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CD5L contributes to the pathogenesis of methicillin-resistant *Staphylococcus aureus*-induced pneumonia

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ABSTRACT

Staphylococcus aureus is a major causative microorganism in community- and healthcare-acquired pneumonia. CD5L is an important protein in the control of immune homeostasis. In this study, we found that patients with *S. aureus* pneumonia displayed increased levels of circulating CD5L. Likewise, mice with *S. aureus* pneumonia had elevated CD5L levels in the lungs. Anti-CD5L antibody protected mice from lethal pneumonia induced by methicillin-resistant *S. aureus*. The survival benefit obtained with antibody against CD5L was associated with an improvement of bacterial clearance and a reduction of pulmonary inflammatory cytokines and chemokines. Conversely, co-injection of recombinant CD5L and *S. aureus* markedly increased the lethality of *S. aureus* pneumonia. These findings suggest that CD5L contributed to the immunopathology of *S. aureus* pneumonia.

1. Introduction

Staphylococcus aureus is an important cause of bacterial pneumonia. In the past few decades, methicillin-resistant *Staphylococcus aureus* (MRSA) has evolved as the most important cause of nosocomial infections, which accounts for 10%–40% cases of hospital-acquired and ventilator-associated pneumonia [1,2]. Even though some antimicrobial agents, such as linezolid and vancomycin, are approved by the U.S. Food and Drug Administration for the treatment of MRSA pneumonia, there remains significant morbidity and mortality associated with MRSA, which accounts for 94, 360 of all reported cases and 18, 650 deaths in the United States annually [3]. The emergence of linezolid resistance in *Staphylococcus* poses significant challenges to the clinical treatment of MRSA infections [4], and the emergence of less-susceptible strains challenges the current role of vancomycin in treating MRSA pneumonia [5]. Moreover, even with appropriate antibiotic treatment, MRSA infections in humans are often associated with poor clinical outcomes [6]. Therefore, it is imperative to gain more insight into host immune responses that could influence the outcome of MRSA pneumonia.

MRSA pneumonia develops when the initial host response fails to control this bacterial infection, resulting in aberrant lung inflammation and injury [7,8]. *S. aureus* infection could activate a variety of proinflammatory signaling cascades, leading to the production of some important inflammatory mediators, which contribute to the immunopathology of MRSA infection [7–9]. For example, IL-17 expression has been found to be essential for host defense against *S. aureus* infection [10], while IL-16 expression has been shown to contribute to the pathogenesis of *S. aureus* pneumonia [11]. Identification of the role of host factors involved in the pathogenesis of MRSA would provide potential immunomodulatory adjuvant therapeutic target for treating MRSA pneumonia.

CD5L (for CD5-like), also known as apoptosis inhibitor of macrophages (AIM), is a member of the scavenger receptor cysteine-rich domain superfamily (SRCR-SF), which is expressed mostly by macrophages and to a lower extent by epithelial cells in the lung [12]. CD5L was initially identified as a contributor to protect macrophages against apoptosis. Recent studies have shown that CD5L is implicated in a variety of inflammatory diseases, such as atherosclerosis [13], chronic kidney disease [14], obesity-associated inflammatory diseases [15], chronic obstructive pulmonary disease (COPD) [16], lung adenocarcinoma [17] and hepatocellular carcinoma in steatosis [18]. Moreover, there have been several reports showing the involvement of CD5L in the pathogenesis of microbial infections, including chronic hepatitis C virus (HCV) infection [19], bacterial and fungal infection [20]. Based on these data, we hypothesized that CD5L may play a role in the

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immunopathology of MRSA pneumonia. We here identified CD5L as a possible deleterious mediator during MRSA pneumonia. Blockade of CD5L using antibody against CD5L represents a potential adjuvant therapy for MRSA pneumonia, as a means to improve host control of infection and to improve survival.

2. Materials and methods

2.1. Human studies

Twenty-five patients with pneumonia caused by *Staphylococcus aureus* and sixteen control subjects were recruited from Children's Hospital of Chongqing Medical University (Supplementary Table 1). Pneumonia due to *Staphylococcus aureus* was defined as culture positivity for *Staphylococcus aureus* from any clinical sample plus persistent infiltrate on chest radiography associated with at least one of the following: fever (temperature ≥ 38.0 °C) or hypothermia (temperature < 35.0 °C), cough, pleuritic chest pain, dyspnea, and altered breath sounds on auscultation [21]. Peripheral blood was collected at the time of patient admission, and serum was then isolated and stored at −80 °C. Patient data such as the counts of white blood cells (WBC), the levels of C-reaction proteins (CRP), and microbial culture results were recorded. Patients with malignancy and organ transplantation, HIV-infected patients, and patients receiving immunosuppressive agents in the past 8 weeks were excluded from the study. Thirty-eight patients without lung disease scheduled to undergo elective surgery served as a control. This protocol was approved by the Clinical Research Ethics Committee of Chongqing Medical University, and informed consent was obtained from all participants according to the Declaration of Helsinki.

2.2. Mice

C57BL/6 mice aged 6–8 weeks were obtained from and raised at Chongqing Medical University. All mice were housed under humidity- and temperature-controlled specific pathogen-free conditions in the animal facility of Chongqing Medical University. All animal experiments were done in accordance with the Institutional Animal Care and Use Committee's guidelines at the Chongqing Medical University.

