Co-cultivation of Conjunctival Epithelial Cells and Chlamydia Trachomatis: Electron Microscopic Findings

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This study used primary culture of rabbit conjunctival epithelial cells to investigate the infection process of chlamydia. The epithelial cells isolated from conjunctiva of rabbit were initially cultured for three weeks. After attaining confluence they were infected with Chlamydia trachomatis (C. trachomatis) serotype D, and after co-cultivation for 24, 48, and 96 hours, electron microscopic study was performed. An inclusion body, a characteristic finding of chlamydial infection, was observed in the vicinity of the nucleus after 24 hours of co-cultivation. It contained a large number of elementary and reticulate bodies and their intermediate forms. Infectious particles known as elementary bodies were noted in the inclusion as 20 to 30 μm sized round bodies with an electron dense core. Reticulate bodies were also noted; they too were round but somewhat pleomorphic and larger than elementary bodies. Some reticulate bodies multiplied actively by means of binary fission. In this study, we observed the characteristic changes of C. trachomatis-infected cells; this in-vitro system might provide a suitable model for the study of some aspects of the pathogenesis of ocular chlamydia infection.

Key words: Chlamydia trachomatis, conjunctival epithelial cell, co-cultivation, inclusion body

INTRODUCTION

Chlamydiae are obligate prokaryotic parasites of eukaryotic cells and initiate their life cycle after entering the cytoplasm of susceptible host cells. The genus Chlamydia comprises three species: C. trachomatis, C. psittaci, and C. pneumoniae. C. trachomatis is a human pathogen responsible for a variety of diseases involving all age groups and populations and can be divided into 15 immunotypes causing various ocular, genital, and systemic infections.1-7

Chlamydiae have a unique developmental cycle with two distinct forms that distinguish them from all other microorganisms.1,6,7 One is the elementary body (EB) that is adapted for extracellular survival and for establishing infection; the other is the metabolically active intracellular form, reticulate body (RB), that is capable of division. Infected cells have a characteristic intracytoplasmic inclusion body containing EB and RB.

The present study was undertaken to determine whether rabbit conjunctival epithelial cells are a suitable model for chlamydial culture and to describe the ultrastructural details of infected cells co-cultivated with C. trachomatis serotype D.
MATERIALS AND METHODS

Strips of bulbar conjunctiva were taken from a rabbit and they were cut into small pieces about 1 × 1 mm² under a dissecting microscope. Thin sheets of conjunctiva were subsequently peeled off the underlying stroma using the fine forceps. The conjunctival epithelium was then inoculated in a culture dish (Nuclon, Denmark) containing DMEM (Dulbecco’s minimal essential medium) (Gibco, U.K.) supplemented with 10% fetal calf serum, 50 μg/ml gentamicin and 2 μg/ml fungizone. The mixture was cultured in a 5% CO₂ chamber and the culture medium was replaced every three days. The growth pattern of cultured conjunctival epithelial cells was observed daily under a phase-contrast microscope.

After conjunctival epithelial cells reached confluence, they were infected with C. trachomatis serotype D, a pathogen of human inclusion conjunctivitis. They were co-cultivated for 24, 48 or 96 hours in preparation for electron microscopy. Co-cultivated infected cells were then fixed in 2% glutaraldehyde for 2 hours, postfixed in osmium tetroxide, dehydrated in ethyl alcohol and embedded in Epon. Specimens were sectioned with an ultramicrotome into 1 μm-thick slices, and after staining with toluidine blue, sections were selected under a light microscope for evaluation of the pathologic effects of infected cells. After double staining with uranyl acetate and lead citrate, a transmission electron microscope (Hitachi-600, Japan) was used for viewing.

RESULTS

Spindle-shaped cultured rabbit conjunctival epithelial cells had formed a confluent monolayer by day 19. After three weeks, they were inoculated with C. trachomatis serotype D, and co-cultivated for 24, 48, or 96 hours. Electron microscopic examination of infected cells 24 hours after co-cultivation showed a marked decrease in cytoplasmic processes as compared to normal conjunctival epithelial cells. Although typical inclusion bodies were not observed at this time, penetrated EBs and some RBs were observed in the cytoplasm of infected cells (Fig. 1).

After co-cultivation for 48-hours, distinct inclusion bodies containing numerous EBs and RBs were observed near the nucleus of cultured epithelial cells. Within the inclusion, the EBs appeared as small round structures, 20-30 nm in diameter and with an electron dense DNA core surrounded by a cytoplasmic membrane. The RBs were also observed within the inclusion as round or relatively pleomorphic in shape and with a diameter of about 1 μm. They were less homogeneous than EBs and also had an outer envelope. In addition, some RBs multiplied actively by means of binary fission (Fig. 2). Accumulations of electron dense glycogen granules were observed adjacent to RBs (Fig. 3).

After 96 hours of co-cultivation, numerous RBs, EBs and glycogen granules were present in the mature inclusion body. The expanding inclusion occupied the bulk of the host epithelial cell cytoplasmic compartment, pushing the nucleus into a small corner (Fig. 4).

