Mode of Entry and Release of Chlamydiae in Infections of Intestinal Epithelial Cells

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The uptake and release of chlamydiae in infections of highly specialized intestinal, absorptive epithelial cells of the ileum of newborn calves were studied. Infection occurred only through the brush border of the enterocytes. The chlamydial elementary bodies were adsorbed onto the microvilli, a process that induced pinocytosis of the elementary bodies and formation of endosomes containing one dense-centered chlamydial form. The endosomes moved towards the supranuclear region of the enterocytes, and the cell membrane entrapping the chlamydial agent gradually dissolved so that inclusions with reticulate and dense-centered forms appeared to be free in the cytoplasm. After replication, chlamydiae were shed into the intestinal lumen in three different ways. Some infected enterocytes ruptured and liberated their contents into the lumen. In other instances, entire infected cells were extruded into the lumen, where their membranes degenerated to release chlamydial forms. In the third mode of release, chlamydiae were liberated in cytoplasmic fragments surrounded by cell membranes.

Materials and Methods

Experimental calves. Three neonatal Hereford calves from chlamydia-free cows were used. The calves were separated from their dams immediately after birth without access to colostrum. They did not excrete any cytopathogenic viruses or chlamydial agents in their feces [4]. All were fed milk that was free of chlamydiae or chlamydial antibodies.

Inoculum. The inoculum used was the LW-613 chlamydial strain, originally isolated from the tarsal joint of a calf afflicted with polyarthritis [5]. The inoculum was in the 12th and 15th yolk-sac passage. The calves drank the inocula from nipple bottles with force-feeding. Each calf received approximately \(4 \times 10^8\) 50% chick-embryo lethal doses (CELD₅₀) of yolk-sac propagated chlamydial agents suspended in sucrose-phosphate buffer. The calves began to excrete chlamydiae in their feces one to three days after inoculation [4].
five days after inoculation. They were stunned by electrocution and bled from brachial vessels. The abdominal viscera were exposed at once, and a 2-mm-wide intestinal ring from the terminal ileum was obtained and placed immediately into a petri dish containing chilled 6.25% glutaraldehyde in phosphate-buffered solution. When the specimen of tissue became rigid after 4–5 min, it was cut with a sharp razor blade parallel to the longitudinal axis of the villi into 1-mm³ pieces. The tissue blocks were then transferred to 5-ml vials in which they were fixed in 6.25% glutaraldehyde and postfixed with 1% osmium tetroxide. Thereafter they were processed routinely for electron-microscopic examination as described previously [6]. The tissue blocks were oriented to obtain sections parallel to the longitudinal axis of the villi. The HS-8 Hitachi electron microscope was used in examining the sections.

Figure 1. Electron photomicrograph of chlamydial elementary body adsorbed onto the border of the microvillus of an intestinal absorptive epithelial cell. Notice the gap between the agent and the cell membrane of the microvillus as well as the electron dense area beneath site of adsorption (uranyl acetate-lead citrate stain, ×20,875; inset ×42,500).

Figure 2. Electron photomicrograph of chlamydial elementary body in an endosome. Notice the cell membrane surrounding the elementary body (top) which disappeared during primary reorganization (right, bottom) (uranyl acetate-lead citrate stain, ×49,500).

Results

Distribution of intestinal infection. Although chlamydiae were reisolated in developing chicken embryos from composite samples of mucosal scrapings and digestive contents of abomasum and the lower portions of the intestine, they were recovered most consistently and in highest titers from the ileum. The electron-microscopic studies were therefore concentrated on this preferential site of intestinal chlamydial infection.

Entry of chlamydia into intestinal epithelial cells. The infectious, dense-centered elementary bodies were adsorbed initially onto the surface of the microvilli (figure 1). This contact was made between the glycocalyx of the microvilli and the surface of the elementary body without formation of a tight union between the surface and the cell
membranes of the microvilli. There was always a gap between the limiting membrane of the elementary bodies and the microvillous cell membrane. The gap was generally filled by material of the glycocalyx. The cytoplasm of the cell beneath the site of adsorption developed a definite increase in electron density in the region of the terminal web (figure 1). After adsorption of the infectious elementary body, intestinal epithelial cells responded by invaginating the membrane to which the particle was adsorbed. This was evidently the second step in pinocytosis of the agent into the cell. The invaginating membrane formed a vesicle or a caveola that enveloped the elementary body. Only one dense-centered chlamydial form per vesicle was seen, although frequently several organisms simultaneously invaded a single cell or two adjacent epithelial cells. Chlamydiae entered the absorptive epithelial cells only through the brush border. No chlamydial agents were seen passing through the lateral junctional complex of the intestinal epithelial cells.

Figure 3. Electron photomicrograph of infected intestinal absorptive epithelial cells containing different chlamydial forms free in the cytoplasm without limiting cell membrane surrounding the inclusions (uranyl acetate-lead citrate stain, \( \times 8,000 \)).

Figure 4 (left). Electron photomicrograph of an intestinal absorptive epithelial cell infected with chlamydiae with bulging apical cytoplasm (uranyl acetate-lead citrate stain, \( \times 8,000 \)).

