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PECULIARITIES OF *RICKETTSIA PROWAZEKII* IN THE CELL CULTURE AS REVEALED BY CRYOULTRAMICROTOMY

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Summary. — Ultrastructure of *Rickettsia prowazekii* has been followed in L-929 cells 4 days post-infection (p.i.) by cryoultramicrotomy. Groups of rickettsiae were present in the cytoplasm outside of vacuoles forming microcolonies. The size of rickettsiae amounted to 400 × 700 nm, the average thickness of the cell wall was 5 nm, that of periplasmic space and cytoplasmic membrane 14 and 6 nm, respectively. Within intracytoplasmic colonies the rickettsiae were tightly packed and their cell walls were closely adjacent to each other. No halo or capsule-like coating around them was detected. No ultrastructural details were observed in the light translucent spaces between cells. Marginal rickettsiae of the microcolonies were often in close contact with the host cell mitochondria.

Key words: Rickettsia prowazekii; ultrastructure; cryoultramicrotomy

Introduction

The anatomy of rickettsiae was thoroughly studied mainly with the help of ultrathin sections of infected eukaryotic cells embedded in epoxy resins (for reviews see Weiss, 1982; Avakyan and Popov, 1984). Long-term treatment of the material by means of this traditional method might bring about artifacts caused by dehydration and embedding of the samples in epoxy resins, as recently exemplified by *Rickettsia rickettsii* (Todd *et al.*, 1983). To avoid such undesired effects we have used cryoultramicrotomy (Bernha and Viron, 1971) to demonstrate the interaction of *R. prowazekii* with the host cell.

The method is based on quick freezing of softly fixed biological objects and their cutting at low temperatures. This allows to avoid artifacts appearing during conventional treatment of the material and to study the ultrastructure of cells under conditions as similar as possible to the in vivo conditions.

Materials and Methods

Rickettsia prowazekii (strain Breinl) was cultivated in monolayer cultures of L-929 cells in 100 ml glass flasks capacity at 36 °C. The cells were grown in medium 199 supplemented with 10 % inactivated bovine serum.

Electron microscopy. After 4 days in culture the cell monolayer was fixed with 1 % solution of glutaraldehyde (GA) in 0.2 M cacodylate buffer (pH 7.2—7.4) for 1 hr at 4 °C, thereafter it was washed with the same buffer for 5 to 24 hr. The cells were mechanically removed from the glass into the same buffer and sedimented by centrifugation (5000—6000 rev/min) for 10—20 min, 10 °C. The sediment was divided into 2 portions: the first one was additionally fixed by a mixture of formaldehyde, glutaraldehyde and picric acid in 0.2 mol/l cacodylate buffer (pH 7.2—7.4) (Ito and Roikihisa, 1981), finally fixed with 1% OsO_4 solution in the same buffer and placed into Araldit M according to a conventional technique; the other portion served for cryoultramicrotomy.

Cryoultramicrotomy. Step-wise sucrose infiltration has been used according to the following scheme: 2.3 mol/l sucrose solution was diluted at 1 : 2 with distilled water, the sediment was kept in it for at least 5 min, then every 5 min 2.3 mol/l sucrose solution was added to reach dilutions 1 : 1 and 1 : 2 and after that the pieces were placed for 5 min in 2.3 mol/l sucrose solution. The infiltration was conducted in concave glasses placed on ice. After impregnation, the sediment was placed on silver holder, moistened with methylcellulose solution (tissue-tek or Ames O.C.T. Compound) and frozen in liquid nitrogen. Cryosections were prepared with Ultratom-III-8800 with Cryokit-14 800 (LKB, Sweden) at sample temperatures —80 °C— —60 °C and knife temperatures —50 °C— —60 °C. The samples were cut with a dry knife. The sections were placed on the drop of oversaturated aqueous sucrose solution. The sections adhering to the drop were placed on the formwar-coated grid and then washed with distilled water. They were negatively contrasted in 4 % silico-tungstate sodium solution (7—10 s, 37 °C) or in 1—2 % phosphotungstic acid solution, pH 7.2 (5—10 s, 37 °C) and air-dried without washing. The sections were examined immediately in electron microscope JEM-100B (Japan) at voltage 80 kV and aperture diaphragm of 50 μm diameter.

Results

The study of the interaction of *R. prowazekii* with the host cell by the help of conventional ultrathin sections has shown that the rickettsiae are loosely arranged in the cell cytoplasm either separately or more often in groups and are surrounded by large light spaces — haloes, corresponding to a slime or a capsule-like coat (Fig. 1). The rickettsial cells had a bacillus-like or, more rarely, oval shape, and their sizes ranged within the limits of 350×900 nm. Average thickness of the cell wall was 10 nm. The periplasmic space (9 nm) was filled with a substance of average electron density, the cytoplasmic membrane was on average 8 nm thick.

