Intrapulmonary Growth of *Staphylococcus aureus* in Rats during Induced Atelectasis

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Intrinsic pulmonary antibacterial defenses are mediated by alveolar macrophages and by noncellular factors. Mechanical ventilation in the resting tidal volume range leads to alterations in the physical characteristics of alveolar surfactant, alveolar instability, regional hypoxia, and systemic hypoxemia. While a number of experimental manipulations diminish the activity of the intrinsic antibacterial defense system, the effects of mechanical ventilation per se have not been systematically evaluated previously. We found that normal rats ventilated without sighing (periodic large breaths) manifested severe defects in pulmonary clearance of *Staphylococcus aureus* during 6-h experiments, such that growth of the inoculum occurred. Addition of a timer-controlled mechanism to cause the animals to sigh every 2 min, without other modifications in the experimental conditions, caused significant improvement in clearance. Analysis of cellular response, compartmentalization of viable bacteria, surfactant quantities and sedimentation characteristics, and protein influx indicated that the defect in clearance paralleled alterations in the physical state of surfactant and alveolar stability but was not strongly correlated with alterations in the other parameters we measured. The data show that defective pulmonary bacterial clearance is rapidly induced by measures which alter alveolar stability and suggest that intrinsic pulmonary defenses require maintenance of normal air-liquid interfaces for optimal function.

The mechanisms responsible for eradication of bacteria which have been inhaled or aspirated into pulmonary alveoli have been the subject of considerable interest during the past 2 decades. Multiple mechanisms are involved in maintaining sterility of the lower airways. Effectors intrinsic to the pulmonary parenchyma, including alveolar macrophages (10, 11, 31), alveolar surfactant and associated fatty acids (3, 5, 15), and cationic peptides, including lysozyme (2, 8, 26, 29), appear to form the initial barrier to bacterial infection of the lungs. These intrinsic mechanisms are sufficient to eradicate inhaled or aspirated *Staphylococcus aureus* (28), as long as the inoculum is not overly large (36). When the intrinsic mechanisms are inadequate to eradicate the microbes, the activity of secondary defense mechanisms, including polymorphonuclear leukocytes (PMNs), serum antibody, and complement becomes pivotal (4, 31, 36, 37).

While it is recognized that the anatomy of the lungs is uniquely adapted to gas exchange and that manipulations which transiently alter the microarchitecture can lead to profound physiologic alterations (19, 20, 22, 35), there has been no evaluation of the possible interaction between transient structural alterations and pulmonary host defense mechanisms. Mechanical ventilation is known to increase the risk of pneumonia in humans (6, 33). Ventilation with a constant tidal volume (CTV) in the resting range results in changes in the physical state of alveolar surfactant and a functional surfactant deficiency that is associated with microatelectasis and regional alveolar hypoxia (19, 20, 35). Similar alterations in surfactant also follow brief exposure to hyperthermia (19). These physical changes are rapidly inducible and readily reversible by periodic large inflations of the lungs (21). Such physical changes could lead to impairment of pulmonary host defenses. In addition to the known ability of hypoxia to suppress pulmonary clearance of *S. aureus* (11, 12) and phagocytosis by alveolar macrophages (34), it is possible that macrophages, as well as other respiratory tract cells and secretions, are adapted to function optimally at the air-liquid interface normally present in the lungs and that disruption of that environment may impair function (30).

In the present work, we studied the pulmonary clearance of *S. aureus* in rats during ventilation at CTV. Pulmonary antibacterial function was profoundly depressed, such that net growth of the inoculum occurred in experiments that lasted only 6 h. Such severe impairment was not observed in our previous experiments with either influenza infection (24) or chronic glucocorticoid administration (25). Altering the exhalation port of the mechanical ventilator to provide periodic large breaths ("sighs") caused significant improvement in pulmonary clearance of *S. aureus* that paralleled the reversal of microatelectasis and associated surfactant alterations. At all sampling points, in all groups, greater than 80% of *S. aureus* appeared to be extracellular. Defective elimination or growth of *S. aureus*, when it occurred, was seen in both extracellular and intracellular populations.

**MATERIALS AND METHODS**

**Animals.** Adult male Fischer 344 rats weighing 200 to 260 g (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) were used in all experiments.

