Interleukin-1 Promotes Coagulation, Which Is Necessary for Protective Immunity in the Lung Against Streptococcus pneumoniae Infection

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Interleukin (IL)-1 is a well-known cytokine for the initiation of innate immunity in bacterial infection. However, the underlying mechanism of IL-1 on the respiratory infection is not fully elucidated. We studied how IL-1 contributes to the host defense against Streptococcus pneumoniae. IL-1R−/− mice showed high mortality, local cytokine storm, and substantial infiltrates in the lower respiratory tract after intratracheal challenge with S. pneumoniae. The IL-1-deficient condition did not suppress the propagation of bacteria in the lung, although the recruitment and the bacteria-killing ability of neutrophils (CD11b+Ly6C+Ly6G+) were not defective compared with wild-type mice. Unexpectedly, we found that the transcription of fibrinogen alpha and gamma genes were highly activated in the lungs of wild-type mice after the infection, whereas no significant changes were found in IL-1R−/− mice. Of note, synthesis of fibrinogen was dependent on the IL-1-IL-6-Stat3 cascade. Treatment with recombinant fibrinogen improved survival and bacterial propagation in the IL-1R−/− mice and blockade of the coagulation increased the susceptibility of wild-type mice to pneumococcal pneumonia. Our findings suggest that IL-1 signaling leads to the synthesis of fibrinogen in the lung after pneumococcus infection and is followed by coagulation, which contributes to the control of bacterial infection in the pulmonary tract.

Keywords. IL-1; S. pneumoniae; coagulation; fibrinogen; innate immunity.

Streptococcus pneumoniae is arguably the most common cause of pneumonia in the world [1, 2]. Colonization with pneumococcus typically induces mild symptoms that are easily cleared in healthy people; however, when risk factors weaken the protective immunity (eg, the patient’s age, diabetes, and human immunodeficiency virus [HIV] infection), pneumococcus can cause serious diseases [2]. Pneumococcal pneumonia frequently arises after infection by a secondary pathogen such as influenza [3]. Moreover, the more recent emergence of antibiotic-resistant pneumococci, termed “superbacteria,” makes patient treatment more difficult [4]. Therefore, alternative therapies are needed to enhance the immune system.

The recognition of S. pneumoniae by the host is mediated by various ways. For instance, pattern recognition receptors such as Toll-like receptors (TLRs) are expressed on airway epithelia and alveolar macrophages [5]. The bacterial cell wall component is recognized by TLR2, and the pneumococcal toxin pneumolysin depends on TLR4 [6]. These TLRs trigger the activation of NFκB, which, in turn, mediates the signaling pathways from interleukin (IL)-1 and the tumor necrosis factor (TNF) receptor superfamilies [5]. The
nuclear translocation of NFκB is sufficient for the recruitment of neutrophils that are crucial for the clearance of pneumococcus [7].

Coagulation systems are important for innate immunity in invertebrates and vertebrates ranging from drosophila to humans [8]. Fibrinogen, the source of clot formation, is synthesized in the liver and circulates in blood vessels in the natural state [9]. Studies of several infectious diseases of the skin and liver reveal that coagulation is the early innate response to bacterial infection and that bacteria are trapped and killed by fibrin formation [8, 10]. Thus, regulation of the coagulation system has become a topic of interest in regard to host response to bacterial infection. However, the contribution of coagulation in other tissues such as lung has not been established well. Here, we found that IL-1-mediated coagulation pathway play a crucial role on host defense against pneumococcal pneumonia infection.

**METHODS**

**Mice**

Wild-type C57BL/6 (B6) and IL-1R−/− mice were purchased from Charles River Laboratories (Orient Bio, Inc, Korea) and Jackson Laboratories (Bar Harbor, ME), respectively. IL-1R−/− mice on the B6 background failed to respond to IL-1 [11]. In sum, 3–5 mice per group for each experiment were used. All mice used in experiments were between 6 and 12 weeks of age. Mice were maintained under pathogen-free conditions in the experimental facility at the International Vaccine Institute (Seoul, Korea), where they received sterilized food and water ad libitum. The experiments were approved by the Institutional Animal Care and Use Committee of the International Vaccine Institute.

