Pulmonary and Systemic Host Response to *Streptococcus pneumoniae* and *Klebsiella pneumoniae* Bacteremia in Normal and Immunosuppressed Mice

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Mortality related to bacteremic pneumonia remains high, and the role of sepsis in inflammation, pulmonary injury, and death remains unclear, mostly in leukopenic states. In the present study, the microbiology, histopathology, and host response to *Streptococcus pneumoniae* and *Klebsiella pneumoniae* infection were determined in an experimental model of bacteremia in immunocompetent and leukopenic mice. Leukocyte depletion by cyclophosphamide did not impair the early clearance of pneumococci from blood but facilitated growth in lungs. By contrast, *klebsiellae* rapidly grew in blood of leukopenic mice. These observations suggest that tissue-based phagocytes and circulating leukocytes, respectively, play prominent roles in *S. pneumoniae* and *K. pneumoniae* eradication. The kinetics of leukocyte recruitment in lungs during *S. pneumoniae* bacteremia suggested early strong inflammation in immunocompetent mice that is associated with tumor necrosis factor alpha release and histological disorders, including cell debris and surfactant in alveolar spaces. Leukocyte depletion further stimulated pulmonary capillary leakage both in *S. pneumoniae* and *K. pneumoniae* bacteremia, which seemed attributable to bacterial virulence factors. Nitric oxide production did not differ significantly among groups. Leukopenia and low platelet counts characterized the late stage of bacteremia for both strains, but only *K. pneumoniae* altered renal function. Understanding the pathogenesis of bacteremia will help establish beneficial therapies for both sepsis and pneumonia.

Bacterial pneumonia is a leading cause of morbidity and mortality in both developed and developing countries, and *Streptococcus pneumoniae* remains the most common pathogen responsible for community-acquired pneumonia throughout the world. It has been reported that human pneumococcal pulmonary infection when complicated with bacteremia results in two to three fold-higher mortality rates (22). Our previous experimental studies pointed out a direct correlation between bacteremia and mortality in immunocompetent mice suffering from pneumococcal pneumonia (2, 3). In fact, hemodynamic and hemostatic changes and progressive multiple organ failure are the most frequently observed adverse effects of bacteremia in humans. Conversely, sepsis also accounts for as many as half of all cases of acute respiratory distress syndrome (ARDS) (14, 17, 37). Lung injury apparently occurs at the very onset of bacteremia, this organ often being the first to fail (37). However, the contribution of sepsis to lung injury in the context of bacteremic bacterial pneumonia remains unclear. In various pathological conditions (5, 8, 11, 14, 19, 24, 28, 33, 39), lung injury induced by bacteremia is characterized by increased microvascular permeability and edema; hence, pathophysiological changes in the alveolar-capillary barrier most likely contribute to mortality resulting from bacteremia.

A comparison of bacteremic community-acquired lobar pneumonia due to *S. pneumoniae* and *Klebsiella pneumoniae* in

an intensive care unit already showed that thrombocytopenia and leukopenia feature with increased frequency in patients with pulmonary infection due to *K. pneumoniae* (9). However, the pathogenesis of lung injury induced by bacteremia in leukopenic patients after exposure to either microorganism has been poorly defined. The use of chemotherapeutic agents for the treatment of cancer and other illnesses has led to an increasing number of patients with profound leukopenia (18). These patients often develop serious infections, such as pneumonia and bacteremia. Our recent experimental data with cyclophosphamide-treated mice suffering from pneumococcal pneumonia showed surprisingly high cytokine levels in blood of leukopenic animals and similar bacterial counts in blood compared with immunocompetent mice infected with the same size of inoculum, suggesting resistance to bacterial dissemination in leukopenic states by means other than circulating leukocytes (E. Wang, M. Simard, N. Ouellet, Y. Bergeron, D. Beauchamp, and M. G. Bergeron, submitted for publication).

A better understanding of host response to bacteremia in the context of immunosuppression is of therapeutic significance for the treatment of pulmonary infections induced by gram-positive and gram-negative bacteria. In the present study, we investigated the course of leukopenic and immunocompetent mouse responses to *S. pneumoniae* and *K. pneumoniae* bacteremia. We sought to determine the effect of leukopenia on the kinetics of bacterial growth and clearance and on the hematological and inflammatory responses, and we tried to determine the potential correlation of these factors to pulmonary vascular permeability and injury as well as to multiple organ failure.

