JOURNAL OF BACTERIOLOGY, Mar. 1988, p. 1389–1392
0021-9193/88/031389-04$02.00/0

Vol. 170, No. 3

Chlamydia Parasitism: Ultrastructural Characterization of the Interaction between the Chlamydial Cell Envelope and the Host Cell

ELLENA M. PETERSON AND LUIS M. DE LA MAZA*

Department of Pathology, University of California Irvine, Irvine, California 92717

Received 2 October 1987/Accepted 1 December 1987

Ultrastructural analysis of the growth cycles of *Chlamydia trachomatis* and *Chlamydia psittaci* showed that the chlamydial cell envelope became rigid and septated at the time of the reorganization from reticulate to elementary body. This process occurred in the immediacy of the inclusion membrane and in close proximity with the mitochondria or the endoplasmic reticulum of the host cell.

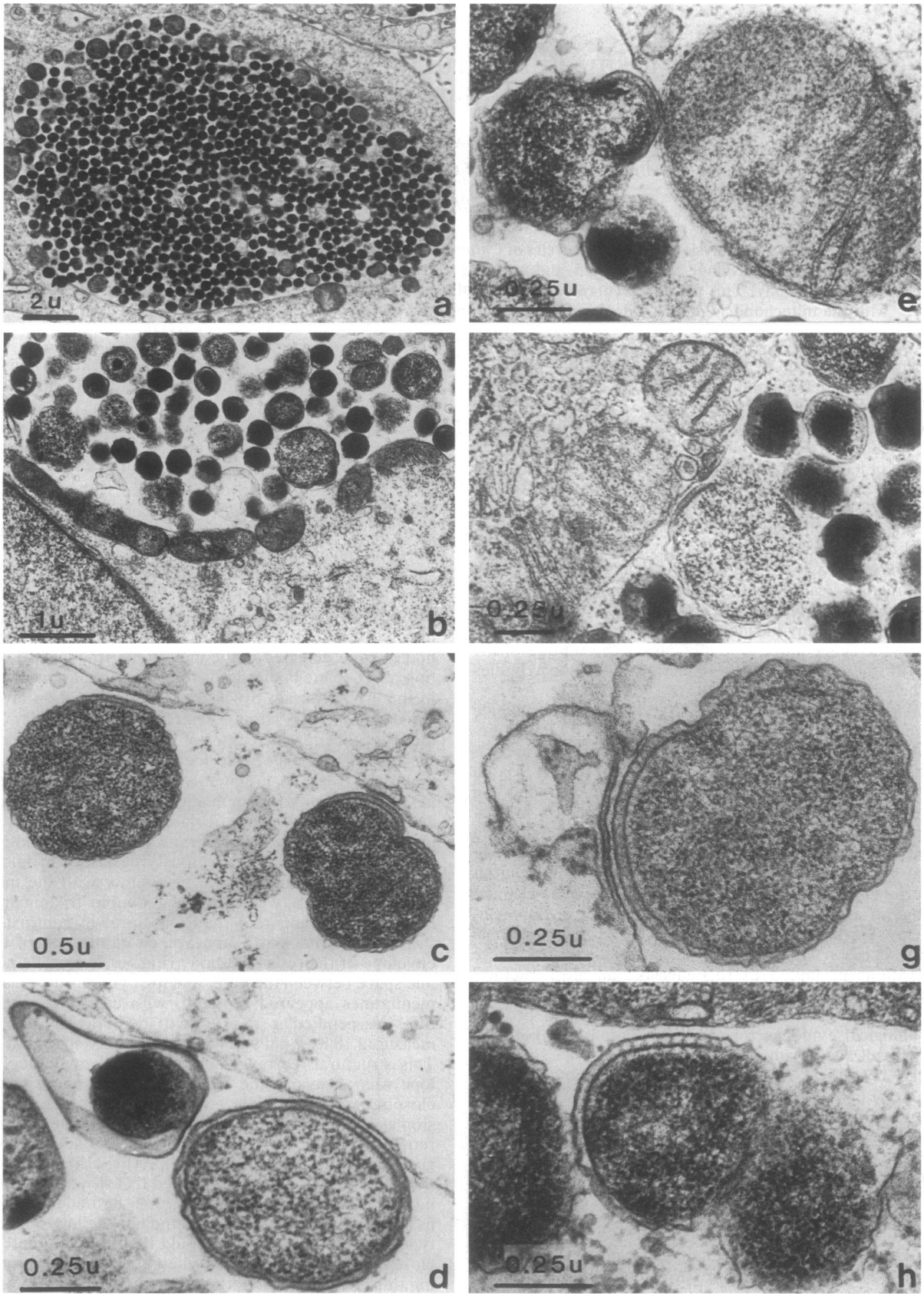
Chlamydiae are one of the most ubiquitous pathogens of humans and higher animals (17). The two species of *Chlamydia*, *C. psittaci* and *C. trachomatis*, share less than 10% DNA homology, but are taxonomically related because of their similarities during the growth cycle (17, 18). Upon infection of a mammalian cell, the elementary body forms an intracytoplasmic inclusion as a result of the invagination of the host cytoplasmic membrane (6, 11, 21). Six to ten hours following infection, the elementary body becomes transformed into a reticulate body that divides by binary fission for the next 12 to 36 h. Following replication, the reticulate bodies mature again into elementary bodies, and the replication cycle of *Chlamydia* spp. is completed by 48 to 72 h. At this point, the chlamydial inclusion occupies most of the host cytoplasmic space and eventually causes the rupture of the cell, with the extrusion of the infectious elementary bodies into the surrounding milieu (6, 11).

