per year.[2)] Despite significant advances in diagnostic and therapeutic approaches, mortality of sepsis ranges from 20%–35% in those with signs of severe sepsis to nearly 50% in patients with septic shock.[3)]

Early identification of patients who have developed sepsis, prompt antibiotic therapy, source control, and maintaining hemodynamic stability are the cornerstones of treatment for sepsis.[4)] However, septic patients can still die of multiorgan dysfunction even when shock is prevented by these strategies. Mortality in patients with sepsis has been attributed to endothelial dysfunction caused by inflammation,[5)] although sepsis bundles by the Surviving Sepsis Campaign Guidelines do not target the inflammatory and oxidative stress caused by sepsis.[6)] Instead, current standard therapies can potentially increase inflammation via the oxidative stress caused by the bactericidal effects of antibiotic administration.[7)] Thus, there is a need for targeted adjunct therapies that reverse the inflammatory and oxidative stress in these patients.

In sepsis, pro-inflammatory mediators cause increased permeability in endothelial layers, which leads to hypotension, hemodynamic instability, and tissue perfusion impairment. Tissue hypoxia directly affects the microcirculation, contributing to shock, multiorgan dysfunction, and death. Specifically, several mediator substances, including tumor necrosis factor (TNF)-α, lipopolysaccharide (LPS), oxidized lipoproteins, and thrombin, disrupt the glycocalyx.[8)] Changes in the structure of the glycocalyx and integrity of the vasculature result in capillary leak, microvascular thrombosis, systemic hypotension, and tissue hypoperfusion. Moreover, hypoxia in the tissues produces large quantities of reactive oxygen and nitrogen species, causing shedding of the glycocalyx and damage to the cell membranes and intercellular junctions.[9)] These effects lead to endothelial dysfunction, refractive vasodilation, and disseminated intravascular coagulation (DIC).

Over the years, more than 100 phases 2 and phase 3 clinical trials have been performed to test various novel pharmacologic agents and therapeutic interventions in an attempt to more effectively treat sepsis and septic shock: however, all of these efforts ultimately failed to directly target the pathophysiologic effects of sepsis. Immunomodulators were initially studied to assess if down-regulating the inflammatory response would lead to improvement in the outcomes of sepsis. However, antibodies against pro-inflammatory mediators (such as anti-LPS antibodies, anti-TNF antibodies, and anti-TNF receptor antibodies) did not improve mortality in patients with sepsis.[10-12] Several studies have also examined blockade of mediators in the immune cascade such as toll-like receptor and interleukin (IL)-1 and found no improvement in outcomes.[13,14] Recombinant human activated protein C was believed to be beneficial due to its fibrinolytic effects and inhibition of thrombosis,[15] although it was later found to be ineffective for treatment of sepsis and was withdrawn from the market.[16]

Uncontrolled recruitment of leukocytes to sites of inflammation also contributes to fatal organ dysfunction during the course of sepsis.[17] Leukocyte extravasation is regulated by a well-coordinated cascade of adhesive events including (i) selectin-mediated rolling and tethering, (ii) chemokine-induced leukocyte activation, (iii) firm adhesion of leukocytes to endothelial cells, and (iv) their transendothelial migration.[18] Firm adhesion of leukocytes to the endothelium and subsequent transendothelial migration are mediated by interactions between leukocyte integrins, such as lymphocyte function antigen (LFA)-1 and macrophage (Mac)-1, and their endothelial counter-receptors, such as intercellular cell adhesion molecule (ICAM)-1.[19,20]

Developmental endothelial locus-1 (Del-1) is a 52 kD glycoprotein that consists of three epidermal growth factor repeats at its N-terminus and two discoidin I-like domains at its C-terminus.[21] Del-1 is secreted by endothelial cells and may associate with the endothelial surface and matrix.[18,21] Del-1 is an endogenous inhibitor of LFA-1-dependent endothelial adhesion of leukocytes. Specifically, it antagonizes the interaction between leukocyte integrin LFA-1 and endothelial ICAM-1[22] as well as the binding of Mac-1 integrin with complement fragment iC3b.[23] Endothelial Del-1 deficiency has been reported to increase LFA-1-dependent leukocyte adhesion in vivo: Del-1-deficient mice showed increased infiltration of neutrophils

in LPS-induced acute lung inflammation[22] and excessive LFA-1-dependent infiltration of neutrophils in the periodontal tissue causing spontaneous periodontitis.[24] Moreover, Del-1 has been implicated to play a protective role against inflammation-mediated adrenal gland dysfunction,[25] pulmonary fibrosis,[26] and neuroinflammation.[27]

Therefore, Del-1 may be an important homeostatic factor for preventing an inflammatory response in the endothelium and subsequent endothelial dysfunction. Given that endothelial dysfunction is one of the main pathophysiology of sepsis and multiorgan dysfunction,[8,9] the present study evaluated the pathophysiologic role of Del-1 in sepsis. Previous studies measured Del-1 expression in various tissues such as lung, brain, gingiva, and adrenal gland,[22,24-27] although data regarding Del-1 level in the blood are limited. Given that Del-1 is deposited in the endothelium and extracellular matrix[28] and that degradation of the endothelial glycocalyx is common in the course of sepsis,[8,9] the study hypothesis was that circulating Del-1 may be increased with increasing severity of sepsis. If so, serum Del-1 could be a useful biomarker of sepsis and dysfunction in other organs.

In this study, serum Del-1 levels were examined in an animal model of sepsis and in humans. The relationship between the serum Del-1 level and the severity and prognosis of sepsis was the main focus of the investigation. To assess whether Del-1 could be a marker of endothelial injury, serum Del-1 levels were measured in mice that undergone cecal ligation and puncture (CLP) and in mice with LPS-induced pneumonia.

MATERIALS AND METHODS

1. Mouse sepsis model

Sepsis was induced in mice using the CLP (non-pulmonary sepsis) and LPS-induced pneumonia (pulmonary sepsis) models (Fig. 1). The CLP procedure was conducted according to the methods described previously.[29] Healthy 6-week-old male C57BL/6 mice were used. The mice were anesthetized with 3%–4% isoflurane with O_2 flow at 2 l/min and restrained in the supine position. The lower half of the abdomen was carefully shaved, and 70% ethanol was used to wipe down the abdomen to remove excess hair. A 1-cm midline incision was made into the skin only; another 1-cm midline cut was then made into peritoneum. After exteriorizing the cecum, the cecum was ligated at a point approximately 2 cm from the cecal tip with a 2-0 silk suture. A 21-gauge needle was used to puncture the cecum twice. In the experiment assessing Del-1 levels according to severity of sepsis induced by varying the size of the enterotomy, the cecum was also punctured twice with a 23-gauge or 25-gauge needle. The ligated and punctured cecum was replaced back into the abdomen and the incision closed. Next, 1 ml of a saline/buprenorphine mixture was injected into the peritoneal cavity.

For the LPS-induced sepsis model, healthy 6-week-old male C57BL/6 mice were used. The mice were anesthetized by intraperitoneal injection of 0.5 ml tribromoethanol (Avertin; Sigma-Aldrich, St. Louis, MO). The neck was carefully trimmed and sterilized using 70% ethanol. A 1-cm midline incision was made into the skin only; when the trachea was exposed, another small incision was made just distal to the larynx. LPS serotype O111:B4 (catalog no: L2630; Sigma-Aldrich) dissolved in 40 μl of sterile 0.9% NaCl was instilled intratracheally via a 23-gauge syringe, followed by 0.15 ml of air. The dose of LPS used was 20 μg/mouse. After intratracheal treatment, the mice were kept in an upright position for 10 min to allow the fluid to spread throughout the lungs.

2. Immunohistochemistry

The glycocalyx and Del-1 were immunolocalized in the septic mice. The mice were anesthetized by intraperitoneal injection of 0.5 ml of Avertin and then perfused with phosphate-buffered saline (PBS) and 4% paraformaldehyde. Their lungs were then removed and fixed in 4% paraformaldehyde for 2 h, followed by sequential incubation in 15% and 30% sucrose. The tissues were then embedded in Optimal Cutting Temperature compound (Siegen) and frozen at -80°C, and 15 μm cryostat sections were prepared. The sections were washed three times with PBS for 5 min, and permeabilized for 15 min with 0.1% Triton X-100 (Sigma-Aldrich). The sections were then incubated overnight at 4°C with primary antibodies (1:100) against Del-1 (AbFrontier, Seoul, Korea), heparan sulfate (Amsbio, Abingdon, UK), E-cadherin (Cell Signaling, Danvers, MA), and CD31 (eBioscience, San Diego, CA), followed by secondary antibodies at room temperature (RT) for 1 h. The glycocalyx was stained with isolectin-fluorescein isothiocyanate (1:100; Sigma-Aldrich) for 1 h. After washing in PBS three times for 10 min, the coverslips were mounted with Fluoromount-G (Electron Microscopy Science, Hatfield, PA). Images were captured using a laser-scanning confocal microscope (LSM 710; Zeiss Microscopy, Jena, Germany).

3. Quantitative real-time polymerase chain reaction

Total RNA was extracted from tissues at various sites in the septic mice using QIAzol reagent (Qiagen, Hilden, Germany), and cDNA was synthesized using the High-Capacity cDNA Archive kit (Applied Biosystems, Foster City, CA). The cDNA was amplified using LightCycler 480 SYBR-Green I Master and a LightCycler 480 machine (Roche, Mannheim, Germany). The polymerase chain reaction conditions were as follows: 95°C for 15 min; 50 cycles of 20 s at 95°C, 20 s at 60°C, and 20 s at 72°C, and 95°C for 15 min. Melting curve analyses were performed to ensure that specific polymerase chain reaction products were generated. The data were analyzed using the comparative threshold (C_T) method,[30] and the mRNA levels were normalized to those of 18S RNA. The primers used were as follows: Del-1, forward: 5'-CCTGTGAGATAAGCGAAG-3' and reverse: 5'-GAGCTCGGTGAGTAGATG-3'; 18S, forward: 5'-CGCGGTTCTATTTTGGT-3' and reverse:

5'-AGTCGGCATCGTTTATGGTC-3'.

