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Alteration of the Pathogenicity of *Pasteurella pneumotropica* for the Murine Lung Caused by Changes in Pulmonary Antibacterial Activity

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Pasteurella pneumotropica is a potential pulmonary pathogen in mice. In healthy animals, this organism was killed rapidly by the normal function of the intrapulmonary phagocytic defense mechanisms. Impairment of this bactericidal activity by the acute renal failure of nephrectomy resulted in multiplication of the *Pasteurella* in the lung, both when the animals were nephrectomized first and then infected, and when the animals were infected first and nephrectomized several hours after the infection. The study demonstrates that the pathogenicity of the *Pasteurella* organisms is governed by the functional state of these pulmonary antibacterial mechanisms.

Recent quantitative studies of mechanisms of nonspecific resistance to pulmonary infection have shown that the rate at which inhaled bacteria are killed by the bronchopulmonary tree reflects accurately the functional state of host resistance in the lung (4, 6). In this respect, environmental agents and pathological states which are associated with increased host susceptibility to pulmonary infection affect adversely the rate at which inhaled nonvirulent bacteria are killed (3, 6, 7).

In preceding studies of nonspecific resistance to infection, nonvirulent bacteria were chosen to avoid induction of pathological responses and to permit study of mechanisms of resistance in the absence of bacterial multiplication. In the present studies, a known potential pathogen for mice, *Pasteurella pneumotropica*, was used as the infecting organism to determine how pathogenic bacteria were killed by the lung. Since even these pathogens were killed rapidly, some of the mice were nephrectomized to depress this bactericidal activity (3).

The data indicate that *P. pneumotropica* is killed rapidly by the lungs of normal mice, but that its death rate is more variable than are the rates at which previously studied nonpathogenic organisms are killed. Nephrectomy impaired this bactericidal activity, and the *Pasteurella* multiplied readily in the lungs of nephrectomized animals. Since multiplication of a pathogen is a critical factor in the pathogenesis of infection, it seems reasonable to assume from the present data that impairment of the antibacterial mechanism of the lung can result in the eventual development of pulmonary disease.

MATERIALS AND METHODS

Male, Swiss, caesarean-originated, barrier-sustained (COBS) mice (20 to 30 g) from Charles River Laboratories were used in all experiments. Animals were housed in plastic cages and fed mouse pellets ad libitum. To minimize contamination, the animals were quarantined, and gown and mask procedures were used in their care.

P. pneumotropica was obtained from the American Type Culture Collection. The bacteria were kept viable by biweekly transfers on a medium made up of 10% sheep's blood in freshly prepared beef heart infusion-agar.

Acute renal failure was induced by nephrectomy performed under ether anesthesia through a longitudinal incision along the lower vertebral column. The kidneys were freed by blunt dissection, the vessels and ureters were doubly ligated, and the kidneys were removed retroperitoneally. The mice appeared to recover from the operation within 30 min. Sham-operated animals were treated in an identical manner except that the ligatures were not tied and the kidneys were left in place. Similar numbers of mice without

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operation and designated as controls were placed in separate cages.

The *P. pneumotropica* was grown for 16 hr in a shaker water bath at 37 C in 25 ml of medium containing 90% Beef Heart Infusion and 10% sheep's blood. The organisms were harvested by centrifugation, washed with phosphate buffer (*p*H 7.4), and resuspended in 8 ml of buffer. A 5-ml amount of this suspension was placed in the aerosol nebulizer.

