Cell Biochem Biophys (2014) 70:795–803
DOI 10.1007/s12013-014-9983-7

ORIGINAL PAPER

Plasma-Derived Human C1-Esterase Inhibitor Does Not Prevent Mechanical Ventilation-Induced Pulmonary Complement Activation in a Rat Model of *Streptococcus pneumoniae* Pneumonia

F. M. de Beer · H. Aslami · J. Hoeksma · G. van Mierlo · D. Wouters · S. Zeerleder · J. J. T. H. Roelofs · N. P. Juffermans · M. J. Schultz · W. K. Lagrand

Published online: 24 April 2014

Abstract Mechanical ventilation has the potential to cause lung injury, and the role of complement activation herein is uncertain. We hypothesized that inhibition of the complement cascade by administration of plasma-derived human C1-esterase inhibitor (C1-INH) prevents ventilation-induced pulmonary complement activation, and as such attenuates lung inflammation and lung injury in a rat model of *Streptococcus pneumoniae* pneumonia. Forty hours after intratracheal challenge with *S. pneumoniae* causing pneumonia rats were subjected to ventilation with lower tidal volumes and positive end-expiratory pressure (PEEP) or high tidal volumes without PEEP, after an intravenous bolus of C1-INH (200 U/kg) or placebo (saline). After 4 h of ventilation blood, broncho-alveolar lavage fluid and lung tissue were collected. Non-ventilated rats with *S. pneumoniae* pneumonia served as controls. While ventilation with lower tidal volumes and PEEP slightly amplified pneumonia-induced complement activation in the lungs, ventilation with higher tidal volumes without PEEP augmented local complement activation more strongly. Systemic pre-treatment with C1-INH, however, failed to alter ventilation-induced complement activation with both ventilation strategies. In accordance, lung inflammation and lung injury were not affected by pre-treatment with C1-INH, neither in rats ventilated with lower tidal volumes and PEEP, nor rats ventilated with high tidal volumes without PEEP. Ventilation augments pulmonary complement activation in a rat model of *S. pneumoniae* pneumonia. Systemic administration of C1-INH, however, does not attenuate ventilation-induced complement activation, lung inflammation, and lung injury.

Keywords Lung injury · Mechanical ventilation · Ventilator-induced lung injury · Complement · C1-esterase inhibitor · Pulmonary inflammation

F. M. de Beer · H. Aslami · J. Hoeksma · N. P. Juffermans · M. J. Schultz · W. K. Lagrand
Laboratory of Experimental Intensive Care and Anesthesiology (L·E·I·C·A), Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

F. M. de Beer (✉) · N. P. Juffermans · M. J. Schultz · W. K. Lagrand
Department of Intensive Care Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands
e-mail: f.m.debeer@amc.uva.nl

G. van Mierlo · D. Wouters · S. Zeerleder
Sanquin Research and Landsteiner laboratory, Department of Immunopathology, Academic Medical Center, Amsterdam, The Netherlands

S. Zeerleder
Department of Hematology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

J. J. T. H. Roelofs
Department of Pathology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Introduction

Patients with severe community-acquired pneumonia frequently need respiratory support [1]. While mechanical ventilation is regarded as a life-saving intervention in these patients, it may also cause additional lung injury via overstretching of aerated lung tissue [2]. The role of complement in the pathogenesis of ventilation-induced lung injury is uncertain. The complement cascade is an evolutionary highly conserved part of the innate immune system that plays an important role in host defense through opsonisation and lysis of bacterial pathogens, and

chemotaxis of leukocytes to the site of infection [3–6]. Although the complement cascade plays a beneficial role in innate immunity, a failure to prevent excessive complement activation and/or its amplification is associated with development of lung injury [7, 8].

C1-esterase inhibitor (C1-INH) is an acute phase protein regulating the initial activation of the complement cascade by inhibition of both the classical pathway and the lectin pathway [9]. During inflammation, complement activation could tilt toward an exaggerated response due to a relative C1-INH deficiency [10]. This deficiency occurs if demands for C1-INH exceed its synthesis due to complex formations and inactivation by proteolysis [11]. As such, abundant stress in the lung may result in "overheated" complement activation causing additional lung injury.

We hypothesized that ventilation augments pulmonary complement activation in rats with pneumonia. We furthermore hypothesized that systemic treatment with C1-INH prevents ventilation-induced pulmonary complement activation, and as such attenuates lung inflammation and lung injury. To test these hypotheses, we pre-treated rats systemically with plasma-derived human C1-INH in a well-established and clinically relevant rat model of ventilation for *Streptococcus pneumoniae* pneumonia.

Methods

Ethics Statement

The animal care and use committee of the Academic Medical Center, Amsterdam, The Netherlands approved the study protocol. All procedures were carried out in compliance with Institutional Standards for Human Care and Use of Laboratory Animals.

Induction of Pneumonia

Fifty-six male Wistar rats (weight 320–400 g; Charles River, Maastricht, The Netherlands) were challenged intratracheally with $\sim 1.0 \times 10^7$ colony-forming units (CFU) of *S. pneumoniae* serotype 3 (ATCC 6303, Rockville, MD, USA) using a trans-oral miniature nebulizer (Penn-Century, Philadelphia, PA, USA) under light anesthesia (3 % isoflurane in oxygen). Thereafter, rats received an intra-peritoneal bolus of 10 mL sterile normal saline (NaCl 0.9 %) after which they were allowed to recover in their cages with food and water ad libitum. At 24 h after challenge, rats received an additional intra-peritoneal bolus of 10 mL sterile saline injection. A flow diagram of the study is presented in Fig. 1.

