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REPRODUCTION OF VACCINE AND VIRULENT *RICKECTSIA PROWAZEKI* STRAINS IN CONTINUOUS CELL LINES AT DIFFERENT TEMPERATURES

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Summary. — The features of intracellular development of the virulent Breinl strain and 3 vaccine E strains of *Rickettsia prowazeki* have been followed in continuous FL, McCoy, and B cell cultures at temperatures of 30, 35, 37, 38.5 and 40 °C. The virulent Breinl strain multiplied well at these temperatures in McCoy and B cells but in had been gradually lost when cultured at 40 °C in FL cells. In contrast to the virulent Breinl strain the vaccine E strains have lost their capacity of long term reproduction at 38.5 °C. At 40 °C the E strains did not multiply in and had been eliminated from the McCoy and B cells; thus the vaccine E strain revealed a ts-phenotype and, accordingly, it was found to represent a ts-mutant.

Key words: Rickettsia prowazeki; vaccine and virulent strains; reproduction in cell cultures; ts-mutant

Introduction

Rickettsiae multiply in cells in vitro at quite a wide temperature range. The optimal conditions range is definitely narrower and varies at different rickettsial species. According to Oaks and Osterman (1979), *Rickettsia conori* multiplies in L cells at temperatures of 26—40 °C showing an optimal growth at 32—38 °C. An intensive development of *R. prowazeki* in cell explants was observed at 32 °C (Pinkerton and Haas, 1932) while cultivation regimen at 35—37 °C proved to be optimal for *R. tsutsugamushi* (Cohn *et al.*, 1959; Hopps *et al.*, 1959). Moreover, the intensity of intracellular development and the level of accumulation of rickettsiae are determined by the cell species used for cultivation (Gudima, 1960), by pH of the medium (Weiss *et al.*, 1972; Oaks and Osterman, 1979), and by the agent strain (Gudima, 1979). The optimal reproduction of certain rickettsial species requires enrichment of the cell culture atmosphere with CO_2 (Kopmans-Gargantiel and Wisseman, 1981).

This paper presents the results of the quantitative study on reproduction of the vaccine and virulent *R. prowazeki* strains in the course of long-term

Table 1. Multiplication of Rickettsia prowazeki as passaged in FL cells

Strain	T (°C)	Weeks post inoculation										Mean titre
		1	2	3	4	5	6	7	8	9	10	
Breinl	30	4.5*	4.5	4.5	4.5	n. d.	4.5	4.5	3.75	4.5	3.75	4.3
	35	5.5	5.5	5.33	5.5	n. d.	4.0	5.5	5.5	5.0	5.5	5.3
	37	5.25	5.0	5.37	4.75	n. d.	5.5	5.37	5.5	5.33	5.5	5.3
	38.5	5.25	5.25	4.5	3.5	n. d.	3.5	3.29	3.0	3.5	2.5	3.8
	40	5.0	3.5	3.75	2.5	n. d.	1.5	0.5	0	0	0	
E-55	30	4.5	n. d.	3.5	4.5	4.5	4.25	4.5	5.25	4.75	5.0	4.5
	35	5.4	n. d.	5.25	4.75	5.5	5.5	n. d.	4.5	5.5	5.5	5.2
	37	3.19	3.22	4.0	n. d.	4.33	n. d.	3.52	3.25	3.0	4.5	3.6
	38.5	3.0	3.17	3.25	n. d.	1.83	n. d.	1.6	2.37	2.0	0.75	***
	40	1.75	1.67	1.0	0	0	0					
E-69	30	2.5	2.5	1.5	2.5	2.5	2.0	3.5	2.0	2.5	2.0	2.4
	35	4.5	4.75	5.5	4.5	4.75	n. d.	5.5	6.0	5.5	5.5	5.2
	37	3.83	4.5	4.75	4.23	n. d.	n. d.	3.5	2.5	2.83	2.5	3.6
	38.5	3.68	4.35	3.55	3.5	3.22	n. d.	2.5	1.0	0	0	
	40	2.78	2.5	2.22	3.25	2.75	n. d.	1.5	0	0	0	
E-Er	30	4.0	4.5	2.75	4.5	5.0	4.5	n. d.	n. d.	4.25	n. d.	4.2
	35	3.67	5.25	5.25	6.5	5.75	5.0	5.25	n. d.	4.25	5.5	5.2
	37	3.33	4.5	3.57	4.36	4.37	3.37	3.22	3.0	n. d.	2.63	3.6
	38.5	3.17	2.68	3.37	3.0	3.5	1.71	2.5	1.0	n. d.	1.25	**
	40	3.25	2.25	0.5	0	0	0					

n.d. = not done.
0 = Rickettsiae not found (eliminated in the course of passaging).
T = Temperature of cultivation.
* Titres expressed in log ID_{50}/ml.
** Rickettsiae eliminated by 13 weeks.
*** Rickettsiae eliminated by 11 weeks.

cultivation in continuous cell lines at different temperatures. This study is another step in the analysis of the E strain population of *R. prowazeki* which is of special interest as a live vaccine.

