細菌性肺炎におけるプロテオグリカンSyndecan-4の役割

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Serum Syndecan-4 as a Possible Biomarker in Patients with Acute Pneumonia

（急性肺炎患者におけるバイオマーカーとしての血清 syndecan-4 の可能性）

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ABSTRACT

Background: Syndecan-4 is a transmembrane heparan sulfate proteoglycan expressed in a variety of cells, and glycosaminoglycan side chains of syndecan-4 bind to several proteins, suggesting several biological functions. However, the role of syndecan-4 in acute bacterial pneumonia has not yet been elucidated.

Methods: Serumsyndecan-4 levels weremeasured in patients with acute pneumonia, and the relationships between serum syndecan-4 levels and clinical parameters were analyzed. Next, we treated wild-type and syndecan-4–deficient mice with Streptococcus pneumoniae intranasally and analyzed the phenotype of syndecan-4–deficient mice.

Results: In the patients with acute pneumonia, serumsyndecan-4 levels were significantly higher than in the healthy volunteers and correlated negatively with the pneumonia severity score. In addition, in patients who improved with short-term antibiotic therapy, serum syndecan-4 levels were higher on admission and gradually increased during antibiotic therapy. Furthermore, in syndecan-4–deficient mice, the survival rate was significantly worse, and total neutrophil counts in bronchoalveolar lavage fluid, bacterial counts in blood, and plasma levels of inflammatory cytokines were significantly higher than in wild-type mice.

Conclusions: These results suggest that syndecan-4 has an anti-inflammatory function in acute pneumonia and could serve as a useful biomarker in these patients.

ABBREVIATION LIST

ADAM17 = a disintegrin and metalloproteinase 17

BAL = bronchoalveolar lavage

CFU = colony forming unit

CRP = C-reactive protein

HSPG = heparan sulfate proteoglycan

GAG = glycosaminoglycan

LPS = lipopolysaccharide

MMP = matrix metalloproteinase

Sdc4 KO = syndecan-4 deficient

WBC = white blood cell count

WT = wild-type

INTRODUCTION

Bacterial pneumonia continues to have a high mortality rate worldwide, with approximately 320 million persons dying each year from viral and bacterial pneumonia, making it the third most frequent cause of death in the world in 2011 [1]. Streptococcus pneumoniae is the most common cause of acute pneumonia, accounting for more than 25% of all cases of bacterial pneumonia. Both bacterial and host factors are involved in the pathogenesis of acute pneumonia. The bacterial factors include capsular polysaccharides, cell wall polysaccharides, and cell surface or intracellular proteins/enzymes. These pathogenic factors are recognized by the innate immune systems, leading to production of proinflammatory mediators and causing exaggerated inflammation and lung injury [2, 3].

Proteoglycans are glycoproteins consisting of a core protein with glycosaminoglycan (GAG) side chains. Several types of proteoglycans exist in the lung as components of extracellular matrices and were previously recognized only as a “simple glue” that provided structural support to tissues. However, growing evidence suggests that proteoglycans have a variety of biological activities and are involved in inflammation, wound healing, development, and homeostasis [4–10]. Heparan sulfate is the most abundant GAG in healthy lungs, and heparan sulfate proteoglycans (HSPGs) play a key role in tissue inflammation. Syndecan is one of the HSPGs and consists of 4 isoforms. Syndecan-1, -2, and -3 specifically are expressed on surfaces of epithelial cells or plasma cells, fibroblasts or endothelium, and nerve cells, respectively. On the other hand, syndecan-4 is expressed on a variety of cells, including alveolar macrophages, epithelial cells, endothelial cells, and fibroblasts [7, 11–14]. Syndecans are also reported to be cleaved from cell surfaces by matrix metalloproteinase 7, metalloproteinase 9, or a disintegrin and metalloproteinase 17 (ADAM17) [15–18] and exist as soluble forms. Heparan sulfate GAG side chains of syndecans bind to various cytokines, chemokines, and growth factors and mediate their biological activities [4, 7, 19, 20].

Previous work by Tanino et al [21] shows that the treatment of syndecan 4–deficient mice (*Sdc4* knockout [KO]) with intratracheal lipopolysaccharide (LPS) significantly increases the recovery of neutrophils and CXC chemokines (Keratinocyte Chemoattractant [KC] and macrophage inflammatory protein [MIP]-2) in bronchoalveolar lavage (BAL) fluid compared with results in wild-type (WT) mice. Although these results suggest that syndecan-4 has an important role in acute lung inflammation [21], the role of syndecan-4 in bacterial pneumonia is not known. It is also not clear whether changes in syndecan-4 levels in biological samples occur in humans with bacterial pneumonia. The goal of this study was to determine whether syndecan-4 actually has a protective role in acute bacterial pneumonia.

MATERIALS AND METHODS

Subjects

We reviewed the records and clinical course of 30 patients with acute pneumonia who had been admitted to our hospital. The patients were evaluated for clinical characteristics and laboratory data on the first day of their admission and treated with suitable intravascular antibiotics. Healthy controls were recruited voluntarily in our study group. The study was conducted after receiving approval of the Fukushima Medical University Ethics Committee.

Measurement of Syndecan-4

Syndecan-4 levels in human and mice were measured using commercially available kits (IBL and Wuhan Huamei Biotech), according to the manufacturers' protocols.

Analysis of Serum Syndecan-4 and Clinical Parameters

At first, serum syndecan-4 levels measured in the patients with acute pneumonia at admission were compared with those in the healthy volunteers. Thereafter, the relationship between serum syndecan-4 levels and clinical parameters (white blood cell count, C-reactive protein level, pneumonia severity score [A-DROP score], and clinical course) in the patients with acute pneumonia were analyzed as described elsewhere [22]. The A-DROP scoring system uses a 6-point scoring scale (0–5) including the following parameters: (1) age (≥70 years in male and ≥75 years in female subjects), (2) dehydration (serum urea nitrogen ≥21 mg/dL), (3) respiratory failure (arterial oxygen saturation ≤90% or partial pressure of oxygen, arterial, ≤60 torr), (4) orientation disturbance (confusion), and (5) low blood pressure (systolic blood pressure ≤90 mm Hg). The scoring system was proposed by the Japanese Respiratory Society and was reported to correlate significantly with the Pneumonia Severity Index of the Infectious Disease Society of America [23–25]. The score ranges from 0 to 5, and higher scores indicate worse clinical status.

Analysis of Serum Syndecan-4 Levels and Clinical Course

We evaluated the changes in serum syndecan-4 levels (days 1, 8, and 15) during the course of antibiotic therapy in the patients with acute pneumonia and analyzed the relationship to severity scores and treatment effects. A-DROP and duration of intravenous antibiotic therapy were used for evaluating severity scores and treatment effects, respectively. Death during the hospital stay was considered the worst prognosis.

