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PROPERTIES IN CULTURE AND PERSISTENCE IN COTTON RATS OF THE *RICKETTSIA PROWAZEKII* VACCINE STRAIN E AND ITS MUTANTS

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Summary. — Cultural properties and the capacity for persistence were studied in spontaneous erythromycin-resistant (E er^rS^M), in induced erythromycin-resistant (E er^rI) mutants and in a virulent revertant (E Vir) of the vaccine strain E, as compared with parent vaccine strain E and standard virulent strain Breinl of *Rickettsia prowazekii*. Cultural properties of the strains were found to differ in passages in chick embryos (CE) and cultures of FL cells. Multiplication indices in CE of mutant E er^rI were significantly lower than those of other strains (E, E er^rS^M, E Vir, Breinl). The multiplication rate in FL cells was found to be high in strains E er^rS^M, Breinl, E Vir, being much lower in strains E er^rI and E. The capacity of the virulent revertant E Vir to persist in cotton rat (CR) was higher as compared with that of standard strain Breinl and significantly higher than that of the parent strain E. Low level carrier state of rickettsia was registered in CR infected with the mutant E er^rI.

Key words: *Rickettsia prowazekii*; *vaccine strain E mutants*; *cultural properties*; *persistence*

Introduction

Studies on the genetic apparatus of rickettsiae on the physiology and structure of the microbial cell as well as studies of different aspects of interactions between rickettsiae and host cell point to the necessity of obtaining marker mutations and determining their biological characteristics. Here we present the results of investigations on the properties in culture and ability to persist in host cells of the antibiotic-resistant mutants and of the isogenous highly virulent mutant of the vaccine strain E in comparison with the parent strain and the standard virulent strain Breinl of *Rickettsia prowazekii*. The isolation of the mutants and their characteristic properties were described earlier (Balaeva and Frolova, 1982; Balaeva *et al.*, 1985; Frolova *et al.*, 1987).

Materials and Methods

Rickettsial strains. Vaccine strain E of *Rickettsia prowazekii* was obtained from prof. Wisseman in 1969; the passage number 282 in chick embryos (CE) was used. Erythromycin-resistant mutant strain E — E er^{r}I was prepared by nitrozoguanidine treatment (Balaeva and Frolova, 1982). Egg culture was 10 times passaged in antibiotic-free CE. Spontaneous erythromycin-resistant mutant of strain E — E er^{r}S^M was selected at the 2nd passage in CE in the presence of 200 mkg/CE erythromycin (Balaeva and Frolova, 1982). Egg culture at passage level 13 in the absence of erythromycin was used. Highly virulent revertant strain E — E Vir — was isolated at the 13th passage from lungs of albino mice (Balaeva, 1969). Egg culture at passage level 6 in CE was used. Standard strain Breinl was applied at its 134th passage in CE.

CE and cell cultures. The ID_{50}CE was determined as well as the rate of rickettsiae accumulation expressed by means of a mean value coefficient. This was determined on the basis of the microscopic analysis of infected yolk sacks as follows: single rickettsiae in the whole preparation were marked as 1, single rickettsiae in each field of view — 2, up to 10 rickettsiae in the field of view — 3, up to 100 rickettsiae — 4, more than 100 rickettsiae — 5, uncountable number of rickettsiae — 6. The mean coefficient was estimated per 1 infected CE out of the total number of rickettsiae in all CE infected with rickettsiae in different doses. From 5 to 8 independent egg cultures of each strain were studied.

To determine the ability of the strains to multiply in FL cells human amniotic cells were γ-irradiated (3000 R) 2 days after seeding. The cells had been grown in medium 199 supplemented with 10% of bovine serum; egg cultures of each strain were 10 times diluted in BHI ("Difco") inoculated into FL cells which were maintained as described earlier (Ignatowich and Gulevskaya, 1970). The cells were observed from 2 to 15 days post-infection. The growth of rickettsiae was assessed by counting of at least 100 cells in each preparation. The percentage of the infected cells was calculated as well as the rate of the rickettsial accumulation. The extent of rickettsial accumulation per cell was expressed as follows: 1 — up to 10, 2 — up to 100, and 3 — over 100 rickettsiae per cell. ID_{50}/cell titre was calculated based on 3 titrations of one egg culture of each strain.