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FIG. 1. Bacterial counts in bloodstream of four infected groups (means plus SD for five mice). *, value higher than that for immunocompetent mice with *S. pneumoniae* bacteremia ($P < 0.01$); §, value significantly higher than that for immunocompetent mice with *K. pneumoniae* bacteremia ($P < 0.001$).

MATERIALS AND METHODS

Animals. Female CD1 Swiss mice (obtained from Charles River, St-Constant, Quebec, Canada) weighing 18 to 20 g were used throughout the study. All protocols using animals were approved by the Laval University Animal Protection Committee. Animals had access to food and water throughout the experiment. They were acclimatized to our animal facilities for 1 week before the beginning of the experiment. Half of the animals were rendered leukopenic by intraperitoneal injections of 150 mg of cyclophosphamide (Charte-Horner Inc., Mississauga, Ontario, Canada) per kg of body weight 3 successive days before and 1 day after the bacterial challenge.

Bacteremia model. Clinically isolated encapsulated *S. pneumoniae* serotype 3 and *K. pneumoniae* strains were first grown on blood agar for 18 h. Freshly grown colonies were suspended in brain heart infusion (supplemented with 5% horse serum for *S. pneumoniae*) and incubated at 37°C overnight. On the day of infection, immunocompetent and leukopenic mice were injected in the tail vein with 100 µl of phosphate-buffered saline containing 10^6 CFU of either *S. pneumoniae* or *K. pneumoniae* cells. The size of inoculum was confirmed by serial dilution and quantitative subculture on blood agar. This inoculum was chosen based on bacterial counts recovered from blood of animals suffering from severe pneumonia (unpublished data). It induced mortality within 24 h in leukopenic mice and within 48 h in immunocompetent mice for either strain. Therefore, all parameters evaluated in the pathogenesis studies were tested within 36 h postinfection.

Experimental protocol. Leukopenic and immunocompetent animals were infected as described above. Five mice per group were sacrificed either before infection (control values) or at 0.5, 2, 4, 12, 24, or 36 h postinfection. The following parameters were determined: viable bacteria in blood and lungs, white blood cells (WBCs), platelets, myeloperoxidase (MPO) as a marker of neutrophil infiltration in lung tissue, nitric oxide (NO), various enzymatic markers in blood as indicators of organ failure, and electron microscopy. At the time of sacrifice, animals were killed by CO₂ inhalation and were immediately exsanguinated by intracardiac puncture. Blood was collected in heparinized tubes for hematological analysis. Bronchoalveolar lavage (BAL) fluid was obtained as previously described (2). Briefly, the trachea was exposed, a catheter was inserted, and then a volume of 0.6 ml of potassium phosphate buffer was infused and recovered; this procedure was repeated three times. BAL fluid was centrifuged at $1,800 \times g$ for 10 min, and supernatant was used to determine the concentration of NO end products. Lungs were removed and homogenized for the dosage of inflammatory mediators. Tissue sections were also made from pulmonary lobes for histological observations. Vascular permeability studies were performed with additional animals (five per group per time point), after injection of Evans blue, as described below.

Bacterial growth and clearance. Blood was collected and lungs were removed together with the heart, as previously described (2). Residual blood in pulmonary capillaries was removed from lungs through the infusion of sterile saline into the right ventricle until the effluent was clear. Lungs were then homogenized with a

FIG. 2. Bacterial counts in lungs from four infected groups (means plus SD for 5 mice). • and *, values significantly higher than those for immunocompetent mice with *S. pneumoniae* bacteremia (P values of <0.05 and <0.01 respectively); §, value significantly higher than that for immunocompetent mice with corresponding *S. pneumoniae* or *K. pneumoniae* bacteremia ($P < 0.001$).

Potter-Elvehjem homogenizer in 2 ml of potassium phosphate buffer (50 mM, pH 6.0) at 4°C. Bacterial counts in blood and lung homogenates were made on serial 10-fold dilutions plated on blood agar and incubated at 37°C for 18 h. The limit of detection was $2 \log_{10}$ CFU per ml of sample.

Hematological parameters. Blood cells (WBCs and platelets) were quantified using a Coulter Counter (SS80; Coulter Electronics, Hialeah, Fla.). Differential WBC counts were made on Wright-stained blood smears. The infiltration of neutrophils in lung tissues was quantified through the measurement of MPO, as previously described (2).

