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PERSISTENCE OF *RICKETTSIA PROWAZEKII* IN COTTON RAT MACROPHAGE CULTURES

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Summary. – *Rickettsia prowazekii* is able to multiply and persist for a long time in cotton rat macrophage culture (29-days observation period). Electron microscopic studies showed that the structure of Rickettsiae remained intact at different intervals post-inoculation (p.i.). In the course of persistence Rickettsiae revealed a reduced capacity to infect chick embryos and guinea pigs, however, the infectious agent could be isolated at all stages of persistence of cultured cells such as fibroblasts of the guinea pig embryo, macrophages of intact cotton rats.

Key words: Rickettsia prowazekii; culture of cotton rat macrophages; chick embryos; guinea pigs

Introduction

In the course of exanthematous typhus the host usually becomes cleared from the infectious agent. However, in some instances the rickettsiae may persist in the body for a long time causing recrudescence of epidemic typhus, an illness known Brill-Zinsser disease. Problems associated with rickettsial persistence still attract attention of investigators, as longterm presence of the agent is potential hazard of exanthematous typhus. The reasons for elimination of rickettsiae on one hand and their persistence in the body on the other hand are unknown. Recently increasing interest has been focused to the role of macrophages in persistence (Kazár, 1988). The result of infectious agent's interaction with macrophages could be one of the factors affecting the fate of rickettsiae in the body. This assumption was based on the analysis of rickettsial interaction with macrophages of different animal species and their strains. We observed different patterns of disease progression in association with interaction of macrophages and *Rickettsia tsutsugamushi* in different inbred mice strains (Kekcheeva *et al.*, 1978). In highly sensitive mouse strains the rickettsiae multiplied in macrophages and developed manifest infection. In low sensitive mouse strains macrophages digested most of rickettsiae and only inappa-

rent infection took place. Similar outcome was noticed in various animal species (white rats, guinea pigs, and cotton rats) following infection with *Rickettsia prowazekii.* White rats develop only asymptomatic infection with a rapid clearance of the infectious agent from the body. In this case, as reported by Ablizin (1974), active digestion of rickettsiae was observed in white rat macrophage culture following inoculation with *Rickettsia prowazekii* (particularly within the first hours after inoculation). Guinea pigs infected with *Rickettsia prowazekii* develop manifest infection with fever and in most instances the host is cleared from the infectious agent within 1 month. Active multiplication of *Rickettsia prowazekii* in intact guinea pig macrophage culture results in their destruction by days 7–9 p.i. Cotton rats are very susceptible to *Rickettsia prowazekii,* and the latter may persist in their body for a long time period (Krasnik, 1963; Ignatovich, 1973). Long term active multiplication of persisting rickettsiae (for the 19 days long observation period) can be seen in macrophage culture of cotton rats, while the structure of a number of macrophages remained intact (Kekcheeva *et al.,* 1983).

Here we attempted to use cotton rat macrophage culture infected with *Rickettsia prowazekii* to follow their persistence. We were interested to describe the state of rickettsiae after their long-term persistence in cotton rat macrophages, regarding their ultrastructure and some inherent biological properties (capacity to infect chick embryos, guinea pigs).

Materials and Methods

Preparation of macrophages. Macrophages were obtained from peritoneal exudate of cotton rats injected intraperitoneally withThioglucol broth 3 days before cell isolation. Cell suspension was seeded into test tubes containing converslips (600 000 cells/tube) or to special tube lacking coverslips for electron microscopy (for details see Popov *et al.,* 1987). Cultivation was performed in medium 199 supplemented with 10 % bovine serum.

Macrophage inoculation. By 48 hr after seeding the macrophage culture was infected with egg suspension of *Rickettsia prowazekii* partially purified by differential centrifugation, the dose of 10^5–10^6 ID_{50}. Microscopic examination of Giemsa-stained prepararions was done using light microscope. To calculate the percentage of rickettsia-infected macrophages 400 cells were viewed. In parallel, the cells were embedded for electron microscopic studies. The capacity of rickettsiae to infect chick embryos was checked by inoculation of the content of test tubes (scraping of the cell mass from tube wells) to 7-day chick embryos. Chick embryos' death was recorded, and microscopic examination of yolk sacks was carried out. „Blind" passage was performed in case of negative results of microscopy. The same suspension was tested in guinea pig males weighing 300–350 g by intraperitoneal inoculation and subsequent registration of body temperature and assays for complement-fixing antibodies in blood sera. The content of tubes was also used for infecting the primary culture of guinea pig embryo fibroblasts and the intact cotton rat macrophage cultures.

