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Murine Complement Interactions with *Pseudomonas aeruginosa* and Their Consequences During Pneumonia

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Abstract

Complement is necessary for defense against lung infection with *Pseudomonas aeruginosa* in mice. We studied *in vitro* interactions between complement and *P. aeruginosa* and *in vivo* effects of complement depletion to better understand this relationship. *In vitro*, *P. aeruginosa* strain UI-18 was resistant to killing by mouse serum. However, C3 opsonized the organism (via the alternative and mannose binding lectin [MBL] pathways), and C5 convertase activity on the bacterial surface was demonstrated. *In vivo*, compared with normal mice, complement-deficient mice experienced higher mortality and failed to sterilize their bronchoalveolar space within 24 h of inoculation. These changes did not seem to be a result of decreased inflammation because complement-deficient mice had normal neutrophil recruitment, greater lung myeloperoxidase content, and, by 24 h, a 35-fold higher level of the CXC chemokine KC. Lung static pressure-volume curves were abnormal in infected animals but were significantly more so in complement deficient mice. These data indicate that although *P. aeruginosa* is resistant to serum killing, C3 opsonization and C5 convertase assembly occur on its surface. This interaction *in vivo* plays a central role in host survival beyond just recruitment and activation of phagocytes and may serve to limit the inflammatory response to and tissue injury resulting from bacterial infection.

Despite advances in diagnosis and treatment, Gram-negative pneumonia is a major health threat in the United States. As of 1999, pneumonia remained the fourth leading cause of death among hospitalized patients, behind the broad categories of cardiovascular disease, cerebrovascular disease, and malignancy (1). For patients developing nosocomial pneumonia, *Pseudomonas aeruginosa* is the most often identified etiologic organism, accounting for 21% of such infections in a recent survey (2). Well-recognized risk factors for infection with this organism include impaired host immunity, including malignancy; endotracheal intubation and mechanical ventilation; severe burns; and prolonged hospitalization (3).

In mouse models of pneumonia, requirements for the cellular and humoral components of the innate immune system have been demonstrated in defense against *P. aeruginosa*. The

presence of neutrophils and the signaling apparatus needed to recruit them into the lung have been shown to be essential for survival during acute infection; native alveolar macrophages alone seem to be insufficient to defend against instilled bacteria (4). The complement system also has been shown to be significant in the response to *Pseudomonas pneumonia*. Genetically C5-deficient mice exhibit impaired clearance of intrapulmonary *P. aeruginosa* (5). Although the formation of the membrane attack complex and subsequent complement-mediated bacteriolysis may contribute to the role of C5, a need for the anaphylatoxin C5a has been demonstrated in C5a-receptor null mice challenged with intratracheal *Pseudomonas* (6).

Given the multiplicity of chemotactic and proinflammatory signals that can arise from an acutely infected lung (e.g., bacterial lipopolysaccharides, formyl peptides, and various host mediators) and the numerous means by which phagocytosis can be stimulated, the necessity of the complement system remains incompletely understood. We therefore have further investigated complement-mediated interactions with this pathogen in a murine model of acute bacterial pneumonia. We first sought to define *in vitro* the interactions between murine complement components and this pathogen by determining its susceptibility to serum-mediated killing and the ability of mouse serum to opsonize this strain with C3 and to assemble functional solid-phase C5 convertase complexes on the bacterial surface. We then examined host-pathogen interactions *in vivo* by first confirming the lethality of complement deficiency (in homozygous C5-deficient or in complement-depleted mice) in the setting of pseudomonal pneumonia. The immune, inflammatory, and functional consequences of hypocomplementemia were quantified using cultures of blood and bronchoalveolar lavage fluid (BALF), lung inflammatory cell and chemokine content, and static lung compliance measurements, respectively. Our findings support a critical role for the complement cascade including and distal to C5 and suggest that the absence of complement during airway challenge with *Pseudomonas* produces an exaggerated host inflammatory response that is incapable of sterilizing the lung and that leads to worsening pulmonary function and increased mortality.