2.3. MRSA lung infection

Methicillin-resistant *S. aureus* (American Type Culture Collection, ATCC43300) purchased from the ATCC was used in these studies. The bacteria were grown overnight in brain-heart infusion broth (Becton Dickinson) and were frozen at −80 °C. For lung infection, mice were anesthetized by intraperitoneal (i.p.) injection with 100 μl of xylazine (20 mg/ml) and ketamine (1 mg/ml) in phosphate-buffered saline (PBS). The anesthetized mice were then intranasally inoculated with 40 μl PBS containing MRSA at a dose of 5×10^8 colony-forming units (CFU). All mice were observed at 2 h intervals for the first 48 h, and survival was monitored for at least 14 days.

2.4. Bacterial burden analysis

At 24 h after bacterial infection, mice were sacrificed and their lungs and blood were harvested. Lungs were homogenized with a glass homogenizer. Serial dilutions of the samples were inoculated on Trypticase soy agar plates and incubated for 24 h at 37 °C to enumerate CFU.

2.5. Histopathology

At 24 h post infection, mice were euthanized, and the lungs were excised and fixed with paraformaldehyde, then embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E). For immunohistochemistry, the left lung was inflated with 10% neutral buffered formalin for 24 h before paraffin embedding. Slides were stained anti-CD5L polyclonal antibody. Controls were performed with secondary antibody only.

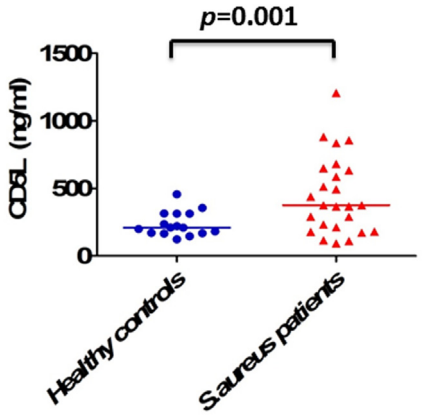

Fig. 1. CD5L was elevated in human pneumonia caused by *Staphylococcus aureus.* CD5L concentrations were measured by ELISA in the sera samples collected from 25 patients with *S. aureus* pneumonia and from 38 healthy controls. Each dot represents a measurement within an individual patient, with horizontal lines showing medians. $p = 0.001$, by the Mann-Whitney U test, for between-group comparison (denoted by horizontal bracket).

2.6. Pathology score (PA) assessment

Mouse lungs were harvested, fixed in 10% buffered formalin, and embedded in paraffin. 4 μm sections were stained with H&E and analyzed by a pathologist blinded for all groups. To score lung inflammation and damage, the entire lung surface was analyzed with respect to the following parameters: bronchitis, edema, interstitial inflammation, intra-alveolar inflammation, pleuritis, endothelialitis and percentage of the lung surface demonstrating confluent inflammatory infiltrate. Each parameter was graded from 0 (absent) to 4 (severe). The total pathology scores for lungs were expressed as the sum of the score for all parameters.

2.7. Quantification of cytokines and chemokines

The concentrations of CD5L were determined by mouse CD5L ELISA kit (My Biosource Inc., San Diego, CA) or human CD5L quantikine ELISA kit (My Biosource Inc.). The levels of IL-1β, TNF-α, IL-6, IL-10, CXCL1, and CCL2 were determined with commercially available ELISA kits (BioLegend Inc., San Diego, CA) according to the manufacturer's instructions.

2.8. Flow cytometric analysis

Single cell suspensions from mouse lungs were blocked with Fcγ blocker (BD Pharmingen, San Jose, CA, USA) and stained with antibodies against extracellular mouse F4/80 (BD Pharmingen). Cells were then incubated with Fix/Perm solution (BD Pharmingen) for 20 min before washing in Perm/Wash buffer (BD Pharmingen), and then stained with goat anti-mouse CD5L antibodies (R&D Systems, Minneapolis, MN, USA) for 60 min followed by FITC-conjugated rabbit anti-goat IgG secondary antibody for another 45 min at 48C in dark. Cells were then washed, resuspended, and subjected to analysis. Expression of intracellular phosphorylated signaling molecules of 10, 000 viable cells was analyzed by flow cytometry (FACSCalibur, BD Biosciences).