Results

As expected, *Rickettsia prowazekii* multiplied in cotton rat macrophage culture very intensively. This finding was fully confirmed in presented studies.

Table 1. The results of studies of *Rickettsia prowazekii*-infected cotton rat macrophage culture

Day of the testing of infected macrophages	Rickettsia-containing macrophages (%)	ID_{50} of rickettsia in the macrophage suspension	
		for chick embryos	for guinea pigs
1	20	$10^{2.5}$	$10^{2.5}$
4-5	50	$10^{5.16}$	$10^{4.5}$
7-8	70	10^{6}	not done
11	90	$10^{4.5}$	$10^{2.5}$
19	85	$10^{1.5}$	$10^{1.5}$
22	90	0	not done
28	90	0	not done

Thus, on day 2 after macrophage inoculation, about 20 % of cells were infected with rickettsiae. By day 5 the percentage of infected macrophages rose to 50 % with subsequent increase up to 70 % by day 7 and up to 90 % by day 11, respectively; the latter rate of infected macrophages persisted up to day 28, i. e. The total observation period (Table 1). The quantity of rickettsiae found in macrophages was extensive (Fig. 1).

Electron microscopic examination revealed a large number of free lying multiplying rickettsiae in macrophage cytoplasm one day later. Further on, rickettsiae continued to multiply abundantly in macrophage cytoplasm forming vast microcolonies and filling up a larger part of cytoplasm. Occasionally phagolysosomes containing altered rickettsiae occurred in the cells. Macrophages did not show any marked features of destruction. Considerable vacuolization and accumulation of intracellular lipid were also seen in control (uninfected) culture. This picture was observed up to day 28 when macrophage destruction occurred with release of rickettsia. Rickettsial structure was typical

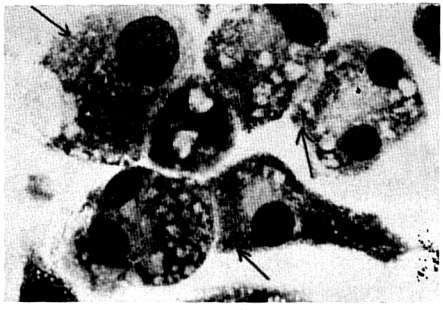


Fig. 1
White rat macrophages on day 21 p.i. with *Rickettsia prowazekii*
Intracellularly located rickettsiae are indicated by arrows. Magn. x 630.

