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COMPARISON OF THE ULTRASTRUCTURE OF SMALL DENSE FORMS OF CHLAMYDIAE AND *COXIELLA BURNETII*

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Summary. — In the course of passaging of *Coxiella burnetii* (*C.b.*) in *Alveonasus lahorensis* ticks, the haemocytes containted cell forms with electrondense cytoplasm, intracytoplasmic lamellar membranes, and a peculiar limiting membrane—25 to 30 nm thick "envelope complex". Similar small forms occurred when *C.b.* had been cultured in the yolk sack of chick embryos. The dense forms of *C.b.* were similar to those of Rickettsiella cells. Dense forms (elementary bodies) surrounded by an "envelope complex" were found also in some chlamydiae cultured in yolk sacs of chick embryos.

Key words: *Coxiella burnetii*; *chlamydiae*; *ultrastructure*

Introduction

The aim of this report was to compare the ultrastructure of chlamydiae and *C.b.* in different biological models — developing chick embryos (CE) and argasid ticks *Alveonasus lahorensis Neuman.* The latter seem suitable for laboratory investigations, because of their size and the possibility of long-term propagation of rickettsiae in them.

Materials and Methods

Strains. The Shorsher strain of *C.b.* (phase I) was isolated from the blood of a patient with Q-fever in Yaroslavl (Kulagin *et al.*, 1958)., 1958). The following strains of chlamydiae were used: AV, B, HA, NA (trachoma agent), LB 1 (paratrochoma agent), LV (lymphogranuloma venereum agent), MP (meningopneumonitis agent), Nigg (mouse pneumonitis agent), PL (agent of the enzootic abortion of ewes), SR-1, AP-23 and OV isolated from a patient with Reiter's syndrome and from human arthritis, respectively, and finally, strain MyB was isolated from enteric infection of cattle. Strains LB-1, MP and MyB were kindly provided by Dr. J. Schachter (WHO Int. Reference Center, San-Francisco, U.S.A.), the strain PL was supplied by prof. J. Storz (Colorado State University, Fort Collins, U.S.A.) and the Nigg strain was obtained from the American Type Culture Collection (U.S.A.). The LV strain was received from the museum of the D. I. Ivanovsky Institute of Virology, Moscow. The rest strains were isolated in the laboratory of prof. A. A. Shatkin.

Propagation of rickettsiae and chlamydiae. *C.b.* and chlamydiae were cultured in the yolk sac epithelium of developing CE. The yolk sacs of 7-day-old embryos were inoculated with 0.5 ml of infectious suspension. The accumulation of the agent was investigated in the yolk sac impression smears stained according to Giemsa-Romanowski or according to the method of Zdrodovsky. Infected yolk sac membranes were fixed at 5—7 days post inoculation at the peak of infection development and examined by electron microscopy.

The *Alveonasus lahorensis* imagos were infected with *C. b.* into the haemocel (Sidorov, 1960). Suspension of infected yolk sac membranes in the 2nd passage was used for primoinfection. Drops of haemolymphs were fixed after a long term tick to tick passaging of *C.b.* Their infectivity was checked by examination of impression smears prepared from the haemolymph and stained according to Zdrodovsky.

Electron microscopy. Fragments of the yolk sac membranes were fixed on a cooled bench (Reichert, Austria) adopted for work with infectious material in the drops of the chilled fixative; then they were immersed for 20 min into 1% glutaraldehyde solution prepared either in 0.2 mol/l cacodylate buffer or in 0.1 mol/l phosphate buffer (pH 7.2) and postfixed in 1% OsO_4 in the same buffer for 2 hr.

The drops of haemolymph were fixed for 5 min in plastic wells coated with 1—2% gelatine (Chebanov and Sidorov, 1972*a,b*) by means of 4% glutaraldehyde in Hank's solution and then postfixed in 1% OsO_4.

The samples were dehydrated in ethanol and absolute acetone and embedded into Araldite M (Luft, 1961) or into Epon-Araldite (Mollenhauer, 1964). Ultrathin sections were cut on Ultrotom III LKB 8800 and viewed in microscope JEM-7 and JEM-100 B (Jeol, Japan). The sections were contrasted with 5% uranylacetate in certain cases followed by lead citrate in alcohol solution (Chebanov, 1971).