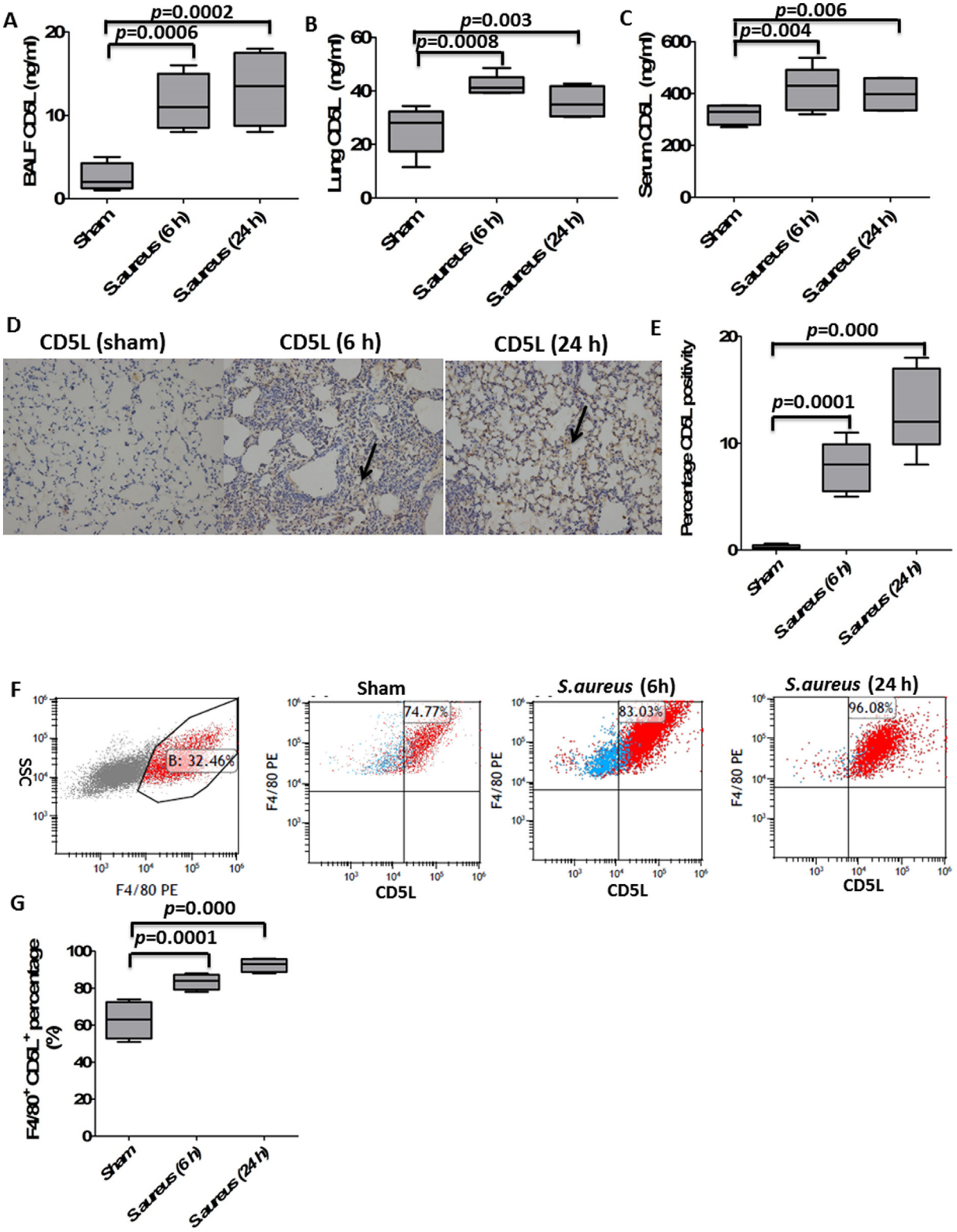

Fig. 2. CD5L was elevated in mice with MRSA pneumonia. Mice (n = 6) were intranasally infected with 5×10^8 *S. aureus* (ATCC43300). (A-C) CD5L levels in bronchoalveolar lavage fluids (BALF, A), whole lung homogenates (B), and sera (C) were determined by ELISA at 6 and 24 h after infection. (D) Immunohistochemistry by standard methods with anti-CD5L antibody on fixed-whole lungs of mice given phosphate-buffered saline (PBS) or infected with 5×10^8 *S. aureus* for 24 h. Black arrows point to areas of CD5L staining (brown). (E) Semi-quantitation of pulmonary CD5L positivity at 6 and 24 h after *S. aureus* infection. (F) Representative intracellular staining analysis for CD5L in F4/80$^+$ macrophages in the lung from mice after *S. aureus* infection. (G) Percentage of F4/80$^+$CD5L$^+$ macrophages in the lung from mice after *S. aureus* infection. Comparisons between groups were analyzed using Kruskal-Wallis test followed by Dunn's multiple comparisons post test. All results are representative of 3 independent experiments. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

