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MULTIPLICATION OF *RICKETTSIA PROWAZEKII* IN COTTON RAT MACROPHAGE CULTURES

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Summary. — The susceptibility of cotton rat macrophages to *Rickettsia* (*R.*) *prowazekii*, the percentage of the affected cells, and the intensity of damage to individual cells by rickettsiae were found to be much higher than those in guinea pig macrophages infected under similar conditions. At the same time, cotton rat macrophages proved to be more resistant to the effect of rickettsiae than guinea pig macrophages. Some common features of infection in cell culture and in animals have been observed. It is suggested that the outcome of interaction of rickettsiae with macrophages of one or another animal species may be important in generating acute or persistent infection.

Key words: *macrophages*; *cotton rats*; *Rickettsia prowazekii*

Introduction

In recent years, the study of the cellular factors of immunity and macrophages has received increasing attention in infectious pathology and immunonology. It has been demonstrated that macrophages and T lymphocytes of guinea pigs and mice play a role in resistance to rickettsial diseases (Catanzaro *et al.*, 1977; Kazár *et al.*, 1977; Nacy *et al.*, 1979; Murphy *et al.*, 1980). As to the cotton rat immunocompetent cells, we have found no information in available literature. Though cotton rats are highly sensitive to *R. prowazekii*, it is in cotton rats where the longest persistence of the *R. prowazekii* is known to occur (Krasnik, 1963; Ignatovich, 1973). It could be expected that immunocompetent cells and macrophages would differ in their behaviour as compared to other animal species. Therefore, it seemed important to investigate the ability of cotton rat macrophages infected with *R. prowazekii* to eliminate the agent in comparison to the guinea pig macrophages more efficient in their clearance function.

Materials and Methods

Methods for the preparation of cotton rat macrophages. The optimal conditions for propagation of these cells were determined. This work included (1) determination of the time and method for deriving macrophages from the peritoneal cavity of cotton rats, (2) selection of the growth medium for the cells and the temperature regimen, (3) determination of the optimal number of

Table 1. The use of different sera for cultivation of cotton rat macrophages

Serum species	Examination of macrophages in culture (days)		
	3	6	9
Bovine	60*	38	40
Cotton rat	40	60	65
Guinea pig	50	77	75

* Percentage of macrophages containing rickettsiae.

cells to be seeded into a tube culture, (4) determination of the proper time and infecting dose for inoculation of the cells with rickettsiae, and (5) intervals and methods for examination of the infected macrophages.

The study of conditions for propagation and inoculation of macrophages showed that an exudate containing macrophages in abundance could be obtained 3 days after intraperitoneal inoculation of thioglycolate broth. As a rule, cotton rat exudates contained approximately 2—5-fold as many cells as those of guinea pigs.

The macrophages were propagated in medium 199 supplemented with 10% bovine, cotton rats or guinea pigs serum (Table 1). The cells grew well at 37 °C. The optimal number of cells seeded into a tube was $5-6 \times 10^5$ cells/ml.

Infection of macrophages. Macrophages were inoculated 48 hr after seeding with an egg culture of *R. prowazekii*. This was partially purified by differential centrifugation, titrated in chick embryos (EID_{50}), suspended in milk and frozen at −60 °C. Along with the high doses equal to those used for guinea pig macrophage inoculations i.e. 10^6-10^7 EID employed in our previous paper (Vovk *et al.*, 1980) also lower doses (10^2 EID_{50}) were used (Table 2). The rickettsiae-infected macrophages were examined microscopically in Giemsa-stained preparations on days 2, 3, 5, 7, 9, 13, 17 and 19 post inoculation. The condition of the cells, the intensity of their affection with rickettsiae, their morphology, and the percentage of the rickettsiae-containing cells were followed. Inoculation of chick embryos and guinea pigs with the contents of the tubes (bioassay) was performed to assess the viability of the agent.

Results

In culturing cotton rat peritoneal macrophages and infecting them with *R. prowazekii* we were interested to assess the viability of cells in tubes and their susceptibility to rickettsiae. The studies showed that peritoneal macrophages of cotton rats might be propagated in tubes for long periods of time. As a rule, in our experiments the observation period was 19—21 days with weekly changes of the medium. Intact as well as infected macrophages could be propagated in the presence of sera of different species: bovine, cotton rat and guinea pig (Table 1). Because the infection rate of macrophages infected with rickettsiae and propagated in the presence of sera from different species was approximately similar, i.e. the rickettsiae multiplied equally well under all the conditions tested, the percentage of infected cells had been quite high. In further experiments cotton rat serum was used.

When the results of infection of macrophages of cotton rats and guinea pigs (a dose of 10^6) were compared, it could be seen that the infection rate of cotton rat macrophages exposed to a high dose (10^6 EID_{50}) of *R. prowazekii* was considerably higher than that of guinea pig macrophages (Table 2). Thus, the portion of affected macrophages of guinea pigs that is, at the peak of infection, by 5—7 days, as a rule, did not exceed 40% in contrast to the cotton rat macrophages, in which in some experiments as many as 90%, i.e.