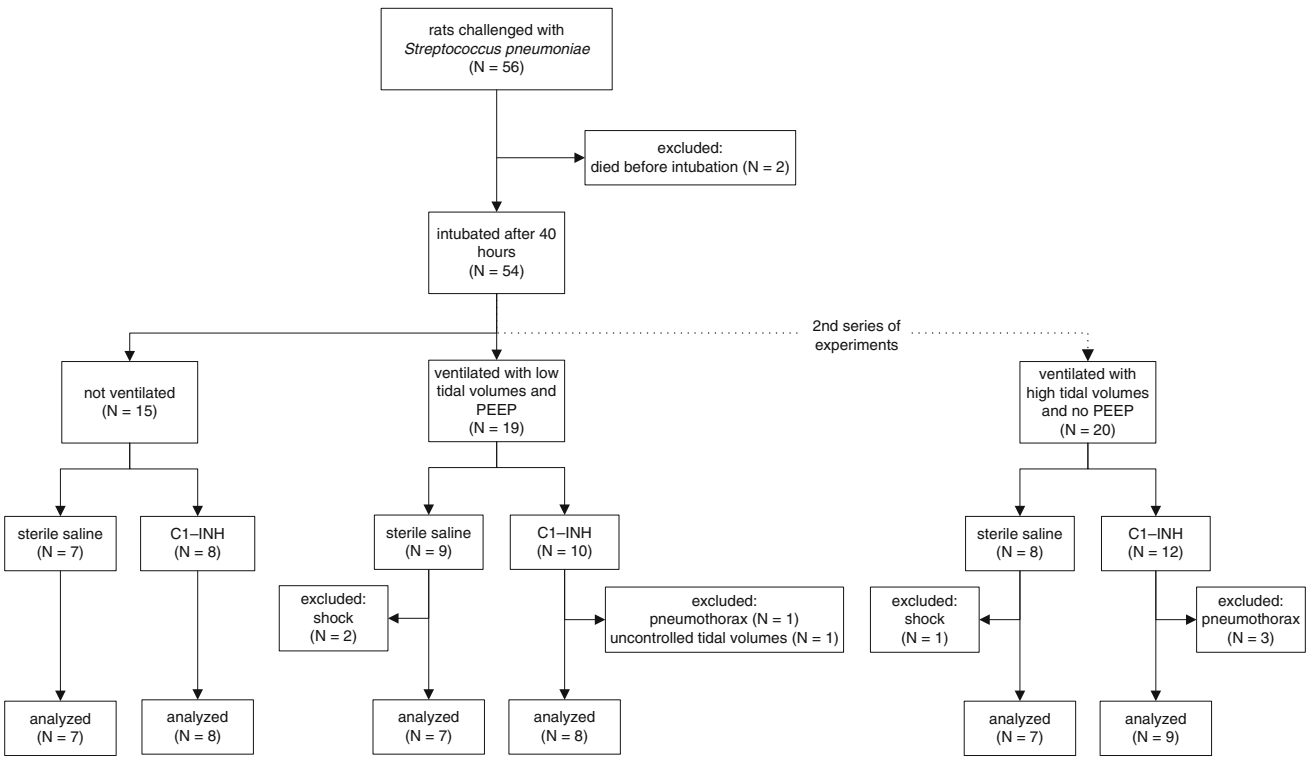

Fig. 1 Consort diagram of the study

Anesthesia and Instrumentation

40 h after administration of *S. pneumoniae* inducing pneumonia, rats were anesthetized with intra-peritoneal injection, receiving 90 mg/kg ketamine (Nimatek, Eurovet Animal Health BV, Bladel, The Netherlands), 0.125 mg/kg dexmedetomidine (Dexdomitor, Janssen Pharmaceutica NV, Beerse, The Netherlands), and 0.05 mg/kg atropine (Atropinesulfate, Centrafarm BV, Etten-Leur, The Netherlands). After a tracheotomy, a sterile metal cannula was inserted into the trachea and connected to a human ventilator (Servo 300; Siemens, Väsby, Sweden). A polyethylene catheter was inserted into the carotid artery for hemodynamic monitoring and access to arterial blood. Anesthesia was maintained by continuous intravenous infusion of ketamine (100 mg/kg/h). All medications were infused via a tail vein catheter (Braun, Vasofix Safety, 24G). The body temperature was maintained at 37 °C using a heating pad.

Mechanical Ventilation

Rats were subjected to ventilation using a pressure-controlled mode of mechanical ventilation with a human ventilator (Servo 900C; Siemens, Väsby, Sweden). In one group, we used lower tidal volumes of 6 mL/kg and positive end-expiratory pressure (PEEP) of 5 cmH_2O ("lung-protective mechanical ventilation," LP-MV), guided by a pneumotachometer (HSE; Harvard Apparatus, Manheim, Germany). Respiratory rate (RR) was set at 40 breaths/min at baseline and was adjusted when necessary to maintain normocapnia (pCO_2 4.5–6.0 kPa). The inspiratory-to-expiratory ratio was set at 1:2 and the inspired oxygen fraction (FiO_2) at 0.4. Rats were mechanically ventilated for a period of 4 h. An additional non-ventilated group was added to provide baseline values of complement activation during pneumonia. These rats received a sham operation and were sacrificed shortly after C1-INH or saline was given. In a second group, rats were ventilated with high tidal volumes of 12 mL/kg without PEEP ("lung-injurious mechanical ventilation," LI-MV). We adjusted RR to 20 breaths/min to maintain equal respiratory minute volumes.

C1-Esterase Inhibitor

C1-esterase inhibitor (Cetor, Sanquin, Amsterdam, The Netherlands) was administered through the tail vein catheter before the start of mechanical ventilation. For this, 200 U/kg C1-INH was administered as a bolus. Rats not receiving C1-INH received a volume-matched infusion of sterile normal saline.