**Bacteria Strain and Infection**

*S. pneumoniae* virulent capsular serotype 3 strain WU2 was cultured in Todd-Hewitt broth (BD Biosciences) plus 0.5% yeast extract (THY medium, BD Biosciences) as described elsewhere [12]. After mice were anesthetized, they were given 5 × 10^5 colony-forming units (CFU) or 5 × 10^3 CFU/30 µL of *S. pneumoniae* diluted in phosphate-buffered saline (PBS) intratracheally. In addition, wild-type *S. pneumoniae* TIGR4 strain (AC535), and its trpG mutant strain (AC1142) were used. TIGR4 ΔtrpG strain has a defect in tryptophan G synthesis and therefore is invasive but cannot proliferate well.

**Treatment In Vivo**

Wild-type B6 mice were treated intraperitoneally with anakinra (0.5 mg/mouse; Amgen, Thousand Oaks, CA), antagonist for IL-1, or PBS every 3 days starting 1 day before infection with *S. pneumoniae* WU2 (5 × 10^5 CFU). Six hours after the IL-1R−/− mice were infected with WU2, they were injected intraperitoneally with penicillin (1 mg/mouse). For the blockade of IL-6 signal, wild-type mice were treated intratracheally with PBS, anti-IL-6Ra antibody (Ab; 0.1 mg/mouse), or NSC 74859 as a Stat3 inhibitor (10 µg/mouse; SellecBio.com, Houston, TX). For the regulation of clotting cascade, IL-1R−/− mice were treated intratracheally with PBS or mouse fibrinogen (0.1 mg/mouse; Haematologic Technologies, Inc, Essex Junction, VT) at 0 and 1 day postinfection. Warfarin (10 µg/mouse; DAE HWA Pharmaceutical Co., Ltd, Korea) was administrated orally to wild-type mice every day beginning 1 day before infection.

**Preparation of Bronchoalveolar Lavage Fluid**

To obtain bronchoalveolar lavage fluid (BALF), tracheas were cannulated after exsanguinations, and lungs were washed twice with 0.5 mL of PBS as described elsewhere [13].

**Cytokine and Fibrinogen Detection**

The levels of MCP-1, IL-6, TNF-α, and interferon γ (IFN-γ) were determined by BD Cytometric Bead Array Mouse Inflammation Kit (BD Biosciences). The levels of KC, a chemotactic for neutrophils, and IL-6 were measured by DuoSet Mouse Enzyme-Linked Immunosorbent Assay (ELISA) Kit (R&D Systems, Minneapolis, MN) and ELISA MAX Standard Sets (Biologic, Inc, San Diego, CA), respectively. The mouse fibrinogen was quantified by Mouse Fibrinogen ELISA (Immunology Consultants Laboratory, Inc, Newberg, OR). All experiments were performed according to each manufacturer’s instructions.

**Determination of Bacterial Load**

Lungs were removed and homogenized in 1 mL of 0.2% Triton X-100 in PBS. Samples were serially diluted in PBS and plated onto TSA II (BD Biosciences, San Jose, CA) for CFU determination.

**Flow Cytometry**

Cells were collected from the BALF and stained with CD11b (M1/70), Ly6C (AL-21), and Ly6G (1A8), which were purchased from BD Biosciences. The neutrophils were determined as CD11b^+Ly6C^−Ly6G^+ cells.

**Microarray Analysis**

Whole lung tissues from wild-type and IL-1R−/− mice were obtained 4 hours postinfection after perfusion. The lungs were then homogenized and RNA was extracted (RNA Isolation Kit, Qiagen, Valencia, CA). We performed complementary DNA microarray analysis using a MouseRef-8 v2 Expression Beadchip Kit (Illumina, Inc, San Diego, CA). The differentially expressed genes in wild-type vs IL-1R−/− (|FC| ≥ 2) were selected and categorized as coagulation, SMAD signal, protein folding, and apoptosis.
Thrombin Activity
Thrombin activity in BALF collected from wild-type or IL-1R−/− mice at 0 or 1 day postinfection was determined by SensoLyte AFC Thrombin Activity Assay Kit (AnaSpec, Inc, Fremont, CA). The experiment was performed according to the manufacturer’s instructions.

