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Electron Microscopy of Cell Cultures Infected with a Chlamydial Agent Causing Polyarthritis of Lambs

RANDALL C. CUTLIP

The National Animal Disease Laboratory, Animal Disease and Parasite Research Division, Ames, Iowa 50010

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McCoy cell cultures infected with the agent of ovine chlamydial polyarthritis were examined with the electron microscope. The agent was seen as small dense particles (250 to 450 nm) with an eccentric nucleoid and a multilaminated cell wall, as large (800 to 1,200 nm) granular particles surrounded by two unit membranes and as intermediate particles. Replication, which occurred throughout the cytoplasm, was initiated by phagocytosis of a small dense particle and terminated by rupture of the plasma membrane. Upon entering a cell, the small dense particles developed into large granular particles which divided by binary fission. Daughter particles either repeated the division or condensed to form new small dense particles.

Ovine chlamydial polyarthritis (OCP) is an infectious disease of lambs caused by a chlamydial (psittacosis - lymphogranuloma - venerum) agent (6, 13). The disease is manifest clinically by lameness of varying severity and duration and pathologically by a serofibrinous to fibrinopurulent polyarthritis (7, 11, 12). This report describes the ultrastructure and developmental cycle of the OCP agent.

MATERIALS AND METHODS

The organism used in this study was isolated from joint exudate of a lamb affected with OCP (11). An inoculum of the OCP agent for exposure of cell cultures was grown in yolk sacs of chicken embryos and partially purified by centrifugation. The chicken embryo LD_{50} of this material after freezing was 0.2 ml of a $10^{-5.6}$ dilution.

McCoy cells were grown and maintained throughout the study at 37 C in medium 199 containing 10% fetal bovine serum, 5% lactalbumin hydrolysate, and dihydrostreptomycin and kanamycin each at 100 μg/ml. When the cells were nearly confluent, 0.2 ml of the OCP inoculum was added. After 1 hr of incubation, the excess inoculum was removed and new medium was added. The cells were examined after 1, 2, 4, 8, 16, 24, 48, 78, and 96 hr of incubation.

Cells were removed from the glass with 0.25% trypsin in saline, washed in sodium cacodylate buffer (0.2 M, *p*H 7.4), fixed in 2.5% glutaraldehyde, embedded in agar, postfixed in 1% osmium tetroxide, dehydrated in ethanol, and embedded in epoxy resin (Epon 812, Shell Chemical Co., San Francisco, Calif.). Ultrathin sections were stained with uranyl acetate and lead hydroxide and examined with a Philips EM 200 electron microscope.

Controls included noninoculated cells and cells inoculated with noninfected egg yolk sac. Control cells were examined after 24 and 96 hr of incubation.

RESULTS

Chlamydial particles of various sizes were found either in the extracellular fluid or in the cell cytoplasm of cultures inoculated with the OCP agent. Structure of the agent varied from small dense particles with apparent rigid cell walls to large granular particles of less rigidity and electron density. Small dense particles were spherical, from 250 to 450 nm in diameter, with an eccentrically placed electron-dense nucleoid and tightly invested by a multilaminated wall (Fig. 1). A variant of the small dense particle (intermediate particle) was structurally similar except the nucleoid was less eccentric and the particle was larger (400 to 600 nm; Fig. 1). The large granular particle varied in size from 600 to 1,500 nm; however, most were from 800 to 1,200 nm in diameter (Fig. 1). Internal structure consisted primarily of small granules. Many also contained electron-dense strands of DNA (Fig. 1; reference 10). A few had internal membranes which appeared either as relatively electron-lucent tubule-like structures (Fig. 2) or as unit membranes which divided the particles into two or three portions. Large granular particles were surrounded by two unit membranes. All particles contained structures characteristic of ribosomes.