Inflammatory mediators. Tumor necrosis factor alpha (TNF- α) production was evaluated in lung homogenates and in serum. Six hundred microliters of phosphate buffer containing aprotinin (20 U) and 3-[3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS, 0.2%) was added to 600 μ l of lung homogenates. After centrifugation of lung homogenates and of blood, TNF- α was measured in the supernatants by sandwich enzyme-linked immunosorbent assays. The release of NO in BAL fluid was evaluated by the colorimetric method of Griess after reduction of nitrate into nitrite metabolites, as previously reported (2).

Lung vascular permeability. The Evans blue permeability assay was used to quantify lung capillary permeability. Evans blue avidly binds to serum albumin and can therefore be used as a tracer for transcapillary flux of macromolecules. The extravasation of Evans blue has frequently been employed to quantify vascular permeability (13, 21, 29, 36). Evans blue (0.2 ml at a concentration of 25 mg/ml) was injected in a tail vein 30 min prior to the sacrifice. Lungs were

homogenized in 2 ml of potassium phosphate buffer. Evans blue was extracted by incubating samples in 4 ml of formamide at 60°C for 24 h, followed by centrifugation at $5,000 \times g$ for 30 min. The concentration of Evans blue was estimated by dual-wavelength (620 and 740 nm) spectrophotometry, which allowed correction of optical densities (E) for contaminating heme pigments. Thus, the following formula was used: $E_{620}(\text{corrected}) = E_{620} - (1.426 \times E_{740} + 0.030)$.

Histopathological examination. Lung injury was observed by standard histology procedures (2). Whole lungs were fixed in 4% formalin, embedded in paraffin, and processed for light microscopy using eosin and hematoxylin stainings. Tissue sections were also fixed in 2.5% glutaraldehyde-0.1 M phosphate buffer (pH 7.4), postfixed in 1% osmium tetroxide, dehydrated, and embedded in Epon for electron microscopy.

Biochemical markers. To further detect potential multiple organ failure, various biochemical markers were quantified in serum (at all time points listed in the experimental protocol), including aspartate transaminase and alanine transaminase for the liver, creatine kinase for the heart, and creatinine and blood urea nitrogen (BUN) for the kidneys. Standard procedures were applied for the dosage of these markers, by routine clinical biochemistry laboratory protocols and devices.

Statistical analyses. Data are presented as means plus standard deviations (SD). Statistical analysis of the data was carried out by two-way analysis of variance for comparison of differences between groups. Fisher tests were used for multiple paired comparisons of data. A P value of <0.05 was considered significant.

FIG. 3. WBCs in peripheral bloodstream of four infected groups (means plus SD for five mice). •, value significantly higher than that for leukopenic mice with *S. pneumoniae* bacteremia ($P < 0.05$); *, value significantly higher than that for leukopenic mice with *K. pneumoniae* bacteremia ($P < 0.01$); §, value significantly higher than that for leukopenic mice with corresponding *S. pneumoniae* or *K. pneumoniae* bacteremia ($P < 0.001$). The value for noninfected immunocompetent mice was $7.2 \times 10^9 \pm 1.0 \times 10^9$ cells/liter; the value for noninfected leukopenic mice was $1.0 \times 10^9 \pm 0.5 \times 10^9$ cells/liter.

RESULTS

Bacterial counts in blood and lungs. The log CFU of *S. pneumoniae* and *K. pneumoniae* in blood and lungs are shown in Fig. 1 and 2, respectively. Shortly after infection (0.5 h), no significant difference was observed in blood CFUs from immunocompetent and leukopenic mice infected with *S. pneumoniae* or *K. pneumoniae* (Fig. 1). From that point, the kinetic of bacterial counts over time showed that maximal CFU in blood were observed at the earliest time point (0.5 h) postinfection in immunocompetent mice infected with either *S. pneumoniae* or *K. pneumoniae*, while maximal CFU were obtained 12 h postinfection in leukopenic mice (for both strains), from which time point animals started to die. Significantly higher *K. pneumoniae* counts were observed in blood of leukopenic mice than in blood of immunocompetent animals at 2, 4, and 12 h postinfection ($P < 0.001$), while only at 12 h could we see a difference in *S. pneumoniae* counts following leukocyte depletion ($P < 0.01$).

Leukocyte depletion rapidly promoted pulmonary invasion by *S. pneumoniae* (Fig. 2), as the log CFU of this strain in lungs

was significantly higher in leukopenic mice than in immunocompetent animals at 0.5, 2, 4, and 12 h postinfection (P values of <0.05 , <0.01 , <0.001 , and <0.001 , respectively). By contrast, *K. pneumoniae* counts in lungs of immunocompetent mice remained low at early times of the experiment, and leukocyte depletion promoted growth only from 4 h postinfection, with a striking difference being observed at 12 h ($P < 0.001$ at 4 and 12 h).

