

Interleukin-1 β Participates in the Development of Pneumococcal Acute Lung Injury and Death by Promoting Alveolar Microvascular Leakage

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Streptococcus pneumoniae (*S. pneumoniae*, also known as pneumococcus) infections are major causes of death worldwide. Despite the development and use of effective antibiotics, high, early mortality due to pneumococcal infections has not been decreased for the last few decades. Recent study found a deadly hemorrhagic acute lung injury (ALI) as a major cause of death at the early stage of severe pneumococcal infections. Interleukin (IL)-1 β was known to play critical roles not only for the development of ALI but also resolution of it. The role of IL-1 β on the pathogenesis of pneumococcal ALI, however, has not been well understood yet. This study aims to investigate the role of IL-1 β on the development of pneumococcal ALI and subsequent death. IL-1 β expression was upregulated in the lungs of pneumococcal ALI in wild-type (WT) mice, but not in the plasma. Despite an increased expression of pulmonary IL-1 β , no inflammatory cell infiltration into airway has been observed. Upregulation of IL-1 β expression was indeed dependent on pneumococcal cytoplasmic toxin pneumolysin and its cell surface receptor Toll-like receptor 4. Deficiency of IL-1R1, a cell surface receptor of IL-1 β , resulted in a markedly reduced hemorrhagic pulmonary edema and early death in pneumococcal ALI. Finally, IL-1 β neutralization in WT mice protects against pulmonary hemorrhagic edema and death. These data suggest that pulmonary expression of IL-1 β exacerbates pneumolysin-induced ALI and death by promoting alveolar hemorrhagic edema.

Key Words: *Streptococcus pneumoniae*, Pneumococcus, Pneumolysin, Acute lung injury, IL-1 β , IL-1R1

INTRODUCTION

Acute lung injury (ALI) is one of the most life-threatening complications of many pulmonary and non-pulmonary diseases (1~4). Despite the heterogeneous causes of injury, ALI is characterized by severe cellular damage and subsequent pulmonary microvascular leak, which result in influx

of protein-rich edema fluid into the airway and respiratory failure (3, 5). Severe pulmonary infections with respiratory pathogens are the most common causes of direct pulmonary injuries and subsequent development of ALI. Among many respiratory pathogens, *Streptococcus pneumoniae* (*S. pneumoniae*, also known as pneumococcus) is a major bacterial pathogen involved in primary and secondary causes of ALI (6~8).

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S. pneumoniae, a gram-positive bacterium, resides on mucosal surfaces in the upper respiratory tract of many healthy individuals without any clinical symptoms (6, 9~11). If the delicate balance between mucosal epithelial resistance and pneumococcal pathogenicity is disturbed, pneumococcus travels down to the trachea and lung and causes low respiratory tract infections (9~12). In some circumstances, pneumococcus disseminates into the blood and causes detrimental disease (6, 9~12). Although deaths due to pneumococcal infections have been greatly decreased since the discovery of penicillin and subsequent development of other effective antibiotics, it is still a major cause of morbidity and mortality worldwide (10, 13). According to the World Health Organization (WHO) report, about 1.6 million people die due to pneumococcal infections annually (13).

Among many detrimental diseases due to *S. pneumoniae*, ALI accompanied with hemorrhagic edema is a major cause of death in early onset of severe infections (14, 15). Pneumolysin (PLY), a cholesterol-binding cytolysin, is produced and localized in the cytoplasm due to lack of secretion signal and released by autolysis (16, 17). Released pneumolysin then binds to cholesterol in the cell membrane, oligomerizes to form transmembrane pore, and causes irreversible damage to the cells. Alveolar epithelial and endothelial damages result in deadly hemorrhagic edema (7, 14, 16, 18). Although inflammatory cytokines and infiltrates have been implicated in the development of ALI, their role in hemorrhagic ALI at the early stage of severe pneumococcal infections has not been well understood yet. Lung tissues from mice and human with pneumococcal hemorrhagic ALI showed no signs of inflammatory infiltrates, and anti-Nuclear factor- κ B (NF- κ B) and anti-Tumor necrosis factor α treatments failed to prevent death from these mice and patients (9, 15). These findings suggest a dispensable role of inflammation and its mediators in the pathogenesis of hemorrhagic ALI and early death during severe *S. pneumoniae* infections.

Our earlier study found that CYLD, a tumor suppressor and negative regulator of inflammation, exacerbates pneumococcal ALI by negatively regulating the expression of type 1 plasminogen activator inhibitor (PAI-1) (15, 19, 20). Because inflammatory cell infiltrations into airways were

not observed in the lungs of pneumococcal ALI and CYLD-deficiency protects mice against pneumococcal ALI independently of its negatively regulatory function on NF κ B, the role of inflammatory mediators on the development of pneumococcal ALI and subsequent death has been dismissed (15). However, since alveolar resident cells, such as alveolar epithelial and endothelial cells, resident macrophages, and also fibroblast, also produce inflammatory mediators in response to various types of pulmonary injuries (21~24), it is still interesting to know if inflammatory mediators, such as pro-inflammatory cytokines, play a role in the development of pneumococcal ALI and death.