In 1962, Moulder postulated that chlamydiae are obligate intracellular parasites as a result of their inability to synthesize ATP (15, 16). Since then, significant evidence supporting this concept has accumulated, and very recently, Hatch et al. (2, 9, 10) showed that chlamydial reticulate bodies can transport ATP and ADP by an ATP-ADP exchange mechanism, while the elementary bodies cannot transport ATP. In the past, several authors have noted that during the chlamydial growth cycle, reticulate bodies tend to be found at the periphery of the chlamydial inclusion, while the elementary bodies are generally located at the center of the inclusion (19). Furthermore, mitochondria have been found in close association with the chlamydial inclusion (8, 20). These observations prompted us to undertake an ultrastructural study in an attempt to gain an understanding of the transition from reticulate body to elementary body and to provide morphological evidence for the interrelationships between the host cell and chlamydiae.

HeLa 229, HEp2, A549, and T24 cells were obtained from the American Type Culture Collection, Rockville, Md., and McCoy cells were purchased from Viromed Laboratories, Inc., Minneapolis, Minn. The five cell lines were grown in Eagle minimal essential medium with 5% fetal calf serum (Irvine Scientific, Irvine, Calif.) and gentamicin (50 μg/ml) with or without cycloheximide (1 μg/ml) at 37°C in a 5% CO_2 incubator (7). The monolayers were grown in glass vials (15 by 45 mm) containing a 12-mm-diameter cover slip and were infected by centrifuging the chlamydial inoculum at 1,000 × *g* for 1 h at room temperature. *C. trachomatis* serovar L2 (strain 434) and *C. psittaci* (Texas Turkey strain) were purchased from the American Type Culture Collection, and the TWAR-183 strain was obtained from The Washington Research Foundation, Seattle, Wash. Monolayers were prepared for electron microscopy at 24, 36, 48, and 72 h postinfection as previously described (7).

At 24 h postinfection, cells infected with *C. trachomatis* serovar L2 or *C. psittaci* (Texas Turkey) contained inclusions with multiple reticulate bodies actively dividing. On the other hand, cells infected with the TWAR strain showed small chlamydial inclusions containing a single or a few reticulate bodies. By 36 to 48 h, the chlamydial inclusions had further increased in size, and particularly in those cells infected with *C. psittaci* or *C. trachomatis*, elementary bodies were detected in the inclusions. In contrast, maturation of reticulate bodies into elementary bodies was detected in the TWAR strain by 48 to 72 h postinfection. With the three *Chlamydia* strains tested, the elementary bodies tended to occupy the center of the inclusion, while most of the reticulate bodies were located at the periphery of the inclusions (Fig. 1a). Frequently, mitochondria were detected in close proximity to the chlamydial inclusion (Fig. 1a, b, e, and f). Analysis of the reticulate bodies that were in proximity to the inclusion membrane demonstrated ultrastructural characteristics not previously described. Some of the reticulate bodies in the area of contact with the inclusion membrane had a well-delineated double trilaminar membrane with an apparent rigid structure, in contrast to the "ruffled-ballerina-skirt" appearance of the rest of the cell envelope of the reticulate body (Fig. 1c to h). The periplasmic space between the outer and the cytoplasmic chlamydial membranes appeared septated, with electron-dense structures perpendicular to the two membranes. The septae measured 18 by 9 nm and had a periodicity spaced at 24 nm. This structural rearrangement of the chlamydial cell envelope was always in the region of the reticulate bodies, in close proximity and adjacent to the membrane of the inclusion as if it had initiated at that point (Fig. 1c to g). In some reticulate bodies, this modification had extended to involve a greater section of the cell envelope (Fig. 1h). Furthermore, the reticulate bodies that displayed this structural rearrangement appeared in many instances to be undergoing binary fission (Fig. 1c, e, g, and h). In the host cell cytoplasm, two types of cellular organelles could be frequently detected in close proximity to the chlamydial reticulate bodies undergoing this alteration. Smooth or rough endoplasmic reticulum vesicles were in close contact with the chlamydial inclusion membrane, resulting in an apparent electron-dense double

* Corresponding author.