WBCs in peripheral bloodstream. Figure 3 shows the WBC counts in blood of infected immunocompetent and leukopenic mice. WBCs in infected leukopenic animals were consistently lower than 1.0×10^9 /liter, with no significant difference at any time point compared to uninfected leukopenic mice ($1.0 \times 10^9 \pm 0.5 \times 10^9$ cells/liter) for either strain tested. WBCs in blood of immunocompetent animals infected with *S. pneumoniae* reached a peak of 11.3×10^9 /liter at 2 h postinfection ($P < 0.001$ compared to noninfected immunocompetent mice, which had $7.2 \times 10^9 \pm 1.0 \times 10^9$ cells/liter), sharply declined thereafter (from 4 h), and remained below the levels in uninfected mice until death ($P < 0.01$ at 4, 12, and 24 h). The same

FIG. 4. MPO in lungs of four infected groups (means plus SD for five mice). §, value significantly higher than that for leukopenic mice with corresponding *S. pneumoniae* or *K. pneumoniae* bacteremia ($P < 0.001$). The value for noninfected immunocompetent mice was 0.5 ± 0.1 U/lung; the value for noninfected leukopenic mice was 0.4 ± 0.1 U/lung.

pattern prevailed with *K. pneumoniae* bacteremia, except that peak levels reached 9.8×10^9 /liter at 0.5 h and that the decline occurred earlier, at 2 h. In fact, the increase at 0.5 h did not differ significantly from that in noninfected immunocompetent mice, while significantly lower counts were observed at 2, 12, 24, and 36 h ($P \leq 0.05$). Significant differences (P values from <0.05 to <0.001) between infected immunocompetent and leukopenic animals were observed throughout the experiment for both strains tested.

MPO activity in lung tissues. Neutrophil recruitment into lung tissues was quantified by the intracellular enzymatic marker MPO (Fig. 4). As expected, MPO levels in lungs of infected leukopenic mice remained unchanged compared to those of noninfected leukopenic animals (0.4 ± 0.1 U/lung). Peak MPO activity was observed at 2 h postinfection in lungs of immunocompetent mice infected with either *S. pneumoniae* or *K. pneumoniae*; it decreased gradually thereafter. Of interest, MPO at 0.5 h was significantly higher in *S. pneumoniae*-infected immunocompetent mice than in *K. pneumoniae*-infected immunocompetent mice ($P < 0.001$).

Platelet counts in peripheral blood. Platelet counts (Fig. 5) were significantly affected by cyclophosphamide injections, de-

creasing from approximately $1,400 \times 10^9$ /liter in noninfected immunocompetent mice to 600×10^9 /liter in noninfected leukopenic mice ($P < 0.001$). Infection of immunocompetent mice with either *S. pneumoniae* or *K. pneumoniae* significantly reduced platelet counts at the latest time points (12, 24, and 36 h) compared to uninfected immunocompetent controls ($P < 0.01$ at 12 h and $P < 0.001$ at 24 and 36 h), but no further reduction in platelet counts was observed in leukopenic mice infected with either strain compared to the respective leukopenic noninfected controls.

Inflammatory mediator levels. TNF- α levels in infected mice are reported in Fig. 6. Early (2-h) peak secretion of TNF- α was seen in lungs (Fig. 6A) and serum (Fig. 6B) of *S. pneumoniae*-infected immunocompetent mice ($P \leq 0.05$ compared to leukopenic mice). TNF- α levels rapidly decreased thereafter. There was no significant difference in TNF- α levels between immunocompetent and immunosuppressed mice infected with *K. pneumoniae*. There was no significant increase in NO levels in BAL fluid of animals after induction of bacteremia with either bacterial strain tested in this experiment (data not shown).

FIG. 5. Platelet counts in peripheral blood of four infected groups (means plus SD for five mice). *, value significantly lower than that for immunocompetent uninfected mice ($P < 0.001$); §, value significantly lower than that for immunocompetent uninfected mice ($P < 0.001$). The value for noninfected immunocompetent mice was $1,380 \times 10^9 \pm 110 \times 10^9$ cells/liter; the value for noninfected leukopenic mice was $580 \times 10^9 \pm 70 \times 10^9$ cells/liter.

Pulmonary vascular permeability. The pulmonary vascular permeability (as evaluated by Evans blue extravasation) showed higher values ($P < 0.01$) in leukopenic mice than in immunocompetent mice 2 h after infection with either *S. pneumoniae* or *K. pneumoniae* (Fig. 7).