The aerosol exposure apparatus and techniques are

TABLE 1. *Retention and clearance of Pasteurella pneumotropica in murine lungs*[a]

Set no.	Bacterial count of nebulizer suspension[b]	Time after exposure	Bacterial count in lungs[c]	Percentage cleared[c] $(N_0 - N_t) \times 100/N_0$
		hr		
1	3.2×10^8	0	3,920 ± 330 (4)	—
		6	1,140 ± 470 (2)	71 ± 12
2	3.5×10^8	0	3,780 ± 320 (5)	
		2	3,210 ± 970 (4)	15 ± 27
		6	1,980 ± 690 (4)	48 ± 19
		24	20 ± 5 (3)	99.5 ± 0.14
3	5.2×10^8	0	10,990 ± 1,580 (3)	
		6	1,360 ± 410 (5)	88 ± 4.7
4	8.2×10^8	0	23,030 ± 3,360 (3)	
		6	7,510 ± 2,230 (4)	67 ± 11
5	8.3×10^8	0	4,560 ± 1,830 (6)	
		2	2,660 ± 1,620 (6)	41 ± 24
		4	2,450 ± 2,190 (5)	46 ± 42
		6	490 ± 260 (6)	89 ± 3.4
6	8.5×10^8	0	9,250 ± 650 (5)	
		2	3,000 ± 610 (5)	68 ± 7.8
		4	1,370 ± 580 (5)	85 ± 1.7
		24	110 ± 90 (6)	99 ± 0.8
7	11.7×10^8	0	4,350 ± 920 (5)	
		20	780 ± 270	82 ± 7.3
8	13.2×10^8	0	25,130 ± 2,240 (4)	
		6	8,240 ± 1,610 (6)	67 ± 8.6
9	26.5×10^8	0	36,000 ± 3,000 (5)	
		18	5,760 ± 750 (4)	84 ± 2.5
10	100.2×10^8	0	59,150 ± 7,080 (6)	
		2	38,500 ± 6,110 (6)	35 ± 14
		4	13,650 ± 7,470 (6)	77 ± 13
		6	3,050 ± 1,360 (6)	95 ± 2.2
11	139.5×10^8	0	85,460 ± 10,690 (6)	
		2	35,530 ± 13,340 (6)	58 ± 16
		4	15,010 ± 3,820 (6)	82 ± 4.6
		6	7,340 ± 6,150 (6)	91 ± 1.5
12	143.5×10^8	0	130,200 ± 14,580 (5)	
		17	30,500 ± 11,240 (5)	76 ± 8.2
13	—	0	61,000 ± 5,850 (2)	
		6	11,280 ± 4,050 (4)	82 ± 7.8

[a] Number in parentheses is the number of animals in each group.
[b] Number of bacteria per milliliter.
[c] Mean ± SE.

the ones previously used in this laboratory. They permit quantitative infection of the lungs of small animals, and produce predictable and reproducible results (8).

In the initial experiments, designed to determine the disappearance rate of *P. pneumotropica* in murine lungs, groups of 24 untreated mice were exposed to a 30-min aerosol of the bacteria. Immediately after exposure (time zero) and at various times thereafter, groups of mice were killed by administration of ether. The lungs from each mouse were removed aseptically and homogenized in broth. A sample of each homogenate was cultured quantitatively on the sheep's blood-Beef Heart Infusion Agar. In this and in each subsequent experiment, the homogenates were also streaked on blood-agar plates to determine the presence of organisms other than *Pasteurella*.

The second series of experiments studied the effect of acute renal failure on the pulmonary antibacterial activity against *P. pneumotropica*. Groups of eight nephrectomized, eight sham-operated, and eight untreated mice were exposed to aerosols of *Pasteurella* 2 to 4 hr after surgery. Half the animals were sacrificed immediately and half 6 hr later. Quantitative cultures of the lungs of these mice were performed. This experiment was repeated twice.

The last series of experiments studied the effect of acute renal failure on the rate of decline of *P. pneumotropica* in mice that had inhaled the *Pasteurella* prior to surgery and were thus well along in the antibacterial process at the time of nephrectomy. In these experiments, groups of 20 mice were exposed to *Pasteurella* aerosols. Five animals were sacrificed immediately (time zero) and quantitative cultures were performed. After 11 or 12 hr, 5 of the remaining 15 mice were nephrectomized and five had sham operations. The group of 15 mice was then killed 6 or 9 hr after surgery, and the lungs were cultured quantitatively.

