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ELECTRON MICROSCOPIC ANALYSIS OF IN VITRO INTERACTION OF *RICKETTSIA PROWAZEKII* WITH GUINEA PIG MACROPHAGES. II. MACROPHAGES FROM IMMUNE ANIMALS

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Summary. — Alike to macrophages from intact animals, reproduction, destruction and formation of spheroplast-like forms were observed in macrophages from immune guinea pigs 2 months post-infection (p.i.) with the virulent Breinl strain of *Rickettsia prowazekii*. Unlike to the former, immune macrophages revealed phagolysosomes which were larger in size and contained more rickettsiae showing morphologic signs of destruction. Spheroplast-like forms occurred more often and were more numerous than in intact animals. Structures morphologically similar to L-forms of gram-negative bacteria and that of chlamydiae were also detected. After adding immune serum, more intact rickettsiae and spheroplasts were found in phagosomes as well as more phagolysosomes contained rickettsiae and spheroplasts with morphologic signs of destruction. It is suggested that clearance of immune macrophages from rickettsiae is mediated by at least two processes: on one hand by destruction of rod-shaped rickettsiae within phagolysosomes and, on the other hand, by formation and subsequent destruction of spheroplast-like forms within vacuoles, which probably also function as phagolysosomes.

Key words: Rickettsia prowazekii; macrophages; reproduction; destruction; spheroplasts; immune guinea pigs

Introduction

In previous communication (Popov *et al.*, 1987) we have described the interaction of *Rickettsia prowazekii* with macrophages from intact guinea pigs. The task of present paper has been to describe the ultrastructure of macrophages from immune animals during their interaction with these rickettsia.

Materials and Methods

Infection. Guinea pigs were infected intraperitoneally with 1 ml of standard egg culture of *Rickettsia prowazekii* (strain Breinl) stored at −60 °C in a dose of 10^4-10^5 ELD_{50}. Peritoneal macrophages have been used 2 months post-infection.

Methods of preparation and infection of macrophage cultures as well as light and electron microscopic techniques were the same as described in previous communication (Popov *et al.*, 1987).

Results

Examination of macrophages from immune animals in the light microscope has shown that 30 min p.i. with rickettsiae which had been treated with normal serum a relatively low number of cells was damaged (up to 26 %). By 4 hr later, this number had remained approximately the same. Within 2 days, the macrophages became destroyed with many (uncountable number) of rickettsiae being inside and outside cells.

When macrophages from immune animals were infected with rickettsiae treated with immune serum, the quantity of damaged cells was rather low (up to 40 %) after 30 min, but it dramatically increased at hr 4 (up to 80 % of the monolayer cells). On day 2, the macrophages were destroyed, and large amounts of rickettsiae were seen both within and outside macrophages.

Control inoculation of chick embryos with the content of the test tubes harvested 2 days after rickettsial infection of cells from either intact or immune animals revealed viable rickettsiae.

Rickettsia prowazekii reproduced free in the cytoplasm of macrophages from immune animals infected with rickettsiae treated with normal serum (Figs. 1 and 2). Electron microscopic examination allowed to observe division of rickettsiae in macrophages even by 30 min after infection (Fig. 1). Destruction and spheroplast-formation was observed more often in macrophages from immune animals as in macrophages from nonimmune ones. Phagolysosomes in the former were larger and often contained several rickettsiae and, in addition, many vesicles, membranes and amorphous material of moderate density (Figs. 3 and 4). Spheroplasts of *Rickettsia prowazekii* in macrophages from immune animals could be small in size, but their cytoplasm had a low density and their nucleoids were loosened (Figs. 5 and 6). In some cells peculiar structures were seen with long membrane evaginations, vacuolized and fragmented cytoplasm. Long protrusions enclosed cytoplasmic fragments limited by single membrane (Fig. 7). These forms were morphologically similar to giant chlamydial bodies which, according to our suggestion, represent a stage of L-transformation of chlamydiae (Popov *et al.*, 1977, 1982; Prozorovsky *et al.*, 1979). Organelles of immune animal macrophages showed a more "activated" appearance: Golgi complex and the granular endoplasmatic reticulum revealed hypertrophy to different extent, and considerable quantity of cytoplasmic vesicles was detected.