Results

Ultrastructure of Coxiella burnetii

Haemocytes of the infected ticks contained 2 types of *C.b.* cells (Fig. 1): 1) about 10—15% of *C.b.* cells comprised the commonly described forms which were 1.5—1.6 μm long, 0.5—0.8 μm wide; 2) the majority of *C.b.* cells was smaller, 0.8—1 μm long, not wider than 0.3 μm in diameter, showed a dense cytoplasm and was surrounded by a 25—30 nm thick "envelope complex". The "envelope complex" resembled to the cell-wall of Gram-positive bacteria and consisted of 5 layers: external osmiophilic, external osmiophobic, thick intermediate osmiophilic and internal osmiophobic layers; the last internal osmiophilic layer was usually masked by the electrondense cytoplasm. The conventional form of cytoplasmic membrane was not found in the latter cells (Fig. 2). In some *C.b.* cells peculiar membrane structures were seen on the periphery of cytoplasm forming electron dense lamellae with a periodicity of 6—7 nm and 3 μm thick osmiophilic layer (Figs. 1, 2). The nucleoid was usually situated in the centre. Occasionally, dense elongated cells were encountered in the stage of binary fission.

Similar small, rounded dense *C.b.* cells 0.2—0.3 μm in diameter surrounded by a thick "envelope complex" (about 17 nm) were sometimes found in the yolk sac epithelium of CE (Fig. 3). These also showed lamellar intracytoplasmic structures. It should be mentioned that *C.b.* cells with such fine structure were not frequent under given conditions.

Ultractructure of chlamydiae

Spore-like forms (elementary bodies) surrounded by a thick membrane were seen also among some strains of chlamydiae (Nigg, PL, MyB, SR-1 and Ov, Figs 4–6), but not among others. Between the cell wall and the cytoplasmic membrane a highly electron dense 6–8 nm thick layer was visible situated on the whole circumference. An impression was gained that the outer wall and the cytoplasmic membrane of elementary bodies were closely connected with each other forming the five-layer 16–20 nm thick "envelope complex" described above. The internal osmiophilic layer in this structure was often not apparent being overshadowed by the dense cytoplasm. The dense nucleoid in elementary bodies was often located excentrically. Between the nucleoid and the "envelope complex" a system of folded, several paralelly packed endoplasmic membranes was found showing a periodicity of 6.5 nm and a 3 μm thick osmiophobic layer (Fig. 4). In contrast, the vegetative forms of chlamydiae (reticulate bodies) had a conventional structure (Fig. 6) in all strains investigated, irrespective of whether their elementary bodies had or had not the "envelope complex" cell-wall strucure.

It should be emphasized that the occurrence of dense forms of *C.b.* and chlamydiae surrounded by the "envelope complex" depended on conditions of cultivation. Among *C.b.* cells they were regularly found in the course of long term passaging in ticks but rarely in chick embryos; among chlamydiae they were seen only in some strains from recently dead chick embryos but not in cell cultures.

Discussion

Electron microscopic investigation showed analogous dense forms in *C.b.* and in some chlamydiae. There was a tendency for fusion of the cytoplasmic membrane with the cell wall into single "envelope complex" formation. This was found also among *C.b.* cells cultured in the organs of susceptible laboratory animals such as spleen of mice and tunica vaginalis of guinea pigs (Gulevskaya and Kudelina, 1968). A similar composition of the "envelope complex" was detected also in smaller dense *C.b.* phase I cells as demonstrated by Wiebe *et al.* (1972). The latter authors had separated two types of cells by means of density gradient centrifugation of the yolk sac homogenates infected with the strain Nine Mile phase I: 1) small, rod shaped dense cells with the "envelope complex" and condensed nucleoid and 2) larger, bacteria-like cells showing loosened nucleoid filaments. Similar "envelope complex" of the dense forms (elementary bodies) was described in *Chlamydia psittaci* (strain 6 BC) from yolk sacs of CE (Poffenroth *et al.*, 1973).