Among many inflammatory mediators, interleukin (IL)-1 β was found to be critically involved not only in the development of ALI but also in the resolution of it (25~28). In this study, we thus investigated the role of IL-1 β on pneumococcal ALI and subsequent death in a mouse model of severe pneumococcal infections and ALI. Expression of IL-1 β was significantly increased in the lungs of wild-type (WT) mice, but not in the plasma of animals following acute hemorrhagic injury with *S. pneumoniae*. Inflammatory cell infiltration, however, was not observed in the lungs of WT mice, which suggests alveolar resident cells as sources of pulmonary expression of IL-1 β . Upregulation of IL-1 β expression was indeed dependent of cytoplasmic toxin pneumolysin and a cell surface PLY receptor, Toll-like receptor (TLR) 4. Furthermore, lungs of IL-1 receptor 1 (IL-1R1)-deficient mice showed significantly lower microvascular leakage and mortality compared to those of WT mice. Moreover, neutralization of pulmonary IL-1 β significantly inhibited *S. pneumoniae*-induced alveolar leakage and death in WT mice. Taken together, present findings suggest that PLY-induced pulmonary expression of IL-1 β participates in the development of pneumococcal ALI and death by promoting alveolar microvascular leakage.

MATERIALS AND METHODS

Bacteria, bacterial lysate and pneumolysin

Pneumococcal strain D39 (serotype 2, NTCC 7466) and PLY-deficient isogenic mutant of D39 (D39-PLN) were used

in these experiments (15, 29~31). Cell-free pneumococcal lysates were prepared as described previously (15, 31~33). Following overnight incubation at 37°C on chocolate agar plate, pneumococcal cells were collected and inoculated into Todd-Hewitt broth (Difco, Franklin Lakes, NJ, USA) supplemented with 0.5% yeast extract (THY). After overnight incubation, pneumococcal lysates were then prepared at the concentration of 1×10^9 colony forming unit (CFU)/ml. Native pneumolysin was purified using Ni-NTA chromatography (Qiagen, Valencia, CA, USA), and any trace of LPS was removed with End-X[®] endotoxin affinity resin (Associates of Cape Cod, East Falmouth, MA) as described previously (15, 34).

Animals and mouse model of pneumococcal ALI

C57BL/6J, BALB/c, Tlr4^{-/-}, and IL-1R1^{-/-} mice were purchased from Jackson Laboratory (Bar Harbor, ME, USA) and Orient Bio (Korea). Mouse model of pneumococcal ALI was established as described previously (15). Briefly, pneumococcal ALI was induced by intratracheal (i.t.) inoculation of pneumococcal lysate equivalent to 5×10^7 CFU or 200 ng pneumolysin. DPBS, a vehicle control used for the preparation of pneumococcal lysate and dilution of pneumolysin, was used as control inoculations. For the experiments recording survival and mortality rate, animals were monitored for the lethality every 2 hours for 12 hours and every 12 hours for 5 days thereafter. For the neutralization of pulmonary IL-1 β , WT mice were i.t. inoculated with 100 μ g of anti-mouse IL-1 β antibody (R&D systems, Minneapolis, MN) 2 hours before pneumococcal lysate inoculation, and normal IgG was inoculated in control mice (24, 35). Animals were cared for in accordance with the Ethical Guidelines for Use of Experimental Animals in Biomedical Research (36). All animal experiments were approved by the Institutional Animal Care and Use Committees (IACUCs) at House Ear Institute and Ewha Womans University School of Medicine.

Alveolar microvascular leakage and hemorrhage

To measure the alveolar microvascular leakage and hemorrhage, animals were sacrificed at 3 hours after pneumo-

coccal lysate or pneumolysin inoculation, and blood was collected from the abdominal vena cava with 3.2% buffered sodium citrate for plasma separation. Plasma was collected and stored at -70°C for the ELISA assay of IL-1 β . Broncho-alveolar lavage (BAL) was conducted as described previously (15, 19, 20, 22). BAL fluid (BALF) was collected by centrifugation at 3,000 g for 10 min, and protein concentration was measured from BALF by Bicinchoninic acid (BAC) assay with Pierce BCA protein assay kit (Life Technologies) following the manufacturer's instruction. Cells of BALF were resuspended with 200 μ l of RBC lysis buffer, and optical density at 414 nm was measured to quantify free hemoglobin (15, 37). To measure the extravascular lung water, lungs were dissected from mice, wet lung weight was measured, and lungs were dried at 85°C for 5 days. Dry lung weight was then measured, and lung water weight was calculated by subtracting dry lung weight from wet lung weight (Wet-to-dry lung weight ratio) (15, 38).