Prior to 8 hr of incubation, particles were found only in extracellular fluid either as discrete small dense particles or as slightly larger particles without detailed internal structure. These particles

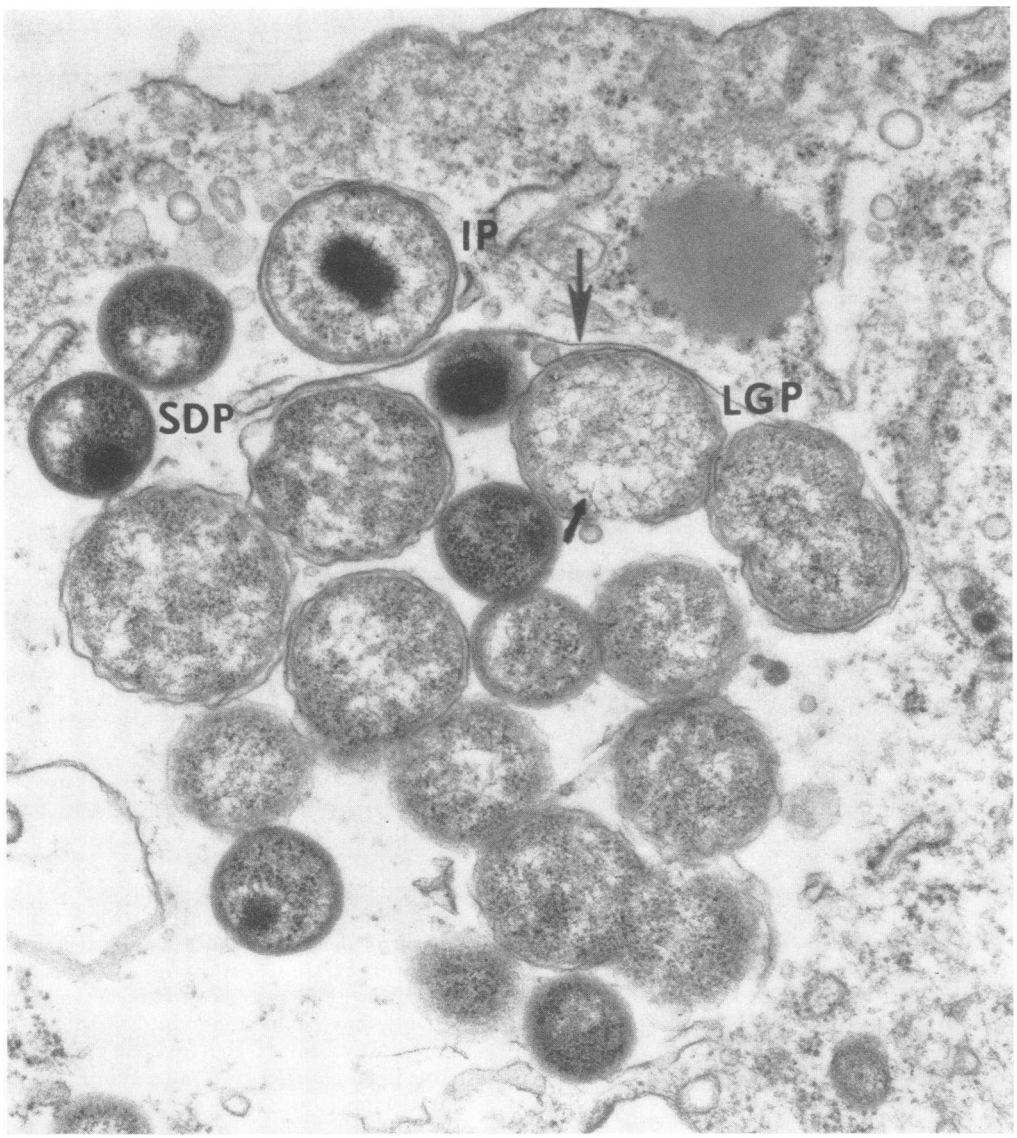

FIG. 1. *Electron micrograph of a chlamydial inclusion in the cytoplasm of a McCoy cell after 48 hr of incubation. The various stages of the developmental cycle are recognizable: small dense particle (SDP), intermediate particle (IP), and large granular particle (LGP), some of which are dividing. The multilaminated nature of the wall of the small dense particles, double unit membrane surrounding the intermediate and large granular particles and fragments of the plasma membrane of the original vesicle (large arrow) are visible. Filaments of deoxyribonucleic acid are visible in several of the large granular particles (small arrow).* × *34,000.*