Animals and serological tests. Male cotton rats weighing 50 g were challenged intraperitoneally with egg cultures of different rickettsial strains in the doses from 10^2 to 10^6 ID_{50}/CE. Their sera were investigated on days 15 and 30 at 2—3 and 4—5 months post-infection by complement fixation (CF) microtest against a soluble antigen of *R. prowazekii* (Zdrodovsky and Golinevich, 1972). In a number of experiments the rickettsia were isolated from the infected CR by biological sampling (Ignatovich, 1973).

Results

The mutants and their parent strains as well as the standard strain Breinl of *R. prowazekii* were cultured in CE and in FL cells. The characteristic features of their multiplication in CE are shown in Fig. 1. ID_{50}/CE and the

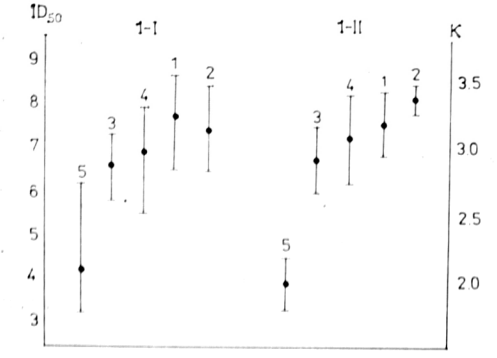

Fig. 1

Multiplication characteristics of *Rickettsia prowazekii* vaccine strain E and its mutants in CE.
1-I: ID_{50}/CE ranges of rickettsia egg cultures, left ordinate — ID_{50}/CE log.
1-II: coefficient of rickettsial accumulation in CE (M $\pm$ M); (right ordinate).
Here and in Figs. 2 and 3: strain E vir — 1, strain Breinl — 2, mutant E er^{r}S^M — 3, strain E — 4, mutant E er^{r}I — 5.

accumulatiou indices of different rickettsial strains differed. High and equal levels of ID_{50}/CE were registered with strains E Vir and Breinl (6.4×10^6 — 5.73×10^8). A little lower values of ID_{50}/CE were obtained for strains E er^rS^M and E (7.67×10^5 — 1.09×10^8). The same correlation has been found between rates of rickettsial accumulation in CE yolk sacks. The accumulation coefficients of rickettsial strains E Vir and Breinl ranged from 3.21 $\pm$ 0.25 to 3.43 $\pm$ 0.1 and those of strains E er^rS^M and E — from 2.94 $\pm$ 0.17 to 3.14 $\pm$ 0.18. The ID_{50}/CE value and coefficient of rickettsial accumulation were much lower with the mutant E er^rI. The ID_{50}/CE ranged from 2.25×10^3 to 1.89×10^6, whereas the mean coefficient of rickettsial accumulation in CE was 2.04 $\pm$ 0.19.

The results of the growth of rickettsial strains E, E er^rI, E er^rS^M, E Vir, and Breinl in FL cells are presented in Fig. 2. Numerical values of ID_{50}/cell of all strains investigated were approximately the same fluctuating within 1 log (1.85×10^7 — 6.41×10^7). However, the values reflecting the intensity of propagation of the agent in FL cells (percentage of the infected cells in culture and accumulation of agent in them) demonstrate the differences in reproductive capacity of the investigated strains. The infection rates of FL cells with strains E and E er^rI were lower with all 7 infectious doses used. The introduction of high doses of rickettsiae (in culture dilution from 10^{-1} to 10^{-3}) of strains E and E er^rI resulted in infection rate from 20.4 to 44% of FL cells, whereas the same concentrations of the agent strains E Vir, E er^rS^M, and Breinl affected from 46.7 to 88.5% of FL cells. With decreasing concentrations of rickettsia in the inoculum, the differences in the percent-

