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In Vitro Interaction Between Normal Cynolmolgus Monkey Alveolar Macrophages and Legionnaires Disease Bacteria

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The interaction between normal cynomolgus monkey alveolar macrophages and Legionnaires disease bacteria was studied by transmission electron microscopy. After ingestion of Legionnaires disease bacteria, 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 (2, 4). The consistent pathological feature noted was acute pneumonia with an infiltrate of polymorphonuclear neutrophils and macrophages (1, 3, 9). Microscopically, dense clusters of organisms which often obscured nuclear detail were observed in lung macrophages (1). Disruption of phagocytic cells accompanied by many extracellular bacteria has also been noted (9). These observations have led to the hypothesis that the organisms resist digestion and replicate within phagocytes (1, 9).

Preliminary studies in our laboratories indicate that cynomolgus monkeys (*Macaca fascicularis*) show clinical signs of illness after an aerosol-induced challenge of the Washington strain of Legionnaires disease bacteria (LDB) (unpublished data).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% IsoVitaleX (Difco Laboratories, Detroit, Mich.) and 1% hemoglobin and incubated at 37°C for 48 h in a humid atmosphere of air containing 5% CO_2. Organisms were scraped off the plates, washed once in Hanks balanced salt solution (HBSS), and resuspended in sufficient Earle 199 medium supplemented with 10% normal cynomolgus serum so that the final suspension contained ca. 10^8 colony-forming units per ml.

Cynomolgus monkeys of both sexes, weighing 2.0 to 3.5 kg, were used in this study. Alveolar macrophages were recovered from anesthetized, normal monkeys by lung lavage (5a). About 25 $\times 10^6$ macrophages in 25 ml of Earle 199 medium supplemented with 10% normal cynomolgus monkey serum were dispensed into petri dishes (60 by 15 mm; Falcon Plastics, Oxnard, Calif.) incubated at 37°C for 3 h in a humid atmosphere containing 5% 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 h, macrophage cultures were washed three times with 25 ml of HBSS to minimize additional phagocytosis. Some cultures were processed for examination by TEM (8), while others were reincubated in fresh medium for an additional 21 h at 37°C. The percentages of macrophages containing ingested bacteria and subsequent fate of ingested organisms are based upon the mean of four replicates for each sample time. About 100 to 200 cells were examined in each experiment. In comparison, uninfected macrophages were processed simultaneously. All TEM preparations were viewed with a Hitachi HU-12 electron microscope operated at 75 kV.

After the 3-h interaction period, ca. 5% of the macrophages showed evidence of intracellular LDB. These cells contained one to three 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. These changes were not observed in uninfected macrophages. LDB are very pleomorphic (Fig. 3). The cell structure of the organisms resembled that of other gram-negative bacteria (Fig. 3), as well as those described by Katz and Nash (6). In Fig. 3 two organisms undergoing binary fission can be seen.