Sampling and Measurements

The carotid arterial line was used for continuous blood pressure monitoring and to obtain hourly blood for arterial blood gas analysis, using a Rapidlab 865 blood gas analyzer (Bayer, Mijdrecht, The Netherlands). At the end of the experiment, rats were bled to death by taking blood from the arterial line. The lungs were removed en bloc followed by bronchoalveolar lavage (BAL) with sterile normal saline (3 × 2 mL) of the left lung. Part of the right lung was used for histopathology examination after being fixed in 4 % buffered formaldehyde and embedded in paraffin. The remainder of the right lung was used for measuring CFU counts and wet-to-dry ratio which was determined by weighing immediately after harvesting and weighing again after being dried for 7 days in a 65 °C stove. Cell counts were determined using a hematocytometer (Z2 Coulter Particle Counter; Beckman Coulter Corporation, Hialeah, FL, USA) in BAL-fluid (BALF). Differential counts were done on cytospin preparations stained with Giemsa stain (Dade Behring AG, Dudingen, Switzerland).

Complement Levels

Activation of complement factor 4 was measured to determine complement activation through classical and lectin pathway, and C1-INH activity was measured to determine total available active fraction of C1-INH using enzyme-linked immuno sorbent assays (ELISA) (Sanquin) as described before [12]. Notably, the assay for activated complement factor 4 does not distinguish C4b from C4bi and C4c, which is therefore referred to as C4b/c.

Bacterial Outgrowth

The number of viable bacteria in lung homogenates was determined by culturing 10–fold dilutions on blood agar plates (AMC, Amsterdam, The Netherlands); plates were incubated at 37 °C in 5 % CO_2; CFUs were counted the following day.

Cytokines and Protein Levels

Levels of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6 and cytokine-induced neutrophil chemoattractant (CINC)-3 in BALF and plasma were measured using rat-specific ELISA according to the manufacturer's instructions (all: R&D Systems, Abingdon, UK). Furthermore, total protein as a determinant for lung vascular permeability was determined in BALF using the Bradford method (Oz Biosciences, Marseille, France).

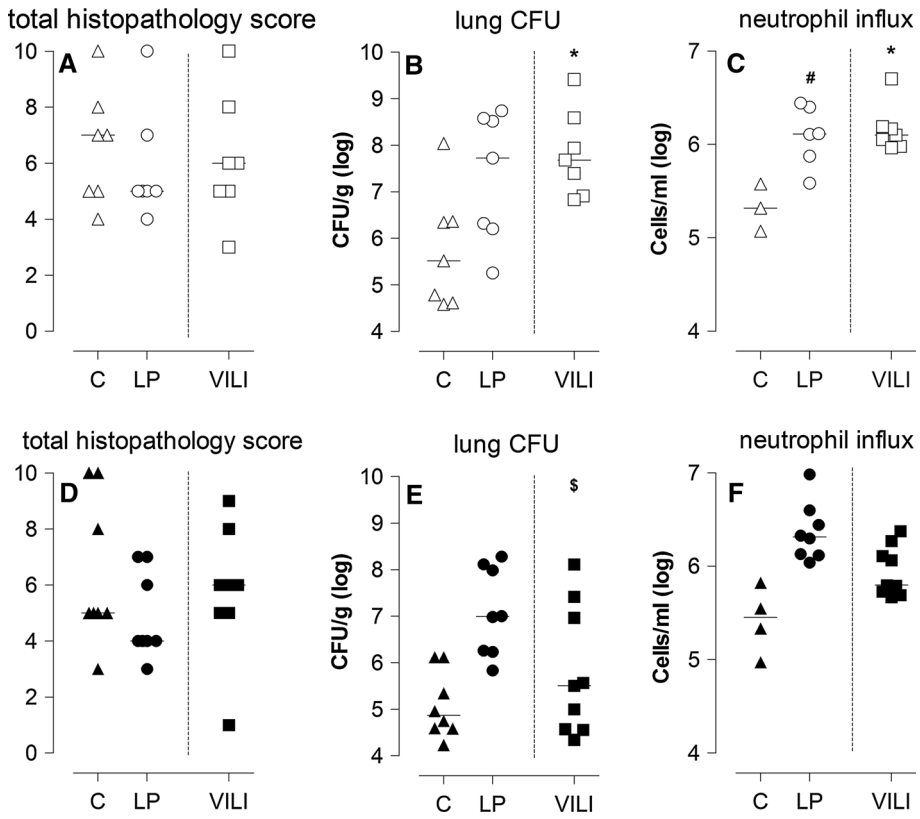

Fig. 2 Total histopathology score (**a** + **d**), bacterial outgrowth in the lung (**b** + **e**) and neutrophil influx (**c** + **f**) in rats infected with *S. pneumoniae* without ventilation (**c**), or after 4 h of lung-protective (LP) or lung-injurious (VILI) ventilation either treated with saline (*clear*) or C1-INH (*black*). *Bars* represent median. #C + saline versus LP + saline, *C + saline versus VILI + saline, $VILI + saline versus VILI + C1-INH $p < 0.05$

Histopathology

4-µm-thick paraffin sections of lung tissue were stained with hematoxylin and eosin. A pathologist who was blinded to group randomization scored lung histopathology. To score lung inflammation and damage, lung sections were analyzed with respect to the following variables: interstitial inflammation, endothelialitis, bronchitis, edema, pleuritis, and thrombus formation, as described previously [13]. Each variable was graded on a scale of 0–4 (0, absent; 1, mild; 2, moderate; 3, severe; 4, very severe). The total histopathology score was expressed as the sum of the scores for all variables.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism version 5 (GraphPad software Inc, La Jolla, CA, USA). To investigate the effect of ventilation on complement activation and to investigate the effect of systemic treatment with C1-INH on pulmonary complement activation, inflammation, and injury, groups were compared using ANOVA with Bonferroni post-hoc test or Kruskall–Wallis test with a Dunn's post-hoc correction according to the data distribution. A P value of <0.05 was considered statistically significant. The data are presented as mean ± standard deviation (SD) or median with interquartile range, where appropriate.