Materials and Methods

Six- to 10-wk-old female C57Bl/6J, homozygous C5-deficient (B10.D2 *Hc*⁰), and congenic C5-sufficient (B10.D2 *Hc*¹) mice were obtained from Jackson Laboratories (Bar Harbor, ME) and maintained in specific pathogen-free conditions. The local animal use committee approved all *in vivo* experimental protocols. *P. aeruginosa* strain UI-18, a smooth, nonmucoid, pyocyanin-producing clinical isolate, was used in all experiments. Bacteria were raised in tryptic soy broth or on tryptic soy blood agar (Difco, Detroit, MI) in room air at 37°C. In all experiments, bacteria at mid-log growth were used. Bacteria suspensions were quantitated turbidimetrically. Antisera and antibodies used included goat anti-mouse C3 antiserum (ICN/Cappel, Aurora, OH), an affinity-purified polyclonal rabbit IgG against the carboxyl-terminal end of human C5a (described below), and HRP-conjugated secondary antibodies (Amersham Biosciences, Piscataway, NJ). C3-depleted human serum and purified human C5, C6, C7, C8, and C9 were obtained from Quidel (San Diego, CA). All other reagents were obtained from Sigma Chemical Co. (St. Louis, MO) unless otherwise noted.

Opsonization of *P. aeruginosa* by Murine C3

Aliquots (50 μ l) containing 10^6 CFU in 20% ethanol were placed in microtiter wells and allowed to dry. The wells were then blocked with 3% bovine serum albumin in phosphate-buffered saline (PBS). Pooled fresh mouse serum diluted 1:3 in a triethanolamine buffer (0.28% triethanolamine in 130 mM NaCl, 150 μ M CaCl₂, 500 μ M MgCl₂, pH 7.35) was applied to bacteria-coated wells, incubated for 30 min at 37°C, and then washed off. Anti-C3 antibody was added for 1 h and washed away, and bound anti-C3 was detected using a horseradish peroxidase (HRP)-conjugated secondary antibody and tetramethyl benzidine substrate (Pierce, Rockford, IL). Inhibitors of complement activation, including 10 mM EDTA (to block all three activating pathways), 10 mM EGTA + 100 mM Mg²⁺ (to selectively block the classical and lectin pathways), and 100 mM mannose or 100 mM N-acetyl glucosamine (GluNAc) (to block the lectin pathway) were also studied. Serum that had been heat inactivated at 57°C for 1 h was used as a negative control. Assays were run in triplicate, and results are presented as percent activity (the difference between the Abs_{450nm} of normal serum and any test condition divided by the difference between normal and heat-treated serum $\times$ 100%).

Solid-Phase Assembly of Murine C5 Convertase

No antibody is currently available that is capable of differentiating murine C5a from the C5 parent molecule from which it is cleaved. Antibodies fulfilling this requirement have been raised against human C5a. Therefore, an analytic strategy in which murine C5 convertase was allowed to act upon purified human C5 was devised. *Pseudomonas*-coated plates were exposed to normal mouse serum as described above. After washing the serum from the plates, purified human C5 (Quidel) in PBS was added and allowed to incubate for 3 h at 37°C. The C5a catalytic product was measured by immunoblot of the reaction supernatants, using an affinity-purified rabbit anti-huC5a as a primary detection antibody. This material was raised against a synthetic peptide comprising carboxyl terminal amino acids 55-74 (CVVASQLRANISHKDMQLGR) and has documented specificity for huC5a without cross-reacting with the parent protein huC5 (7). A chemiluminescence detection system (ECL Plus; Amersham Biosciences) was used to detect anti-C5a. As a positive control, huC5 was incubated with phorbol myristate acetate-stimulated rat macrophages as previously described (7). Results of these experiments are reported as representatives of at least triplicate experiments.

In Vitro Serum Bactericidal Activity

Susceptibility to complement-mediated killing was determined by quantitatively culturing *P. aeruginosa*, which had been incubated with normal and heat-inactivated human serum (initial dose, 10^7 CFU/100 μ l) and mouse serum (initial dose, 10^3 CFU/100 μ l) for 60 min. Additional experiments were conducted using hyperimmune mouse serum, which was raised in animals receiving subcutaneously 50 μ g (dry weight) of heat-killed *P. aeruginosa* in Freund's complete adjuvant (Pierce, Rockford, IL) (initial injection) or incomplete adjuvant (on Days 3, 17, 21, and 24) per published protocols (8, 9); this procedure yielded anti-pseudomonal titers in excess of 1:250,000. Titers of normal human and normal mouse sera were undetectable (i.e., < 1:20).