Histology. This bacteremia model did not induce major injuries to the lungs, in contrast to pneumonia models. Therefore, light microscopy did not reveal major differences among groups (data not shown) whereas electron microscopy did reveal otherwise undetectable tissue injuries. Electron microscopy (Fig. 8) showed slight neutrophil infiltration in perivascular areas of *S. pneumoniae*-infected (Fig. 8D) and *K. pneumoniae*-infected (Fig. 8F) lungs in immunocompetent mice. By contrast, no neutrophils were observed in lung interstitium of leukopenic mice (Fig. 8C and E). Cellular debris and surfactant in alveolar spaces were more abundant in *S. pneumoniae*-infected mice (Fig. 8C and D) (mostly the leukopenic group [Fig. 8C]) than in *K. pneumoniae*-infected animals (Fig. 8E and F). Pneumococci were also seen in capillaries of leukopenic mice (Fig. 8C) despite blood removal at the time of sacrifice. Lungs from *K. pneumoniae*-infected immunocompe-

tent mice (Fig. 8F) appeared to be less affected than those from any other infected group.

Biochemical function. No significant difference was observed among groups in serum aspartate transaminase, serum alanine transaminase, and creatine kinase levels throughout the experiment, with values of 215, 182, and 393 U/liter, respectively, in normal mice. However, BUN levels increased in *K. pneumoniae*-infected immunocompetent mice at 24 h (12.3 versus 6.4 mmol/liter, $P < 0.01$ compared to those in normal uninfected mice) while serum creatinine increased at 36 h in the same group (28.8 versus 11.0 μ mol/liter, $P < 0.01$ compared to those in normal uninfected mice). Infection with *S. pneumoniae* did not alter BUN and creatinine levels in this experiment.

DISCUSSION

Septicemia is known to contribute to the high mortality rate associated with pneumonia (3). However, the contribution of bacteremia to pulmonary capillary leakage, lung injury, and ARDS is still poorly defined. It is also not clear how blood and lungs respond to pneumococcal bacteremia in leukopenic

FIG. 6. TNF- α levels in lungs (A) and serum (B) of leukopenic and immunocompetent mice suffering from bacteremia induced by either *S. pneumoniae* or *K. pneumoniae* (means $\pm$ SD, for five mice). *, value significantly higher than that for leukopenic mice with *S. pneumoniae* bacteremia ($P < 0.01$). •, value significantly higher than that for leukopenic mice with *S. pneumoniae* bacteremia and that for noninfected immunocompetent mice ($P < 0.05$). The values for noninfected immunocompetent mice were 27 ± 30 pg/ml (lungs) and in 110 ± 50 pg/ml (serum); the values for noninfected leukopenic mice were 20 ± 18 pg/ml (lungs) and 160 ± 90 pg/ml (serum).

FIG. 7. Pulmonary vascular permeability in four infected groups (means plus SD for five mice). *, value significantly higher than that for immunocompetent mice with corresponding *S. pneumoniae* or *K. pneumoniae* bacteremia ($P < 0.01$). The value for noninfected immunocompetent and leukopenic mice was 1.4 ± 0.5 U.

states. Our recent observations made with leukopenic mouse models of pneumonia suggested that high levels of cytokines and chemokines may be produced in lungs and blood of bacteremic mice despite severe leukocyte depletion (Wang et al., submitted). The goal of the present study was to investigate the microbiological and inflammatory events that characterize host response and pulmonary injury during bacteremia induced by either *S. pneumoniae* or *K. pneumoniae* in leukopenic mice and to compare them with those observed in immunocompetent mice.

The kinetics of bacterial growth and clearance suggested that leukocyte depletion did not impair the host capacity to control *S. pneumoniae* in blood over the first hours following infection, although it facilitated growth in lungs. These data support our observations with leukopenic mice suffering from pneumococcal pneumonia, which suggested that host defense mechanisms responsible for removal of *S. pneumoniae* from bloodstream differed from those in lungs (Wang et al., submitted). In contrast, leukocyte depletion rapidly impaired the clearance of *K. pneumoniae* from both the bloodstream and the lungs. These data suggest that circulating leukocytes are key

mediators for *K. pneumoniae* clearance but that tissue-based phagocytes might play a more prominent role in the clearance of *S. pneumoniae*. Our results also suggest that lungs are pre-disposed targets for the proliferation of *S. pneumoniae* while the bloodstream would preferentially be susceptible to *K. pneumoniae* growth in immunosuppressed hosts.