Inflammatory cell migration analysis

For the analysis of polymorphonuclear neutrophil migration into airway, alveolar inflammatory cells were collected from BALF by centrifugation at 3,000 g for 10 min, and cell pellets were resuspended with 200 μ l of DPBS. Cell numbers in BALF were counted using C-CHIP (inCyto, Cheonan-si, Korea) (15, 19, 20, 22).

Real-time quantitative RT-PCR (Q-PCR) assay

Q-PCR analysis of mouse IL-1 β was conducted as follows. Lungs snap frozen in liquid nitrogen were crushed in liquid nitrogen and washed twice with DPBS. Lung tissues were then lysed with TRIzol reagent (Life Technologies), and total RNA was isolated following the manufacturer's instructions. The reverse transcription reaction was performed using TaqMan reverse transcription reagents (Life Technologies), and PCR amplification was performed with Fast SYBR Green Universal Master Mix (Life Technologies). Reaction was amplified and quantified by using StepOne Plus (Life Technologies) according to the manufacturer's instructions as described previously (15, 31, 39, 40). Relative quantity (RQ) of mouse IL-1 β mRNA expression was obtained by

using the comparative threshold cycle (Ct) Method and was normalized using mouse glyceraldehydes-3-phosphate dehydrogenase (GAPDH) as endogenous control. Primers for mouse IL-1 β and GAPDH were described previously (39, 40).

Enzyme-linked immunosorbent (ELISA) assay for mouse IL-1 β

Protein concentration of mouse IL-1 β was measured from the BALF and plasma of mice by using Mouse IL-1 β /IL-1F2 Quantikine ELISA kit (R&D systems) following the manufacturer's instructions.

Statistical analysis

Differences in survival rate between experimental groups were determined by Kaplan-Meier analysis and evaluated by log-rank test. Non-survival data were presented as Mean \pm S.D. and analyzed by Student's *t*-test. A value of $p < 0.05$ was considered significantly different.

RESULTS

Expression of IL-1 β is upregulated in the lungs of pneumococcal ALI, but not in the plasma of animals

Pneumococcal ALI is characterized by severe hemorrhagic pulmonary edema and early death (15). Despite critical roles of inflammatory infiltrates and mediators in the pathophysiology of ALI, the roles of inflammation on pneumococcal ALI are not fully understood yet. Among many inflammatory mediators, IL-1 β was known to play important roles not only on the development of ALI but also for the resolution of ALI and subsequent development of pulmonary fibrosis (21, 24, 25). Furthermore, in less severe pneumococcal infections characterized by profound inflammatory responses rather than hemorrhagic tissue injury, ligands of IL-1 family play important roles on resistance to pneumococcal infections (41, 42). Among three ligands of IL-1 family, IL-1 β was found to play a major role in resistance to pneumococcal infections (42). Although no significant inflammatory cell infiltration has been observed in the lungs of pneumococcal ALI, resident pulmonary cells

such as resident alveolar macrophages, alveolar epithelial cells, and microvascular endothelial cells may also be important sources of IL-1 β (21, 43, 44).

It is thus important to know if IL-1 β participates in the development of pneumococcus-induced hemorrhagic pulmonary edema and early death as it does in less severe inflammatory infections. To determine the role of IL-1 β in pneumococcal ALI, we first investigated if IL-1 β expression is regulated in the lungs of pneumococcal ALI. As shown in Fig. 1A and 1B, mRNA expression of IL-1 β was upregulated in the lungs of mice models of pneumococcal ALI (Fig. 1A), and pneumococcus upregulated mRNA expression of IL-1 β in a dose-dependent manner (Fig. 1B). Consistent with upregulated expression of IL-1 β mRNA, upregulation of IL-1 β protein expression was also detected in the BALF of pneumococcal ALI. Circulatory level of IL-1 β however has not been changed (Fig. 1C). Further study found no significant inflammatory cell migration in the lungs of pneumococcal ALI, and even decrease in alveolar cell count has been observed (Fig. 1D).