DISCUSSION

Using rabbit conjunctival epithelium, we showed in this report that an in vitro culture system can be infected with C. trachomatis, which will then multiply. This biologic phenomenon appears to be a common characteristic of C. trachomatis infection and may be relevant to complex in vivo pathogenesis related to this organism. Because its exact nature and pathogenesis are uncertain, chlamydia infections have over the years posed diagnostic and therapeutic challenges for ophthalmologists. For many years, these organisms were thought to be viruses, but since they have many of the characteristics of bacteria, they are now considered to be a specific type of bacteria. There are currently 15 recognized C. trachomatis serotypes (A, B, Ba, C-K, L1-L3). In the present study the authors used C. trachomatis serotype D for in vitro infection of rabbit conjunctival epithelial cells, and studied C. trachomatis-host cell interactions to evaluate the sequential pathologic process of host cell.

Two main structures, adapted for either intra- or extracellular survival are alternate in the chlamydial growth cycle; EBs, which represent infectious stage of the growth cycle; and RBs, which are concerned
solely with multiplication of the chlamydial population within an infected cell.\textsuperscript{5,8} While RBs are highly labile and do not survive outside the host cell, EBs are relatively stable in extracellular environments. The infection of host cells is known to be initiated by the close adhesion of EBs to the host cell surface, and thereafter, entry of EBs into host cells is caused by endocytosis. In our study EBs and RBs were observed 24 hours after infection, though typical inclusion within the cytoplasm was not detected. These findings suggest that metabolically inactive EBs enter the host cell, where within 24 hours, some kind of recognition transforms them into actively dividing RBs. As our results showed, mature RBs may be pleomorphic, and have a definite outer envelope similar to that of Gram-negative bacteria.

Within 48 hours of infection, typical inclusion bodies containing numerous EBs and RBs can be observed; they occupy a large proportion of the cytoplasm of infected cells. The increased size of an inclusion body within the infected cell may be related to the active multiplication of RBs. These large particles are metabolically active, synthesize macromolecular material, and multiply by binary fission within infected cells. At this time the authors observed well-defined electron-dense glycogen granules, as shown in Fig. 3, around the RBs; 96 hours after co-cultivation, these became less electron dense granules which may reflect the active infectious process and provide an energy source in
actively multiplying RBs within infected host cells. In contrast to C. psittaci, C. trachomatis forms an inclusion containing glycoprotein granules, and this allows detection by iodine staining of infected cell cultures. These granules begin to appear at 30-40 hours, peaking approximately 48-72 hours after infection. The source as well as the significance of glycoprotein granules is still unknown.

According to some reports, a small inclusion body appeared within the cytoplasm of a host cell about 18 hours after infection, and through continuing multiplication of RBs became two to three times larger within 24 hours. About 48 hours after infection the mature inclusion body might occupy some three-quarters of the host cell volume and push the host cell nucleus to the margin of the cytoplasm. After 70 hours of infection most RBs changed into new EBs within the mature inclusion body. Ultimately, release of EBs outside the cell occurred by autolysis of infected host cells about 100 hours after infection. Although the detailed time sequence was not recorded during our study, we observed similar findings. Within 96 hours of cocultivation, a fully developed inclusion body containing all stages of growth cycle was observed; as shown in Fig. 4, it occupied most of the area of the infected host cells. At this time, however, EBs predominated within the inclusion body, and these infectious forms might be released into extracellular space by autolysis of the infected host cell.

The penetration of an EB into its host cell is known to occur by means of receptor-mediated endocytosis. Once in the cell, the organism remains inside the phagosome throughout the growth cycle, and chlamydia antigens may prevent fusion of the phagosome with the lysosome until late in the developmental cycle. With regard to the morphology of EBs and RBs, our results were similar to those of a previous study. EBs were spherical rigid structures, 0.2–0.3 μm in diameter and packed tightly with DNA that renders the core electron dense when seen under the electron microscope. A trilaminar cytoplasmic membrane surrounds this dense DNA core. RBs also have a trilaminar outer envelope and are relatively pleomorphic in shape and size (0.5–1.0 μm).

Several cell types have been used for the isolation of C. trachomatis. The most widely used lines are the McCoy (mouse fibroblast) and the HeLa (human cervical epithelioid carcinoma). In addition, some animal and human conjunctival epithelial cells were recently used for in vitro culture of C. trachomatis. In the present study we used rabbit conjunctival epithelial cells co-cultivated with C. trachomatis serotype D; this in vitro cocultivation can be readily employed for studies of C. trachomatis-host cell interactions. It may thus be that studies of C. trachomatis-conjunctival cell interactions will provide new insights into the several aspects of ocular chlamydial infections that remain poorly understood. To clarify the complete physiologic and biochemical aspects of the extracellular and intracellular developmental cycle of C. trachomatis, further investigation will be needed.

REFERENCES