4. Enzyme-linked immunosorbent assay

An enzyme-linked immunosorbent assay (ELISA) was performed to analyze Del-1 and other biomarkers in the serum and bronchoalveolar lavage fluid of septic mice and to analyze levels of Del-1 and inflammatory cytokines in human blood. To measure Del-1 concentrations in mouse and human, a MaxiSorp 96-well plate (Nunc A/S, Roskilde, Denmark) was coated with 50 μl of 200 ng/ml L-α-phosphatidylserine (Avanti Polar Lipids, Alabaster, AL) at 4°C for 12 h.[31] After washing with 0.05% PBS-Tween-20 (PBST) three times, diluted samples were added, and the plate was incubated at RT for 3 h. Serial dilutions of recombinant human Del-1 protein (R&D Systems, Minneapolis, MN) were added as the standard. The plate was then washed, incubated with a rabbit anti-Del-1 antibody (catalog no: 12580-1-AP; Proteintech, Rosemont, IL) at RT for 2 h, washed four times with PBST, and incubated with the horseradish peroxidase (HRP)-conjugated anti-rabbit IgG (Jackson ImmuneResearch, West Grove, PA) at RT for 1 h. After five washes with PBST, the plate was incubated with tetramethylbenzidine substrate (BD Biosciences, San Diego, CA). Absorbance at 650 nm was read on a Synergy HT Microplate Reader (BioTek Instruments, Winooski, VT). To measure other biomarkers in mice, the capture antibodies were diluted in PBS and coated on a Maxisorp plate, and then incubated at 4°C for 12 h. In some ELISA, serum was diluted and added to a Maxisorp plate that was not coated with capture antibodies, and then analyzed using detection antibodies. The antibodies used for mouse serum were as follows: anti-receptor for advanced glycation end products (RAGE) (catalog no: MAB11795; R&D Systems), anti-intercellular adhesion molecule (ICAM)-1 (catalog no: 116113; Biolegend, San Diego, CA), anti-syndecan-1 (catalog no: 142503; Biolegend), anti-interleukin (IL)-6 (catalog no: 14-7061-68; eBioscience), anti-IL-17 (catalog no: 14-7175-68B; eBioscience), and anti-tumor necrosis factor (TNF)-α (catalog no: 14-7423-68A; eBioscience). After three washes in 0.1% PBST, the plate was blocked with PBST containing 0.3% skim milk for 1 h at RT. The plate was then washed five times with 0.1% PBST, incubated with samples and the standard at 4°C for 12 h, washed again, and incubated

with the respective detection antibodies and HRP-conjugated secondary antibodies (Cell Signaling) at RT for 1 h. After five washes, the plate was incubated with tetramethylbenzidine solution and the reaction was stopped with 1 N HCl. Absorbance was read at 450 nm on a microplate reader (BioTek Instruments). A Magnetic Luminex Performance Assay (catalog no: FCST03; R&D Systems) was used for quantification of IL-1β, TNF-α, and IL-6 in the human blood.

5. Study subjects

Eighty-four patients diagnosed with sepsis or septic shock between March 2011 and January 2013 were enrolled. All patients (54 male, 30 female) were older than 18 years of age (median 70 [range, 58–76] years) and had been admitted to the medical intensive care unit (ICU) of a university-affiliated tertiary care hospital in Seoul, Korea. Blood samples were collected after informed written consent was obtained from all patients and subject or their next of kin within the first day of admission to the ICU. Twenty healthy individuals (10 male, 10 female) who had voluntarily undergone a private health examination at the same hospital in April 2014 were recruited as controls.

The baseline demographic and clinical characteristics that were collected were age, sex, comorbidities based on the Charlson Comorbidity Index,[32] source of sepsis, presence of bacteremia, septic shock, acute respiratory distress syndrome, and/or DIC, and the status of the patient within 24 h of admission to the ICU, namely, whether the patient was being treated with mechanical ventilation and vasopressors. In addition, the severity of illness at the time of ICU admission was recorded; this was assessed using the APACHE (Acute Physiology and Chronic Health Evaluation) II score[33] and the SOFA (Sequential Organ Failure Assessment) score.[34] Laboratory data included a complete blood cell count, coagulation profile, C-reactive protein, procalcitonin, and serum lactate. Overt DIC was defined according to the criteria of the International Society on Thrombosis and Haemostasis.[35] Sepsis and septic shock were defined using the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3).[1] Acute respiratory distress syndrome was diagnosed by a consensus definition.[36]

6. Statistical analysis

Continuous variables are presented as mean ± standard error of the mean, as mean ± standard deviation, or median and interquartile range. Categorical variables are presented as percentages. The two groups were compared in terms of continuous variables using the Mann-Whitney *U* or Student's *t*-test, and in terms of categorical variables using the chi-square or Fisher's exact tests. The optimal cutoff value for serum Del-1 predicting 90-day mortality was identified by receiver-operating characteristic curve analysis. Kaplan-Meier estimates were built to predict mortality, and the curves were compared using the log-rank test. A Cox proportional hazards regression model using the forward conditional method was used to identify factors associated with time to 90-day mortality in the study patients. All analyses were performed using SPSS version 22.0 for Windows (IBM, Armonk, NY). All tests of significance were two-tailed. *P*-values < 0.05 were considered statistically significant.

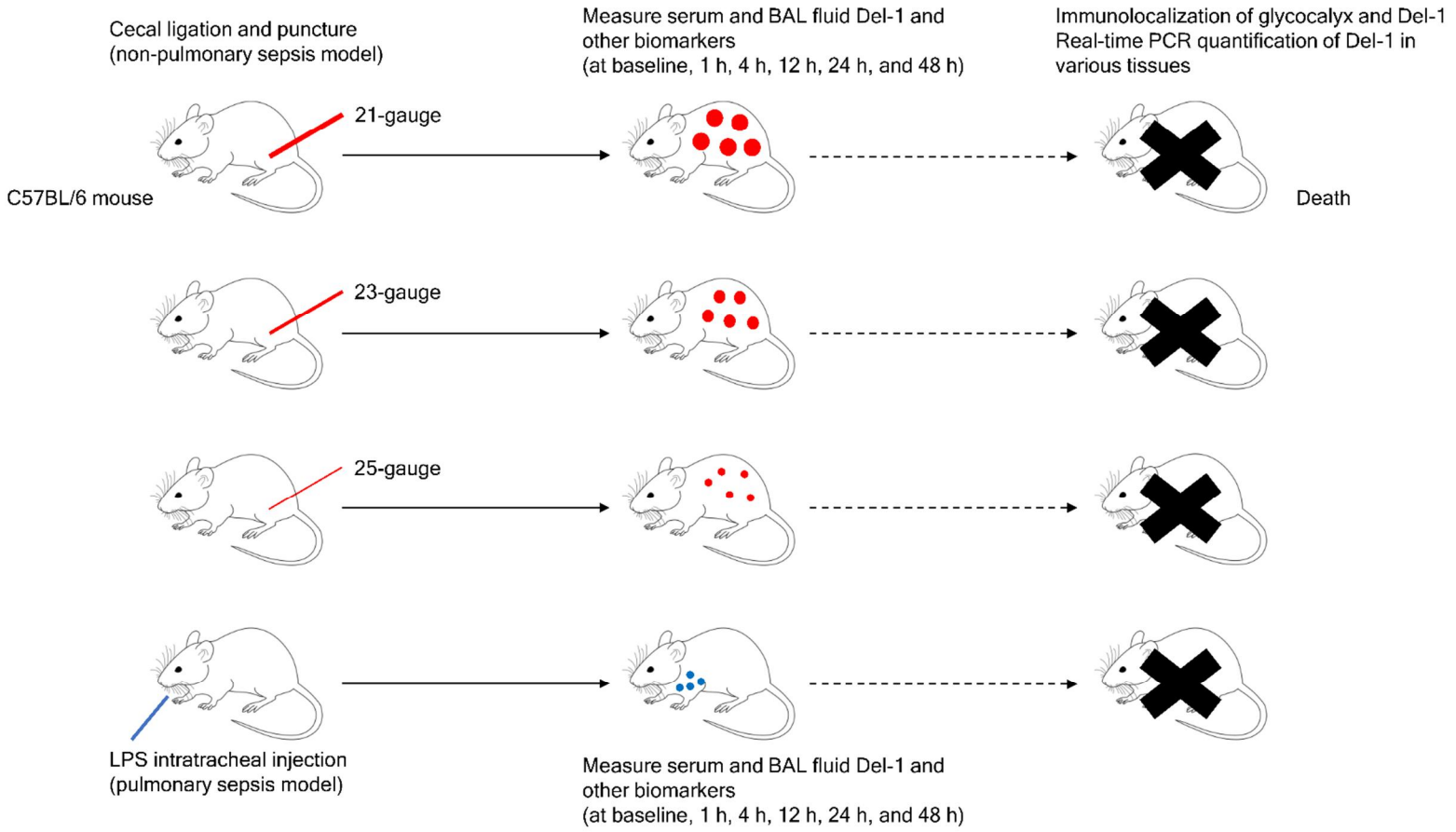


Fig. 1. Schematic flow chart of the experiment. BAL, bronchoalveolar lavage; Del-1, developmental endothelial locus-1; LPS, lipopolysaccharide.