Statistics
To compare differences between 2 experimental groups, we used a paired 2-sample t test for analysis, except for survival data for which we used Kaplan-Meier analysis. In addition, *P < .05, **P < .01, and ***P < .001 were assumed to be statistically significant.

RESULTS
IL-1R−/− Mice Are More Susceptible to Pulmonary S. Pneumoniae Infection than Wild-Type Mice
To clarify the role of IL-1 on bacterial infection of the lung, we infected both wild-type B6 and IL-1R−/− mice intratracheally with sublethal doses of S. pneumoniae WU2 mouse virulent strain. All IL-1R−/− mice died within 7 days postinfection when given 5 × 10^5 CFU of WU2 strain while all wild-type mice survived (Figure 1A). Even the lower dose (5 × 10^3 CFU) killed about 80% of the IL-1R−/− mice (Figure 1A). IL-1 receptor antagonist in the infected wild-type mice resulted in deaths in half of the mice, but all mice that were not treated survived (Supplementary Figure 1A). Total protein in the BALF of IL-1R−/− mice was much higher at 3 days postinfection with 5 × 10^5 CFU of WU2 strain than found in the wild-type mice (Supplementary Figure 1B). Next, we compared proinflammatory cytokine levels in BALF at 3 days postinfection in IL-1R−/− and wild-type mice. We found that TNF-α, IFN-γ, IL-6, and MCP-1 were significantly higher in the BALF of IL-1R−/− mice than in the wild-type mice (Figure 1B). In a histologic study, the lungs of IL-1R−/− mice were more severe inflammation at 3 days postinfection, resulting in a significantly higher clinical score than in the wild-type mice (Figure 1C). When viewed together, these results indicate that IL-1R−/− mice were susceptible to sublethal intratracheal challenge with pneumococcus because of severe inflammation in the lung.

Bacterial Proliferation Is Not Restrained in IL-1R−/− Mice
We next assessed the relationship between severe inflammation and bacterial load in the IL-1R−/− mice. There were significantly more bacteria in the lungs of IL-1R−/− mice at 4 hours after infection with 5 × 10^5 CFU of WU2 than in the wild-type mice; however, bacteria colonies gradually declined in the lungs of wild-type mice by 1 day postinfection (Figure 2A). Yet bacteria in peripheral blood were hardly detectable in both mice at 3 days after infection. Further, confocal microscopy at 1 day postinfection showed more red fluorescence-labeled WU2 strain in the lungs of IL-1R−/− mice than in wild-type mice (Figure 2B). We speculated that the severe inflammation in IL-1R−/− mice might be caused by uncontrolled bacterial propagation but not by absence of feedback regulation of IL-1. To further examine this hypothesis, we performed 2 experiments in which the initiation of immunity against bacterial infection might be established, but the propagation of bacteria was inhibited. We infected IL-1R−/− mice with either the TIGR4 ΔtrpG or the mother TIGR4 strain as a control and determined bacterial load in the lung and survival rate. When pneumococcal propagation was suppressed, the IL-1R−/− mice survived (Figure 2C). Next, we treated IL-1R−/− mice with penicillin 6 hours after infection with 5 × 10^5 CFU with WU2. Penicillin treatment led to total clearance of pneumococia by 1 day postinfection and survival of the IL-1R−/− mice (Figure 2D). These results suggest that the lethal effects of pneumococcal infection in IL-1R−/− mice might be driven by uncontrolled bacterial propagation in the lung in the IL-1 signaling-deficient condition.

