Streptococcus pneumoniae and *Pseudomonas aeruginosa* pneumonia induce distinct host responses

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Objective: **Pathogens that cause pneumonia may be treated in a targeted fashion by antibiotics, but if this therapy fails, then treatment involves only nonspecific supportive measures, independent of the inciting infection. The purpose of this study was to determine whether host response is similar after disparate infections with similar mortalities.**

Design: **Prospective, randomized controlled study.**

Setting: **Animal laboratory in a university medical center.**

Interventions: **Pneumonia was induced in FVB/N mice by either *Streptococcus pneumoniae* or two different concentrations of *Pseudomonas aeruginosa.* Plasma and bronchoalveolar lavage fluid from septic animals was assayed by a microarray immunoassay measuring 18 inflammatory mediators at multiple time points.**

Measurements and Main Results: **The host response was dependent on the causative organism as well as kinetics of mortality, but the pro-inflammatory and anti-inflammatory responses were independent of inoculum concentration or degree of bacteremia. Pneumonia caused by different concentrations of the same bacteria, *Pseudomonas aeruginosa,* also yielded distinct inflammatory responses; however, inflammatory mediator expression did not directly track the severity of infection. For all infections, the host response was compartmentalized, with markedly different concentrations of inflammatory mediators in the systemic circulation and the lungs. Hierarchical clustering analysis resulted in the identification of five distinct clusters of the host response to bacterial infection. Principal components analysis correlated pulmonary macrophage inflammatory peptide-2 and interleukin-10 with progression of infection, whereas elevated plasma tumor necrosis factor sr2 and macrophage chemotactic peptide-1 were indicative of fulminant disease with >90% mortality within 48 hrs.**

Conclusions: **Septic mice have distinct local and systemic responses to *Streptococcus pneumoniae* and *Pseudomonas aeruginosa* pneumonia. Targeting specific host inflammatory responses induced by distinct bacterial infections could represent a potential therapeutic approach in the treatment of sepsis. (Crit Care Med 2010; 38:223–241)**

Key Words: sepsis; pneumonia; *Streptococcus pneumoniae*; *Pseudomonas aeruginosa*; host response; cytokine

Sepsis is treated using a combination of specific and nonspecific therapies. Antibiotic treatment directed against a specific pathogen is a targeted therapeutic approach and results in a favorable outcome in many patients. Simultaneously, generalized supportive care is often initiated. However, when antimicrobial therapy is unsuccessful, patients receive the same nonspecific therapy regardless of the inciting infection.

The majority of published sepsis trials use entry criteria that do not distinguish between differing types of infection (Gram-positive, Gram-negative) but instead rely on a 17-yr-old nonspecific definition of sepsis that includes heart rate, respiratory rate, white blood cell count, and temperature (1, 2). Implicit in the entry criteria for most sepsis trials is the assumption that host response is similar after different inciting infections. In this paradigm, a pathogen-associated molecular pattern (3) or danger-associated molecular pattern (4) is detected by a pattern recognition receptor (5). Even though different pathogen or danger-associated molecular patterns bind to and activate different Toll-like receptors, the implication is that a common host response exists, independent of the triggering organism or signaling pathway. Supporting this position is a recent study demonstrating no differences in genome-wide microarray analysis of circulating neutrophils or peripheral blood mononuclear cells between septic patients infected with Gram-positive or Gram-negative infections (6, 7), as well as earlier studies showing a common host response to sepsis (8, 9). The view that host response is independent of inciting organism has been termed the generic septic response (10). In theory, a common host response could be altered via mediator blockade, but this has not proven successful to date in the treatment of human sepsis (11).

The generic septic response theory is not universally accepted because some experimental studies have demonstrated differences in gene expression between Gram-positive and Gram-negative sepsis (12, 13). One useful way to study this is to interrogate the host either on a genome-wide RNA level or on a more targeted

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protein level. This approach has been useful in preliminary studies to discriminate septic patients from those with systemic inflammation (14), and it has also been successful in distinguishing between causative organisms in acutely infected children, suggesting a series of mediators can be used for diagnostic purposes (15). Additionally, human volunteers administered lipopolysaccharide have a reproducible host response that evolves over the course of 24 hrs as subjects go from healthy to sick to healing (16). These studies suggest that the clinical entity "sepsis" may represent a spectrum of related infection-initiated immunologic disorders that progress to multiple organ failure and death, and each of these disorders may have a characteristic host response and temporal progression.

To test the hypothesis that there are discrete responses to individual infections as opposed to a common, generic response, we assayed mice given pneumonia with either *Streptococcus pneumoniae* (*S. pneumoniae*) or *Pseudomonas aeruginosa* (*P. aeruginosa*), the most common cause of Gram-positive community-acquired pneumonia and most common cause of Gram-negative hospital-acquired pneumonia, respectively (17, 18).

MATERIALS AND METHODS

Pneumonia Models

P. aeruginosa (ATCC strain 27853) was placed in trypticase soy broth with constant shaking overnight. The resulting culture was centrifuged at 6,000*g*, washed twice with saline, and re-suspended to a density of 0.1 (low-dose), or 0.3 (high-dose) $A_{600\ nm}$. *S. pneumoniae* (strain 99.55, capsular subtype 6A) was placed on 5% blood agar plates overnight, washed, and re-suspended to an absorbance of 0.5 $A_{600\ nm}$.

Under halothane anesthesia, mice received an intratracheal injection via a midline cervical incision of one of the following: 20 μL of *P. aeruginosa* at 0.1 $A_{600\ nm}$ ($2–4 \times 10^6$ colony-forming units [CFU]), 40 μL *P. aeruginosa* at 0.3 $A_{600\ nm}$ ($2–4 \times 10^7$ CFU), or 60 μL *S. pneumoniae* at 0.5 $A_{600\ nm}$ ($2–4 \times 10^7$ CFU). Sham animals were treated identically but received an intratracheal injection of 40 μL saline. Unless otherwise indicated, all experiments were performed on FVB/N mice. After incision closure, mice received 1 mL of saline via subcutaneous injection for fluid resuscitation. All animal studies were preformed in accordance with National Institutes of Health Guidelines and approved by the Washington University Animal Studies Committee.

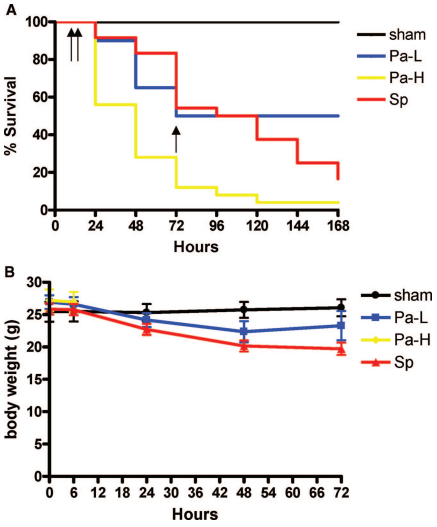

Figure 1. Mortality and body weights of mice administered different pneumonia models. *A*, Animals were followed-up for survival for 7 days after intratracheal injection of *S. pneumoniae* (*red*, n = 24), low-dose *P. aeruginosa* (*blue*, n = 20), high-dose *P. aeruginosa* (*yellow*, n = 25), or saline (*black*, n = 5). The kinetics of mortality are very similar in the first 72 hrs between *S. pneumoniae* and low-dose *P. aeruginosa*. Whereas mice administered high-dose *P. aeruginosa* have a higher death rate in the first 72 hrs than those administered *S. pneumoniae*, their eventual 7-day mortality is similar. *Arrows* indicate time points when cytokine samples were taken in subsequent experiments. Of note, samples taken at 6 or 12 hrs were drawn at a time point before there was any mortality and samples taken at 72 hrs were drawn at a time point when there is a similar 50% mortality in *S. pneumoniae* and low-dose *P. aeruginosa*. *B*, Body weights of animals (n = 7–20 mice/experimental group, 5 shams) 6, 24, 48, and 72 hrs after induction of pneumonia. There were no statistically significant differences in body weights between mice administered sham pneumonia (black) and animals administered low-dose *P. aeruginosa* (blue) or high-dose *P. aeruginosa* (yellow). Weights were lower in mice administered *S. pneumoniae* (red) 48 and 72 hrs after induction of pneumonia. Note, x-axis for survival in (*A*) is 7 days, but x-axis for body weights in (*B*) is 3 days because all subsequent experiments were performed using time points ranging from 6 to 72 hrs.

Survival Studies

Pneumonia was induced by a single investigator, and animals were followed-up for survival for 7 days. The high-dose *P. aeruginosa* model has been extensively used in our laboratory (19, 20), whereas the *S. pneumoniae* and the low-dose *P. aeruginosa* models were developed for this article. Because of ethical concerns of performing surgery on animals to regenerate a survival curve in a model that has been reproducible by the authors, the portion of the survival curve for the high-dose *P. aeruginosa* in Figure 1 comes from a previous publication from our laboratory (21; reprinted with permission from *JAMA*).

Studies examining the influence of tumor necrosis factor (TNF)-α were performed on animals administered 300 μg of anti-TNF-α antibody TN3 19.12 (a generous gift from Robert Schreiber, Washington University; 22) 3 hrs before induction of pneumonia. To study the effects of macrophage chemotactic peptide (MCP)-1 on survival, additional experiments were required to generate survival curves in C57Bl/6 mice that had similar kinetics and 7-day mortality as FVB/N mice shown in Figure 1. Doses used were 20 μL of *P. aeruginosa* at 0.2 $A_{600\ nm}$, 30 μL *P. aeruginosa* at 0.3 $A_{600\ nm}$, or 20 μL *S. pneumoniae* at 0.1 $A_{600\ nm}$. Once these experiments were completed, survival studies on MCP-$1^{-/-}$ mice (Jackson Laboratories, Bar Harbor, ME) were performed.

Cytokines

Mice subjected to the three pneumonia models and sham pneumonia were killed at 6, 12, or 72 hrs. To collect bronchoalveolar lavage (BAL) samples, the trachea was cannulated with a 22-gauge angiocatheter, and lungs were lavaged with 1 mL of phosphate-buffered saline. BAL and blood samples from each mouse were centrifuged for 5 mins at 6,000*g*. The supernatants were removed and analyzed for soluble inflammatory mediator concentration using a microarray immunoassay measuring interleukin (IL)-1b, macrophage inflammatory peptide (MIP)-2, MCP-1, Eotaxin, IL-18, interferon (IFN)-γ, MIP-α, TNF-α, IL-6, IL-1ra, IL-10, TNFsr-I, TNFsr-II, IL-2, IL-5, IL-12, IL-13, and RANTES (23). There were no sham values of IL-18 in either blood or BAL. There were no sham values of IL-1ra in either blood or BAL. There were no sham values of Eotaxin in BAl (but there were sham values of Eotaxin in blood).

Cultures

BAL and blood samples were taken from mice once killed and then diluted serially in saline and plated on blood agar plates. After incubation at 37°C, plates were examined after 24 and 48 hrs for colony counts. Log-transformation of calculated colony counts was then used for further analysis (24).

Pattern Analysis

Cytokine abundance data were analyzed after importation into SpotFire Decision Site 8.2.1 (Tibco Spotfire, Palo Alto, CA). Six individual mice were censored from analysis because of technical issues resulting in missing values of either all serum or BAL cytokines. These included three mice administered *S.* pneumoniae at 6 hrs, one mouse administered low-dose *P. aeruginosa* at 6 hrs, and two mice administered high-dose *P. aeruginosa* at 6 hrs.

No individual data points were excluded. Values that were below or above the detection limits of the assay were replaced with the lower or upper detection limits to allow for numerical analysis. Hierarchical clustering was performed on BAL and blood cytokine measurements individually and also together using correlation as the similarity measure. Principal component analysis was performed on cytokine abundance data from three data sets: BAL, blood, and combined data. Principal components were calculated using all mice analyzed for hierarchical clustering and averages and SE were calculated using either the treatment group identifier or the hierarchical clustering group assignment. Pearson's correlation between individual cytokines and each principal component were calculated to determine whether any of the measured quantities could serve as a surrogate for the principal component.

Blood Counts

White blood counts were performed using a Coulter counter (Baker 9000) using 50 μL of whole blood. Differential cell count was performed by counting 100 leukocytes on a smear with Wright's stain.

Myeloperoxidase Assay

Twelve hours after induction of pneumonia, the pulmonary vasculature was perfused with 1 mL phosphate-buffered saline, and lungs were frozen in liquid nitrogen (25). Right lower lobe sections were subsequently thawed, weighed, and homogenized in 4 mL of 20 mM potassium phosphate buffer with 0.5 g/dL hexadecyltrimethyl ammonium bromide. After sonication for 90 secs, sections were incubated for 2 hrs in a 60°C water bath. Samples were then centrifuged and 100 μL of supernatant placed into 2.9 mL of 50 mM potassium phosphate buffer (pH 6.0) with 0.167 mg/mL O-dianisidine and 0.0005% hydrogen peroxide. Absorbance at 460 nm was measured for 3 mins. Myeloperoxidase activity per gram of protein was calculated using the rate of change in absorbance over 3 mins and the protein content of the sample was determined by a modified Bradford assay (26).

Statistics

Data analysis was performed using Prizm version 4.0 (GraphPad) and SAS version 9.1. Data are presented as mean ± SEM. Survival curves were compared using chi-square analysis. Cytokines, quantitative cultures, blood counts, and myeloperoxidase activity were first analyzed using Kruskal-Wallis one-way analysis of variance by ranks. *Post hoc* pair-wise comparisons were conducted using the Mann-Whitney *U* test. Cytokine data at each time point was compared for all possible groups (i.e., sham vs. high-dose *P. aeruginosa*, sham vs. low-dose *P. aeruginosa,* sham vs. *S. pneumoniae,* high-dose *P. aeruginosa* vs. *S. pneumoniae,* low-dose *P. aeruginosa* vs. *S. pneumoniae,* high-dose *P. aeruginosa*, and low-dose *P. aeruginosa*). Individual cytokine levels were not compared between different models at different time points. A $p < .05$ was accepted as statistically significant.