RESULTS

1. Endothelium and Del-1 in sepsis

The localization pattern of Del-1 was first examined by Del-1 staining in the pulmonary vascular endothelium of wild-type (WT) and Del-1$^{-/-}$ mice. WT vessels displayed abundant Del-1 staining (Fig. 2A). As expected, no Del-1 staining was detected in Del-1$^{-/-}$ vessels (Fig. 2B). Fluorescence microscopic photographs illustrating the endothelial glycocalyx and Del-1 are shown in Fig. 3. The relatively restricted pattern of glycocalyx staining in the surface layer of the pulmonary vascular endothelium is contrasted with Del-1 staining involving the endothelial surface layer, an endothelial cell, and the extracellular matrix. The spatial colocalization of the glycocalyx and Del-1 suggests that Del-1 is linked with the glycocalyx. Sections of lung from the CLP and control mice were stained for heparan sulfate, Del-1, and glycocalyx. In the control mice, a mostly intact glycocalyx and spatial distribution of heparan sulfate and Del-1 were verified by immunostaining (Fig. 4A). Lower levels of glycocalyx, heparan sulfate, and Del-1 staining were detected in the CLP mice (Fig. 4B).

2. Del-1 levels in various tissues in the septic animal model

Del-1 expression in various septic mouse tissues was assessed using quantitative real-time polymerase chain reaction (Fig. 5). After 24 h of CLP, expression of Del-1 was significantly decreased in the adrenal gland, heart, brain, and lung tissues (Fig. 5A, 5C, 5D, 5E).

3. Serum Del-1 levels in the animal model according to severity of sepsis

Fig. 6 shows the changes in serum Del-1 levels in the CLP mice according to the needle puncture size. Mice that underwent CLP with a 25-gauge needle died within 5 days of surgery and those that underwent CLP with a 21-gauge needle died within 2 days of surgery (Fig. 6A). Serum Del-1 levels were significantly higher 24 hours after CLP in the CLP mice than in the

control mice and were significantly increased in the CLP mice with increasing severity of sepsis (Fig. 6B).

4. Del-1 levels in serum and BAL fluid in pulmonary and non-pulmonary sepsis

Del-1 levels in serum and BAL fluid were measured in the mice with LPS-induced lung inflammation (pulmonary sepsis) and in the CLP mice (non-pulmonary sepsis; Fig. 7). In the pulmonary sepsis group, the serum Del-1 level rapidly increased after 1 h, started to decrease at 4 h, and had returned to the basal level by 48 h after administration of LPS (Fig. 7A); the findings for the Del-1 level in BAL fluid were similar (Fig. 7B). However, in the non-pulmonary sepsis group, there was a steady increase in Del-1 in both serum and BAL fluid in the course of CLP-induced sepsis (Fig. 7A, 7B).

5. Other serum biomarker levels in pulmonary and non-pulmonary sepsis

The serum levels of various biomarkers in the pulmonary and non-pulmonary sepsis models are shown in Fig. 8. In the pulmonary sepsis group, LPS-induced serum levels of syndecan-1, ICAM-1, and RAGE rapidly increased after 1h, started to decrease at 4 h, and had returned to the basal level by 48 h (Fig. 8A, 8B, 8C). Meanwhile, serum levels of syndecan-1, ICAM-1, and RAGE increased in the non-pulmonary sepsis model (Fig. 8A, 8B, 8C). These findings were largely similar for IL-17, IL-6, and TNF-α levels (Fig. 8D, 8E, 8F).

6. Patient characteristics

The median serum Del-1 levels in the 84 patients and 20 healthy control subjects were 174.0 (range, 113.7–534.4) μg/ml and 88.2 (range, 73.5–120.5) μg/ml, respectively ($P = 0.001$; Fig. 9). The optimal cutoff serum Del-1 level that predicted 90-day mortality in the patients was 375.96 μg/ml (sensitivity 47%; specificity 83%).

The patients were divided into a low Del-1 group (serum Del-1 < 375.96 μg/ml; n = 57) and

a high Del-1 group (serum Del-1 ≥ 375.96 μg/ml; n = 27). The baseline characteristics and clinical outcomes in these two groups are shown in Table 1. The groups were similar in terms of age, sex, and Charlson Comorbidity Index. Patients in the low Del-1 group were significantly more likely to have pulmonary sepsis (72% vs. 44%; $P = 0.02$) whereas those in the high Del-1 group tended to be more likely to have non-pulmonary sepsis (23% vs. 41%; $P = 0.09$). The high Del-1 group had a significantly higher SOFA score (10 [range, 7–12] vs. 12 [range, 10–14]; $P = 0.02$) and DIC score (3 [range, 2–4] vs. 4 [range, 3–6]; $P = 0.02$) when admitted to the ICU. Likewise, there were trends towards a higher APACHE II score, a higher proportion of patients with septic shock, and a higher proportion of patients receiving mechanical ventilation or vasopressors in the high Del-1 group. The high Del-1 group had a significantly lower platelet count and prothrombin time (%). Initial serum lactate was also higher in the high Del-1 group, but the difference was not statistically significant ($P = 0.08$).

Table 1. Baseline characteristics and clinical outcomes in the low Del-1 and high Del-1 groups

Variable	Low Del-1 group (n = 57)	High Del-1 group (n = 27)	*P*-value
Baseline characteristics			
Age, years	70 (62–76)	65 (54–74)	0.29
Male sex	35 (61)	19 (70)	0.42
Charlson Comorbidity Index	3 (1–4)	3 (2–6)	0.21
Source of sepsis			
Pulmonary	41 (72)	12 (44)	0.02
Non-pulmonary	13 (23)	11 (41)	0.09
Bacteremia	15 (26)	10 (37)	0.32
Septic shock on admission to ICU	44 (77)	24 (89)	0.20
ARDS on admission to ICU	9 (16)	4 (15)	>0.99

Table 1. Continued

Variable	Low Del-1 group (n = 57)	High Del-1 group (n = 27)	*P*-value
APACHE II score on admission to ICU	23 (18–27)	25 (21–29)	0.26
SOFA score on admission to ICU	10 (7–12)	12 (10–14)	0.02
DIC score on admission to ICU	3 (2–4)	4 (3–6)	0.02
Overt DIC*	12 (21)	11 (41)	0.059
Use of mechanical ventilation in day 1	34 (60)	20 (74)	0.20
Use of a vasopressor on day 1	38 (67)	21 (78)	0.30
Laboratory data on day 1			
White cell count, 1000/mm^3	14.8 (10.7–21.2)	13.3 (5.4–18.8)	0.13
Platelet count, 1000/mm^3	134 (70–243)	93 (49–136)	0.02
Prothrombin time, %	64 (55–80)	43 (37–62)	<0.001
C-reactive protein, mg/dl	18.8 (6.8–26.2)	10.8 (6.3–20.0)	0.10
Procalcitonin, ng/ml	4.4 (0.6–26.2)	5.4 (0.7–19.5)	0.96
Lactate, mmol/l	2.5 (1.2–3.9)	4.0 (1.7–7.3)	0.08
Outcomes			
Length of stay, d			
ICU	4 (2–16)	5 (2–12)	0.77
Hospital	21 (10–34)	32 (14–55)	0.07
Mortality			
28-day	12 (21)	11 (41)	0.059
90-day	23 (40)	20 (74)	0.004

The data are presented as the median (interquartile range) or number (percentage) of patients. The *P*-values indicate the results of comparing the low Del-1 and high Del-1 groups using the Mann-Whitney *U*, chi-square, or Fisher's exact test. *A DIC score of five or more. APACHE,

Acute Physiology and Chronic Health Evaluation; ARDS, acute respiratory distress syndrome; Del-1, developmental endothelial locus-1; DIC, disseminated intravascular coagulation; ICU, intensive care unit; SOFA, Sequential Organ Failure Assessment.

7. Clinical outcomes in the low Del-1 and high Del-1 groups

There was no difference in the resuscitation or infection goal achieved between the groups (Table 2); however, the 90-day mortality rate was significantly higher in the high Del-1 group than in the low Del-1 group (40% vs. 74%; $P = 0.004$; Table 1). Moreover, there were trends towards a higher 28-day mortality rate and a longer hospital stay in the high Del-1 group (Table 1). Subgroup analysis that did not include pulmonary sepsis showed a significant difference in the 90-day mortality rate in favor of the high Del-1 group (19% vs. 67%; $P = 0.007$; Table 3). Fig. 10 shows the Kaplan-Meier survival curves for the low and high Del-1 groups ($P = 0.004$).

Table 2. Resuscitation and infection goals achieved

Variable	Low Del-1 group (n = 57)	High Del-1 group (n = 27)	*P*-value
Central venous pressure ≥ 8 mmHg	39/49 (80)	16/22 (73)	0.55
Mean arterial pressure ≥ 65 mmHg	43/49 (88)	22/24 (92)	>0.99
Central venous oxygen saturation ≥ 70%	17/42 (41)	8/20 (40)	0.97
Adequate antimicrobial therapy*	47 (83)	22 (82)	>0.99

The data are presented as the number (percentage) of patients. *P*-values indicate the results of comparing the low and high Del-1 groups by chi-square or Fisher's exact test. *Defined as when the regimen included one or more antimicrobial agents to which the causative pathogen was susceptible *in vitro*. Del-1, developmental endothelial locus-1.

Table 3. Clinical outcomes in the low and high Del-1 groups that did not include pulmonary sepsis

Variable	Low Del-1 group (n = 16)	High Del-1 group (n = 15)	*P*-value
Length of stay, d			
ICU	2 (1–6)	3 (2–8)	0.24
Hospital	19 (11–44)	34 (16–50)	0.28
Mortality			
28-day	1 (6)	5 (33)	0.08
90-day	3 (19)	10 (67)	0.007

The data are presented as the median (interquartile range) or number (percentage) of patients. *P*-values indicate the results of comparing the low and high Del-1 groups using the Mann-Whitney *U*, chi-square, or Fisher's exact test. Del-1, developmental endothelial locus-1; ICU, intensive care unit.