Results

Animals

Two rats died shortly after instillation of bacteria, probably due to larynx edema. Four rats died as a result of pneumothorax after tracheal cannulation, and three suffered from irreversible shock. This left us with 47 rats that completed the whole experiment. In one rat, tidal volumes could not be controlled during the experiment; data collected in this animal were not used in the final analysis. Thus, in total, data from 46 animals were analyzed (Fig. 1).

Complement Activation with Pneumonia and Mechanical Ventilation

Tracheal instillation of *S. pneumoniae* resulted in pneumonia, as demonstrated by bilateral macroscopic lung infiltrates with histopathological analysis showing clear signs of pneumonia (Fig. 2a). During 4 h of ventilation, rats had stable hemodynamic and ventilation parameters (Fig. 3). Ventilation augmented pulmonary levels of

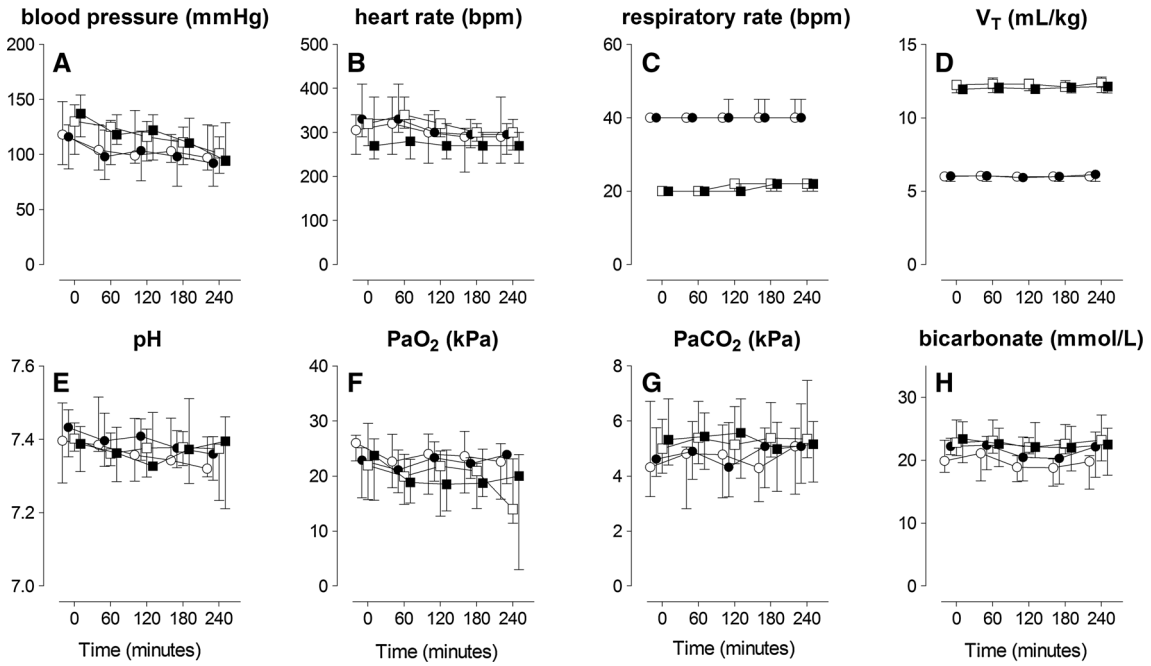

Fig. 3 Mean arterial pressure (**a**), heart rate (**b**), respiratory rate (breaths/min) (**c**), tidal volumes (**d**), pH (**e**), PaO_2 (**f**), $PaCO_2$ (**g**) and bicarbonate (**h**) during 4 h of lung-protective (*circles*) or lung-injurious (*squares*) mechanical ventilation in rats infected with *Streptococcus pneumoniae* either treated with saline (*clear*) or C1-INH (*black*). Data are median with range

complement activation product C4b/c, though differences with non-ventilated animals were only significant after injurious ventilation (Fig. 4a).

Inflammation and Injury After 4 h of Lung-Protective Mechanical Ventilation

LP-MV did neither affect total histopathology scores, nor bacterial outgrowth from lung homogenates, though a significant increase in neutrophil influx in the pulmonary compartment was found (Fig. 2a–c). Notably, LP-MV did not affect lung wet-to-dry ratios (Fig. 5a) and only tended to increase pulmonary vascular leakage as demonstrated by a non-significant rise of total protein levels in BALF (Fig. 5b). While LP-MV did not result in higher levels of TNF-α in BALF, LP-MV was associated with significantly higher levels of IL-1β, IL-6, and CINC-3 in BALF (Fig. 6a–d).

Systemic treatment with C1-INH resulted in a significant increase of C1-INH protein levels, both systemically and in the pulmonary compartment. Active C1-INH fraction measurements suggested functional C1-INH protein availability both in the systemic compartment and in the lung (Fig. 4e–f). Systemic treatment with C1-INH was, however, not able to reduce C4b/c levels in the pulmonary compartment (Fig. 4d). Furthermore, the intervention with C1-INH did not affect total histopathology scores, bacterial outgrowth, neutrophil influx (Fig. 2d–f), and lung wet–to–dry ratios (Fig. 5c), nor total protein levels in BALF (Fig. 5d), and levels of cytokines in BALF (Fig. 6e–h).