2u
a
0.25u
e
1u
b
0.25u
f
0.5u
c
0.25u
g
0.25u
d
0.25u
h

inclusion membrane (Fig. 1c, g, and h). In other instances, mitochondria could be seen in intimate contact with the reticulate bodies (Fig. 1a, b, e, and f). These observations were independent of the *Chlamydia* serovar or species, the cell line, or the presence or absence of cycloheximide in the medium.

We describe here for the first time the presence of electron-dense structures in the periplasmic space of the chlamydiae. In the past, several authors have discussed the existence of this empty space as proof of the lack of peptidoglycan in the chlamydial envelope. Costerton et al. (5) described the presence of an electron-dense material in the periplasmic space, but they though that it was caused by the plasmolysis undergone by the elementary bodies during extraction. We do not know at this point either the molecular structure or the function of the electron-dense septae. The periodicity of these septae, however, may be based on the hexagonal array of the chlamydial membranes described by Matsumoto and Manire (14) for *C. psittaci* and by Chang et al. (3) for *C. trachomatis*. The hexagonal unit cell measures 17 to 20 nm in diameter, closely correlating with the 24-nm periodicity of the septae. According to Chang et al. (3), this hexagonal protein array is composed of six subunit domains made up of dimers of the major outer membrane protein around a depression measuring 10 nm in diameter and 8 nm in depth. The septae could also correspond to the "button" or the "crater" structure described by Matsumoto (13) and Louis et al. (12), respectively, in the cytoplasmic membrane of *C. psittaci*. These craterlike formations measure 27 nm in diameter and are spaced approximately 40 to 50 nm apart, and in the model proposed by Louis et al. (12), the center of the crater has a canal that is continued through the cell wall. Alternatively, the septae may be unique structures formed during a very specific stage of the growth cycle of the chlamydiae to facilitate the transfer of specific metabolites from the host cell or to provide structural rigidity to the cell envelope. The morphological changes were observed mainly at 36 and 48 h postinfection, the point at which the reticulate bodies are reorganizing into elementary bodies. A signal or a metabolic component may be transferred from the host cell and result in the final division of the reticulate body before conversion into an elementary body. The observation that in most instances the morphologically modified reticulate body is in close contact with endoplasmic reticulum or mitochondria supports the concept that there is a transfer of metabolic components, most likely including ATP, between the host cell and chlamydiae. In addition, the membrane changes may represent new-membrane assembly by the microorganism, perhaps using the host cell inclusion membrane as a primer.

Bavoil et al. (1) proposed that the reorganization of the reticulate body into an elementary body occurs when the level of ATP and the reducing power, in the form of NADPH or reduced glutathione, significantly decrease. As a result, these authors suggested that there will be rigidification and closing of the pores in the outer membrane and oxidation of free sulfhydryls into disulfides. It is then not surprising that to facilitate the transfer of metabolites, the reticulate bodies get into close physical contact with the mitochondria and the endoplasmic reticulum. Further studies will be needed to understand the structure of the chlamydial cell envelope. It appears, however, that the cell envelope is very pliant and is rearranged to meet the structural and metabolic requirements of the microorganisms at the different stages of the growth cycle.

LITERATURE CITED

1. **Bavoil, P., A. Ohlin, and J. Schachter.** 1984. Role of disulfide bonding in outer membrane structure and permeability in *Chlamydia trachomatis*. Infect. Immun. **44**:479–485.
2. **Ceballos, M. M., and T. P. Hatch.** 1979. Use of HeLa cell guanine nucleotides by *Chlamydia psittaci*. Infect. Immun. **25**: 98–102.
3. **Chang, J. J., K. Leonard, T. Arad, and T. Pitt.** 1982. Structural studies of the outer envelope of *Chlamydia trachomatis* by electron microscopy. J. Mol. Biol. **161**:579–590.
4. **Chi, E. Y., C. C. Kuo, and J. T. Grayston.** 1987. Unique ultrastructure in the elementary body of *Chlamydia* sp. strain TWAR. J. Bacteriol. **169**:3757–3763.
5. **Costerton, J. W., L. Poffenroth, J. C. Wilt, and N. Kordova.** 1975. The effect of purification on the ultrastructure and infectivity of egg-attenuated *Chlamydia psittaci* (6BC). Can. J. Microbiol. **21**:1448–1463.
6. **de la Maza, L. M., and E. M. Peterson.** 1982. Scanning electron microscopy of McCoy cells infected with *Chlamydia trachomatis*. Exp. Mol. Pathol. **36**:217–226.
7. **de la Maza, L. M., M. J. Plunkett, E. J. Carlson, E. M. Peterson, and C. W. Czarniecki.** 1987. Ultrastructural analysis of the anti-chlamydial activity of recombinant murine interferon-γ. Exp. Mol. Pathol. **47**:13–25.
8. **Friis, R. R.** 1972. Interaction of L cells and *Chlamydia psittaci*: entry of the parasite and host responses to its development. J. Bacteriol. **110**:706–721.
9. **Hatch, T. P., E. Al-Hossainy, and J. A. Silverman.** 1982. Adenine nucleotide and lysine transport in *Chlamydia psittaci*. J. Bacteriol. **150**:662–670.
10. **Hatch, T. P., I. Allan, and J. H. Pearce.** 1984. Structural and polypeptide differences between envelopes of infective and reproductive life cycle forms on *Chlamydia* spp. J. Bacteriol. **157**:13–20.
11. **Higashi, N.** 1955. Electron microscope studies on the mode of reproduction of trachoma virus and psittacosis virus in cell cultures. Exp. Mol. Pathol. **4**:24–39.
12. **Louis, C., G. Nicolas, F. Eb, J. F. Lefebvre, and J. Orfila.** 1980.