8. Association between the serum Del-1 level and mortality

The results for the Cox proportional hazards regression model of risk factors associated with 90-day mortality are shown in Table 4. Multivariate analysis revealed a significant association of a low platelet count with higher mortality (adjusted odds ratio, 0.996; 95% confidence interval, 0.99–1.00; $P = 0.04$). A high serum Del-1 level tended to be associated with higher mortality ($P = 0.052$); subgroup analysis showed that this relationship was only statistically significant in the non-pulmonary sepsis group (adjusted odds ratio, 4.37; 95% confidence interval, 1.19–16.00; $P = 0.03$) (Table 5).

Table 4. Cox regression model with 90-day mortality as the outcome

Variable	Adjusted HR (95% CI)	*P*-value
Platelet count	0.996 (0.99–1.00)	0.04
High Del-1	1.87 (0.995–3.50)	0.052

Multivariate analyses were adjusted for the Charlson Comorbidity Index, APACHE II score, SOFA score, and whether or not coagulopathy was present on admission to the intensive care unit, use of mechanical ventilation within the first 24 h, platelet count and prothrombin time on day 1, length of ICU stay, and a high Del-1 level. APACHE, Acute Physiology and Chronic Health Evaluation; CI, confidence interval; Del-1, developmental endothelial locus-1; HR, hazard ratio; SOFA, Sequential Organ Failure Assessment.

Table 5. Cox regression model with 90-day mortality as the outcome in the low and high Del-1 groups that did not include pulmonary sepsis

Variable	Adjusted HR (95% CI)	*P*-value
Platelet count	0.99 (0.97–0.999)	0.03
High Del-1	4.37 (1.19–16.00)	0.03

Multivariate analyses were adjusted for the platelet count on day 1 and a high Del-1 level. CI, confidence interval; Del-1, developmental endothelial locus-1; HR, hazard ratio.

9. Inflammatory cytokines and serum Del-1

The correlations between serum Del-1 and inflammatory cytokine levels in the human samples are shown in Table 6. The IL-6 level correlated weakly with the serum Del-1 level ($\gamma = 0.24$; $P = 0.03$). The serum IL-6 level was significantly higher in the high Del-1 group than in the low Del-1 group (126.2 [range, 41.9–795.3] pg/ml vs. 360.5 [range, 91.9–3349.6] pg/ml; $P = 0.02$; Table 7).

Table 6. Correlation between serum Del-1 and inflammatory cytokines

Variable		IL-1β	TNF-α	IL-6
Del-1	γ	-0.07	0.11	0.24
	P-value	0.52	0.31	0.03

Del-1, developmental endothelial locus-1; IL, interleukin; TNF, tumor necrosis factor.

Table 7. Inflammatory cytokine levels in the low and high Del-1 groups

Variable	Low Del-1 group (n = 57)	High Del-1 group (n = 27)	*P*-value
IL-1β, pg/ml	10.1 (3.2–34.5)	6.3 (2.5–26.7)	0.36
TNF-α, pg/ml	17.6 (9.2–54.4)	19.1 (8.8–59.8)	0.70
IL-6, pg/ml	126.2 (41.9–795.3)	360.5 (91.9–3349.6)	0.02

The data are presented as the median (interquartile range). *P*-values indicate the results of comparing the low and high Del-1 groups using the Mann-Whitney *U* test. Del-1, developmental endothelial locus-1; IL, interleukin; TNF, tumor necrosis factor.

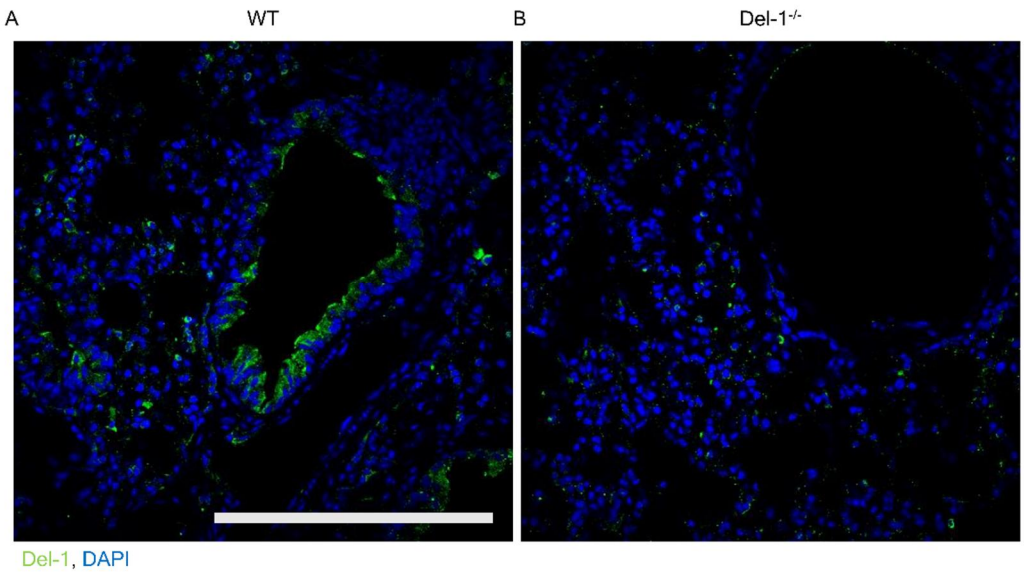


Fig. 2. Fluorescent confocal images of pulmonary vascular endothelium from 6-week-old WT and Del-1$^{-/-}$ mice stained for Del-1 to assess Del-1 expression. Scale bars, 200 μm. (A) Expression of Del-1 was prominent in the WT vessels. (B) As expected, no positive Del-1 staining was detected in Del-1$^{-/-}$ vessels. DAPI, 4',6-diamidino-2-phenylindole; Del-1, developmental endothelial locus-1; WT, wild-type.

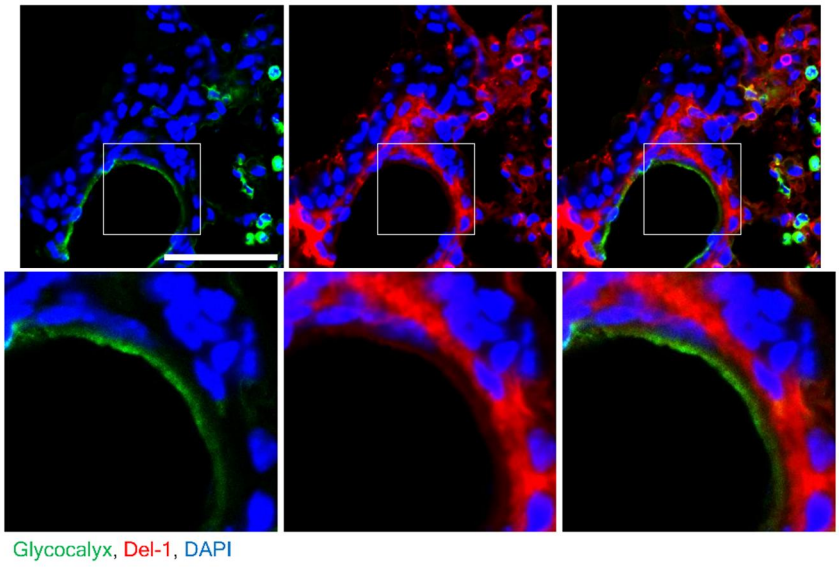


Fig. 3. Sections of pulmonary vascular endothelium from WT mice stained for glycocalyx (left) or Del-1 (middle); right, merged images. Bottom row, ×630 enlargement of areas outlined above. Expression in the glycocalyx was localized to the endothelial surface layer, although Del-1 staining in the confocal image involved the endothelial surface layer, the endothelial cell, and the extracellular matrix. DAPI, 4',6-diamidino-2-phenylindole; Del-1, developmental endothelial locus-1; WT, wild-type.

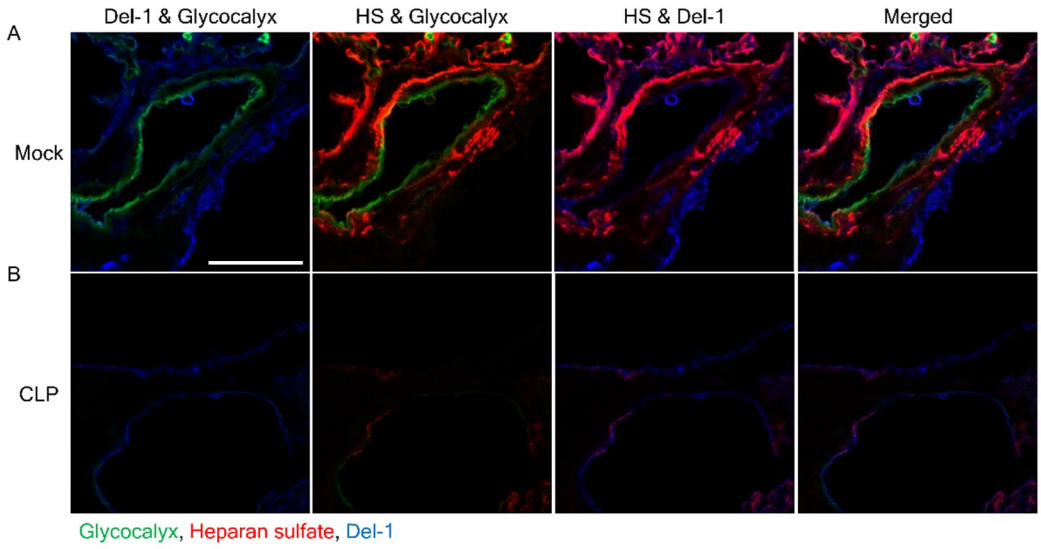


Fig. 4. Sections of lung from the CLP and control mice stained for heparan sulfate, Del-1, and glycocalyx. Scale bars, 50 μm. (A) The endothelial glycocalyx was mostly intact, and heparan sulfate and Del-1 were spatially distributed in the endothelial cell and extracellular matrix. (B) After 24 h of CLP, the endothelial glycocalyx was almost completely degraded with less formation of heparan sulfate and Del-1. CLP, cecal ligation and puncture; Del-1, developmental endothelial locus-1; HS, heparan sulfate.