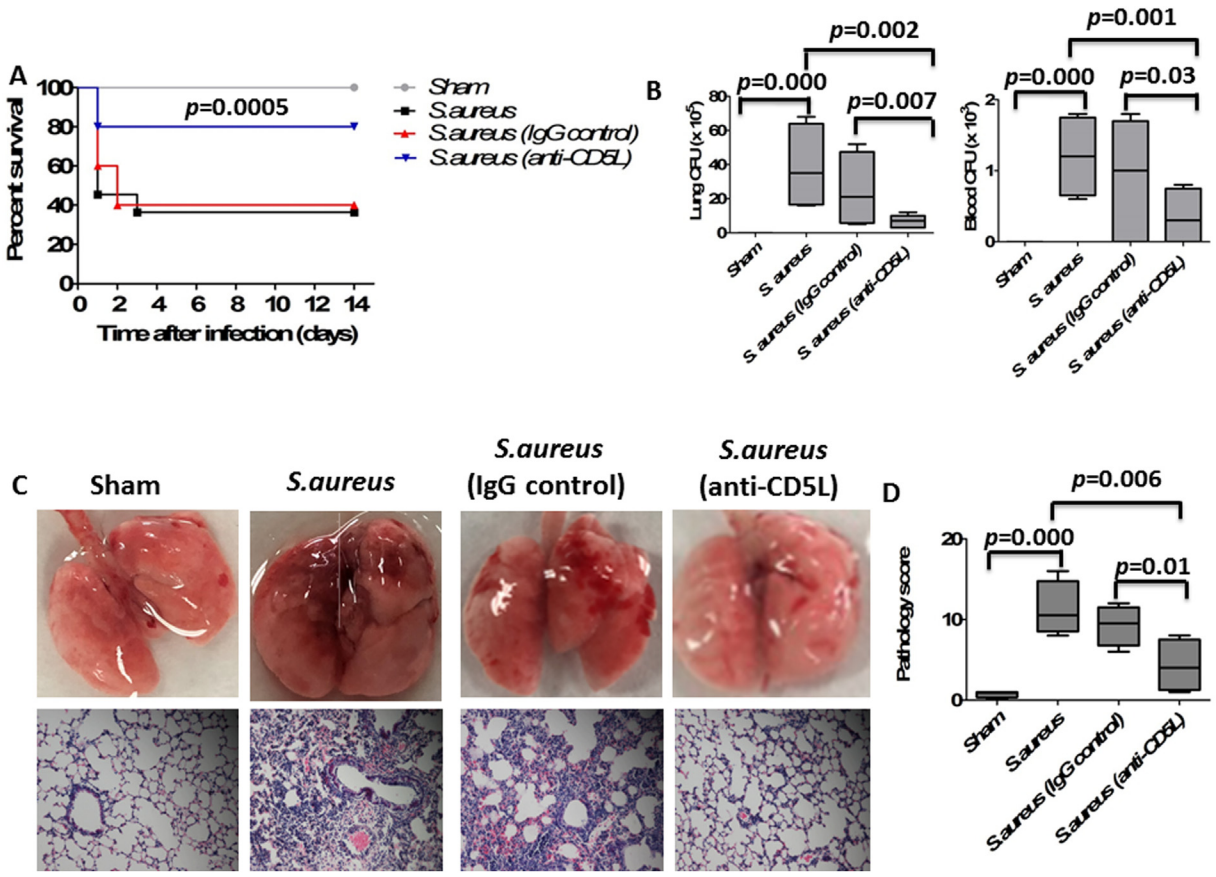

Fig. 3. Antibody against CD5L protected mice against lethal MRSA pneumonia. 20 μg of anti-CD5L antibodies or IgG isotype was intranasally injected immediately after intranasal infection with 5×10^8 *S. aureus* (ATCC43300). (A) Survival of mice ($n = 16$) after intranasal infection with *S. aureus* (ATCC43300). $p = 0.0005$, by Kaplan-Meier analysis followed by log-rank tests, compared with mice treated with isotypical IgG control. (B) Bacterial loads in the lung and blood at 24 h after intranasal inoculation with *S. aureus* ($n = 5$). Comparisons between groups were analyzed using Kruskal-Wallis test followed by Dunn's multiple comparisons post test. (C) Representative pathological and histological analysis of the lungs from mice (n = 5) treated with or without anti-CD5L antibody at 24 h after intranasal infection with *S. aureus* (ATCC43300). (D) Histological scores for lungs from mice treated with or without anti-CD5L antibody. Comparisons between groups were analyzed using Kruskal-Wallis test followed by Dunn's multiple comparisons post test. All results are representative of 3 independent experiments.

2.9. In vivo blockade of CD5L

For the neutralization of CD5L, 20 μg of anti-CD5L antibodies (R&D systems) or IgG isotype antibodies were intranasally injected immediately after bacterial infection.

2.10. In vivo administration of recombinant murine CD5L

Recombinant murine CD5L (R&D systems) was injected intranasally at the time of bacterial infection (1 μg/injection), and PBS was delivered in a similar fashion as control solutions.

2.11. Phagocytosis and intracellular killing assays

Alveolar macrophages were collected as described previously [22], and samples from 4 to 6 mice were pooled. Cells were seeded at 10^5 cells/well onto flat-bottomed 96-well plates in Roswell Park Memorial Institute 1640 medium supplemented with 10% fetal calf serum. Plates were cultured overnight to separate plastic adherent cells, and non-adherent cells were removed by replacement of culture medium with antibiotic-free medium. Neutrophils were purified from the bone marrow by magnetic cell sorting (Miltenyi Biotec, Bergisch Gladbach, Germany).

Adherent alveolar macrophages (1×10^5 cells) were infected with live *S. aureus* (multiplicity of infection, 10) at 37 °C for 1 h, and they were washed with buffer containing tobramycin (100 μg/ml) for 30 min to remove extracellular bacteria and were lysed with lysis buffer (Promega Corp, Madison, USA). Live intracellular bacteria were quantified by culture of lysates for determination of bacterial uptake ($t = 0$ h) and intracellular killing ($t = 4$ h). Killing was calculated from the percentage of colonies present at t = 4 h as compared to t = 0 h, as follows: 100 − [number of CFUs t = 4 h/number of CFUs t = 0 h]. In another experiment, neutrophils (1×10^6 cells) were infected with *S. aureus* at an multiplicity of infection ratio of 1:100 at 37 °C for 30 min, and cells were resuspended in medium containing 100 μg/ml tobramycin for 30 min to remove extracellular bacteria, and then lysed in PBS containing 0.1% Triton 100 for assessment of uptake ($t = 0$ h), and additional samples were incubated for 1 additional hour ($t = 1$ h) to assess bacterial killing as described above. In some experiments, macrophages and neutrophils were pretreated with recombinant murine CD5L (200 ng/ml, R&D systems) before infection by live *S. aureus*.