**Ventilation.** All rats were anesthetized with intraperitoneal ketamine and xylazine, and the trachea was exposed. Animals selected for mechanical ventilation had a plastic endotracheal tube inserted through an anterior tracheal incision. After the endotracheal tube was inserted, anesthe-
tized rats were mechanically ventilated at CTV with warmed, humidified air delivered by a Harvard small-animal-volume-cycled ventilator (Harvard Apparatus Co., Inc., S. Natick, Mass.). Ventilated rats were kept anesthetized throughout the experiments by repeated administration of ketamine plus xylazine. Control rats were allowed to recover from the initial anesthesia and were fully active within 30 min. The nomogram of Kleinman and Radford (14) was used to determine the tidal volume appropriate for the body weight of the animal and a respiratory rate of 50 breaths per min. “Sighed” rats received a two- to threefold tidal volume inflation once every 2 min (CTV+S). Rectal temperature of ventilated rats was continuously monitored and maintained at 37 or 40°C with a heating pad controlled by a rectal thermometer probe. Without use of the heating pad, rectal temperature remained at approximately 34°C. Control rats had rectal temperatures at sacrifice of 36 to 38°C without a heating pad and were not constantly monitored.

Determination of clearance of bacteria. S. aureus 502A was maintained on brain heart infusion agar plates and grown overnight in tryptic soy broth for use. The bacteria were washed and suspended in sterile phosphate-buffered saline (PBS). Washed bacterial suspensions were diluted to approximately 2 × 10⁷ to 3 × 10⁸ CFU/ml for most experiments. The numbers of viable organisms in each inoculum were quantitated by plate counts of serial 10-fold dilutions. After ventilation was established, 0.1 ml of a PBS suspension of washed bacteria was injected via a 25-gauge needle placed into the tracheal cannula and ventilation was immediately resumed. Each ventilated animal was matched with a spontaneously breathing control animal, inoculated by injection into the exposed trachea. Wound clips were used to close the neck incisions. The bacterial counts in the inoculum were assumed to represent t = 0 counts for clearance rate estimation.

Animals were sacrificed by aortic exsanguination 1 or 6 h after intratracheal injection. Lungs were removed and ground in sterile glass tissue grinders. Serial dilutions of lung homogenates were quantitatively cultured, and the fraction of the initial inoculum remaining at each time was determined.

In separate experiments, we determined the fraction of total viable organisms present in the lavage fluid and within macrophages, as well as the numbers remaining in lungs after lavage. At the time of sacrifice, lungs were first lavaged with 50 ml of PBS containing 1.25 mM EDTA in 10-ml portions. Samples of lavage fluid were diluted in sterile, cold water and plated. Cellular elements in the lavage were collected by centrifugation (150 × g for 10 min) and incubated with 10 U of lysozyme per ml in PBS for 10 min at 37°C to eliminate cell-associated extracellular S. aureus (23). Incubation of our stock of S. aureus 502A with 10 U of lysozyme per ml killed >99.5% of a suspension with 10⁶ CFU/ml in 10 min. The lysozyme-treated cellular elements were then washed twice with PBS and suspended in 1 ml of ice-cold H₂O for macrophage lysis and plating. After washing, no residual lysozyme activity could be detected against a plated test inoculum of 502A.

In vitro macrophage bactericidal activity. For these experiments, rats were inoculated by intratracheal injection of S. aureus. After 1 h, to allow in vivo phagocytosis of a portion of the inoculum, the animals were sacrificed and the lungs were lavaged with PBS. After centrifugation at 150 × g, the pelleted cells were suspended in tissue culture medium (medium 199 plus 10% newborn calf serum) containing lysozyme (10 U/ml) and incubated at 37°C to eliminate extracellular bacteria. Monolayers of infected cells were established in glass Leighton tubes, which were then gassed with either air and 5% CO₂ or nitrogen and 5% CO₂, and the tubes were tightly stoppered (34). After incubation for 1 h, nonadherent cells and debris were removed with two rinses of warm medium 199. Tissue culture medium containing penicillin G (10 U/ml) was replaced, and the tubes were gassed with air or nitrogen. Although anaerobiosis was not obtained in this manner, the po₂ was ≤40 torr (1 torr = 133.3 Pa), as determined by mass spectrometry (Perkin-Elmer 1100) in the tubes gassed with nitrogen, a level of oxygenation comparable with that of mixed venous blood. At t = 0 and t = 24 h, quantitative cultures of distilled water lysates of penicillinase-treated macrophage monolayers were performed.