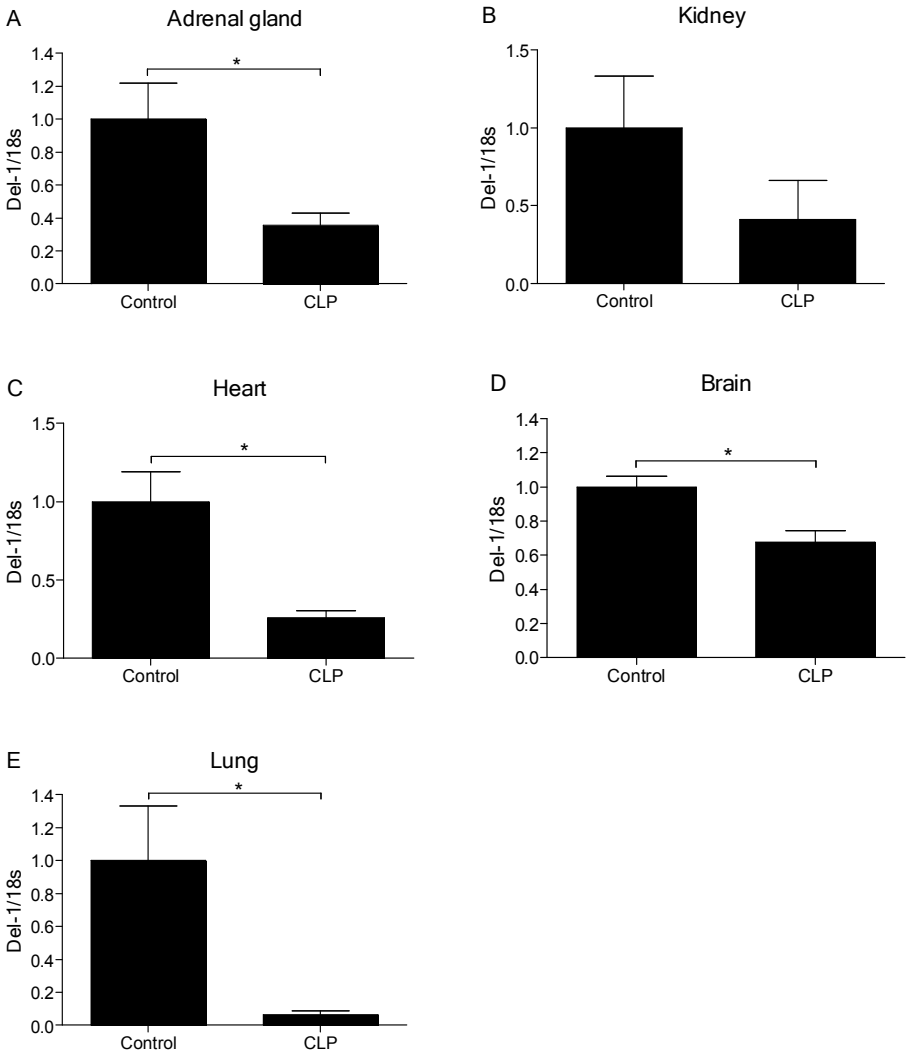


Fig. 5. Real-time polymerase chain reaction quantification of Del-1 mRNA expression in various mouse tissues during sepsis. After 24 h of CLP, relative quantification of Del-1 expression shows that CLP significantly decreased Del-1 mRNA expression in adrenal gland, heart, brain, and lung tissue. The data are shown as a ratio of the control, which is set as 1.0. The data are shown as the mean ± standard error of the mean (n = 5 mice per experimental group and n = 3 mice per control group). $^{*}P < 0.05$, Mann-Whitney *U* test (Student's *t*-test in Figure D). CLP, cecal ligation and puncture; Del-1, developmental endothelial locus-1.

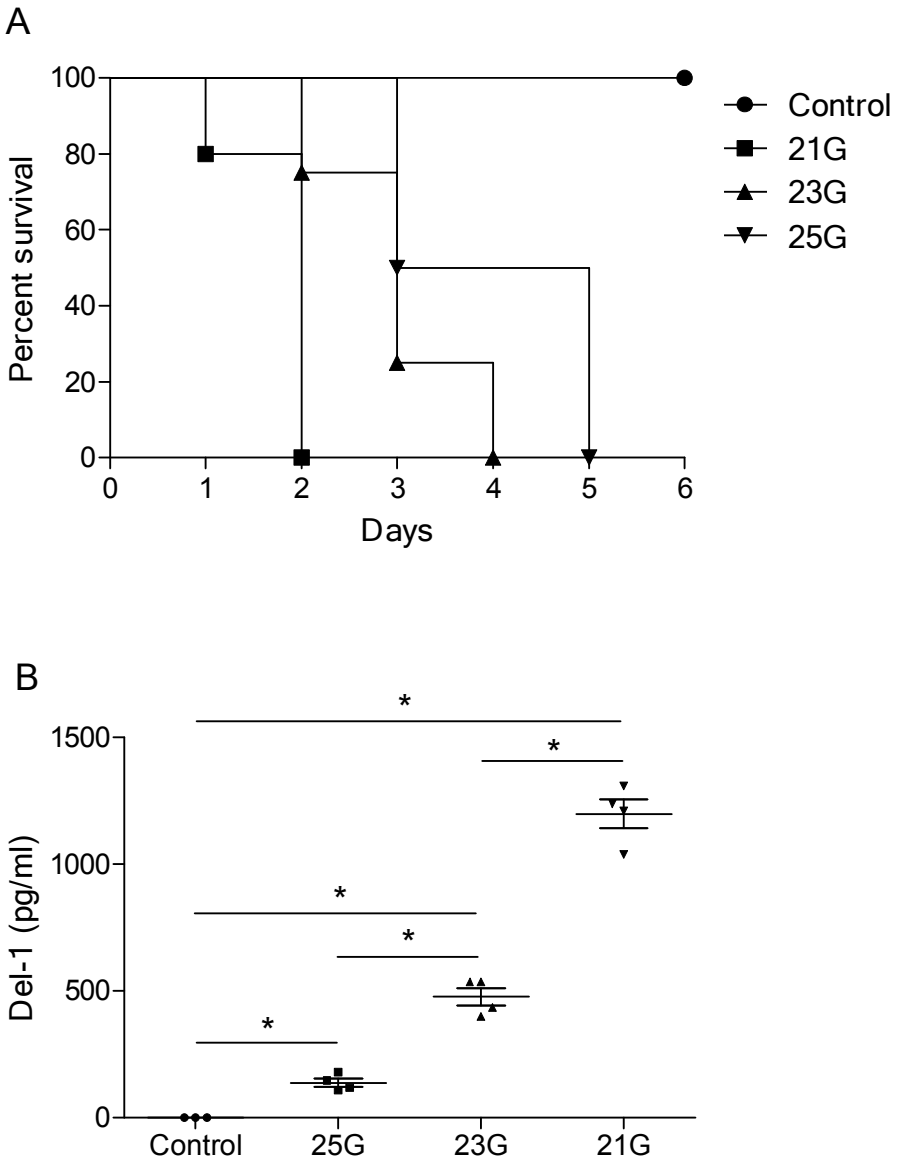


Fig. 6. Serum Del-1 levels according to severity of sepsis. (A) The CLP mice with less severe sepsis (25-gauge needle puncture size) died within 5 days whereas those with more severe sepsis (21-gauge needle puncture size) died within 2 days (n = 4–5 mice per experimental group and n = 3 mice per control group; $P = 0.01$, log-rank test, compared by Kaplan-Meier estimates). (B) After 24 h of CLP, serum Del-1 levels were significantly increased in accordance with the severity of sepsis. The data are shown as the mean ± standard error of the mean (n = 4–5 mice per experimental group and n = 3 mice per control group). $^{*}P < 0.01$, Student's *t*-test. CLP, cecal ligation and puncture; Del-1, developmental endothelial locus-1.

