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RESTRICTION FRAGMENT LENGTH POLYMORPHISM OF THE DNA OF TYPHUS GROUP RICKETTSIAE

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Summary. - The DNA of 10 strains of *Rickettsia prowazekii*, 5 strains of *Rickettsia typhi* and 1 strain of *Rickettsia canada* was investigated by restriction fragment length polymorphism analysis. Interspecies differences were characterized by a great number of noncomigrating bands. Using the endonuclease *Hind*III and *Pst*I fragments comigration as a quantitive criterion, genetic similarity coefficient was calculated for the pair *Rickettsia prowazekii/Rickettsia typhi*-32.0 %, for *Rickettsia prowazekii/Rickettsia canada*-22.7 %, and for *Rickettsia typhi/Rickettsia canada*-23.5 %. Intraspecies differences expressed are very subtle and concern 1-2 noncomigrating fragments. The investigated strains of *Rickettsia prowazekii* and *Rickettsia typhi* can be divided into 2 groups without any correlation to the source and period of isolation, or to strain passage history.

Key words: rickettsiae; restriction fragment length polymorphism analysis; DNA; genotypic differentiation

Introduction

Classical methods of species differentiation of pathogenic rickettsiae (seroimmunologic parameters, antigenic and other biologic characteristics) are practically non effective for intraspecies (strain) differentiation. Restriction fragment length polymorphism (RFLP) of DNA in combination with the use of molecular probes is believed to be very promising for this purpose (Regnery *et al.*, 1983-1986; Wood *et al.*, 1984). During earlier RFLP analysis 6 strains of *Rickettsia prowazekii* were investigated (Balayeva *et al.*, 1989; Balayeva, 1989; Rydkina *et al.*, 1990). Here we report on investigations of typhus group rickettsiae, including 10 strains of *R. prowazekii*, 5 strains of *R. typhi* and 1 strain of *R. canada*, using RFLP.

Materials and Methods

R. prowazekii strains: Breinl, Katsynian, Ananiev, Vladyko, and Kuzina were isolated from typhus patients; strains G and Chernikova - from Brill disease patients. The standard strain Breinl was isolated in Poland, all the other strains in the European part of the U.S.S.R., except of strain Katsynian, isolated in Armenia. Low virulent strain E of *R. prowazekii* was selected during passaging of the virulent strain Madrid-1 (Spain), its virulent revertant strain EVir was derived in the U.S.S.R. (Balayeva *et al.*, 1989).

R. typhi were human isolates (strain Wilmington, U.S.A.; strains Ger and Museibov, U.S.S.R.) as well as the *Rattus norvegicus* isolate (strain B-1, U.S.S.R.) and the *Xenopsylla cheopis* isolate (srain X, U.S.S.R.). Strain 2678 *R. canada* was isolated from ticks *Haemaphysalis leporispalustris,* Canada.

Rickettsiae were cultivated in yolk sacs of chicken embryos, then inactivated with 0.1 % formalin at 6 °C for 24 hr (Regnery *et al.*, 1983). The biomass of rickettsiae was purified according to Aniskovich *et al.* (1989), using differential centrifugation and verografin density gradient.

The total rickettsial DNA was isolated according to the method of Priefer *et al.* (1984). RFLP analysis was carried out as recommended by Maniatis *et al* (1982).

Results and Discussion

The restriction endonuclease analysis of DNA of 3 species of typhus group rickettsiae revealed considerable and various distinctions of the DNA restriction patterns. These are clearly evidenced in the high molecular weight zone that allows to discriminate easily and reliably the size of fragments (Fig. 1A). Side by side with the fragments that differed in their electrophoretic mobility, there were many comigrating fragments. This fact confirmes the genetical relationship between the compared rickettsial species. According to the quantitative criterion of the presence of coincident DNA fragments in electrophoregrams, while using endonucleases *Hind*III and *Pst*I, the genetic similarity coefficient of rickettsial species was calculated. The highest coefficient of genetic similarity was revealed for the pair *R. prowazekii/R. typhi* - 32.0 %. The coefficients for the pairs *R. prowazekii/R. canada* and *R. typhi/R. canada* were approximately similar being 22.7 % and 23.5 %, respectively.

The differences between strains of the same rickettsial species were more subtle and concerned 1-2 fragments of altered mobility for the strains of *R. prowazekii* and only 1 fragment for the strains of *R. typhi* (Fig. 1B, Fig. 1C). Strains of *R. prowazekii* were divided into 2 groups by the criterion of presence or absence of such fragments after cleavage of the DNA with endonucleases *Bam*HI, *Cfr*13I, *Eco*RI, *Hind*III, *Msp*I, *Mva*I, *Pst*I, *Xho*I (Table 1). This groups were conditionally called as Breinl strain group (Breinl, Ananiev, G, L, Vladyko, Chernikova, Kuzina) and E strain group (E, EVir, Katsynian). It must be emphasized that rickettsiae of one group gave identical restriction DNA patterns for every of endonuclease consumed.