Bronchoalveolar lavage phospholipids, protein, and cells. To assess phagocytic cell response and alterations in phospholipids, the lungs of control rats, ventilated rats inoculated with 502A, and ventilated, uninfected rats were lavaged with 0.15 M NaCl at 4 h. Recovery of lavage fluid was identical and was >90% in all animals. Lavage fluid was centrifuged at 150 × g for 10 min to separate cellular and noncellular components. The cell pellet was suspended in PBS for determination of cell count by hemacytometry and of differential count by microscopic assessment of Diff-Quik-stained cytopsin preparations. Physical properties of alveolar surfactant were assessed as originally described by Thet et al. (35). Briefly, the supernatant fluid was centrifuged at 1,000 × g for 20 min, and the concentration of disaturated phosphatidylcholine (DSPC), used as a marker for alveolar surfactant phospholipid, in the sediment was compared with that in the lyophilized 1,000 × g supernatant. Lipids were extracted from both fractions by the method of Folch et al. (9). DSPC, separated with osmium tetroxide (18) and isolated on silica gel thin layer chromatography, was quantitated by the lipid phosphorus assay of Roux et al. (32). The amount in the pellet was expressed as a proportion of the total amount of DSPC present in the lavage returns. Total protein concentration in the low-speed supernatant fluid was determined by the method of Lowry et al. (17).

Physiologic parameters. Static respiratory compliance was determined at the onset and after 4 h of ventilation. Airway pressure was estimated by water manometry over 5 s while the lung was inflated with the volume of air selected as the tidal volume (14). The 4-h value was expressed as a fraction of the initial value. In addition, abdominal aortic blood samples were obtained from some animals just before sacrifice for determination of arterial pO₂, pCO₂, and pH, measured with a Corning 178 analyzer.

Histology. Other animals were ventilated and inoculated as described above, and the lungs were removed to allow histological examination of the alveolar architecture. After the animals were sacrificed by exsanguination, the lungs were removed and inflated with buffered 10% Formalin at a constant pressure of 8 to 10 cm H₂O for 16 h. Tissue sections were stained with hematoxylin-eosin and the Brown-and-Brenn stain.

Statistical methods. Results are expressed as means ± standard error. Student’s t test, analysis of variance with Tukey’s test for significance of differences between pairs of means, and multivariate regression analysis were carried out with Systat software (Systat Inc., Evanston, Ill.). To test the contributions of the major experimental variables to clearance, multivariate analysis was carried out using the following equation: Residual S. aureus CFU (log₁₀) in lungs at 6 h = constant + sigh (0 or 1) + temperature (34, 37, or 40°C) + S. aureus CFU (log₁₀) in inoculum.
TABLE 1. Survival of *S. aureus* after intratracheal inoculation in animals ventilated with and without sighing and simultaneous controls<sup>a</sup>