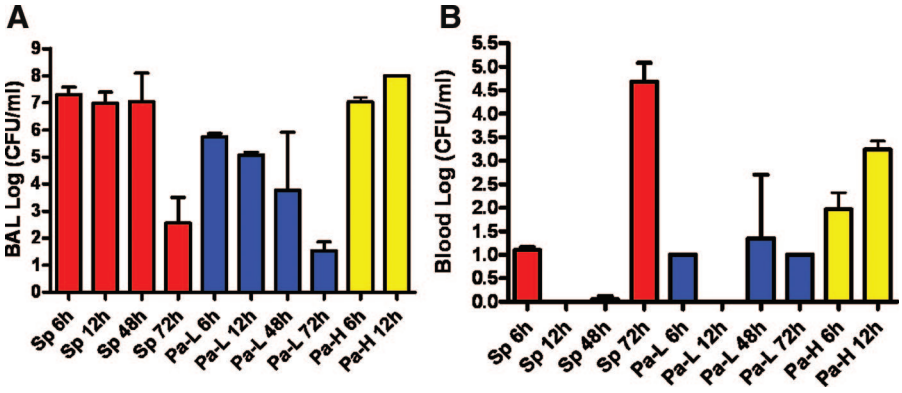

Figure 2. Quantitative bronchoalveolar lavage (*BAL*) and blood cultures. *A*, Bacterial counts are similar at early time points in BAL fluid from animals administered *S. pneumoniae* or high-dose *P. aeruginosa*, whereas counts are lower after injection of low-dose *P. aeruginosa*. Bacterial concentration decline markedly in BAL fluid between 48 and 72 hrs, consistent with animals clearing pulmonary infection. Data has been log-transformed for presentation to allow graphical representation of 100,000-fold decrease in lung bacterial burden between these time points. *B*, Animals administered *S. pneumoniae* or low-dose *P. aeruginosa* have similar low degrees of bacteremia at early time points. There is a marked increase in bacteremia in animals administered *S. pneumoniae* between 48 and 72 hrs without any change in systemic bacterial concentrations in those administered low-dose *P. aeruginosa*. Note, the differences in scale on y-axis between (*A*) and (*B*), with substantially higher bacterial concentrations in the lungs than the blood at all points measured except in *S. pneumoniae* at 72 hrs.

RESULTS

Survival

Animals were administered one of two doses of *P. aeruginosa* that caused either 96% or 50% 7-day mortality, or a dose of *S. pneumoniae* that resulted in a 84% 7-day mortality (Fig. 1*A*). Animals died faster after high-dose *P. aeruginosa* than *S. pneumoniae*. Mortality after low-dose *P. aeruginosa* and *S. pneumoniae* was similar in the first 3 days ($p = .59$). There were no statistically significant differences in body weights in mice subjected to any model of pneumonia at either 6 or 24 hrs (Fig. 1*B*). Mice administered *S. pneumoniae* pneumonia had lower body weights than animals administered sham pneumonia 48 and 72 hrs after intratracheal injection of bacteria ($p = .02$ and .007, respectively), although no statistically significant differences were seen between animals administered low-dose *P. aeruginosa* and sham animals at any time point.

BAL and blood cultures were taken from animals at 6, 12, 48, or 72 hrs after infection. Mice administered *S. pneumoniae* had approximately 10^7 CFU/mL in their airways 48 hrs after onset of pneumonia, declining precipitously between days 2 and 3 (Fig. 2*A*). Mice administered high-dose *P. aeruginosa* had similar bacterial loads in their lungs at 6 hrs and a ten-fold increase at 12 hrs ($p < .05$ compared to *S. pneumoniae*). Cultures were not measured at later time points in this model because of high levels of mortality at 48 hrs. Animals administered low-dose *P. aeruginosa* had ten-fold fewer bacteria 6 hrs after the onset of pneumonia (10^6 CFU/mL, $p < .05$ compared to the other infections at the same time points). Similar to animals administered *S. pneumoniae*, mice infected with low-dose *P. aeruginosa* had a substantial decline in pulmonary bacterial load between 48 and 72 hrs.

In contrast to local microbial concentrations, animals administered *S. pneumoniae* or low-dose *P. aeruginosa* had only trace amounts of bacteria detectable in their blood 6, 12, or 48 hrs after the onset of pneumonia (Fig. 2*B*). After 72 hrs, however, mice infected with *S. pneumoniae* had significant bacteremia ($>10^4$ CFU/mL blood), whereas animals infected with low-dose *P. aeruginosa* had essentially no blood-borne bacteria. Animals administered high-dose *P. aeruginosa* had similar low levels at 6 hrs, with a ten-fold increase in bacterial load 12 hrs after onset of pneumonia ($p < 0.05$ compared to both other infections at same time point).

Table 1. Bronchoalveolar lavage

Cytokine	Time, hrs	Sham, pg/mL	Sp, pg/mL	Pa-L, pg/mL	Pa-H, pg/mL
IL-1β	6	15.1 ± 2.3 (n = 4) $p = .02$ vs. Sp $p = .006$ vs. Pa-L $p = .004$ vs. Pa-H	12.1 ± 0.1 (n = 9) $p = .0002$ vs. Pa-L $p < .0001$ vs. Pa-H	1809.9 ± 392.2 (n = 7) $p = .0003$ vs. Pa-H	24,524.2 ± 2862.5 (n = 8)
	12	n/a	575.5 ± 227.8 (n = 11) $p = .77$ vs. Pa-L $p = .0003$ vs. Pa-H	24.9 ± 0.9 (n = 8) $p = .0002$ vs. Pa-H	52,405.3 ± 9531.6 (n = 8)
	72	116.1 ± 101.7 (n = 4) $p = .006$ vs. Sp $p = .29$ vs. Pa-L	1807.0 ± 551.9 (n = 7) $p = .002$ vs. Pa-L	28.4 ± 4.4 (n = 5)	n/a
MIP-2	6	145.5 ± 55.0 (n = 4) $p = .002$ vs. Sp $p = .006$ vs. Pa-L $p = .004$ vs. Pa-H	5092.6 ± 610.1 (n = 10) $p = .0002$ vs. Pa-L $p < .0001$ vs. Pa-H	38,807.0 ± 9162.6 (n = 7) $p = .15$ vs. Pa-H	18,988.1 ± 2301.2 (n = 8)
	12	n/a	3120.1 ± 1024.4 (n = 11) $p = .88$ vs. Pa-L $p = .0003$ vs. Pa-H	5573.8 ± 2806.5 (n = 9) $p = .002$ vs. Pa-H	28,003.7 ± 4115.1 (n = 8)
	72	127.0 ± 29.4 (n = 4) $p = .006$ vs. Sp $p = .11$ vs. Pa-L	304,097.6 ± 35,058.0 (n = 7) $p = .002$ vs. Pa-L	24.2 ± 0.2 (n = 5)	n/a
MCP-1	6	7.4 ± 7.4 (n = 4) $p = .05$ vs. Sp $p = .006$ vs. Pa-L $p = .004$ vs. Pa-H	54.4 ± 25.4 (n = 10) $p = .0002$ vs. Pa-L $p < .0001$ vs. Pa-H	322.1 ± 26.4 (n = 7) $p = .0003$ vs. Pa-H	1524.6 ± 277.6 (n = 8)
	12	n/a	218.1 ± 64.8 (n = 11) $p = .49$ vs. Pa-L $p = .0003$ vs. Pa-H	223.1 ± 27.8 (n = 9) $p = .0003$ vs. Pa-H	1448.1 ± 131.2 (n = 8)
	72	0.0 ± 0.0 (n = 4) p = n/a vs. Sp p = n/a vs. Pa-L	1479.6 ± 470.6 (n = 6) $p = .009$ vs. Pa-L	264.3 ± 51.1 (n = 6)	n/a
Eotaxin	6	n/a	12.1 ± 0.1 (n = 9) $p = .0002$ vs. Pa-L $p = .48$ vs. Pa-H	624.8 ± 124.1 (n = 7) $p = .0003$ vs. Pa-H	11.9 ± 0.1 (n = 8)
	12	n/a	11.9 ± 0.1 (n = 11) $p = .0002$ vs. Pa-L $p = .0003$ vs. Pa-H	1781.3 ± 289.6 (n = 9) $p = .02$ vs. Pa-H	915.6 ± 296.7 (n = 8)
	72	n/a	394.6 ± 81.7 (n = 7) $p = .07$ vs. Pa-L	113.5 ± 57.3 (n = 6)	n/a
IL-18	6	n/a	11.9 ± 0.1 (n = 10) $p = .0001$ vs. Pa-L $p < .0001$ vs. Pa-H	3483.7 ± 110.2 (n = 7) $p = .0003$ vs. Pa-H	1715.0 ± 226.0 (n = 8)
	12	n/a	12.1 ± 0.1 (n = 11) $p < .0001$ vs. Pa-L $p < .0001$ vs. Pa-H	3180.1 ± 145.3 (n = 9) $p = .04$ vs. Pa-H	5674.5 ± 1029.0 (n = 8)
	72	n/a	729.2 ± 340.6 (n = 7) $p = .23$ vs. Pa-L	876.5 ± 539.2 (n = 6)	n/a

Table 1.—*Continued*

Cytokine	Time, hrs	Sham, pg/mL	Sp, pg/mL	Pa-L, pg/mL	Pa-H, pg/mL
IFN-γ	6	7.6 ± 4.4 (n = 4) $p = 1.00$ vs. Sp $p = .06$ vs. Pa-L $p = 1.00$ vs. Pa-H	11.9 ± 0.1 (n = 10) $p = .0001$ vs. Pa-L $p = .89$ vs. Pa-H	874.2 ± 158.7 (n = 7) $p = .0006$ vs. Pa-H	11.9 ± 0.1 (n = 7)
	12	n/a	11.9 ± 0.1 (n = 11) $p = .0002$ vs. Pa-L $p = .93$ vs. Pa-H	548.4 ± 93.28 (n = 9) $p < .0001$ vs. Pa-H	11.9 ± 0.1 (n = 8)
	72	15.6 ± 8.5 (n = 4) $p = .006$ vs. Sp $p = .01$ vs. Pa-L	703.6 ± 115.5 (n = 7) $p = .53$ vs. Pa-L	642. ± 108.0 (n = 6)	n/a
MIP-1α	6	426.3 ± 15.3 (n = 4) $p = .04$ vs. Sp $p = .06$ vs. Pa-L $p = .04$ vs. Pa-H	38,314.5 ± 4160.6 (n = 9) $p = .0002$ vs. Pa-L $p = .11$ vs. Pa-H	11,007.7 ± 1428.5 (n = 7) $p = .0003$ vs. Pa-H	48,162.1 ± 1247.1 (n = 8)
	12	n/a	27,057.2 ± 5863.7 (n = 11) $p = .048$ vs. Pa-L $p = .08$ vs. Pa-H	6071.8 ± 1435.3 (n = 9) $p < .0001$ vs. Pa-H	42,358.0 ± 3306.3 (n = 8)
	72	889.2 ± 235.2 (n = 4) $p = .06$ vs. Sp $p = .07$ vs. Pa-L	4069.7 ± 628.4 (n = 7) $p = .001$ vs. Pa-L	39.8 ± 15.8 (n = 6)	n/a
TNF-α	6	42.5 ± 3.6 (n = 4) $p = .003$ vs. Sp $p = .006$ vs. Pa-L $p = .004$ vs. Pa-H	17,225.9 ± 4665.5 (n = 9) $p = .01$ vs. Pa-L $p = .89$ vs. Pa-H	4519.0 ± 1365.1 (n = 7) $p = .001$ vs. Pa-H	13,156.4 ± 1549.7 (n = 8)
	12	n/a	4792.2 ± 785.0 (n = 11) $p = .32$ vs. Pa-L $p = .06$ vs. Pa-H	3617.3 ± 740.0 (n = 9) $p = .01$ vs. Pa-H	7986.4 ± 1374.7 (n = 8)
	72	62.2 ± 9.8 (n = 4) $p = .006$ vs. Sp $p = .07$ vs. Pa-L	1790.1 ± 114.6 (n = 7) $p = .001$ vs. Pa-L	36.2 ± 7.2 (n = 6)	n/a
IL-6	6	290.8 ± 182.1 (n = 4) $p = .002$ vs. Sp $p = .006$ vs. Pa-L $p = .004$ vs. Pa-H	2562.7 ± 475.2 (n = 10) $p = .0004$ vs. Pa-L $p < .0001$ vs. Pa-H	7486.8 ± 914.2 (n = 7) $p = .002$ vs. Pa-H	12,694.1 ± 909.8 (n = 8)
	12	n/a	3763.8 ± 852.2 (n = 11) $p = .03$ vs. Pa-L $p = .0003$ vs. Pa-H	8129.9 ± 1282.1 (n = 9) $p < .0001$ vs. Pa-H	43,991.7 ± 4996.4 (n = 8)
	72	29.5 ± 21.6 (n = 4) $p = .006$ vs. Sp $p = .29$ vs. Pa-L	2965.5 ± 558.8 (n = 7) $p = .002$ vs. Pa-L	24.2 ± 0.2 (n = 5)	n/a
IL-1ra	6	n/a	5212.0 ± 1015.8 (n = 9) $p = .005$ vs. Pa-L $p = .14$ vs. Pa-H	12,244.7 ± 2092.0 (n = 7) $p = .04$ vs. Pa-H	7340.5 ± 1037.7 (n = 8)
	12	n/a	33,621.2 ± 5382.4 (n = 11) $p = .45$ vs. Pa-L $p = .39$ vs. Pa-H	39,829.1 ± 5655.4 (n = 9) $p = .07$ vs. Pa-H	25,691.0 ± 4642.4 (n = 8)
	72	n/a	44,033.0 ± 9500.9 (n = 7) $p = .008$ vs. Pa-L	13,157.5 ± 3331.5 (n = 6)	n/a