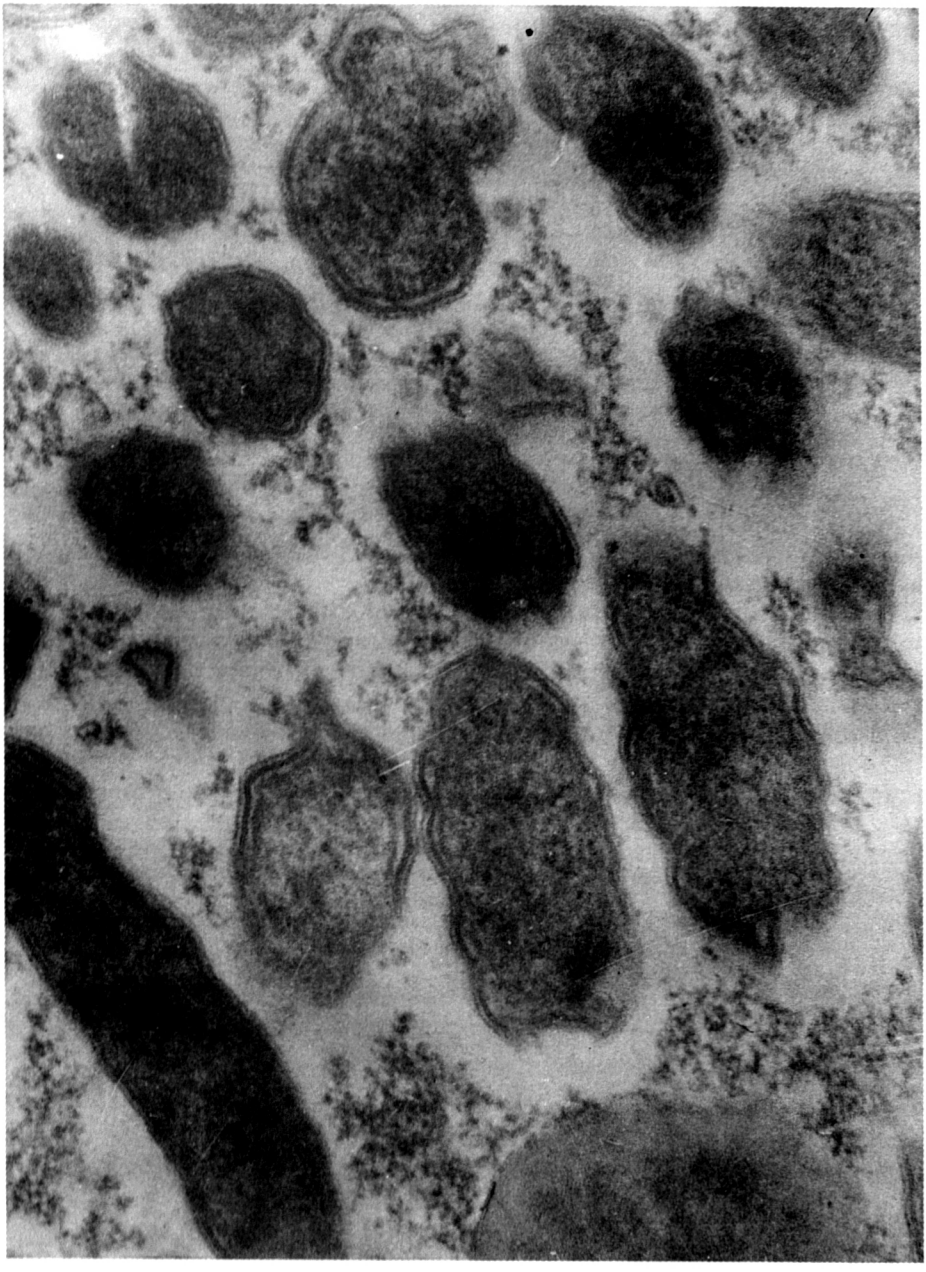


Table 2. The ability of *Rickettsia prowazekii*-infected macrophage cultures to infect guinea pig embryonic fibroblasts and intact white rat macrophages

Day of examination of infected macrophages	Dilution of rickettsiae suspension in macrophages	Rickettsiae in indicator cells (days)						
		guinea pig fibroblasts				white rat macrophages		
		5	8	5	9	16	22	22
1	10^{-1}	++++	++++	-	-	+	++++	
	10^{-2}	not done		-	-	-	single	
	10^{-1}	++	++++	++	+	+++	destructed cells	
4	10^{-2}	not done		-	-	+++	+++	+++
	10^{-1}	++	+++	-	-	single	+++	destr. cells
11	10^{-2}	not done				+	not done	
	10^{-1}	++	++++	single		+	not done	+++
28	10^{-2}	not done		-	-	+	not done	+++

Footnote: ++++ uncountable number of rickettsiae in the field of view
+++ more than 100 rickettsiae in the field of view
++ 50-100 rickettsiae in the field of view
+ less than 50 rickettsiae in the field of view

and characteristic for alive microorganisms (Fig. 2). Consequently, electron microscopic studies showed that ultrastructure of rickettsiae was not changed during their persistence in cotton rat macrophage culture, and no signs indicated any loss of viability of the infectious agent.