Normal Neutrophil Recruitment Was Detected in the IL-1-Deficient Condition
Because the role of neutrophils in the susceptibility of bacterial infection in the IL-1-deficient condition is controversial [7, 14], we assessed the characteristics of neutrophils in the lungs of IL-1R−/− mice following intratracheal infection with 5 × 10^5 CFU of WU2. As shown in Figure 3A and 3B, at 1 day postinfection the lungs of IL-1R−/− and wild-type mice had comparable numbers of CD11b+Ly6C+Ly6G+ neutrophils. Moreover, at 3 days postinfection, there were significantly more neutrophils in the lungs of IL-1R−/− mice than in wild-type mice (Figure 3A and 3B). The level of KC in the BALF of wild-type and IL-1R−/− mice was similar at 1 day postinfection but was substantially enhanced in IL-1R−/− mice at 3 days postinfection (Figure 3C). Moreover, the bactericidal activity of peritoneal neutrophils from IL-1R−/− and wild-type mice was comparable (Figure 3D). An earlier study showed that phagocytosis by alveolar macrophages also plays a critical role in clearing bacteria from the lung [15]; therefore, we further compared the phagocytosis of alveolar macrophages in both mice at 1 day postinfection. The CD11b+CD11c+ alveolar macrophages captured red fluorescence-labeled WU2 strains in a normal way in both wild-type and IL-1R−/− mice (Supplementary Figure 2). Overall, we did not find any defect of infiltration or killing ability of neutrophils and phagocytosis of alveolar macrophages in IL-1R−/− mice. Thus, there may be other underlying mechanisms that affect the control of bacterial propagation in IL-1R−/− mice.
Fibrinogen Synthesis in the Lung Is Down-Regulated in the IL-1 Signal-Deficient Condition

To determine which genes are regulated by IL-1 signaling, at 4 hours after intratracheal infection with $5 \times 10^5$ CFU of WU2, we used microarray analysis to assess gene expression patterns in the lungs of wild-type and IL-1R$^{−/−}$ mice and compared the results to those of mice given PBS. We unexpectedly found that there were little expressions of fga and fgg messenger RNA (mRNA) in the lungs of IL-1R$^{−/−}$, whereas mRNA expression of fga and fgg were significantly enhanced in the lungs of wild-type mice 4 hours after infection with WU2 (Figure 4A). To confirm this result, we performed quantitative polymerase chain reaction of fga and fgg. We found that mRNA expression levels of fga and fgg were greatly increased in the lungs of wild-type mice after infection, but there were no significant changes in the IL-1R$^{−/−}$ mice (Figure 4B).

Figure 1. Interleukin (IL)-1R$^{−/−}$ mice are more susceptible to Streptococcus pneumoniae infection than wild-type mice. (A) Wild-type C57BL/6 (B6) and IL-1R$^{−/−}$ mice of B6 background were infected intratracheally with S. pneumoniae WU2 ($5 \times 10^5$ or $5 \times 10^3$ CFU) and monitored daily for survival (n = 8 mice per group). (B) Proinflammatory cytokine levels in the bronchoalveolar lavage fluid (BALF) of wild-type and IL-1R$^{−/−}$ mice were measured 3 days postinfection (n = 3 mice per group). (C) Pathophysiologic study of the lung was performed at 3 days postinfection by H&E staining. Representative pictures and histopathological scores are shown as mean ± SD of mice per group (n = 10). Data are representative of 3 experiments. **P < .01; ***P < .001.
Likewise, protein levels of fibrinogen were significantly higher in BALF of wild-type mice at 1 day postinfection than at 4 hours postinfection, but no changes were observed in the IL-1R−/− mice (Figure 4C). On the other hand, fibrinogen levels in blood of both wild-type and IL-1R−/− mice increased in a time-dependent manner after infection (Supplementary Figure 3). These results demonstrate that fibrinogen can be expressed in lung after pulmonary pneumococcal infection and that local synthesis of fibrinogen might be induced in the lung in an IL-1-dependent manner.