Table 1.—*Continued*

Cytokine	Time, hrs	Sham, pg/mL	Sp, pg/mL	Pa-L, pg/mL	Pa-H, pg/mL
IL-10	6	1.8 ± 0.0 (n = 4) p = n/a vs. Sp p = n/a vs. Pa-L p = n/a vs. Pa-H	11.9 ± 0.1 (n = 10) p = .0001 vs. Pa-L p = .96 vs. Pa-H	23.9 ± 0.1 (n = 7) p = .0003 vs. Pa-H	11.9 ± 0.1 (n = 8)
	12	n/a	11.9 ± 0.1 (n = 11) p = .0002 vs. Pa-L p = .0003 vs. Pa-H	23.9 ± 0.1 (n = 9) p < .0001 vs. Pa-H	988.2 ± 343.0 (n = 8)
	72	40.0 ± 35.4 (n = 4) p = .006 vs. Sp p = .26 vs. Pa-L	11311.5 ± 3182.5 (n = 7) p = .001 vs. Pa-L	23.8 ± 0.2 (n = 6)	n/a
TNFsrI	6	335.9 ± 60.9 (n = 4) p = .003 vs. Sp p = .65 vs. Pa-L p = .68 vs. Pa-H	123.7 ± 15.4 (n = 9) p = .001 vs. Pa-L p < .0001 vs. Pa-H	281.3 ± 30.1 (n = 7) p = 0.28 vs. Pa-H	337.6 ± 26.9 (n = 8)
	12	n/a	172.8 ± 21.5 (n = 11) p = .01 vs. Pa-L p = .0003 vs. Pa-H	344.6 ± 54.5 (n = 9) p = .0006 vs. Pa-H	994.4 ± 241.6 (n = 8)
	72	279.8 ± 57.3 (n = 4) p = .01 vs. Sp p = .48 vs. Pa-L	470.5 ± 19.2 (n = 7) p = .001 vs. Pa-L	200.8 ± 23.3 (n = 6)	n/a
TNFsrII	6	1930.8 ± 300.5 (n = 4) p = .01 vs. Sp p = .006 vs. Pa-L p = 1.00 vs. Pa-H	1184.8 ± 183.8 (n = 10) p = .23 vs. Pa-L p = .003 vs. Pa-H	879.8 ± 12.7 (n = 7) p = .0003 vs. Pa-H	1735.7 ± 66.5 (n = 8)
	12	n/a	1762.8 ± 182.8 (n = 11) p = .004 vs. Pa-L p = .006 vs. Pa-H	1001.6 ± 30.4 (n = 8) p < .0001 vs. Pa-H	2449.6 ± 142.4 (n = 8)
	72	2228.5 ± 564.1 (n = 4) p = .006 vs. Sp p = .11 vs. Pa-L	463.8 ± 11.5 (n = 7) p = .001 vs. Pa-L	1229.9 ± 166.8 (n = 6)	n/a
IL-2	6	13.8 ± 0.0 (n = 4) p = n/a vs. Sp p = n/a vs. Pa-L p = n/a vs. Pa-H	12.0 ± 0.0 (n = 10) p = n/a vs. Pa-L p = n/a vs. Pa-H	24.0 ± 0.0 (n = 7) p = n/a vs. Pa-H	12.0 ± 0.0 (n = 8)
	12	n/a	12.0 ± 0.0 (n = 11) p = n/a vs. Pa-L p = vs. Pa-H	24.0 ± 0.0 (n = 9) p = n/a vs. Pa-H	12.0 ± 0.0 (n = 8)
	72	13.8 ± 0.0 (n = 4) p = n/a vs. Sp p = n/a vs. Pa-L	227.8 ± 139.3 (n = 7) p = n/a vs. Pa-L	24.0 ± 0.0 (n = 6) p = n/a vs. Pa-H	n/a
IL-5	6	14.4 ± 4.1 (n = 4) p = .64 vs. Sp p = n/a vs. Pa-L p = .02 vs. Pa-H	33.0 ± 15.8 (n = 10) p = n/a vs. Pa-L p = .02 vs. Pa-H	12.0 ± 0.0 (n = 7) p = n/a vs. Pa-H	99.8 ± 15.5 (n = 8)
	12	n/a	35.8 ± 11.6 (n = 11) p = n/a vs. Pa-L p = .39 vs. Pa-H	12.0 ± 0.0 (n = 9) p = n/a vs. Pa-H	18.8 ± 6.8 (n = 8)
	72	23.1 ± 16.2 (n = 4) p = n/a vs. Sp p = n/a vs. Pa-L	12.0 ± 0.0 (n = 7) p = n/a vs. Pa-L	12.0 ± 0.0 (n = 6)	n/a

Table 1.—*Continued*

Cytokine	Time, hrs	Sham, pg/mL	Sp, pg/mL	Pa-L, pg/mL	Pa-H, pg/mL
IL-12	6	4.0 ± 0.0 (n = 4) p = n/a vs. Sp p = n/a vs. Pa-L p = n/a vs. Pa-H	12.0 ± 0.0 (n = 10) p = n/a vs. Pa-L p = n/a vs. Pa-H	219.4 ± 126.7 (n = 7) p = n/a vs. Pa-H	12.0 ± 0.0 (n = 8)
	12	n/a	16.5 ± 4.5 (n = 11) p = n/a vs. Pa-L p = n/a vs. Pa-H	24.0 ± 0.0 (n = 9) p = n/a vs. Pa-H	12.0 ± 0.0 (n = 8)
	72	32.5 ± 16.6 (n = 4) p = 1.00 vs. Sp p = .76 vs. Pa-L	12.4 ± 0.4 (n = 7) p = .001 vs. Pa-L	54.4 ± 30.4 (n = 6)	n/a
IL-13	6	9.1 ± 0.0 (n = 4) p = n/a vs. Sp p = n/a vs. Pa-L p = n/a vs. Pa-H	461.3 ± 176.4 (n = 10) p = 1.00 vs. Pa-L p = .83 vs. Pa-H	6200.3 ± 5336.3 (n = 7) p = .46 vs. Pa-H	401.0 ± 97.1 (n = 8)
	12	n/a	393.3 ± 164.4 (n = 11) p = .76 vs. Pa-L p = .15 vs. Pa-H	24.1 ± 0.1 (n = 9) p = .008 vs. Pa-H	98.2 ± 86.2 (n = 8)
	72	107.8 ± 24.7 (n = 4) p = .22 vs. Sp p = .29 vs. Pa-L	1605.3 ± 502.2 (n = 7) p = .07 vs. Pa-L	133.8 ± 109.8 (n = 6)	n/a
RANTES	6	427.9 ± 0.0 (n = 4) p = n/a vs. Sp p = n/a vs. Pa-L p = n/a vs. Pa-H	35.0 ± 21.7 (n = 9) p = .0007 vs. Pa-L p = .004 vs. Pa-H	503.3 ± 206.4 (n = 7) p = .004 vs. Pa-H	119.4 ± 26.2 (n = 8)
	12	n/a	20.4 ± 5.0 (n = 11) p = .0002 vs. Pa-L p = .0003 vs. Pa-H	581.1 ± 178.4 (n = 9) p = .09 vs. Pa-H	277.8 ± 69.3 (n = 8)
	72	470.9 ± 119.3 (n = 4) p = .23 vs. Sp p = .01 vs. Pa-L	669.2 ± 118.2 (n = 7) p = .001 vs. Pa-L	27.4 ± 3.4 (n = 6)	n/a

IL, interleukin; MIP, macrophage inflammatory peptide; MCP, macrophage chemotactic peptide; IFN, interferon; TNF, tumor necrosis factor; RANTES, regulated on activation, normal T-cell expressed and probably secreted.

Cytokine Analysis

BAL and blood samples were taken from animals at 6, 12, and 72 hrs after onset of pneumonia to measure the local and systemic host response, respectively. At 6 and 12 hrs after infection, all animals in all groups were alive, regardless of the ultimate mortality of the pneumonia model used. Animals that received high-dose *P. aeruginosa* did not undergo sampling at 72 hrs because of >80% mortality by this time point. Cytokine levels for all models of pneumonia as well as animals that underwent sham operation are listed in Table 1 (BAL) and Table 2 (blood).

To determine whether there were different cytokine levels between pneumonia models, the experimental design allowed for three distinct comparisons of the host response to infection: (1) low-dose *P. aeruginosa* and *S. pneumoniae*—animals with similar kinetics of mortality over the 3 days when samples were obtained; (2) high-dose *P. aeruginosa* and *S. pneumonia*—animals that would eventually have 96% and 84% 7-day mortality, respectively; and (3) high-dose *P. aeruginosa* and low-dose *P. aeruginosa*—animals receiving the identical pathogen but at doses that cause differing mortalities.

Pathogens Causing Similar Kinetics of Mortality Have Distinct Local and Systemic Cytokine Profiles

Local cytokine production is higher in animals administered low-dose *P. aeruginosa* in BAL fluid 6 hrs after the onset of pneumonia compared to animals administered *S. pneumoniae* (Fig. 3*A*; pro-inflammatory cytokines at top of figure, anti-inflammatory at bottom). This is not a result of LPS in Gram-negative bacteria causing a greater increase in TNF-α, because this was one of only two cytokines that was higher in mice infected with *S. pneumoniae* at 6 hrs. There is a marked temporal shift in the local response to the two infections such that by 72 hrs, the majority of cytokines are higher in mice subjected to *S. pneumoniae* pneumonia. Importantly, the inflammatory response is not correlated to bacterial colony counts in the lung. At 6 and 12 hrs, there are more *S. pneumoniae* bacteria in the lungs than *P. aeruginosa* but higher pro-inflammatory and anti-inflammatory cytokine concentrations are seen with the latter organism (compare Fig. 2*A* to 3*A*). Additionally, the late shift toward relative higher cytokine abundance in animals in-

Table 2. Blood

Cytokine	Time, hr	Sham, pg/mL	Sp, pg/mL	Pa-L, pg/mL	Pa-H, pg/mL
IL-1β	6	106.2 ± 25.1 (n = 4) p = .57 vs. Sp p = .004 vs. Pa-L p = .48 vs. Pa-H	438.5 ± 266.2 (n = 8) p = .80 vs. Pa-L p = .34 vs. Pa-H	21.00 ± 3.000 (n = 8) p = .23 vs. Pa-H	1108.8 ± 422.1 (n = 6)
	12	n/a	890.2 ± 424.2 (n = 11) p = .90 vs. Pa-L p = .64 vs. Pa-H	21.00 ± 3.000 (n = 8) p = .72 vs. Pa-H	4408.7 ± 2636.0 (n = 8)
	72	115.5 ± 19.3 (n = 4) p = .006 vs. Sp p = n/a vs. Pa-L	10.3 ± 1.7 (n = 7) p = n/a vs. Pa-L	24.0 ± 0.0 (n = 6)	n/a
MIP-2	6	183.1 ± 49.0 (n = 4) p = .21 vs. Sp p = 1.00 vs. Pa-L p = .01 vs. Pa-H	542.3 ± 134.2 (n = 8) p = .72 vs. Pa-L p = .0007 vs. Pa-H	599.9 ± 306.6 (n = 8) p = .0007 vs. Pa-H	4785 ± 962.5 (n = 6)
	12	n/a	800.8 ± 179.7 (n = 11) p = .04 vs. Pa-L p = .0005 vs. Pa-H	1897 ± 435.9 (n = 8) p = .01 vs. Pa-H	13,469 ± 5852 (n = 8)
	72	194.1 ± 44.4 (n = 4) p = .65 vs. Sp p = .01 vs. Pa-L	2931 ± 1840 (n = 7) p = .23 vs. Pa-L	24.17 ± 0.17 (n = 6)	n/a
MCP-1	6	38.9 ± 10.6 (n = 4) p = .21 vs. Sp p = .004 vs. Pa-L p = .02 vs. Pa-H	104.2 ± 23.7 (n = 8) p = .003 vs. Pa-L p = .002 vs. Pa-H	237.9 ± 23.1 (n = 8) p = .002 vs. Pa-H	50,000.0 ± 0.0 (n = 5)
	12	n/a	58.1 ± 13.4 (n = 11) p = .20 vs. Pa-L p = .0003 vs. Pa-H	87.2 ± 9.4 (n = 8) p = .002 vs. Pa-H	50,000.0 ± 0.0 (n = 8)
	72	0.0 ± 0.0 (n = 4) p = n/a vs. Sp p = n/a vs. Pa-L	78.3 ± 26.3 (n = 7) p = .94 vs. Pa-L	70.9 ± 13.0 (n = 6)	n/a
Eotaxin	6	7856.2 ± 3276.7 (n = 4) p = .004 vs. Sp p = .008 vs. Pa-L p = .01 vs. Pa-H	525.6 ± 284.7 (n = 8) p = .003 vs. Pa-L p = .01 vs. Pa-H	2218.9 ± 348.6 (n = 8) p = .85 vs. Pa-H	2013.8 ± 184.5 (n = 6)
	12	n/a	292.6 ± 138.3 (n = 11) p = .05 vs. Pa-L p = .0005 vs. Pa-H	1111.5 ± 486.1 (n = 8) p = .007 vs. Pa-H	3915.1 ± 873.6 (n = 8)
	72	3751.8 ± 1058.6 (n = 4) p = .01 vs. Sp p = .07 vs. Pa-L	805.8 ± 249.5 (n = 7) p = .84 vs. Pa-L	1262.7 ± 579.5 (n = 6)	n/a
IL-18	6	n/a	11.9 ± 0.1 (n = 8) p = .0002 vs. Pa-L p = .41 vs. Pa-H	23.9 ± 0.1 (n = 8) p = .04 vs. Pa-H	72.9 ± 60.9 (n = 6)
	12	n/a	420.9 ± 276.4 (n = 11) p = .006 vs. Pa-L p = .06 vs. Pa-H	1090.6 ± 521.1 (n = 8) p = .72 vs. Pa-H	1820.3 ± 773.5 (n = 8)
	72	n/a	12.1 ± 0.1 (n = 7) p = .001 vs. Pa-L	23.8 ± 0.2 (n = 6)	n/a