Table 2. Results of infection of cotton rat and guinea pig macrophages with different doses of R. prowazekii

Animal species	Infecting dose (ID)	Days						
		2	5	7	9	12	14	19
Cotton rat	10^6	30*	90	55	90			47
	10^5	7	90	58	57	38	50	78
	10^2	0	0	0	0	1	3	4.4
Guinea pig	10^6	3	38	21	Cells destroyed			
	10^2	0	0	0	0	0	Cells destroyed	

* Percentage of cells containing rickettsiae.

virtually all cells were filled with tremendous numbers of rickettsiae. Apart from a high percentage of the affected cotton rat cells, noteworthy was a long-term persistencs of rickettsiae in cell culture without degeneration of cells. Large numbers of rickettsiae were found viable during the 19 days observation period, as determined in bioassays using chick embryos and guinea pigs. At the same time, the multiplication of rickettsiae in guinea pig macrophages proceedeed for 7—9 days only. Then the cells became destroyed and the rickettsiae died. Because such multiplication feature of *R. prowazekii* in cotton rat peritoneal macrophages was observeed at high infecting doses (10^6 to 10^7 EID), there was of interest to determine the sensitivity of cells to infection with low doses. The data in Table 2 indicate that cotton rat macrophages may be infected with low doses, while guinea pig macrophages could not. The susceptibility of cotton rat macrophages to the *R. prowazekii* is higher than that of guinea pig macrophages. The same data testify to a peculiar process of rickettsia multiplication in cotton rat cells. Thus, after inoculation of a small dose (10^2 EID_{50}), no rickettsiae could be detected in the culture for 9 days, but further observations and bioassays in chick embryos showed that the agent was present in the cells. This fact may be explained either by the presence of rickettsia in very small numbers or in an unconventional form undetectable by the microscopic methods used.

These observations also indicate that rickettsiae may persist in small numbers for long periods in cotton rat macrophages escaping destruction. Inoculation of macrophages with large doses of rickettsiae led to infection of a great number of cells, in some cases up to 90%. The dynamics of macrophage infection, however, varied in different tests as shown by the different numbers of affected cells (Tables 1, 2). In general, however, cells were highly infected from the very first days post inoculation and remained so throughout. Morphologically, rickettsiae were observed like rods of various sizes in different amount (Figs 1—2). Later on, long filamentous forms appeared (Fig. 3). This change of morphological forms was not absolute for each experiment, but was quite typical. In the course of propagation, some macrophages were disintegrated displaying short rod forms (Fig. 4).

Discussion

Analysing the experimental results and comparing them with the results of the studies with guinea pig macrophages, it seems obvious that the pattern of interaction of *R. prowazekii* with cotton rat macrophages differs from that in guinea pig macrophage cultures. First, the high susceptibility of cotton rat cells is noteworthy. Cotton rat macrophages could be infected with such a small number of rickettsiae which did not affect guinea pig macrophages. Despite of the abundance of rickettsiae in cotton rat macrophages, they were more resistant to the effect of rickettsiae, the majority of them remaining viable for a long time. This kind of interaction of cotton rat macrophages with *R. prowazekii* differs from their interaction with guinea pig macrophages. In the latter case death of macrophages occurred in the early stages of infection. Thus, cotton rat macrophage cultures were characterized by a high susceptibility of cells to the *R. prowazekii*, an intensive spread of the agent in the cell population, and by a long-term viability of both rickettsiae and macrophages.

These features of interaction of *R. prowazekii* with cotton rat macrophages seemed to be analogous to the course of experimental typhus infection in the animals. Cotton rats are known to be highly susceptible to *R. prowazekii* and, at the same time, the agent is capable of long-term persistence in them. The cotton rat macrophage culture was also found to be highly susceptible to this rickettsial species and at the same time capable of prolonged survival in their presence. It seems important, in our opinion, that not only large but also minimal amounts of the agent can persist for long time in cotton rat macrophages. Guinea pigs are less susceptible to *R. prowazekii* than cotton rats and they eliminate the agent sooner. Their macrophage cultures were also less susceptible to *R. prowazekii* but the infection of the cells was more acute. Thus, conclusion can be drawn that the result of interaction of rickettsia with macrophages of one or another animal species may be important for generation of acute or persistent infection. The participation of other factors, however, can not be completely excluded.

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Explanation of Micrographs (Plate XXXI):

Figs. 1—4. Morphological forms of rickettsiae in cotton rat macrophages (× 540).
1 — Large number of rod-shaped forms.
2 — Small number of rod-shaped forms.
3 — Long filamentous forms.
4 — Short rod-shaped forms.