Fibrinogen Synthesis Is Activated by IL-1-Dependent IL-6 Signal

We have next tried to identify how IL-1 signal transduction induced the synthesis of fibrinogen in the lung after *Streptococcus pneumoniae* infection [16, 17]. We examined the IL-6 levels in the BALF from wild-type and IL-1R−/− mice 4 hours after infection. The IL-6 levels were increased in both wild-type and IL-1R−/− mice, although the amount of IL-6 in IL-1R−/− mice was significantly less than in wild-type mice (Figure 5A). The IL-6 signaling pathway activates Stat3, which acts as a transcription factor and binds to the IL-6 motif on human fibrinogen γ promoter [18]. Thus, we investigated the alteration of fibrinogen production by the blockade of IL-6 or Stat3 activation [19]. The transcriptional levels of fga and fgg genes were significantly reduced in wild-type mice treated with anti-IL-6 Ab or Stat3 inhibitor (Figure 5B). Likewise, the fibrinogen levels in the BALF of wild-type mice decreased after in vivo treatment with anti-IL-6 Ab or Stat3 inhibitor (Figure 5C). The reduced fibrinogen levels after treatment were similar to those of nontreated IL-1R−/− mice at 1 day postinfection (Figure 5C). In addition, the thrombin activity, which cleaves...
the fibrinogen to fibrin for the initiation of clotting, was also down-regulated in BALF of IL-1R−/− mice compared with wild-type mice (Figure 5D). Overall, our results clearly indicate that the synthesis of fibrinogen in lungs with bacterial infections is tightly regulated by the IL-1-IL-6-Stat3 cascade.

Coagulation in the Lung Is Critical to Control Bacterial Propagation

We next addressed whether the defect in the lung coagulation system, especially fibrinogen synthesis in the lung of IL-1R−/− mice after pneumococcal infection, has a direct relationship to controlling bacteria. First, we administered recombinant mouse fibrinogen in vivo into infected IL-1R−/− mice. Survival significantly improved with fibrinogen treatment (Figure 6A), and at 2 days postinfection, bacterial burden in the lung had declined in the fibrinogen-treated IL-1R−/− mice but not in those given PBS (Figure 6A). Next, warfarin-treated mice for anticoagulation approximately 60% died within 14 days postinfection and bacterial load in the lung significantly increased (Figure 6B). Overall, we found that fibrinogen in the lung
played an indispensable role in the control of pneumococcus in IL-1R−/− mice and that treatment with an anticoagulant drug exacerbated pneumococcal pneumonia in wild-type mice.

**DISCUSSION**

Innate immunity is the immediate host defense against infections [5]. IL-1, consisting of α and β subtypes, is the initial cytokine that accompanies TNF-α to induce inflammation [20]. In the murine pneumococcal pneumonia model, high levels of IL-1, TNF-α, and IL-6 were determined in lung exudates within 4 hours of infection [21, 22]. IL-1, which is mainly produced by resident alveolar macrophages, can bind to IL-1 receptor on pulmonary epithelial cells [23, 24]. The IL-1-deficient condition has been presumed to be vulnerable in infectious diseases. In a previous study, the lack of IL-1 impaired bacterial clearance and induced high mortality in pneumococcal meningitis [25]. IL-1β−/− and IL-1α/β−/− mice are particularly susceptible to pneumococcal infection, but not IL-1α−/− and IL-1R antagonist−/− mice [26]. Here, we demonstrated that IL-1R−/− mice have higher mortality and more severe inflammation than wild-type mice when infected intratracheally with *S. pneumoniae*. Our results suggest that IL-1 is critical for protection against pneumococcal infection.

Neutrophils are thought to be the first innate immune cells recruited after infection. Within a few hours of infection, they give rise to powerful protective immunity, especially against extracellular bacteria [27]. Several reports have suggested that insufficient recruitment of neutrophils may explain the susceptibility of IL-1-deficient mice [14, 26]. A recent study noted that IL-1β and its related signaling pathways play a central role in the production of CXCL8, a potent neutrophil chemoattractant, in rhinovirus infection [28]. However, it seems likely that IL-1R signals are activated via MyD88, a common adaptor molecule of TLRs, and that activation of MyD88-mediated
NFκB is a general transcription factor for the induction of inflammation that can also be activated by TNF-α [29]. Others propose that TNF-α can compensate for IL-1-mediated NFκB activation [7, 30]. There are no differences in the recruitment of neutrophils in the absence of IL-1 or TNF-α alone, but triple-mutant mice deficient in TNFR1, TNFR2, and IL-1RI are defective in the recruitment of neutrophils [7, 30]. In our study, we wondered why the number of CD11b^+Ly6C^-Ly6G^- neutrophils recruited into the lung was similar or even higher in IL-1R^-/- mice than in wild-type mice. Further, bacterial killing ability of peritoneal neutrophils from IL-1R^-/- mice was identical to those of wild-type mice. Overall, the bactericidal ability of neutrophils in the IL-1R^-/- mice in our study seemed to operate normally.