In clinical settings, the rate of bacteremia due to gram-negative bacteria and fungi dramatically increases in leukopenic patients. For instance, an eightfold increase in the rate of gram-negative bacteremia (such as *K. pneumoniae*, *Escherichia coli*, or *Pseudomonas aeruginosa*) was observed in patients with absolute neutrophil counts lower than or equal to 500 cells/ml for a period of less than 1 week (23). It is generally assumed that pneumococcal bacteremia in this population is uncommon (16, 27). However, most pneumococcal bacteremias that develop in neutropenic patients with cancer are associated with the development of pneumococcal pneumonia (4, 6, 7). Therefore, in the prevention and treatment of *S. pneumoniae* and *K. pneumoniae* infections in leukopenic hosts, lung tissue and the bloodstream, respectively, should be considered privileged sites.

FIG. 8. Histopathology of lung tissues in leukopenic (A, C, and E) and immunocompetent (B, D, and F) mice which were either not infected (A and B) or sacrificed 4 h after infection with either *S. pneumoniae* (C and D) or *K. pneumoniae* (E and F). Slight neutrophil infiltration was noted in perivascular areas of *S. pneumoniae*-infected (D) and *K. pneumoniae*-infected (F) lungs in immunocompetent mice. By contrast, no neutrophils were observed in lung interstitium of leukopenic mice (C and E). Cellular debris and surfactant in alveolar spaces were more abundant in *S. pneumoniae*-infected mice (C and D) (mostly the leukopenic group [C]) than in *K. pneumoniae*-infected animals (E and F). Pneumococci were also seen in capillaries of leukopenic mice (C). A, alveoli; B, bacteria; N, neutrophil; P₂, type 2 pneumocyte; S, surfactant. Bar = 5 μ m.

The kinetics of leukocyte counts in blood and MPO activity in lung tissues that we observed in *S. pneumoniae*-infected immunocompetent mice showed maximal availability of circulating leukocytes in blood and neutrophil sequestration in lungs within 2 h postinfection. Cell activity was associated with a high pulmonary capillary permeability index compared to that in uninfected mice and with maximal TNF- α release at 2 h, followed at 4 h by histological anomalies such as debris and surfactant in alveolar spaces. In recent years neutrophils were shown to participate in lung injury and edema (1, 10, 31, 38). The concept was largely derived from the fact that neutrophil depletion protected against the increase in pulmonary vascular permeability which occurred in various animal models of acute lung injury induced by nonbacterial stimulating agents (15, 32). However, many aspects of this concept extrapolated from non-bacterial models remained unverified in bacteremia. In our experiment, a significantly higher ($P < 0.05$ at 2 h) permeability index was observed in leukopenic mice than in corresponding immunocompetent infected mice. Since intrapulmonary neutrophil sequestration was not seen in leukopenic animals and nitrogen intermediates (NO levels) remained low in lungs, bacterial virulence factors (e.g., teichoic acid and pneumolysin for *S. pneumoniae* and endotoxin for *K. pneumoniae*) may have contributed to the enhanced permeability and lung tissue injuries. In vitro, the binding of bacterial cell walls to endothelia leads to the separation of contiguous endothelial cells, with a resulting loss of endothelium barrier function (12). The same phenomenon could have occurred in bacteremia, thus contributing to the observed increase in vascular permeability. Therefore, the putative role of leukocytes as inducers of edema may be misleading in bacteremia. In fact, most leukopenic patients with bacteremia subsequently develop diffuse lung injury or ARDS (20, 25, 26, 30, 34, 35).

As for the reduction in blood WBCs and fall in platelet counts observed in the late stage of both *S. pneumoniae*- and *K. pneumoniae*-induced bacteremia in immunocompetent mice, they might be related to cell recruitment to the lungs and disseminated intravascular coagulation, respectively. While hematological disorders, ultrastructural changes to the lungs, and rapid mortality characterized bacteremia in both immunocompetent and leukopenic mice, biochemical analyses showed no major effects on other organs in this model, and mice apparently did not die from multiple organ failure. Only the BUN and creatinine levels were shown to be affected shortly before death in the *K. pneumoniae*-infected immunocompetent group, suggesting that renal dysfunction most likely was induced by vasoactive mediators triggered by bacterial components. Despite the complexity of the pathophysiologic changes and toxicity reactions that together contribute to morbidity and mortality in sepsis, advances in our understanding of the factors that ultimately affect the outcome of bacteremia and pneumonia will allow us to improve the therapy of these threatening infections.