were structurally identical to organisms in the inoculum and were partially enveloped by cell membrane in 8-hr cultures (Fig. 3). Intracellular particles were found after 16 hr of incubation in phagocytic vacuoles or partially surrounded by membranous projections. At 24 hr, large granular particles, either singular or in clusters of two to three, were scattered throughout the cytoplasm. Intact or broken remnants of plasma membrane of the phagocytic vacuole surrounded the inclusion. After 48 hr of incubation, inclusions, found in many cells, were composed predominantly of large granular particles, many of which were in various stages of division (Fig. 1). Small and intermediate particles were present in fewer numbers. The particles were free in the cytoplasm

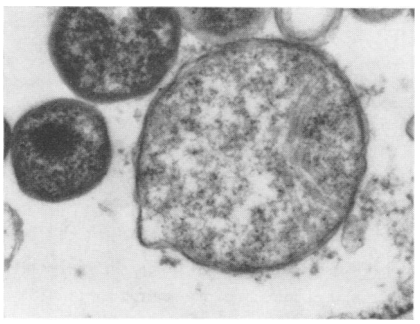

FIG. 2. *Electron micrograph of intracytoplasmic large granular particle-containing membranous structures probably related to mesosomes of other bacteria. Lead hydroxide and uranyl acetate.* × *34,000.*

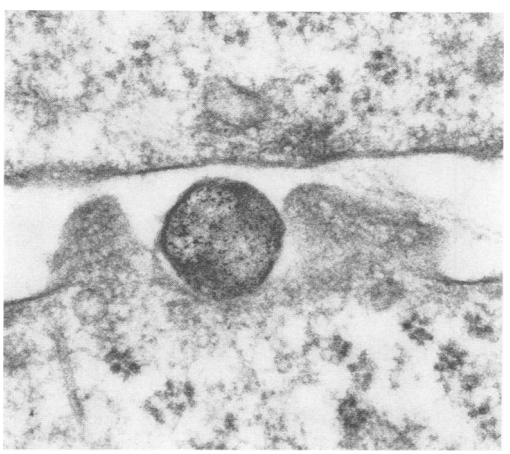

FIG. 3. *Small dense chlamydial particle partially surrounded by cytoplasmic projections of McCoy cell after 8 hr of incubation.* × *58,000.*

with only remnants of the plasma membrane remaining. At 72 and 96 hr postinoculation, particles intermingled with remnants of plasma membrane, and cytoplasmic constituents were diffusely distributed throughout the cytoplasm. The small dense particle was the major type, but many large and intermediate particles were present. Rents in the plasma membrane with loss of cytoplasmic constituents and chlamydial particles were evident in many cells. In a few cells incubated for 96 hr, small inclusions of large granular particles similar to those in 24-hr cultures were found.

DISCUSSION

Electron microscopic observations indicated that replication of the OCP agent is identical to that reported for other chlamydiae (1, 5, 9). Small dense particles entered the cell cytoplasm by phagocytosis and developed into large granular particles which divided by binary fission; the daughter particles condensed to form new dense particles which were released by rupture of the cell membrane. It appeared that shortly after dense particles entered a cell, or possibly upon contact with the cytoplasmic membrane, they began to enlarge and developed into large granular particles without loss of morphological identity. This enlargement was associated with dispersal of the dense chromatin nucleoid, rearrangement of the multilaminated wall into two unit membranes, and tremendous growth.

