ANESTHESIOLOGY

Neutralizing Complement C5a Protects Mice with Pneumococcal Pulmonary Sepsis

Holger Müller-Redetzky, M.D., Ute Kellermann, M.D., Sandra-Maria Wienhold, D.V.M., Birgitt Gutbier, D.V.M., Jasmin Lienau, D.V.M., Katharina Hellwig, Katrin Reppe, D.V.M., Eleftheria Letsiou, Ph.D., Thomas Tschernig, M.D., Markus Scholz, Ph.D., Peter Ahnert, Ph.D., Christian Maasch, Ph.D., Kai Hoehlig, Ph.D., Sven Klussmann, Ph.D., Axel Vater, Ph.D., Theresa C. Firsching, D.V.M., Judith Hoppe, D.V.M., Norbert Suttorp, M.D., Martin Witzenrath, M.D.

ANESTHESIOLOGY 2020; 132:795–807

EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- Pneumonia, sepsis, and immune dysregulation cause morbidity and mortality
- C5a is a component of the complement system and a proinflammatory mediator that modulates the innate immune response in critical illness
- Disruption of the C5a receptor axis with antibodies or antagonists was previously protective in various animal sepsis models

What This Article Tells Us That Is New

- In hospitalized patients with community-acquired pneumonia, serum C5a concentrations were 1.4-fold higher compared to healthy subjects
- In two mouse models of pneumonia and sepsis, NOX-D19, a C5a-neutralizing L-RNA-aptamer, caused lower pulmonary hyperpermeability and sepsis-related acute liver injury

ABSTRACT

Background: Community-acquired pneumonia and associated sepsis cause high mortality despite antibiotic treatment. Uncontrolled inflammatory host responses contribute to the unfavorable outcome by driving lung and extrapulmonary organ failure. The complement fragment C5a holds significant proinflammatory functions and is associated with tissue damage in various inflammatory conditions. The authors hypothesized that C5a concentrations are increased in pneumonia and C5a neutralization promotes barrier stabilization in the lung and is protective in pneumococcal pulmonary sepsis.

Methods: The authors investigated regulation of C5a in pneumonia in a prospective patient cohort and in experimental pneumonia. Two complementary models of murine pneumococcal pneumonia were applied. Female mice were treated with NOX-D19, a C5a-neutralizing L-RNA-aptamer. Lung, liver, and kidney injury and the inflammatory response were assessed by measuring pulmonary permeability (primary outcome), pulmonary and blood leukocytes, cytokine concentrations in lung and blood, and bacterial load in lung, spleen, and blood, and performing histologic analyses of tissue damage, apoptosis, and fibrin deposition (n = 5 to 13).

Results: In hospitalized patients with pneumonia (n = 395), higher serum C5a concentrations were observed compared to healthy subjects (n = 24; 6.3 nmol/l [3.9 to 10.0] *vs.* 4.5 nmol/l [3.8 to 6.6], median [25 to 75% interquartile range]; difference: 1.4 [95% CI, 0.1 to 2.9]; $P = 0.029$). Neutralization of C5a in mice resulted in lower pulmonary permeability in pneumococcal pneumonia (1.38 ± 0.89 *vs.* 3.29 ± 2.34, mean ± SD; difference: 1.90 [95% CI, 0.15 to 3.66]; $P = 0.035$; n = 10 or 11) or combined severe pneumonia and mechanical ventilation (2.56 ± 1.17 *vs.* 7.31 ± 5.22; difference: 4.76 [95% CI, 1.22 to 8.30]; $P = 0.011$; n = 9 or 10). Further, C5a neutralization led to lower blood granulocyte colony-stimulating factor concentrations and protected against sepsis-associated liver injury.

Conclusions: Systemic C5a is elevated in pneumonia patients. Neutralizing C5a protected against lung and liver injury in pneumococcal pneumonia in mice. Early neutralization of C5a might be a promising adjunctive treatment strategy to improve outcome in community-acquired pneumonia.

(ANESTHESIOLOGY 2020; 132:795–807)

Community-acquired pneumonia is a significant cause of morbidity and mortality worldwide, and *Streptococcus pneumoniae* is the most prevalent causative pathogen.[1,2] Despite effective antibiotic therapy, dysregulated inflammatory pathogen–host interactions may evoke pulmonary and

This article is featured in "This Month in Anesthesiology," page 1A. Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text and are available in both the HTML and PDF versions of this article. Links to the digital files are provided in the HTML text of this article on the Journal's Web site (www.anesthesiology.org). This article has a video abstract. Part of the work presented in this article has been presented at the American Thoracic Society 2014 International Conference in San Diego, California, May 16 to 21, 2014; the European Respiratory Society International Congress in Munich, Germany, September 6 to 10, 2014; and the 37th Annual Conference of the Shock Society, Charlotte, North Carolina, June 7 to 10, 2014.

Submitted for publication December 11, 2018. Accepted for publication December 20, 2019. Published online first on February 20, 2020. From the Division of Pulmonary Inflammation (H.M.-R., S.-M.W., B.G., J.L., K.H., K.R., E.L., M.W.) and the Department of Infectious Diseases and Respiratory Medicine (H.M.-R., U.K., N.S., M.W.), Charité - University Medicine Berlin (Charité - Universitätsmedizin Berlin), corporate member of Free University of Berlin (Freie Universität Berlin), Humboldt University of Berlin (Humboldt-Universität zu Berlin), and Berlin Institute of Health, Berlin, Germany; Institute of Anatomy and Cell Biology, Saarland University, Homburg/Saar, Germany (T.T.); Institute for Medical Informatics, Statistics and Epidemiology, University of Leipzig, Leipzig, Germany (M.S., P.A.); NOXXON Pharma AG, Berlin, Germany (C.M., K.H., S.K., A.V.); Institute of Veterinary Pathology, Free University of Berlin (Freie Universität Berlin), Berlin, Germany (T.C.F., J.H.); and German Center for Lung Research, Giessen, Germany (associate members N.S., M.W.). Current Position: Takeda GmbH, Oranienburg, Germany (C.M.); and APTARION biotech AG, Berlin, Germany (K.H., S.K., A.V.).

 Anesthesiology 2020; 132:795–807. DOI: 10.1097/ALN.0000000000003149

<zdoi;. DOI: 10.1097/ALN.0000000000003149>

extrapulmonary organ failure.[3,4] Besides antibiotic therapy and supportive strategies in case of respiratory failure and septic shock, no effective adjuvant therapy is available to improve the outcome so far. Particularly vascular hyperpermeability, the hallmark of acute respiratory failure in pneumonia, can be attributed to both direct effects of pathogenic factors and an uncontrolled host response.[5]

C5 is a component of the complement system, cleaved into C5a and C5b. While C5b is a component of the membrane attack complex that is crucial for lysis of invading pathogens, C5a acts as a proinflammatory anaphylatoxin after binding to its receptors C5aR1 and C5aR2. Notably, both receptors are relevant for C5a to exert its effects.[6–8] C5a modulates the innate immune response in critical illness. It leads to rapid activation of neutrophils, which likely contributes to tissue injury, while prolonged exposure with high concentrations of C5a lead to anergy of neutrophils, rendering the host immunocompromised.[9–12] Taken together, C5a might be a significant driver of inflammation-related tissue injury and immunoparalysis in sepsis. For an overview of the role of C5a in the inflammatory response, see figure S1, Supplemental Digital Content 1 (http://links.lww.com/ALN/C198).

Pulmonary administration of C5a alone can induce significant lung injury in mice,[13] and in different sepsis models in various species disruption of the C5a/C5a receptor axis was protective.[7,14–19] Further, C5a contributes to lung injury in H7N9 influenza and Middle East respiratory syndrome coronavirus models.[20,21] In pneumococcal pneumonia, however, the functional role of C5a and its potential as a target for adjuvant pharmacotherapy have not been investigated so far.

Targeting the C5a/C5a receptor axis therapeutically may be achieved by C5a antibodies, C5a receptor antagonists, or prevention of C5 cleavage. In this study, we chose a novel approach using a C5a specific L-RNA-aptamer[22] termed NOX-D19 to neutralize C5a. L-RNA-aptamers are mirror-image structured (L)-oligonucleotides binding and neutralizing a target conceptually similar to monoclonal antibodies. The nonnatural chirality makes L-aptamers resistant to nucleases that are prevalent in biologic fluids and prevents activation of Toll-like receptors. NOX-D19 has previously shown protective effects in mouse models of tracheal allograft transplantation and sepsis.[16,23] By directly neutralizing C5a, the generation of a functional membrane attack complex is not hampered, as C5b generation is not affected,[16] and signaling through both C5a receptors is disrupted, which cannot be achieved by single currently known receptor-binding small molecules or antibodies.[16]

To test the hypothesis that C5a concentrations are increased in pneumonia and C5a neutralization promotes barrier stabilization in the lung and is protective in pneumococcal pulmonary sepsis, we accompanied a human clinical study with *in vivo* mice experiments. C5a was quantified in serum of hospitalized pneumonia patients. Therapeutic neutralization of C5a was performed in mice with severe pneumococcal pneumonia and concomitant sepsis and in a complex combination model of severe pneumococcal pneumonia and mechanical ventilation, applying NOX-D19 for early and delayed therapeutic interventions.