Syndecan-4 Deficient Mice

WT and *Sdc4* KO mice (provided by T. Kojima, MD, PhD, University of Nagoya) were maintained under specific pathogenfree conditions [26]. To confirm *Sdc4* messenger RNA (mRNA) expression in lung tissues of WT and *Sdc4* KO mice, reversetranscription polymerase chain reaction (PCR) was performed using the following primers, as described elsewhere [27]: forward, 5′-CGAGAGACTGAGGTCATCGAC-3′; reverse, 5′- GCGGTA GAACTCATTGGTGG-3′.

Preparation of Bacteria

Streptococcus pneumoniae D39 (the National Collection of Type Cultures [NCTC 7466]) stocked in 10% skimmed milk at −80°C was inoculated onto blood agar, followed by incubation for 20 hours in a carbon dioxide incubator at 37°C, and the colonies were collected and suspended in brain-heart infusion broth (Nikken Biomedical Laboratory), as described elsewhere [28]. With reading at 600 nm, the number of bacteria in the solution was calculated as 1×10^6 colony-forming units (CFUs)/μL at an optical density of 38, and we prepared bacterial solution of the adequate density for further studies.

Mouse Model of Acute Bacterial Pneumonia

Mice were instilled with live *S. pneumoniae* intranasally, as described elsewhere [28]. Mice were anesthetized with pentobarbital, and 20 μL of bacteria solution (5×10^6 CFUs per mouse) in brain-heart infusion broth was pipetted onto the nose of each mouse. In the preliminary experiment, survival of WT mice was assessed using various doses of *S. pneumoniae*. Survival rates at 7 days after *S. pneumoniae* instillation of 3.3×10^6, 6.6×10^6, and 1.0×10^7 CFUs were 100%, 42.9%, and 20.0%, respectively. From these results, we decided to instill 5.0×10^6 CFUs of *S. pneumoniae* for this study.

After determining the dosage, we first injected *S. pneumonia* intranasally into WT mice to evaluate the expression of syndecan-4. Next, we compared survival rates between the WT and *Sdc4* KO mice. Furthermore, to clarify the role of syndecan-4 in this model, we performed BAL in the left lungs and obtained right lungs and plasma 24 hours after instillation. The Animal Research Committees of Fukushima Medical University approved all animal experiments.

Isolation of RNA and Measurement of mRNA

RNA was isolated with the Absolutely RNA Miniprep Kit (Stratagene). Genomic DNA was digested with DNase I (Ambion), and RNA was reverse transcribed with the High Capacity cDNA Archive Kit (Applied Biosystems), as described elsewhere [21]. Quantitative PCR was performed using Power SYBR Green PCR Master Mix and an ABI PRISM 7000 (Applied Biosystems). The threshold cycle was calculated using threshold cycles for the target genes and 18S. Relative mRNA expression was expressed as fold increase over values obtained from RNA from normal lungs, untreated cells, or human reference total RNA (Stratagene).

Measurement of the Levels of Total Protein and Inflammatory Cytokines

The levels of total protein in BAL fluid were measured using the BCA Protein Assay Kit (Thermo Scientific). Multiplex Luminex assay (mouse cytokine/chemokine magnetic bead panel; EMD Millipore) was used to measure the levels of inflammatory cytokines (tumor necrosis factor α, interleukin 1β and 6, KC, and MIP-2) in BAL fluid and plasma, according to the manufacturer’s protocol.

Analysis of Bacterial Counts

Analysis of bacterial counts in lung tissues and blood was performed as described elsewhere [28]. Briefly, after collection of the right lungs, they were weighted and homogenized in 900 µL of brain-heart infusion broth. Viable bacteria counts were determined by inoculating the serial dilution of the lung homogenates on blood agar. Viable bacteria counts of blood were analyzed in the same way for lung homogenates.

Pathological Evaluation of Lung Sections

For pathological evaluation, the lungs were excised by opening the chest. The lungs were fixed by inflation at 25 cm H2O with a phosphate buffer (10 mmol/L; pH 7.4) containing 10% formalin for 24 hours and then embedded in paraffin. A 5-µm-thick tissue section was prepared from the midportion of paraffinembedded whole lung tissue and stained with hematoxylineosin, as described elsewhere [29].

Statistical Analysis

Data are expressed as means with standard errors of the mean (SEM), unless otherwise stated. We used t tests to compare syndecan-4 levels between the 2 groups. Pearson’s correlation coefficient was used to analyze correlations between serumsyndecan-4 levels and clinical parameters. Analysis of variance was used to analyze the time course of serum syndecan-4 levels. Survival curves were made using Kaplan–Meier methods, and survival rates were analyzed with the log-rank test. Unless otherwise indicated, theMann–Whitney U test or analysis of variance with Fisher’s least significant difference was used to compare the groups. Differences were considered statistically significant at P

< .05.

RESULTS

Relationship between Serum Syndecan-4 Levels and Clinical Parameters

Table 1 shows clinical characteristics of the patients with acute bacterial pneumonia (n = 30) in this study. Their mean age was 67.1 years old. Laboratory data showed increased inflammatory response, although A-DROP was relatively low. The causative organism was identified in 19 of the patients (S. pneumoniae [5], Haemophilus influenzae [6], Moraxella catarrhalis [2], Klebsiella pneumoniae [1], Enterococcus faecalis [1], Mycoplasma pneumoniae [1], and anaerobic bacteria [3]). However, the causative agent was not determined in the remaining 11 patients. Serum syndecan-4 levels at admission were significantly higher in all the patients (mean [SEM], 20.3 [8.9] ng/mL) compared with the healthy volunteers (15.1 [2.6] ng/mL) (Figure 1A).

In the patients with acute pneumonia, there was a significant negative correlation between serum syndecan-4 levels and the A-DROP score (Figure 1B) but not age ($r = -0.184$; $P = .33$), white blood cell count ($r = -0.017$; $P = .58$), C-reactive protein level ($r = -0.032$; $P = .62$), or duration of hospitalization ($r = -0.175$; $P = .36$).

When serum syndecan-4 levels were evaluated according to severity of acute pneumonia, the patients with mild pneumonia (A-DROP score, 0–1) showed significantly higher serum syndecan-4 levels (mean [SEM], 24.7 [9.2] ng/mL) compared with the healthy volunteers (15.1 [2.6] ng/mL). Interestingly, however, there was no difference in serum syndecan-4 levels between the patients with moderate or severe pneumonia (A-DROP 2–5; 14.5 [4.0] ng/mL) and the healthy volunteers (Figure 1C).

Serum Syndecan-4 Levels and Clinical Outcome

We next investigated the relationship between serum syndecan-4 levels and clinical course after admission. Serum syndecan-4 levels in the patients who improved with short-term antibiotic therapy (<14 days) were significantly higher (mean [SEM], 20.7 [9.9] ng/mL) than in the healthy volunteers (15.1 [2.6] ng/mL); however, in the patients who required long-term antibiotic therapy (≥14 days), serum syndecan-4 levels (19.3 [6.9] ng/mL) were not different from those in the healthy subjects (Figure 1D).