REFERENCES

- Baird, B. R., J. C. Cheronis, R. A. Sandhaus, E. M. Berger, C. W. White, and J. E. Repine. 1986. O₂ metabolites and neutrophil elastase synergistically cause edematous injury in isolated rat lungs. *J. Appl. Physiol.* **61**:2224–2229.
- Bergeron, Y., N. Ouellet, A. M. Deslauriers, M. Simard, M. Olivier, and M. G. Bergeron. 1998. Cytokine kinetics and other host factors in response to pneumococcal pulmonary infection in mice. *Infect. Immun.* **66**:912–922.
- Bergeron, Y., and M. G. Bergeron. 1999. Why does pneumococcus kill? *Can. J. Infect. Dis.* **10**(Suppl. C):49C–60C.
- Bernard, C., G. Mombelli, and J. Klastersky. 1981. Pneumococcal bacteremia in patients with neoplastic diseases. *Eur. J. Cancer Clin. Oncol.* **17**:1041–1046.
- Bernard, G. R., A. Artigas, K. L. Brigham, J. Carlet, K. Falke, L. Hudson, M. Lamy, J. R. LeGall, A. Morris, and R. Spragg. 1994. The American-European Consensus Conference on ARDS. Definitions, mechanisms, relevant outcomes, and clinical trial coordination. *Am. J. Respir. Crit. Care Med.* **149**:818–824.
- Carratala, J., A. Marron, A. Fernandez-Sevilla, J. Linares, and F. Gudiol. 1997. Treatment of penicillin-resistant pneumococcal bacteremia in neutropenic patients with cancer. *Clin. Infect. Dis.* **24**:148–152.
- Chou, M. Y., A. E. Brown, A. Blevins, and D. Armstrong. 1983. Severe pneumococcal infection in patients with neoplastic disease. *Cancer* **51**:1546–1550.
- Downey, G. P., Q. Dong, J. Kruger, S. Dedhar, and V. Cherapanov. 1999. Regulation of neutrophil activation in acute lung injury. *Chest* **116**(Suppl. 1):46S–54S.
- Feldman, C., J. M. Kallenbach, H. Levy, J. R. Thorburn, M. D. Hurwitz, and H. J. Koornhof. 1991. Comparison of bacteraemic community-acquired lobar pneumonia due to *Streptococcus pneumoniae* and *Klebsiella pneumoniae* in an intensive care unit. *Respiration* **58**:265–270.
- Flick, M. R., A. Perel, and N. C. Staub. 1981. Leukocytes are required for increased lung microvascular permeability after microembolization in sheep. *Circ. Res.* **48**:344–351.
- Fulkerson, W. J., N. MacIntyre, J. Stamler, and J. D. Crapo. 1996. Pathogenesis and treatment of the adult respiratory distress syndrome. *Arch. Intern. Med.* **156**:29–38.
- Geelen, S., C. Bhattacharyya, and E. Tuomanen. 1993. The cell wall mediates pneumococcal attachment to and cytopathology in human endothelial cells. *Infect. Immun.* **61**:1538–1543.
- Green, T. P., D. E. Johnson, R. P. Marchessault, and C. W. Gatto. 1988. Transvascular flux and tissue accrual of Evans blue: effects of endotoxin and histamine. *J. Lab. Clin. Med.* **111**:173–183.
- Hasleton, P. S., and T. E. Roberts. 1999. Adult respiratory distress syndrome. *Histopathology* **34**:285–294.
- Heffin, A. C., Jr., and K. L. Brigham. 1981. Prevention by granulocyte depletion of increased vascular permeability of sheep lung following endotoxemia. *J. Clin. Invest.* **68**:1253–1260.
- Howard, M. W., R. G. Strauss, and R. B. Johnston, Jr. 1977. Infections in patients with neutropenia. *Am. J. Dis. Child.* **131**:788–790.
- Hudson, L. D., and K. P. Steinberg. 1999. Epidemiology of acute lung injury and ARDS. *Chest* **116**(Suppl. 1):74S–82S.
- Kerr, K. G. 1999. The prophylaxis of bacterial infections in neutropenic patients. *J. Antimicrob. Chemother.* **44**:587–591.
- Kollef, M. H., and D. P. Schuster. 1995. The acute respiratory distress syndrome. *N. Engl. J. Med.* **332**:27–37.
- Laufe, M. D., R. H. Simon, A. Flint, and J. B. Keller. 1986. Adult respiratory distress syndrome in neutropenic patients. *Am. J. Med.* **80**:1022–1026.
- Lundberg, J. M., and A. Saria. 1983. Capsaicin-induced desensitization of airway mucosa to cigarette smoke, mechanical and chemical irritants. *Nature* **302**:251–253.
- Mannes, G. P., W. G. Boersma, C. H. Baur, and P. E. Postmus. 1991. Adult respiratory distress syndrome (ARDS) due to bacteraemic pneumococcal pneumonia. *Eur. Respir. J.* **4**:503–504.
- Mathews, W. C., J. Caperna, J. G. Toerner, R. E. Barber, and H. Morgenstern. 1998. Neutropenia is a risk factor for gram-negative bacillus bacteremia in human immunodeficiency virus-infected patients: results of a nested case-control study. *Am. J. Epidemiol.* **148**:1175–1183.
- Matthay, M. A. 1990. The adult respiratory distress syndrome. Definition and prognosis. *Clin. Chest Med.* **11**:575–580.
- Maunder, R. J., R. C. Hackman, E. Riff, R. K. Albert, and S. C. Springmeyer. 1986. Occurrence of the adult respiratory distress syndrome in neutropenic patients. *Am. Rev. Respir. Dis.* **133**:313–316.
- Ognibene, F. P., S. E. Martin, M. M. Parker, T. Schlesinger, P. Roach, C. Burch, J. H. Shelhamer, and J. E. Parrillo. 1986. Adult respiratory distress syndrome in patients with severe neutropenia. *N. Engl. J. Med.* **315**:547–551.
- Pincus, S. H., L. A. Boxer, and T. P. Stossel. 1976. Chronic neutropenia in childhood. Analysis of 16 cases and a review of the literature. *Am. J. Med.* **61**:849–861.
- Rinaldo, J. E., and R. M. Rogers. 1982. Adult respiratory distress syndrome: changing concepts of lung injury and repair. *N. Engl. J. Med.* **306**:900–909.
- Rinkema, L. E., K. G. Bemis, and J. H. Fleisch. 1984. Production and antagonism of cutaneous vascular permeability in the guinea pig in response to histamine, leukotrienes and A23187. *J. Pharmacol. Exp. Ther.* **230**:550–557.
- Sivan, Y., C. Mor, S. Jundi, and C. J. Newth. 1990. Adult respiratory distress syndrome in severely neutropenic children. *Pediatr. Pulmonol.* **8**:104–108.
- Tate, R. M., and J. E. Repine. 1983. Neutrophils and the adult respiratory distress syndrome. *Am. Rev. Respir. Dis.* **128**:552–559.
- Till, G. O., K. J. Johnson, R. Kunkel, and P. A. Ward. 1982. Intravascular activation of complement and acute lung injury. Dependency on neutrophils