Materials and Methods

The full methods description is available in Supplemental Digital Content 2 (http://links.lww.com/ALN/C199).

Clinical Study

This was an ancillary study of PROGRESS (Pneumonia Research Network on Genetic Resistance and Susceptibility for the Evolution of Severe Sepsis),[24] an observational, prospective cohort study enrolling consecutive patients with community-acquired pneumonia as approved by central and local ethics committees (ethics committee of the University of Jena, Jena, Germany, registration number 2403-10/08). Data and laboratory samples were obtained as controls from healthy volunteers, as approved by the local ethics committee. Written informed consent was obtained from each patient and volunteer, or each patient's legal representative. Samples of patients with community-acquired pneumonia (18 yr or older) enrolled within 48 h of hospitalization were provided. Serum C5a/C5a-desArg concentrations were quantified by enzyme-linked immunosorbent assay according to the manufacturer's instructions (HK349; Hycult, The Netherlands).

Mice Study

Animal procedures were approved by the animal ethics committee of Charité - University Medicine Berlin (Charité - Universitätsmedizin Berlin, Berlin, Germany) and local governmental authorities (State Office for Health and Social Affairs Berlin, Berlin, Germany). Animal housing and experimental procedures complied with the Federation of European Laboratory Animal Science Associations guidelines and recommendations for the care and use of laboratory animals. For all experiments, female C57Bl/6N mice (8 to 11 weeks old, weighing 18 to 23 g; Charles River, Germany) were used and randomly assigned to experimental groups as illustrated in figure S2 in Supplemental Digital Content 1 (http://links.lww.com/ALN/C198).

Pneumococcal Pneumonia and NOX-D19 Treatment

S. pneumoniae (serotype 3, strain NCTC7978) was grown to mid–log phase. Mice were anesthetized and transnasally inoculated with 5×10^6 colony-forming units of *S. pneumoniae* in 20 μl sterile phosphate-buffered saline.[25] Sham-infected control mice received 20 μl of sterile phosphate-buffered saline.

In the first pneumonia model (fig. S2A, Supplemental Digital Content 1, http://links.lww.com/ALN/C198), at time of infection (0 h) and 24 h postinfection, mice were intraperitoneally treated with anti-C5a L-aptamer

NOX-D19 (20 mg/kg in 220 µl 5% glucose) or solvent (5% glucose; n = 13 each group). The dosage was based on previous studies determining the pharmacokinetic profile of NOX-D19 (unpublished data) and NOX-D20,[16] a very close relative of NOX-D19. Twenty-four hours (all groups) or 48 h postinfection (only *S. pneumoniae*–infected groups), mice were anesthetized and exsanguinated. A subset of animals (n = 18) was subjected to assessment of specific murine pneumonia symptoms (clinical signs) at time of euthanization (24 h postinfection, sham-infected group; 48 h postinfection, *S. pneumoniae*–infected groups) as described previously[26] and detailed in table S1 in Supplemental Digital Content 3 (http://links.lww.com/ALN/C200). C5a/C5a-desArg concentrations in bronchoalveolar lavage fluid and plasma of *S. pneumoniae*–infected and control mice were quantified by plasmon resonance measurements.

In the second model of pneumonia combined with mechanical ventilation (fig. S2B, Supplemental Digital Content 1, http://links.lww.com/ALN/C198), mice received a single intraperitoneal injection of NOX-D19 (20 mg/kg in 220 µl 5% glucose) or solvent (5% glucose) 23 h postinfection and were subjected to mechanical ventilation for 6 h starting 24 h postinfection, as described in detail below under "Mechanical Ventilation," when severe pneumonia had developed (n = 11 each group). In both models of pneumonia, mice were randomly assigned to the different groups by simple randomization.

Mechanical Ventilation

Twenty-four hours after infection, mice were subjected to mechanical ventilation as previously described.[27,28] Briefly, mice were anesthetized, and body temperature was maintained at 37°C by a body temperature–controlled heating pad. Mice were tracheotomized, intubated, and ventilated with tidal volume of 12 ml/kg, respiratory rate of 120 per min, fraction of inspiratory oxygen of 0.75, and 2 cm H_2O positive end-expiratory pressure. A carotid artery catheter was placed for blood pressure monitoring and infusion of a balanced electrolyte solution. Mice were ventilated for 6 h by using a special rodent ventilation system, which continuously recorded airway pressure, respiratory rate, and tidal volume (flexiVent; Scireq, Canada). All mice survived the protocol. At termination of the experiment, mice were euthanized by exsanguination *via* the carotid artery catheter. "Nonventilated mice" that served as controls were subjected to identical preparation procedures 30 h after infection and were euthanized after 5 min of mechanical ventilation (n = 5 each group).

Lung Permeability

Human serum albumin was injected intravenously 60 min (pneumonia alone model) or 90 min (combined model of pneumonia and mechanical ventilation) before lung preparation. The human serum albumin concentration in bronchoalveolar lavage fluid and plasma was determined by enzyme-linked immunosorbent assay. The permeability index was defined as the calculated ratio[27,29] of the human serum albumin concentration in bronchoalveolar lavage fluid and plasma ($x10^{-3}$).

Further Analyses in Mice

Leukocytes in bronchoalveolar lavage fluid and blood were differentially quantified by fluorescence-activated cell sorting analysis (FACS Calibur; BD Biosciences, Germany). Cytokines in bronchoalveolar lavage fluid and plasma were quantified using a multiplex assay (BioRad, USA). Bacterial load was determined by plating serial dilutions of bronchoalveolar lavage fluid, blood, and spleen homogenate on blood agar and incubating at 37°C under 5% CO_2 for 24 h to count colony-forming units. Aspartate transaminase and blood urea nitrogen were quantified 48 h postinfection by routine laboratory tests. For histologic analysis, liver tissue was immersion-fixed in 4% buffered formalin, embedded in paraffin and cut into 2-µm-thick sections. For analysis of apoptosis, liver sections were stained for caspase 3a and fibrin, and counterstained with hemalaun. The tissue sections were analyzed and scored (0, no signal; 1, signal) by an independent investigator blinded to the study groups (n = 8 per group).

Statistical Analysis

No statistical power calculation was conducted before the study. Sample size was based on our past experience with pneumococcal pneumonia models in mice and published papers. Four mice were excluded from the study due to technical reasons (1 *S. pneumoniae*/solvent 24 h, 2 *S. pneumoniae*/solvent 48 h, 1 *S. pneumoniae*/NOX-D19 48 h). Further, data were lost for final analysis due to technical errors during preparation or measurements. Exact sample sizes for each group are provided in each figure legend. Data are presented as mean with SD or boxplots depicting median, quartiles, and range excluding outliers (open circles), with N representing the number of animals or human subjects. Lung permeability was defined as primary outcome, all other investigated parameters as secondary outcomes. The difference between means and 95% CI was calculated for the primary outcome. Preselected pairs of groups were compared using one-way ANOVA and Sidak's multiple comparisons test for data normally distributed, or two-tailed Mann–Whitney U tests followed by Bonferroni correction for nonnormally distributed data. Analyses were performed using GraphPad Prism 6.05 (USA). *P* values less than 0.05 were considered significant.

In the PROGRESS study cohort, C5a concentrations were compared between pneumonia patients and controls using two-tailed Mann–Whitney U test, and the Hodges–Lehmann median difference and its 95% CI were calculated. C5a values were logarithmized to obtain normally distributed values. Batch effect of time of measurement was removed by linear mixed model analysis. C5a values in selected patient groups were compared using two-tailed *t*

tests. Correlation analyses were performed using Spearman's rank correlation coefficients.

Results

C5a Concentrations Are Elevated in Community-acquired Pneumonia and Murine Pneumococcal Pneumonia

The PROGRESS patient cohort comprised 395 hospitalized patients with community-acquired pneumonia. Table 1 shows clinical parameters and characteristics of patients and volunteers. Pneumonia patients had higher serum concentrations of C5a (6.3 nmol/l [3.9 to 10.0]; median [25 to 75% interquartile range]) compared to healthy volunteers (4.5 nmol/l [3.8 to 6.6]; difference: 1.4 [95% CI, 0.1 to 2.9]; $P = 0.029$; fig. 1A). C5a concentrations were not different between patients with or without mechanical ventilation, sepsis, or septic shock (table S2, Supplemental Digital Content 3, http://links.lww.com/ALN/C200). Further, C5a concentrations were positively correlated with inflammatory markers such as procalcitonin, C-reactive protein, and interleukin-6, and did not correlate with other clinical parameters and characteristics shown in table S3 in Supplemental Digital Content 3 (http://links.lww.com/ALN/C200). Consistent with the clinical study, in murine pneumonia, C5a concentrations in plasma as well as in bronchoalveolar lavage fluid were elevated, with highest concentrations 24 h after infection (fig. 1, B and C).