In macrophages from immune animals infected with rickettsiae pretreated with immune serum and then cultivated in its presence, most of

rickettsiae, either bacillus-shaped or spheroplast-like, were situated within vacuoles at 30 min p.i. (Figs. 5 and 6). These vacuoles reached a large size with semilunar invaginations into the nucleus (Fig. 8). Most rickettsiae within vacuoles were destroyed. This was evident from regular detection of bacillus-shaped and spheroplast-like cells in the course of destruction inside vacuoles (Figs. 4 and 6). The destruction of bacillus-shaped forms, alike to macrophages from nonimmune animals, started by bleaching and destruction of nucleoid zones (Fig. 4). In spheroplast-like rickettsiae, both the

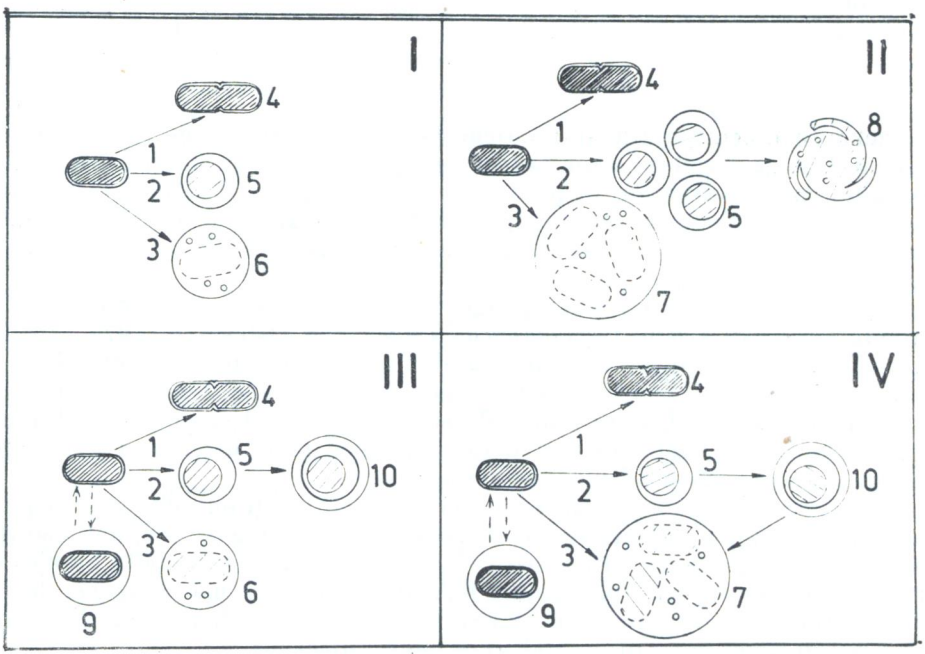

Fig. 10

Scheme of interaction of macrophages from intact and immune guinea pigs with virulent strain of *Rickettsia prowazekii*.

1 — reproduction of rickettsiae, 2 — formation of spheroplast-like forms, 3 — destruction in phagolysosomes, 4 — binary fission of rickettsial cells, 5 — spheroplast-like rickettsiae, 6 — phagolysosome with a rickettsial cell showing signs of morphologic destruction 7 — large phagolysosomes produced in macrophages from immune animals with many rickettsiae showing signs of morphologic destruction, 8 — rickettsial forms similar to L-forms of gram-negative bacteria and chlamydiae, 9 — vacuoles with intact rickettsiae treated with immune serum, 10 — vacuoles with spheroplast-like rickettsiae in experiments with immune serum.
I — nonimmune macrophages; normal serum treatment.
II — immune macrophages; normal serum treatment.
III — nonimmune macrophages; immune serum treatment.
IV — immune macrophages; immune serum treatment.

nucleoid and cytoplasmic membranes were destroyed. In the meantime, the cells could attain the most unusual shapes (Figs. 4 and 6). In addition to a large number of rickettsiae found in vacuoles, bacillus-shaped and spheroplast-like cells occurred free in the cytoplasm (Figs. 4 and 6).

At hr 4 p.i., in addition to giant vacuoles containing large quantities of rickettsiae, microcolonies of *Rickettsia prowazekii* freely reproducing in the cytoplasm were found in the macrophage cytoplasm (Fig. 9).

After 24 hr p.i. most of the monolayer cells were destroyed and only single cellular fragments (nuclei, cytoplasmic organelles) sometimes with adsorbed rod-shaped and spheroplast-like rickettsial forms were observed in the electron microscope.