Pneumococcal PLY induces IL-1 β upregulation via Toll-like receptor (TLR) 4

Since we found that IL-1 β expression is upregulated in the lungs of pneumococcal ALI, we next sought to determine pneumococcal pathogenic factor responsible for IL-1 β upregulation. Because pneumococcal cytoplasmic toxin PLY is a key pathogenic factor for the development of pneumococcal ALI (7, 14~16, 45), we first determined if PLY is responsible for the upregulation of IL-1 β expression. mRNA expression of IL-1 β was upregulated in the lungs of mice inoculated pneumococcal D39 lysate, but not in the lungs of mice inoculated with D39-PLN (Fig. 2A), which suggest that PLY plays a critical role for the induction of IL-1 β mRNA expression. Purified pneumolysin indeed upregulated mRNA expression of IL-1 β following i.t. inoculation in the lungs (Fig. 2A). Consistent to the findings from the mRNA expression analysis, protein expression of IL-1 β was also upregulated by pneumococcal D39 lysate and PLY, but not by D39-PLN lysate (Fig. 2B). Expression level of circulatory IL-1 β was not affected (Fig. 2C). Although PLY

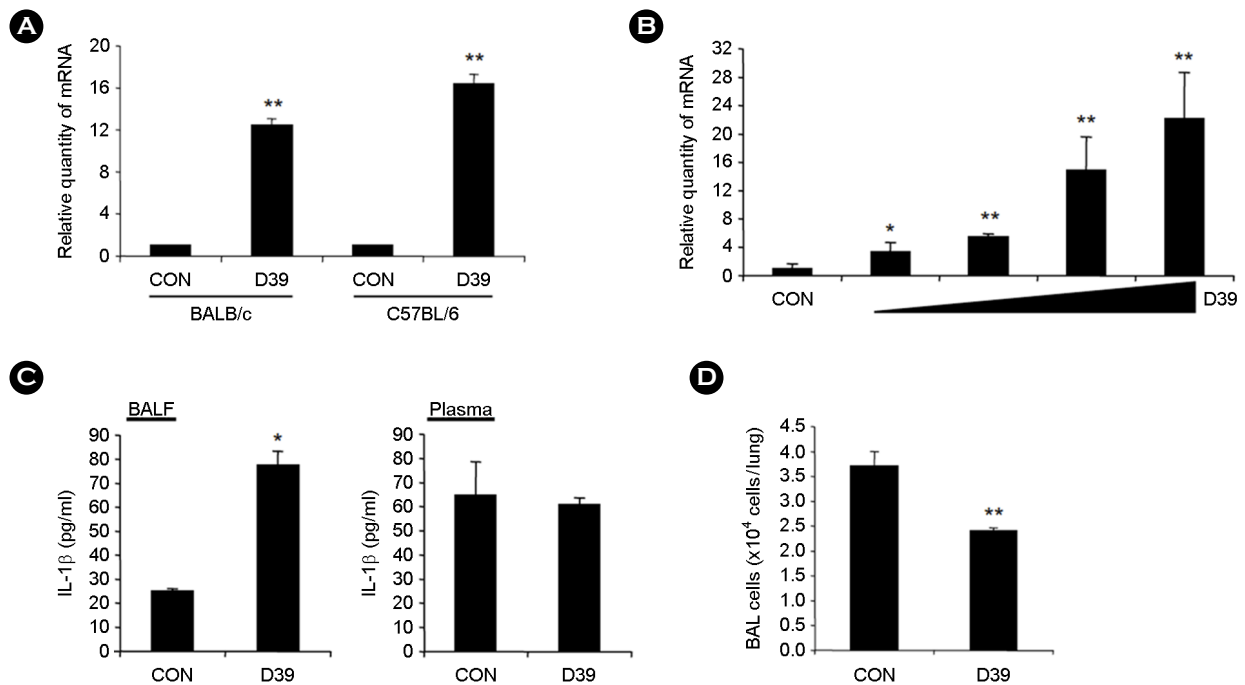


Figure 1. IL-1 β expression is upregulated in the lungs of pneumococcal ALI, but not in the plasma. **A**, BALB/c and C57BL/6 mice were intratracheally (i.t.) inoculated with pneumococcal D39 lysate equivalent to the 5×10^7 colony forming unit (CFU), and mRNA expression of IL-1 β was measured from the lungs of mice 3 hours after i.t. inoculation by real-time quantitative PCR (Q-PCR) assay. **B**, C57BL/6 mice were i.t. inoculated with various amount of pneumococcal D39 lysate (equivalent to $0.625 \sim 5 \times 10^7$ CFU), and mRNA expression of IL-1 β was measured from the lungs of mice 3 hours after i.t. inoculation by Q-PCR assay. **C**, BALB/c mice were i.t. inoculated with pneumococcal D39 lysate, and protein expression of IL-1 β was measured from the BALF and plasma of mice 3 hours after i.t. inoculation by ELISA assay. **D**, C57BL/6 mice were i.t. inoculated with pneumococcal D39 lysate, and bronchoalveolar lavage (BAL) was conducted 3 hours after i.t. inoculation. Cell numbers in the BAL fluid (BALF) were counted. Data are the means \pm SD. *, $p < 0.05$ compared with CON; **, $p < 0.01$ compared with CON. CON, control.

exerts many pathological effects by binding cell membrane cholesterol, Toll-like receptor (TLR) 4 has been found as a receptor of PLY, especially in the transcriptional regulation by PLY (12, 15, 34). To determine if PLY also regulates transcription of the IL-1 β via TLR4, TLR4 $^{-/-}$ and genetic-background control BALB/c mice were i.t. inoculated with pneumococcal D39 lysate, and mRNA expression of IL-1 β was measured in the lungs of mice. mRNA expression of IL-1 β was significantly lower in the lungs of TLR4 $^{-/-}$ mice compared to that of control BALB/c mice (Fig. 2D). These data suggest PLY as a key pathogenic factor for pulmonary expression of IL-1 β .