C5a Neutralization in Mice Led to a Lower Pulmonary Permeability and Disease Severity in Pneumonia without Affecting the Pulmonary Immune Response and Bacterial Load

Pneumonia-induced hyperpermeability was lower after treatment with anti-C5a L-aptamer NOX-D19 48 h after infection (1.38 ± 0.89 *vs.* 3.29 ± 2.34, mean ± SD; difference: 1.90 [95% CI, 0.15 to 3.66]; $P = 0.035$; fig. 2A), and mice treated with NOX-D19 displayed less clinical symptoms of severe disease than solvent-treated mice at 48 h postinfection (fig. S3, Supplemental Digital Content 1, http://links.lww.com/ALN/C198). C5a neutralization by NOX-D19 had no impact on pulmonary leukocyte recruitment and leukocyte subset distribution (fig. 2B; fig. S4 in Supplemental Digital Content 1, http://links.lww.com/ALN/C198) and did not affect either pulmonary inflammatory cytokine release upon pneumonia (fig. S5, Supplemental Digital Content 1, http://links.lww.com/ALN/C198) or bacterial load in the bronchoalveolar lavage fluid compared to solvent treatment (fig. 2C). Complementary histopathologic analyses confirmed the substantial extent of cellular infiltration with no differences between NOX-D19– and solvent-treated infected mice (data not shown).

Table 1. Clinical Parameters and Characteristics of the PROGRESS Study Cohort

Parameter/Characteristic	Patients (n = 395)	Healthy Controls (n = 24)
Median age, yr (interquartile range)	62 (46–73)	58 (53–62)
Sex, male/female	237/158	12/12
C-reactive protein, mg/l, median (interquartile range)	174.2 (97.7–248.7)	
Leukocytes, cells/nl, median (interquartile range)	11.7 (9.0–16.6)	
Urea, mmol/l, median (interquartile range)	6.0 (4.2–8.8)	
Procalcitonin, ng/ml, median (interquartile range)	0.30 (0.12–3.14)	
Length of hospital stay, d, median (interquartile range)	8 (6–12)	
Physical examination findings, yes/no, n		
Confusion	30/285	
Respiratory rate > 30 breaths/min	69/194	
Pao_2/Fio_2 ratio, median (interquartile range)	252 (190–322)	
Mechanical ventilation, yes/no, n	25/291	
Systolic blood pressure < 90 mmHg or diastolic blood pressure < 60 mmHg, yes/no, n	83/304	
Previous antibiotics, yes/no, n	57/338	
CRB-65, 0/1/2/3/4, n	153/168/58/15/1	
CURB-65, 0/1/2/3/4/5, n	130/124/90/39/11/1	
Pneumonia severity index (PSI), 1/2/3/4/5, n	86/91/82/92/44	
Sequential Organ Failure Assessment (SOFA) score, median (interquartile range)	3 (2–4)	
Sepsis (SOFA ≥ 2), yes/no, n	314/81	
Septic shock, yes/no, n	8/387	
Multilobar consolidations, yes/no, n	170/219	

The study cohort comprised consecutive patients with community-acquired pneumonia enrolled within 48 h of hospitalization. Clinical data were not available from all patients: confusion (315 available), respiratory rate greater than 30 breaths/min (263 available), mechanical ventilation (316 available), systolic blood pressure less than 90 mmHg or diastolic blood pressure less than 60 mmHg (387 available), and multilobar consolidations (389 available).

CRB-65 (Confusion, Respiratory rate, Blood pressure, age 65 yr or older) and CURB-65 (Confusion, Urea, Respiratory rate, Blood pressure, age 65 yr or older) are pneumonia severity scores. Fio_2, fraction of inspiratory oxygen.

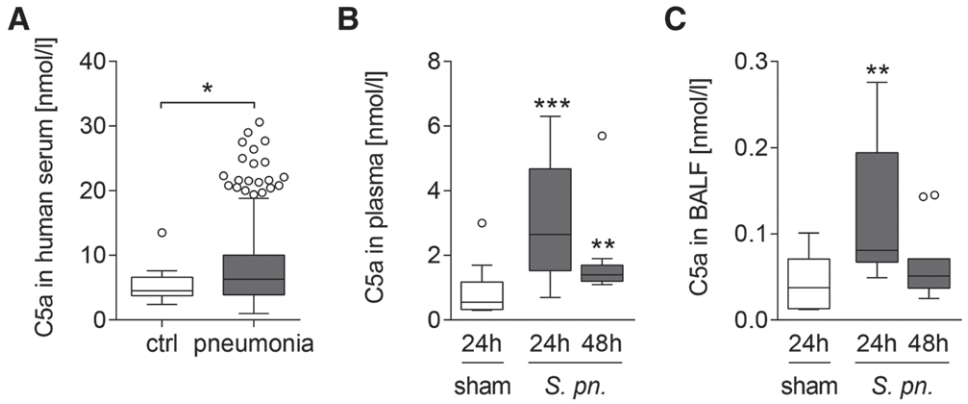

Fig. 1. C5a concentrations are elevated in human and murine pneumonia. (*A*) C5a was quantified in serum of patients with community-acquired pneumonia enrolled within 48 h of hospitalization (n = 395) and in healthy controls (ctrl, n = 24). Pneumonia patients had higher serum concentrations of C5a. (*B* and *C*) Mice were transnasally infected with *Streptococcus pneumoniae* (*S. pn.*; 5×10^6 colony-forming units/mouse) or sham-infected with phosphate-buffered saline, and 24 h or 48 h after infection C5a concentrations in plasma (*B*) and bronchoalveolar lavage fluid (BALF; *C*) were measured. In (*B*), n = 12 (sham, *S. pneumoniae* 24 h) or n = 11 (*S. pneumoniae* 48 h); in (*C*), n = 10 (sham) or n = 11 (*S. pneumoniae*). (*A–C*) Data are represented as *box plots* depicting median, quartiles, and ranges excluding outliers (*open circles*). (*A*) $^*P < 0.05$ (Mann–Whitney U test). (*B and C*) $^{**}P < 0.01$, $^{***}P < 0.001$ *vs.* sham-infected group (multiple Mann–Whitney U tests with Bonferroni correction for multiple comparisons).

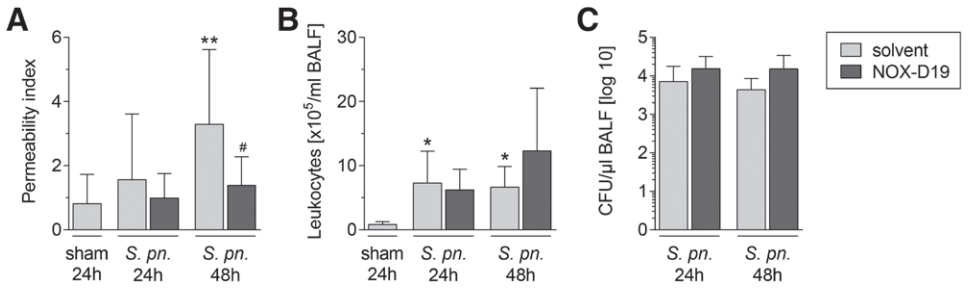

Fig. 2. Treatment with anti-C5a L-aptamer NOX-D19 led to a lower pulmonary permeability in *Streptococcus pneumoniae*–infected mice without affecting pulmonary leukocyte recruitment and bacterial load. Mice were transnasally infected with *S. pneumoniae* (*S. pn.*; 5×10^6 colony-forming units [CFU]/mouse) or sham-infected with phosphate-buffered saline, and intraperitoneally treated with NOX-D19 or solvent at time of infection (0 h) and 24 h postinfection. (*A*) Human serum albumin was administered intravenously 23 h or 47 h after infection, and 24 h or 48 h postinfection, the human serum albumin concentration in bronchoalveolar lavage fluid and plasma determined to quantify permeability. The permeability index was defined as the ratio of the human serum albumin concentration in bronchoalveolar lavage fluid (BALF) and plasma ($x10^{-3}$). Pneumonia-induced hyperpermeability was lower upon NOX-D19 treatment 48 h postinfection. (*B* and *C*) Pulmonary leukocyte recruitment (*B*), and number of CFUs in BALF (*C*) quantified 24 h and 48 h after infection were not altered by NOX-D19 treatment. Values are given as mean and SD. In (*A*), n = 13 (sham, *S. pn.*/NOX-D19 24 h) or n = 12 (*S. pn.*/solvent 24 h) or n = 10 (*S. pn.*/solvent 48 h) or n = 9 (*S. pn.*/NOX-D19 48 h); in (*B*), n = 13 (sham, *S. pn.*/NOX-D19 24 h) or n = 12 (*S. pn.*/solvent 24 h) or n = 10 (*S. pn.*/solvent 48 h) or n = 11 (*S. pn.*/NOX-D19 48 h); in (*C*), n = 12 (*S. pn.*/solvent 24 h) or n = 13 (*S. pn.*/NOX-D19 24 h) or n = 10 (*S. pn.*/solvent 48 h) or n = 11 (*S. pn.*/NOX-D19 48 h). $^*P < 0.05$, $^{**}P < 0.01$ *vs.* sham-infected, solvent-treated group, #$P < 0.05$ *vs. S. pneumoniae*–infected, solvent-treated group at the respective time point (one-way ANOVA and Sidak's multiple comparisons test).

