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Short Communication. Role of Agar Beads in the Pathogenicity of *Pseudomonas aeruginosa* in the Rat Respiratory Tract

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Summary

Sterile agar beads plus *Pseudomonas aeruginosa* injected intratracheally produced local infection in rats, similar to that described for the injection of agar beads containing the same pathogen. It is suggested that it is not necessary for *P. aeruginosa* to be inside the beads to induce lung infection.

Abbreviation

cfu, colony forming unit

In 1979, Cash and coworkers (2) described a rat model for chronic pulmonary *Pseudomonas aeruginosa* infection that consisted of intratracheal injection of agar beads containing the pathogen. Other investigators have used a similar system to produce models in guinea pigs (1) and cats (8). Different mechanisms have been proposed to explain enhanced Pseudomonas pathogenicity, not only in the Cash rat model, but also in human disorders like cystic fibrosis. There has been some speculation, for example, that coating of Pseudomonas by mucoid substances may provide survival advantages in the respiratory tract by protecting the microorganisms from phagocytic cells, antibodies and even antibiotics (4, 5, 6). The importance of bacterial adherence to the epithelia, before replication and microcolony formation has also been recognized (7). In this report we present histopathologic and bacteriologic data obtained from rats injected intratracheally either with agar beads containing *P. aeruginosa*, agar beads mixed with *P. aeruginosa* or *P. aeruginosa* alone.

MATERIALS AND METHODS

Young adult outbred Wistar rats, 140–160 g in weight, were anesthetized with ether, and a small midline cervical incision was made to expose the trachea. Then, 0.20 ml of the inoculum was injected intratracheally. One group was injected with agar beads containing 10^5 cfu of *P. aeruginosa*; a second group received 10^5 cfu of *P. aeruginosa*; and the last group was injected with sterile agar beads plus 10^5 cfu of *P. aeruginosa*, mixed immediately before injection. *P. aeruginosa* ATCC 15152 was cultured overnight in 15 ml tryptic soy broth at 37°C; the pelleted cells were washed with cold saline, and resuspended in phosphate buffered saline, pH 7.2. Agar beads were prepared with or without *P. aeruginosa* as described previously (2). Groups of animals were sacrificed with a single dose of Nembutal (Abbott Laboratories, North Chicago, IL). The lungs were excised and the gross pathologic features recorded. The left lung was fixed by immersion in 10% buffered formalin and stained with hematoxylin-eosin. The right lung was homogenized in 5 ml of cold distilled water in a Potter-Elvehjem homogenizer and appropriate dilutions of the homogenates plated for quantitation purposes in tryptic soy agar. Homogenate samples were also cultured in blood agar and eosin-methylene blue agar plates. Infecting flora were identified in each case by standard clinical laboratory procedures.

Table 1. *Rat lung histopathology after intratracheal injection of* P. aeruginosa *within, mixed with, and without agar beads**

Pathology (degree)	Intratracheal injection of: Pseudomonas in agar beads (a)	Pseudomonas plus agar beads (b)	Pseudomonas alone (c)
No signs (−)	3	2	4
Discrete inflammation (+)	1	1	4
Leukocyte infiltration in peribronchial and bronchial regions (++)	1	2	2
Leukocyte infiltration with extention to parenchyma and alveoli (+++)	5	4	0
Sample size	10	9	10
	c *vs* a, b: $P < 0.01$ and a *vs* b: N.S.†		

* Animals were sacrificed at 5-d intervals for 20 d.
† Significance levels for a χ^2 analysis.

RESULTS

Gross inspection of the lungs revealed foci of necrosis and inflammation, and tan-white nodules of fibrous lesions were occasionally observed. Eleven of 29 rats had lungs with normal macroscopic features; the lungs of two of these 11 had histopathologic changes, one with discrete inflammation and the other with leukocyte infiltration extended to parenchyma and alveoli. All lungs with gross alterations also had histopathologic changes. The results of the microscopic examinations are shown in Table 1. The seven rats without histopathologic changes were found throughout the time of the experiment, and those animals injected with *P. aeruginosa* alone tended to show the highest degree of inflammation only in the 5-d period after injection.

P. aeruginosa was isolated from lung homogenates of seven of 10 rats injected with agar beads containing the pathogen. Three negative cultures corresponded to the three rats with normal lungs. Similarly, seven of nine cultures were positive for those rats injected with the mixture of agar beads plus *P. aeruginosa*, and the two negative cultures corresponded again to those rats with normal lungs. *P. aeruginosa* was isolated from animals that had been injected with *P. aeruginosa* alone only in the group sacrificed 24 h after injection. The lung of only one of all the rats used in the experiment was colonized with a distinctive flora. *Proteus mirabilis* was isolated from the lungs of an animal injected with *P. aeruginosa* plus agar beads and sacrificed 15 d after injection. The number of cfu of *P. aeruginosa* isolated from lungs of animals injected with *P. aeruginosa* inside or plus agar beads varied from 2×10^6 to 5×10^8 per right lung, and the results of the counts for both groups overlapped.

DISCUSSION

The precise role of agar beads in the enhancement of Pseudomonas pathogenicity is still unclear. Agar beads, however, *a)* can induce pulmonary inflammation which lasts for up to 7 d (2), *b)* may provide an appropriate surface for bacterial adherence and growth (3), and *c)* may obstruct small airways (2), which in turn may lead to infections. In this study, we found that a single intratracheal injection, either with agar beads containing *P. aeruginosa* or agar beads mixed with *P. aeruginosa*, produced similar patterns. These two groups of animals were different from that injected with *P. aeruginosa* alone. Costerton *et al.* (3) have presented data showing that Pseudomonas encased in agar beads are protected from host lung defense by the agar matrix until they grow out of the beads, produce their own protective exopolysaccharide matrix, and colonize the alveolar surfaces. In conclusion, we present data supporting the enhancing role of agar beads in *P. aeruginosa* pathogenicity and showing that it is not necessary for Pseudomonas to be inside the agar bead in order to replicate and produce chronic respiratory infection.

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