IL-1R1 deficiency protects mice against pneumococcal ALI and early death

Despite an increase in pulmonary expression of IL-1 β in pneumococcal ALI, it is not clear if pulmonary IL-1 β plays a role on the development of ALI and subsequent death. Previously it has been reported that transient expression of IL-1 β in the lung induces ALI and chronic repair leading to pulmonary fibrosis (25). However, it is also found that higher pulmonary IL-1 β expression in patients with ALI is beneficial for alveolar epithelial repair (46). By considering such controversial results, it is interesting to know if IL-1 β plays role on pneumococcal ALI and death.

IL-1 β is a pro-inflammatory cytokine, which is expressed by many cells, and exerts its biological activities primarily

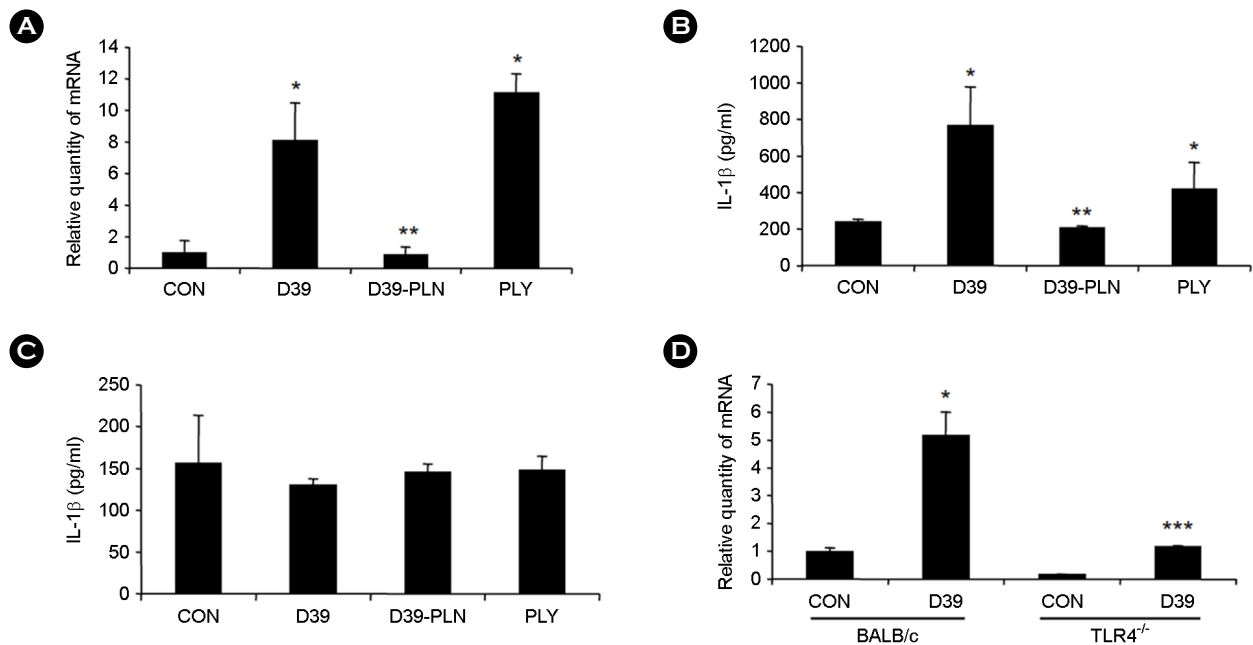


Figure 2. Pneumococcal pneumolysin-induced upregulation of pulmonary IL-1 β is dependent on a cellular TLR4. A, C57BL/6 mice were i.t. inoculated with pneumococcal D39 lysate, D39-PLN, or pneumolysin (PLY), and mRNA expression of IL-1 β was measured from the lungs of mice 3 hours after i.t. inoculation by Q-PCR assay. B & C, C57BL/6 mice were i.t. inoculated with pneumococcal D39 lysate, D39-PLN, or PLY, and protein expression of IL-1 β was measured from the BALF (B) and plasma (C) of mice 3 hours after i.t. inoculation by ELISA assay. D, TLR4^{-/-} mice were i.t. inoculated with pneumococcal D39 lysate, and expression levels of IL-1 β mRNA were compared to those of genetic background control BALB/c mice by Q-PCR assay. Data are the means \pm SD. *, $p < 0.01$ compared with CON; **, $p < 0.05$ compared with D39 (A-C); ***, $p < 0.05$ compared with D39 BALB/c mice. CON, control; PLY, pneumolysin.

