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LEGIONNAIRES' DISEASE BACTERIA: REPLICATION IN ALVEOLAR MACROPH--ETC(U)
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(6) Legionnaires' Disease Bacteria: Replication in Alveolar Macrophages of Cynomolgus Monkeys,

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The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on the Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

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Abstract. The interaction between normal cynomolgus monkey alveolar macrophages and Legionnaires' disease bacteria (LDB) was studied by transmission electron microscopy. Following ingestion of LDB, the organisms replicated within macrophages and destroyed the phagocytic cell.

A severe outbreak of pneumonia involving 182 individuals with 29 deaths occurred at an American Legion convention in Pennsylvania in the summer of 1976 (1, 2). The consistent pathologic feature noted was acute pneumonia with an infiltrate of polymorphonuclear neutrophils and macrophages (3-5). Microscopically, dense clusters of organisms which often obscured nuclear detail were observed in lung macrophages (3). Disruption of phagocytic cells accompanied by many extracellular bacteria has also been noted (5). These observations have led to the hypothesis that the organisms resist digestion and replicate within phagocytes (3, 5).

Preliminary studies in our laboratories indicate that cynomolgus monkeys (Macaca fascicularis) show clinical signs of illness following an aerosol-induced challenge of the Washington strain of Legionnaires' disease bacteria (LDB) (unpublished data). The objective of the current study was to examine by transmission electron microscopy (TEM) in vitro interactions between LDB and alveolar macrophages obtained from normal cynomolgus monkeys.

The Washington strain of LDB which is virulent for guinea pigs was cultured on Mueller-Hinton agar supplemented with 2 percent Isovitalex (6) and 1 percent hemoglobin and incubated at 37°C for 48 hours in a humid atmosphere of air containing 5 percent CO_2. Organisms were scraped off the plates, washed once in Hank's balanced salt solution (HBSS), and resuspended in sufficient Earle 199 medium supplemented with 10 percent normal cynomolgus serum so that the final suspension contained approximately 10^8 colony forming units/ml.

Cynomolgus monkeys of both sexes, weighing 2.0-3.5 kg were used in this study. Alveolar macrophages were recovered from anesthetized,

normal monkeys by lung lavage (7). Approximately 25 x 10^6 macrophages in 25 ml Earle 199 medium supplemented with 10 percent normal cynomolgus monkey serum were dispensed into 60 x 15 mm petri dishes (8), incubated at 37°C for 3 hours in a humid atmosphere containing 5 percent CO_2, then washed twice with HBSS to remove nonadherent cells. Antibiotics were excluded in this study. Macrophage cultures were inoculated with LDB at a ratio of 100 organisms per macrophage. After incubation at 37°C for 3 hours, macrophage cultures were washed three times with 25 ml of HBSS. Some cultures were processed for examination by TEM (9), while others were re-incubated in fresh medium for an additional 21 hours at 37°C. All TEM preparations were viewed with a Hitachi HU-12 electron microscope operated at 75 kV.

After the 3-hour interaction period, approximately 5 percent of the macrophages showed evidence of intracellular LDB. These cells contained 1-3 organisms which appeared to be contained within a membrane-bound cytoplasmic vesicle (Fig. 1A). Twenty-four hours later, many macrophages contained distended vacuoles filled with LDB (Fig. 1B). Multiplication of organisms appeared to be so rapid and extensive that the entire cytoplasmic compartment of some cells became filled with vesicles containing LDB (Fig. 2) and ultimately the phagocytes were destroyed. Figure 3 shows that LDB are very pleomorphic. The cell structure of the organisms resembled that of other gram-negative bacteria (Fig 3), as well as those described by Katz and Nash (10). In Fig. 3 two organisms undergoing binary fission can be seen.

Often the outcome of the interaction between invading organisms and alveolar macrophages determines the extent of infection (11). If organisms are killed by the macrophage, disease is averted; replication of the invading organism within the phagoctye causes the host to be compromised.