Because initial bacterial killing assays also revealed differences in bactericidal activity between human and mouse sera, additional experiments examined bacterial killing by mouse serum supplemented with C3-depleted human serum or with all of the human terminal complex components. Purified C5, C6, C7, C8, and C9 were added to normal mouse serum for a final concentration of 10 µg/ml of each component.

Murine Pneumonia Model

Mice were intraperitoneally anesthetized with 85 mg/kg ketamine HCl (Fort Dodge Animal Health, Fort Dodge, IA) and 15 mg/kg xylazine HCl (Vedco, St. Joseph, MO), and 30-µl inoculates containing 10⁵ CFU were delivered intratracheally using a pipetter. To discriminate between the immune and purely inflammatory roles of complement in this system, some animals received intratracheal injections of 10⁵ heat-killed bacteria.

In Vivo Complement Depletion

Mice were complement depleted using cobra venom factor (CVF) from *Naja naja kaouthia* as previously described (10, 11). Doses of CVF (30 U/kg) were administered intraperitoneally 36, 24, and 12 h before experimentation. Control animals received the saline vehicle. The depth and duration of complement depletion using this strategy was assessed in two ways. First, serum was drawn from depleted animals at the conclusion of the CVF treatment protocol (i.e., 12 h after the final CVF dose), and 48 and 96 h later ($n = 5$ at each time point). Opsonization of plate-bound *Pseudomonas* was assayed as described above. Second, bronchoalveolar lavage (BAL) was performed to measure airspace C3 levels. Cell-free BALF from each group was pooled and assayed for protein concentration. Fifteen micrograms of material from each time point along with 15 µg of normal mouse BALF and normal mouse serum were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis under reducing conditions and examined by immunoblot for murine C3. The appearance of 115-kD and 75-kD bands corresponding to α - and β -chains was considered evidence of C3 (12).

Lethality Among C5^{-/-} and Complement-Depleted Animals

Because the availability of C5^{-/-} mice is limited, most of our endpoints examined complement-depleted rather than genetically deficient animals. Conducting survival studies on deficient and depleted animals tested the validity of this strategy. We performed 96-h survival studies in C5^{-/-}, congenic C5^{+/+}, and CVF-depleted and vehicle-treated mice, with a census of surviving animals being taken at least every 12 h.

Lung Bacterial Clearance and Development of Bacteremia

Twenty-four-hour post-inoculation quantitative cultures of blood and BALF were performed. Given the dilutions used, the theoretical detection limit for the method was 100 CFU/ml.

Lung Neutrophil Content and Chemokine Concentration

Neutrophil migration and activation were measured in two ways. Bronchoalveolar cellularity was assessed by cytologic analysis of BALF 12 h after infection. The cells contained within

200 μ l of BALF were deposited on a microscope slide using a cytologic centrifuge and stained with Dif-Quik (Dade Behring, Newark, Delaware). The relative content of mononuclear and polymorphonuclear leukocytes was determined by counting five high-power fields or 150 cells, whichever was greater. Whole-lung myeloperoxidase (MPO) activity was measured as previously described (10, 13). BAL levels of the CXC chemokine KC were determined using a commercially available kit and following the manufacturer's protocol (R&D Systems, Minneapolis, MN). Although treatment with intraperitoneal CVF generally a mild systemic stimulus, experiments were performed to confirm the impact of our complement-depletion strategy on BAL KC levels. These experiments revealed no demonstrable effect of CVF treatment (normal: 29 ± 14 pg/ml; CVF-treated: 19 ± 9 pg/ml; $P = 0.18$; $n = 5$ per group).