2.12. Statistical analysis

Human data were expressed as scatter dot plots with medians. Mice data were expressed as box plots, showing the smallest observation, the lower quartile, the median value, the upper quartile, and the largest observation, or as median values with interquartile ranges. Comparisons between groups were analyzed using the Mann-Whitney *U* test or Kruskal-Wallis test followed by Dunn's multiple comparisons post test as appropriate. For survival studies, Kaplan- Meier analyses followed by log-rank tests were performed. For correlation studies, the

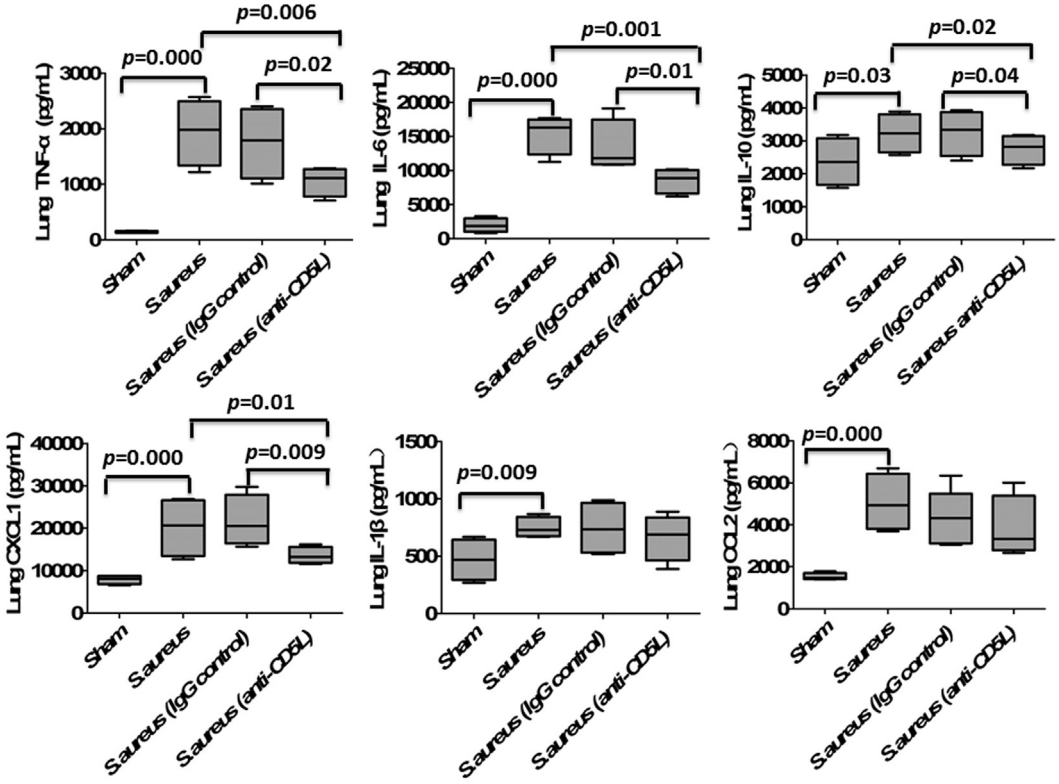

Fig. 4. Antibody against CD5L down-regulated the production of cytokines and chemokines during MRSA pneumonia. Cytokine and chemokine concentrations in the lungs from 5 mice treated with or without anti–CD5L antibodies were determined by ELISA at 24 h after intranasal infection with 5×10^8 *S. aureus* (ATCC43300). Comparisons between groups were analyzed using Kruskal-Wallis test followed by Dunn's multiple comparisons post test. All results are representative of 3 independent experiments.

Spearman rank correlation test was used. All analyses were done using GraphPad Prism version 5.01 (GraphPad Software, San Diego, CA, USA). p values of < 0.05 were considered statistically significant.

3. Results

3.1. Patients with S. aureus pneumonia show elevated serum CD5L levels

To obtain a first insight into CD5L release during *S. aureus* pneumonia, we measured CD5L levels in the sera from 24 pneumonia patients caused by *S. aureus* infection. Patients with *S. aureus* pneumonia displayed a significant ($p < 0.01$) increase of circulating CD5L levels (median 368 ng/ml for patients) as compared with healthy controls (median 221 ng/ml for controls; Fig. 1).

3.2. S. aureus induced CD5L production in the murine lung

We next used a well-established bacterial pneumonia model in mice, produced by intranasal infection with a methicillin-resistant *S. aureus* strain, ATCC43300. After infection, significantly ($p < 0.01$) higher levels of CD5L were recovered from the bronchoalveolar lavage fluids (BALF) and whole-lung homogenates of C57BL/6 mice infected with MRSA (Fig. 2A and B). In the blood, CD5L was also significantly ($p < 0.01$) enhanced after *S. aureus* infection (Fig. 2C). We also performed immunohistochemical staining for CD5L on lung tissue slides from naive and infected mice. While murine control lung tissue samples showed a small number of cells expressing CD5L, lung tissue from mice infected with MRSA demonstrated a strong increase in CD5L staining (Fig. 2D and E). Furthermore, flow cytometry analysis showed that the intracellular expression of CD5L was significantly ($p < 0.0005$) increased in F4/80$^+$ macrophages from lungs of mice after *S. aureus* infection (Fig. 2F and G). Together, these data indicate that murine *S. aureus* pneumonia resembles human *S. aureus* pneumonia with regard to increased local and systemic expression of CD5L.

3.3. Antibody against CD5L protected against MRSA pneumonia

To examine the role of CD5L in MRSA pneumonia, we first studied the effect of CD5L blockade using anti-CD5L polyclonal antibody in the mouse model of MRSA pneumonia. Treatment of MRSA-challenged mice with a single dose of 20 μg of anti-mouse polyclonal antibodies against CD5L increased survival from 40% to 80% (Fig. 3A). Antibody against CD5L resulted in an approximately 3 times-reduced median bacterial load in the lung and 6 times-reduced median bacterial load in the blood as compared with IgG isotype control (Fig. 3B). There was no significant difference for the bacterial load in the mice infected with *S. aureus* alone as compared with *S. aureus*-infected mice treated with IgG control. Furthermore, CD5L blockade resulted in attenuated lung pathology, with less hemorrhage, endothelialitis, and edema (Fig. 3C), which was reflected by significantly ($p < 0.05$) lower lung pathology scores in the mice treated with anti-CD5L antibody (Fig. 3D).