FIG. 1. (a) HeLa cells infected with *C. psittaci* 48 h postinfection. Note the reticulate bodies mainly located at the periphery of the inclusion, while the elementary bodies are in the center. Several mitochondria can be seen in close proximity and indenting the inclusion. (b) HeLa cells infected with *C. psittaci* 48 h postinfection. Part of the chlamydial inclusion is surrounded by mitochondria. (c) Two reticulate bodies with modified cell envelopes in close contact with the inclusion membrane. One of the reticulate bodies is undergoing division. Reticulum endothelium-like vesicles appear in the vicinity of the chlamydiae. HeLa cells infected with *C. trachomatis* L2, 48 h postinfection. (d) HeLa cells infected with *Chlamydia* TWAR strain at 72 h postinfection. The cell envelope of the reticulate body has septae in the periplasmic space that is in the vicinity of the inclusion membrane. Note the typical pear-shaped appearance of the TWAR elementary body, recently described by Chi et al. (4). (e) Reticulate body of *C. psittaci* at 48 h postinfection in HeLa cells. There is reorganization of the reticulate body envelope that is in intimate contact with mitochondria. (f) Reticulate body with modified envelope in close contact with the chlamydial inclusion membrane and mitochondria. *C. psittaci* 48 h postinfection in HeLa cells. (g) *C. trachomatis* L2 serovar 48 h postinfection in HeLa cell. The reticulate body is undergoing division. The modified region of the chlamydial envelope appears rigid and with electron-dense septae in the periplasmic space. The endoplasmic reticulum adjacent to the chlamydiae results in the apparent double inclusion membrane. (h) Reticulate body with septae in approximately 25% of its envelope undergoing binary fission. *C. trachomatis* L2 serovar at 48 h postinfection in HeLa cells.

Modifications of the envelope of *Chlamydia psittaci* during its developmental cycle: freeze-fracture study of complementary replicas. J. Bacteriol. **141**:868–875.
13. **Matsumoto, A.** 1973. Fine structures of cell envelopes of *Chlamydia* organisms as revealed by freeze-etching and negative staining techniques. J. Bacteriol. **116**:1355–1363.
14. **Matsumoto, A., and G. P. Manire.** 1970. Electron microscopic observations of the fine structure of cell walls of *Chlamydia psittaci*. J. Bacteriol. **104**:1332–1337.
15. **Moulder, J. W.** 1962. The biochemistry of intracellular parasitism, p. 122-124. The University of Chicago Press, Chicago.
16. **Moulder, J. W.** 1970. Glucose metabolism of L cells before and after infection with *Chlamydia psittaci*. J. Bacteriol. **104**:1189–1196.
17. **Moulder, J. W., T. P. Hatch, C. C. Kuo, J. Schachter, and J. Storz.** 1984. Genus *Chlamydia*, p. 729–739. *In* N. R. Krieg (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore.
18. **Peterson, E. M., and L. M. de la Maza.** 1983. Characterization of *Chlamydia* DNA by restriction endonuclease cleavage. Infect. Immun. **41**:604–608.
19. **Phillips, D. M., C. E. Swenson, and J. Schachter.** 1984. Ultrastructure of *Chlamydia trachomatis* infection of the mouse oviduct. J. Ultrastruct. Res. **88**:244–256.
20. **Spears, P., and J. Storz.** 1979. Changes in the ultrastructure of *Chlamydia psittaci* produced by treatment of the host cell with DEAE-dextran and cycloheximide. J. Ultrastruct. Res. **67**:152–160.
21. **Tamura, A. A., A. Matsumoto, G. P. Manire, and N. Higashi.** 1971. Electron microscopic observations on the structure of the envelopes of mature elementary bodies and development reticulate forms of *Chlamydia psittaci*. J. Bacteriol. **105**:355–360.