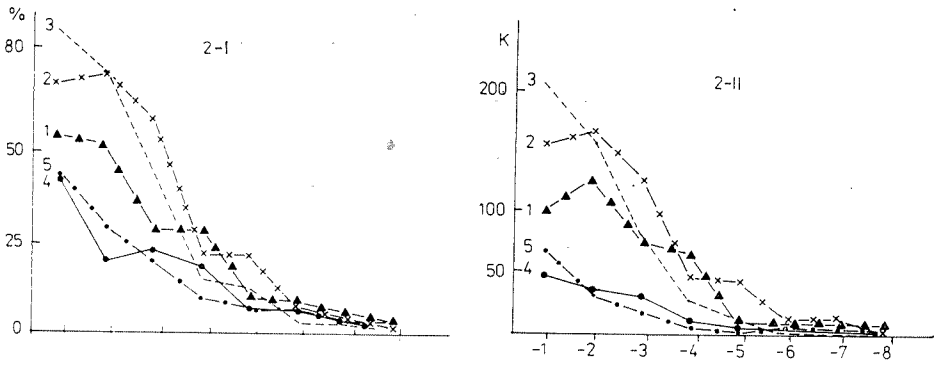

Fig. 2

Multiplication characteristics of *R. prowazekii* vaccine strain E and its mutants in FL cells
2-I: percentage of infected cells at different infection doses; ordinate — the number of infected cells; abscissa — infectious doses for FL cells (logarithms of dilution reciprocals).
2-II: Rickettsial accumulation coefficients in FL cells at various infection doses; ordinate — values of rickettsial accumulation coefficients; abscissa — infection doses for FL cells in reverse logarithms of rickettsia suspension dilutions.
ID_{50}/cell of the studied rickettsia strains (mutants): E Vir — 6.41×10^7; Breinl — 1.02×10^7; E er^rS^M — 2.67×10^7; E er^rI — 1.85×10^7; E — 2.05×10^7.

Table 1. Indices of multiplication of the R. prowazekii strains in FL cells

Index	Strain				
	E	E er^{r}I	E Vir	E er^{r}S^{M}	Breinl
Number of infected cells (%)	14.9*	15.4	25.3	31.1	32.8
Rickettsial accumulation coefficients	19.7**	19.8	53.8	64.8	71.8

* Mean arithmetic values of the percentage of infected FL cells.
** Rickettsial accumulation coefficients derived from summarized data in all cultures investigated independently of the infection dose.

age of infected cells by various strains remained, though they were less evident. In FL cells inoculated with the culture dilutions from 10^{-4} to 10^{-8} the rickettsiae were detected in 0.1 — 19.1% of the cells (strains E and E er^{r}I) and in 0.6 — 33.3% (strains E Vir, E er^{r}S^{M}, Breinl). The same relationships were observed comparing the coefficients of rickettsial accumulation in FL cells. Their levels were lower with strains E and E er^{r}S^{M}, and Breinl. The reproduction rates of rickettsial strains E and E er^{r}I showed coefficients 17 to 73.9 after maximal infection doses (dilution of rickettsial suspension from 10^{-1} to 10^{-3}) then revealed a significant decrease ranging from 0.25 to 8.81. With the rest of the investigated strains high accumulation coefficient was recorded in FL cells (29.6 — 213.2) when rickettsiae were inoculated in dilutions up to 10^{-4}. Even when FL cell cultures were infected with dilutions 10^{-5} — 10^{-6} the strains E Vir, E er^{r}S^{M}, and Breinl had accumulation coefficients of 18.1 to 42.3. Summing up, the reproduction indices of rickettsial strains E and E er^{r}I in FL cells were found to be low and practically indistinguishable. These indices were significantly higher with three other strains (E Vir, E er^{r}S^{M}, Breinl). They coincided with strains E er^{r}S^{M} and Breinl being a little lower in E Vir due to insignificant decrease of the indices in cultures infected with concentrated doses (culture dilutions from 10^{-1} to 10^{-2}). The data are presented in Table 1.

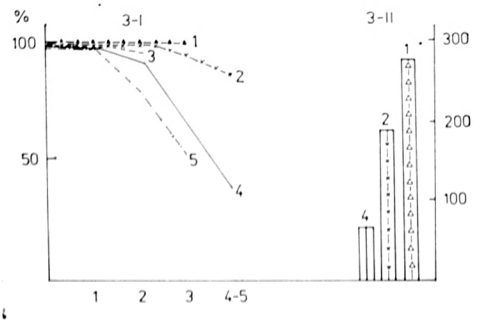

Fig. 3
Indices of persistence of different rickettsial strains in the infected CR
3-I: The time course of reduction of the number of CF-seropositive animals (in %) in different groups of infected CR; left ordinate — number of CF-seropositive animals (%); abscissa — intervals of investigation (months).
3-II: Maximal intervals of rickettsia isolation; right ordinate — intervals of rickettsia isolation (days); abscissa — groups of animals.