C5a Neutralization Led to Lower Systemic G-CSF Concentrations but Did Not Affect the Cellular Systemic Immune Response and Bacterial Dissemination

S. pneumoniae–induced leukopenia and leukocyte subset distribution in blood were not altered by C5a neutralization (fig. 3A; fig. S6 in Supplemental Digital Content 1, http://links.lww.com/ALN/C198). Granulocyte colony-stimulating factor was statistically significantly lower 48 h postinfection in the NOX-D19– compared to the solvent-treated group, while further measured cytokines remained largely unaffected by C5a neutralization (fig. S7, Supplemental Digital Content 1, http://links.lww.com/ALN/C198).

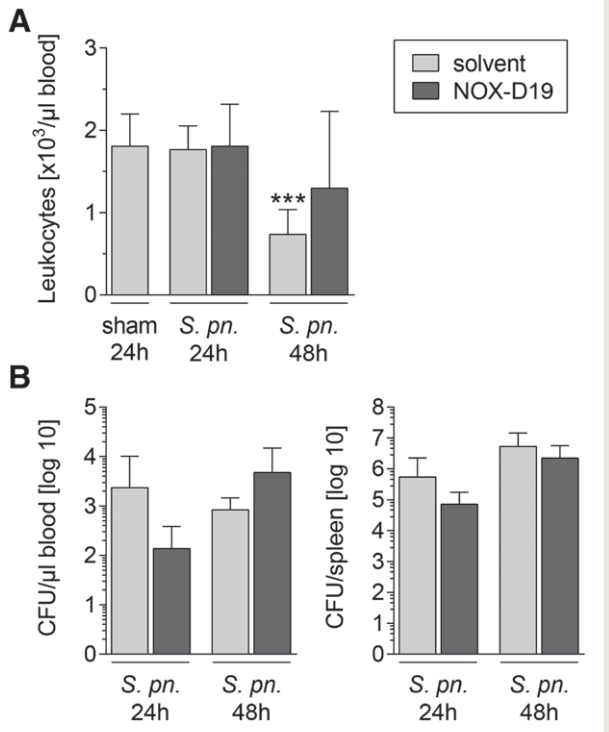

Fig. 3. Treatment with anti-C5a L-aptamer NOX-D19 did not affect the systemic cellular immune response and bacterial dissemination. Mice were transnasally infected with *Streptococcus pneumoniae* (*S. pn.*; 5×10^6 colony-forming units [CFU]/mouse) or sham-infected with phosphate-buffered saline, and intraperitoneally treated with NOX-D19 or solvent at time of infection (0 h) and 24 h postinfection. Twenty-four hours or 48 h after infection, blood leukocytes (*A*) as well as blood and spleen bacterial load (*B*) were quantified. (*A*) Leukocyte number was not significantly altered by NOX-D19 treatment. (*B*) Number of CFUs in blood and spleen was not affected by NOX-D19 treatment. Values are given as mean and SD. In (*A*), n = 11 (sham, *S. pn.*/solvent 24 h) or n = 12 (*S. pn.*/NOX-D19 24 h) or n = 10 (*S. pn.* 48 h); in (*B*), n = 12 (*S. pn.*/solvent 24 h) or n = 13 (*S. pn.*/NOX-D19 24 h) or n = 10 (*S. pn.*/solvent 48 h) or n = 11 (*S. pn.*/NOX-D19 48 h). ***$P < 0.001$ *vs.* sham-infected, solvent-treated group (one-way ANOVA and Sidak's multiple comparisons test).

Further, blood and splenic bacterial loads were not affected by C5a neutralization if compared to the solvent-treated group at both time points (fig. 3B).

C5a Neutralization Protected Mice from Development of Pneumonia-induced Liver Injury

To assess liver and kidney injury, liver sections were stained for caspase 3a and fibrin, and aspartate aminotransferase and blood urea nitrogen were quantified in plasma. Extended liver injury shown by necrotic areas and induction of hepatic apoptosis was observed in pneumococcal-infected mice 48 h postinfection, but undetectable in mice treated with NOX-D19 (fig. 4A). In mice with pneumonia, fibrin deposition in liver tissue was detected, implying a severe disruption of the local microcirculation, which was absent in NOX-D19–treated animals (fig. 4B). The immunohistochemistry scoring frequency for caspase and fibrin is displayed in figure S8 in Supplemental Digital Content 1 (http://links.lww.com/ALN/C198). Aspartate aminotransferase concentrations 48 h after infection were assessed, matching the histologic findings, with significant elevation of aspartate aminotransferase concentrations in mice with pneumonia after 48 h, which was prevented by NOX-D19 treatment. Blood urea nitrogen concentrations were elevated in plasma 48 h postinfection but were not significantly affected by NOX-D19 treatment (fig. 4C).

Delayed C5a Neutralization in Mice Protected Pulmonary Barrier Function and Lung Mechanics in Combined Severe Pneumonia and Mechanical Ventilation without Affecting the Pulmonary Cellular Immune Response

In pneumococcal pneumonia, mechanical ventilation leads to a dramatically higher lung permeability. Hyperpermeability was substantially lower after single administration of NOX-D19 23 h after infection (2.56 ± 1.17 *vs.* 7.31 ± 5.22, mean ± SD; difference: 4.76 [95% CI, 1.22 to 8.30]; $P = 0.011$; fig. 5A). Hyperpermeability causes lung edema, which then reduces lung compliance. Under the applied volume-controlled mechanical ventilation, an increase of mean airway pressure indicates a decrease of lung compliance. In line with the permeability data, mechanical ventilation led to higher airway pressure in mice with pneumonia receiving solvent. This was prevented by NOX-D19 treatment (fig. S9, Supplemental Digital Content 1, http://links.lww.com/ALN/C198). Mechanical ventilation leads to a dramatic exacerbation of the pulmonary inflammatory response in pneumococcal pneumonia. Similar to the model of pneumococcal pneumonia, the delayed C5a neutralization by NOX-D19 used here had no impact on pulmonary leukocyte recruitment and leukocyte subset distribution (fig. 5B; fig. S10 in Supplemental Digital Content 1, http://links.lww.com/ALN/C198) and did not affect pulmonary inflammatory cytokine release in bronchoalveolar lavage fluid compared to solvent treatment. Only lower concentrations of the cytokine CC chemokine ligand 3 were detected 30 h postinfection after C5a neutralization (fig. S11, Supplemental Digital Content 1, http://links.lww.com/ALN/C198).

Discussion

Improving the outcome of severe community-acquired pneumonia and associated sepsis is of utmost importance regarding the still high mortality of the disease. However, novel adjunctive pharmacologic treatment strategies are missing. Here we show that C5a concentrations are elevated in human and murine pneumonia. Neutralizing C5a by the