<table>
<thead>
<tr>
<th>Group and temp (°C)</th>
<th>n&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Inoculum&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Ventilated CFU&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Fraction remaining&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Concurrent control CFU</th>
<th>Fraction remaining&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTV (34)</td>
<td>7</td>
<td>6.03 ± 0.12</td>
<td>5.95 ± 0.07</td>
<td>1.01 ± 0.21</td>
<td>5.24 ± 0.13</td>
<td>0.24 ± 0.06</td>
</tr>
<tr>
<td>CTV + S (34)</td>
<td>6</td>
<td>6.15 ± 0.14</td>
<td>6.50 ± 0.13</td>
<td>1.05 ± 0.18</td>
<td>5.61 ± 0.24</td>
<td>0.20 ± 0.08</td>
</tr>
<tr>
<td>CTV (37)</td>
<td>8</td>
<td>6.52 ± 0.13</td>
<td>6.39 ± 0.12</td>
<td>0.64 ± 0.10</td>
<td>6.01 ± 0.13</td>
<td>0.26 ± 0.05</td>
</tr>
<tr>
<td>CTV + S (37)</td>
<td>9</td>
<td>6.64 ± 0.08</td>
<td>6.34 ± 0.19</td>
<td>1.47 ± 0.35</td>
<td>5.67 ± 0.14</td>
<td>0.30 ± 0.08</td>
</tr>
<tr>
<td>CTV (40)</td>
<td>8</td>
<td>6.27 ± 0.11</td>
<td>6.14 ± 0.14</td>
<td>0.46 ± 0.10</td>
<td>5.66 ± 0.20</td>
<td>0.19 ± 0.06</td>
</tr>
<tr>
<td>CTV + S (40)</td>
<td>6</td>
<td>6.54 ± 0.14</td>
<td>6.28 ± 0.09</td>
<td>1.18 ± 0.15</td>
<td>5.52 ± 0.11</td>
<td>0.25 ± 0.04</td>
</tr>
<tr>
<td>Mean CTV</td>
<td>23</td>
<td>6.29 ± 0.08</td>
<td>6.14 ± 0.09</td>
<td>0.56 ± 0.06</td>
<td>5.70 ± 0.12</td>
<td>0.24 ± 0.04</td>
</tr>
<tr>
<td>Mean CTV + S</td>
<td>21</td>
<td>6.47 ± 0.08</td>
<td>6.21 ± 0.07</td>
<td>0.86 ± 0.10</td>
<td>5.60 ± 0.08</td>
<td>0.24 ± 0.03</td>
</tr>
<tr>
<td>Mean CTV-CTV + S</td>
<td>44</td>
<td>6.38 ± 0.06</td>
<td>6.21 ± 0.07</td>
<td>0.86 ± 0.10</td>
<td>5.60 ± 0.08</td>
<td>0.24 ± 0.03</td>
</tr>
</tbody>
</table>

<sup>a</sup> Control animals received intratracheal injections of *S. aureus* at the same time as the ventilated animals with which they were paired. After recovery from the anesthesia, the controls were awake, spontaneously breathing, and normothermic (36 to 38°C just before sacrifice).

<sup>b</sup> Number of animals in each ventilated or control group: data from experiments using 88 animals are included in this table.

<sup>c</sup> Bacterial counts are log<sub>10</sub> of the mean ± standard error of the mean.

<sup>d</sup> Mean ± standard error of the mean of CFU at 6 h CFU delivered in inoculum.

RESULTS

Clearance. The clearance data are summarized in Table 1. After direct intratracheal injection of 10<sup>6.38 ± 0.00</sup> *S. aureus*, 24 ± 3% of the initial inoculum remained viable at 6 h in spontaneously breathing control rats. While the anesthetized, ventilated rats (CTV or CTV+S) exhibited abnormal clearance of *S. aureus* when compared with the spontaneously breathing, awake controls (P < 0.001), the most profound defect in bacterial clearance was induced by mechanical ventilation without sighing. In rats ventilated at CTV, no net clearance of *S. aureus* occurred, with the 6-h viable bacterial counts being 118 ± 15% of the original inoculum (n = 23). Addition of an external sighing mechanism, which resulted in inflation of the lungs to two- to threefold tidal volume every 2 min, caused significant improvement in clearance, such that 56 ± 6% of the inoculum remained at 6 h. Thus, while this mechanical intervention did not completely normalize clearance rates, net reduction in bacterial viability occurred, and in only 1 of 21 animals was the 6-h colony count greater than the inoculum. While there was no overall statistically significant effect of varying the temperature from 34 to 40°C, all animals in which the inoculum doubled within the 6-h period were in the unsighed group maintained at 40°C, a temperature which is known to diminish alveolar stability (19).

There were several variables that could have influenced the numbers of viable *S. aureus* cultured from the lungs at 6 h: the ventilation status (including intubation, anesthesia, and sighing), the size of the inoculum, and the temperature of the animals. As already noted, none of the ventilated, anesthetized groups eliminated the challenge organisms as well as the spontaneously breathing, awake animals did. When all the data for the ventilated animals in the experiments summarized in Table 1 were analyzed by multivariate regression analysis for the effects of ventilation mode, inoculum size, and temperature, residual counts at 6 h were highly correlated with inoculum size (P ≤ 0.001) and with whether the animal was signed during the experiment (P ≤ 0.003); the influence of temperature was not significant (P ≤ 0.3). For the simultaneous controls, numbers of *S. aureus* recovered from the lungs at 6 h were significantly correlated only with inoculum size (P ≤ 0.001). As expected, since the grouping reflects only the status of the animals with which the controls were paired, there was no significant variation related to temperature (P ≤ 0.4) or sighing status (P ≤ 0.8) (of the ventilated partner).