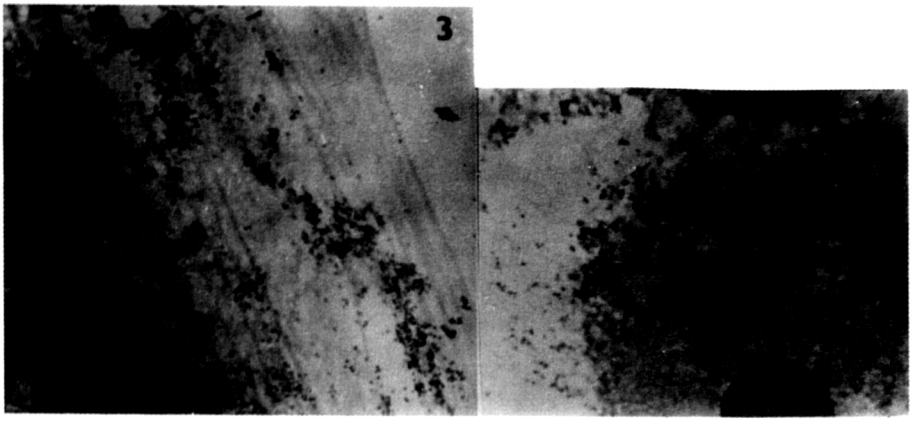
The ability of rickettsiae persisting on white rat macrophage culture to infect chick embryos was examined on days 1, 4, 8, 11, 19, 22, and 28 p. i. The results of these experiments are summarized in Table 1. It is evident that the rickettsiae were isolated up to day 19; on days 1–4 and 8 p. i. all the probes were positive, and the rickettsiae could be islolated in chick embryos in a comparatively high titre without a „blind" passage. On day 11 p. i. the rickettsial titre in the probes was somewhat lowered. On day 19 after infection not all the probes proved to be positive, and the rickettsial titre diminished markedly. Moreover, on days 11 and 19 in some samples the rickettsiae could be isolated only after a „blind" passage. On days 22 and 28 neither direct infection nor a „blind"

Fig. 2
Rickettsia prowazekii in cotton rat macrophages on day 25 after p.i.
Preserved struture of rickettsiae as revealed by electron microscopy. Magn. x 50 000.

passage could detect rickettsiae in chick embryos. As indicated above, rickettsial concentration in the macrophages was very high as shown by light microscopy while electron microscopic examination revealed no alterations of rickettsial structure.

Similar results were obtained in infected macrophages of guinea pigs (Table 1). Upon inoculation of guinea pigs with the macrophages preinfected with rickettsiae 1 or 4 days earlier, the animals developed typical infection with fever and characteristic increase of antibody titres (maximum level on week 2). The highest infectious titre in the culture was noted on day 4 after macrophage inoculation. On day 11 the infectious titre of the culture decreased and the antibody titres in infected guinea pigs somewhat increased by week 4. On day 19 p. i. the infectious titre in macrophage culture decreased even more.

Thus, during persistence of *Rickettsia prowazekii* in cotton rat macrophage culture their ability to infect chick embryos and guinea pigs decreased in spite of their presence in abundant amounts in cells and preserved ultrastructure (Table 1). The viability of such rickettsiae in the samples of infected macrophages was tested on days 1, 4, 11, and 28 p. i. using two types of cell culture: guinea pig embryo fibroblasts and intact cotton rat macrophages. Guinea pig embryo fibroblasts appear to be very susceptible to rickettsial infection as shown by I. N. Kokorin. According to our data (Kekcheeva *et al.*, 1983) cotton rat macrophages are very sensitive to *Rickettsia prowazekii* infection. The results of cell culture inoculation with the material under study (Table 2) showed that in all samples (days 1–28 after macrophage infection) the rickettsiae were seen in both guinea pig embryo fibroblasts and in cotton rat macrop-



Figs. 3–4

Fig. 3. Guinea pig embryonic fibroblast culture on day 5 p.i. with infected macrophage suspension (day 28 p.i.). Coccal-like forms. Magn. x 630.

