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ULTRASTRUCTURE OF *RICKETTSIA SIBIRICA* DURING INTERACTION WITH THE HOST CELL

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Summary. — *Rickettsia sibirica* (strain Netsvetaev) was found within large translucent spaces in the cytoplasm of L-929 cell monolayer cultures on day 6 postinfection (p. i.). These rickettsiae were rod shaped 0.3 × 10 μm in size encircled by a halo of up to 200 nm wide corresponding to a capsule-like coat. Directly on the cell wall was a 12 nm thick microcapsule in which subunit structures with 12 nm thick spacing could be recognized. The cell wall membrane was 14 nm thick with a wider internal layer occasionally in a section found split into two electron-dense lamels; the internal layer corresponded to peptidoglycan. The periplasmic space with an average thickness of 5 nm separated the cell wall from a 7 nm thick cytoplasmic membrane. The ultrastructure of *R. sibirica* was similar to that of other rickettsiae, although the capsule-like coat was thicker than in spotted fever (SF) group rickettsiae.

Key words: *Rickettsia sibirica*; *ultrastructure*; *host cell*

Introduction

The ultrastructure of most rickettsiae, especially basic representatives of 3 groups of *Rickettsia* genus, has been thoroughly studied (Avakyan and Popov, 1984). Recently, rather a broad variety of spotted fever (SF) group rickettsiae has been demonstrated, and thus, several new species isolated from *Ixodes* ticks have been described: *Rickettsia rhipicephali* in the North America (Burgdorfer *et al.*, 1978), *R. slovaca* (Úrvölgyi and Brezina, 1978), and *R. helvetica* (Peter *et al.*, 1984) in Europe. However, as far as the SF group rickettsiae of European circulation are concerned, it is unclear whether they form a separate species, or are members of one, widely spread species of *R. sibirica* (Makarova *et al.*, 1978; Jablonskaya, 1978; Makarova and Tarasevich, 1982). For tackling of this problem a thorough study is needed, in particular, characterization of ultrastructural peculiarities, first and foremost of the *R. sibirica* species. However, the data on submicroscopic

structure of these rickettsiae are very scarce. The present communication is devoted to anatomical description of *R. sibirica* in the ultrathin sections during their cultivation in continuous cell line L-929.

Materials and Methods

Rickettsia strains. R. sibirica strain Netsvetaev was serially maintained in chick embryo yolk sacs.

Infection of cells. Cells L-929 were grown in medium 199 containing 10% bovine serum. During passages the cells were removed from the glass with 0.02% versene. Cell suspension ($2 \times \times 10^5$ cells per 1 ml) was distributed into glass flat-bottomed test-tubes (1 ml per tube) 13 mm in diameter and 45 mm high; some of them contained cover glasses of 11—12 mm in diameter. Cell monolayers were cultivated in vertically-positioned test tubes at 37 °C.

The cells were infected with 10% suspensions of yolk sac membranes of developing chick embryos in medium 199, partially purified from debris and insoluble lipids by centrifugation. Initial titre of infectious material was 10^{-6} ELD_{50}. One-day-old cell monolayers were infected with 0.5 ml of initial yolk rickettsiae containing suspensions by centrifugation at 2500 rev/min (1 hr, 20 °C) in CLS-3IM centrifuge with horizontal rotor. After centrifugation, the inoculate was removed and the monolayer was twice washed with the medium 199, 1 ml growth medium was then added and the cells were cultivated at 35 °C. The growth medium consisted of medium 199 and 2% foetal calf serum without antibiotics, pH 7.2—7.4. As soon as the pH was shifted, the medium was replaced by the fresh one.

For the estimation of the reproduction of rickettsiae in the monolayer by light microscope, the cells were stained on cover glasses according to Zdrodovsky.

Electron microscopy. Cell monolayers were fixed with the mixture of glutaraldehyde, paraformaldehyde and picric acid in 0.2 mol/l cacodylate buffer with pH 7.2 (Ito and Rikihisa, 1981) for 1 hr at 20 °C, postfixed for 1 hr with 1% OsO_4 in the same buffer. The material was dehydrated in cool ethanol and absolute acetone, and embedded into Araldit M. The embedded material was removed from the glass by dipping of the test tubes into liquid nitrogen. Ultrathin sections were prepared in Ultratom-III LKB-8800 (Sweden), contrasted by 5% aqueous solution of uranylacetate (30 min) and lead citrate (10 min). The grids were examined in microscope JEM-100B (Japan) at voltage of 80 kV and objective aperture of 50 μm diameter.

Results

Six days postinfection (p. i.) in many infected cells, as a rule, the rickettsiae were arranged in groups in the cytoplasm. The described model was characterized by the presence of large light spaces round such groups of rickettsiae, free of the host-cell organelles (Fig. 1). Occasionally the rickettsiae were located as if at the margin of giant translucent spaces of 1.3—2.8 μm in diameter, which looked like "vacuoles" in the light microscope.

R. sibirica cells are rod shaped 0.3 × 1.0 μm in size, or shorter ovoid cells (Fig. 1). Single rickettsiae in the cytoplasm were encircled by distinct haloes up to 200 nm wide (Fig. 2). Directly on the cell surface could be seen a microcapsule forming a 12 nm thick uniform layer. The cell wall membrane 14 nm thick had an asymetric profile — its internal layer was thicker than the external one (8 nm versus 3 nm). In some areas this layer was divided into two ones; first lamel seemed to correspond to the internal membrane as such, while second to the rigid peptidoglycan layer. Periplasmic space was rather narrow (5 nm), uniform throughout the cell perimeter, with moderate electron density. Cytoplasmic membrane was 7 nm thick and quite distinct in most of the cells.