- and toxic oxygen metabolites. *J. Clin. Invest.* **69**:1126–1135.
33. **Toews, G. B.** 1999. Cellular alterations in fibroproliferative lung disease. *Chest* **116**(Suppl. 1):112S–116S.
34. **Vansteenkiste, J. F., and M. A. Boogaerts.** 1989. Adult respiratory distress syndrome in neutropenic leukemia patients. *Blut* **58**:287–290.
35. **Ward, P. A., and K. J. Johnson.** 1987. Adult respiratory distress syndrome and neutropenia. *N. Engl. J. Med.* **316**:413.
36. **Wei, E. T., J. G. Kiang, P. Buchan, and T. W. Smith.** 1986. Corticotropin-releasing factor inhibits neurogenic plasma extravasation in the rat paw. *J. Pharmacol. Exp. Ther.* **238**:783–787.
37. **Wheeler, A. P., and G. R. Bernard.** 1999. Treating patients with severe sepsis. *N. Engl. J. Med.* **340**:207–214.
38. **Xie, J., R. Li, P. Kotovuori, C. Vermot-Desroches, J. Wijdenes, M. A. Arnaout, P. Nortamo, and C. G. Gahmberg.** 1995. Intercellular adhesion molecule-2 (CD102) binds to the leukocyte integrin CD11b/CD18 through the A domain. *J. Immunol.* **155**:3619–3628.
39. **Zimmerman, G. A., K. H. Albertine, H. J. Carveth, E. A. Gill, C. K. Grissom, J. R. Hoidal, T. Imaizumi, C. G. Maloney, T. M. McIntyre, J. R. Michael, J. F. Orme, S. M. Prescott, and M. S. Topham.** 1999. Endothelial activation in ARDS. *Chest* **116**(Suppl. 1):18S–24S.

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