Quantitation of Lung Mechanics

Static lung compliance was used to determine the extent of pulmonary injury and was measured immediately after killing. The trachea was isolated and intubated with a 21-gauge catheter attached to a calibrated syringe and pressure transducer (Transpac IV; Abbott Critical Care, North Chicago, IL) interfaced to a data acquisition system (MP100; Biopac Systems, Santa Barbara, CA). Lung compliance curves were generated for inflation and exhalation by recording the change in airway pressure observed with serial injection (or for exhalation, serial removal) of 100- μ l aliquots of air to a total of 600 μ l.

Statistical Methods

Results are reported as means $\pm$ SEM unless otherwise noted. For continuous variables, analysis of variance (ANOVA) was followed by post-hoc comparisons using the Tukey method. ANOVA for repeated measures was used to compare the static lung compliance curves. In these analyses, overall significance only was determined; post-hoc comparisons at single pressure-volume points (e.g., comparing airway pressures between groups at any single inflation volume) were not performed. Survival curves were compared using proportional hazards modeling. All statistical calculations were performed using SAS 8 software (SAS Institute, Cary, NC).

Results

Serum Resistance of *P. aeruginosa* UI-18 and *In Vitro* Complement Assays

Strain UI-18 was found to be susceptible to killing by human serum, sustaining roughly a 3-log decrease (i.e., 99.9%) in viable organisms after 60 min of exposure, an effect that was heat labile (Table 1). Exposure of this organism to serum from C57Bl/6J mice resulted in no detectable reduction in bacterial counts. *Pseudomonas* was equally resistant to hyperimmune mouse serum. Supplementation of murine serum with C3-depleted human serum or human complement components C5 through C9 did not render murine serum bactericidal. Nevertheless, murine C3 opsonized microtiter plate-affixed bacteria (Figure 1). Mannose and GluNAc reduced opsonization by roughly 40%, demonstrating a substantial role for the lectin pathway in this system. However, selective binding of Ca^{2+} while preserving Mg^{2+} (a strategy that inhibits function of C1q and MBL) preserved 80% of opsonic activity, indicating that the alternative pathway alone is largely effective in initiating the opsonization

of *P. aeruginosa*. Nonselective divalent cation chelation with EDTA completely inhibited the complement-mediated attack.

The C3-opsonized bacterial surface possessed functional C5 convertase activity against human C5 (Figure 1), an effect that was also heat labile. The appearance of C5a above the opsonized surface demonstrates that C5b is also produced by this system. These results indicate that the resistance of *P. aeruginosa* to mouse serum stems from defective assembly or ineffective insertion of C5b-9 (membrane attack complex) into the pseudomonal cell wall rather than from failure of the complement terminal sequence to be initiated on the pathogen's surface.

Effectiveness and Duration of Complement Depletion by CVF

After complement depletion with CVF, the bacterial opsonic activity of murine serum was reduced 97% from baseline (Figure 2). By 96 h, this activity had recovered. Compared with serum, normal murine BALF also had significantly less antigenic C3 as measured by immunoblot (Figure 2). The complement depletion strategy rendered C3 initially undetectable; recovery of BAL C3 paralleled the time course of serum opsonic activity.

Lethality of Complement Deficiency

All C5-deficient mice challenged with *P. aeruginosa* died between 18 and 48 h, whereas 73% of the C5^{+/+} animals survived the entire 96-h observation period ($P < 0.01$) (Figure 3). Lethality among the CVF-treated animals followed a similar pattern during the first 36 h after exposure, although ultimately 25% of CVF-treated animals recovered ($P < 0.01$). Although there was no statistically significant difference in the poor survival between C5^{-/-} and CVF-treated mice ($P = 0.82$), the CVF-treated animals that did survive may have done so as a result of rebounding serum and BAL C3 levels during the course of the experiment.

Bacterial Clearance

Complement-intact mice uniformly sterilized their bronchoalveolar space 24 h post-exposure (no bacterial growth from any of four sampled animals), whereas complement-depleted mice failed to do so (mean 10^7 CFU/ml; range 5×10^3 to 4×10^7 ; $n = 4$; $P < 0.05$). Blood cultures drawn 24 h after intratracheal inoculation were negative in all animals in all groups.