Inflammation and Injury after 4 h of Lung-Injurious Mechanical Ventilation

LI-MV resulted in unchanged total histopathology scores compared to non-ventilated controls. Bacterial outgrowth from lung homogenate and neutrophil influx, however, were significantly increased upon LI-MV (Fig. 2b, c). LI-MV was furthermore associated with a significant increase of total protein levels in BALF, though lung wet-to-dry ratios remained unaffected (Fig. 5a, b). Similar to LP-MV, LI-MV did not result in higher levels of TNF-α, but did significantly increase levels of IL-1β, IL-6, and CINC-3 in BALF (Fig. 6a–d) compared to non-ventilated rats.

C1-INH active fraction measurements suggested functional C1-INH protein availability both in the systemic compartment and in the lung (Fig. 4e, f). Although the intervention was unable to prevent a further rise in C4b/c levels in the pulmonary compartment during LI-MV (Fig. 4d), bacterial outgrowth in the lung was significantly reduced upon systemic treatment with C1-INH (Fig. 2e). The decrease in bacterial outgrowth was not accompanied by a reduction in lung injury following systemic treatment with C1-INH, as histopathology scores (Fig. 2f), lung wet-

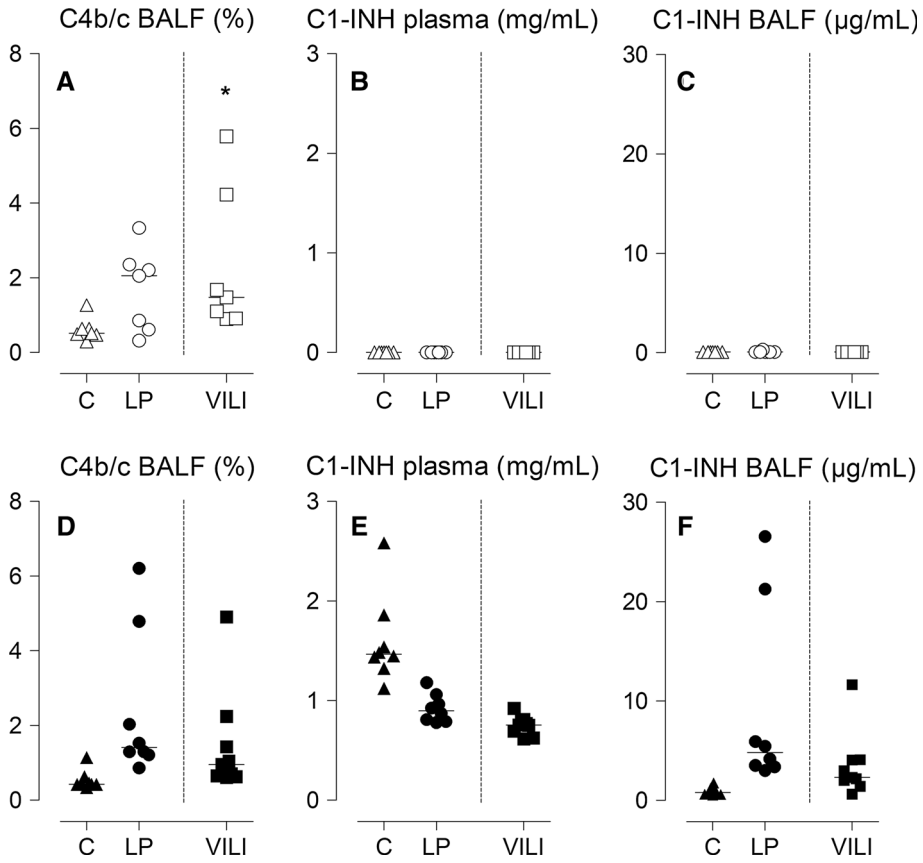

Fig. 4 Percentage of C4b/c in BALF compared to maximal activated C4b/c in plasma (**a** + **d**), functional human C1-INH in plasma (**b** + **e**) and functional human C1-INH in BALF (**c** + **f**) in rats infected with *S. pneumoniae* without ventilation (**c**), or after 4 h of lung-protective (LP) or lung-injurious (VILI) ventilation either treated with saline (*clear*) or C1-INH (*black*). *Bars* represent median. *C + saline versus VILI + saline, $p < 0.05$

to-dry ratios (Fig. 5c), total protein levels in BALF (Fig. 5d), and levels of pro-inflammatory cytokines TNF-α IL-1β, IL-6, and CINC-3 in BALF (Fig. 6e–h) were unaltered.

Discussion

This study shows that ventilation in rats with pneumonia is associated with an increase of the classical and/or lectin complement activation product C4b/c in the lungs. The extent of complement activation is dependent on the chosen ventilation strategy, as only lung ventilated with high tidal volumes without PEEP showed a significant increase of C4b/c levels. Systemic treatment with C1-INH does neither affect ventilation-induced pulmonary complement activation nor lung inflammation or lung injury. Finally, lung-injurious mechanical ventilation is associated with an increased bacterial outgrowth as compared to non-ventilated rats, and C1-INH reduces bacterial outgrowth in this setting.

The findings of this study confirm findings from two previous investigations [14, 15]. Activated complement component C3 was suggested to play a role in a mouse model for ventilator-induced lung injury using very high tidal volumes (i.e., 35 ml/kg) [15]. In another study of ventilation-induced lung injury in rats, the soluble terminal complement complex SC5-b9 was found to increase vascular permeability [14]. In the present study, we found an increase in pulmonary C4b/c, indicating a possible classical and/or lectin pathway involvement. This activation did seem to be tidal volume size-dependent. Thus, the extent of pulmonary complement activation is dependent on the chosen ventilation strategy. Compared to the previous investigations, our model is more comparable to the clinical scenario in severely ill pneumonia patients in need of mechanical ventilation. The tidal volumes we investigated are more realistic to those used in the clinical setting.