Materials and Methods

Rickettsiae. Egg cultures of *R. prowazeki* were used — the virulent Breinl strain and three variants if the vaccine E strain, received from U.S.A., namely in 1955 (E-55), in 1969 (E-69) and the erythromycin resistant E strain (E-Er) (Weiss and Dressler, 1960). Rickettsial replication was followed in continuous FL (human amnion) cells (Fogh and Lund, 1957), in McCoy (human synovial) cells (Fernandez, 1959) and in B cells (derived by us from C57BL/6 mouse embryo tissue). The cells were propagated in tubes situated on horizontal racks at 35 °C in medium 199 (produced by the Moscow Research Institute of Virus Preparations, U.S.S.R. Ministry of Public Health), supplemented with 10% native bovine serum in absence of antibiotics.

Propagation. For primary inoculation of the cell cultures (1—2 days after seeding), the rickettsiae were partially purified by differential centrifugation and used in doses of $10^{3.0}$—$10^{3.6}$ ID_{50}/ml. After adsorption for 2 hr at 35 °C the inoculum was removed, tubes were washed and growth medium was added. The inoculated cell cultures were incubated at 30, 35, 37, 38.5, or 40 °C. The infected cells were subcultured 3—5 days post inoculation (p.i.) depending on the status of the cell sheet. The cells were removed from the glass surface with a rubber policeman, suspended in fresh medium and seeded into new tubes at dilutions 1 : 5 or 1 : 10. The rickettsial concentrations

Table 2. Multiplication of Rickettsia prowazeki as passaged in McCoy cells

Strain	T (°C)	Weeks post inoculation										Mean titre
		1	2	3	4	5	6	7	8	9	10	
Breinl	30	4.0	4.25	4.0	3.0	n. d.	4.5	4.5	4.29	n. d.	3.5	4.0
	35	5.0	6.0	6.0	5.0	n. d.	4.25	5.5	5.25	5.5	4.75	5.3
	40	4.0	5.5	6.0	5.25	n. d.	5.5	5.25	5.33	5.25	5.0	5.2
E-55	30	2.5	1.5	1.25	2.25	1.67	2.5	2.0	1.5	2.0	2.5	2.0
	35	2.25	0.75	1.5	1.77	2.5	2.5	n. d.	2.75	1.52	1.75	1.9
	40	1.52	0.5	0	0	0						
E-69	30	3.5	1.5	n. d.	2.5	n. d.	2.5	3.25	3.5	2.5	1.0	2.5
	35	3.5	1.25	2.5	2.5	n. d.	2.25	2.33	2.5	1.5	2.5	2.3
	40	0.5	0	0	0							
E-Er	30	2.67	1.25	2.5	1.5	1.5	1.67	2.33	1.5	2.33	n. d.	1.9
	35	3.5	0.5	0.5	0.5	0	0	0				
	40	1.5	0	0	0							

Legends in Table 1

were determined weekly by titrations of 2—3 infected tube contants in 6—8-day-old chick embryos (Ignatovich and Salagova, 1974). Tenfold dilutions of infected materials were prepared in medium 199 lacking serum. The results of titrations were recorded 8—9 days p.i. of chick embryos and expressed in log ID_{50}/ml values.

Results

The virulent Breinl strain of *R. prowazeki* (Tables 1—3) intensively multiplied at 35 °C in FL, McCoy, and B cells (mean titres log 5.3). At 30 °C, a temperature, which is quite suitable for the vital activity of louse vectors and for the multiplication of *R. prowazeki* in them (Zdrodovsky and Golinevich, 1972) the average titres of the rickettsiae were 5—10—30% of those attained at 35 °C in McCoy, FL, and B cells, respectively. The greatest differences in reproduction of the Breinl strain were observed at 40 °C. In McCoy and B cells this strain multiplied with equal intensity at both 35° and 40 °C, whereas in FL cells at 40 °C it was gradually eliminated in the course of long-term cultivation. Similar results were also obtained in infected FL cells cultivated for several weeks at 35 °C and then shifted up to 40 °C. In addition, experiments were carried out in which the rickettsiae intensively multiplying in McCoy or B cells at 40 °C had been transferred to FL cells and subsequently propagated at 40 °C. In these experiments the virulent Breinl strain was exhausted in the course of passages too.