Figure 5 (right). Electron photomicrograph illustrating ruptured intestinal absorptive epithelial cell infected with chlamydiae and release of its contents into the gut lumen (uranyl acetate-lead citrate stain, \( \times 3,000 \)).
The vesicles containing chlamydial forms pinched off or detached from the surface of the cell and took a position in the apical cytoplasm of the cell. Once within the cell, the chlamydiae remained enclosed for some time by the cell membrane, which formed an endosome but remained separated from the chlamydial surface (figure 2). Inside the intestinal absorptive epithelial cells, the chlamydial agents remained viable and replicated by going through the developmental cycle as established from studies on infected cultured animal cells [1–3, 7]. After pinocytosis and primary reorganization and division of reticulate forms, the cell membrane entrapping the chlamydial inclusion dissolved gradually so that inclusions containing reticulate and dense-centered forms were free within the cytoplasm (figure 3).

Chlamydial release from intestinal epithelial cells. The chlamydial agents were liberated from intestinal epithelial cells into the gut lumen by one of three mechanisms: rupture of infected cells (figures 4 and 5), extrusion of whole infected cells (figures 6 and 7), or as membrane-bound cytoplasmic fragments (figures 8 and 9).

When chlamydiae were released through rupture of infected cells, the chlamydial inclusion had matured to occupy most of the apical cytoplasm. The chlamydial inclusion seemed to create pressure in infected cells to cause bulging and rupturing of the apical cell membrane (figures 4 and 5). As soon as the apical cell membrane ruptured, chlamydial forms as well as fragments of the cytoplasmic matrix and its constituents were released into the lumen (figure 5).

The second mechanism by which chlamydiae were released into the gut lumen was by extrusion of whole infected cells. The mechanism of cell extrusion consisted of two steps. The infected cells first lost their normal, close apposition to the basal lamina due to increased intercellular fluid at this location. Second, neutrophilic leuko-
cytes migrated into this intercellular space, leading to detachment of the lateral junctional complex of the cell membranes (figure 6). This resulted in complete detachment of infected cells from the adjacent epithelial cells, a phenomenon that was frequently observed. After extrusion of infected epithelial cells into the gut lumen, the cell membrane degenerated and fragmented, leading to release of the agent into the lumen (figure 7).

The third mechanism was seen occasionally. The apical cytoplasm of infected cells flowed toward the apical cell surface, pushing the cell membrane ahead of it and forming a pseudopod-like structure that bulged into the lumen (figure 8). Chlamydial were present in this cytoplasmic protuberance, the membrane of which constricted to a narrow neck. Then the cell membranes fused, recombined, and pinched off from infected cells as cytoplasmic fragments containing chlamydiae. In some sections the cell membranes surrounding these cytoplasmic fragments were further broken down (figure 9).

**Discussion**

In intestinal infections, chlamydiae infected enterocytes solely through the brush border of the cells forming the mucosal epithelium. Chlamydiae were never seen to invade the intestinal epithelial lining through the lateral junctional complex between epithelial cells. This is in contrast to reports that certain enterobacteria invade the intestinal epithelial lining through the lateral junctional complex [8].

The chlamydial elementary bodies adsorbed specifically to the microvilli of the brush border of enterocytes and were then taken up into the cytoplasm of the cells by a process of pinocytosis. There was no degeneration of the microvilli or the apical cytoplasm of the enterocytes as chlamydial elementary bodies adsorbed to the microvilli. Although the contact between the surface of the chlamydial elementary body and the cell membranes of the microvilli never formed a tight union, it appeared to be specific enough to cause

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**Figure 8.** Electron photomicrograph illustrating a pseudopod-like protrusion interrupting the brush border and extending into the gut lumen. Notice the presence of chlamydial forms in cytoplasmic matrix (uranyl acetate-lead citrate stain, ×7,200).

**Figure 9.** Electron photomicrograph of a membrane-bound cytoplasmic fragment lying free in the gut lumen and containing three chlamydiae. The cell membrane is undergoing fragmentation (uranyl acetate-lead citrate stain, ×24,750).
a definite increase in density in the underlying region of the terminal web. In spite of the lining of the glycocalyx, complementary bonding between chlamydial agents and the cell membrane of the microvilli was sufficient and specific enough to initiate the process of pinocytosis. The resultant vacuole containing the chlamydial form was termed an endosome [9]. During the process of primary reorganization of the dense-centered chlamydial form and its subsequent division, the cell membrane initially formed the endosome gradually dissolved. At the stage of secondary reorganization, the different developmental chlamydial forms were free in the cytoplasm of the infected cells. Loss of the cell membrane surrounding the chlamydial inclusions may be attributed to the enlarging contents of the endosomes.

Infectious chlamydiae were released from infected enterocytes by three different mechanisms. In two of these modes of release, the chlamydiae remained in extruded cells or cytoplasmic fragments surrounded by cell membranes. Thus, the chlamydiae had a protective cover. This finding may explain the fact that colostrum containing chlamydial antibodies did not prevent infection of intestinal cells [10].

The occasional epithelial pseudopods that were seen as one of the modes of chlamydial release probably reflect increased intracytoplasmic pressure in the infected cell within the confinement of the intestinal epithelium. It also may reflect the ability of replicating chlamydiae to stimulate infected cells to modify the surface of the cell locally for pseudopod formation.

In the process of extrusion of whole infected cells, invasion of neutrophilic leukocytes into the intercellular spaces of intestinal epithelial cells effected detachment of the lateral junctional complex of enterocytes. The leukocytic invasion is preceded by increasing amounts of intercellular fluid that caused detachment of infected enterocytes from the basal lamina. Enterocytes infected with chlamydiae attracted neutrophilic leukocytes.

In studies of chlamydial replication in cultured cells, the actual mechanism of release has not been clearly identified. The mechanisms described here are probably more diverse than events occurring in tissue-culture cells since they reflect the interaction of chlamydiae with highly specialized cells in the living host.

References