C1-INH administration is a well-established intervention for hereditary angioedema, which is caused by a deficiency or dysfunction of this protein [16]. Also, plasma-derived human C1-INH is used as a complement inhibitor in various animal models, including models in rats, because of its property to regulate initial complement activation of the classical and lectin pathway in animals as well [17–20]. In models of sepsis and ischemia–reperfusion injury, systemic treatment with plasma-derived human C1-INH showed to have beneficial effects [17–27]. Based on these earlier studies, we hypothesized C1-INH to be able to attenuate pulmonary complement activation in a model of ventilation for pneumonia, and as such to attenuate lung inflammation and lung injury. While C1-INH increases BALF levels of

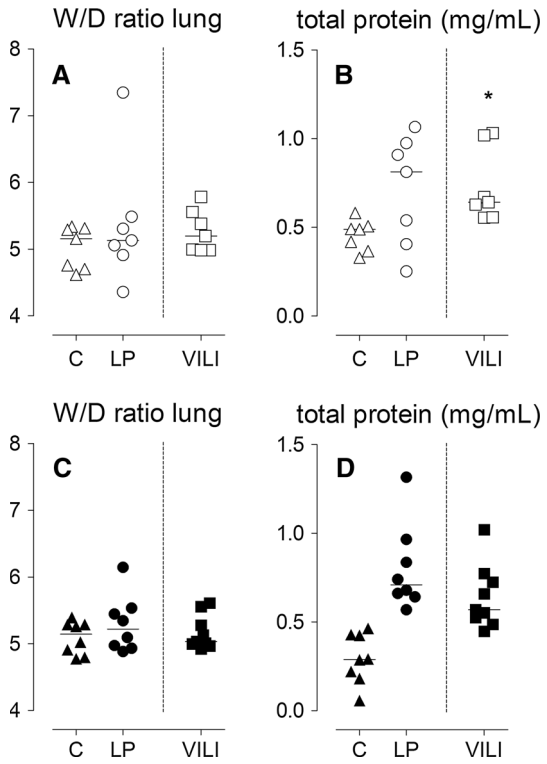

Fig. 5 Wet-to-dry ratio lung (**a** + **c**) and total protein BALF (**b** + **d**) in rats infected with *S. pneumoniae* without ventilation (**c**), or after 4 h of lung-protective (LP) or lung-injurious (VILI) ventilation either treated with saline (*clear*) or C1-INH (*black*). *Bars* represent median. *C + saline versus VILI + saline, $p < 0.05$

C1-INH in the present experiment, the intervention does not affect the local complement activation.

The inability of C1-INH to prevent further complement activation in the lung could be related to various reasons. First, the dosage of C1-INH could simply have been too low. Although the dosage used in the present experiments is comparable to those used in various preclinical models in which protective effects of C1-INH were observed [23–25], local levels could still be too low to have an effect. An increased inactivation of C1-INH causing a lack of effect seems unlikely, since the C1-INH activity assay showed no considerable discrepancy in C1-INH protein availability and its functional fraction in BALF. Second, a diminished specificity of human C1-INH for its substrate in rats could be a reason for the absence of complement inhibition. This is unlikely, since previous studies in rats have shown plasma-derived human C1-INH to work successfully [17–20, 23–25]. Another explanation could be that inhibition of complement activation by C1-INH is, partly or completely, compensated for. Indeed, while C1-INH acts high in the complement cascade, it cannot prevent activation lower in the cascade [9]. Complement activation lower in the cascade could be the result of increased proteolytic activity by e.g., neutrophil elastases [28]. Thus, increased levels of C4b/c could still be found in the lungs, irrespective of C1-INH administration. This could be important in planning new intervention studies that focus on the role of complement in lung injury models.