Table 2.—*Continued*

Cytokine	Time, hr	Sham, pg/mL	Sp, pg/mL	Pa-L, pg/mL	Pa-H, pg/mL
IFN-γ	6	108.8 ± 22.9 (n = 4) $p = .57$ vs. Sp $p = .048$ vs. Pa-L $p = .76$ vs. Pa-H	161.0 ± 80.4 (n = 8) $p = .002$ vs. Pa-L $p = .34$ vs. Pa-H	838.2 ± 142.8 (n = 8) $p = .02$ vs. Pa-H	349.2 ± 147.5 (n = 6)
	12	n/a	146.1 ± 63.4 (n = 10) $p < .0001$ vs. Pa-L $p = .96$ vs. Pa-H	948.3 ± 137.6 (n = 8) $p = .01$ vs. Pa-H	246.9 ± 134.2 (n = 8)
	72	77.5 ± 13.6 (n = 4) $p = .16$ vs. Sp $p = .01$ vs. Pa-L	214.5 ± 64.5 (n = 7) $p = .04$ vs. Pa-L	508.0 ± 84.2 (n = 6)	n/a
MIP-1α	6	1274.4 ± 361.1 (n = 4) $p = .004$ vs. Sp $p = .004$ vs. Pa-L $p = .76$ vs. Pa-H	12.1 ± 0.1 (n = 8) $p = .0002$ vs. Pa-L $p = .06$ vs. Pa-H	6409.7 ± 1104.9 (n = 8) $p = .005$ vs. Pa-H	1659.4 ± 579.1 (n = 6)
	12	n/a	930.3 ± 501.9 (n = 11) $p = .05$ vs. Pa-L $p = .21$ vs. Pa-H	1561.9 ± 427.4 (n = 8) $p = 1.00$ vs. Pa-H	2704.5 ± 1124.2 (n = 8)
	72	1136.2 ± 195.6 (n = 4) $p = .41$ vs. Sp $p = .11$ vs. Pa-L	841.8 ± 335.4 (n = 7) $p = .53$ vs. Pa-L	340.3 ± 202.9 (n = 6)	n/a
TNF-α	6	40.4 ± 8.3 (n = 4) $p = .28$ vs. Sp $p = .46$ vs. Pa-L $p = .01$ vs. Pa-H	24.2 ± 5.6 (n = 8) $p = .01$ vs. Pa-L $p = .0007$ vs. Pa-H	65.2 ± 11.4 (n = 8) $p = .0007$ vs. Pa-H	203.9 ± 35.4 (n = 6)
	12	n/a	126.5 ± 67.6 (n = 11) $p = .39$ vs. Pa-L $p = .02$ vs. Pa-H	42.2 ± 6.8 (n = 8) $p = .007$ vs. Pa-H	278.7 ± 130.8 (n = 8)
	72	32.1 ± 0.0 (n = 4) p = n/a vs. Sp p = n/a vs. Pa-L	61.3 ± 26.6 (n = 7) $p = .73$ vs. Pa-L	26.9 ± 2.9 (n = 6)	n/a
IL-6	6	231.3 ± 16.1 (n = 4) $p = .004$ vs. Sp $p = .004$ vs. Pa-L $p = .01$ vs. Pa-H	916.1 ± 204.6 (n = 8) $p = .007$ vs. Pa-L $p = .001$ vs. Pa-H	2570.6 ± 568.1 (n = 8) $p = .28$ vs. Pa-H	2771.8 ± 318.5 (n = 6)
	12	n/a	319.0 ± 95.4 (n = 11) $p = .39$ vs. Pa-L $p = .0003$ vs. Pa-H	487.9 ± 173.2 (n = 8) $p = .0002$ vs. Pa-H	14,851.3 ± 5323.2 (n = 8)
	72	36.1 ± 3.1 (n = 4) $p = .79$ vs. Sp $p = .01$ vs. Pa-L	109.1 ± 58.3 (n = 7) $p = .73$ vs. Pa-L	24.2 ± 0.2 (n = 6)	n/a
IL-1ra	6	n/a	361.4 ± 110.4 (n = 7) $p = .0003$ vs. Pa-L $p = .14$ vs. Pa-H	13,519.0 ± 4691.1 (n = 8) $p = .003$ vs. Pa-H	1061.5 ± 359.6 (n = 6)
	12	n/a	851.5 ± 465.1 (n = 11) $p = .01$ vs. Pa-L $p = .0006$ vs. Pa-H	4460.5 ± 1394.9 (n = 8) $p = .08$ vs. Pa-H	20624.3 ± 9818.5 (n = 8)
	72	n/a	12,423.0 ± 5756.0 (n = 7) $p = .07$ vs. Pa-L	672.8 ± 349.2 (n = 6)	n/a

Table 2.—*Continued*

Cytokine	Time, hr	Sham, pg/mL	Sp, pg/mL	Pa-L, pg/mL	Pa-H, pg/mL
IL-10	6	309.5 ± 80.8 (n = 4) p = .006 vs. Sp p = .004 vs. Pa-L p = .76 vs. Pa-H	19.2 ± 7.2 (n = 8) p = .02 vs. Pa-L p = .01 vs. Pa-H	23.9 ± 0.1 (n = 8) p = .04 vs. Pa-H	403.7 ± 150.7 (n = 6)
	12	n/a	162.3 ± 57.98 (n = 11) p = .77 vs. Pa-L p = .005 vs. Pa-H	23.9 ± 0.1 (n = 8) p = .01 vs. Pa-H	3859.0 ± 1366.6 (n = 8)
	72	247.5 ± 38.9 (n = 4) p = .32 vs. Sp p = .01 vs. Pa-L	845.0 ± 537.7 (n = 7) p = .23 vs. Pa-L	23.8 ± 0.2 (n = 6)	n/a
TNFsrI	6	352.7 ± 38.6 (n = 4) p = .02 vs. Sp p = .004 vs. Pa-L p = .01 vs. Pa-H	984.2 ± 150.5 (n = 8) p = .88 vs. Pa-L p = .28 vs. Pa-H	991.6 ± 59.8 (n = 8) p = .14 vs. Pa-H	1417 ± 262.9 (n = 6)
	12	n/a	464.1 ± 98.2 (n = 11) p = .17 vs. Pa-L p = .0008 vs. Pa-H	654.8 ± 133.9 (n = 8) p = .002 vs. Pa-H	1788 ± 307.6 (n = 8)
	72	336.2 ± 48.2 (n = 4) p = .11 vs. Sp p = .61 vs. Pa-L	146.2 ± 63.5 (n = 7) p = .01 vs. Pa-L	449.1 ± 73.4 (n = 6)	n/a
TNFsrII	6	2009.8 ± 190.6 (n = 4) p = 1.00 vs. Sp p = .004 vs. Pa-L p = .02 vs. Pa-H	2034.3 ± 250.6 (n = 7) p = .0003 vs. Pa-L p = .002 vs. Pa-H	992.6 ± 19.7 (n = 8) p = .002 vs. Pa-H	100000.0 ± 0.0 (n = 5)
	12	n/a	917.8 ± 122.6 (n = 11) p = .23 vs. Pa-L p = .0003 vs. Pa-H	996.3 ± 47.5 (n = 8) p = .0002 vs. Pa-H	51162.8 ± 18458.9 (n = 8)
	72	1738.2 ± 272.3 (n = 4) p = .006 vs. Sp p = .02 vs. Pa-L	452.4 ± 22.0 (n = 7) p = .001 vs. Pa-L	936.7 ± 50.0 (n = 6)	n/a
IL-2	6	13.8 ± 0.0 (n = 4) p = n/a vs. Sp p = n/a vs. Pa-L p = n/a vs. Pa-H	12.0 ± 0.0 (n = 8) p = n/a vs. Pa-L p = n/a vs. Pa-H	399.2 ± 245.6 (n = 8) p = n/a vs. Pa-H	12.0 ± 0.0 (n = 6)
	12	n/a	110.0 ± 98.0 (n = 11) p = n/a vs. Pa-L p = n/a vs. Pa-H	24.0 ± 0.0 (n = 8) p = n/a vs. Pa-H	12.0 ± 0.0 (n = 8)
	72	13.8 ± 0.0 (n = 4) p = n/a vs. Sp p = n/a vs. Pa-L p = n/a vs. Pa-H	12.00 ± 0.0 (n = 7) p = n/a vs. Pa-L p = n/a vs. Pa-H	24.0 ± 0.0 (n = 6) p = n/a vs. Pa-H	n/a
IL-5	6	80.8 ± 13.9 (n = 4) p = .048 vs. Sp p = n/a vs. Pa-L p = .17 vs. Pa-H	29.7 ± 11.6 (n = 8) p = n/a vs. Pa-L p = .85 vs. Pa-H	12.0 ± 0.0 (n = 8) p = n/a vs. Pa-H	37.8 ± 18.1 (n = 6)
	12	n/a	23.5 ± 11.19 (n = 11) p = n/a vs. Pa-L p = .83 vs. Pa-H	12.0 ± 0.0 (n = 8) p = n/a vs. Pa-H	19.5 ± 5.2 (n = 8)
	72	61.5 ± 5.5 (n = 4) p = n/a vs. Sp p = n/a vs. Pa-L	12.0 ± 0.0 (n = 7) p = n/a vs. Pa-L	12.0 ± 0.0 (n = 6)	n/a

Table 2.—*Continued*

Cytokine	Time, hr	Sham, pg/mL	Sp, pg/mL	Pa-L, pg/mL	Pa-H, pg/mL
IL-12	6	132.5 ± 34.3 (n = 4) p = n/a vs. Sp p = .02 vs. Pa-L p = n/a vs. Pa-H	12.0 ± 0.0 (n = 8) p = n/a vs. Pa-L p = n/a vs. Pa-H	36.4 ± 12.4 (n = 8) p = n/a vs. Pa-H	12.0 ± 0.0 (n = 6)
	12	n/a	112.4 ± 100.4 (n = 11) p = n/a vs. Pa-L p = n/a vs. Pa-H	24.0 ± 0.0 (n = 8) p = n/a vs. Pa-H	12.0 ± 0.0 (n = 8)
	72	111.7 ± 15.8 (n = 4) p = n/a vs. Sp p = n/a vs. Pa-L	12.0 ± 0.0 (n = 7) p = n/a vs. Pa-L	24.0 ± 0.0 (n = 6)	n/a
IL-13	6	620.0 ± 126.4 (n = 4) p = .008 vs. Sp p = .03 vs. Pa-L p = .11 vs. Pa-H	69.4 ± 48.4 (n = 8) p = .0499 vs. Pa-L p = .66 vs. Pa-H	146.6 ± 84.1 (n = 8) p = .23 vs. Pa-H	179.9 ± 108.3 (n = 6)
	12	n/a s	107.6 ± 79.9 (n = 11) p = .03 vs. Pa-L p = .90 vs. Pa-H	24.1 ± 0.1 (n = 8) p = .01 vs. Pa-H	222.8 ± 210.8 (n = 8)
	72	567.0 ± 64.6 (n = 4) p = .32 vs. Sp p = .01 vs. Pa-L	359.6 ± 217.5 (n = 7) p = .73 vs. Pa-L	24.2 ± 0.2 (n = 6)	n/a
RANTES	6	8999.8 ± 1951.6 (n = 4) p = .004 vs. Sp p = .004 vs. Pa-L p = .01 vs. Pa-H	41.7 ± 25.1 (n = 7) p = .03 vs. Pa-L p = .73 vs. Pa-H	137.3 ± 69.8 (n = 8) p = .02 vs. Pa-H	31.5 ± 19.5 (n = 6)
	12	n/a	13.0 ± 1.0 (n = 11) p = .0003 vs. Pa-L p = .90 vs. Pa-H	164.4 ± 71.4 (n = 8) p = .001 vs. Pa-H	16.9 ± 4.9 (n = 8)
	72	7612.5 ± 490.7 (n = 4) p = .07 vs. Sp p = .01 vs. Pa-L	66.0 ± 49.8 (n = 6) p = .18 vs. Pa-L	52.0 ± 21.5 (n = 6)	n/a

IL, interleukin; MIP, macrophage inflammatory peptide; MCP, macrophage chemotactic peptide; IFN, interfron; TNF, tumor necrosis factor; RANTES, regulated on activation, normal T-cell expressed and probably secreted.

fected with *S. pneumoniae* pneumonia occurs in the setting of a 5-log decrease in bacteria recovered from BAL samples.