The lung is recognized as one of the most frequently involved organs in Legionnaires' disease (1, 3, 4), but the pathogenesis has not been elucidated. Our data indicate that LDB are not avidly phagocytized by the alveolar macrophage. However, when ingested, the organisms replicated rapidly and destroyed the phagocyte. Alveolar macrophages from normal cynomolgus monkeys would not appear to provide a defense against initial infection with this organism. This observation suggests that LDB can abrogate one of the primary defense mechanisms of the host. Additional studies are necessary to elucidate the role of other defense mechanisms such as polymorphonuclear leukocytes, specific antibody, and cell-mediated immunity.

References and Notes

1. D. W. Fraser, T. R. Tsai, W. Orenstein, W. E. Parkin, H. J. Beecham, R. G. Sharrar, J. Harris, G. F. Mallison, S. M. Martin, J. E. McDade, C. C. Shepard, P. S. Brachman, the Field Investigation Team, N. Engl. J. Med. 297, 1189 (1977).
2. Center for Disease Control, Morbidity and Mortality Weekly Report 25, 244 (1976).
3. J. A. Blackmon, M. D. Hicklin, F. W. Chandler, the Special Expert Pathology Panel, Arch. Pathol. Lab. Med. 102, 337 (1978).
4. F. W. Chandler, M. D. Hicklin, J. A. Blackmon, N. Engl. J. Med. 297, 1218 (1977).
5. W. C. Winn, Jr., F. L. Glavin, D. P. Perl, J. L. Keller, T. L. Andres, T. M. Brown, C. M. Coffin, J. E. Sensecqua, L. N. Roman, J. E. Craighead, Arch. Pathol. Lab. Med. 102, 344 (1978).
6. Purchased from *Difco Laboratories, Detroit*, Mich.
7. M. D. Kastello, A. D. Emmert, R. F. Denson, R. A. Kishimoto, Am. J. Vet. Res., in press (1979).
8. Purchased from Falcon Plastics, Oxnard, Calif.
9. R. A. Kishimoto, B. J. Veltri, P. G. Canonico, F. G. Shirey, J. S. Walker, Infect. Immun. 14, 1087 (1976).
10. S. M. Katz and P. Nash, Am. J. Pathol. 90, 701 (1978).
11. E. Goldstein, W. Lippert, D. Warshauer, J. Clin. Invest. 54, 519 (1974).

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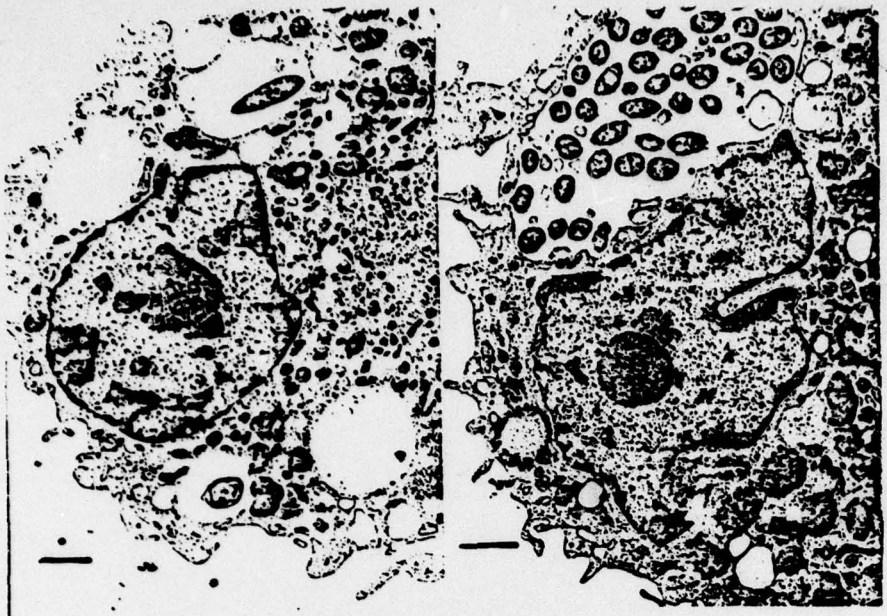
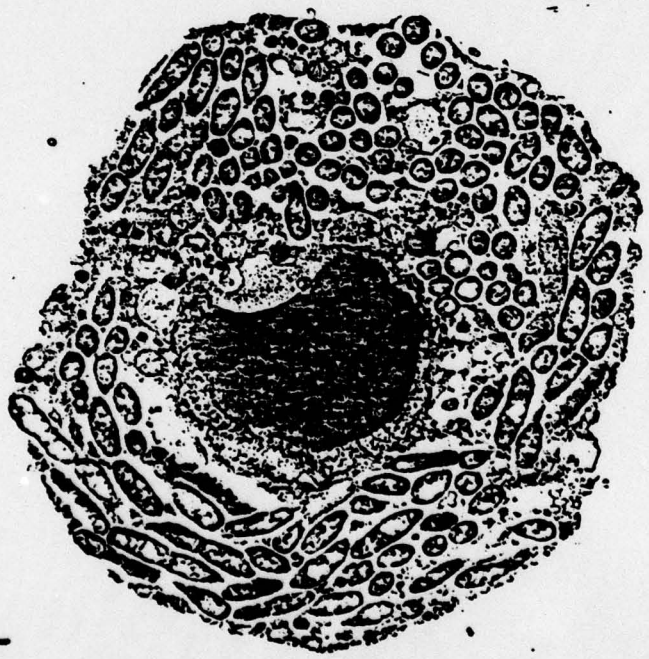
Figure Legends