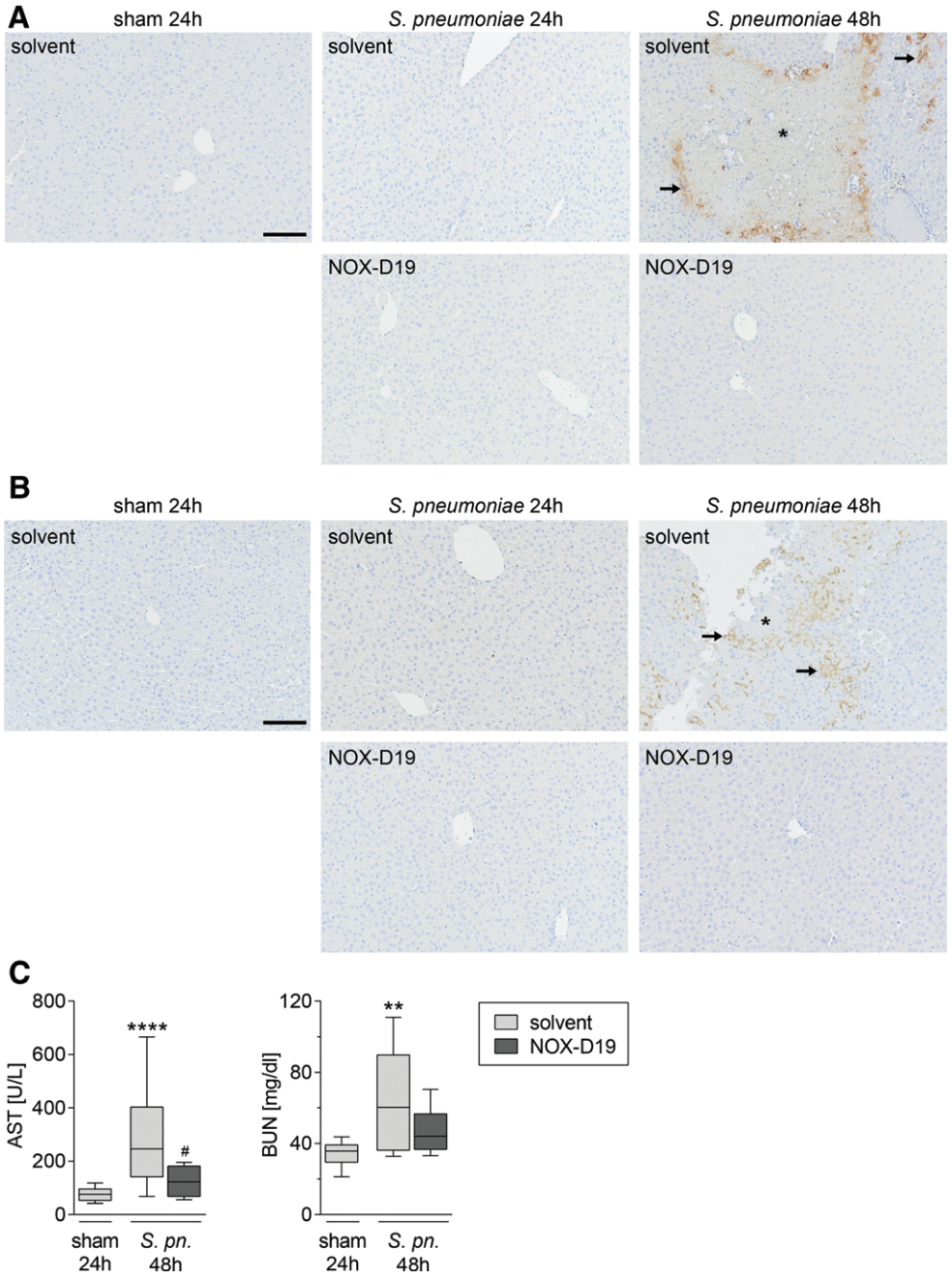

Fig. 4. Treatment with anti-C5a L-aptamer NOX-D19 protected mice from development of pneumonia-induced liver injury. Mice were transnasally infected with *Streptococcus pneumoniae* (*S. pn.*; 5×10^6 colony-forming units/mouse) or sham-infected with phosphate-buffered saline, and intraperitoneally treated with NOX-D19 or solvent at time of infection (0 h) and 24 h postinfection. (*A* and *B*) Twenty-four hours or 48 h after infection, liver sections were stained for caspase 3A (*A*) or fibrin (*B*) and counterstained with hemalaun. In the *S. pneumoniae*–infected, solvent-treated group, liver necrosis (*asterisks*) surrounded by caspase 3a–positive apoptotic cells (*arrows*, *A*) and fibrin deposition (*arrows*, *B*) was seen 48 h postinfection. Liver injury and concomitant microcirculatory failure were absent under NOX-D19 treatment. (*C*) Forty-eight hours after infection, liver aspartate aminotransferase (AST) quantified in plasma was reduced after NOX-19 treatment, while blood urea nitrogen (BUN) concentrations in plasma were not altered upon treatment. (*A* and *B*) Representative images are shown for each group (n = 8). *Scale bar* = 100 μm (valid for all photomicrographs). (*C*) Data are represented as *boxplots* depicting median, quartiles and range; for AST n = 13 (sham, *S. pn.*/solvent) or n = 10 (*S. pn.*/NOX-D19); for BUN n = 12 (sham) or n = 10 (*S. pn.*/solvent) or n = 8 (*S. pn.*/NOX-D19). $^{**}P < 0.01$, $^{****}P < 0.0001$ *vs.* sham-infected, solvent-treated group, $\#P < 0.05$ *vs. S. pneumoniae*–infected, solvent-treated group (multiple Mann–Whitney U tests with Bonferroni correction).

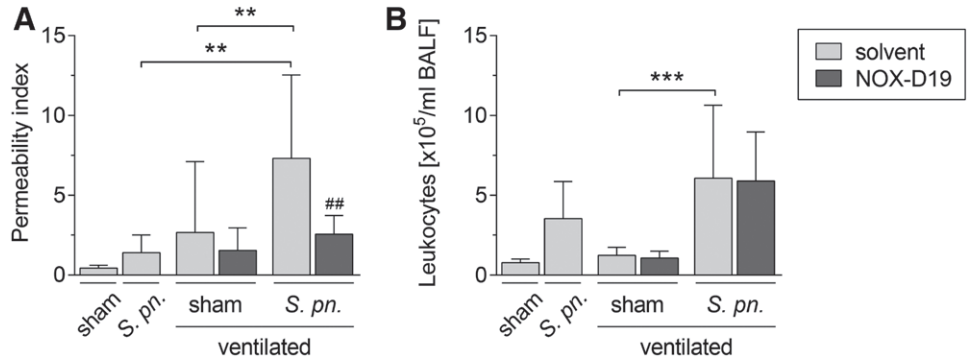

Fig. 5. Treatment with anti-C5a L-aptamer NOX-D19 protected pulmonary barrier function in combined severe pneumonia and mechanical ventilation without affecting pulmonary leukocyte recruitment. Mice were transnasally infected with *Streptococcus pneumoniae* (*S. pn.*; 5 × 10^6 colony-forming units/mouse) or sham-infected with phosphate-buffered saline, and intraperitoneally treated with NOX-D19 or solvent 23 h postinfection. One hour later (24 h postinfection), mechanical ventilation was performed for 6 h. Ventilated and nonventilated mice were euthanized 30 h after infection. (*A*) Human serum albumin was applied 90 min before termination of the experiment, and the human serum albumin concentration in bronchoalveolar lavage fluid (BALF) and plasma determined. The permeability index was defined as the ratio of the human serum albumin concentration in BALF and plasma ($x10^{-3}$). Lung hyperpermeability induced by pneumonia and mechanical ventilation was lower upon NOX-D19 treatment. (*B*) Pulmonary leukocyte recruitment was not altered by NOX-D19 treatment. Values are given as mean and SD. In (*A*), n = 5 (nonventilated) or n = 11 (solvent/ventilated) or n = 10 (NOX-D19/ventilated); in (*B*), n = 5 (nonventilated) or n = 11 (solvent/ventilated) or n = 10 (sham/NOX-D19/ventilated) or n = 9 (*S. pn.*/NOX-D19/ventilated). $^{**}P < 0.01$, $^{***}P < 0.001$ between indicated groups, $^{\#\#}P < 0.01$ *v. S. pneumoniae*–infected, solvent-treated, ventilated group (one-way ANOVA and Sidak's multiple comparisons test).

L-RNA-aptamer NOX-D19 in mice was highly protective against lung and septic liver injury induced by *S. pneumoniae* pneumonia and mechanical ventilation, suggesting that C5a might be a promising target for adjuvant therapy in community-acquired pneumonia.

C5a concentrations in cohorts of sepsis patients were unchanged or even reduced compared to healthy controls.[30–32] In hospitalized patients with community-acquired pneumonia, we found elevated C5a concentrations. This may suggest a prominent role of C5a in community-acquired pneumonia, but also might be an effect of timing. In mice, C5a was elevated early in pneumonia and declined in the further course of the disease. The patient samples analyzed were collected within 48 h of hospitalization, and pneumonia was the leading cause of hospitalization. In contrast, patients in the septic cohorts were by definition in an advanced state of their severe infection with a prolonged activated immune response, in which C5a concentrations might already have decreased. Notably, low C5a concentrations may still be relevant, as downregulation of C5a receptors 1 and 2 on neutrophils of septic patients was mediated by C5a and correlates with an unfavorable outcome in sepsis.[12] Although C5a is elevated early in pneumonia, our correlation analyses revealed no potential for C5a as biomarker for the diagnosis or prognosis of community-acquired pneumonia.

To investigate if neutralization of C5a may reduce organ dysfunction in severe pneumonia, we employed two different mouse models of pneumococcal pneumonia.[25,33] In both, mice were transnasally infected with *S. pneumoniae*. In the first pneumonia model, 24 h after infection, severe pneumonia developed, and mice became septic 48 h postinfection, as demonstrated by systemic hyperinflammation and multiple organ dysfunction. Analogous to the clinical cohort, C5a concentrations were found to be elevated in blood as well as in bronchoalveolar lavage fluid in mice with pneumococcal pneumonia. The second applied model combined severe pneumococcal pneumonia with mechanical ventilation, mimicking the clinical scenario of respiratory failure in patients with community-acquired pneumonia. The combination of these two noxious stimuli leads to severe respiratory failure.[33] In both models, C5a neutralization exhibited a comparable protective function on pulmonary hyperpermeability and further sepsis-related acute liver injury. Notably, in the combination model, treatment was initiated 23 h after infection when pneumonia was already established, which corresponds to the time community-acquired pneumonia is usually diagnosed in patients. Thus, these findings provide robust evidence for the efficacy of C5a neutralization in reducing pulmonary hyperpermeability, the hallmark of acute respiratory failure. Targeting pulmonary permeability as an adjuvant therapy for severe pneumonia seems to be rational, as elevated pulmonary permeability, which can be assessed in patients by calculating extravascular lung water index or pulmonary permeability index using the transpulmonary thermodilution technique, is correlated with an unfavorable outcome in critically ill patients.[34,35] Furthermore, the manifestation

of organ dysfunction is also correlated with a poor outcome in these patients.[36] In the current study, C5a neutralization improved both pulmonary permeability and liver injury in the applied experimental models. The severity of the disease, assessed by clinical scoring of mice, was also reduced by C5a neutralization. Thus, C5a neutralization improved outcome-relevant surrogate parameters in the current experimental setting. Notably, in a cecal ligation and puncture model of sepsis, we have already shown improved survival of mice treated with C5a-neutralizing L-RNA-aptamers.[16]