In addition, we evaluated the relationship between the time course of serum syndecan-4 levels after admission and duration of antibiotic therapy. Because this study was retrospectively performed, no specific protocol regarding completion of antibiotic therapy had not been prepared. The practicing physicians decided whether antibiotic therapy should be continued or stopped by evaluating clinical parameters, such as vital signs, oxygenation, blood inflammatory markers, and chest images. In the patients who improved with short-term antibiotic therapy (<14 days), serum syndecan-4 levels gradually increased after admission (mean [SEM], 14.2 [5.6], 16.4 [7.6], and 23.0 [11.2] ng/mL on days 1, 8, and 15, respectively) (Figure 2). In contrast, serum syndecan-4 levels remained at similar levels after admission in the patients who required long-term antibiotic therapy (≥14 days) (15.3 [3.3], 14.6 [6.6], and 14.8

[1.4] ng/mL on days 1, 8, and 15, respectively). There was no difference in an initial A-DROP severity score and positive rate of causative pathogens between short- and long-term antibiotic therapy groups.

Phenotype of Syndecan-4–Deficient Mice in Acute Bacterial Pneumonia

The results from the patients with acute pneumonia suggest that syndecan-4 has an anti-inflammatory property. To clarify the role of syndecan-4 in acute pneumonia, we used a murine model of acute bacterial pneumonia. At first, we instilled live *S. pneumoniae* intranasally into WT mice to evaluate the expression of syndecan-4 in the lungs and plasma. At 24 hours after instillation, mRNA expression and plasma levels of syndecan-4 in the WT mice were significantly higher than in the control mice (Figure 3).

After we confirmed the lack of *Sdc4* expression in *Sdc4* KO lungs (data not shown), we instilled *S. pneumoniae* intranasally into the WT or *Sdc4* KO mice and compared the survival rates. As shown in Figure 4, the *Sdc4* KO mice had a significantly higher mortality rate than the WT mice.

To clarify the mechanism responsible for the higher mortality rate in the *Sdc4* KO mice, we evaluated the extent of inflammation and bacterial burden in the lungs and blood 24 hours after infection. Although bacterial counts in lung tissues did not differ between the 2 groups, total cell and neutrophil counts in BAL fluid were significantly higher (Figure 5A), and pulmonary inflammation was more pathologically severe (Figure 5B) in the *Sdc4* KO mice than in the WT mice. Blood bacterial counts were also higher in the *Sdc4* KO mice than in the WT mice (Figure 5C). Furthermore, analysis of inflammatory cytokines showed that the plasma levels of interleukin 6, MIP-2, and KC were significantly higher in the *Sdc4* KO mice than in the WT mice (Figure 6). On the other hand, the levels of inflammatory cytokines in BAL fluid did not differ between the 2 groups (data not shown).

DISCUSSION

We have demonstrated 5 major findings in this study: (1) patients with acute pneumonia had significantly higher serum syndecan-4 levels than healthy volunteers; (2) there was a negative correlation between serum syndecan-4 levels and severity score and significantly higher serum syndecan-4 levels in patients with acute pneumonia whose severity score was low or who improved with short-term antibiotic therapy; (3) there was a gradual increase of serum syndecan-4 levels in patients who improved with short-term antibiotic therapy; (4) the mortality rate was significantly higher in *Sdc4* KO mice than in WT mice after intranasal instillation of S. pneumoniae; and (5) *Sdc4* KO mice had more severe pulmonary and systemic inflammation with higher blood bacterial counts than WT mice. Taken together, these findings suggest that syndecan-4 has a protective role in acute bacterial pneumonia.

Syndecan-4 is an HSPG expressed on surfaces of a variety of cells and consists of a core protein and heparan sulfate GAG side chains, which bind several cytokines and growth factors, leading to a variety of biological activities [7, 11, 13]. Ishiguro et al [30] reported that syndecan-4 expression in vascular endothelial cells and monocytes was up-regulated in WT mice after intraperitoneal LPS injection, and mortality rates were significantly worse in *Sdc4* KO mice than in the WT mice. Tanino et al [21] reported elsewhere that *Sdc4* mRNA expression was significantly increased in lungs of mice treated with LPS and showed that the lack of syndecan-4 resulted in significantly more pulmonary inflammation and injury. Although these results suggested that syndecan-4 was involved in the pathogenesis of acute pulmonary inflammation, there was no previous information about the role of syndecan-4 in humans with acute pneumonia.

Our findings of elevated serum syndecan-4 levels in patients with acute pneumonia are consistent with previous reports showing increased *Sdc4* expression in lungs of mice after intratracheal LPS instillation and in murine endothelial cells and monocytes after intraperitoneal LPS injection [21, 30]. Although the precise mechanism of increased syndecan-4 expression in acute pneumonia is not certain, stimulation of Toll-like receptor-2, -4, and -5 was reported to increase syndecan-4 expression via NF-κB in the gastric epithelium [14, 31]. These results suggest that syndecan-4 expression is increased through a stimulation of Toll-like receptors 2 and 4 by bacterial pathogenassociated molecular patterns, such as LPS or peptidoglycan in acute pneumonia.

Syndecan-4 is expressed on the surfaces of several types of cells in membrane-bound forms and can be cleaved from cell surfaces by metalloproteinase 7 or 9 or ADAM17, producing soluble forms [32]. The serum syndecan-4 measured in this study was a soluble form. This study did not show the precise source(s) of serum syndecan-4, but there are several possibilities, including lung tissues and other organs/cells apart from the lungs. Prior studies have shown that LPS stimulation increases syndecan-4 expression in the lungs and several other organs (eg, intestine, kidney, and liver) [21]. Moreover, endothelial cells, neutrophils, and lymphocytes are reported to express syndecan-4 [33]. To clarify the precise source(s) of syndecan-4 in acute pneumonia, further studies need to be conducted.

Interestingly, we found that serum syndecan-4 levels in patients with mild pneumonia, but not severe pneumonia, were higher than in the healthy volunteers. Furthermore, serum syndecan-4 levels gradually increased in patients who improved with short-termantibiotic therapy. These results suggest the possibility that serumsyndecan-4 can be used as a biomarker which predicts the clinical outcome of patients with acute pneumonia. Tanino et al [21] have reported elsewhere that pretreatment of recombinant syndecan-4 inhibits LPS-induced CXCL8 up-regulation in bronchial epithelial cells. We also showed that the mortality rate was significantly worse in the *Sdc4* KO mice than in the WT mice after intranasal bacterial instillation. Furthermore, pulmonary and systemic inflammation was more severe, and blood bacterial counts were significantly higher in *Sdc4* KO mice than in WT mice. These results suggest that syndecan-4might have a previously unrecognized anti-inflammatory effect in acute pneumonia.