As an indication of the extent of renal failure present in the nephrectomy experiments, blood-urea nitrogen determinations were performed on heart's blood obtained at the time of death (3) from nine nephrectomized, seven sham-operated, and five untreated mice. Sections of lung for histological examination were also obtained at this time.

Pulmonary antibacterial activity was determined by the previously described group mean clearance method (6). The mean bacterial count of each group of mice at each time period was calculated. Bacterial clearance was then expressed as the number of viable bacteria present immediately after the aerosol exposure (N_0) minus the number present at time t (N_t) divided by the initial number of bacteria (N_0): $(N_0 - N_t)/N_0$.

These calculations allow the computation of mean pulmonary bactericidal rates. Because negative values occurred in the experiments where multiplication occurred, the percentage of organisms remaining at time t was calculated N_t/N_0. Standard errors were determined by the statistical methods previously described (6).

RESULTS

The rate of inactivation of *P. pneumotropica* in murine lungs was determined from the data of Table 1, which shows in several selected experiments the concentrations of bacteria in the nebulizer and the number of viable *Pasteurella* remaining in the lungs of mice at various intervals after the 30-min exposure period. The numbers of inhaled bacteria correlated generally with the concentration of *Pasteurella* in the nebulizer suspension over a 40-fold range, 3.5×10^8 to 139.5×10^8 bacteria per ml. The bacterial killing rates were relatively independent of the size of the inhaled inocula, although there was a distinct tendency to increased killing rates at higher inocula. Figure 1 shows the pulmonary bactericidal curve constructed from all the data. At 2 hr, the average killing was 45%; at 4 hr, 65%; at 6 hr, 79%; at 17 hr, 89%; and at 24 hr, 99%. In each experiment, the number of viable *Pasteurella* cells declined rapidly.

Acute renal failure was induced by nephrectomy prior to aerosolization of *P. pneumotropica* in 39 mice. None died during the experimental periods. The animals appeared normal at the time of exposure to the aerosol, but many were listless at the time of sacrifice. The data of Table 2 show that the 6-hr pulmonary bactericidal rates for the untreated mice were 67 to 82%. The numbers of viable *Pasteurella* which could be cultured from the lungs of nephrectomized mice increased markedly during the 6-hr postaerosol period, indicating that intrapulmonary bacterial multiplication had occurred. Although multiplication was found in 11 of 13 nephrectomized mice, the large standard error indicates that there was considerable individual variation in the extent of multiplication. There was no regular correlation between the number of bacteria inhaled and the extent of subsequent bacterial multiplication. The nephrectomized mice appeared to have inhaled greater numbers of bac-

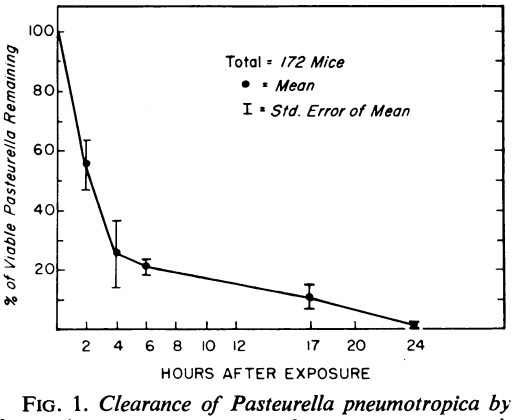

FIG. 1. *Clearance of Pasteurella pneumotropica by lungs of mice at various times after exposure to aerosols of Pasteurella.*

teria than did either of the other two groups. However, previous studies with radio-labeled staphylococci have demonstrated that the increased numbers of bacteria present in nephrectomized mice immediately after an aerosol exposure represent decreased bacterial killing during the exposure period rather than increased retention (3). The mice that underwent sham pro-

TABLE 2. *Retention and clearance of Pasteurella pneumotropica in murine lungs after bilateral nephrectomy*[a]