Rickettsial cytoplasm was of uniform density and contained DNA fibrils which did not form a marked nucleoid zone. The cytoplasm was rather dense in most cells, however, lighter cells also occurred (Fig. 1); the latter were, as a rule, somewhat shorter and thicker, than the dense ones.

No rickettsiae were found in the nuclei of infected cells. In some rickettsiae the division was in progress by binary fission (Fig. 2).

Host cell fragments encircled by plasmalemma, but completely devoid of organelles and containing *R. sibirica* only, were occasionally found in the monolayer (Fig. 3). In these fragments the rickettsiae were situated in an electron translucent space and some of them had irregular shape. The cytoplasm of infected host cells contained somewhat enlarged cisterns of granular endoplasmatic reticulum, some mitochondriae were in the state of swelling. Vacuolization of cytoplasm was observed in the light spaces containing rickettsiae. In some cells the rickettsia-containing translucent spaces occupied the most part of the cell.

Discussion

The comparison of *R. sibirica* anatomy with the structure of other rickettsiae has revealed a typical general trend in cellular structure. *R. sibirica* are ultrastructurally similar to other representatives of this genus with respect to all parameters except for *R. tsutsugamushi* (Silverman and Wisseman, 1978*a*; Avakyan and Popov, 1984). The method of whole cell negative contrasting allowed to describe 2 types of fimbriae in *R. sibirica*: those 10—12 nm in diameter, often arranged as acluster at one pole of the cell (often longer than the rickettsia itself); and thinner pili (4—5 nm) forming a kind of fringe along the cell perimeter (Gudima and Kokorin, 1968). No structure similar to rickettsial fimbriae or pili has been found in ultrathin sections.

Light halo around some intracytoplasmic rickettsiae seems to correspond to their capsule-like coat, or slime layer (Silverman and Wisseman, 1978*b*; Silverman *et al.*, 1978). This coating seems to be thicker in intracellular *R. sibirica* than in other SF group rickettsiae, since the halo is 200 nm wide. Probably, in the course of rickettsia reproduction their capsule-like coats became fused, enlarged and formed large light intracytoplasmic spaces, in which rickettsiae were found.

The microcapsule was regularly found in all rickettsial cells. In some cases its fibrils were regularly spaced and the interval between them was about 12 nm, which consisted with the earlier reported data on *R. rickettsii* and *R. prowazekii* (Popov and Ignatovich, 1976; Silverman and Wisseman, 1978*b*; Hayes and Burgdorfer, 1982). In some areas fo the cell wall a five-layer structure could be recognized; the most likely explanation of such structure was the close association with the internal lamel of the peptidoglycan rigid layer.

The capsule-like coat of *Rickettsia rickettsii* grown in cell culture was 125 nm thick (Silverman and Wisseman, 1978*a*). In the tick body the capsule-like coat of rickettsiae is distinct only in engorged ticks, whereas in hungry

ones it essentially disappears with the microcapsule (Hayes and Burgdorfer, 1979; 1982). At he same time the coat is thinner than in the cell culture of warm-blooded, — i. e. in the salivary gland cells it is 80 nm thick (Hayes and Burgdorfer, 1979). In other recently described SF group members the capsule-like coat is thinner, as evident from the presented electronograms: about 30 nm in *R. rhipicephali* (Hayes and Burgdorfer, 1979) up to 60 nm in *R. helvetica* (Burgdorfer *et al.*, 1979) and *Rickettsia WB*-8-2 (Burgdorfer *et al.*, 1981*b*) and in East-side agent (Burgdorfer *et al.*, 1981*a*). In *R. slovaca* it is less expressed or completly absent (Čiampor *et al.*, 1978; Burgdorfer *et al.*, 1979; Hayes *et al.*, 1980). Capsule-like coat and the microcapsule are the first rickettsial surface structures that interact with the host cell. Therefore, the structural dissimilarities and/or differences in the expression of these structures are important for understanding of the mechanism of such interactions.

A characteristic cytopathology has been described for the chick fibroblast secondary cultures infected with *Rickettsia rickettsii*: dramatic vacuolization of cells resulting from hypertrophied granular endoplasmic reticulum leading to encircling of rickettsiae by the host membranes (Silverman and Wisseman, 1979). In L-cells infected with *R. sibirica* the vacuolization of cytoplasm was less prominent and intracellular membranes did not appear to be involved. We observed neither the release of rickettsiae nor their engulfment by host cell membranes. The revealed differences are likely to be related to the differences of cellular systems, and also to the fact that *Rickettsia rickettsii* were studied in the course of infection.

Thus, intensive development of capsule-like coating in *R. sibirica* and their specific behaviour in the host-cell cytoplasm represents a differential feature as compared to *Rickettsia rickettsii*. However, a comparative study of different SF group members, and especially of newly isolated rickettsiae, is needed for the elucidation of their taxonomic relations.

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Explanation to Figures (Plates LVII—LVIII)

Fig. 1. R. sibirica in the cytoplasm of L-cell, day 6 p. i. (magn. × 55 000).

Fig. 2. Division of *R. sibirica* in the cell cytoplasm. The light halo encircling rickettsial cells and the corresponding capsule-like coat are well visible (magn. × 70 000).

Fig. 3. R. sibirica in a destroyed cell (magn. × 40 000).