The period of rickettsial carrier state in the infected CR was followed by a CF test based on the previous observation (Ignatovich, 1978) that negative seroconversion points to elimination of the agent. In a number of experiments the persistence of rickettsiae was confirmed by rickettsial isolation using biological sampling (Ignatovich, 1973). Fig. 3 shows the persistence indices of different strains. According to CF results the elimination of the agent was accelerated in a population of CR infected with rickettsial strain E er^rI. As soon as 2 months post-infection, 20.6% of animals became negative. The same elimination level was observed at later intervals. After 3 months in additional 27.3% of remaining animals no antibodies were detected. Negative seroconversion of the analogous intensity rate was noticed in a population of CR infected with rickettsiae of strain E but at a more remote interval, namely after 3 months and more. After 2 months the share of CF-negative CR infected with rickettsial strains E and E er^rS^M was 9.1 and 4.2%, respectively. Different results were obtained upon studies on CR infected with the virulent rickettsiae strains. In a group of animals infected with standard strain Breinl the first negative seroconversion result was registered 3 months post-infection (7.3%), without changes for as long as 5 months. The CR infected with virulent revertant E Vir rickettsiae were not found to show negative response within the observation period of 3 months. Random investigations on direct isolation of rickettsiae from organs and tissues of the infected animals also indicated variations between the strains as to the length of persistence. Thus, maximal period during which rickettsia of strains E and Breinl could be isolated was 63 days and 189 days, respectively, whereas with E Vir rickettsiae this period lasted for 282 days.

Discussion

In the course of the studies the differences in cultural properties and persistence were revealed between strain E mutants and both parent and standard strain Breinl of *R. prowazekii*. Erythromycin-resistant mutant E er^rI obtained upon the rickettsia treatment with nitrozoguanidine differed from all the strains studied by lower level of reproduction in CE. This strain was found to have low ranges of ID_{50}/CE and significantly reduced coefficient of rickettsial accumulation in CE (Fig. 1). As to the rate of reproduction in FL cells, mutant E er^rI did not differ from the parent strain E, the investigated culture of which was characterized by a low reproduction level in FL cells. A distinctive feature of the mutant E er^rI was rapid elimination from the host organism. In a group of animals infected with this strain the highest percentage (20.6%) of seroconversion was found according to CFA already after 2 months, while CR infected with rickettsia of vaccine strain E were found to reach the same level of negative seroconversion only after 3 months or later.

Spontaneous erythromycin-resistant mutant E er^rS^M was found to have multiplication characteristics close to that of parent strain E. However, the multiplication indices in FL cells were close to those of virulent E Vir

and Breinl, though exceeding the values found for the investigated culture of strain E. It is difficult to assess the capacity for persistence of rickettsiae of strain E er^rS^M since the observations were limited by the period of 2 months, though it should be noted that negative seroconversion value in the animals after this interval resembled the value among animal sera obtained from DR infected with strain E (4.2 and 9.1%, respectively).

Highly virulent revertant of strain E — strain E Vir was found to have indices of multiplication in CE and FL cells equal to those of standard virulent strain Breinl of *R. prowazekii*. As to the length of persistence in organisms of infected CR, this strain exceeded the standard strain (280 days compared with 189 days).

The biological properties of both the vaccine strain E and the standard virulent strain Breinl are known from a number of publications. In the course of the present studies a slight decrease in the level of rickettsia accumulation in CE was observed in strain E, if compared with that of the virulent strains E Vir and Breinl. The growth rate of strain E rickettsia in FL cells was less than that of strains E Vir, E er^rS^M, and Breinl, being comparable with that of strain E er^rI. In this connection it should be noted that a considerable discrepancy of the data obtained by a number of investigators working at different laboratories with regard to the cultural vaccine strain E (Ignatovich and Gulevskaya, 1970; Gambrill and Wisseman, 1973; Ignatovich, 1973; 1976; Wisseman and Waddell, 1975; Gudima, 1979; 1982; Turco and Winkler, 1982; Winkler and Dougherty, 1983) seems to be due to the heterogeneity of rickettsial populations (Balaeva, 1968; *et al.*, 1978; Pshenichnov *et al.*, 1985) which is preserved among different sublines despite cloning.

In conclusion, it is necessary to point at a distinctive feature, namely at the discrepancy between the values of ID_{50}/cell and the intensity rate of rickettsia multiplication in FL cells. As mentioned above, the ranges of ID_{50}/cell in all strains were within 1 log ($1.85 \times 10^7 - 6.41 \times 10^7$). Along with variations of the percentage of infected cells in culture and the intensity rate of rickettsial accumulation in a single cell (Table 1), these indices were considerably reduced with strain E er^rI and strain E. Therefore, the capacity for adsorption and penetration into cell among the investigated rickettsial strains seemed approximately the same, and only the reproduction stage in strains E er^rI and E was restricted due to metabolic changes (Austin *et al.*, 1987).

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