3.4. Blocking CD5L decreased TNF-α, IL-6, IL-10 and CXCL1 levels during MRSA pneumonia

The effect of CD5L blockade on MRSA-induced cytokines and chemokines in the lung was evaluated at 24 h after MRSA pneumonia. MRSA pneumonia was associated with dramatic increases in TNF-α, IL-1β, IL-6, IL-10, CXCL1 and CCL2 in the lung. Treatment with anti-CD5L antibody blunted this inflammatory response in the MRSA pneumonia model, and resulted in a significant ($p < 0.05$) decrease in TNF-α (median 991 pg/ml for anti-CD5L-treated mice; median 1825 pg/ml for IgG-treated mice), IL-6 (median 8163 pg/ml for anti-CD5L-treated mice; median 11,082 pg/ml for IgG-treated mice), IL-10 (median

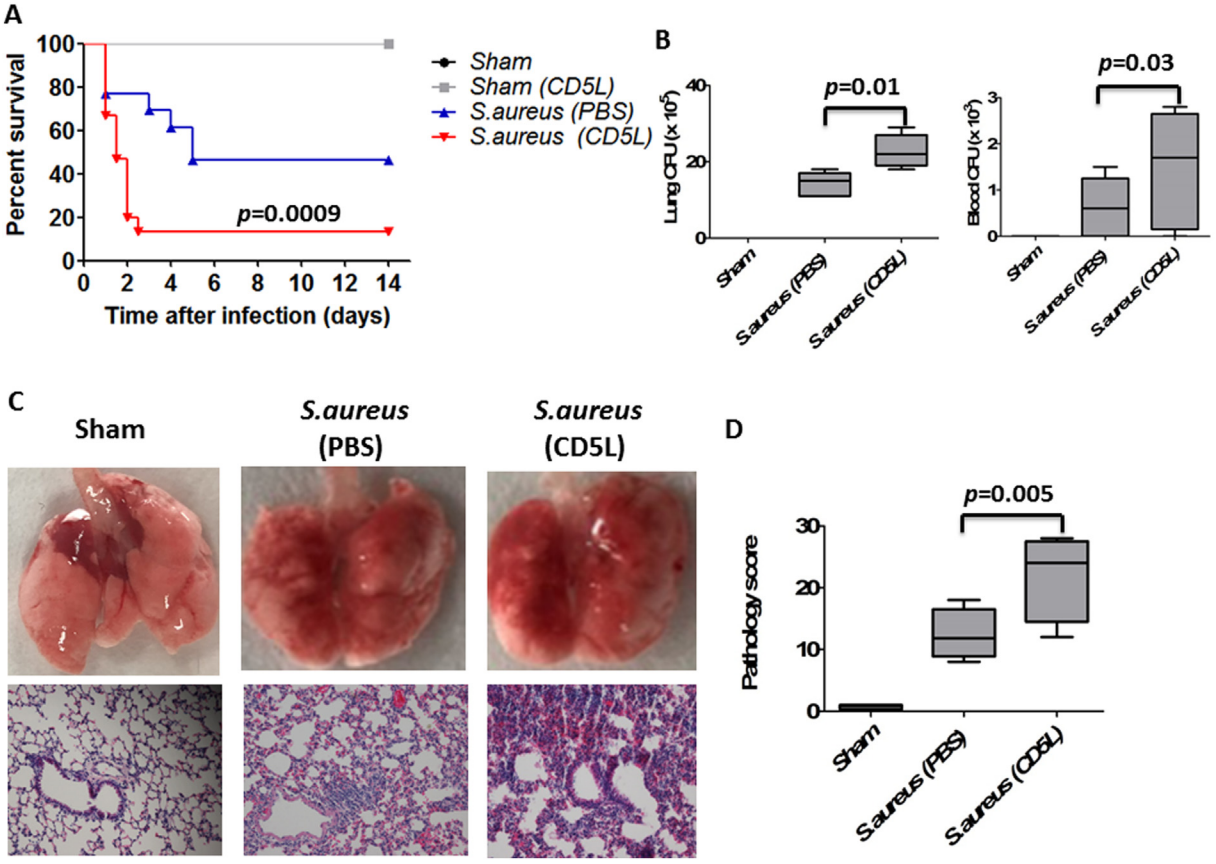

Fig. 5. CD5L potentiated lethality of MRSA pneumonia. Mice were injected intranasally with 5×10^8 *S. aureus* (ATCC43300) given either alone or in combination with 1 μg of recombinant mouse CD5L. (A) Survival of mice with MRSA pneumonia (n = 16) following CD5L supplementation. $p < 0.0009$, by Kaplan-Meier analysis followed by log-rank tests, compared with mice treated with PBS control. (B) Bacterial loads in the lung and blood at 24 h after intranasal inoculation with *S. aureus* (n = 5). Comparisons between groups were analyzed using Kruskal-Wallis test followed by Dunn's multiple comparisons post test. (C) Representative pathological and histological analysis of the lungs from mice (n = 5) treated with or without recombinant CD5L protein at 24 h after intranasal infection with *S. aureus* (ATCC43300). (D) Histological scores for lungs from mice treated with or without CD5L protein at 24 h after intranasal infection with *S. aureus*. Comparisons between groups were analyzed using Kruskal-Wallis test followed by Dunn's multiple comparisons post test. All results are representative of 3 independent experiments.