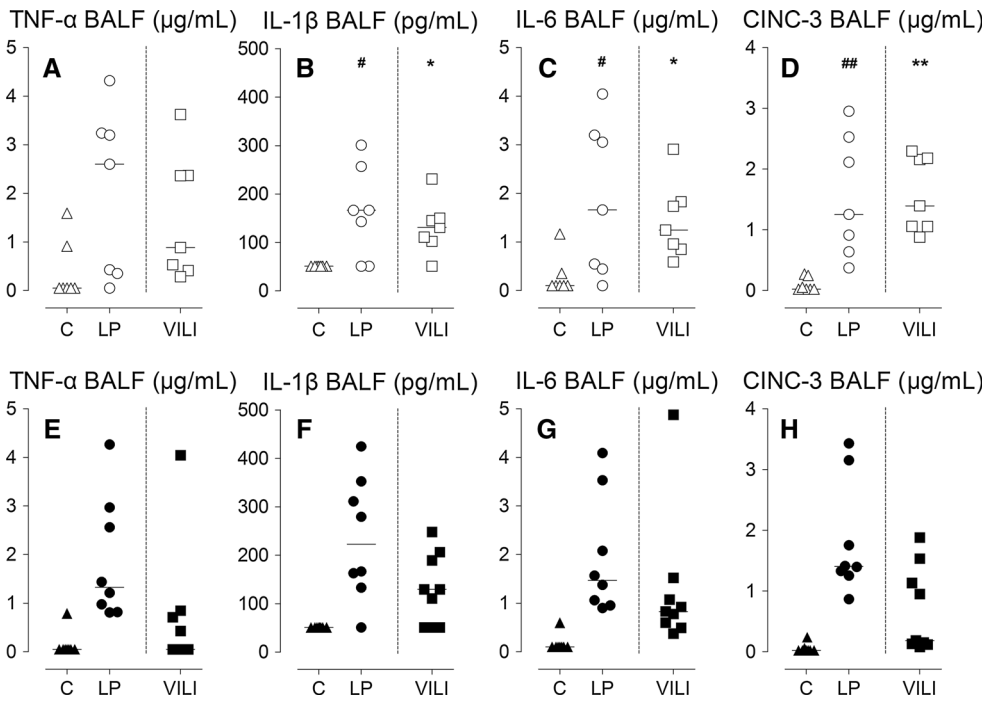

Fig. 6 TNF-α (**a** + **e**), IL-1β (**b** + **f**), IL-6 (**c** + **g**) and CINC-3 (**d** + **h**) in BALF in rats infected with *S. pneumoniae* without ventilation (**c**), or after 4 h of lung-protective (LP) or lung-injurious (VILI) ventilation either treated with saline (*clear*) or C1-INH (*black*). *Bars* represent median. #C + saline versus LP + saline, *C + saline versus VILI + saline, $p < 0.05$. ##C + saline versus LP + saline, **C + saline versus VILI + saline, $p < 0.01$

Timing of the intervention could also be crucial. Complement activation probably starts early after inoculation with *S. pneumoniae* with development of pneumonia. Of course, C1-INH could never attenuate complement activation that is already present at the moment of administration. But at least, it must be concluded that systemic treatment with C1-INH is not able to attenuate further activation of the complement cascade in the lungs elicited by ventilation. We specifically chose to administer C1-INH before start of ventilation, and not before inoculation of bacteria, because we consider this a more realistic clinical scenario.

Notably, the results of this study suggest treatment with C1-INH to inhibit bacterial outgrowth from lungs. This is in line with an experimental *S. pneumoniae* meningitis model in rats, in which C1-INH treatment resulted in reduced bacterial outgrowth from cerebrospinal fluid [20]. In the present experiment, the inhibitory effect of C1-INH on bacterial outgrowth is not consistent, since the number of CFU is not affected in the experiments using LP-MV. This could suggest an interaction between ventilation-induced lung injury and host response. In both, complement is involved. We are uncertain about the reason for these observed differences, and this needs confirmation in future studies.

As mentioned, the present experiment tried to mimic a clinical scenario, which is a strength of this study. The present experiment also knows several limitations. First, young and healthy rats may not mimic the population at risk for severe pneumonia. Second, this study used a *S. pneumoniae* pneumonia model, and C1-INH could have beneficial effects in ventilation models with other pathogens, which we missed in this model. Third, we used systemic administration of C1-INH while inflammation and injury is mostly compartmentalized in pneumonia. Local treatment (e.g., by means of nebulization) could be more efficacious, because higher concentration could possibly be reached.

Finally, C1-INH only intervenes with the classical and lectin pathway of the complement cascade, while leaving the alternative pathway unaltered. Therefore, no activity markers for the alternative pathway have been measured in this experiment. This could possibly mask an additional role for the alternative pathway.

In conclusion, ventilation for pneumonia is associated with additional complement activation. Systemic C1-INH administration prior to ventilation, however, is unable to attenuate ventilation-induced pulmonary complement activation, lung inflammation, and lung injury.