The determine more exactly the influence of temperature on the reproduction of Brainl strain in FL cells infected cells were, cultivated at 37° and 38.5 °C, respectively. The agent was found to multiply at 37 °C as intensively as at 35 °C, whereas at 38.5 °C its reproduction decreased being approximately as low as 3% of mean titres at 35—37 °C.

Like the virulent Breinl strain, the attenuated E strain intensively multiplied in FL cells at 35 °C (Tables 1—3). The level of reproduction of the E-55

Table 3. Multiplication of Rickettsia prowazeki as passaged in B cells

Strain	T (°C)	Weeks post inoculation										Mean titre
		1	2	3	4	5	6	7	8	9	10	
Breinl	30	3.75	4.37	5.0	5.5	n. d.	4.55	4.4	5.0	5.25	5.25	4.8
	35	3.67	5.5	5.33	5.5	n. d.	5.5	5.37	5.5	5.5	5.5	5.3
	40	3.75	5.33	5.37	5.37	n. d.	4.75	4.75	5.5	5.5	6.5	5.2
E-55	30	3.0	2.55	1.25	1.5	0	0	0				
	35	3.0	2.25	1.0	1.5	1.0	0.5	0	0	0		
	40	0.5	0	0	0							
E-69	30	3 25	n. d.	3.25	3.5	n. d.	n. d.	2.67	2.75	2.5	2.5	2.9
	35	2.75	2.63	0.62	0.5	0	0	0				
	40	0.5	0	0	0							
E-Er	30	3.0	3.25	3.75	2.75	2.5	3.33	3.75	n. d.	3.0	n. d.	3.2
	35	2.33	4.25	3.05	3.0	3.33	1.75	n. d.	2.75	n. d.	2.75	3.0
	40	2.5	0	0	0							

Legends in Table 1.

and E-Er variants at 30 °C was similar to that of the virulent strain but for the E-69 variant the mean titres were lower approximately by two orders. In contrast to the virulent strain, reproduction of the vassine E strain in FL cells is markedly decreased already at 37 °C (approximately to 2.5% of the level at 35 °C) and at 38.5 °C they were eliminated within 8—13 weeks. In FL cells at 40 °C both vaccine and virulent strains have been eliminated althouhg this process is completed later with the virulent strain. The temperature of 35 °C was found oprimal for the development of the vaccine E strain in FL cells.

In McCoy cells at 30 °C, all three variants of the vaccine E strain multiplied to low levels (mean titres log 1.9—2.5). At 35 °C these rickettsiae replicated poorly or they had been eliminated during the period of 10 weeks passaging. Similar results were obtained in B cells at both 35 °C and 30 °C.

The temperature of 40 °C has been found absolutely restrictive for all three variants of the vaccine E strain in both McCoy and B cells. No infectious rickettsiae could be already detected by the 1st week of cultivation. This observation was confirmed in numerous tests. In contrast, multiplication of the virulent Breinl strain under these conditions has continued for the several months observation period.

Discussion

Significant differences have been found in the patterns of intracellular development of various *R. prowazeki* strains under identical conditions of cultivation. The virulent Breinl strain multiplied in McCoy and B cells at temperatures of 30—40 °C; its maximal accumulation was observed at 35 °C.. In FL cells the virulent strain multiplied at 30—38.5 °C, the highest titres

being reached at 35—37 °C. At 40 °C in FL cells the agent has been eliminated within 10-weeks. Further studies will show whether this is a typical property of the virulent *R. prowazeki* strains in general or of the Breinl strain alone. The presented experiments have clearly demonstrated that the damaging effect of the supraoptimal temperature (40 °C) on the intracellular development of the Breinl strain is largely host cell mediated, for the effect observed in FL cells did not occur in McCoy cells; thus it probably does not reflect a true thermal sensitivity of the agent.

As distinct from the virulent Breinl strain, the vaccine E strain of *R. prowazeki* has lost its capacity for long-term reproduction in FL cells at a temperature of 38.5 °C. It did not multiply at 40 °C in McCoy, B, and FL cells, that is, has shown the ts-phenotype and may be defined as a ts mutant. In our previous work (Gudima, 1979) the E strain was found to be a conditional lethal host-dependent (hr) mutant. The discovery of two new genetic markers (hr and ts) of the vaccine E strain is of principal importance for the elucidation of the nature of attenuation or virulence of *R. prowazeki*. The approaches used in this study extend the possibilities of rickettsial population analysis and are practically important in search for a stable vaccine strain suitable for specific prophylaxis.

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