Although not directly measured in the current study, but considering the results from previous dosing and binding studies of the L-RNA-aptamer NOX-D19, we assume that C5a concentrations in blood and bronchoalveolar lavage fluid were efficiently neutralized.[16] C5a is involved in the recruitment of granulocytes to inflamed tissues, which was reported to be a major mechanism of the protective effect of C5a/C5a receptor axis disruption.[13,18,20,21,37] However, in this study, C5a neutralization did not affect the recruitment of granulocytes to the lungs, excluding this mechanism as explanatory for the observed protective effects. Notably, C5a directly contributes to the development of barrier dysfunction by activating endothelial cells and neutrophils.[16,38,39] As we did not observe an effect on neutrophil recruitment and cytokine concentrations by C5a neutralization, a direct effect on vascular permeability is likely to be the main underlying mechanism of C5a neutralization in pneumonia-related pulmonary barrier failure in this study. With the current experimental setting, however, we cannot estimate to what extent the observed protective effect on pulmonary barrier function is mediated by systemic or alveolar effects of C5a neutralization, as after injection, circulating NOX-D19 quickly penetrates into the alveolar space[16] (personal communication with NOXXON AG; Axel Vater, Ph.D., Chief Scientific Officer APTARION biotech AG, Berlin, Germany [former Vice President Drug Discovery and Preclinical Research NOXXON AG, Berlin, Germany], December 2016, written and verbal communication).

Due to its proinflammatory functions, C5a/C5a receptor axis disruption had been reported to result in reduced cytokine concentrations in different sepsis models.[16,18] In our model, C5a neutralization had no impact on pulmonary cytokine concentrations except for lowering concentrations of CC chemokine ligand 3. This is in line with an earlier report in which intratracheal administration of C5a led to increased CC chemokine release in the lungs, which contributed to the overall C5a-induced lung injury.[13] Thus, for the pulmonary compartment, the beneficial effect of C5a neutralization-mediated barrier protection does only partially seem to be related to a modulation of the local inflammatory response. In the circulation, the concentration of granulocyte colony-stimulating factor was lower due to blocking C5a signaling. Notably, granulocyte colony-stimulating factor concentrations are directly influenced by C5a/C5a receptor 1 interaction, as studies on mouse peritoneal-elicited macrophages have reported recently.[6] As further measured cytokines remained largely unaffected by C5a neutralization, the modulation of the systemic inflammatory response does not seem to be a major underlying mechanism of NOX-D19–mediated organ protection according to the findings in the lung.

After cleavage of C5, the C5b component initiates the assembly of the membrane attack complex, which mediates lyses of cells or microbes. Accordingly, interfering with C5 cleavage, for example due to treatment with the C5 antibody eculizumab, increases the susceptibility to infections with *Neisseria meningitidis*. NOX-D19 specifically binds to C5a, leaving C5b and thereby the membrane attack complex unaffected, as we recently demonstrated.[16] Nevertheless, interfering with the regulation of inflammation in the innate immune response toward infections bears the risk of rendering the host immunosuppressed. We measured bacterial burden in the lung, spleen, and blood and did not detect an impact of C5a neutralization on bacterial outgrowth, which is an important finding when considering C5a neutralization as adjunctive treatment for patients with pneumonia. Although we did not detect any adverse effects of L-RNA-aptamer–mediated C5a neutralization in this and previous studies, we cannot exclude potentially rare side effects in humans, which requires clinical studies with a sufficient number of patients.

In the described experimental model of pneumococcal pneumonia, 48 h after infection, animals became septic. Along with the observed systemic inflammatory response displayed by significantly increased cytokine concentrations in the circulation, extrapulmonary organ dysfunction developed. We documented an increase in blood urea nitrogen representing acute renal failure, significant signs of acute liver injury, and associated disturbed local microcirculation in the liver. C5a neutralization almost completely prevented liver injury and associated failure of the local microcirculation. This is in line with previous reports in which C5a had been shown to contribute to inflammation-associated liver injury in models of acute liver injury and polymicrobial sepsis.[40] One underlying mechanism might be the modulation of the systemic inflammation as it has been reported for a NOX-D19 follow-up in a sepsis model and in other studies;[16,18] however, in the current study, we found no clear impact on systemic cytokine concentrations by NOX-D19 treatment. Notably, C5a is also involved in the regulation of coagulation; thus, interaction with local and systemic activation of the coagulation cascade might also have contributed to the observed protection against severe liver injury by C5a neutralization.[41] Since it was beyond the scope of the study to further dissect the detailed underlying mechanisms of the observed protective effect of C5a neutralization, studies are needed to investigate the interaction of C5a with the coagulation system and systemic vascular barrier function.

There are limitations of the current study that need to be acknowledged. First, we did not study the impact of C5a

neutralization on survival in the applied model of severe pneumococcal pneumonia. In this model, all infected mice die in the short course of severe infection due to septic shock and multiple organ failure. According to our experience with this model, this can only be avoided by initiation of early antibiotic treatment in the phase of localized pneumonia after approximately 24 h postinfection. Later antibiotic treatment (48 h postinfection) does not rescue mice from death.[26] If an antimicrobial therapy capable of rescuing the mouse is initiated in this model, the animals recover quickly, and pulmonary permeability and further organ failure do not occur. Thus, survival analysis in this model applying a barrier-stabilizing adjunctive therapy is not effective. Second, we performed a single-sex experimental study in female mice, and our results cannot be extrapolated to male counterparts. Third, our experiments were limited to an inbred mouse strain and previously healthy individuals of the same age corresponding to a young adult, not representing the much more heterogeneous collective of patients with community-acquired pneumonia.

In summary, systemic C5a was significantly elevated in community-acquired pneumonia patients. In two mouse models of severe pneumococcal pneumonia, C5a neutralization by the L-aptamer NOX-D19 protected mice against pulmonary hyperpermeability and acute liver injury without impairing the local, protective host response. Early targeting of C5a as an adjunctive treatment strategy in severe community-acquired pneumonia might be a promising novel approach to ameliorate the severity of the disease.

Acknowledgments

The excellent technical assistance of Marfa Polikarpova (assistance during operations), Denise Barthel, Xiaohui Jiang, Carolin Ehrler, D.V.M., and Jeniffer Viernickel, D.V.M. (preparation of human serum samples; all Department of Infectious Diseases and Respiratory Medicine, Charité – University Medicine Berlin [Charité - Universitätsmedizin Berlin], Berlin, Germany), is greatly appreciated. The authors thank Achim D. Gruber, D.V.M., Ph.D. (Institute of Veterinary Pathology, Free University of Berlin [Freie Universität Berlin], Berlin, Germany), for performing lung histology. Parts of this work are incorporated into the doctoral thesis of Ute Kellermann, M.D. (Department of Infectious Diseases and Respiratory Medicine, Charité – University Medicine Berlin [Charité - Universitätsmedizin Berlin]). The authors thank the following members of the PROGRESS Study Group for contributing to patient sample and data collection within the PROGRESS study: Christoph Arntzen, M.D., Jan Pluta, M.D. (Hospital Angermünde [Krankenhaus Angermünde], Angermünde, Germany); Walter Knüppel, M.D. (Hospital Bad Arolsen GmbH [Krankenhaus Bad Arolsen GmbH], Bad Arolsen, Germany); Karsten Hartung, M.D., Barbara Wagener, M.D. (Lung Clinic Ballenstedt/Harz gGmbH [Lungenklinik Ballenstedt/Harz gGmbH], Ballenstedt, Germany); Stefan Angermair, M.D., Michael Benzke, M.D., Petra Creutz, M.D., Ulrike Föllmer, M.D., Carmen Garcia, M.D., Andreas Hocke, M.D., Charlotte Keller, M.D., Agata Mikolajewska, M.D., Michaela Niebank, M.D., Mirja Ramke, M.D. (Charité - University Medicine Berlin [Charité - Universitätsmedizin Berlin], Berlin, Germany); Achim Lies, M.D., Wulf Pankow, M.D., Dorina Thiemig, M.D. (Vivantes Hospital Neukölln [Vivantes Klinikum Neukölln], Berlin, Germany); Josefa Lehmke, M.D. (Vivantes Humboldt Hospital [Vivantes Humboldt-Klinikum], Berlin, Germany); Sven Gläser, M.D., Henning Kahnert, M.D., Markus Niesen, M.D. (Vivantes Hospital Spandau [Vivantes Klinikum Spandau], Berlin, Germany); Christian Grah, M.D. (Havelhöhe Community Hospital [Gemeinschaftskrankenhaus Havelhöhe], Berlin, Germany); Barbara Hauptmeier, M.D., Deborah Wehde, M.D. (University Hospital Bergmannsheil GmbH [Berufsgenossenschaftliches Universitätsklinikum Bergmannsheil GmbH], Bochum, Germany); Martin Buchenroth, M.D., Oliver Kanwar, M.D. (Protestant Hospital Bonn [Evangelische Kliniken Bonn], Bonn, Germany); Judith Pannier, M.D., Mathias Plauth, M.D., Marianne Schelle, M.D. (Dessau Municipal Hospital [Städtisches Klinikum Dessau], Dessau-Roßlau, Germany); Frederik Hempel, M.D., Kalina Popkirova, M.D., Bernhard Schaaf, M.D., Markus Unnewehr, M.D. (Hospital Dortmund gGmbH [Klinikum Dortmund gGmbH], Dortmund, Germany); Martin Kolditz, M.D. (University Hospital Carl Gustav Carus, Technical University Dresden [Universitätsklinikum Carl Gustav Carus, Technische Universität Dresden], Dresden, Germany); Holger Flick, M.D., Gudrun Wakonigg, M.D. (University Hospital Graz [Landeskrankenhaus-Universitätsklinikum Graz], Graz, Austria); Tim Oqueka, M.D. (University Medical Center Hamburg-Eppendorf [Universitätsklinikum Hamburg-Eppendorf], Hamburg, Germany); Julia Freise, M.D., Jessica Rademacher, M.D. (Hannover Medical School [Medizinische Hochschule Hannover], Hannover, Germany); Brigitte Mayer, M.D. (Heidenheim Hospital [Kliniken Heidenheim], Heidenheim, Germany); Carola Hobler, M.D., Simone Hamberger, M.D., Thomas Müller, M.D. (Main Taunus district clinics [Kliniken des Main-Taunus-Kreises], Hofheim, Germany); Robert Bals, M.D., Christian Lensch, M.D. (Saarland University Medical Center [Universitätsklinikum des Saarlandes], Homburg/Saar, Germany); Katrin Ludewig, M.D., Frank Bloos, M.D., Daniel Thomas-Rüddel, M.D., Anne Moeser, M.D., Mathias Pletz, M.D. (Jena University Hospital [Universitätsklinikum Jena], Jena, Germany); Michael Simpfendörfer, M.D. (Saint Vincent's Hospital gAG [Sankt Vincentius-Kliniken gAG], Karlsruhe, Germany); Lorenz Balke, M.D. (University Medical Center Schleswig-Holstein [Universitätsklinikum Schleswig-Holstein] – Campus Kiel, Kiel, Germany); Lena Kappauf, M.D. (Protestant Hospital Kalk gGmbH [Evangelisches Krankenhaus Kalk gGmbH], Köln, Germany); Lea Deterding, M.D., Eva Koch, M.D., Hubert Wirtz, M.D. (University of Leipzig,