by binding to cell surface type 1 IL-1R (IL-1R1) (43, 44). We thus investigated the role of IL-1 β on pneumococcal ALI by using IL-1R1^{-/-} mice. IL-1R1^{-/-} and genetic-background control C57BL/6 mice were i.t. inoculated with pneumococcal D39 lysate, and pulmonary microvascular leakage was measured. As shown in Fig. 3, lung extravascular water weight (Fig. 3A), alveolar microvascular leakage of protein (Fig. 3B), and alveolar hemorrhage (Fig. 3C) were significantly decreased in the lungs of IL-1R1^{-/-} mice compared to those of control C57BL/6 mice. In addition, IL-1R1^{-/-} mice showed much lower mortality compared to that of control C57BL/6 mice following pneumococcal D39 lysate inoculation (Fig. 3D).

Neutralization of IL-1 β protects mice against pneumococcal ALI

Although we found pulmonary IL-1 β expression is

induced and IL-1R1-deficiency protects mice against pneumococcal ALI and subsequent early death, it is still not clear if IL-1 β actually participates in pneumococcal ALI and deficiency of IL-1R1 exerts protective effects by blocking the action of IL-1 β . We thus decided to investigate the role of IL-1 β on pneumococcal ALI by assessing the effect of IL-1 β neutralization in the lungs of WT mice. Since increase in IL-1 β expression is limited in the lungs of pneumococcal ALI, we decided to neutralize pulmonary IL-1 β by i.t. inoculation of anti-mouse IL-1 β antibody. As shown in Fig. 4A, neutralization of IL-1 β in C57BL/6 mice resulted in a significantly reduced mortality compared to that of control IgG inoculated mice (Fig. 4A). Moreover, lung extravascular leakage of water (Fig. 4B), alveolar protein permeability (Fig. 4C), and hemorrhage (Fig. 4D) were significantly inhibited by i.t. treatment of anti-mouse IL-1 β antibody. These results support above findings from IL-1R1^{-/-} mice