In the sections prepared by cryoultramicrotomy *R. prowazekii* were tightly arranged in the cells, their cell walls were often adjacent to each other over a long distance (Fig. 2). The rickettsiae had most often an oval shape, sometimes irregular, repeating the outlines of the adjacent cells. Most of the cells were not surrounded by a distinct halo, their sizes were 400×700 nm. The cell wall (5 nm thick) was clearly seen in the cryosections and was separated from the cytoplasmic membrane (6 nm thick) by a periplasmic space about 14 nm wide.

No surface structures were observed among adjacent rickettsiae or in the translucent light spaces near the cells. Some rickettsiae, especially those at the edge of the colony were in close contact to host mitochondria by their cell wall surface (Fig. 3). The comparison of the findings obtained in ultrathin

sections and by cryoultramicrotomy revealed the following ultrastructural differences in interaction of *R. prowazekii* with the host cell. While in ultrathin sections the rickettsiae were arranged loosely in the cell cytoplasm, as a rule, at a considerable distance from each other, and were surrounded by a light halo, in the cryosections they were closely adjacent to each other and in contact by their cell walls. No slime layer or capsule-like coat have been found. In the cryosections *R. prowazekii* cells were of a rounder shape and their sizes varied within narrower ranges, as compared to cells in conventional ultrathin sections. The cell wall thickness of the rickettsiae was in the latter case nearly two-fold thinner (5 nm), whereas the periplasmic space was wider (an average of 14 nm) in contrast to the ultrathin sections where it did not exceed 9 nm. The least ultrastructural differences have been found in the cytoplasmic membrane. It was somewhat less thick in cryosections as compared to the ultrathin sections (cryosections — 6 nm, ultrathin sections — — 8 nm), however, in the range of possible measurements mistake.

Discussion

The method of cryoultramicrotomy which makes unnecessary dehydration of tissues and their embedding in the epoxy resins which may cause a compression of cells (Luft, 1973), allows to keep the biological objects unchanged and most similar to in vivo conditions (Bernhard and Viron, 1971). This suggests that *R. provazekii* cells are indeed closely adjacent to each other by their cell walls within a cytoplasmic colony, as we have observed in our experiments. In the conventional ultrathin sections *R. prowazekii* have a typical rickettsial structure previously described in detail by different authors and similar to the anatomy of gram negative bacteria (see the review of Avakyan and Popov, 1984). In ultrathin sections not all rickettsiae are surrounded by haloes and if they are, these have different sizes. It has been also found that the contrast of the halo is dependent on the chosen fixation and embedding media (Ito *et al.*, 1975, 1978). The correspondence of halo to the capsule-like, or slime coating has been clearly demonstrated during treatment of rickettsiae with specific antiserum (Silverman *et al.*, 1978), but the host cell was inevitably destroyed and the interactions between its organelles and rickettsiae could be disrupted. In the cryosections large light spaces were occasionally observed round the rickettsiae situated on the edge of the colony. These spaces could correspond to the capsule-like coat (Fig. 2), which was washed out in the course of the treatment of the sections. Another cryotechnique, namely freezing-etching (Ito *et al.*, 1975, 1978), used for the study of *Rickettsia typhi* in the intestinal cells of vectors (fleas and lice) failed to reveal close contacts between rickettsial cells, probably because they were not tightly arranged in the tested samples.

The study of *R. ricketsii* in high-voltage electron microscope detected haloes larger than conventional ultrathin sectioning (Todd *et al.*, 1983). Within these areas fibrils consisting either of outgrowths of cell wall membrane or corresponding to microfilaments of the host cells have been described.

in these experiments the rickettsiae were not embedded in epoxy resins, but were dehydrated in ethanol. The comparison of these data with our own suggests that the substance of the capsule-like coating is sensitive to dehydration and is unwrapped when the host cell cytoplasm is damaged. It seems that microcolonies of rickettsiae developing in the cytoplasm are characterized by close contacts of their cells. With *R. prowazekii* most part of the cell surface is involved in these contacts. Close contacts of intracytoplasmic rickettsiae, both lateral and those of the cell poles, have also been described for *R. rickettsii* studied in high-voltage electron microscope (Todd *et al.*, 1983). Close contacts of developing organisms within one microcolony have been observed among other obligate intracellular parasites — the Chlamydiae (Popov *et al.*, 1978, 1979). In this case they were also detected in ultrathin cryosections unlike to conventional ones embedded in epoxy resins.

Thus, cryoultramicrotomy allowed us to obtain new facts concerning the structure of *R. prowazekii* cells which need additional comparison with other results obtained by adequate methods.

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Explanation of Figures (Plates LII—LIV):

Fig. **1**. L-929 cells infected by *R. prowazekii* 4 days p.i. Conventional treatment. Magn. 25,000×; the bar in all Figures has 0.5 μm.

Fig. **2**. Tight packing of *R. prowazekii* in the host cell cytoplasm, 4 days p.i.; ultrathin cryosection. Magn. 60,000×.

Fig. **3**. Ultrathin cryosections of L-929 cells infected with *R. prowazekii*. Contacts of marginal cells in intracytoplasmic rickettsial colony with the host cell mitochondria; magn. 50,000×.