The investigated *R. typhi* strains could also be divided into 2 groups (Table 1). One group was represented by strain Wilmington, other group included strains

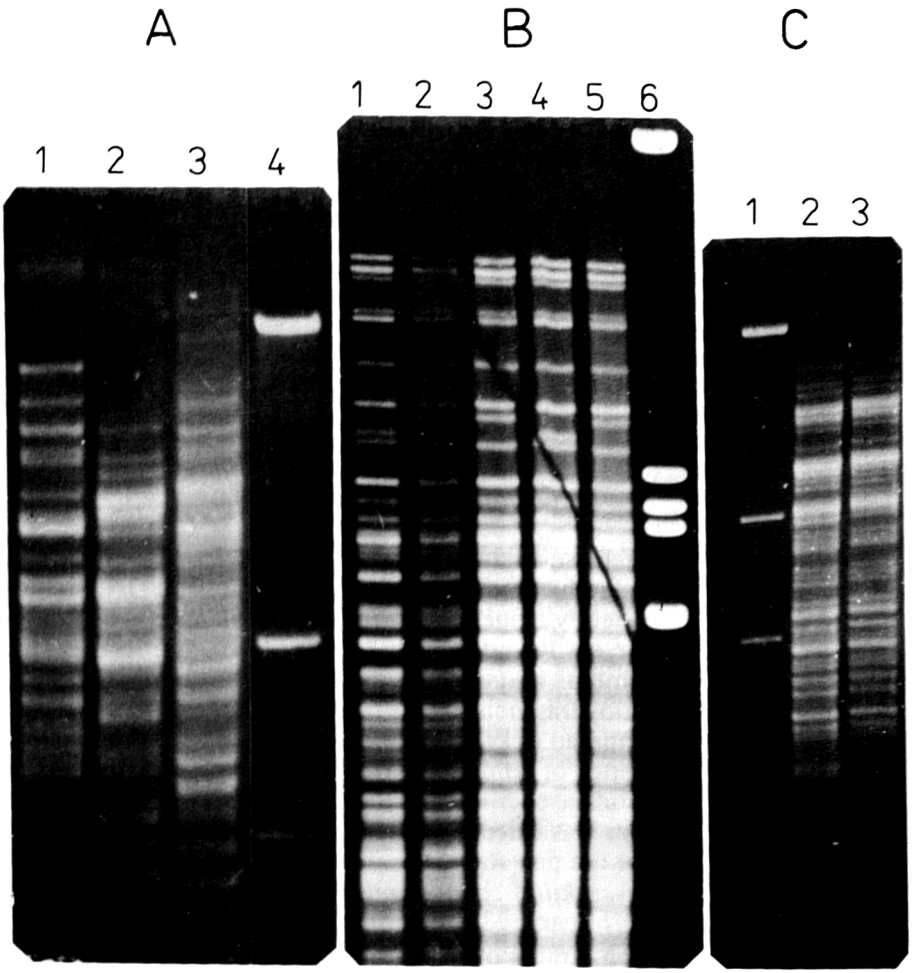

Fig. 1

Restriction patterns of typhus group rickettsiae, digested by endonucleases. After restriction endonuclease digestion, the various DNAs were electrophoresed and photographed

A. DNA of typhus group rickettsiae, digested by *Pst*I: *R. prowazekii* strain Breinl (1), *R. typhi* strain Wilmington (2), *R. canada* strain 2678 (3). *Hind*III-digested λDNA (4) was used as a molecular weight standard.

B. DNA of *R. prowazekii* strains, digested by *Msp*I: EVir (1), Breinl (2), Ananiev (3), Katsynian (4), G (5). *Eco*RI-digested λ DNA (6) was used as a molecular weight standard.

C. DNA of *R. typhi* strains, digested by *Pst*I: Wilmington (2) and B-1 (3). *Hind*III digested λDNA (1) was used as a molecular weight standard.

B-1, Ger, X, Museibov. The 12 kb fragments could be seen only in *Pst*I and *Msp*I restrictograms, (Table 1, Fig. 1C). Strain Wilmington had an additional band in the *Msp*I restrictogram, and the B-1 group strains after cleavage with *Pst*I.

Our present investigations confirm the data about evident genetic differences between typhus group rickettsiae species (Regnery *et al.*, 1983; 1986). As

Table 1. Comparison of typhus group rickettsiae restrictograms

Restriction endonuclease	Number of visually discerned DNA bands		Range of fragments (kb)	Size of additional fragments (kb)
	Strain of *R. prowazekii*			
	Breinl, G, Ananiev, L, Chernikova, Vladyko, Kuzina	E. EVir Katsynian		
*Bam*HI	8	9	7-50	9.5
*Cfr*13I	36	35	1-15	8
*Eco*RI	40	41	2-25	18.10
*Hind*III	39	38	1-15	9.6
*Msp*I	50	50	0.5-15	9.8
*Mva*I	46	45	1-20	18
*Pst*I	50	51	1-30	20.15
*Xho*I	12	13	6-50	15
	Strain of *R. typhi*			
	Wilmington	Ger, B-1 Museibov, X		
*Msp*I	31	30	1-12	12
*Pst*I	46	47	1-20	12
*Eco*RI	42	42	1-30	
*Hind*III	40	40	1-14	
*Xba*I	35	35	1-30	
	R. canada strain 2678			
*Hind*III	25		4-15	
*Pst*I	32		4-30	

for intraspecies strain differentiation, data found in literature concern only *R. prowazekii* strains and show clear distinctions in strain E and Breinl DNA structure (Regnery *et al.*, 1983; 1984; Wood *et al.*, 1984). Other investigated *R. prowazekii* strains are difficult to classify using currently available characteristics (Regnery *et al.*, 1984; 1985). *R. prowazekii* strains, isolated from flying squirrels, by our opinion, must be attributed to a specific group, not only because of its DNA structure (Regnery *et al.*, 1986), but because of its ecologo-epidemiologic peculiarities. *R. typhi* strains were also divided into two groups.

Strains of *R. prowazekii* and *R. typhi* were divided into groups without any correlation to the source and period of isolation and strain passage history. However, the limited number of investigated strains gives us only a few data concerning possible connections of DNA structure with geographic points of strain isolation.

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