Li et al demonstrated that neutrophil migration into the alveolar space was impaired (ie, neutrophils remained in the interstitium and did not advance into the alveolar space) after intratracheal bleomycin instillation in matrilysin-deficient mice compared with WT mice [15]. This was associated with impaired formation of a CXC chemokine gradient because of the lack of syndecan-1 shedding by matrilysin. This study showed the importance of syndecan-1 and CXC chemokine binding for neutrophil migration into the alveolar space in injured lungs [11].Moreover, Xu et al [34] reported that shedded syndecan-1 inhibited pulmonary allergic inflammation by inhibiting T-cell migration by binding to CC chemokines. These results show that binding of syndecan-1 to chemokines contributes to inflammatory cell migration into the lung. In addition, Tanino et al [35] demonstrated that intratracheal instillation of mutant CXCL8, whose binding to GAG was weakened, showed increased migration of neutrophils into the lung compared with WT CXCL8. These data show that the binding of chemokine with GAG has critical roles in neutrophil migration into the lung.

Although the precise mechanism by which syndecan-4 inhibits acute lung inflammation is not clear, the GAG side chains of syndecan-4 expressed on alveolar macrophages, epithelial cells, and in the interstitium may bind to inflammatory mediators and regulate their activities. In previous studies [36–39], *Sdc4* KO mice had higher sensitivity than WT mice to renal damage and sclerosis, hepatic damage, lung injury and fibrosis, and heart rupture after myocardial infarction, as well as showing delayed wound repair of skin [40]. These results suggest that syndecan-4 has important roles in inflammation and repair processes of injured organs. In this study, we showed that the mortality rate was significantly worse in *Sdc4* KO mice after *S. pneumoniae* instillation. In addition, pulmonary and systemic inflammation was more severe in *Sdc4* KO mice.

Interestingly, blood bacterial counts were significantly higher in *Sdc4* KO mice.

Although more severe pulmonary inflammation may lead to higher blood bacterial counts, the lack of syndecan-4 in the lungs might accelerate the movement of bacteria into the systemic circulation. Tanino et al [35] have demonstrated that the binding of CXCL8 to GAG regulated its movement from the alveolar space to systemic circulation, when CXCL8 was instilled into WT mice intratracheally. Because bacterial pathogens such as *S. pneumoniae* bind to heparan sulfate, a part of syndecan-4 [41], the lack of syndecan-4 in the lungs may allow the pathogens to move easily from the alveolar space to systemic circulation. Although further studies are necessary to clarify the exact mechanism, our results in the current study show that syndecan-4 plays a critical role in modulating the inflammatory response in bacterial pneumonia.

It has been demonstrated that intravenous administration of danaparoid, which is composed mainly of HSPG, attenuates LPS-induced proinflammatory cytokine production and organ dysfunction, including acute lung injury, and reduces mortality rates in rats [42, 43]. In addition, a synthesized GAG analogue was reported to inhibit delayed-type hypersensitivity and allergen-induced arthritis [44]. It was also reported that intraperitoneal administration of endogenous glucosamine, used for biosynthesis of GAG, inhibited LPS-induced acute lung injury [45]. Overexpression of *Sdc4* in rats has been reported to reduce the decline of heart function after myocardial infarction [46]. These data suggest that GAGs and proteoglycans, such as syndecan-4, might have therapeutic uses in various inflammatory diseases. Taken together, these observations lead us to conclude that syndecan-4 has a previously unrecognized protective effect in acute bacterial pneumonia and that serum syndecan-4 might be useful as a biomarker to predict the clinical course in patients with acute pneumonia.

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CONTRIBUTIONS

T. N., Y. T., and M. M. contributed to the study design, data acquisition, data analysis, and data interpretation and drafting of the manuscript. X. W. contributed to the study design, data acquisition, and data analysis. S. S., K. M., N. F., Y. S., A. F., M. U., and Y. S. contributed to sample collection and data interpretation. Y. E. contributed to study design, data acquisition, data analysis, and interpretation for animal experiments and drafting of the manuscript. T. K., M. T., K. T., and I. K. contributed to study design, data analysis, and interpretation for animal experiments. C. W. F. contributed to data analysis, data interpretation, and critical revision of the manuscript. All authors approved the final version of the manuscript. M. M. is guarantor of the manuscript.

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FIGURE LEGENDS

Figure 1: *A*, Serum syndecan-4 levels in healthy volunteers (HVs) and patients with acute pneumonia. Serum syndecan-4 levels were significantly higher in patients with acute pneumonia (n=30) than in HVs (n=11) *P=.006. *B*, Correlation of serum syndecan-4 levels with A-DROP score in acute pneumonia. Serum syndecan-4 levels correlated negatively with A-DROP score (r=−0.391; P=.03). *C*, Relationship between serum syndecan-4 levels and severity of acute pneumonia at admission. Serum syndecan-4 levels in patients with mild pneumonia (A-DROP score, 0 or 1; n=17), but not those with moderate or severe

pneumonia (A-DROP score, 2–5; n=13), were significantly higher than in HVs (n=11). **P*=.001 (vs HVs); †*P*<.001 (vs moderate/severe pneumonia). *D*, Relationship between serum syndecan-4 levels and duration of antibiotic therapy. Serum syndecan-4 levels in patients with acute pneumonia who improved with short-term antibiotic therapy (<14 days; n=20), but not in those who required long-term antibiotic therapy (≥14 days; n=10), were significantly higher than in HVs (n=11). **P* =.02 (vs HVs). Values represent means with standard errors of the mean.

Figure 2: Time course of serum syndecan-4 levels in patients with acute pneumonia who improved with short-term antibiotic therapy. Levels were gradually increased in patients with acute pneumonia who improved with short-term antibiotic therapy (<14 days). *P = .005.

Figure 3: Syndecan-4 expression in the lungs after intranasal *Streptococcus pneumoniae* instillation. Messenger RNA (mRNA) expression of syndecan-4 in lung tissues (A) and the levels of syndecan-4 in bronchoalveolar lavage (BAL) fluid (B) were significantly higher in *Sdc4* knockout mice than in wild-type mice 24 hours after instillation. **P*<.05.

Figure 4: Survival of wild-type (WT) and *Sdc4* knockout (KO) mice afterintranasal instillation of *Streptococcus pneumoniae*. The mortality rate in *Sdc4* KO mice was significantly worse than that in WT mice after intranasal instillation of *S. pneumoniae* (5.0 × 106 colony-forming units) (P = .04).

