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PERSISTENCE OF *RICKETTSIA PROWAZEKII* WITH DIFFERENT INITIAL BIOLOGICAL PROPERTIES IN INFECTED COTTON RATS

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Summary. — Evidence has been obtained indicating the association of certain biological properties of *Rickettsia prowazekii* with their capacity for persistence. Highly virulent rickettsial cultures inducing increased levels of complement-fixing antibodies were shown to cause a high percentage of rickettsial carriers in infected cotton rats. Within an interval of 133—189 days after inoculation with the virulent Breinl strain, rickettsial carrier-state was still demonstrable in 20% of the animals. The vaccine strain E of *R. prowazekii* had a low capacity for persistence in cotton rats, because 64 days after inoculation all animals were found free of rickettsiae.

Key words: *Rickettsia prowazekii*; *virulence*; *antigenicity*; *persistence*

Introduction

Many aspects of the formation and course of carrier-state in various rickettsioses remain still obscure, even though persistence in different hosts and vectors is the ecological basis for existence of pathogenic rickettsiae (Ormsbee, 1969; Zdrodovsky and Golinevich, 1972; Řeháček, 1978; Weiss, 1978; Weyer, 1978). The role of biological properties of the agent in establishment of the carrier-state has not been elucidated either. This paper presents experimental data on persistence of *R. prowazekii* with different initial characteristics. The study was done on the model of typhus infection in cotton rats.

Materials and Methods

Animals and chick embryos. Male cotton rats weighing 50 g, male guinea pigs weighing 300 g and 6-day-old chick embryos were used.

Rickettsial strains and cultures. Two strains of *R. prowazekii*, Breinl and E, were used. The Breinl strain had an unknown quinea pig passage history and 61—99 passages in chick embryos (CE). The E strain underwent 262—274 passages in CE. Before used, the rickettsial cultures had been characterized by their infectivity for guinea pigs, cotton rats and CE. Guinea pigs were examined for febrile reaction and periorchitis from 3 to 21 days post-infection (p.i.);

Table 1. Biological properties of Rickettsia prowazekii cultures used to infect cotton rats

Experimental model	Property tested	Breinl strain		E strain
		I	II	III
Guinea pigs	Febrile reaction	39.9—41.0* (3—13 days,	39.6—40.0* (1—7 days)	39.6—40.0*** (1—4 days)
	Periorchitis	absent	absent	absent
	CF antibody[1])	452.0*	130.0*	82.3***
	ID_{50}^{ser} [2])	$10^6—10^8$	$10^6—10^7$	10^3
Cotton rats	Death	10—20%**	1—10%***	0%***
	CF antibody	647.8*	333.3*	260.0*
	ID_{50}^{ser}	$10^8—10^9$	$10^8—10^9$	$10^7—10^8$
Chick embryos	ID_{50}	$10^6—10^8$	$10^6—10^7$	$10^6—10^7$

EID_{50} used for inoculation of animals: * $10^4—10^5$; ** $10^7—10^8$; *** $10^5—10^6$; [1]) mean titre; [2]) ID_{50}^{ser} was determined by the development of CF antibodies.

complement-fixing (CF) antibodies were determined at 21—30 days p.i. In cotton rats, mortality and CF antibody levels were recorded at 5—21 days and at 15—21 days p.i., respectively. The ID_{50}^{ser} was calculated from seroconversions determined at 21 days. Egg infectious doses (EID_{50}) were estimated by the results of microscopic examination of yolk sacs of CE dying within or surviving up to 13 days p.i. Table 1 summarizes the results of studies on the biological properties of the rickettsial cultures used. The Breinl strain cultures of *R. prowazekii* were found to differ in biological properties and could be divided conditionally into 2 groups. Group I included 2 cultures with higher virulence for guinea pigs and cotton rats and with a higher antigenic activity with regard to CF antibody induction in animals, group II, 3 cultures with lower virulence and lower antigenicity. Rickettsial cultures of the E strain were typical and homogeneous in their properties (column 3 of Table 1).

Determination of rickettsial carrier-state in infected cotton rats. Experimental cotton rats were divided into 3 groups according to biological characteristics of the rickettsial cultures used for infection. Cotton rats were inoculated intraperitoneally with 0.5 ml volumes containing 10^4 to 10^5 ID_{50} of rickettsiae for CE. Organs of the animals were examined for the presence of rickettsiae, beginning from 30 days for animals of groups I and II and from 21 days for group III, respectively. To increase the rickettsial isolation rate, cotton rats were treated with cortisone in a total dose of 22.5 mg (Ignatovich, 1973). The animal organs (brains, lungs, spleens, kidneys, livers) were pooled before examination. Suspensions were prepared from the organs in a minimal dilution (1 : 5 to the pool mass). The test material was inoculated into 3—4 intact cotton rats, the sera of which were three times tested during 30—45 days for presence of CF antibodies. A specimen was considered to be positive if at least one animal developed antibody. Altogether, bioassays with organs from 117 infected cotton rats were performed. In addition, the results of studies on rickettsial carrier-state included animals in which antibodies had disappeared (negative seroconversion). Such cotton rats were considered to be free of rickettsiae at the time of testing. As shown previously (Ignatovich, 1978), negative CF antibody seroconversion reflected elimination of the agent. The results of rickettsial carrier-state testing in infected cotton rats were treated statistically by grouping the data according to the principle of constant decrements using the least interval.