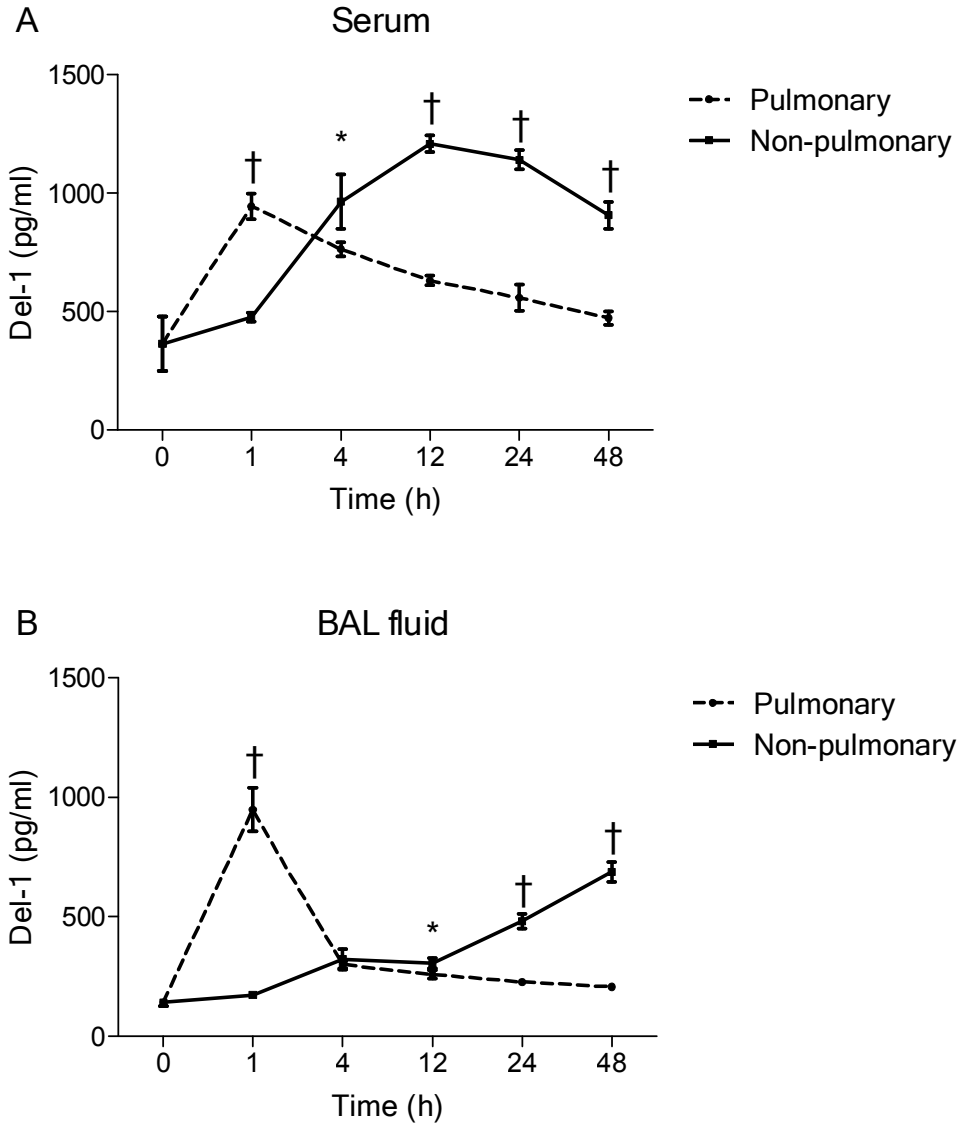
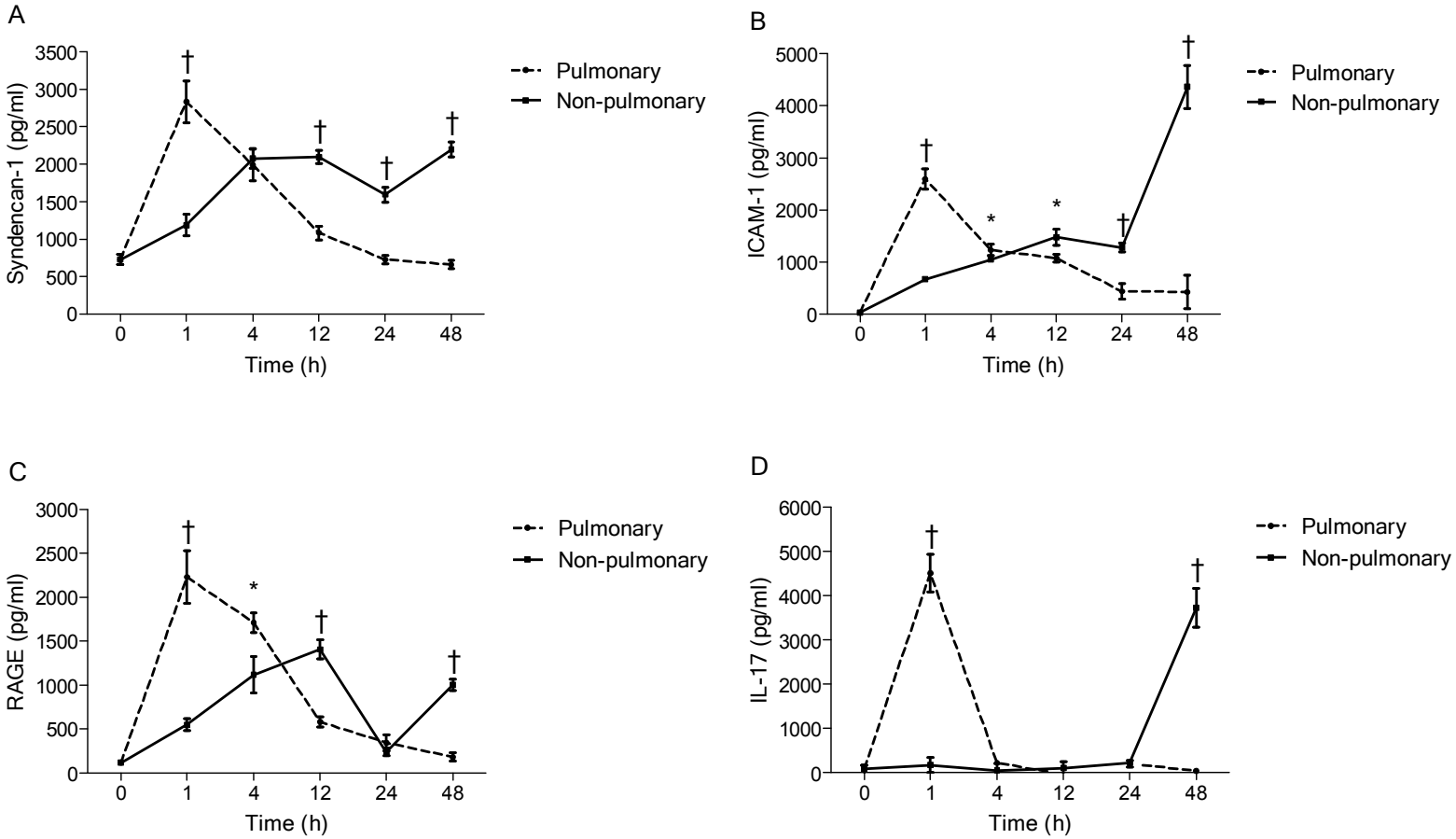


Fig. 7. Del-1 levels in serum and BAL fluid in the pulmonary (LPS mice, dashed line) and non-pulmonary (CLP mice, solid line) sepsis models. (A) The serum Del-1 level rapidly increased after 1 h, started to decrease at 4 h, and had returned to the basal value by 48 h in the pulmonary sepsis group but increased steadily in the non-pulmonary sepsis group. (B) The findings were similar in BAL fluid. The data are shown as the mean ± standard deviation (n = 6 mice per group). $^{*}P < 0.05$ and $^{\dagger}P < 0.01$, pulmonary sepsis vs. non-pulmonary sepsis, Student's *t*-test. BAL, bronchoalveolar lavage; CLP, cecal ligation and puncture; Del-1, developmental endothelial locus-1; LPS, lipopolysaccharide.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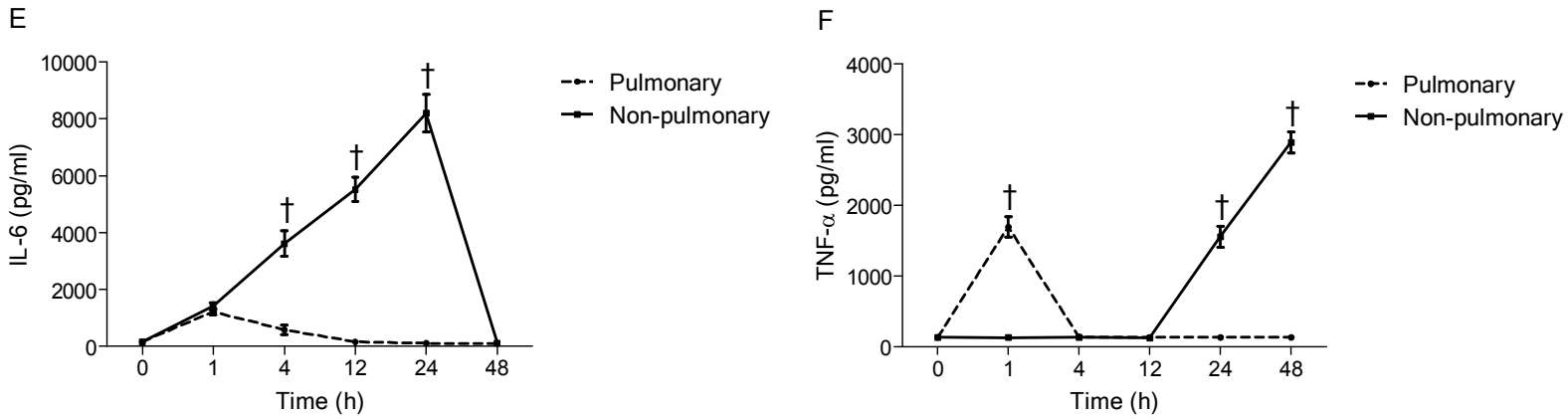

Fig. 8. Biomarker levels in pulmonary (LPS mice, dashed line) and non-pulmonary (CLP mice, solid line) sepsis models. (A–C) Serum levels of syndecan-1, ICAM-1, and RAGE rapidly increased after 1 h, started to decrease at 4 h, and had returned to the basal level by 48 h in the pulmonary sepsis group. Increased serum protein levels were observed in non-pulmonary sepsis group. (D–F) These findings were largely similar for inflammatory biomarkers (IL-17, IL-6, and TNF-α). The data are shown as the mean ± standard deviation (n = 6 mice per group). $^{*}P < 0.05$ and $^{\dagger}P < 0.01$, pulmonary sepsis vs. non-pulmonary sepsis, Student's *t*-test. CLP, cecal ligation and puncture; ICAM, intercellular cell adhesion molecule; IL, interleukin; LPS, lipopolysaccharide; RAGE, receptor for advanced glycation end products; TNF, tumor necrosis factor.

