

BIOLOGICAL PROPERTIES OF *RICKETTSIA PROWAZEKII* ON LONG-TERM PERSISTENCE IN INFECTED COTTON RATS

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Summary. — The biological properties of *Rickettsia prowazekii* were found to change in the course of long-term persistence in infected cotton rats. The persistent rickettsial populations proved to be heterogeneous with respect to several biological characters. Along with populations possessing typical biological properties, lines of *R. prowazekii* with lowered antigenicity and virulence and lost ability to be passed in animals were obtained. The latter property was related with rickettsial antigen inducing the formation of complement-fixing antibody. No such relationship was found with antigen inducing the formation of antihaemolysins.

Key words: *Rickettsia prowazekii*; variability; persistence

Introduction

Persistence of *Rickettsia prowazekii* in the host cell represents a form of existence of the agent established in the course of evolution. *R. prowazekii* may persist in man for 10—50 years or even longer (Kratochvíl *et al.*, 1960; Zdrovovsky, 1965; Kostrzewski, 1966; Fališevac *et al.*, 1970; Milošovičová *et al.*, 1970; Tokarevich and Krasnik, 1975; Buzdugan, 1978; Mittermayer, 1978). All strains of *R. prowazekii* isolated from patients with Brill's disease have been reported as virulent (Murray and Snyder, 1951; Mosing, 1952; Zdrovovsky, 1965; Zatulovsky and Shkolnik, 1967; Krasnik, 1975). But many authors explained the mild or intermediate course of recurrent infection as compared with the primary one by a lowered pathogenicity of the agent. However, no evidence of variability of *R. prowazekii* was presented.

The present experiments were aimed at a study of biological properties of *R. prowazekii* at various stages of persistence in infected cotton rats.

Materials and Methods

Rickettsiae. The uncloned Breinl strain of *R. prowazekii* was used in the form of egg cultures which were subjected to standards tests.

Animals. Chronic infection of cotton rats was induced as described (Ignatovich, 1973, 1978). At intervals after inoculation rickettsiae were isolated from the animals' organs (brains, kidneys,

spleens, livers and lungs) and their biological properties were examined. The organ suspensions were immediately inoculated in cotton rats and guinea pigs because a single egg passage was found (Ignatovich and Grokhovskaya, 1972) to cause reversion of the properties of rickettsial populations if the changes were of a phenotype character. The test suspensions were diluted not more than 1 : 5 with respect to the mass of the organ. The use of such concentrated samples was necessary because of the low concentration of rickettsiae in the animals' organs in the course of latency. Preliminary tests showed that the persisting rickettsiae reached titres not surpassing $10^{0.25} - 10^1$ ID₅₀/ml for cotton rats.

Tests. In cotton rats-recipients the dynamics of complement-fixing (CF) antibody and anti-haemolysins and their titres were determined. In guinea pigs-recipients, the temperature and the CF antibody titres were determined. Passaging of the rickettsiae was tested by carrying out 1-2 subpassages in intact cotton rats and guinea pigs.

Results

The properties of rickettsiae isolated from organs of 23 cotton rats 25-189 days after inoculation were investigated. Almost all cultures obtained up to the 90th day of the carrier state proved to be typical as to the markers tested. Changes in biological properties were found in 7 of 15 populations of *R. prowazekii* from 8 cotton rats examined at remote intervals (4-6 months).

Selected data on the seroimmunological responses of animals - recipients on the inoculation of the rickettsial populations tested and on their passaging are presented in Table 1. In 3 cotton rats of a group (Nos 190, 194, 199), rickettsiae were found on the 48th day only in the spleens (Nos 194, 190) or on both the spleen and kidneys (No. 199). The persisting rickettsiae in rat No. 190 showed high antigenicity and could be passaged. Three rickettsial sublines from the other two rats showed a distinct, changed antigenicity which was manifested by a lowered level or complete absence of CF antibody in cotton rats-recipients. The function of rickettsial haemolysin was preserved, the antihaemolysin titres in the animals were rather high (80-160). These lines could not be passaged, the rickettsiae disappeared in the 2nd subpassage.