Bronchoalveolar Cytology, Lung MPO Content, and Chemokine Secretion

Infection dramatically changed the cellular composition of BALF within 12 h of inoculation (Table 2 and Figure 4). However, there was no difference in the relative neutrophil content of normal and complement-depleted mice. In contrast, CVF-treated animals had higher and sustained MPO content at 12 and 24 h post-exposure (Figure 5), suggesting an increase in neutrophil numbers in the lung outside of the airspace or an increase in MPO expression by the neutrophils of complement-depleted animals. Bronchoalveolar KC levels were sharply elevated within 4 h of inoculation of live organism (Figure 6). Levels were higher in CVF-treated animals at 4 h and at subsequent time points, although this difference was statistically significant only at 24 h, by which time KC levels in the CVF-treated mice were 35 times higher than in vehicle-treated mice ($P < 0.01$).

Challenge with Heat-Killed Bacteria

The inflammatory response to 10^5 CFU heat-killed *P. aeruginosa* was much milder than that seen after challenge with live organisms. Lung MPO content was higher in complement-depleted animals through the first 12 h ($P < 0.05$ at baseline, 4 h, and 12 h), with falling levels seen in both groups with no statistically significant difference by 24 h post-exposure (Figure 4). BAL KC levels were roughly 10-fold lower after challenge with dead bacteria compared with exposure to live organisms, with no differences seen between groups at any time point (Figure 6).

Lung Injury After Inoculation

Mechanical performance of the lung as measured by static lung compliance was impaired during acute infection. Complement depletion significantly worsened compliance (Figure 7).

Discussion

The current studies underscore the complexity of complement immunity in host defense during lung infection with *P. aeruginosa* and that *in vitro* resistance to complement-mediated killing does not exclude an essential role for complement *in vivo*. Although murine serum does not kill UI-18, it successfully opsonizes it and assembles working C5 convertases on its surface. Cleavage of C5 in the absence of bactericidal activity implies that the terminal complement complex is being activated but is failing to kill the bacteria. This has been noted in studies of human complement, in which a defect in C9 insertion into the *P. aeruginosa* cell wall has been found against some serum-resistant strains (20). One role of C5b-9 in the killing of Gram-negative bacteria may be to permit access by lysozyme and other defense proteins across the outer membrane and into the periplasmic space (21). Our findings imply species-specific differences in membrane attack complex (MAC) function or differences in complement-cooperative bactericidal enzymes capable of capitalizing upon cell wall defects produced by MAC. Incubation of bacteria simultaneously with murine serum and C3-depleted human serum or with murine serum supplements with human C5, C6, C7, C8, and C9 produced no demonstrable bacterial killing, which suggests that factors other than the membrane attack complex may be participating in bacterial killing.

Alternative and lectin pathways participate in the initial attack, which suggests that constituents of these pathways are present on the bacteria surface. These factors are known to serve as ligands for numerous receptors on immune cell lines, including the MBL receptor, CR1 (CD35), CR3 (CD11b/CD18), and CR4 (CD11c/CD18) (14–18). *In vivo*, all of these should be available to the host during early infection, and none requires C5. Nevertheless, alone they are insufficient to protect mice against this infection; C5-deficient mice have increased susceptibility to lung challenge with *P. aeruginosa* in our system and others (19). A recent study using C5a-receptor null mice strongly suggested that it was the anaphylatoxin, rather than C5b, that played the crucial role in host protection (6). The critical contribution made by C5a in a model system already possessing many other chemoattractants and pro-inflammatory mediators of pathogen and host origin remains unclear.

An important issue to be settled is whether the mechanism of complement activation in the airway is the same as that seen in serum. Because of a lack of useful antibodies against most murine complement proteins, progress in this regard has been slow. In the current work, we found antigenic evidence of C3 in the BALF of normal mice. On a gram-per-gram basis, the amount of C3 found in BALF was significantly less than in serum, which is not the case in fluid recovered from human lungs (22). In preliminary experiments, we have been unable to demonstrate C3 deposition on *P. aeruginosa* by airway proteins recovered by BAL (data not shown) and are unable to assess other complement components in murine BAL. However, in humans, Factor B levels have been reported to be ~ 16% of serum values, raising the possibility that the classical or MBL pathways may be more active in the airway. Indirect evidence points to an important role for the MBL pathway; it has been noted that in patients with cystic fibrosis, certain MBL mutant alleles correlate to worse outcome related to infection with *P. aeruginosa* (23).