Figure 5: Pulmonary inflammation and bacterial burden in blood and lung tissues. A, Total neutrophils in bronchoalveolar lavage (BAL) fluid was significantly higher in *Sdc4* knockout (KO) mice than in wild-type (WT) mice. B, Pathological findings showed more severe inflammation in *Sdc4* KO mice (×200, Hematoxylin and Eosin Staining). Each photomicrograph is the representative of 4 mice. C, D, Bacterial counts in blood (C), but not lung tissues (D), were significantly higher in *Sdc4* KO mice 24 hours after instillation. **P*<.05. Abbreviations: CFUs, colony-forming units; NS, not significant.

Figure 6: Plasma levels of inflammatory cytokines. *A*, *B*, *C*, Interleukin 6 (IL-6) (A), Keratinocyte Chemoattractant (KC) (B), and macrophage inflammatory protein (MIP)-2 (C) levels were significantly higher in *Sdc4* knockout (KO) mice than in wild-type (WT) mice 24 hours after instillation. *D*, Tumor necrosis factor (TNF) α levels did not differ between the groups. **P*<.05. Abbreviation: NS, not significant.

Table 1. Clinical Characteristics of Healthy Volunteers and Patients with Acute Pneumonia

	Healthy volunteer	Acute pneumonia	P value
Subjects (n)	11	30	
Age (yrs)	50.1 ± 4.8	67.1 ± 3.1*	$P < 0.05$
Gender (M/F)	6/5	20/10	NS
WBC (/µL)	NA	12626 ± 630.4	NS
CRP (mg/dl)	NA	20.1 ± 1.4	NS
A- DROP	0±0	1.57 ± 0.25	$P < 0.05$
PF ratio	NA	1444 ± 241	NS

The A-DROP scoring system is a 6-point scoring scale (0–5) and considers the following parameters: 1) Age (male ≥ 70years, female ≥ 75 years), 2) Dehydration (BUN ≥ 21 mg/dL), 3) Respiratory failure (SaO_2 ≤ 90% or PaO_2 ≤ 60 Torr), 4) Orientation disturbance (confusion) and 5) Low blood pressure (systolic blood pressure ≤ 90 mm Hg). * vs HV,

Figure 1

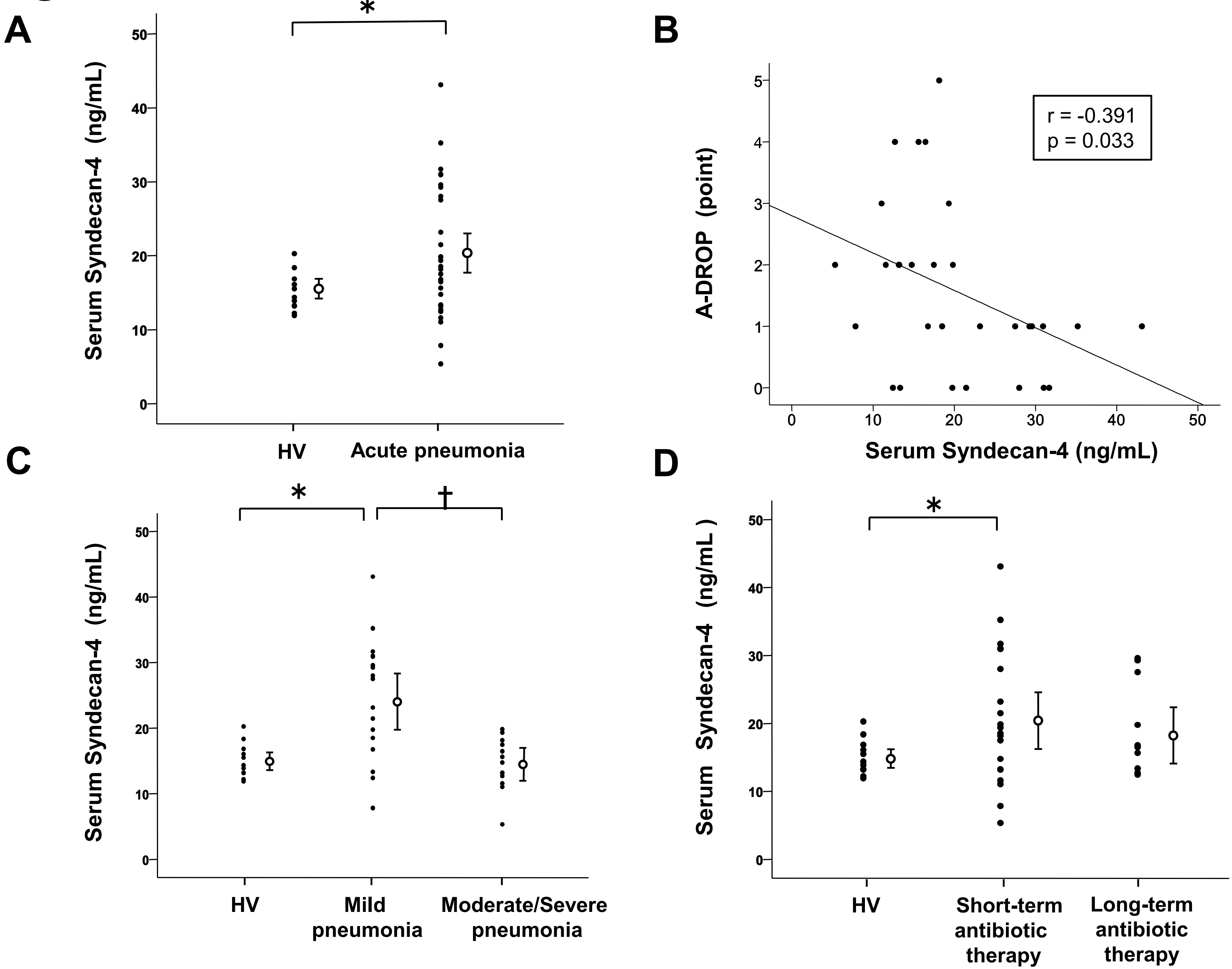

A
*
Serum Syndecan-4 (ng/mL)
0
10
20
30
40
50
HV
Acute pneumonia
B
r = -0.391
p = 0.033
A-DROP (point)
0
1
2
3
4
5
0
10
20
30
40
50
Serum Syndecan-4 (ng/mL)
C
*
†
Serum Syndecan-4 (ng/mL)
0
10
20
30
40
50
HV
Mild pneumonia
Moderate/Severe pneumonia
D
*
Serum Syndecan-4 (ng/mL)
0
10
20
30
40
50
HV
Short-term antibiotic therapy
Long-term antibiotic therapy