Discussion

The fate of intracytoplasmic rickettsiae in macrophages from immune guinea pigs (as well as from intact ones) could follow 3 different paths: reproduction, destruction or production of spheroplast-like forms (Fig. 10). According to electron microscopic findings, rickettsiae divided equally well in macrophages from nonimmune and immune animals, and the division could start even 30 min p.i.

Specifically stimulated macrophages (immune) failed to maintain the reproduction of *Rickettsia tsutsugamushi* (Nacy and Osterman, 1979). In the case of *R. typhi* no differences in the ability to maintain their reproduction has been observed between macrophages from immune donors and nonimmune macrophages (Gambrill and Wisseman, 1973). Phagosomes containing rickettsiae in the course of destruction occurred more often and had larger size in the macrophages from immune animals.

After adding of immune serum to macrophages from immune guinea pigs, not only the number of rickettsiae increased within phagosomes, but also the number of phagosomes with destroyed rickettsiae. This especially applies to spheroplast-like forms. Spheroplast formation in these macrophages developed in the same way as in untreated ones, however, spheroplast-like forms occurred in them more often and were more numerous.

In addition to spheroplasts, immune macrophages sometimes also contained unusual rickettsial forms (Fig. 4) similar to cells observed after L-transformation of chlamydiae (Prozorovsky *et al.*, 1979) and gram-negative bacteria (Prozorovsky *et al.*, 1981). Since spheroplast formation can be regarded as the initial stage of bacterial L-transformation, and taking into account the production of the unusual forms mentioned, it can be suggested that the process of rickettsial L-transformation goes farther in immune macrophages than in nonimmune ones and is more intensive. Morphological data are not enough for estimation of the viability of rickettsial L-like forms and their subsequent fate. However, it can be asserted that in macrophages from immune animals most of the spheroplasts die, and that this process can be regarded for a mode of protection of the immune organism against rickettsial infection.

The described peculiarities of the interaction of *Rickettsia prowazekii* with macrophages are summarized in Fig. 10. The differences between 2 types of cells are mainly quantitative: the process of destruction and spheroplast formation is observed more often in macrophages from immune animals. A significant destruction of spheroplasts also takes place within vacuoles. With respect to ultrastructure, the interaction of the guinea pig peritoneal macrophages with *Rickettsia prowazekii* is similar to their interaction with other obligate intracellular agents such as chlamydiae (Popov *et al.*, 1973, 1974). However, the rickettsiae are more resistant to the destroying effect of macrophages and fail to produce as many L-like forms as chlamydiae do, although these forms are destroyed in immune macrophages.

Peculiarities of the interaction of rickettsiae with macrophages are species-dependent. For instance, according to Kekcheeva *et al.* (1983), as well as to our preliminary electron microscopic findings, the fate of *Rickettsia prowazekii* is different in peritoneal macrophages of cotton rats. High multiplicity of infection and mild treatment allowed us to observe the typhus pathogen in macrophages in the electron microscope during the first hours p.i. These rickettsiae seem to have high "aggressiveness" and their intensive reproduction leads to fast destruction of macrophages. At final stage of our electron microscopic analysis (24 hr) both reproducing rickettsiae and spheroplasts were present in the macrophage cytoplasm (outside phagosomes), i.e. the rickettsiae were not completely destroyed at that time. It was impossible to observe the subsequent fate of rickettsiae in macrophages because of cell death. However, in our previous work (Vovk *et al.*, 1980) we observed the interaction of rickettsiae with macrophages over several days, when rickettsiae purified by differential centrifugation were added in smaller quantities. These observations have shown that most of macrophages from immune animals in the presence of immune serum appeared to be free of rickettsiae, which was confirmed by biological tests on chick embryos.

Presented investigations demonstrated that clearance of immune macrophages from the pathogen seems to proceed by at least two processes. On one hand, the destruction of rod-shaped rickettsiae within phagolysosomes and, on the other, production and subsequent destruction of spheroplast-like forms within vacuoles, the latter probably representing also phagolysosomes.

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References

Gambrill, M. R., and Wisseman, C. L., Jr. (1973): Mechanisms of immunity in typhus infections. III. Influence of human immune serum and complement on the fate of *R. mooseri* within human macrophages. *Infect. Immun.* **8**, 631–640.