Despite having similar (low) levels of bacteremia, systemic concentrations of pro-inflammatory and anti-inflammatory mediators are higher in animals administered low-dose *P. aeruginosa* compared to animals administered *S. pneumoniae* at 6 hrs (Fig. 3*B*). However, despite a marked increase in bacteria in the blood 72 hrs after onset of pneumonia in animals administered *S. pneumoniae*, plasma cytokine concentrations are generally similar between both groups. Even though levels of bacteria are >3 logs higher in animals administered *S. pneumoniae,* there is not a single mediator that is higher in these animals (compare Fig. 2*B* to 3*B*). The local response is therefore markedly different from the systemic response at 72 hrs (compare Fig. 3*A* to 3*B*).

Pathogens Causing High 7-Day Mortality Have Distinct Early Cytokine Profiles

Both pro-inflammatory and anti-inflammatory cytokines are generally higher in animals administered high-dose *P. aeruginosa* in BAL fluid 6 and 12 hrs after the onset of pneumonia compared to animals administered *S. pneumoniae* (Fig. 3*C*). This local effect is TNF-α-independent because concentrations of this cytokine are similar in mice subjected to either infection. The inflammatory response is also independent of pulmonary bacterial load because there was not a single cytokine measured that was statistically higher in the lungs of mice infected with *S. pneumoniae* despite having similar concentrations of bacteria at 6 hrs (compare Fig. 2*A* to 3*C*).

There is a similar trend in systemic cytokines. Both pro-inflammatory and anti-inflammatory cytokines are generally higher in the blood of animals administered high-dose *P. aeruginosa* 6 and 12 hrs after the onset of pneumonia, and at no time is the relative abundance of *S. pneumoniae* higher for any cytokine (Fig. 3*D*), despite having statistically similar low levels of bacteremia at 6 hrs in each group (Fig. 2*B*). Of note, even when the trend for cytokine abundance is similar between BAL and blood, both the absolute values and ratios may be markedly different between groups. An example is TNFsr-2, which is higher in mice administered high-dose *P. aeruginosa* in BAL and blood at 6 hrs (Fig. 3, *C* and *D*). TNFsr-2 concentrations in BAL fluid are 1,736 and 1,185 pg/mL in animals administered high-dose *P. aeruginosa* or *S. pneumoniae*, respectively (ratio 1.5:1),

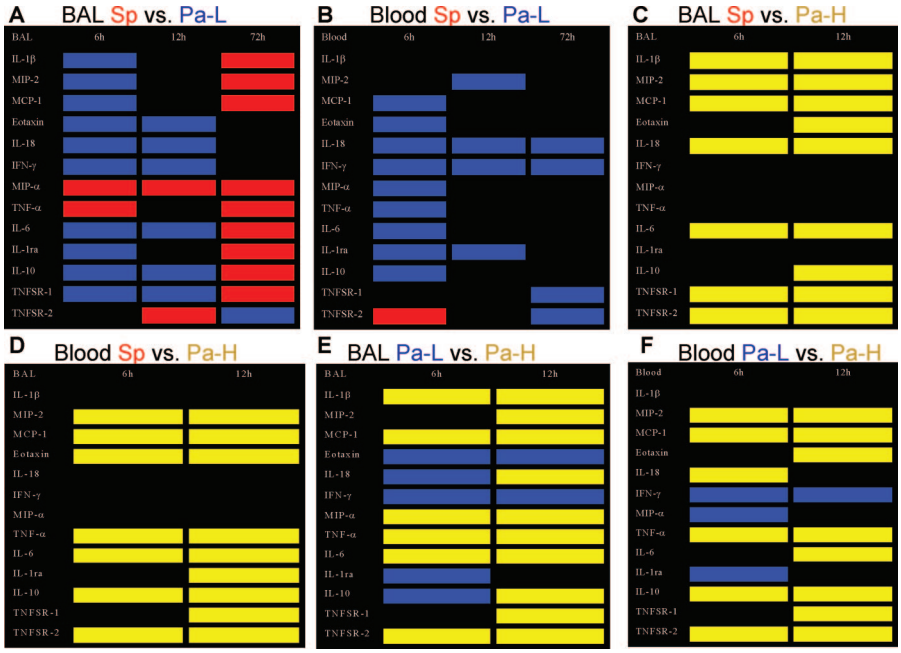

Figure 3. Relative cytokine abundance in bronchoalveolar lavage (*BAL*) and blood. All panels compare cytokine concentrations between two groups of animals (n = 6–11/group/time point) administered different models of pneumonia at various time points. The presence of a colored horizontal bar indicates that there was a statistically higher level of the measured cytokine in animals administered *S. pneumoniae* (*red*), low-dose *P. aeruginosa* (*blue*), or high-dose *P. aeruginosa* (*yellow*) compared to the other group examined. When no colored horizontal bar is present, cytokine abundance was statistically similar between the two groups examined. Data presented represent 13 of 18 cytokines measured. The five mediators not shown in this figure (interleukin [*IL*]-2, IL-5, IL-12, IL-13, and regulated on activation, normal T cell expressed and probably secreted) were excluded either because there were no differences between animals with pneumonia and sham animals or because the majority cytokine levels were below the limit of detection. Raw data for all cytokine levels are shown in Tables 1 and 2. *A*, Despite having similar mortality at all time points measured, the abundance of most pro-inflammatory and anti-inflammatory cytokines in BAL fluid is higher in mice administered low-dose *P. aeruginosa* than those administered *S. pneumoniae* at 6 hrs. However, the pattern reverses nearly completely by 72 hrs. *B*, Similar to BAL, systemic cytokines are generally higher in mice administered low-dose *P. aeruginosa* at 6 hrs. However, despite the relative increase in cytokine abundance in BAL at 72 hrs and the increase in bacteremia seen solely in animals administered *S. pneumoniae,* no relative increase in systemic cytokines is noted at this time point. *C* and *D*, Despite similar 7-day mortality, relative cytokine abundance is generally higher in animals administered high-dose *P. aeruginosa.* Not a single cytokine measured was significantly higher in either compartment in animals administered *S. pneumoniae*. Cytokine patterns are generally similar between BAL and blood; however, differences exist in multiple mediators such as tumor necrosis factor (TNF)-α, IL-18, and IL-1b. It should be noted that interferon (*IFN*)-γ levels are at the lower limit of detection in animals administered either high-dose *P. aeruginosa* or *S. pneumoniae*. *E*, BAL samples in animals administered either high-dose or low-dose *P. aeruginosa.* Although there is a higher bacterial load in the lungs of those that received a higher dose, in 5 of 11 cytokines levels in which a difference was detected between groups, they were more elevated in those that received low-dose bacteria. *F*, Although the blood from animals that received high-dose or low-dose *P. aeruginosa* was more homogeneous than BAL, IFN-γ concentrations were higher in animals that received a lower inoculum of bacteria. *MIP*, macrophage inflammatory peptide; *MCP*, macrophage chemotactic peptide.

whereas serum concentrations in the same animals are >100,000 pg/mL and 2,034, respectively (ratio >50:1).

Differing Doses of *P. Aeruginosa* Cause Distinct Early Cytokine Profiles

Although the bacterial load is higher in the lungs of mice administered high-dose *P. aeruginosa* compared to low-dose, it does not directly correlate to local cytokine abundance (compare Fig. 2*A* to Fig. 3*E*). BAL cytokine concentrations are nearly as likely to be higher 6 hrs after low-dose *P. aeruginosa* as they are after high-dose *P. aeruginosa*. Blood concentrations were higher after high-dose *P. aeruginosa* for 6 cytokines, higher after low-dose *P. aeruginosa* cytokines for five cytokines, and were not statistically different between the two models for two cytokines (Fig. 3*F*). In contrast to the 6-hr time point, by 12 hrs cytokine levels were consistently higher in both BAL and blood in high-dose *P. aeruginosa.*

Hierarchical Clustering of Cytokine Expression

Figure 3 illustrated that there were statistically significant differences between most cytokines in the different models at each time point, and that the magnitude of these differences varied depending on the cytokine, the infection, the body fluid sampled, and the time point examined. Whereas these data are instructive on a population basis, they do not examine the heterogeneity of the individual response to each challenge. To examine relationships between individual animals, hierarchical clustering of cytokine abundance data from the individual mice was performed (Fig. 4*A*). Although eight groups of animals were included in the analysis (two infections at three time points and one infection at two time points), only five major nodes were identified.

The principal node separates ten animals in cluster A from the remaining 50 mice. The animals in this group include 5 of 6 mice administered high-dose *P. aeruginosa* at 6 hrs, 4 of 8 of mice administered high-dose *P. aeruginosa* at 12 hrs, and 1 of 7 mice administered *S. pneumoniae* at 6 hrs. Animals in cluster A are characterized by elevated concentrations of pro-inflammatory cytokines (IL-1b, IL-6, IL-18, MIP-α, TNF-α, and TNFsr2) in BAL fluid, as well as elevated concentrations of Eotaxin, TNFsr2, TNFsr1, and MCP-1 in the blood.

The second node separates 15 mice in cluster B from the remaining 35 animals. These animals include all six mice administered low-dose *P. aeruginosa* at 6 hrs, 1 out of 8 mice administered low-dose *P. aeruginosa* at 12 hrs, and all seven mice administered *S. pneumoniae* at 72 hrs. These cohorts of mice have a 50% to 60% mortality rate within 72 hrs of the sampling time. However, there is no apparent cytokine or combination of cytokines that subdivides either the *P. aeruginosa*-infected or *S. pneumoniae*-infected mice, suggesting that local and systemic cytokines at this time point do not have the capacity to predict which animals will go on to recover or die. Interestingly, the dendrogram on the left of Figure 4 shows that the seven *S. pneumoniae*-infected mice have the most similar cytokine expression profiles of all groups of mice in