Figure 2

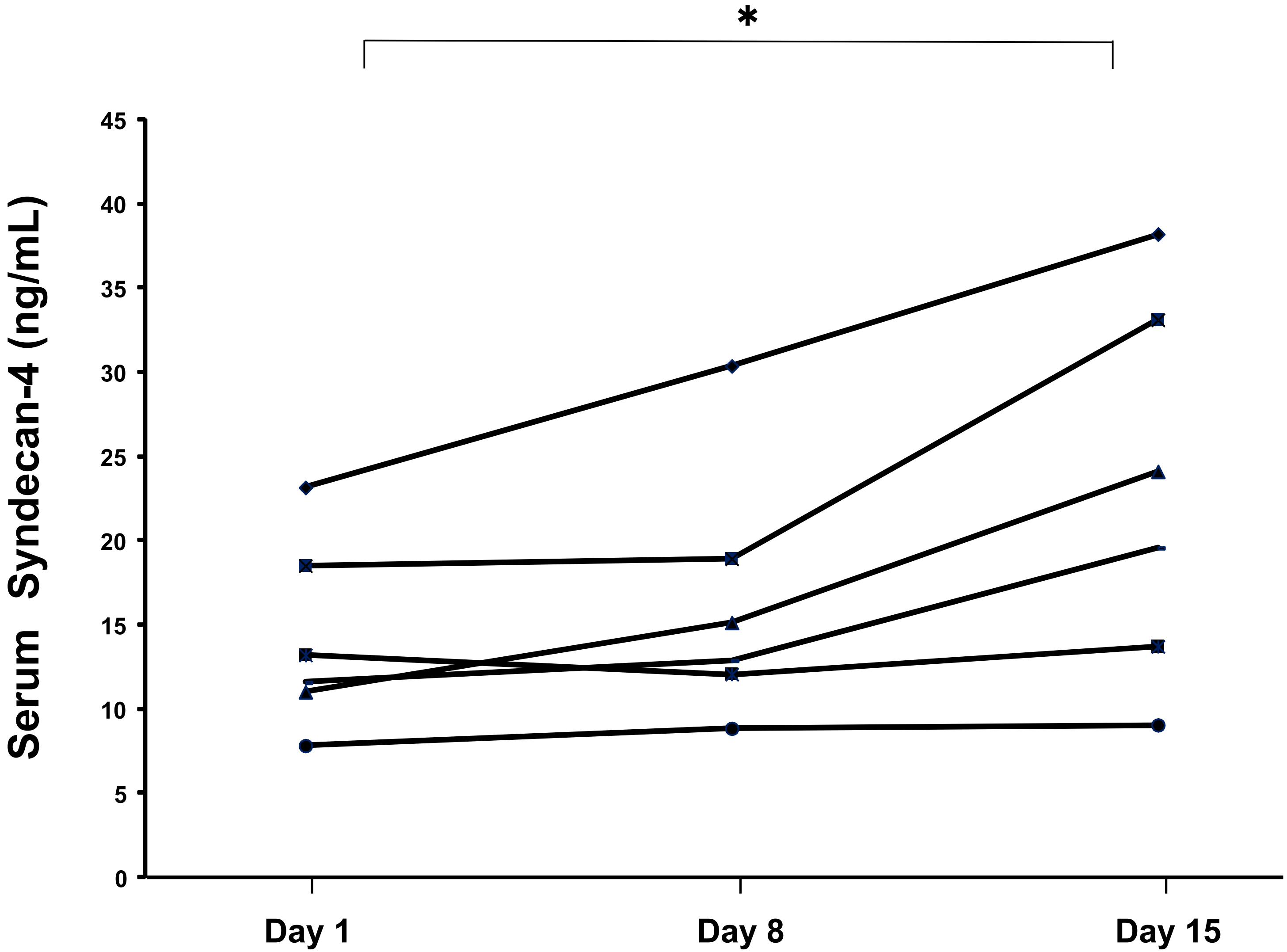


Figure 3

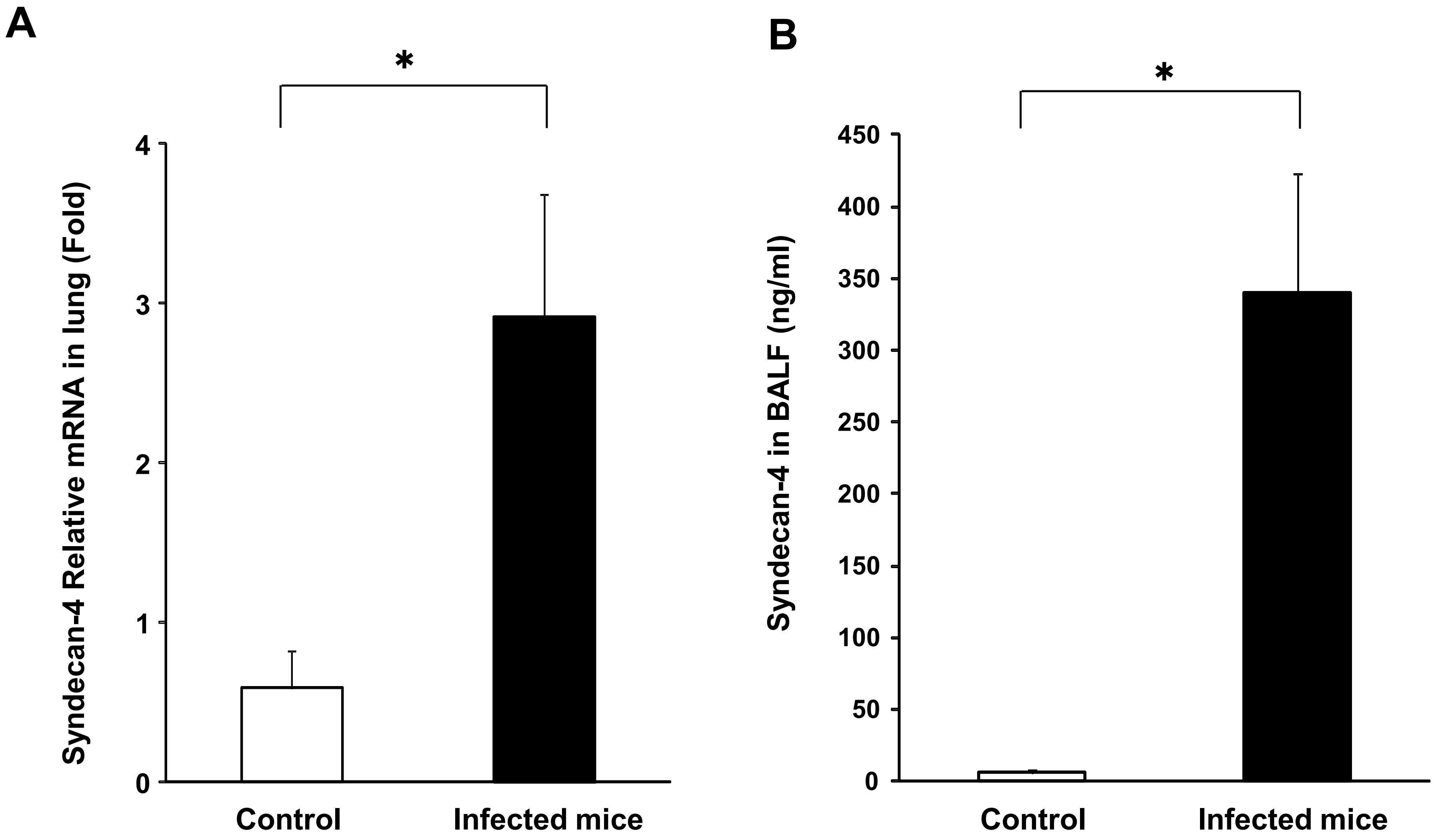
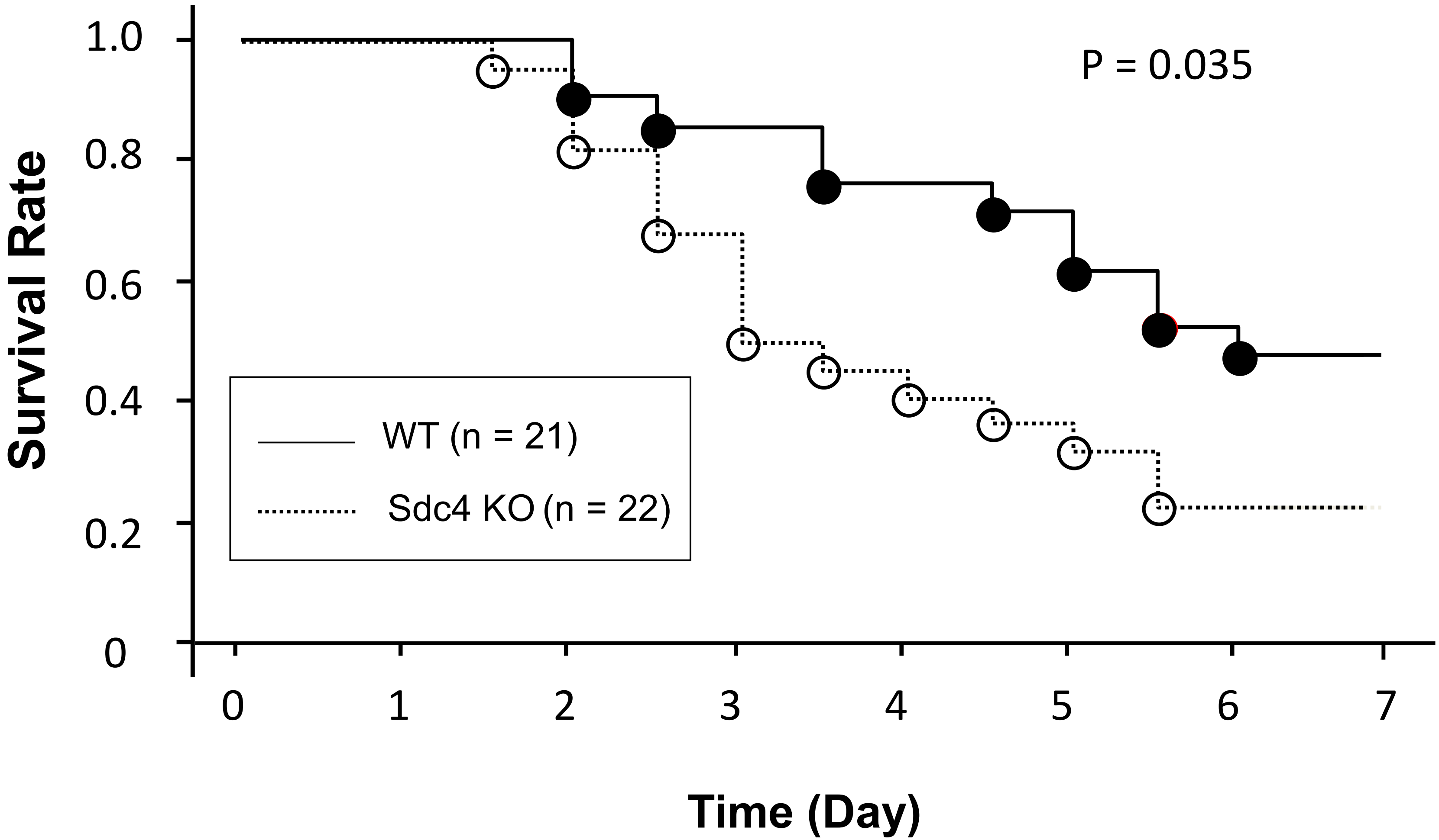

A
*
Syndecan-4 Relative mRNA in lung (Fold)
4
3
2
1
0
Control
Infected mice
B
*
Syndecan-4 in BALF (ng/ml)
450
400
350
300
250
200
150
100
50
0
Control
Infected mice

Figure 4



P = 0.035
1.0
0.8
0.6
0.4
0.2
0
Survival Rate
0
1
2
3
4
5
6
7
Time (Day)
WT (n = 21)
Sdc4 KO (n = 22)