Kekcheeva, N. G., Vovk, O. A., Chereshkova, E. A., and Abrosimova, G. E. (1983): Reproduction of *Rickettsia prowazekii* in cotton rat macrophage cultures. *Acta virol.* **27**, 268–272.

Nacy, C., and Osterman, J. (1979): Host responses in experimental scrub typhus: role of normal and activated macrophages. *Infect. Immun.* **26**, 744–750.

Popov, V. L., Martynova, V. R., Shatkin, A. A. (1973): Interaction of venereal lymphogranuloma agent with macrophages in vitro (in Russian). *Vop. Virus.* **18**, 175–181.

Popov, V. L., Martynova, V. R., and Shatkin, A. G. (1974): Interaction of venereal lymphogranuloma agent with macrophages from immune animals in vitro (in Russian). *Vop. Virus.* **12**, 678—683.

Popov, V. L., Shatkin, A. A., Avakyan, A. A., and Prozorovsky, S. V. (1977): Peculiar small forms in the developmental cycle of *halprowiae* (*Chlamydiae*) and their probable relationship L-transformation phenomena (inRussian). Zh.Mikrobiol. (Mosk.) **1977** (3), 42—46.

Popov, V. L., Shatkin, A. A., and Prozorovsky, S. V. (1982): Urogenital chlamydioses (halprowioses). Atypical pathogen forms and their probable significance in the etiology and pathogenesis of infection (in Russian). *Vestn. Vener. Derm.* **1982** (2), 26—28.

Popov, V. L., Prozorovsky, S. V., Vovk, O. A., Kekcheeva, N. K., Smirnova, N. S., and Barkhatova, O. I. (1987): Electron microscopic analysis of in vitro interaction of *Rickettsia prowazekii* with guinea pig macrophages. Macrophages from nonimmune animals. *Acta virol.* **31**, 53—58.

Prozorovsky, S. V., Beskina, S. R., Popov, V. L., and Barkhatova, O. I. (1979): Morphological, changes of halprowiae (Chlamydiae) proceeding on the pattern of bacterial L-transformation (in Russian), pp. 22—26. In O. V. Baroyan (Ed.): *Halprowiozy* (*Chlamidiozy*) *Cheloveka i Zhivotnykh*, Moskva.

Prozorovsky, S. V., Kats, L. N., and Kagan, C. Ya. (1981): *Bacterial L-forms* (mechanisms of formation, structure, contribution to pathology). Meditsina, Moskva, p. 240.

Vovk, O. A., Kekcheeva, N. G., and Abrosimova, G. E. (1980): In vitro study of the function of macrophages from intact and immune animals during experimental rickettsial infection. *Zh. Mikrobiol.* (*Mosk.*) **1980** (2), 41—45.

Legends to Figures (Plates XII—XVII):

Fig. 1. Division of rickettsiae in the cytoplasm of immune macrophage 30 min p.i. Normal serum treatment. Magn. ×75,000. Here and thereafter the scale bar 0.5 μm.

Fig. 2. Rickettsia prowazekii free in the macrophage cytoplasm 24 hr p.i. Normal serum treatment. Magn. ×55,000.

Fig. 3. A large phagolysosome containing rickettsial cells in the state of morphologic descruction, vesicles, membranes and amorphous material, 4 hr p.i. Normal serum treatment. Magn. ×5,000.

Fig. 4. Macrophage cytoplasm with phagolysosomes containing rickettsiae and spheroplasts at different stages of digestion 2 hr p.i. Immune serum treatment. Magn. ×40,000.

Fig. 5. Area of immune macrophage cytoplasm with phagolysosomes containing spheroplast-like rickettsial forms 30 min p.i. Immune serum treatment. Magn. ×90,000.

Fig. 6. Area of immune macrophage containing free spheroplasts, spheroplasts within vacuoles in the course of destruction and intact rickettsia inside the vacuole 2 hr p.i. Immune serum treatment. Magn. ×80,000.

Fig. 7. An L-like rickettsial form 4 hr p.i. Normal serum treatment. Magn. ×70,000.

Fig. 8. A large vacuole containing *Rickettsia prowazekii* 30 min p.i. Immune serum treatment. Magn. ×70,000.

Fig. 9. A fragment of macrophage cytoplasm 4 hr p.i. containing a microcolony of free *Rickettsia prowazekii* and a large vacuole (indicated by arrows) with numerous rickettsiae. Immune serum treatment. Magn. ×50,000.