Inasmuch as uncontrolled proliferation of pneumococcus cannot be fully explained by the recruitment and killing of neutrophils, we turned to fibrinogen production, a hallmark of the coagulation cascade that plays a critical role in the formation and resolution of the pleurisy in pneumococcal pneumonia [4].

Figure 5. Interleukin (IL)-1-mediated IL-6 synthesis is responsible for fibrinogen production in the lung. (A) IL-6 levels in bronchoalveolar lavage fluid (BALF) from wild-type or IL-1R^-/- mice were measured by enzyme-linked immunosorbent assay (ELISA) at 0 and 4 h after infection with Streptococcus pneumoniae WU2 (5 x 10^8 CFU). (B) Wild-type mice were treated intratracheally with phosphate-buffered saline (PBS), anti-IL-6 Ab (0.1 mg/mouse), or Stat3 inhibitor (NSC 74859; 10 µg/mouse) following infection. Quantitative polymerase chain reaction (PCR) was used to determine messenger RNA (mRNA) levels of fibrinogen alpha (Fga) and gamma (Fgg) in the lung 4 h after infection. The mRNA expression levels were adjusted to the expression levels of β-actin. Values are mean ± SD of 3 independent experiments carried out in triplicate. (C) Fibrinogen levels measured in BALF by ELISA. Significance is vs 1 day postinfection. (D) Thrombin activity in BALF were measured. Values are mean relative fluorescence units (RFU) ± SED of 5 mice per group. Data are representative of 3 independent experiments. *p < .05; **, p < .01; ***, p < .001.

Coagulation by IL-1 in Pneumonia • JID 2013:207 (1 January) • 57
ability of neutrophils in the IL-1R−/− mice, we used microarray analysis to investigate another factor that might be altered in an IL-1-dependent manner after pneumococcal infection. The SMAD signal, protein folding, and apoptosis-related genes were highly expressed in the lungs of IL-1R−/− mice compared with those of wild-type mice (Figure 4A). Both Fos and Jun are transcription factors induced by TGFβ and SMAD proteins during pneumocystis pneumonia [31]. A recent study showed that pneumonia infection enhances endoplasmic reticulum stress and induces the protein folding-related genes [32]. In addition, apoptosis is a well-known feature of S. pneumoniae infection [33]. We also found the coagulation factors, transcripts of fga and fgg genes, were downregulated in lung tissue from IL-1R−/− mice compared with wild-type mice at 4 hours after infection. The fibrinogen is usually synthesized in the liver and is abundant in circulating blood [9, 34]. However, previous studies indicated that constitutive expression of the 3 fibrinogen genes (fga, fgb, and fgg) were found in cultured pulmonary epithelial cells and lung epithelium [35, 36]. Importantly, fibrinogen can be induced in the lung epithelium by respiratory infection such as pneumocystis pneumonia or inflammatory cytokines such as IL-6, which is regulated by IL-1 receptor-mediated NFκB activation [17, 36–38]. A recent study suggested a scenario in which phosphorylated Stat3, by IL-6 signaling cascade, binds to IL-6 motifs on the promoter region of fibrinogen genes and expresses 3 fibrinogen chains [39]. In our current study, we found less IL-6 secretion was induced in IL-1R−/− mice than in wild-type mice at early time points after infection. This was followed by lack of fibrinogen in the BALF. Likewise, blockade of IL-6 or Stat3 reduced the secretion of fibrinogen in the lungs of infected wild-type mice. We therefore suggest that IL-6 secretion induced by IL-1 signal plays an indispensable role in the synthesis of fibrinogen in the lung against pneumococcal infection.