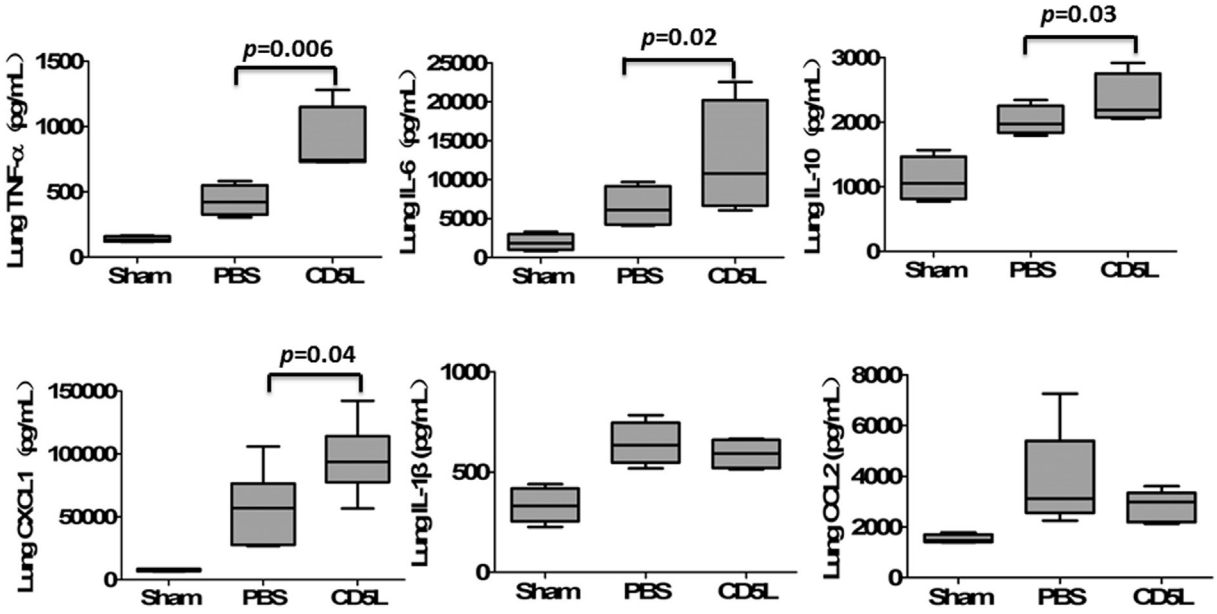

Fig. 6. CD5L administration up-regulated the production of cytokines and chemokines during MRSA pneumonia. Cytokine and chemokine concentrations in the lungs from 5 mice treated with or without recombinant CD5L (1 μg/injection) were determined by ELISA at 24 h after intranasal infection with 5×10^8 *S. aureus* (ATCC43300). Comparisons between groups were analyzed using Kruskal-Wallis test followed by Dunn's multiple comparisons post test. All results are representative of 3 independent experiments.

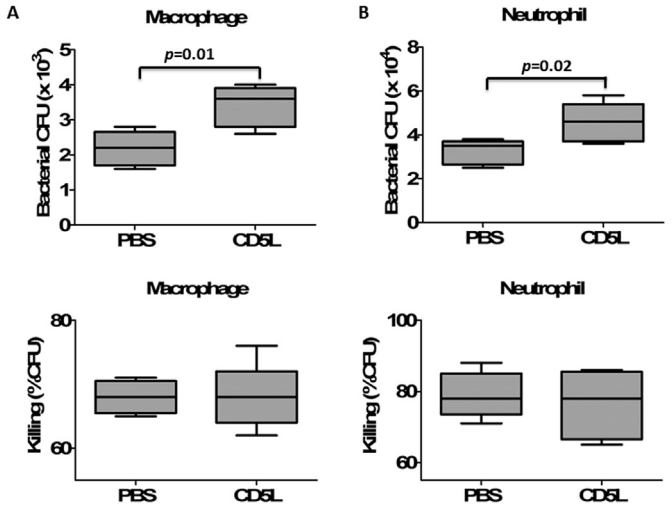

Fig. 7. Effects of CD5L on bacterial phagocytosis and killing by phagocytes. (A) Mouse alveolar macrophages ($n = 6$) were pretreated with recombinant CD5L (200 ng/ml) for 1 h, and then were infected with *S. aureus* (multiplicity of infection, 10). Extracellular bacteria were then removed by washing with tobramycin. Cells were lysed with lysis buffer, and live intracellular bacteria were quantified by culture of lysates for determination of bacterial uptake ($t = 0$ h) and intracellular killing ($t = 4$ h). (B) Neutrophils (n = 6) were stimulated with recombinant CD5L at the concentration of 200 ng/ml for 1 h and then infected with *S. aureus* (multiplicity of infection, 100). Cells were washed with buffer containing tobramycin to remove extracellular bacteria and were lysed. Live intracellular bacteria were quantified by culture of lysates for determination of bacterial uptake (t = 0) and intracellular killing ($t = 1$ h). Comparisons between groups were analyzed using the Mann-Whitney *U* test. All results are representative of 3 independent experiments.

2692 pg/ml for anti-CD5L-treated mice; median 3043 pg/ml for IgG-treated mice) and CXCL1 (median 13,809 pg/ml for anti-CD5L-treated mice; median 20,189 pg/ml for IgG-treated mice) levels in the lung (Fig. 4). In addition, there was no significant difference for pulmonary TNF-α, IL-1β, IL-6, IL-10, CXCL1 and CCL2 levels in the mice infected with *S. aureus* alone as compared with *S. aureus*-infected mice treated with IgG control.