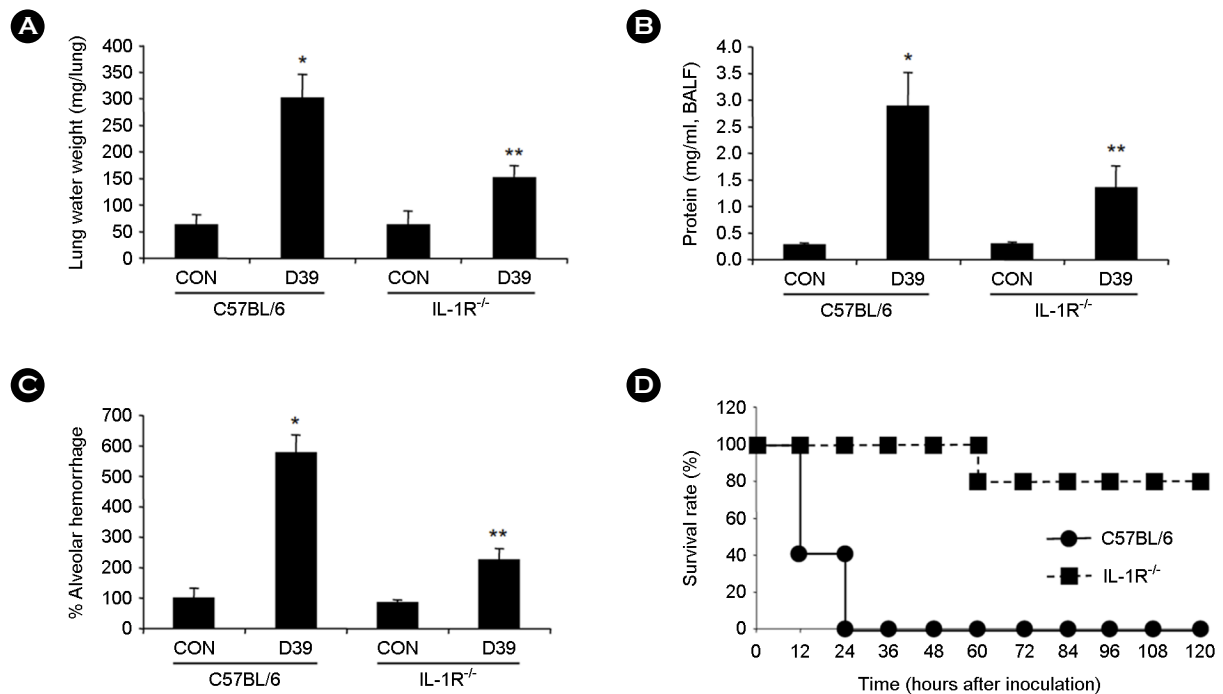


Figure 3. Deficiency of IL-1R1 protects mice against pneumococcal ALI and death. A-C, IL-1R1^{-/-} and control C57BL/6 mice were i.t. inoculated with pneumococcal D39 lysate, and lung extravascular water weight (A), alveolar protein permeability (B), and alveolar hemoglobin (C) were measured from the lungs of mice 3 hours after i.t. inoculation. D, IL-1R1^{-/-} and control C57BL/6 mice were i.t. inoculated with pneumococcal D39 lysate, and survival rates were recorded for 5 days. Viability in D was assessed by using Kaplan-Meier survival analysis and compared by long-lank test. Data are the means \pm SD. *, $p < 0.01$ compared with CON in C57BL/6; **, $p < 0.05$ compared with D39 in C57BL/6 mice. CON, control.

and certainly suggest that PLY-induced pulmonary IL-1 β contributes to the development of hemorrhagic ALI and death, and deficiency of IL-1R1 may protects mice against early mortality by blocking IL-1 β -mediated hemorrhagic ALI.

DISCUSSION

Although IL-1 β is a hematopoietic cytokine mediating neutrophil recruitment to the site of infection and injury either directly or indirectly by inducing other mediators of chemotaxis, we did not observe a significant increase in pulmonary inflammatory cell infiltrates in the lung tissues of pneumococcal ALI. Previously it has been reported that neutrophil infiltration into pneumococcal infection sites is not affected by the absence of IL-1 and its receptor IL-1R1 at the early stage of infection. However, failure in early

defense against pneumococcal infections results in pneumococcal outgrowth and subsequent influx of neutrophils to the site of infection (42). Unlike in inflammation profound pneumococcal infection model with sub-lethal dose of live pneumococcal infection, lacks of pneumococcal outgrowth in lungs and expression of circulatory IL-1 β in pneumococcal ALI may partly explain the findings of no neutrophil infiltration into the airway. Alveolar macrophages are known to help to maximize the expression of CXCL8 chemokine in the lung in response to pulmonary IL-1 β (47). Cytotoxicity to the alveolar macrophage is a well-known immune evasion mechanism of pneumococcus (48~50), and a considerable decrease in an alveolar macrophage number was found in the lungs of pneumococcal ALI. In this regard, decrease in alveolar macrophages in the lungs of pneumococcal ALI may also play a role in no neutrophil migration into the airway at the early stage of pneumococcal ALI. Our