Figure 5

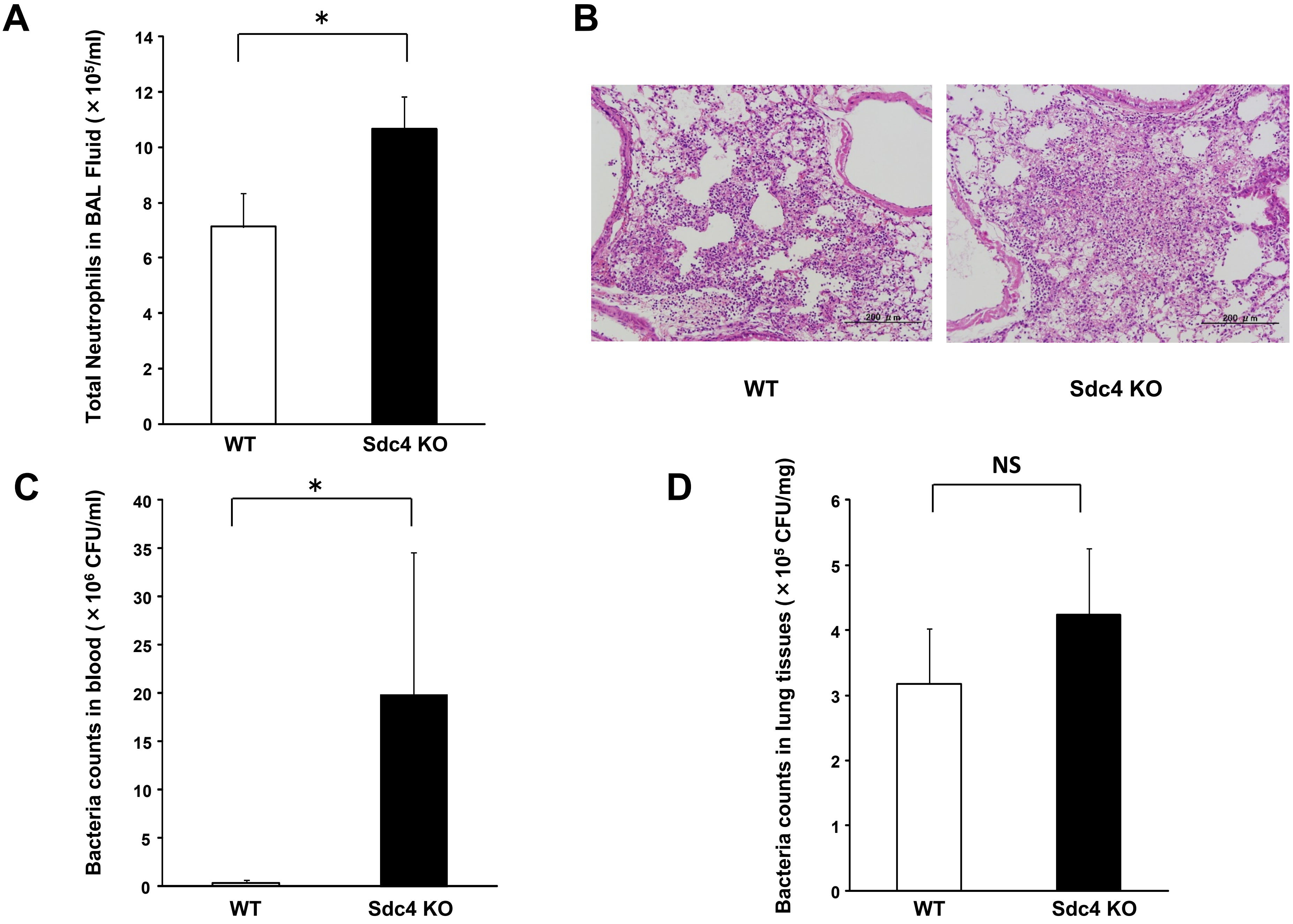
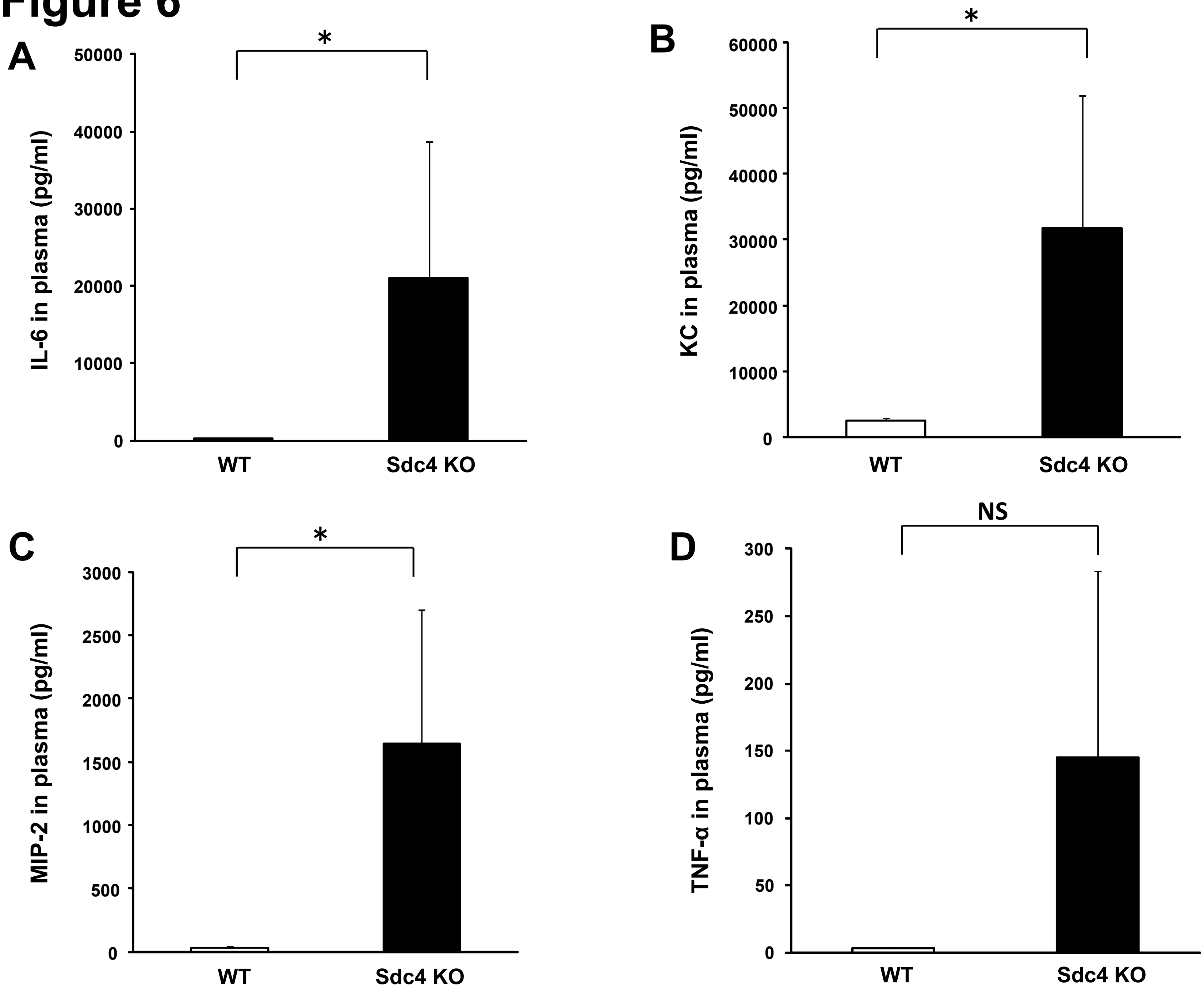

A
*
Total Neutrophils in BAL Fluid (×10⁵/ml)
0
2
4
6
8
10
12
14
WT
Sdc4 KO
B
200 μm
200 μm
WT
Sdc4 KO
C
*
Bacteria counts in blood (×10⁶ CFU/ml)
0
5
10
15
20
25
30
35
40
WT
Sdc4 KO
D
NS
Bacteria counts in lung tissues (×10⁵ CFU/mg)
0
1
2
3
4
5
6
WT
Sdc4 KO

Figure 6


A
IL-6 in plasma (pg/ml)
50000
40000
30000
20000
10000
0
*
WT
Sdc4 KO
B
KC in plasma (pg/ml)
60000
50000
40000
30000
20000
10000
0
*
WT
Sdc4 KO
C
MIP-2 in plasma (pg/ml)
3000
2500
2000
1500
1000
500
0
*
WT
Sdc4 KO
D
TNF-α in plasma (pg/ml)
300
250
200
150
100
50
0
NS
WT
Sdc4 KO