Leipzig, Germany); Bernhard Ruf, M.D., Nils Keller, M.D. (Saint Georg Hospital gGmbH [Klinikum Sankt Georg gGmbH], Leipzig, Germany); Jan Rupp, M.D. (University Medical Center Schleswig-Holstein [Universitätsklinikum Schleswig-Holstein] – Campus Lübeck, Lübeck, Germany); Andreas Liebrich, M.D. (Saint Vincenz and Elisabeth Hospital [Sankt Vincenz und Elisabeth Hospital], Mainz, Germany); Ursula Hoffmann, M.D., Tetyana Shchetynska-Marinova, M.D. (University Medical Center Mannheim [Universitätsmedizin Mannheim], Mannheim, Germany); Phillipp Mayer, M.D., Christine Krollmann, M.D., Thomas Spinner, M.D. (Hospital München-Neuperlach [Krankenhaus München-Neuperlach], München, Germany); Claus Peckelsen, M.D., Patrick Schmidt-Schridde, M.D. (Munich Municipal Hospital GmbH – Harlaching Hospital [Städtisches Klinikum München GmbH – Klinikum Harlaching], München, Germany); Felix Rosenow, M.D., Lena-Maria Makowski, M.D. (University Hospital Münster [Universitätsklinikum Münster], Münster, Germany); Oleg Dmitriev, M.D., Hermann Druckmiller, M.D., Galina Schott, M.D. (Christian Hospital Quakenbrück e.V. [Christliches Krankenhaus Quakenbrück e.V.], Quakenbrück, Germany); Ayhan Berber, M.D., Nicolas Dickgreber, M.D. (Mathias Hospital Rheine [Mathias-Spital Rheine], Rheine, Germany); Tom Schaberg, M.D., Iris Hering, M.D. (Diaconia Hospital Rotenburg [Wümme] gGmbH [Diakoniekrankenhaus Rotenburg [Wümme] gGmbH], Rotenburg/Wümme, Germany); Cornelia Kropf-Sanchen, M.D., Stefan Rüdiger, M.D. (Ulm University Hospital [Universitätsklinikum Ulm], Ulm, Germany); Matthias Held, M.D., and Barbara Schröder, M.D. (Würzburg Hospital Missioclinic gGmbH [Klinikum Würzburg Mitte-Standort MissioKlinik] gGmbH, Würzburg, Germany).

Research Support

This study was supported by the German Research Foundation, Bonn, Germany (SFB-TR84 projects C3, C6, C7 to Drs. Müller-Redetzky and Witzenrath), by the German Federal Ministry of Education and Research, Bonn, Germany (e:Med CAPSyS-FKZ 01ZX1304A/B and 01ZX1604B to Drs. Scholz, Suttorp, and Witzenrath), by the German Center for Lung Research, Giessen, Germany (to Dr. Ahnert), and by NOXXON Pharma AG, Berlin, Germany (to Dr. Witzenrath). Dr. Letsiou received the Marie Sklodowska-Curie ERS/EU-RESPIRE 2 grant 9019/2015 (ERS, Lausanne, Switzerland).

Competing Interests

Drs. Maasch, Hoehlig, Klussmann, and Axel Vater are coinventors of NOX-D19 (PCT/EP2013/000056). Dr. Hoehlig is employee of, and Drs. Klussmann and Axel Vater are shareholders of, Aptarion Biotech AG, Berlin, Germany, which owns the intellectual property rights related to NOX-D19. The other authors declare no competing interests. Dr. Scholz receives funding from Pfizer Inc. for a research project unrelated to this work.

Correspondence

Address correspondence to Dr. Witzenrath: Division of Pulmonary Inflammation, and Department of Infectious Diseases and Respiratory Medicine, Charité – Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany. martin.witzenrath@charite.de. Information on purchasing reprints may be found at www.anesthesiology.org or on the masthead page at the beginning of this issue. Anesthesiology's articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

References

1. Mizgerd JP: Acute lower respiratory tract infection. N Engl J Med 2008; 358:716–27
2. WHO: The top 10 causes of death. Updated May 2018. Available at: http://www.who.int/en/news-room/fact-sheets/detail/the-top-10-causes-of-death. Accessed August 15, 2018.
3. Angus DC, van der Poll T: Severe sepsis and septic shock. N Engl J Med 2013; 369:840–51
4. Opal SM, van der Poll T: Endothelial barrier dysfunction in septic shock. J Intern Med 2015; 277:277–93
5. Matthay MA, Ware LB, Zimmerman GA: The acute respiratory distress syndrome. J Clin Invest 2012; 122:2731–40
6. Bosmann M, Haggadone MD, Zetoune FS, Sarma JV, Ward PA: The interaction between C5a and both C5aR and C5L2 receptors is required for production of G-CSF during acute inflammation. Eur J Immunol 2013; 43:1907–13
7. Rittirsch D, Flierl MA, Nadeau BA, Day DE, Huber-Lang M, Mackay CR, Zetoune FS, Gerard NP, Cianflone K, Köhl J, Gerard C, Sarma JV, Ward PA: Functional roles for C5a receptors in sepsis. Nat Med 2008; 14:551–7
8. Ward PA, Gao H: Sepsis, complement and the dysregulated inflammatory response. J Cell Mol Med 2009; 13:4154–60
9. Halbgebauer R, Schmidt CQ, Karsten CM, Ignatius A, Huber-Lang M: Janus face of complement-driven neutrophil activation during sepsis. Semin Immunol 2018; 37:12–20
10. Conway Morris A, Kefala K, Wilkinson TS, Dhaliwal K, Farrell L, Walsh T, Mackenzie SJ, Reid H, Davidson DJ, Haslett C, Rossi AG, Sallenave JM, Simpson AJ: C5a mediates peripheral blood neutrophil dysfunction in critically ill patients. Am J Respir Crit Care Med 2009; 180:19–28