Division of the large granular particles appeared to be principally by simple binary fission (Fig. 1). Internal membranes found within a few large granular particles may represent unequal fission into three or more daughter cells or faulty attempts at binary fission. No particles containing internal membranes were believed to represent multiplication by budding or endosporulation as has been reported for other chlamydiae (3, 8). Membranous structures apparently unrelated to division found within other particles (Fig. 3) are of unknown significance. As suggested by Erlandson and Allen (2), membranes of this type may be related to mesosomes of other bacteria in which mitochondrial-type oxidation-reduction enzymes are located.

After division, daughter particles either enlarged and continued to divide or became mature small dense particles. This maturation process was accomplished by condensation of the chromatin, which at first was located centrally and finally excentrically in the particle and by rearrangement of the limiting membranes into a rigid cell wall. Higashi (4) reported that the trachoma agent matured by forming a new membrane around a shrunken central core with disappearance of the former external membrane. A similar mechanism was not found with the OCP agent.

Division and maturation of the intracellular particles continued until the plasma membrane of the cell ruptured and the particles were released into the extracellular fluid. This cycle, which may be excessive in time because of the possibility of ruptured cells being missed with the electron microscope, required 48 to 72 hr for completion. Also, the use of nonpurified inoculum resulting in unsynchronized infection may have influenced the apparent replication time.

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LITERATURE CITED

1. Armstrong, J. A., and S. E. Reed. 1964. Nature and origin of initial bodies in lymphogranuloma venereum. Nature (London) **201**:371–373.
2. Erlandson, R. A., and E. G. Allen. 1964. The ultrastructure of meningopneumonitis. Virology **22**:410–418.
3. Gaylord, W. H., Jr. 1954. Intracellular forms of meningopneumonitis virus. J. Exp. Med. **100**:575–580.
4. Higashi, N. 1964. The mode of reproduction of the psittacosis-lymphogranuloma-trachoma (PLT) group viruses. Int. Rev. Exp. Pathol. **3**:35–64.
5. Litwin, J., J. E. Officer, A. Brown, and J. W. Moulder. 1961. A comparative study of the growth cycles of different members of the psittacosis group in different host cells. J. Infec. Dis. **109**:251–279.
6. Mendlowski, B., W. H. Kraybill, and D. Segre. 1960. Polyarthritis in sheep. II. Characterization of the causative virus. Amer. J. Vet. Res. **21**:74–80.
7. Mendlowski, B., and D. Segre. 1960. Polyarthritis in sheep. I. Description of the disease and experimental transmission. Amer. J. Vet. Res. **21**:68–73.
8. Mitsui, Y., M. Kajima, A. Nishimura, and K. Konishi. 1962. Morphology of trachoma agent in conjunctiva and chick embryo. Ann. N.Y. Acad. Sci. **98**:131–144.
9. Mitsui, Y., T. Kitamuro, and M. Fumimoto. 1967. Adaptation of TRIC agents to tissue culture and characteristics of tissue culture adapted variants. Amer. J. Ophthalmol. **63**: 165–179.
10. Nass, M. M., S. Nass, and B. A. Afzelius. 1965. The general occurrence of mitochondrial DNA. Exp. Cell Res. **37**:516–539.
11. Page, L. A., and R. C. Cutlip. 1968. Chlamydial polyarthritis in Iowa lambs. Iowa Vet. **39**:10–18.
12. Shupe, J. L., and J. Storz. 1964. Pathologic study of psittacosislymphogranuloma polyarthritis of lambs. Amer. J. Vet. Res. **25**:943–951.
13. Storz, J., J. L. Shupe, L. F. James, and R A. Smart. 1963. Polyarthritis of sheep in the intermountain region caused by a psittacosis-lymphogranuloma agent. Amer. J. Vet. Res. **24**:1201–1206.