References

1. Ewig, S., & Torres, A. (1999). Severe community-acquired pneumonia. *Clinics in Chest Medicine, 20*, 575–587.
2. Slutsky, A. S. (1999). Lung injury caused by mechanical ventilation. *Chest, 116*, 9S–15S.
3. Brown, J. S., Hussell, T., Gilliland, S. M., Holden, D. W., Paton, J. C., Ehrenstein, M. R., et al. (2002). The classical pathway is the dominant complement pathway required for innate immunity to *Streptococcus pneumoniae* infection in mice. *Proceedings of the National Academy of Sciences of the United States of America, 99*, 16969–16974.
4. Ricklin, D., Hajishengallis, G., Yang, K., & Lambris, J. D. (2010). Complement: A key system for immune surveillance and homeostasis. *Nature Immunology, 11*, 785–797.
5. Sunyer, J. O., & Lambris, J. D. (1998). Evolution and diversity of the complement system of poikilothermic vertebrates. *Immunological Reviews, 166*, 39–57.
6. Yancey, K. B., Lawley, T. J., Dersookian, M., & Harvath, L. (1989). Analysis of the interaction of human C5a and C5a des Arg with human monocytes and neutrophils: Flow cytometric and chemotaxis studies. *Journal of Investigative Dermatology, 92*, 184–189.
7. Meade, P., Shoemaker, W. C., Donnelly, T. J., Abraham, E., Jagels, M. A., Cryer, H. G., et al. (1994). Temporal patterns of hemodynamics, oxygen transport, cytokine activity, and complement activity in the development of adult respiratory distress syndrome after severe injury. *Journal of Trauma, 36*, 651–657.
8. Zilow, G., Joka, T., Obertacke, U., Rother, U., & Kirschfink, M. (1992). Generation of anaphylatoxin C3a in plasma and bronchoalveolar lavage fluid in trauma patients at risk for the adult respiratory distress syndrome. *Critical Care Medicine, 20*, 468–473.
9. Zeerleder, S. (2011). C1-inhibitor: More than a serine protease inhibitor. *Seminars in Thrombosis and Hemostasis, 37*, 362–374.
10. Igonin, A. A., Protsenko, D. N., Galstyan, G. M., Vlasenko, A. V., Khachatryan, N. N., Nekhaev, I. V., et al. (2012). C1-esterase inhibitor infusion increases survival rates for patients with sepsis*. *Critical Care Medicine, 40*, 770–777.
11. Nuijens, J. H., Abbink, J. J., Wachtfogel, Y. T., Colman, R. W., Eerenberg, A. J., Dors, D., et al. (1992). Plasma elastase alpha 1-antitrypsin and lactoferrin in sepsis: Evidence for neutrophils as mediators in fatal sepsis. *Journal of Laboratory and Clinical Medicine, 119*, 159–168.
12. Bos, I. G., Van Mierlo, G. J., Bleeker, W. K., Rigter, G. M., te Velthuis, H., Dickneite, G., et al. (2001). The potentiation of human C1-inhibitor by dextran sulphate is transient in vivo: Studies in a rat model. *International Immunopharmacology, 1*, 1583–1595.
13. Choi, G., Hofstra, J. J., Roelofs, J. J., Rijneveld, A. W., van der Zee, J. S., Florquin, S., et al. (2008). Antithrombin inhibits bronchoalveolar activation of coagulation and limits lung injury during *Streptococcus pneumoniae* pneumonia in rats. *Critical Care Medicine, 36*, 204–210.
14. Liu, K., Mao, Y. F., Zheng, J., Peng, Z. Y., Liu, W. W., Liu, Y., et al. (2013). SC5b-9-induced pulmonary microvascular endothelial hyperpermeability participates in ventilator-induced lung injury. *Cell Biochemistry and Biophysics, 67*(3), 1421–1431.
15. Takahashi, K., Saha, D., Shattino, I., Pavlov, V. I., Stahl, G. L., Finnegan, P., et al. (2011). Complement 3 is involved with ventilator-induced lung injury. *International Immunopharmacology, 11*, 2138–2143.
16. Gadek, J. E., Hosea, S. W., Gelfand, J. A., Santaella, M., Wickerhauser, M., Triantaphyllopoulos, D. C., et al. (1980). Replacement therapy in hereditary angioedema: Successful treatment of acute episodes of angioedema with partly purified C1 inhibitor. *New England Journal of Medicine, 302*, 542–546.
17. Begieneman, M. P., Kubat, B., Ulrich, M. M., Hahn, N. E., Stumpf-Stolker, Y., Tempelaars, M., et al. (2012). Prolonged C1 inhibitor administration improves local healing of burn wounds

and reduces myocardial inflammation in a rat burn wound model. *Journal of Burn Care & Research, 33*, 544–551.
18. Heydenreich, N., Nolte, M. W., Gob, E., Langhauser, F., Hofmeister, M., Kraft, P., et al. (2012). C1-inhibitor protects from brain ischemia-reperfusion injury by combined antiinflammatory and antithrombotic mechanisms. *Stroke, 43*, 2457–2467.
19. Tei, R., Kaido, T., Nakase, H., & Sakaki, T. (2008). Protective effect of C1 esterase inhibitor on acute traumatic spinal cord injury in the rat. *Neurological Research, 30*, 761–767.
20. Zwijnenburg, P. J., van der Poll, T., Florquin, S., Polfliet, M. M., van den Berg, T. K., Dijkstra, C. D., et al. (2007). C1 inhibitor treatment improves host defense in pneumococcal meningitis in rats and mice. *Journal of Infectious Diseases, 196*, 115–123.
21. Buerke, M., Murohara, T., & Lefer, A. M. (1995). Cardioprotective effects of a C1 esterase inhibitor in myocardial ischemia and reperfusion. *Circulation, 91*, 393–402.
22. Caliezi, C., Zeerleder, S., Redondo, M., Regli, B., Rothen, H. U., Zurcher-Zenklusen, R., et al. (2002). C1-inhibitor in patients with severe sepsis and septic shock: beneficial effect on renal dysfunction. *Critical Care Medicine, 30*, 1722–1728.
23. Croner, R. S., Lehmann, T. G., Fallsehr, C., Herfarth, C., Klar, E., & Kirschfink, M. (2004). C1-inhibitor reduces hepatic leukocyte–endothelial interaction and the expression of VCAM-1 in LPS-induced sepsis in the rat. *Microvascular Research, 67*, 182–191.
24. Fu, J., Lin, G., Zeng, B., Wu, Z., Wu, Y., Chu, H., et al. (2006). Anti-ischemia/reperfusion of C1 inhibitor in myocardial cell injury via regulation of local myocardial C3 activity. *Biochemical and Biophysical Research Communications, 350*, 162–168.
25. Heijnen, B. H., Straatsburg, I. H., Padilla, N. D., Van Mierlo, G. J., Hack, C. E., & van Gulik, T. M. (2006). Inhibition of classical complement activation attenuates liver ischaemia and reperfusion injury in a rat model. *Clinical and Experimental Immunology, 143*, 15–23.
26. Liu, D., Zhang, D., Scafidi, J., Wu, X., Cramer, C. C., & III Davis, A. E. (2005). C1 inhibitor prevents Gram-negative bacterial lipopolysaccharide-induced vascular permeability. *Blood, 105*, 2350–2355.
27. Zeerleder, S., Caliezi, C., van Mierlo, G., Eerenberg-Belmer, A., Sulzer, I., Hack, C. E., et al. (2003). Administration of C1 inhibitor reduces neutrophil activation in patients with sepsis. *Clinical and Diagnostic Laboratory Immunology, 10*, 529–535.
28. McGuire, W. W., Spragg, R. G., Cohen, A. B., & Cochrane, C. G. (1982). Studies on the pathogenesis of the adult respiratory distress syndrome. *Journal of Clinical Investigation, 69*, 543–553.