Serological examination of infected cotton rats. CF antibody titres were determined in all 220 infected cotton rats at 15—30 days postinfection and then monthly up to the time of sacrifice.

Table 2. The dynamics of rickettsial carrier-state in cotton rats infected with R. prowazekii of different initial biological properties

Rickettsial strain	Animal group	Carrier state		CF antibody titres (M ± m)	Rate of antibody decrease (%)
		Detection of rickettsiae on days p.i.	Rats carrying rickettsiae (%)		
Breinl	I	30— 43	80.0	396.9 ± 3.6	
		48— 85	66.7	196.4 ± 3.1	
		97—122	31.6	169.0 ± 3.1	1.9
		133—189	20.0	190.0 ± 3.3	
		195—235	0.0	96.0 ± 3.4	
Breinl	II	30— 41	60.0	135.5 ± 2.9	
		47—105	26.7	71.0 ± 2.7	16.0
		112—195	0.0	48.9 ± 2.5	
E	III	23— 30	33.3	162.7 ± 3.0	
		48— 63	20.0	90.6 ± 2.7	20.8
		64— 90	0.0	76.0 ± 3.0	
		91—160	0.0	25.4 ± 2.0	

Notice: Animal group numbers correspond to numbers of rickettsial cultures used for inoculation of cotton rats.

Results

Table 2 presents the level and duration of rickettsial carrier-state as well as the dynamics of CF antibodies in three groups of experimental animals. It may be seen that the magnitude of the established carrier-state, the duration of persistence of *R. prowazekii* and the dynamics of CF antibody titres differed significantly between individual groups. Thus, in group I, the rate of carrier-state in the early period of latency (up to 45 days) was 80%. The high rickettsial isolation rate (66.7 — 31.6%) was proved during first 4 months p.i. and even at the latest interval tested (189 days) the rickettsiae were found still in 20% of animals. In group II, the general level of carrier-state was lower. It reached 60% in the early period of latency (up to 90 days), then decreased to 26.7% (rickettsiae persisted for 105 days only). Contrasting results, particularly as compared with those in group I, were obtained in cotton rats infected with the E strain of *R. prowazekii*. The initial proportion of rickettsiae-carrying cotton rats (33.3%) established up to 30 days p.i. markedly decreased by 2 months (20.0%), a maximum duration of rickettsial persistence being 63 days p.i. The data under consideration are based on isolation from the donor animals of typical virulent and vaccine strains. The results of bioassays for rickettsiae varying in their biological properties (Ignatovich, 1980) are not included.

The dynamics of CF antibody titres showed a trend of correlation with the level of rickettsial carrier-state in infected cotton rats. In group I, the

highest mean level of CF antibody titres (396.9 ± 3.6) was found during first 90 days p.i. the titres remaining high (190.0 ± 3.3) also by 6 months of observation. In group II, antibody levels were lower at the same periods. Thus, the maximum titre at 30—41 days was 135.5 ± 2.9 declining by 3 months p.i. to 71.0 ± 2.7. In group III (E strain) in the first 3 months the level of CF antibody titres was approximately similar to that in group II, then sharply declining to the lowest levels observed (25.4 ± 2.0). Hence, a noteworthy difference in disappearance of CF antibody in different groups of cotton rats was observed, its rate increasing from group I to group III. Thus, in group I only 1.9% of the animals became seronegative by 235 days, whereas in groups II and III 16.0% and 20.8%, respectively. These data also attest to differences in the process of elimination of the typhus agent.

Discussion

Studies of latency on the model of typhus infection in cotton rats revealed quantitative differences in the rate of formation and duration of *R. prowazekii* carrier-state. Special investigations revealed an association between the capacity of *R. prowazekii* for persistence in immune animals with other properties of this agent such as virulence and antigenic activity. Only those rickettsial cultures possessing sufficiently high virulence and marked CF antibody inducing capacity were shown to produce the carrier-state in the majority of infected cotton rats. The longest duration of the carrier-state was recorded also in the animals infected with these rickettsiae. Weakening of these properties in the virulent strain of *R. prowazekii* resulted in a decrease of percentage of rickettsia-carrying rats and in a shortening of the period of persistence. Rickettsiae of the vaccine E strain had a low capacity for establishment of the carrier-state, although in occasional animals they could be detected 2 months after inoculation.

In our opinion, the results obtained are of epidemiological importance. It is conceivable that formation of a potential reservoir of typhus infection in a region and its further development would be conditioned by the biological properties of the circulating typhus agent. With no disregard of the natural resistance of macroorganism in the phenomenon of latency, it should be emphasized that in perpetuation of *R. prowazekii* as a species the leading role appears to be played by virulent populations of the agent capable of overcoming the defence factors and therefore persisting in immune hosts for a long time.

In the process of latency the phenotype of the persisting agent may change and its virulence and antigenicity decrease. However the genotype of virulent rickettsial populations must be retained for maintenance of *R. prowazekii* circulation in the man — louse — man chain.

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