No doubt, dense forms of *C.b.* and chlamydiae are rigid cells although electron micrographs did not show a definite peptidoglycan layer. It is interesting to mention the assumption of Kordová (1978) that vegetative forms of rickettsiae and chlamydiae had a defective cell wall and that in this respect they are analogous to the instabile L-forms of bacteria, while, the spore-like forms are wrapped by a complete cell wall. When comparing our observations on *C.b.* and on chlamydiae with those carried out on arthropod pathogens – the rickettsiellae (Devauchelle *et al.*, 1972; Louis *et al.*, 1977) a striking analogy in their ultrastructure can be noticed. The similarity of findings can be summarized as follows: 1) Both microorganisms are growing in intracellular vacuoles limited by a membrane. 2) The vacuoles contain 2 types of cells: smaller ones, with dense cytoplasm and nucleoid surrounded by a five-layer "envelope complex" and larger ones, in their ultrastructure similar to "bacteria-like" rickettsiae previously described. 3) The small dense forms of Rickettsiella and *C.b.* are of similar size, shape and ultrastructure with a dense cytoplasm and nucleoid their five-layer "envelope complex" resembling to that of grampositive bacteria.

The replication cycle of rickettsiellae has been described to proceed in alternation of dense and bacteria-like forms. During this cycle, the dense cells may transfer their genetic material to other cells (Devauchelle *et al.*, 1972). Unfortunately our current knowledge on the life cycle of rickettsiellae is unsatisfactory. Therefore, no final conclusion on the role of dense forms of *C.b.* can be made. It can be stated only that both forms may be infectious (Wiebe *et al.*, 1972). In rickettsiellae, occasionally, enlarged dense cells were seen but without signs of division. The dense forms of rickettsiellae did not contain membrane structures in their cytoplasm (Devauchelle *et al.*, 1972).

According to our opinion, the analogy of *C.b.* and rickettsiellae while growing in the arthropod vector is much more marked, than the similarity between the structure of rickettsiellae and chlamydiae. No doubt, some chlamydiae had "envelope complex" membranes limiting the elementary bodies (i.e. the dense spore-like forms). The elementary bodies of chlamydiae are always round-shaped, while the dense Rickettsiella cells are rather elongated. Finally, the vegetative forms of chlamydiae are not infectious. The discovery of related forms of rickettsiellae and *C.b,* in arthropods is of special interest in the light of the view that arthropods were the primary hosts of rickettsiae and that rickettsiae pathogenic for the vertebrates had originated from rickettsiae which were symbiotic or pathogenic for invertebrates (Balashov *et al.*, 1971).

In order to contribute to the taxonomy of *Rickettsiales* and *Chlamydiales* further should be done comparing the fine structure and biology of these agents. During the preparation of our manuscript, the paper of McCaul and Williams (1981) has appeared describing the fine structure of dense forms of the american *C.b.* strains. Their findings are in accordance with ours. The authors postulated a special life cycle for *C.b.* considering the dense forms for endospores in the course of sporogenic differentiation.

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Explanation of Electron Micrographs (Plates XX—XXII):

Fig. 1. Two types of *C.b.* cells in tick haemocytes: dense rounded or elongated (I) and "bacterium-like" (II); icm = intracytoplasmic membrane structures.

Fig. 2. Dense cells of *C.b.* with a thick „envelope complex" in tick haemocytes. icm = intracytoplasmic membrane structures.

Fig. 3. Small dense *C.b.* cells with a thick "envelope complex" and intracytoplasmic membrane structures (icm) in the yolk sac membranes of developing chick embryos (I); II — rickettsiae showing the conventional "bacterium-like" fine structure.

Fig. 4. Elementary body (dense cell) of the chlamydia of mouse pneumonia surrounded with and "envelope complex"; icm = intracytoplasmic membrane structures.

Fig. 5. Elementary body of the chlamydia of enzootic abortion of ewes surrounded with an "envelope complex". In the cytoplasm stellate glycogen granules.

Fig. 6. A fragment of an intracytoplasmic inclusion body of chlamydia isolated from Reiter's syndrome (strain SR-I) in the epithelium of chick embryo yolk sac; elementary (dense forms) and reticulate (vegetative forms) bodies; icm — intracytoplasmic membrane structures.

The length of the scale line in all Figures is 100 nm.