In an attempt to better characterize the mechanisms whereby ventilation altered pulmonary clearance of *S. aureus*, bronchoalveolar lavage fluid was evaluated by differential centrifugation and quantitative cultures to assess changes in viable intracellular and extracellular bacteria (Fig. 1). For the 1-h sample, the total numbers of viable *S. aureus* and the proportion in each fraction did not differ significantly (P > 0.3) among the three groups of animals. It should be noted that this observation supports the assumption we made in the previous set of experiments that equivalent inocula were delivered to all three groups. However, at 6 h, the values for each compartment were significantly (P < 0.05) higher in the CTV animals than in the other two groups. The values in the CTV+S group were also significantly higher than those of the controls for total and lavage fluid counts but were of borderline significance (P ≤ 0.1) for the macrophage fraction. In all groups, the changes in *S. aureus* viability in each fraction paralleled that of bacteria from the total lung samples. In contrast to the decline in viability of *S. aureus* seen in the other two groups, net growth occurred in the lungs and lavage fluid of animals ventilated at CTV. At 1 h, similar numbers of *S. aureus* were lysostaphin resistant and presumably intracellular in all three groups; because there was no decline in these numbers in the cells of the CTV animals, there were significantly more cell-associated organisms by 6 h in that group.

Lavage cells, protein, and DSPC. Both mechanically ventilated groups of animals had increased PMNs in lavage fluid compared with spontaneously breathing control rats given the same inoculum (Table 2). However, the PMN influx in the CTV and CTV+S groups did not differ from each other. Lavage protein was likewise elevated in both ventilated groups when compared with control or normal, un inoculated rats, but the concentrations were similar in CTV and CTV+S animals. The expected changes in amount and
sedimentation characteristics of recoverable alveolar surfactant (20, 35) were found in these experiments (Table 2). Animals ventilated at CTV had less DSPC in lavage fluid than controls (Table 2). In addition, a higher percentage of the total lavage DSPC was pelleted by centrifugation at 1,000 × g after CTV ventilation than in either of the other groups. We found the same increase in pelleted DSPC in animals which did not receive a bacterial inoculum and were ventilated at CTV, indicating that this change resulted from mode of ventilation and not from bacterial inoculation.

Compliance and blood gases. Paralleling the changes in physical properties of alveolar surfactant, static compliance decreased in both mechanically ventilated groups but the decrease was greater in the CTV than the CTV+S group. After 4 h of ventilation, approximately midway through the clearance experiments, lung compliance (84 ± 5% of initial values) and arterial pO2 (84 ± 4 torr) were significantly better in sighed animals than in unsighed animals (compliance = 55 ± 3% of initial values; arterial pO2 = 68 ± 5 torr) (P ≤ 0.05 for both comparisons). Arterial pH (7.35 ± 0.02) and pCO2 (42 ± 2 torr) in unsighed animals were not significantly different from those of sighed animals (pH = 7.38 ± 0.02, pCO2 = 38 ± 2 torr).

In vitro bactericidal activity of hypoxic macrophages. Because it appeared possible that focal alveolar collapse and consequent alveolar hypoxia may have led to defective pulmonary antibacterial activity by inhibiting the bactericidal action of macrophages, the effect of hypoxia on the ability of alveolar macrophages to kill previously ingested S. aureus was tested in vitro. After phagocytosis in vivo in the lungs of CTV rats (n = 5) or spontaneously breathing rats (n = 5), intracellular bactericidal activity of alveolar macrophages cultivated in an atmosphere of ≥95% nitrogen was not significantly impaired compared with that in air. Macrophages from the CTV animals were indistinguishable from those from controls. In these experiments, the cell-associated bacterial count was (2.34 ± 0.9) × 10⁶ CFU/10⁷ macrophages. After 24 h of incubation in vitro, there was a ≥99% reduction in viable bacterial counts in all monolayers, with no intergroup comparison approaching statistical significance by paired t tests.

Histology. As expected from known effects of ventilation without sighing and alveolar surfactant changes on pulmonary architecture (20), lungs of animals ventilated at CTV demonstrated microatelectasis histologically (Fig. 2). With the inocula used in these studies, bacteria could be found only after considerable searching, and conclusions regarding histological correlates of antibacterial activity could not be drawn.