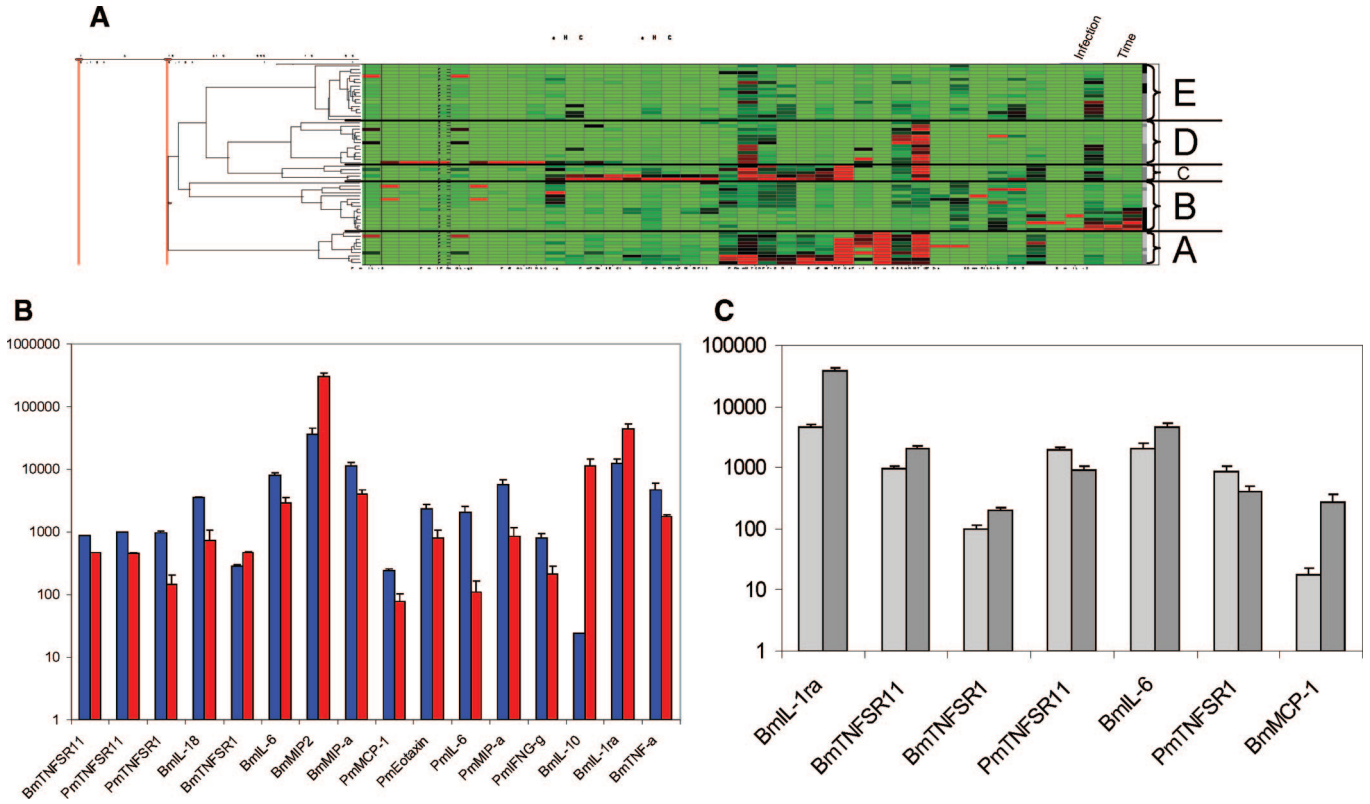

Figure 4. Hierarchical clustering of cytokine abundance. *A*, All mice with complete cytokine data sets were analyzed simultaneously. Each individual mouse is represented as a row across the figure, showing the abundance of each cytokine in both bronchoalveolar lavage (BAL) and blood. Each column represents a single cytokine measured either in the plasma (*P*) or BAL (*B*). The columns were ordered based on Pearson's correlation, although no significant relationships among the profiles of individual cytokines were apparent. The columns represent the following cytokines in this order: P interleukin (*IL*)-5; PIL-2;PIL-12; PIL-13; P interferon (*IFN*)-γ; P macrophage inflammatory peptide (*MIP*)-α; PIL-18; P tumor necrosis factor (*TNF*)-α; PIL-1b; PIL-10; BTNFsr1; PIL-6; PMIP-2; PIL-1ra; Peotaxin; BTNFsrII; PTNFsr1; BIL-18; BIL-1b; BIL-6; P macrophage chemotactic peptide (*MCP*)-1; BIL-5; PTNFsrII; BTNF-α; BMIP-a; BIL-12; BIFN-γ; BIL-13; B regulated on activation, normal T-cell expressed and probably secreted (*RANTES); BIL-13; BRANTES; Beotaxin; BMCP-1; PRANTES; BIL-2; BIL-1ra; BIL-10; and BMIP-2. Horizontal bars separate five distinct nodes (A–E*) that encompass the eight treatment groups. Mice receiving *S. pneumoniae* are denoted in *red* whereas mice receive *P. aeruginosa* are denoted by *blue* (low-dose) or *yellow* (high-dose). The time points at which plasma and BAL were acquired are encoded in gray-scale saturation. The 6-hr time point is represented by 25% saturation (*light gray*), the 12-hr time point is indicated by 50% saturation (*dark gray*), and the 72-hr time point is indicated by *black*. Cytokine expression ranged from below limit of detection (*green*) through the mean value for that cytokine (*black*) to the highest abundance for that cytokine (*red*) and as a result this visualization tool is only semi-quantitative. The dendrogram on the left indicates the similarity of adjacent samples. This visualization tool demonstrates the intrinsic variability of the host immunologic response to different infections over time and suggests the identity of cytokines that differentiate across groups. *B*, Cytokine abundance data that differentiate the two groups in cluster B. Cytokines were ranked based on p-value from a Student's *t* test comparing the two groups identified in cluster B by hierarchical cluster analysis. Those with $p < 0.05$ are shown. Average ± SEM are shown for mice infected with *P. aeruginosa* at early time points (*blue*) and *S. pneumoniae* at 72 hrs after infection (*red*). *C*, Cytokine abundance data that differentiate the two groups within cluster D. Cytokines were ranked based on p-value from a Student's *t* test comparing the two groups identified in cluster D by hierarchical cluster analysis and those with $p < 0.05$ are shown. Average ± SEM are shown for mice infected with samples taken at 6 hrs (*light gray*) and 12 hrs after infection (*dark gray*).

the study. Because of the homogeneity in the *S. pneumoniae*-infected mice, we identified significant differences between *P. aeruginosa*-infected animals and *S. pneumoniae*-infected animals in cluster B (Fig. 4*B*). As expected based on their pulmonary bacterial burden (Fig. 2), the *P. aeruginosa*-infected mice in cluster B had higher pro-inflammatory cytokines in BAL fluid than did *S. pneumoniae*-infected mice. Four measured proteins were higher in the BAL of *S. pneumoniae*-infected mice—MIP2, IL-10, TNFsr2, and IL-1ra. Surprisingly, although the only mice in cluster B that had bacteremia were *S. pneumonia*-infected mice (Fig. 2), plasma cytokines were higher in *P. aeruginosa*-infected mice.

The remaining 35 mice were clustered into three separate groups. Cluster C contained the five remaining animals that received high-dose *P. aeruginosa* at 6 and 12 hrs that were not in cluster A. Of the 36 measured cytokines, the only one that was significantly different between clusters A and C was systemic TNFsr2.

Cluster D contained 13 mice, all of which were infected with *S. pneumoniae* at either 6 hrs (6 of 7 animals) or 12 hrs (7 of 11 animals) after infection. With the exception of a single animal, the clustering algorithm separated the samples obtained at 6 hrs from those obtained 12 hrs after infection. A small panel of measured components separated these two groups, including marked increases in soluble IL-1 and TNF antagonists in BAL fluid (Fig. 4*C*). Similar to mice infected with high-dose *P. aeruginosa* (clusters A and C), all animals in cluster D had elevated BAL concentrations of MIP-α.

The final 17 mice in cluster E contained 7 out of 8 mice infected with

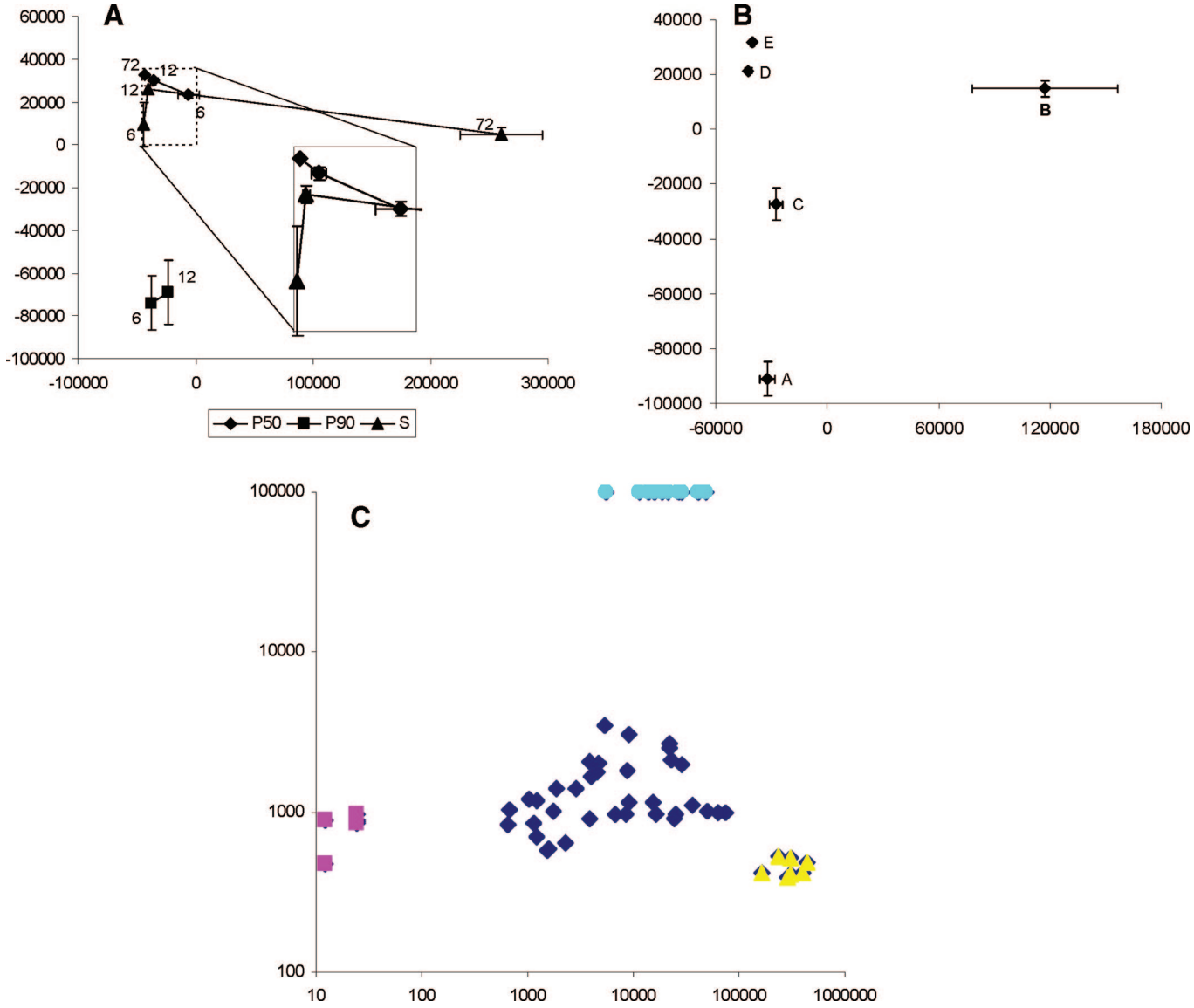

Figure 5. Principal component analysis of cytokine abundance data. *A*, Samples were grouped according to their infection group and time point. *x-axis*: PC1 (arbitrary units); *y-axis*: PC3 (arbitrary units). Points plotted are the mean value for each principal component ± the SEM and are labeled with the time point when samples were collected. Lines connecting the points for the same infection are shown over time to propose a hypothetical trajectory of cytokine expression that occurs during each infection. *Inset*, Close proximity of the low-dose *P. aeruginosa* trajectory and the *S. pneumoniae* trajectory. *B*, Each cluster (*A–E*) identified in Figure 4 was also plotted along the same axes as were used in (*A*). In this instance, the principal components between (*A*) and (*B*) are identical because principal components were calculated using the same data. *C*, Local macrophage inflammation peptide (MIP)2 and systemic tumor necrosis factor (TNF)sr2 abundance classify individual mice into four distinct groups (discussed in the text), effectively separating mice destined to die (high systemic TNFsr2, *blue*, or high local MIP-2, *yellow*) from mice that have cleared infection (low local MIP-2 and low systemic TNFsr2, *pink*). The remaining mice (*black*) cannot be separated into clinically relevant groups using these criteria.

low-dose *P. aeruginosa* at 12 hrs, all 6 mice infected with low-dose *P. aeruginosa* at 72 hrs, and 4 out of 11 mice infected with *S. pneumoniae* at 12 hrs. No clear pattern in cytokine expression separated these animals despite their markedly different prognoses.

Principal Component Analysis

Principal component analysis (PCA) is a computational technique that reduces multidimensional data from one axis per variable into a lower dimensional representation of that data set viewed from its most informational viewpoint. PCA can reveal the internal structure of a data set to best explain the variance in the data, assuming the data conform to three key assumptions: (1) linearity; (2) that the mean and covariance of the data are important; and (3) that large variances have important dynamics. If the observed data have a high signal-to-noise ratio, then the principal components with larger variance usually correspond to interesting dynamics, whereas PCA with lower variance corresponds to noise. Data visualization via PCA can illuminate informative dynamics within time-dependent data sets (27).

One particularly informative visualization of PCA of the data outlined is from principal components one and three of the combined analysis (Fig. 5*A*). Principal component one separated *S. pneumoniae*-infected animals at 72 hrs from

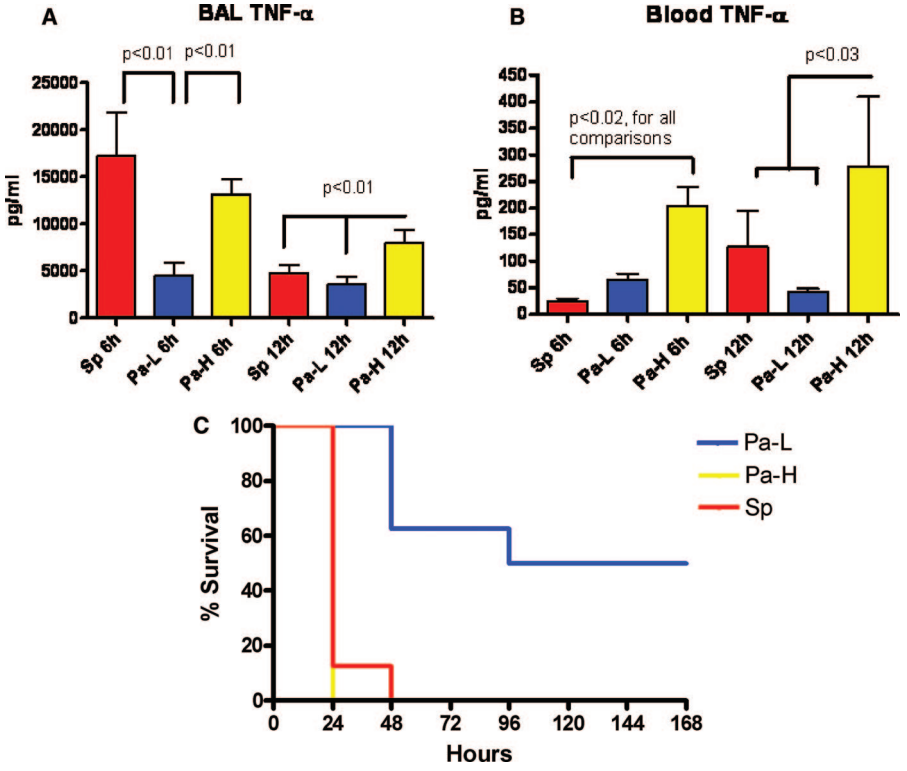

Figure 6. Tumor necrosis factor (*TNF*)-α and survival. The pro-inflammatory cytokine TNF-α is elevated in (*A*) bronchoalveolar lavage (*BAL*) of *S. pneumoniae* and high-dose *P. aeruginosa* and (*B*) blood of high-dose *P. aeruginosa*. *C*, Anti-TNF-α accelerates mortality in animals in which pneumonia induces elevated local or systemic levels of the cytokine but has no impact on survival in animals administered low-dose *P. aeruginosa* (n = 8/group).