At remote intervals of the carrier state, the heterogeneity of the persisting rickettsial populations was examined. According to their biological character, only 2 sublines of *R. prowazekii* from cotton rat No. 124 (examined on day 175) proved to be homogeneous. Their antigenicity was lowered and they could not be passaged. The rickettsial populations obtained from cotton rats Nos 253, 74 and 61 were heterogeneous. Rickettsial lines possessing low virulence (absent febrile reaction in guinea pigs), lowered antigenicity and lost capacity for passaging (lines from the brain of cotton rat No. 253 and from the lungs of cotton rat No. 61) were isolated from some organs. Changes in antigenicity involved both the CF antigen and rickettsial haemolysin (lines from cotton rats Nos 74 and 61). At the same time in other organs there persisted a virulent population of rickettsiae (lines from the brain and liver of cotton rats Nos. 61 and 74, respectively). An analysis of the correlation of the markers showed that the capacity for passaging was related with the activity of the antigen inducing CF antibody. Upon lowering of the activity of this antigen the capacity for passaging was lost (rickettsial populations

Table 1. Biological properties of populations of *R. prowazekii* isolated from cotton rats-carriers

Rat donor No.	Day p. i.	Donor's response ¹⁾		Rickettsiae isolated from	Titre ²⁾	Seroimmunological response of animals-recipients				Capacity for passaging ³⁾
		CFA	AHL			CFA		AHL		
						day 15	day 30	day 15	day 30	
190	48	320	320	spleen	1.0	2560	1280	2560	640	+
194	48	320	40	spleen	0.5	10	20	320	80	-
199	48	80	10	spleen	0.5	< 5	< 5	80	160	-
				kidneys	0.25	< 5	< 5	40	160	ND
253	152	160	80	brain	1.0	20	20	80	80	-
				kidneys	0.5	320	640	40	40	ND
74	165	1280	160	brain	0.3	< 5	< 5	10	40	ND
				spleen	1.0	320	640	10	40	+
				liver	0.5	640	1280	1280	1280	+
124	175	80	ND	brain	1.0	20	10	ND	ND	-
				kidneys	1.0	40	10	ND	ND	-
61	189	160	160	brain	1.0	320	640	640	80	+
				kidneys	0.5	320	320	< 5	< 5	+
				lungs	0.5	20	10	< 5	< 5	-

1) Seroimmunological response on the indicated day after inoculation (p. i.). Titres of CF antibody (CFA) and antihemolysins (AHL).

2) log ID₅₀/ml in the given sample.

3) The isolated rickettsiae could (+) or could not (-) be passaged.

ND = not done.

from the lungs of cotton rat No 61 and the population from the brain of cotton rat No. 253). No such correlation was found between the capacity for passaging and haemolysin. Irrespective of a high activity of the latter antigen (cotton rats Nos. 199 and 194) the capacity for passaging was lost, like in the absence of antihaemolysins. But at high levels of CF antibody the capacity for passaging was demonstrated (rickettsial line from the kidneys of cotton rat No. 61).

Discussion

The present results concerning *R. prowazekii* isolated from cotton rat carriers cannot be explained either by quantitative parameters or variations in the individual susceptibility of animals-recipients or by the presence in the samples of dead rickettsiae. First, as mentioned above, the concentration of persisting rickettsiae in the organs was about the same throughout the period of observation, the titres varying from $10^{0.25}$ to 10^1 ID₅₀/ml for cotton rats. Second, the changed seroimmunological responses of animal recipients occurred only after inoculation of isolates obtained at remote stages of convalescence. Third, the assumption that dead rickettsiae were present in the samples was ruled out by the peculiar dynamics and long-term formation of antibody in recipients, which is characteristic of a live agent with low pathogenicity.

To my opinion, the data obtained suggest that changes occurred in the biological properties of the persistent populations of *R. prowazekii*. These changes involved antigenicity and virulence of rickettsial populations as well as their capacity for passaging. A peculiarity of the changes observed in several populations was a marked heterogeneity of sublines isolated from different organs of the same animal. Along with rickettsiae possessing typical biological properties, rickettsial sublines showing deviations from one or another property were obtained. Especially important appears the isolation of rickettsial populations that had lost the capacity to be passaged from one animal to another. The epidemiological significance of this finding is evident. Transmission of such rickettsiae presumably would become difficult. Unfortunately, rickettsial sublines with lowered antigenicity and virulence could not be preserved for further study.

The course of variability observed in rickettsial populations in the course of long-term persistence in cotton rats to a certain degree explains the differences in the clinically epidemiological features of Brill's disease and epidemic typhus. The mostly mild or moderately severe course of Brill's disease, changes in the seroimmunological characteristics of this disease and the considerable difficulties in isolating *R. prowazekii* apparently are connected not only with the immunological status of the patient with recurrent disease but also with changes in the biological properties of the population of the persisting rickettsiae. An explanation can also be offered of the fact that only highly virulent strains of *R. prowazekii* are isolated from patients with Brill's disease.

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