3.5. CD5L supplementation exacerbated MRSA pneumonia

Because the administration of antibody against CD5L was found to protect mice from MRSA pneumonia, we performed the reverse experiment and examined the effect of an intranasal injection of recombinant mouse CD5L given at the onset of intranasal MRSA infection. CD5L supplementation in the absence of MRSA pneumonia had no effect on survival at a dose of 1 μg as compared with sham group (both of the two groups showed 100% survival). Co-injection of MRSA and recombinant CD5L significantly ($p < 0.001$) increased mortality in mice (Fig. 5A), thus confirming the harmful effect of an excessive amount of CD5L during MRSA pneumonia. Addition of CD5L to MRSA-challenged mice also resulted in an approximately 1.5 times-increased median bacterial load in the lung and 2 times-increased median bacterial load in the blood as compared with PBS control (Fig. 5B). Furthermore, histological analysis showed that administration of recombinant CD5L in mice with MRSA pneumonia resulted in aggravated lung pathology with more advanced signs of alveolar hemorrhage (Fig. 5C and D).

3.6. CD5L increased TNF-α, IL-6, IL-10 and CXCL1 levels during MRSA pneumonia

We further examined whether CD5L modulated the production of cytokines and chemokines in the lungs of mice at 24 h when they were intranasally challenged with MRSA. CD5L injection resulted in a significant ($p < 0.05$) increase in pulmonary TNF-α, IL-6, IL-10 and CXCL1 levels during MRSA pneumonia (Fig. 6).

3.7. CD5L influenced bacterial phagocytosis by phagocytes

To investigate whether CD5L influences intracellular uptake and killing of *S. aureus* in infected phagocytes, we studied bacterial uptake and killing capacities of neutrophils and alveolar macrophages. *S. aureus* uptake was significantly ($p < 0.05$) up-regulated by addition of extrinsic CD5L in macrophages and neutrophils (Fig. 7A and B). However, preincubation with recombinant CD5L did not have direct effects on intracellular killing of *S. aureus* by macrophages or neutrophils (Fig. 7A and B).

4. Discussion

CD5L has been implicated in the modulation of many important aspects of inflammatory conditions. Although previous studies have uncovered an important role of CD5L as a pattern recognition receptor (PRR) of bacterial components [12], the contribution of CD5L to *S. aureus* pneumonia remains unknown. In the present report, we identified CD5L to be centrally involved in the pathogenesis of pneumonia caused by MRSA. We observed elevated CD5L levels in the course of clinical and experimental *S. aureus* pneumonia. Blockade of the CD5L activity with antibody against CD5L produced substantial survival benefit in a mouse model of MRSA pneumonia.

It has been reported that CD5L gene expression in the liver was enhanced after *Corynebacterium parvum* infection in mice [23], and the mRNA levels of CD5L in the lung tissue of mice were significantly increased after lipopolysaccharide (LPS) administration [24]. Knowledge about CD5L expression during human pneumonia is highly limited. We here demonstrated for the first time that blood CD5L levels were elevated in the patients with *S. aureus* pneumonia. In addition, CD5L release occurred in the mouse model of MRSA pneumonia, and pulmonary F4/80 macrophages were important cellular source of CD5L. Taken together, these studies suggest that CD5L may play a potential role in the pathogenesis of MRSA pneumonia.

A recent report has shown that CD5L played an orchestrating role in the resolution of acute lung injury, and that the resolution of lung injury was accelerated in CD5L-deficient mice [24]. Deletion of CD5L also attenuated the inflammatory response and infarct size in acute myocardial infarction of mice [25]. Here we found that treatment with antibody against CD5L could protect mice against MRSA pneumonia, and intranasal administration of recombinant CD5L could worsen outcome of MRSA pneumonia. Thus, CD5L can be a potential adjunctive therapeutic target for the treatment of pulmonary MRSA-induced mortality.

The survival benefit obtained with antibody against CD5L was associated with improved bacterial clearance, as shown by a decrease of bacterial loads from lung and blood, which provided a lower inflammatory stimulus, thus resulting in a decreased inflammatory response during MRSA. We also found that blocking CD5L could decrease, while CD5L supplementation could increase pulmonary TNF-α, IL-6, IL-10 and CXCL1 levels during MRSA pneumonia. However, we did not find evidence for a direct cause and effect relationship between CD5L and bacterial clearance. CD5L did not directly influence bacterial killing by macrophages and neutrophils, and it could enhance the uptake of *S. aureus* by macrophages and neutrophils. Our data are therefore consistent with previous report that CD5L was able to bind to and aggregate Gram-negative and-positive bacteria [20]. In fact, in vitro studies have demonstrated that *S. aureus* could survive for long periods of time inside both macrophages and neutrophils isolated from different animals and humans [26–28]. Besides, aggregation and survival of *S. aureus* inside various cells, including macrophages and neutrophils, has been proposed as a mechanism for persistence of this microorganism [28]. From these data, we think that CD5L release during MRSA

pneumonia might favor the uptake of *S. aureus* by macrophages and neutrophils, leading to persistence of *S. aureus* in the host, which contributes to the pathogenesis of MRSA pneumonia. Further experimentation will be required to determine whether this occurs during the course of natural MRSA infection in the lung.

The present study is the first to document that CD5L release occurs in *S. aureus* pneumonia. It is interesting that treatment with antibody against CD5L can protect against MRSA, which was associated with an improvement of bacterial clearance and a reduction of inflammatory response. Therefore, treatment with antibody against CD5L may offer a new strategy for the management of patients with *S. aureus* infections.

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Disclosure statement

All authors declare no conflicts of interest.

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