The absence of a functional complement system has been associated with a paradoxical increase in inflammation in the lung during pneumonia in previous studies (5, 6, 19). In the current work, migration of neutrophils into the airway was unaffected by the absence of complement, and lung homogenate MPO activity was double that observed in normal mice. This may be evidence of increased MPO production by over-stimulated neutrophils or the presence of neutrophils outside of the airspace. The sustained CXC chemokine production seen in complement-depleted animals could contribute to either of these scenarios. Additionally, the inflammatory response seen during bacterial infection in the current studies (Figures 4–6) may be due to unfettered proliferation of bacteria, which can generate large amounts of chemotactic peptides. That inoculation of mice with heat-killed (i.e., nonproliferating) bacteria produced far less of an effect altogether, and a much more modest differential effect between complement-intact and complement-deficient mice suggests that failure to sterilize the bronchoalveolar space is an important determinant of the exaggerated inflammatory response seen in complement-deficient animals.

In a rodent model of cecal ligation and puncture, anti-C5a antibodies have been shown to improve survival, likely by preventing over-stimulation of neutrophils required to contain the intra-abdominal infection (24). The differential effects of complement blockade in an intra-abdominal model of infection initiated by normal gut flora and our model of intrapulmonary infection with a more virulent organism highlight the complexity of the interplay between complement's immune and inflammatory roles.

Limited human experience with anti-complement agents exists, but early results in carefully selected patients have been promising. It has recently been shown that an antibody to human C5, when administered to humans before cardiopulmonary bypass, decreases the need for post-operative blood products and improves early neurocognitive function, presumably as a result of decreased complement activation by the plastic components of the bypass circuit (25). Significant risk of infection to our knowledge has not been noted thus far in clinical trials targeting complement activation. However, this may reflect the careful selection in clinical trials to date of patients unlikely to encounter infectious complications. As anti-complement strategies for acute illness become a clinical reality, we feel it will be important to fully understand the implications of complement blockade on host defense against

pathogens likely to be encountered by acutely ill patients. Further study of complement interactions with hospital-acquired pathogens, such as *P. aeruginosa*, and other Gram-negative organisms is needed.

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Abbreviations

ANOVA	analysis of variance
BAL	bronchoalveolar lavage
BALF	bronchoalveolar lavage fluid
CVF	cobra venom factor
GluNAc	N-acetyl glucosamine
HRP	horseradish peroxidase
MPO	myeloperoxidase
PBS	phosphate-buffered saline

Figure 1.

Complement interactions with microtiter plate-affixed *P. aeruginosa*. (A) Adherent murine C3 was detected using polyclonal goat anti-C3 and a HRP-based ELISA strategy (see text for details). NMS, normal mouse serum; GluNAc, N-acetyl glucosamine. Bars represent mean $\pm$ SEM; $n = 3$ per group. (B) Solid-phase C5 convertase enzymatic activity in the same system. Murine complement-opsonized bacteria were incubated with purified huC5. The supernatants of this reaction were then assayed with immunoblotting using anti-huC5a antibodies. The results indicate that normal mouse serum exposed to *P. aeruginosa* assembled C5 catalytic complexes, an effect that was heat labile. MS, heat-inactivated NMS. Blots are representative of triplicate experiments.

Figure 2.

Loss and recovery of C3 after serial injections of CVF. (A) Opsonic activity of mouse serum in normal mice and mice 0, 48, and 96 h after complement depletion with serial CVF injections. Bars represent mean $\pm$ SEM; $n = 3$ for each condition. * $P < 0.01$. (B) Immunoblot of normal murine serum, normal murine bronchoalveolar lavage fluid (NMBAL), and BALF 0, 48, and 96 h after complement depletion with serial CVF injections. Anti-murine C3 detected bands at ~ 75 and 115 kD, consistent with reduced α - and β -subunits of C3. Together, panels A and B indicate that CVF nearly abolished C3 function in the serum and C3 protein in the airway of treated mice.