**DISCUSSION**

These experiments were undertaken to assess pulmonary antibacterial activity during well-characterized changes in alveolar microenvironment. These changes, which are readily inducible and reversible, have been the subject of numerous basic and clinical studies insofar as they affect pulmonary physiology and mechanics but have not been analyzed for effects on pulmonary host defenses. CTV ventilation is known to induce a variety of changes, including the formation of surfactant aggregates with altered sedimentation properties, alveolar collapse, and hypoxia (20, 35). Some of the experimental manipulations necessary to evaluate the effects of abnormal ventilation, including the placement of a tracheal cannula and anesthesia, could have influenced pulmonary bacterial clearance. Indeed, it is likely

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**TABLE 2. Bronchoalveolar lavage cells, protein, and phospholipids at 4 h**

<table>
<thead>
<tr>
<th></th>
<th>Cells</th>
<th>Protein</th>
<th>DSPC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total no. (10⁶) (n)</td>
<td>% PMNs</td>
<td>(mg)</td>
</tr>
<tr>
<td><strong>Spontaneously breathing</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uninfected</td>
<td>1.1 ± 0.4 (6)</td>
<td>1 ± 0.3b</td>
<td>2.5 ± 0.2</td>
</tr>
<tr>
<td>Infected</td>
<td>1.2 ± 0.4 (12)</td>
<td>6 ± 2</td>
<td>2.4 ± 0.7</td>
</tr>
<tr>
<td><strong>Ventilated and infected</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTV+S</td>
<td>1.3 ± 0.3 (6)</td>
<td>16 ± 6b</td>
<td>3.7 ± 0.8b</td>
</tr>
<tr>
<td>CTV</td>
<td>1.4 ± 0.3 (6)</td>
<td>20 ± 5b</td>
<td>4.0 ± 0.5b</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Notes</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>Number of animals for determination of cells and protein or number of animals for determination of phospholipids.</td>
</tr>
<tr>
<td>b</td>
<td>P ≤ 0.05 compared with spontaneously breathing, infected rats.</td>
</tr>
<tr>
<td>c</td>
<td>ND, Not determined</td>
</tr>
<tr>
<td>d</td>
<td>P ≤ 0.05 compared with CTV + S rats. (A significant difference was found between CTV and CTV + S rats only in the percentage of DSPC which sedimented at 1,000 × g.)</td>
</tr>
</tbody>
</table>
that these factors were largely responsible for the failure of sighing to cause the clearance of *S. aureus* to be equivalent to normal clearance in either of the ventilated groups. However, these conditions were present in both ventilated groups (CTV and CTV + S), but a severe defect in pulmonary clearance was seen only in the CTV animals. Addition of a valve to the exhalation port to sighed, ventilated animals every 2 min diminished the changes in the physical state of surfactant and maintained alveolar stability but had no significant effect on bronchoalveolar lavage fluid cells or protein (Table 2) and caused improvement in pulmonary defenses against *S. aureus*. Since CTV and hyperthermia (19) have been shown to cause similar alterations in alveolar surfactant sedimentation, we had suspected that hyperthermia would worsen the clearance abnormalities. However, we could not demonstrate a statistically significant effect of temperature on clearance by ventilated animals, although the most strikingly abnormal clearances were seen during hyperthermia.