Because fibrinogen plays a key role in clotting, we investigated the relationship between coagulation and pulmonary infection in vivo. Coagulation is one of the serine protease cascades and the ancestral innate immune system [40]. Coagulation not only contributes to arresting hemorrhage but also participates in controlling bacterial growth in the skin, vascular system, and liver [41, 42]. When coagulation is prevented by treatment with an anticoagulant or as seen in fibrinogen-deficient mice, the propagation of the pathogen is not limited and mice have higher mortality [43]. In the case of pneumonia, several coagulation factors were elevated in BALF, and the

Figure 6. Coagulation in the lung is associated with control of bacterial load and survival. (A) Survival plots of interleukin (IL)-1R−/− mice treated intratracheally with phosphate-buffered saline (PBS; n = 8) or mouse fibrinogen (Fib; 0.1 mg/mouse, n = 11) at days 0 and 1 postinfection with Streptococcus pneumoniae WU2 (5 × 10⁵ colony-forming units [CFU]). Lung bacterial load was determined at 2 days postinfection. (B) Survival plots for wild-type mice (n = 9 mice per group) treated intra-gastrically with phosphate-buffered saline (PBS) or warfarin (10 µg/mouse) every day from 1 day before infection with WU2 (5 × 10⁵ CFU). Lung bacterial load was determined at 5 days postinfection. Graphs for bacterial CFU are shown as mean ± SD (n = 4 mice per group). Data are representative of 3 independent experiments. *P < .05; **, P < .01; ***; P < .001.
inhibition of coagulation pathway affect the bacterial growth at early time point [44]. In this study, thrombin activity in the BALF of infected IL-1R−/− mice was reduced compared with wild-type mice as were decreased fibrinogen levels. In vivo treatment of exogenous fibrinogen decreased the bacterial burden and delayed death. In addition, oral administration of warfarin dramatically decreased survival and escalated the bacterial burden in the lung following S. pneumoniae infection. This result supports the notion that coagulation plays an indispensable role in regulation of bacterial growth in the lung.

IL-1 might also regulate coagulation via neutrophil activation. Administration of IL-1α in baboons after lethal challenge with *Escherichia coli* induced the formation of thrombin and treatment of IL-1 receptor antagonist attenuated the release of neutrophil elastase [45]. A recent study revealed that neutrophil serine proteases promote tissue factor- and factor XII-dependent coagulation and help retain GAS in blood vessels [10]. Hence, we hypothesize that IL-1 might have various means to regulate coagulation: These include induction of fibrinogen directly and activation of neutrophil serine proteases for enhancing thrombus formation indirectly. If so, the diminished thrombin activity in IL-1R−/− mice could be caused by the impaired activation of neutrophil serine proteases but this requires further study.

As for application to clinical cases, IL-1R−/− mice mimic humans treated with anakinra. Patients given anakinra or other IL-1 antagonist drugs are thought to be more vulnerable to infectious diseases [46, 47]. In addition, treatment with anticoagulants such as warfarin also increases susceptibility to infection [42]. The treatment with fibrinogen ameliorates the resistance to illness.

In summary, IL-1R−/− mice are highly susceptible to pulmonary infection with *S. pneumoniae*. The bacterial burden in the lung could not be controlled in the IL-1-deficient condition even though the recruitment and the bacteria-killing function of CD11b+Ly6C+Ly6G+ neutrophils were normal. Of note, fibrinogen was synthesized locally in the lung after pneumococcal infection [44]. In this study, thrombin activity in the BALF of infected IL-1R−/− mice was reduced compared with wild-type mice as decreased fibrinogen levels. In vivo treatment of exogenous fibrinogen decreased the bacterial burden and delayed death. In addition, oral administration of warfarin dramatically decreased survival and escalated the bacterial burden in the lung following *S. pneumoniae* infection. This result supports the notion that coagulation plays an indispensable role in regulation of bacterial growth in the lung.

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### Supplementary Data

Supplementary materials are available at the *Journal of Infectious Diseases* online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

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