Figure 3.

Lethality of complement deficiency during acute *P. aeruginosa* pneumonia. (A) Survival in C5^{-/-} and C5^{+/+} mice after intratracheal inoculation with 10⁵ CFU. (B) Same experiment in mice complement-depleted with C56. Differences in survival between complement-deficient and completed-depleted animals may be a result of recovery of C3 activity during the experimental protocol. Vehicle group received intraperitoneal saline. *P* < 0.01 for each panel as determined by proportional hazards survival modeling.

Figure 4.

Bronchoalveolar cytology 12 h after inoculation with *P. aeruginosa*. Normal and CVF-treated animals were inoculated with vehicle (*left column*) or 10⁵ CFU *P. aeruginosa* and harvested for BAL cytology 12 h later. Cell density represents one high-powered (100×) field of a cytologic preparation loaded with 200 μl of BALF and stained with xanthene/thiazine. Images are representative of triplicate measurements in each. See Table 2 for quantitative analysis.

Figure 5.

Whole-lung MPO activity 24 h post-inoculation with *P. aeruginosa*. (A) Serial MPO levels in vehicle-treated or CVF complement-depleted mice after intratracheal injection of 10⁵ live organisms. (B) Same experiment using heat-killed bacteria. *Open bars*, vehicle-treated; *solid bars*, CVF-treated. *n* = 4 animals per group per time point. * *P* < 0.05 by ANOVA.

Figure 6.

Bronchoalveolar KC concentration activity 24 h post-inoculation with *P. aeruginosa*. (A) Serial KC levels in vehicle-treated or CVF complement-depleted mice after intratracheal injection of 10⁵ live organisms. (B) Same experiment using heat-killed bacteria. *Open bars*, vehicle-treated; *solid bars*, CVF-treated. $n = 4$ animals per group per time point. * $P < 0.05$ by ANOVA. Note y axis displayed as log scale.

Figure 7.

Effect of complement deficiency on lung compliance during *P. aeruginosa* pneumonia. Curves reported as mean $\pm$ SD for each volume point; $n \geq 4$ in each group. All three curves are distinct statistically from one another as determined by repeated-measures ANOVA. Open circles, normal; solid circles, *P. aeruginosa*; multi symbols, *P. aeruginosa*, CVF-treated. See text for details of curve analysis.

Table 1
Serum bactericidal activity against P. aeruginosa strain UI-18

Condition	Viable Bacteria Remaining after 60 min Exposure to Serum	
	Human (Initial Inoculum 10^7 cfu)	Mouse (Initial Inoculum 10^3 cfu)
Normal serum	$3.0 \times 10^3 - 2.0 \times 10^4$ *	$5.0 \times 10^3 - 9.0 \times 10^3$
Heat-inactivated serum	$2.0 \times 10^8 - 1.2 \times 10^9$	$3.0 \times 10^3 - 6.0 \times 10^3$
Hyperimmune serum	—	$8.0 \times 10^2 - 8.0 \times 10^2$
Supplemented with huC5, 6, 7, 8, and 9	—	$3.0 \times 10^3 - 3.1 \times 10^3$
Supplemented with C3-depleted human serum	—	$1.4 \times 10^3 - 2.4 \times 10^3$

$n = 3$ for each cell, numbers represent range of values observed.

* $P < 0.01$ compared with other groups by ANOVA. No other comparisons were statistically significant.

Table 2
Bronchoalveolar neutrophil content, as percent of total BAL leukocytes, 12 h after intratracheal inoculation with *P. aeruginosa*

	Intratracheal Injection		
	Vehicle	<i>P. aeruginosa</i> 10 ⁵ cfu	<i>P</i> value
Normal mice	0.3 ± 0.6%	95.1 ± 1.5%	< 0.01
Complement-depleted mice	0.3 ± 0.5%	89.7 ± 7.3%	< 0.01

n = 3 for each group. *P* values represent comparison between vehicle- and bacteria-exposed mice. No statistically significant differences were seen between normal and complement-depleted mice in either the vehicle-treated or the bacterially infected condition.