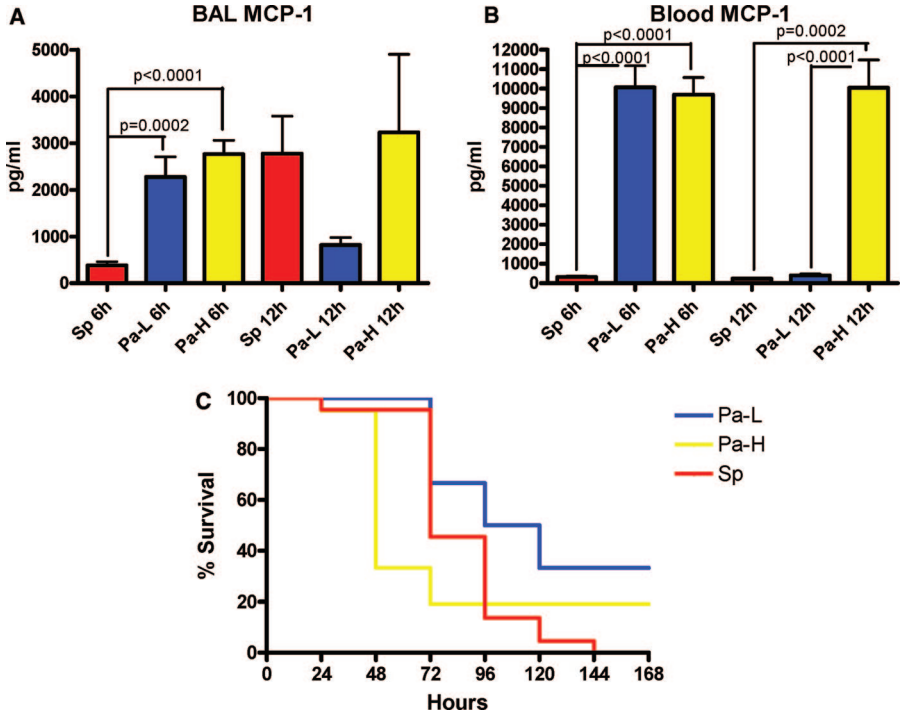

Figure 7. Macrophage chemotactic peptide (*MCP*)-1 and survival. *A*, MCP-1 levels in bronchoalveolar lavage (*BAL*) of all groups. *B*, Blood MCP-1 levels are markedly elevated in both low-dose and high-dose *P. aeruginosa* but not in *S. pneumoniae*. *C*, Survival is unaffected in MCP-$1^{-/-}$ mice administered either low-dose or high-dose *P. aeruginosa* but is accelerated in those subjected to *S. pneumoniae* (n = 21–22/group).

the other groups, whereas principal component three separated the 90% 7-day lethality of *P. aeruginosa*-infected animals from the other groups. Different time points from the same infection were connected by lines to illustrate hypothetical trajectories of disease progression based on local and systemic cytokine abundance. Interestingly, the hypothetical *S. pneumoniae* and 50% mortality of *P. aeruginosa* trajectories become indistinguishable between 6 and 12 hrs after infection. Principal components were also calculated by grouping mice based on which cluster they belonged to in the hierarchical clustering analysis (Fig. 5*B*). PCA of any cytokine data sets identified statistically significant differences between groups whether classified as treatment groups or clusters (data not shown).

Principal component one showed a high correlation with the abundance of MIP-2 ($r^2 = 0.998$) and IL-10 ($r^2 = 0.72$) in BAL (the cytokines whose abundance defines cluster B), whereas principal component three correlated with TNFsr2 ($r^2 = 0.84$) and MCP-1 ($r^2 = 0.70$) in the blood and IL-1b in the BAL ($r^2 = 0.64$). By examining BAL MIP2 and plasma TNFsr2 abundance together, four groups emerge (Fig. 5*C*): (1) mice that have recovered from infection (i.e., low-dose *P. aeruginosa*-infected mice 72 hrs after onset of pneumonia) have essentially none of these cytokines; (2) mice that have high BAL MIP-2 (*S. pneumonia*-infected mice 72 hrs after onset of pneumonia); (3) mice with high-plasma TNFsr2 (*P. aeruginosa*-infected mice that will ultimately have a 90% 7-day mortality); and (4) all other animals (mice with intermediate abundances of local MIP-2 in the BAL and systemic TNFsr2).

Survival Studies

To assess the functional significance of relative differences in cytokine levels, survival studies were performed. TNF-α levels were markedly elevated in BAL at 6 hrs in *S. pneumoniae* and in both BAL at 6 hrs and blood at 12 hrs in high-dose *P. aeruginosa* (Fig. 6*A* and *B*). Treating animals subjected to pneumonia with anti-TNF-α antibody resulted in a marked hastening of mortality in animals in which TNF-α levels were high (Fig. 6*C*, compare kinetics of mortality to Fig. 1), but had no affect in low-dose *P. aeruginosa*, for which local and systemic cytokine levels were lower.

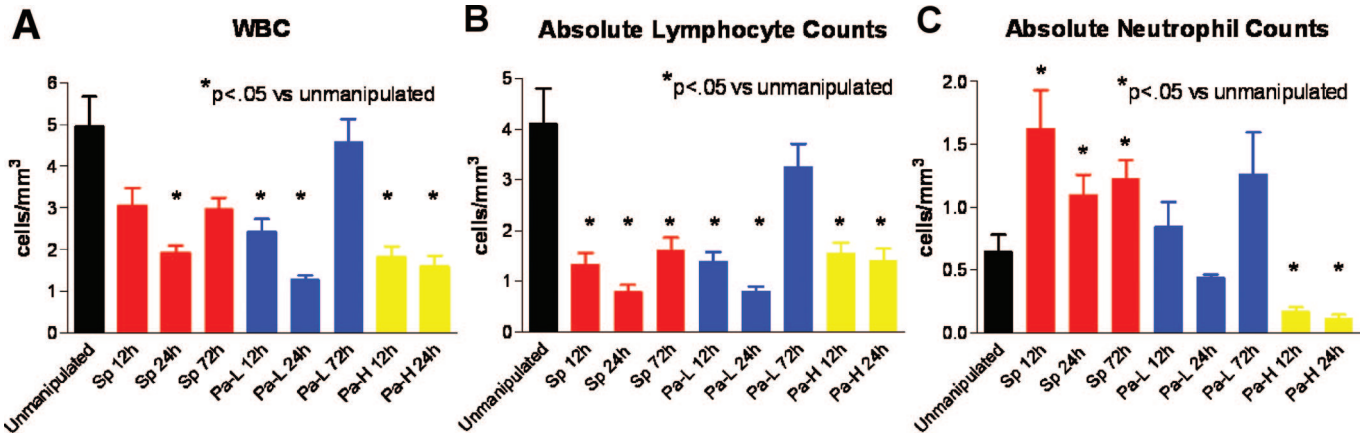

Figure 8. Systemic leukocyte response to pneumonia. *A*, Total white blood cell (*WBC*) counts (n = 6–9) are decreased after infection at 12 and 24 hrs and increase to basal values 72 hrs after low-dose *P. aeruginosa*. *B*, Absolute lymphocyte counts have a similar trend to total WBC counts, with decreases in all groups at all time points except 72 hrs after low-dose *P. aeruginosa*. *C*, Absolute neutrophil counts are increased after *S. pneumoniae,* are not significantly changed with low-dose *P. aeruginosa*, and are markedly decreased after high-dose *P. aeruginosa*.

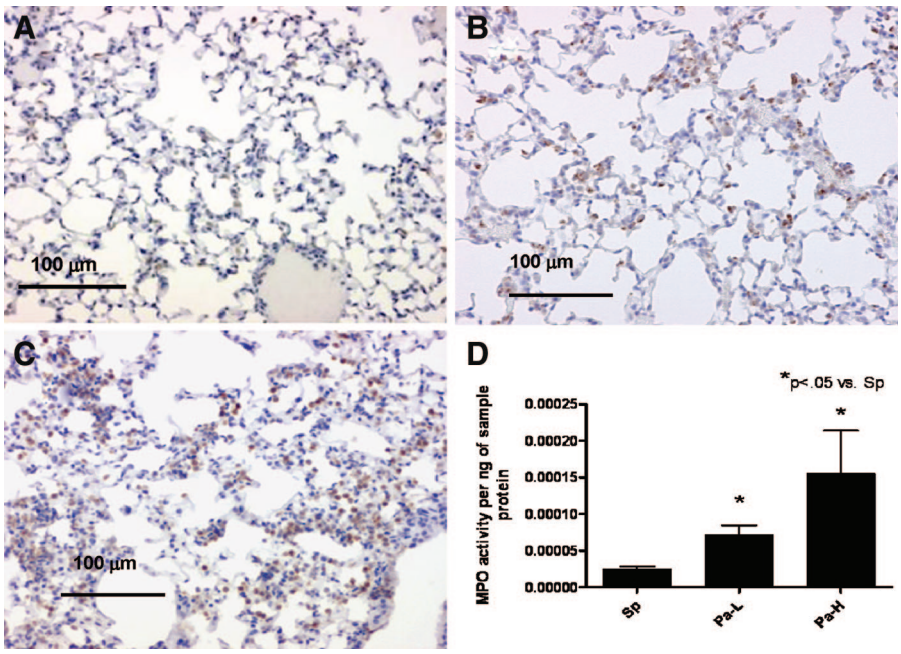

Figure 9. Pulmonary myeloperoxidase (*MPO*) by immunohistochemistry and quantitative assay. Representative histology (n = 5–6) in animals administered (*A*) *S. pneumoniae*, (*B*) low-dose *P. aeruginosa*, or (*C*) high-dose *P. aeruginosa* show comparative increasing staining for MPO activity in the three models, respectively, 12 hrs after onset of pneumonia, which is confirmed by quantitative assay (*D*). Micrographs were taken at 200×.

Survival studies were also performed on MCP-1$^{-/-}$ mice, based on the significance of the cytokine in both hierarchical clustering and PCA studies. Cytokine levels were markedly different between BAL fluid and blood (Fig. 7*A* and *B*). MCP-1 was markedly elevated in the blood of both high-dose and low-dose *P. aeruginosa* but nearly undetectable in *S. pneumoniae* (Fig. 7*B*). Despite these differences, there was no survival affect in MCP-1$^{-/-}$ mice administered either high-dose or low-dose *P. aeruginosa* (in which systemic levels were elevated) and a hastening of mortality in MCP-1$^{-/-}$ mice administered *S. pneumoniae* (even though systemic levels were not elevated, Fig. 7*C*).

Circulating Leukocytes

To further define the host response, circulating white blood cell counts were analyzed (Fig. 8*A*). By 24 hrs, all animals with pneumonia had similar decreases in their leukocyte counts despite marked differences in cytokine production ($p < 0.05$ compared to unmanipulated mice (compare Fig. 8*A* to Fig. 3*B*, *D*, and *F*). Of note, total circulating white blood cells were lowest when animals had minimal bacteremia, and leukocyte counts returned to normal by 3 days, even in the setting of marked *S. pneumoniae* bacteremia (compare Fig. 2*B* to 8*A*). The initial decrease in total white blood cell count was in large part attributable to a decrease in absolute lymphocyte count in all groups at 12 and 24 hrs, independent of type of bacterial infection (Fig. 8*B*).

In contrast to the similarities in absolute lymphocyte count, there were marked differences in absolute neutrophil counts (Fig. 8*C*). Mice administered *S. pneumoniae* had increased circulating neutrophils, mice administered low-dose *P. aeruginosa* had little change in circulating neutrophils, and mice administered high-dose *P. aeruginosa* had a marked decrease in circulating neutrophils. To determine whether this could be explained by differential infiltration of neutrophils into lungs of animals with pneumonia, pulmonary MPO assay was performed (Fig. 9). Whether assessed by histology or quantitative MPO activity, there was a substantial increase in pulmonary neutrophils in mice administered high-dose *P. aeruginosa*, minimal pulmonary neutrophilic infiltration in mice administered *S. pneumoniae*, and intermediate levels in animals administered low-dose *P. aeruginosa*.

DISCUSSION

This study demonstrates that genetically inbred animals have distinct host

responses to pneumonia. The inflammatory response is dependent on kinetics of mortality as well as ultimate 7-day mortality. Different inocula of the same microbe also cause distinct early host responses, but not in a monotonic fashion that might be predicted, because higher bacterial concentrations do not directly correlate to the severity of the inflammatory response. Additionally, the host response is compartmentalized, with substantial variation between local (BAL) and systemic (blood) cytokine profiles.

There is a fundamental disconnect between our results, current patient care, and therapeutic targets of the majority of sepsis clinical trials. Current therapy in sepsis is individualized only as far as targeting specific microbes; however, once antibiotics fail, treatment is nonspecific in keeping with the concept of a "generic septic response." However, if broad-based host responses to infections exist, targeting them may be a rational approach to sepsis therapy that can be undertaken simultaneously to targeting the initiating microbe with antibiotic therapy. To examine this possibility, hierarchical cluster analysis was performed, which allowed us to identify five distinct host response profiles within the eight different groups of animals examined. These clusters may have prognostic significance and potential utility for development of targeted therapeutics or diagnostic assays. For instance, all animals that received high-dose *P. aeruginosa* were in clusters A and C, with the sole difference between the two being >50-fold difference in systemic TNFsr2. Whereas there was a high ultimate mortality in each of these groups of animals, it is possible that that the difference in TNFsr2 concentrations was linked to rapidity of death. Additionally, a vigorous local inflammatory response appears to correlate with rapid death because animals in clusters A and C would be expected to die in <48 hrs based on the survival curves shown in Figure 1. Additionally, all animals in cluster D were infected with *S. pneumoniae* and all had elevated BAL concentrations of MIP-α. We speculate that these mice may be the ones destined to die because 13 out of 18 mice administered this bacteria at 6 or 12 hrs were in this cluster, and this was very close to the percentage of animals that ultimately died after *S. pneumoniae* pneumonia. The remaining mice infected with *S. pneumonia* in cluster E had low MIP-α concentrations. Interestingly, local BAL production of MIP-α ceased by 72 hrs in animals with *S. pneumoniae* pneumonia (cluster B), even though the majority of those animals would go on to die as well. It is also remarkable that on the PCA, the most similar cytokine profiles in the entire experiment were from mice subjected to *S. pneumoniae* pneumonia 72 hrs after infection. Whereas approximately half of these animals die within 72 hrs, there were no differences noted within this entire group of animals.