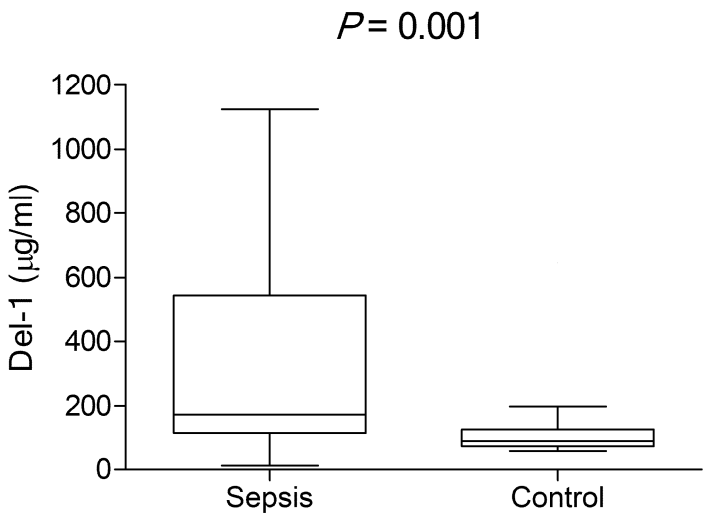

Fig. 9. Comparison of serum Del-1 levels in patients with sepsis and those in controls. The boxplots show the median with the 25th and 75th percentiles. The whiskers show the 5th and 95th percentiles (n = 84 in the sepsis group and n = 20 in the control group). The *P*-value indicates the result of the Mann-Whitney *U* test. Del-1, developmental endothelial locus-1.

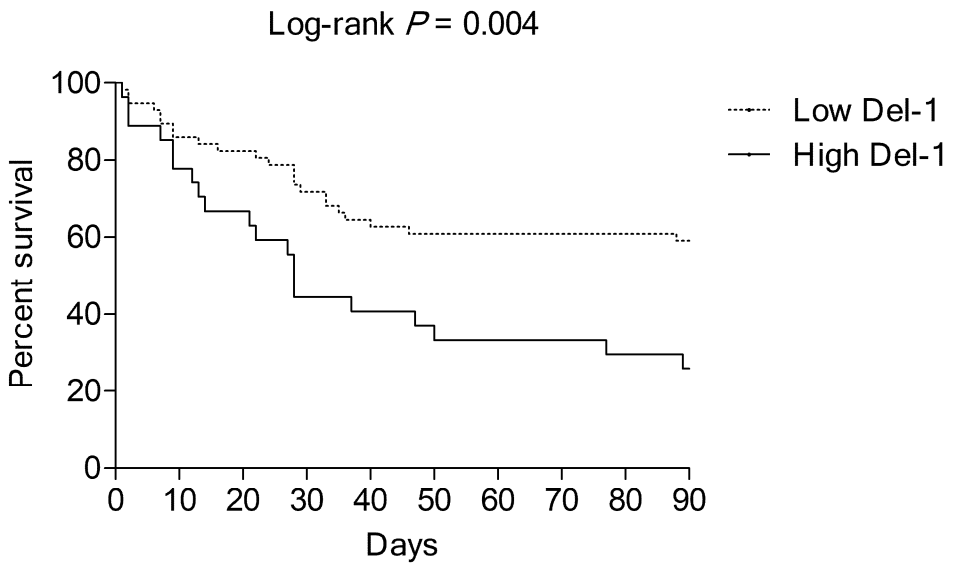

Fig. 10. Kaplan-Meier survival curves for the low Del-1 and high Del-1 groups. Del-1, developmental endothelial locus-1.

DISCUSSION

The main findings of the present study are as follows. First, shedding of the endothelial glycocalyx and washout of Del-1 were observed in an animal model of sepsis. Second, serum Del-1 was increased in the animal model according to the severity of sepsis, and particularly so in animals with non-pulmonary sepsis. Third, serum Del-1 was higher in septic patients than in controls, and high serum Del-1 was associated with more severe sepsis and higher mortality. This study confirms and expands the findings of previous reports suggesting that Del-1 plays an important role in both acute and chronic inflammation.[22,24-27)]

Several pathophysiologic processes have been associated with structural and functional derangement of the glycocalyx. Rehm et al. showed an increase of the main components of the glycocalyx, syndecan-1 and heparan sulfate, in the blood of vascular surgical patients with global or regional ischemia.[37)] Bruegger et al. described similar findings in the arterial blood of patients undergoing coronary artery bypass surgery.[38)] The strong association between degradation of the glycocalyx and disease may exist for sepsis, given that increased serum syndecan-1 levels in patients with sepsis have been found to be positively correlated with increased mortality.[39,40)] The present study demonstrated that together with membrane-bound molecules such as glycocalyx and heparan sulfate, Del-1 forms the endothelium. In the septic condition, the expression of these molecules is less evident, suggesting shedding of the glycocalyx. Moreover, serum Del-1 increased with increasing severity of sepsis and was associated with the prognosis. Endothelial tight junctions are critical for vascular integrity (or permeability) and maintenance of vascular function.[41)] Dysfunction of these junctions occurs in response to a variety of inflammatory stimuli and also during ischemia, leading to tissue edema and damage. In this study, the expressions of zonula occludens-1, occludin-1, and claudin-1, the proteins that form tight junctions between endothelial cells,[42-44)] were also decreased after 24 h of CLP (data not shown). These findings are consistent with the results of the aforementioned studies, supporting the notion that endothelial dysfunction has a prominent role in the pathophysiology of sepsis.

The inflammatory response contributes to life-threatening multiorgan dysfunction in the course of sepsis.[17] As shown in Fig. 11, Del-1 acts as a gatekeeper of inflammation by inhibiting LFA-1-dependent adhesion of leukocytes to the endothelium. Several studies have demonstrated that pro-inflammatory stimuli (e.g., LPS, TNF-α, and IL-17) lead to increased expression of inflammation-promoting adhesion receptors (e.g., LFA-1, Mac-1, and ICAM-1) and to reduced expression of inflammation-inhibiting signals (e.g., Del-1).[22-25,27,45] In this study, there was a decrease in Del-1 expression in various tissues in septic mice when compared to control mice. This finding is also consistent with previous reports,[22,24-27,45] supporting the notion that expression of Del-1 in the tissues decreases in the course of inflammation. Interestingly, Del-1 expression decreased in the adrenal gland tissue from septic mice. Pro-inflammatory cytokine-induced adrenal Del-1 deficiency may promote accumulation of leukocytes in the adrenal gland and subsequently affect adrenal function in the course of sepsis.[25] The Prevalence of adrenal gland dysfunction can be as high as 50%–60% in septic shock and has a high mortality rate.[46,47] Therefore, disruption of endothelial homeostasis in the adrenal gland and subsequent adrenal insufficiency may have contributed to the increased mortality for patients with high serum Del-1 in the present study. However, confirmatory studies are required.

Although there are several reports on expression of Del-1 in body tissues,[22,24-27] there are no reports on Del-1 levels in blood. The issues concerning tissue biomarkers include the problems of obtaining repeated samples and their cost. Unlike other tissues, blood samples can be easily obtained. In this study, Del-1 was found to be increased in blood depending on the severity of sepsis. The present study demonstrated that disruption and shedding of the glycocalyx during sepsis may liberate Del-1 from endothelial cells and the extracellular matrix, allowing it to enter into the bloodstream and resulting in an increased serum Del-1 level. This finding is concordant with previous observations that serum levels of syndecan-1 and circulating glycosaminoglycans, which are glycocalyx degradation markers, are increased in patients with critical illness.[40,48,49] Further investigations in this regard are warranted.

To further translate the current results from animals to humans, blood samples were obtained from critically ill patients with sepsis and from healthy controls. The data showed that serum

Del-1 was significantly higher in the patients with sepsis than in the control subjects. Moreover, high serum Del-1 was associated with more severe disease, organ dysfunction, and mortality. Interestingly, a higher DIC score, a lower platelet count, and a lower prothrombin time (%) were observed in patients with high serum Del-1. Although it was not possible in this observational study to determine an association between coagulopathy and a high serum Del-1 level, there are several possible mechanisms. Formation of microparticles accompanied by externalization of phosphatidylserine has an essential procoagulant role,[50] and Del-1 participates in the clearance of phosphatidylserine-expressing microparticles.[51] Therefore, endothelial damage causing Del-1 depletion (indicated by a high Del-1 level in blood) may contribute to the procoagulant state, with pathologically increased generation of microparticles. Formation of leukocyte-platelet aggregates is accompanied by increased expression of inflammatory genes and procoagulant effects.[52] Del-1 also inhibits formation of leukocyte-platelet aggregates by blocking the interaction between Mac-1 and glycoprotein Ib.[53] Finally, shedding of the glycocalyx alone causes coagulopathy in patients with severe trauma and hemorrhagic shock.[54] Overall, these findings indicate that endothelial dysfunction may be associated with the severity of sepsis and, possibly, with sepsis-induced coagulopathy.

The present study showed that Del-1 levels in serum and BAL fluid increased steadily in the CLP mice, suggesting that degradation of the endothelial glycocalyx may be more prominent in the pathophysiology of non-pulmonary sepsis. Moreover, high serum Del-1 was more common in patients with non-pulmonary sepsis than in those with pulmonary sepsis, and the association between serum Del-1 level and mortality became more pronounced when only the patients with non-pulmonary sepsis were analyzed. These findings are consistent with previous reports that levels of biomarkers of endothelial injury, including heparan sulfate fragments, angiopoietin-2, von Willebrand factor antigen, and syndecan-1, are higher in patients with acute respiratory distress syndrome caused by non-pulmonary sepsis[48,49,55] and support the notion that Del-1 can be a useful biomarker of endothelial dysfunction. Similarly, serum levels of syndecan-1, ICAM-1, IL-17, IL-6, and TNF-α, which are markers of endothelial injury and inflammation, were higher in mice with non-pulmonary sepsis. Of note, RAGE, which reflects lung epithelial injury, was also increased in blood of non-pulmonary sepsis model. Apart from

being a pattern recognition receptor expressed at highest levels on alveolar type 1 epithelial cells,[56] RAGE also has an important role in the innate immune response.[57] Due to RAGE's pro-inflammatory role in innate immunity, it may be less useful in discrimination of pulmonary from non-pulmonary sepsis.