Set no.	Bacterial count of nebulizer suspension[b]	Exptl group	Bacterial count in lungs		Percentage remaining,[d] 0-hr count × 100/ 6-hr count
			At 0 hr[c]	At 6 hr[c]	
1	3.2×10^8	Untreated	39.2 ± 3.3 (4)	11.4 ± 4.7 (2)	29 ± 12
		Sham	26.3 ± 3.5 (3)	30.8 ± 25.4 (3)	117 ± 22
		Nephrectomy	45.2 ± 4.6 (2)	282.1 ± 120.8 (4)	625 ± 337
2	8.2×10^8	Untreated	230.0 ± 33.6 (3)	75.1 ± 22.3 (4)	33 ± 11
		Sham	335.8 ± 144.5 (3)	214.3 ± 130.9 (4)	64 ± 22
		Nephrectomy	312.8 ± 157.3 (3)	8,205.0 ± 1,175.0 (4)	2,623 ± 899
3	—	Untreated	610.0 ± 58.5 (2)	112.8 ± 40.5 (4)	18 ± 7.8
		Sham	685.0 ± 50.0 (2)	462.8 ± 225.8 (4)	68 ± 35
		Nephrectomy	772.0 ± 84.0 (4)	1,320.0 ± 188.0 (4)	171 ± 30
Mean[e]		Untreated			26.7 ± 4.5
		Sham			83.0 ± 15.6
		Nephrectomy			1,140.0 ± 320.0

[a] Number in parentheses is the number of animals in each group.
[b] Number of bacteria per milliliter.
[c] Mean ± SE × 10^2.
[d] Mean ± SE.
[e] Mean clearance values of all groups. *P* values of differences were as follows: untreated versus sham, <0.05; untreated versus nephrectomy, <0.01; sham versus nephrectomy, <0.01.

TABLE 3. *Effect of nephrectomy on the clearance of Pasteurella pneumotropica previously deposited within murine lungs*[a]

Set no.	Bacterial count of nebulizer suspension[b]	Exptl group	Postaerosol time of nephrectomy	Bacterial count in lungs[c]		Percentage remaining,[d] 0-hr count × 100 postnephrectomy count
				At 0 hr	Postnephrectomy	
			hr			
1	143.5×10^8	Untreated	11	1,302 ± 145 (5)	305 ± 112 (5)	24.0 ± 7.9
		Sham			94 ± 23 (4)	6.3 ± 3.6
		Nephrectomy			2,194 ± 158 (5)	169 ± 66
2	26.5×10^8	Untreated	12	360 ± 30 (5)	57.6 ± 7.5 (4)	16 ± 2
		Sham			158 ± 93 (4)	44 ± 13
		Nephrectomy			183 ± 78 (5)	52 ± 14
3	11.7×10^8	Untreated	11	43.5 ± 9.2 (5)	7.8 ± 3.7 (4)	18.0 ± 7.3
		Sham			1.3 ± 0.3 (4)	2.3 ± 0.96
		Nephrectomy			210 ± 123 (4)	483 ± 301
Mean[e]		Untreated				19.3 ± 3.6
		Sham				17.5 ± 13.5
		Nephrectomy				235.0 ± 103.0

[a] Number in parentheses is the number of animals in each group.
[b] Number of bacteria per milliliter.
[c] Mean ± SE × 10^2. Postnephrectomy counts were at 6 hr in sets 1 and 2 and at 9 hr in set 3.
[d] Mean ± SE.
[e] Mean clearance values of all groups. *P* values of differences were as follows: untreated versus sham, >0.5; untreated versus nephrectomy, $0.05 < P < 0.10$; sham versus nephrectomy, $0.05 < P < 0.10$.

cedures either did not kill the inhaled bacteria (set 1, Table 2), or killed them at a slower rate than did control animals (sets 2 and 3, Table 2).