11. Ward PA: The harmful role of c5a on innate immunity in sepsis. J Innate Immun 2010; 2:439–45
12. Xu R, Lin F, Bao C, Huang H, Ji C, Wang S, Jin L, Sun L, Li K, Zhang Z, Wang FS: Complement 5a receptor-mediated neutrophil dysfunction is associated with a poor outcome in sepsis. Cell Mol Immunol 2016; 13:103–9
13. Russkamp NF, Ruemmler R, Roewe J, Moore BB, Ward PA, Bosmann M: Experimental design of complement component 5a-induced acute lung injury (C5a-ALI): A role of CC-chemokine receptor type 5 during immune activation by anaphylatoxin. FASEB J 2015; 29:3762–72
14. Hellerud BC, Orrem HL, Dybwik K, Pischke SE, Baratt-Due A, Castellheim A, Fure H, Bergseth G, Christiansen D, Nunn MA, Espevik T, Lau C, Brandtzæg P, Nielsen EW, Mollnes TE: Combined inhibition of C5 and CD14 efficiently attenuated the inflammatory response in a porcine model of meningococcal sepsis. J Intensive Care 2017; 5:21
15. Herrmann JB, Muenstermann M, Strobel L, Schubert-Unkmeir A, Woodruff TM, Gray-Owen SD, Klos A, Johswich KO: Complement C5a receptor 1 exacerbates the pathophysiology of *N. meningitidis* sepsis and is a potential target for disease treatment. MBio 2018; 9:pii:e01755–17
16. Hoehlig K, Maasch C, Shushakova N, Buchner K, Huber-Lang M, Purschke WG, Vater A, Klussmann S: A novel C5a-neutralizing mirror-image (l-)aptamer prevents organ failure and improves survival in experimental sepsis. Mol Ther 2013; 21:2236–46
17. Kalbitz M, Fattahi F, Grailer JJ, Jajou L, Malan EA, Zetoune FS, Huber-Lang M, Russell MW, Ward PA: Complement-induced activation of the cardiac NLRP3 inflammasome in sepsis. FASEB J 2016; 30:3997–4006
18. Keshari RS, Silasi R, Popescu NI, Patel MM, Chaaban H, Lupu C, Coggeshall KM, Mollnes TE, DeMarco SJ, Lupu F: Inhibition of complement C5 protects against organ failure and reduces mortality in a baboon model of *Escherichia coli* sepsis. Proc Natl Acad Sci USA 2017; 114:E6390–9
19. Tuboly E, Futakuchi M, Varga G, Érces D, Tőkés T, Mészáros A, Kaszaki J, Suzui M, Imai M, Okada A, Okada N, Boros M, Okada H: C5a inhibitor protects against ischemia/reperfusion injury in rat small intestine. Microbiol Immunol 2016; 60:35–46
20. Jiang Y, Zhao G, Song N, Li P, Chen Y, Guo Y, Li J, Du L, Jiang S, Guo R, Sun S, Zhou Y: Blockade of the C5a-C5aR axis alleviates lung damage in hDPP4-transgenic mice infected with MERS-CoV. Emerg Microbes Infect 2018; 7:77
21. Sun S, Zhao G, Liu C, Fan W, Zhou X, Zeng L, Guo Y, Kou Z, Yu H, Li J, Wang R, Li Y, Schneider C, Habel M, Riedemann NC, Du L, Jiang S, Guo R, Zhou Y: Treatment with anti-C5a antibody improves the outcome of H7N9 virus infection in African green monkeys. Clin Infect Dis 2015; 60:586–95
22. Vater A, Klussmann S: Turning mirror-image oligonucleotides into drugs: The evolution of Spiegelmer(®) therapeutics. Drug Discov Today 2015; 20:147–55
23. Khan MA, Maasch C, Vater A, Klussmann S, Morser J, Leung LL, Atkinson C, Tomlinson S, Heeger PS, Nicolls MR: Targeting complement component 5a promotes vascular integrity and limits airway remodeling. Proc Natl Acad Sci USA 2013; 110:6061–6
24. Ahnert P, Creutz P, Scholz M, Schütte H, Engel C, Hossain H, Chakraborty T, Bauer M, Kiehntopf M, Völker U, Hammerschmidt S, Loeffler M, Suttorp N; PROGRESS study group: PROGRESS - prospective observational study on hospitalized community acquired pneumonia. BMC Pulm Med 2016; 16:108
25. Witzenrath M, Schmeck B, Doehn JM, Tschernig T, Zahlten J, Loeffler JM, Zemlin M, Müller H, Gutbier B, Schütte H, Hippenstiel S, Fischetti VA, Suttorp N, Rosseau S: Systemic use of the endolysin Cpl-1 rescues mice with fatal pneumococcal pneumonia. Crit Care Med 2009; 37:642–9
26. Berger S, Goekeri C, Gupta SK, Vera J, Dietert K, Behrendt U, Lienau J, Wienhold SM, Gruber AD, Suttorp N, Witzenrath M, Nouailles G: Delay in antibiotic therapy results in fatal disease outcome in murine pneumococcal pneumonia. Crit Care 2018; 22:287
27. Müller HC, Hellwig K, Rosseau S, Tschernig T, Schmiedl A, Gutbier B, Schmeck B, Hippenstiel S, Peters H, Morawietz L, Suttorp N, Witzenrath M: Simvastatin attenuates ventilator-induced lung injury in mice. Crit Care 2010; 14:R143
28. Müller-Redetzky HC, Kummer W, Pfeil U, Hellwig K, Will D, Paddenberg R, Tabeling C, Hippenstiel S, Suttorp N, Witzenrath M: Intermedin stabilized endothelial barrier function and attenuated ventilator-induced lung injury in mice. PLoS One 2012; 7:e35832
29. Witzenrath M, Gutbier B, Hocke AC, Schmeck B, Hippenstiel S, Berger K, Mitchell TJ, de los Toyos JR, Rosseau S, Suttorp N, Schütte H: Role of pneumolysin for the development of acute lung injury in pneumococcal pneumonia. Crit Care Med 2006; 34:1947–54
30. Gardinali M, Padalino P, Vesconi S, Calcagno A, Ciappellano S, Conciato L, Chiara O, Agostoni A, Nespoli A: Complement activation and polymorphonuclear neutrophil leukocyte elastase in sepsis. Correlation with severity of disease. Arch Surg 1992; 127:1219–24
31. Gressner OA, Koch A, Sanson E, Trautwein C, Tacke F: High C5a levels are associated with increased mortality in sepsis patients–No enhancing effect by actin-free Gc-globulin. Clin Biochem 2008; 41:974–80
32. Stöve S, Welte T, Wagner TO, Kola A, Klos A, Bautsch W, Köhl J: Circulating complement proteins in patients with sepsis or systemic inflammatory response syndrome. Clin Diagn Lab Immunol 1996; 3:175–83
33. Müller-Redetzky HC, Will D, Hellwig K, Kummer W, Tschernig T, Pfeil U, Paddenberg R, Menger MD,

Kershaw O, Gruber AD, Weissmann N, Hippenstiel S, Suttorp N, Witzenrath M: Mechanical ventilation drives pneumococcal pneumonia into lung injury and sepsis in mice: Protection by adrenomedullin. Crit Care 2014; 18:R73

34. Chung FT, Lin SM, Lin SY, Lin HC: Impact of extravascular lung water index on outcomes of severe sepsis patients in a medical intensive care unit. Respir Med 2008; 102:956–61
35. Kushimoto S, Endo T, Yamanouchi S, Sakamoto T, Ishikura H, Kitazawa Y, Taira Y, Okuchi K, Tagami T, Watanabe A, Yamaguchi J, Yoshikawa K, Sugita M, Kase Y, Kanemura T, Takahashi H, Kuroki Y, Izumino H, Rinka H, Seo R, Takatori M, Kaneko T, Nakamura T, Irahara T, Saito N; PiCCO Pulmonary Edema Study Group: Relationship between extravascular lung water and severity categories of acute respiratory distress syndrome by the Berlin definition. Crit Care 2013; 17:R132
36. Raith EP, Udy AA, Bailey M, McGloughlin S, MacIsaac C, Bellomo R, Pilcher DV; Australian and New Zealand Intensive Care Society (ANZICS) Centre for Outcomes and Resource Evaluation (CORE): Prognostic accuracy of the SOFA Score, SIRS Criteria, and qSOFA Score for in-hospital mortality among adults with suspected infection admitted to the intensive care unit. JAMA 2017; 317:290–300
37. Bosmann M, Grailer JJ, Ruemmler R, Russkamp NF, Zetoune FS, Sarma JV, Standiford TJ, Ward PA: Extracellular histones are essential effectors of C5aR- and C5L2-mediated tissue damage and inflammation in acute lung injury. FASEB J 2013; 27:5010–21
38. Mahajan SD, Parikh NU, Woodruff TM, Jarvis JN, Lopez M, Hennon T, Cunningham P, Quigg RJ, Schwartz SA, Alexander JJ: C5a alters blood-brain barrier integrity in a human *in vitro* model of systemic lupus erythematosus. Immunology 2015; 146:130–43
39. Schraufstatter IU, Trieu K, Sikora L, Sriramarao P, DiScipio R: Complement c3a and c5a induce different signal transduction cascades in endothelial cells. J Immunol 2002; 169:2102–10
40. Lei YC, Lu CL, Chen L, Ge K, Yang LL, Li W, Wu YH: C5a/C5aR pathway is essential for up-regulating SphK1 expression through p38-MAPK activation in acute liver failure. World J Gastroenterol 2016; 22:10148–57
41. Laudes IJ, Chu JC, Sikranth S, Huber-Lang M, Guo RF, Riedemann N, Sarma JV, Schmaier AH, Ward PA: Anti-c5a ameliorates coagulation/fibrinolytic protein changes in a rat model of sepsis. Am J Pathol 2002; 160:1867–75