Several potential explanations for the apparent absence of pulmonary antibacterial activity in animals ventilated at CTV must be considered. These include altered phagocyte function and changes in extracellular factors which may be involved in pulmonary defenses in the nonimmune animal. At the inocula we used, pulmonary clearance of *S. aureus* in rodents appears to be mediated by intrinsic antibacterial factors and proceeds normally in PMN-depleted animals (31). All ventilated animals had increased PMN numbers in lavage fluid. While it may be postulated that the PMN influx reflects various degrees of failure of intrinsic defenses in ventilated compared with spontaneously breathing animals, such a consideration does not contribute to understanding the differences between sighed and unsighed animals. The marked difference in antibacterial activity in those animals occurred in the face of similar cellular responses. While PMN function appears generally unrelated to clearance of *S. aureus* in rodents and provides no explanation for our findings, it is possible that alveolar macrophage function was altered by the experimental interventions. Phagocytosis by alveolar macrophages is impaired by hypoxia (34). We found no additional effect of hypoxia on intracellular killing of previously ingested *S. aureus*. To serve as the sole or primary explanation for the severe defect in clearance observed within the short time frame of these experiments, the phagocytic defect would have to be severe and of rapid onset. In our experiments, although markedly abnormal clearance was evident within 6 h, in vivo phagocytosis during the first hour of ventilation, as estimated from accumulation of viable *S. aureus* (Fig. 1), was not inhibited. Lysostaphin-resistant populations at 1 h were equivalent among all experimental groups. Microatelectasis and the attendant focal hypoxia after the initiation of constant low-
volume ventilation is known to occur rapidly, within this 1-h time frame (22, 35). Our data indicate that, although hypoxic inhibition of phagocytosis may contribute to the overall delay in bacterial elimination, the magnitude of the defect in vivo appears to be an insufficient sole explanation for the observed absence of net clearance (and growth) of S. aureus in animals ventilated without sighs. Since it is not possible to study alveolar macrophages in vitro in an environment that mimics the alveolar airspaces, we cannot exclude the possibility that alterations in alveolar architecture could result in production of an environment hostile to normal alveolar macrophage intracallular and extracellular antibacterial activities. The possibility that access of alveolar macrophages to S. aureus is facilitated in expanded and inhibited in collapsed alveoli seems reasonable but is not directly testable.

Intrapulmonary growth of S. aureus has rarely been observed in experimental models of pulmonary clearance. Onofrio et al. found that S. aureus proliferated in mice only when an inoculum of 10^8 organisms was localized to about 20% of the total lung volume (28). If clearance is mediated primarily through intracellular killing, then total cessation of both phagocytosis and intracellular killing would be required for growth to be manifested. We found no evidence for the existence of such a profound defect in macrophage function, either in vivo or in vitro. However, even with modest suppression of macrophage bactericidal function, proliferation could occur if extracellular factors that normally inhibit the growth of, but do not necessarily kill, S. aureus were adversely affected. These factors may be secreted by alveolar macrophages or other cells and may require the presence of alveolar macrophages for their optimal effect. It has long been accepted that other surfaces exposed to environmental contamination (e.g., gut and skin) are protected by nonspecific extracellular defenses. Polypeptides and fatty acids are found in alveolar lavage fluid to various degrees depending on the species (3). In some experimental systems, these substances appear to contribute to intrapulmonary antibacterial activity (7, 16, 26, 27, 29); in others, no bacte- ricidal activity is evident (13). Rat alveolar fatty acids are lethal for pneumococci (3, 5) and other gram-positive micro- bres, including S. aureus (15), and lysozyme has been determined to be important in defense against experimental infection with Micrococcus species (2). In addition to direct bactericidal activity, extracellular factors could act in normal animals to inhibit growth, prevent attachment, or supplement the action of phagocytic cells against microbes.

It is conceivable that changes in mode of ventilation caused diffuse abnormalities in clearance mechanisms. How- ever, it seems more reasonable to postulate that the clearance abnormalities paralleled the known structural abnor- malities depicted in Fig. 2. We did not measure the volume of a collapsed lung, although previous work in ventilated dogs indicates it would be proportionate to the reduction in compliance (22). All the parameters reflecting alveolar stability (surfactant sedimentation, compliance, and arterial oxygenation) that we measured showed changes compatible with the assumption that sighing resulted in a less extensive abnormality of pulmonary architecture than that seen after unisighed ventilation. As an intermediate effect after surfactant alterations, we propose that microatelectasis led not only to the physiologic consequences of reduced lung compliance, focal alveolar hypoxia, and systemic hypoxe- mia but also caused focal impairment of pulmonary antibac- terial defenses.

In humans, as in rats, atelectasis is often transient. When associated with constant-volume ventilation, it may be reversed by one or two large breaths. However, when sus- tained, after acute lung injury, low-volume ventilation in the setting of impaired consciousness or in the presence of obstructed airways has long been recognized as a risk factor predisposing to the development of pneumonia (1, 6, 33). We have shown that merely changing the pattern of ventilation rapidly alters pulmonary clearance of S. aureus. These observations suggest that, in addition to the already charac- terized effectors of pulmonary antibacterial defenses, the presence of normal air-liquid interfaces is somehow essential for maintenance of optimal pulmonary antibacterial activity.

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LITERATURE CITED