The association of high serum Del-1 with sepsis might have implications for the diagnosis and treatment of the disease. The diagnosis of sepsis is based on clinical criteria and does not rely on knowledge of the underlying pathophysiology.[1] Therefore, current definitions identify a very heterogeneous group of patients with sepsis that may have different responses to therapy. For instance, Famous et al. identified distinct subphenotypes of acute respiratory distress syndrome that responded differently to fluid management.[58] The present study findings provide further evidence of the heterogeneity of sepsis in animals and humans, as defined by the extent of disruption of the glycocalyx. If these preliminary results can be validated in the future, serum Del-1 levels might be used to identify subgroups of patients with sepsis for therapy targeted at protection or restoration of the glycocalyx. Several studies in animal models of hemorrhagic shock or ischemia-reperfusion injury have shown that administration of fresh frozen plasma, sevoflurane, or hydrocortisone reduces shedding of the glycocalyx.[59-61] Soluble Del-1 has potential as a treatment for neutrophil-mediated inflammatory diseases. Previous studies showed that administration of Del-1 efficiently reversed the increased neutrophil infiltration[22,24,27] and blocked the activation of coagulation and the generation of platelet-monocyte aggregates.[53] A recent study identified Del-1 to have a role in the induction and maintenance of granulopoiesis.[62] This may be important in patients with sepsis, given that neutropenia is a major cause of morbidity and mortality. Del-1 may be a promising approach for the diagnosis and treatment of sepsis but requires further study.

This study has several limitations. First, although the association between high serum Del-1 and severity of sepsis was robust, it does not fully elucidate the mechanisms via which endothelial dysfunction leads to increased serum Del-1, organ dysfunction, and mortality. However, it is plausible that circulating Del-1 at least partly originate from shedding of the endothelial glycocalyx. This hypothesis is supported by the present study finding that near complete degradation of the glycocalyx was accompanied by less Del-1 expression in the

endothelium in septic mice. Second, the study had a retrospective design, which increases the risk of selection bias, and the low power of the study because of the relatively small sample size is likely to be responsible for some of the non-significant results. Third, it is possible that the low and high Del-1 groups were not similarly treated, although no differences were observed in the resuscitation and infection goals achieved between the groups.

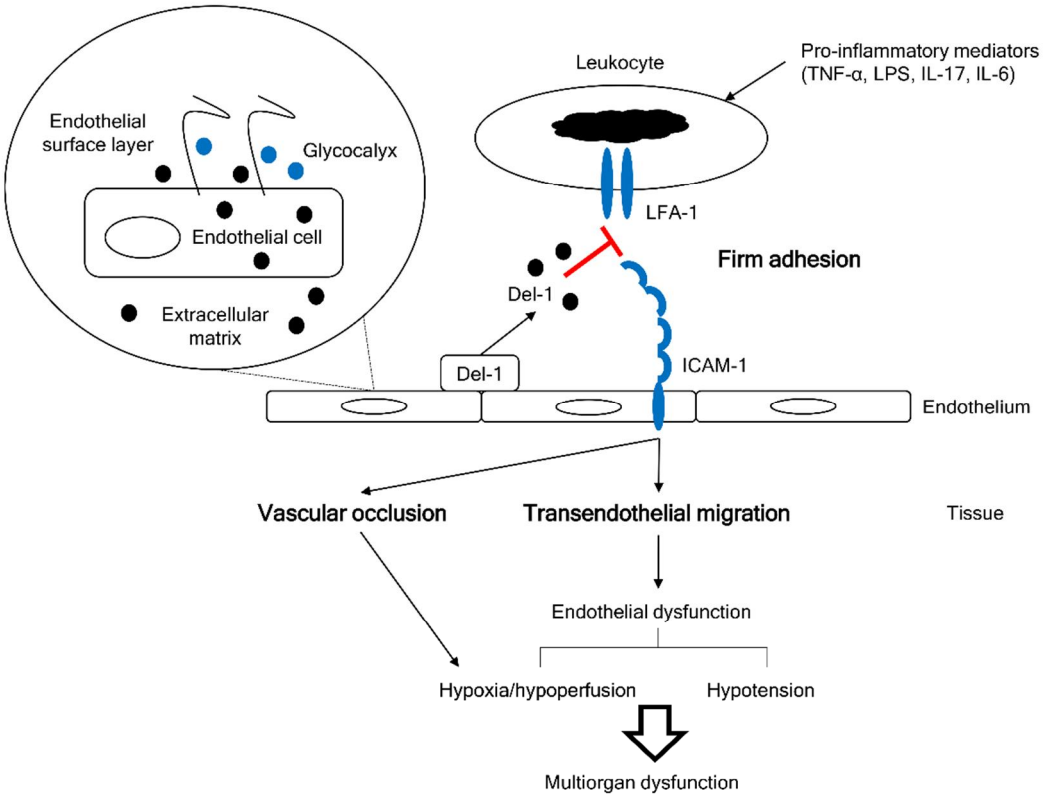

Fig. 11. Proposed mechanism by which Del-1 acts as a gatekeeper for leukocyte-mediated organ dysfunction in sepsis. Del-1 is distributed in the endothelial surface layer, endothelial cell, and extracellular matrix. Del-1, developmental endothelial locus-1; ICAM, intercellular cell adhesion molecule; IL, interleukin; LFA, lymphocyte function antigen; LPS, lipopolysaccharide; TNF, tumor necrosis factor.

CONCLUSION

This study investigated whether serum Del-1 levels increase with increasing severity of sepsis. Shedding of the glycocalyx and washout of Del-1 were observed in an animal model of sepsis. Serum Del-1 was increased according to disease severity, particularly in animals with non-pulmonary sepsis. The serum Del-1 level was increased in humans with sepsis, and high serum Del-1 was associated with more severe sepsis, organ dysfunction, and mortality. Therefore, serum Del-1 could be a useful biomarker of endothelial dysfunction, sepsis, and sepsis-induced organ dysfunction. Further studies are required to assess whether administration of Del-1 could prevent or treat sepsis.

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국문 초록

동물 및 인간 패혈증 모델에서 혈청 Developmental endothelial locus-1 (Del-1)과 중증도 및 예후와의 상관성

의학과 김 원 영

연구배경: 내피세포에 존재하는 glycocalyx의 파괴는 패혈증의 주 병태생리 중 하나이며 Develpmental endothelial locus-1 (Del-1)은 내피세포에서 유래한 항염증인자이다. 패혈증 시 glycocalyx의 분해가 일어남으로써 혈청 Del-1 농도가 증가할 수 있다. 본 연구는 혈청 Del-1 농도와 패혈증의 중증도 및 예후와의 상관성을 조사하였다.

대상 및 방법: 6주령의 수컷 C57BL/6 생쥐를 마취 후 cecal ligation and puncture (CLP)를 시행하여 non-pulmonary 패혈증 모델을 유도하였고 lipopolysaccharide (LPS)를 기관 내 투여하여 pulmonary 패혈증 모델을 유도하였다. CLP군과 대조군에서 면역조직화학염색 후 내피세포 내 glycocalyx 및 Del-1의 발현을 관찰하였다. 또한, 패혈증 시 여러 조직에서의 Del-1 발현을 real-time polymerase chain reaction 방법으로 정량화하였고 대조군과 비교하였으며 CLP 및 LPS군에서 혈청 Del-1과 기타 바이오마커를 효소결합면역흡착검사를 이용하여 측정하였고 대조군과 비교하였다. 마지막으로, 84명의 패혈증 또는 패혈증 쇽 환자와 20명의 건강 대조군에서 혈청 Del-1 농도를 비교하였다.

결과: CLP 시행 24시간 후, 내피세포의 glycocalyx는 거의 완전 분해되었고 대조군에 비해 내피세포 내 Del-1의 발현이 감소하였다. 패혈증 시 여러 조직에서 Del-1 발현은 감소하였고 혈청 Del-1 농도는 패혈증의 중증도에 따라 유의하게 증가하였다. LPS 투여 후 혈청 Del-1, syndecan-1, intercellular adhesion molecule-1, receptor for advanced glycation end products, interleukin-17, interleukin-6 및 tumor necrosis factor-α 농도는 1시간째 급격히 증가하였다가 48시간째 기저 수준으로 회복된 반면 CLP 유도하 패혈증에서는 이들 농도가 꾸준히 증가하였다. 패혈증 환자의 혈청 Del-1 농도의 중앙값은 건강 대조군에 비해 유의하게 높았고 (174.0 μg/ml vs.

88.2 μg/ml; $P < 0.001$) 높은 Del-1군 (혈청 Del-1 ≥ 375.96 μg/ml) 환자들이 낮은 Del-1군 (혈청 Del-1 < 375.96 μg/ml) 환자들에 비해 질병 중증도 점수 및 장기 부전의 빈도가 높았으며 90일 사망률이 높았다 (74% vs. 40%; $P = 0.004$). 다변량 분석에서 높은 혈청 Del-1은 90일 사망을 예측하는 경향을 보였다 (adjusted odds ratio, 1.87; 95% confidence interval, 0.995–3.50; $P = 0.052$).

결론: 패혈증에서 혈청 Del-1 농도는 증가하였고 질병 중증도, 장기 부전 및 사망률과 관련이 있었다. 내피세포의 glycocalyx 분해는 non-pulmonary 패혈증에서 더 두드러질 수 있다. Del-1은 패혈증의 진단을 위한 유용한 접근법일수 있으며 보다 많은 연구가 필요하다.