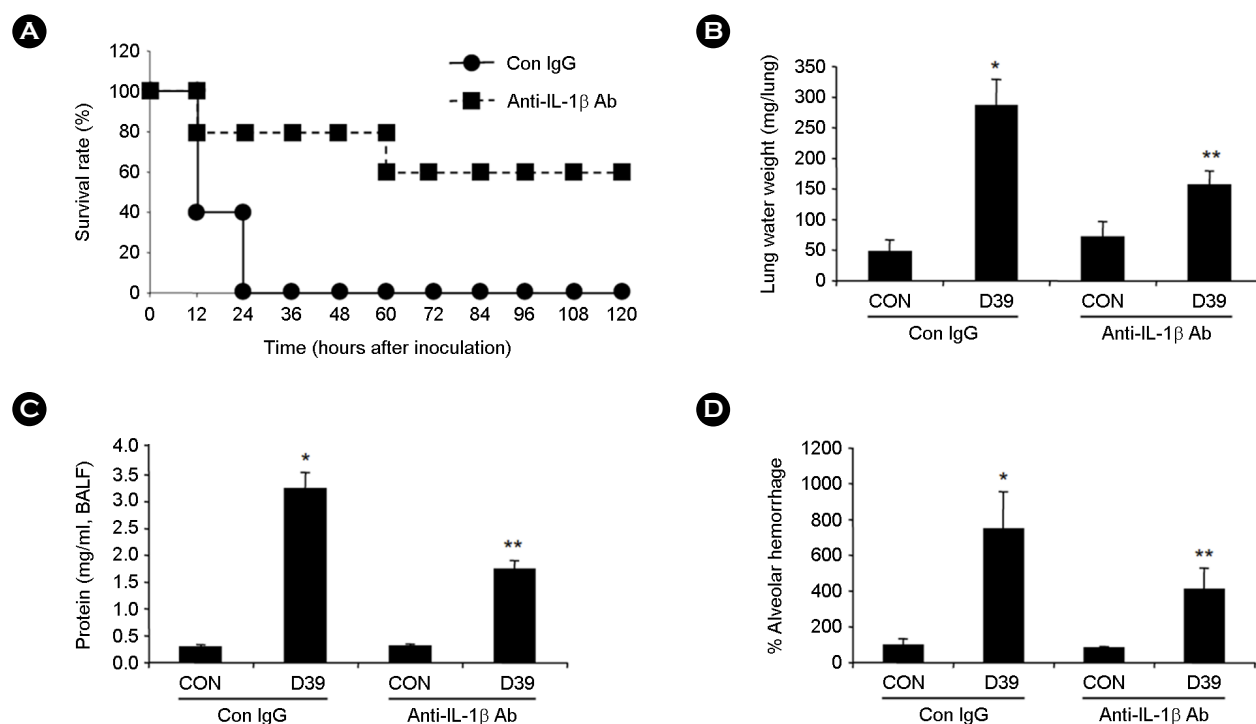


Figure 4. Pulmonary neutralization of IL-1 β inhibited pneumococcal ALI and death in WT mice. **A**, C57BL/6 mice were i.t. inoculated with 100 μ g of anti-mouse IL-1 β antibody or control IgG, and pneumococcal D39 lysate were i.t. inoculated 2 hours after antibody inoculation. Survival rates were recorded for 5 days, and viability was assessed by using Kaplan-Meier survival analysis. **B-D**, C57BL/6 mice were i.t. inoculated with 100 μ g of anti-mouse IL-1 β antibody for 2 hours followed by i.t. inoculation of pneumococcal D39 lysate. Lung extravascular water weight (**B**), alveolar protein permeability (**C**), and alveolar hemoglobin (**D**) were measured from the lungs of mice 3 hours after i.t. inoculation of D39. Data in **B - D** are the means \pm SD. *, $p < 0.01$ compared with CON in WT mice treated with Con IgG; **, $p < 0.05$ compared with D39 in WT mice treated with Con IgG. CON, control.

current finding, together with previous reports, suggest that pulmonary resident cells such as alveolar epithelial cells and alveolar microvascular endothelial cells may be a major source of pulmonary IL-1 β in the lungs of pneumococcal ALI.

Many clinical studies and also experimental animal studies found IL-1 β as the most biologically active cytokine in the lungs early after the onset of ALI (51~53), and alveolar IL-1 β contributes to the development and resolution processes of ALI in many ways (28, 54~56). Notably, IL-1 β causes the activation of Transforming growth factor (TGF)- β via RhoA/ α v β 6 integrin-dependent, which results in increased protein permeability across lung endothelial cell monolayers (28). Furthermore, pretreatment of IL-1R antagonist (IL-1Ra) significantly decreased protein permeability and

pulmonary edema in a rat model of ventilator-induced ALI (27). Our current findings, together with clinical and experimental results from other models of ALI, suggest that IL-1R1-deficiency protects against hemorrhagic ALI and subsequent death in mice, probably by blocking IL-1 β -mediated microvascular permeability and subsequent edema.

We previously reported that a tumor suppressor CYLD promotes PLY-induced ALI by inhibiting PAI-1, which is critical for protection against alveolar microvascular leakage and hemorrhage (15). CYLD protected mice against pneumococcal ALI and subsequent death independently of its negative regulatory function on NF κ B, the most active regulator of inflammation, and inflammatory cell infiltration into the airway was very limited in the lungs of pneumococcal ALI. Therefore, the role of inflammatory mediators,

such as inflammatory cytokines, has not been carefully evaluated. This study found that IL-1 β may coordinate with PLV to promote alveolar microvascular leakage.

Interestingly, IL-1 β was found to enhance alveolar epithelial and endothelial permeability in a mouse model of ALI via RhoA/ α v β 6 integrin-dependent activation and secretion of TGF- β (28). Our recent study found that CYLD negatively regulates TGF- β signaling pathway, and thus CYLD-deficient mice showed enhanced activation of TGF- β signaling pathway (31). Despite an enhanced TGF- β signaling pathway, CYLD-deficient mice are highly resistant to pneumococcal ALI and death. It is probably because TGF- β is not produced/expressed in the lungs of pneumococcal ALI at the early stage of infections and injuries (31). IL-1 β -induced recruitment of neutrophils into the lung also makes a significant contribution to the pathophysiology of ALI. However, at the early stage of pneumococcal ALI, IL-1 β regulatory effects on alveolar microvascular permeability are neither dependent on inflammatory infiltrates nor TGF- β , but rather direct effects via its receptor IL-1R1.

Collectively, although PLV plays a major role for hemorrhagic ALI at the early stage of infections, other physiological factors such as IL-1 β may participate to exacerbate the injury. Therefore, addition of anti-IL-1 β treatment to the therapeutic strategy for pneumococcal ALI and early mortality may be a good strategy to enhancing therapeutic effect for pneumococcal ALI.

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