The data from the three experiments in which nephrectomy was performed 11 or 12 hr after the aerosol exposure are shown in Table 3. The untreated mice had bacterial killing rates of 76, 82, and 84% for the three aerosol procedures. In two of the experiments (sets 1 and 3, Table 3), multiplication of viable organisms must have occurred in the nephrectomized mice, since greater numbers of bacteria were cultured at the end of the experiment than were initially present. In one experiment (set 2, Table 3) nephrectomy may have delayed or reversed the killing process, but the terminal bacterial culture counts did not exceed the number of organisms cultured from mice killed immediately after aerosol exposure. In sets 1 and 3, mice that had sham operations demonstrated an increased rate of bactericidal activity when compared with controls. A delay or reversal of the killing process occurred in set 2. No known change in the procedure accounts for this variation in the results. Although the nephrectomized group of animals clearly represents a different population of animals from controls in regard to the pulmonary bactericidal phenomenon, the wide individual variation prevents a meaningful statistical analysis.

The blood urea nitrogen levels for the nephrectomized mice increased with the length of the interval after nephrectomy. At 4 to 5 hr, the blood urea nitrogen levels were 55 to 60 mg per 100 ml, at 6 to 8 hr, about 80 mg per 100 ml, and at 10 hr equal to or greater than 110 mg per 100 ml, the upper limit for the method used. The blood urea nitrogen levels of the sham-operated and untreated mice varied between 10 and 30 mg per 100 ml.

DISCUSSION

The initial host-parasite encounter is a critical event in the determination of pulmonary bacterial disease. If the entering organisms are killed by host defense mechanisms, or are removed mechanically, the potential infection is averted, If neither of these events occurs, the bacteria may multiply and invade the pulmonary parenchyma.

Previous investigations have established the importance of a nonspecific intrapulmonary phagocytic system in the initial defense of the lung against inhaled bacteria (5). These studies suggested that impairment of this system would result in pulmonary infection. The present experiments offer further evidence in support of this supposition by demonstrating that impairment of the system by the induction of acute renal failure allows a pathogen, which ordinarily is rapidly removed from the lungs, to multiply. Furthermore, reversal of this antibacterial mechanism occurred not only in animals that had inhaled the organism after induction of renal failure, but also in those that had inhaled the pathogen and were well along in the bactericidal process at the time nephrectomy was performed. Following such inhibition, the lung became vulnerable both to newly inhaled pathogens and to pathogens which were already present. Whether this enhanced vulnerability would eventually have led to pulmonary disease cannot be determined from our data, since the nephrectomized mice did not survive long enough for the development of definitive pathological changes. However, it is a reasonable assumption that multiplication of *Pasteurella*, a common pulmonary pathogen of mice, would have resulted, at least in some animals, in demonstrable pulmonary disease.

The pulmonary bactericidal curve for *P. pneumotropica* resembles that previously described for *Proteus mirabilis* (7), and is less steep than that of nonpathogenic strains of *Staphylococcus aureus* or *S. albus*. In most mice, *Pasteurella* organisms were slightly more resistant to host antibacterial defenses than was previously found with staphylococci. However, unlike *S. aureus*, which is killed in a relatively uniform manner, mice rid themselves of inhaled *Pasteurella* with more variation from animal to animal. It is likely that the greater variance observed in the *Pasteurella* experiments produces a quantitative expression of small differences in the capacity of the alveolar macrophages of the host animal to phagocytize and inactivate the *Pasteurella*.

The findings in mice with acute renal failure and in the sham-operated animals suggest some clinical relevance. Pulmonary infection is recognized as a major complication of acute renal failure (1, 9). The present experiments imply that the metabolic abnormalities present in renal failure affect adversely the intrapulmonary antibacterial defense system and allow the multiplication of bacteria which ordinarily are killed by the lungs. The defect may lie in the pulmonary macrophage, a cell which plays a major role in the initial bactericidal process (5) and is suceptible to metabolic disturbance (2, 10).

The observation that sham-operated mice manifest slight but distinct impairment of the antibacterial activity has been noted in similar experiments with *S. aureus* (3). These decreases are probably due to a combination of operative trauma and postoperative metabolic alterations. It is possible that similar changes occur in man

and are in part responsible for postoperative pulmonary infections.

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