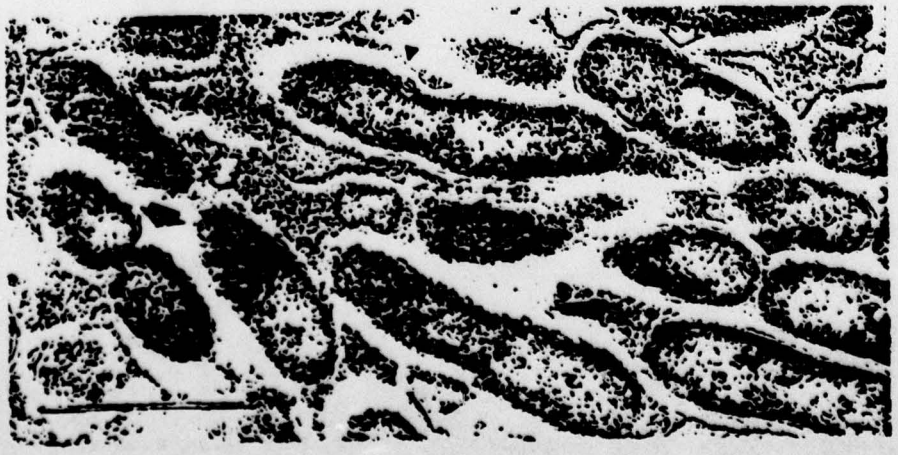
Fig. 1. Alveolar macrophages containing Legionnaires' disease bacteria. A. Single organism in phagocytic vesicle at 3 hours (X8000). Bar, 1.0 µm. B. Large numbers of bacteria within a distended vesicle at 24 hours (X8,000). Bar, 1.0 µm.

Fig. 2. Alveolar macrophage at 24 hours. The cytoplasm is filled with numerous small vesicles containing pleomorphic forms of Legionnaires' disease bacteria (X8,000). Bar, 1.0 µm.

Fig. 3. Cytoplasm of alveolar macrophage containing Legionnaires' disease bacteria. Note evidence of binary fission (◀▶), cell wall of bacterium (⌓), and vesicular membrane (▲) (X30,000). Bar, 1.0 µm.







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The interaction between normal cynomolgus monkey alveolar macrophages and Legionnaires' disease bacteria (LDB) was studied by transmission electron microscopy. Following ingestion of LDB, the organisms replicated within macrophages and destroyed the phagocytic cell.

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