Often the outcome of the interaction between

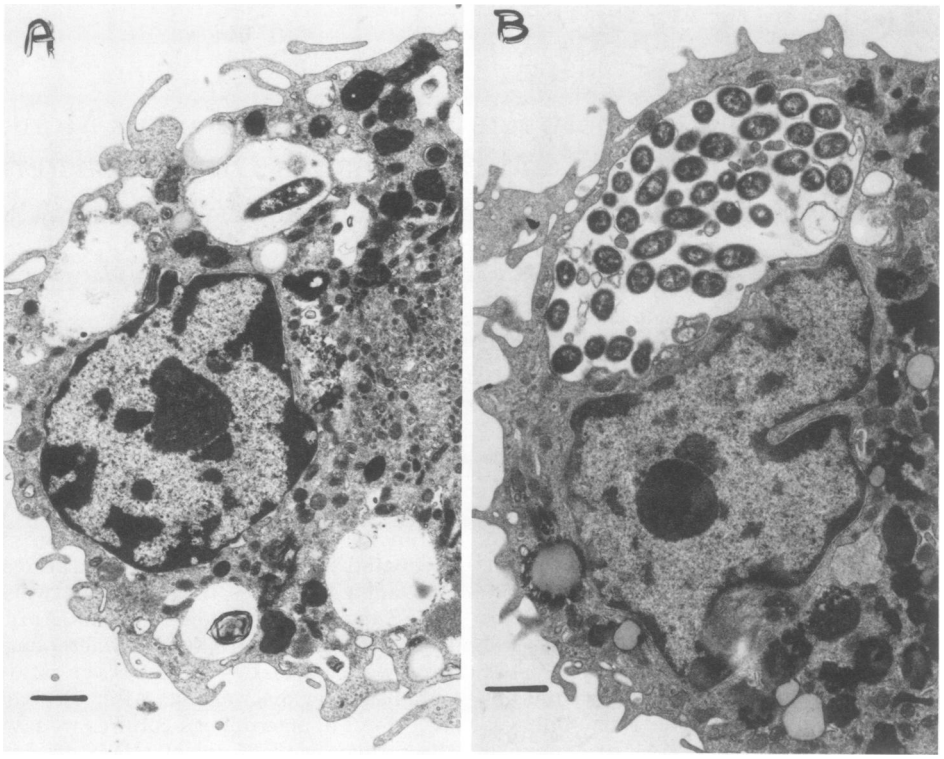

FIG. 1. *Alveolar macrophages containing LDB. (A) Single organism in phagocytic vesicle at 3 h (×8,000). Bar, 1.0 μm. (B) Large numbers of bacteria within a distended vesicle at 24 h (×8,000). Bar, 1.0 μm.*

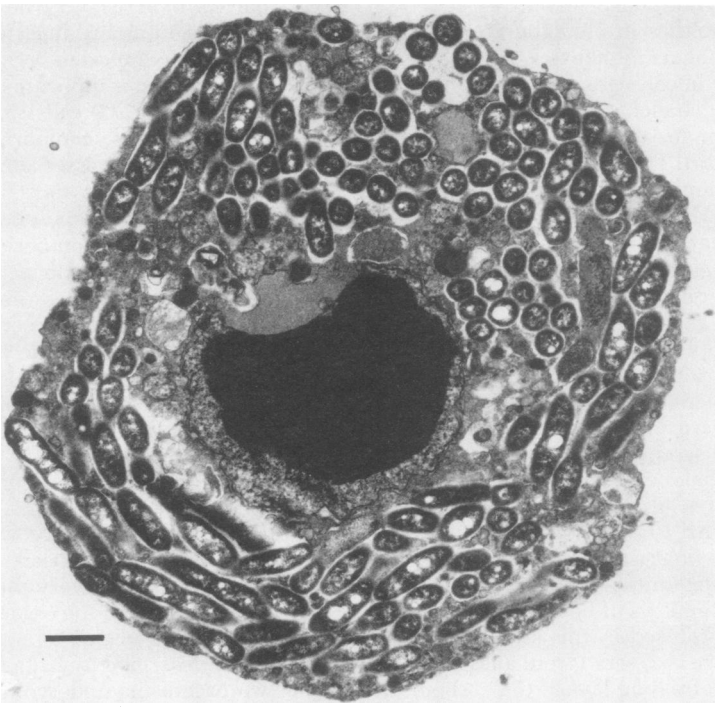

FIG. 2. *Alveolar macrophage at 24 h. The cytoplasm is filled with numerous small vesicles containing pleomorphic forms of LDB (×8,000). Bar, 1.0 μm.*

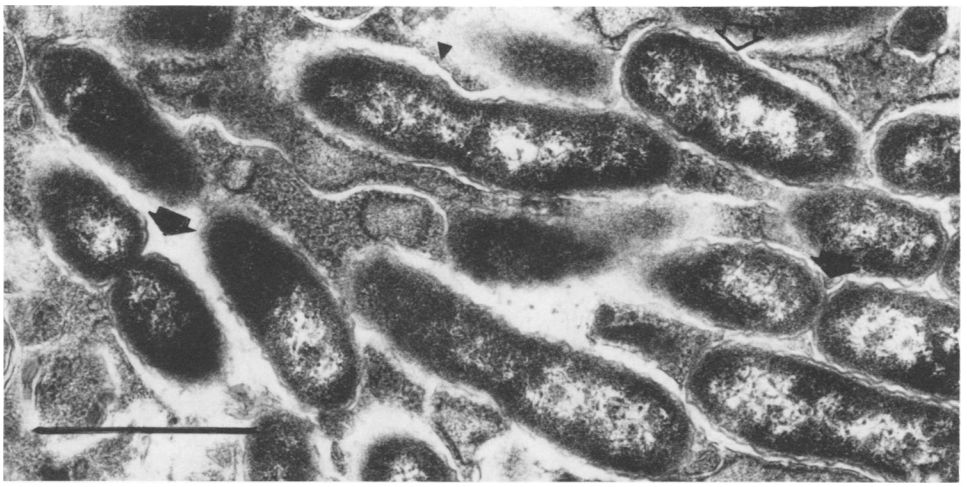

FIG. 3. *Cytoplasm of alveolar macrophage containing LDB. Note evidence of binary fission (◠), cell wall of bacterium (◡), and vesicular membrane (▲) (×30,000). Bar, 1.0 μm.*

invading organisms and alveolar macrophages determines the extent of infection (5). If organisms are killed by the macrophage, disease is averted; replication of the invading organism within the phagocyte 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.

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