The lack of correlation between bacterial concentration and host response was surprising. Mice infected with *S. pneumoniae* had a higher pulmonary bacterial load at early time points, but mice infected with low-dose *P. aeruginosa* had higher cytokine abundance. By 72 hrs there was a 10,000-fold decrease in pulmonary bacterial load in mice administered *S. pneumoniae*, but despite this decrease, local cytokine abundance increased compared to low-dose *P. aeruginosa*. Examining blood from the same animals showed higher concentrations of cytokines in mice with low-dose *P. aeruginosa* at early time points despite similar low levels of bacteremia in both. However, a marked increase in bacteremia in animals administered *S. pneumonia* alone was not accompanied by a change in relative cytokine abundance. The lack of correlation between local and systemic bacterial concentration and the inflammatory response in either compartment suggests that although microbes initiate the host response, it is subsequently modulated, at least partially, independent of the inciting infection and continued presence of infection. A more direct way to examine the correlation between bacterial burden and host response was to compare high-dose and low-dose *P. aeruginosa*. It was reasonable to predict that giving a substantially higher dose of the same bacteria would lead to a more pronounced inflammatory response, at least in the lungs, where the infection was initiated. However, 11 cytokines were different in the two groups 6 hrs after the onset of pneumonia, with a near-even split—six higher in the high-dose group, five higher in the low-dose group. This means that it is at least partially incorrect to assume that the greater the bacterial burden, the more severe the inflammatory response, which has clear implications if attempting to modulate the immune response for therapeutic gain. It should be noted, however, that bacterial counts are only a crude measure of the complex relationship that exists between pathogen and host in sepsis and, in fact, bacterial phenotype may not be an invariant trait, but rather one that undergoes dynamic changes.

The survival experiments demonstrated both the promise and limitations of targeting therapy based on the host response. Whereas targeting TNF-α and knocking out MCP-1 failed to improve survival in any group regardless of cytokine levels, we were able to identify groups that had worse outcomes with these interventions. If a single model of sepsis alone were used in preclinical trials (cecal ligation and puncture often is used for this purpose), it is possible that harmful effects of a therapy in certain subgroups would not be identified. Further, our results show that not only do different organisms lead to different host responses, but severity of illness can also have a profound influence on how the host responds to a specific therapy, even if the inciting organism is the same. It has been postulated that one reason why clinical sepsis trials fail is that animal studies tend to use high mortality models, whereas patient studies use a population that is less sick, which would be expected to behave differently (28). The anti-TNF-α experiments in this study demonstrate a marked worsening of survival in both high mortality groups, with minimal effect in the intermediate mortality group. These results correlate well to two prospective randomized trials of anti-TNF-α antibody in patients, which have shown minimal or no benefit in a population with a baseline mortality of approximately 50% (29, 30). However, our results raise the concern that treating those infected with either *S. pneumoniae* or *P. aeruginosa* with a high risk of death may actually be harmful. Of note, previous studies have shown that anti-TNF-α antibody improves survival in rats subjected to lower mortality models of *Escherichia coli* or *Staphylococcus aureus* pneumonia, and we view our results as complimentary to these because we did not examine models of *S. pneumoniae* or *P. aeruginosa* with mortalities of <50% (31).

We do not have a clear explanation for the survival studies in MCP-$1^{-/-}$ mice. These animals have worsened survival after polymicrobial sepsis (32), and the only effect seen in this study was a hastening of death in animals that did not have a significant increase in sepsis-induced MCP-1. It is possible that different levels of this chemoattractant are

necessary depending on the infecting organism or disease severity. However, it is difficult to know if the results seen in a knockout animal with lifelong MCP-1 deletion accurately replicate what would happen if the mediator were targeted in an acute setting.

We do not believe these results are inconsistent with genome-wide RNA microarray analyses of either circulating neutrophils or peripheral blood mononuclear cells that demonstrates no difference between those infected with Gram-positive and Gram-negative infections (6, 7). This is because studies performed specifically on neutrophils or peripheral blood mononuclear cells would not identify changes in other cell types that might be responsible for the marked differences seen in this study. Further, those studies were performed on a transcriptome level, which would not necessarily identify the changes we found on a translational level. Finally, the mortality rate of patients infected with Gram-positive or Gram-negative bacteria ranged from 11% to 37% in those studies, which is significantly lower than the mortality in all groups examined herein.

Our study has several limitations. Antibiotics were not used in this study because they have been demonstrated to alter the host immune response in both *S. pneumonia* and *P. aeruginosa* pneumonia, which would have complicated interpretation of our results (33, 34). However, antibiotics are standard of care in the treatment of sepsis, and their absence limits the clinical relevance of our results. Additionally the host response to sepsis is a dynamic process (27, 35), and it is possible that critical information was missed by sampling at only three time points. Whereas our study used *S. pneumonia* and *P. aeruginosa* pneumonia as prototypical Gram-positive and Gram-negative infections, respectively, because of their prevalence in septic patients (36), there are marked differences in susceptibility to *S. pneumonia* infections based on different capsular subtypes and nearly 2,000 species of *P. aeruginosa* have been isolated from patients; therefore, it is difficult to determine whether our results are generalizable to either these infections or Gram-positives and Gram-negatives in general (37, 38). It also does not study the host response in the absence of overt signs of infection, which may be very significant in light of recent work demonstrating that patients with a high burden of *P. aeruginosa* who do not meet clinical criteria for ventilator associated pneumonia have increased mortality compared to patients with a high burden of *P. aeruginosa* who have evidence of pneumonia (39). Also, the anti-TNF survival curves were performed at a different time than the survival curves in Figure 1 without concurrent untreated controls. We therefore cannot exclude the possibility that the results in Figure 6 are simply attributable to the fact that survival can vary between models from week to week, independent of the effect of anti-TNF. Finally, the experiments were performed in mice. Whereas the study allowed for examination of the host response without the confounder of genetic variability and allowed for the ability to precisely titrate each variability examined (kinetics of mortality, 7-day mortality, bacterial concentration), how these results translate to humans is unknown.

Despite these limitations, these results demonstrate that individual infections induce unique host responses. The current paradigm of treating septic patients with supportive care clearly improves outcome in individual patients, but the disease still has an unacceptably high mortality rate. Our results suggest that the inflammatory host response to sepsis is, at a minimum, dependent on the inciting organism, the kinetics and severity of infection, the concentration of inoculum, and the time the host response is interrogated. Although there is significant complexity to sepsis as a clinical entity, there appear to be well-orchestrated host responses to infection. The meaning of these responses is yet to be determined.

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REFERENCES

1. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Crit Care Med* 1992; 20:864–874
2. Bernard GR, Vincent JL, Laterre PF, et al: Efficacy and safety of recombinant human activated protein C for severe sepsis. *N Engl J Med* 2001; 344:699–709
3. Elson G, Dunn-Siegrist I, Daubeuf B, et al: Contribution of Toll-like receptors to the innate immune response to Gram-negative and Gram-positive bacteria. *Blood* 2007; 109: 1574–1583
4. Foell D, Wittkowski H, Vogl T, et al: S100 proteins expressed in phagocytes: a novel group of damage-associated molecular pattern molecules. *J Leukoc Biol* 2007; 81: 28–37
5. Hoebe K, Janssen E, Beutler B: The interface between innate and adaptive immunity. *Nat Immunol* 2004; 5:971–974
6. Tang BM, McLean AS, Dawes IW, et al: Gene-expression profiling of gram-positive and gram-negative sepsis in critically ill patients. *Crit Care Med* 2008; 36:1125–1128
7. Tang BM, McLean AS, Dawes IW, et al: Gene-expression profiling of peripheral blood mononuclear cells in sepsis. *Crit Care Med* 2009; 37:882–888
8. Deutschman CS, Konstantinides FN, Tsai M, et al: Physiology and metabolism in isolated viral septicemia. Further evidence of an organism-independent, host-dependent response. *Arch Surg* 1987; 122:21–25
9. Fry DE, Pearlstein L, Fulton RL, et al: Multiple system organ failure. The role of uncontrolled infection. *Arch Surg* 1980; 115: 136–140
10. Fry DE: The generic septic response. *Crit Care Med* 2008; 36:1369–1370
11. Rittirsch D, Hoesel LM, Ward PA: The disconnect between animal models of sepsis and human sepsis. *J Leukoc Biol* 2007; 81:137–143
12. Yu SL, Chen HW, Yang PC, et al: Differential gene expression in gram-negative and gram-positive sepsis. *Am J Respir Crit Care Med* 2004; 169:1135–1143
13. Feezor RJ, Oberholzer C, Baker HV, et al: Molecular characterization of the acute inflammatory response to infections with gram-negative versus gram-positive bacteria. *Infect Immun* 2003; 71:5803–1583
14. Johnson SB, Lissauer M, Bochicchio GV, et al: Gene expression profiles differentiate between sterile SIRS and early sepsis. *Ann Surg* 2007; 245:611–621
15. Ramilo O, Allman W, Chung W, et al: Gene expression patterns in blood leukocytes discriminate patients with acute infections. *Blood* 2007; 109:2066–2077
16. Calvano SE, Xiao W, Richards DR, et al: A network-based analysis of systemic inflammation in humans. *Nature* 2005; 437: 1032–1037
17. National Nosocomial Infections Surveillance (NNIS): System report, data summary from January 1990-May 1999, issued June 1999. *Am J Infect Control* 1999; 27:520–532
18. Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. *Am J Respir Crit Care Med* 2005; 171:388–416
19. Coopersmith CM, Stromberg PE, Davis CG, et al: Sepsis from Pseudomonas aeruginosa pneumonia decreases intestinal proliferation and induces gut epithelial cell cycle arrest. *Crit Care Med* 2003; 31:1630–1637

20. Vyas D, Robertson CM, Stromberg PE, et al: Epithelial apoptosis in mechanistically distinct methods of injury in the murine small intestine. *Histol Histopathol* 2007; 22: 623–630
21. Coopersmith CM, Stromberg PE, Dunne WM, et al: Inhibition of intestinal epithelial apoptosis and survival in a murine model of pneumonia-induced sepsis. *JAMA* 2002; 287: 1716–1721
22. Sheehan KC, Ruddle NH, Schreiber RD: Generation and characterization of hamster monoclonal antibodies that neutralize murine tumor necrosis factors. *J Immunol* 1989; 142:3884–3893
23. Knight PR, Sreekumar A, Siddiqui J, et al: Development of a sensitive microarray immunoassay and comparison with standard enzyme-linked immunoassay for cytokine analysis. *Shock* 2004; 21:26–30
24. Wells C, Hess D, Erlandsen S: Impact of the indigenous flora in animal models of shock and sepsis. *Shock* 2004; 22:562–568
25. Magnotti LJ, Upperman JS, Xu DZ, et al: Gut-derived mesenteric lymph but not portal blood increases endothelial cell permeability and promotes lung injury after hemorrhagic shock. *Ann Surg* 1998; 228:518–527
26. Bradford MM: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; 72: 248–254
27. McDunn JE, Husain KD, Polpitiya AD, et al: Plasticity of the Systemic Inflammatory Response to Acute Infection during Critical Illness: Development of the Riboleukogram. *PLoS ONE* 2008; 3:e1564
28. Eichacker PQ, Parent C, Kalil A, et al: Risk and the efficacy of antiinflammatory agents: retrospective and confirmatory studies of sepsis. *Am J Respir Crit Care Med* 2002; 166:1197–1205
29. Panacek EA, Marshall JC, Albertson TE, et al: Efficacy and safety of the monoclonal anti-tumor necrosis factor antibody F(ab′)2 fragment afelimomab in patients with severe sepsis and elevated interleukin-6 levels. *Crit Care Med* 2004; 32:2173–2182
30. Reinhart K, Menges T, Gardlund B, et al: Randomized, placebo-controlled trial of the anti-tumor necrosis factor antibody fragment afelimomab in hyperinflammatory response during severe sepsis: The RAMSES Study. *Crit Care Med* 2001; 29:765–769
31. Karzai W, Cui X, Mehlhorn B, et al: Protection with antibody to tumor necrosis factor differs with similarly lethal *Escherichia coli* versus *Staphylococcus aureus* pneumonia in rats. *Anesthesiology* 2003; 99:81–89
32. Gomes RN, Figueiredo RT, Bozza FA, et al: Increased susceptibility to septic and endotoxic shock in monocyte chemoattractant protein 1/cc chemokine ligand 2-deficient mice correlates with reduced interleukin 10 and enhanced macrophage migration inhibitory factor production. *Shock* 2006; 26:457–463
33. Nau R, Eiffert H: Minimizing the release of proinflammatory and toxic bacterial products within the host: a promising approach to improve outcome in life-threatening infections. *FEMS Immunol Med Microbiol* 2005; 44:1–16
34. Coopersmith CM, Amiot DM, Stromberg PE, et al: Antibiotics improve survival and alter the inflammatory profile in a murine model of sepsis from Pseudomonas aeruginosa pneumonia. *Shock* 2003; 19:408–414
35. Hotchkiss RS, Karl IE: The pathophysiology and treatment of sepsis. *N Engl J Med* 2003; 348:138–150
36. Branger J, Knapp S, Weijer S, et al: Role of Toll-like receptor 4 in gram-positive and gram-negative pneumonia in mice. *Infect Immun* 2004; 72:788–794
37. Gingles NA, Alexander JE, Kadioglu A, et al: Role of genetic resistance in invasive pneumococcal infection: identification and study of susceptibility and resistance in inbred mouse strains. *Infect Immun* 2001; 69: 426–434
38. Prince AS, Mizgerd JP, Wiener-Kronish J, et al: Cell signaling underlying the pathophysiology of pneumonia. *Am J Physiol Lung Cell Mol Physiol* 2006; 291:L297–L300
39. Zhuo H, Yang K, Lynch SV, et al: Increased mortality of ventilated patients with endotracheal Pseudomonas aeruginosa without clinical signs of infection. *Crit Care Med* 2008; 36:2495–2503