Fig. 4. White rat macrophage culture on day 22 with infected macrophage suspension (day 28 after macrophage infection). Coccal-like forms of rickettsiae. Magn. x 630.

hages (Figs. 3 and 4). In fibroblast culture a large amount of rickettsiae was observed on day 5 p. i. (the first observation day), their amount rose even higher on day 8 (period of observation). In cotton rat macrophage culture rickettsiae accumulated in smaller amounts and at later terms, however, finally their amount was very high. To prove that the rickettsiae seen were *Rickettsa prowazekii* and not other microorganisms, the content of tubes with fibroblast culture (sample of day 28 after macrophage inoculation) was used to infect guinea pigs which were subsequenthy examined serologically. Moreover, guinea pigs were infected with fibroblasts following two passages in these cells. In the first case serological examination yielded negative results, in the second, i. e. after two passages, serum of infected guinea pigs revealed antibodies in a titre of 1:40. The identity of *Rickettsia prowazekii* was proven by immunofluorescent assay (direct examination of cell culture). First, it was proven that the microorganisms isolated from cell culture were *Rickettsia prowazekii,* and second, it was shown that the last ability of rickettsiae to induce infection in guinea pigs was restored following passaging in guinea pig embryo fibroblast culture.

Discussion

The following conclusion can be drawn summarizing the presented data. *Rickettsia prowazekii* is able to persist in white rat macrophage culture for 28 days. A great amount of rickettsiae fill up the cells, but their ultrastructure is not altered. The majority of macrophages also remain practically intact, but may be destructed. This process is very different from that noted in guinea pig macrophage culture where abundant multiplication of rickettsiae is associated with mass destruction of macrophages (days 7–9 p. i.). As shown in specially designed studies, rickettsiae in white rat macrophages remain viable. The question arises whether most of macrophage-persisting rickettsia are viable or the amount of viable rickettsiae is markedly reduced so that only their small portion preserves viability. Our assumption that a major proportion of the infectious agent remains viable is based on the following findings: first, on results of electron microscopic studies showing no alterations in rickettsial structure at all intervals of investigation (up to day 28); second, infectivity of rickettsiae persisting in macrophages on days 1, 4, 11, and 28 p. i. proved to be practically the same for guinea pig embryonic fibroblasts (Table 2). Thus, upon infection of these cells, rickettsiae multiplied intensively both using the samples taken on day 1 p. i. as on day 28. The results of the inoculation with this material of intact white rat macrophages also demonstrate the presence of high amounts of viable rickettsiae at all time thoughout the persistence (Table 2). For example, in inoculated macrophages with the material from day 28, the rickettsiae were present at day 5 which is typical for the high inoculation dose. As shown by our previous studies, cotton rat macrophages inoculated in the

small dose with egg cultured *Rickettsia prowazekii* showed the presence of rickettsiae at later intervals (days 12–14).

Despite a large amount of rickettsiae at all periods of persistence, their ability to infect chick embryos and guinea pigs has markedly decreased. We consider this process to be the result of alterations in some biological properties of persisting rickettsia. These alterations are not constant so that the original properties of rickettsiae may recover during passaging in guinea pig embryo fibroblasts (other cell cultures were not tested). Similar results were obtained by Ignatovich and Grokhovskaya (1972) following long-term cultivation of *Rickettsia prowazekii* in ticks. In some cases decreased virulence and antigenic activity of rickettsia were noted. Single passage of rickettsiae with altered properties in chick embryos led to reversion of their original properties.

It is difficult to made a complete analogy between what occurs in cell cultures and in an organism. We do not know how long rickettsiae may persist in macrophages of the host and in what particular cells they persist for the longest. It is not known whether birological properties of rickettsiae may change in the course of their persistence and if this can promote long-term persistence. If one assumes that altered forms of rickettsiae may exist in the human organism then we can explain the difficulties associated with the attempts of rickettsial isolation from the body at distant terms after infection since the isolation procedure usually involves chick embryos and guinea pigs. It is reasonable to suggest that other cultures of sensitive cells should be applied in addition to chick embryos and guinea pigs to isolate the rickettsiae from the body; in this case both typical and altered types could be obtained. In this respect, cell cultures seem to have advantage because they lack protecting factors.

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