SEQUENCE ANALYSIS OF A FRAGMENT OF ROMPA GENE OF SEVERAL ISOLATES OF SPOTTED FEVER GROUP RICKETTSIAE FROM CHINA

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Summary. – The nucleotide sequence of rOmpA gene fragment of three Chinese isolates of spotted fever group rickettsiae (SFGR) (BJ-90, Ha-91 and HLJ-054) was determined. The obtained nucleotide and putative amino acid sequences were compared with those of another Chinese SFGR isolate (HL-93) and several prototype SFGR strains. This comparison showed that the isolates BJ-90 and Ha-91 are closely related to each other and *R. sibirica* but different from the isolates HLJ-054 and HL-93. We assume that with exception of the isolates HLJ-054 and HL-93 that represent new, unique members of SFGR, the isolates BJ-90 and Ha-91 are closely related to *R. sibirica*, one of the prototype SFGR strains.

Key words: spotted fever group rickettsiae; rOmpA gene; sequence analysis

The SFGR, obligate intracellular bacteria, represent a multitude of various strains and serotypes. Recently, several new serotypes of SFGR have been described (Pretzman *et al.*, 1994; Stenos *et al.*, 1994). The identification and classification of SFGR has been initially based on phenotypic characterisation and their interaction with the environment. However, these approaches no longer meet the current demands. Therefore, genotypic characterisation, namely the primary structure (sequence) of the rOmpA gene and/or its protein product that is presumably involved in the pathogenesis of SFGR infection and the respective immune response, has been recently introduced (Regnery *et al.*, 1991). Amplification of a 533 bp fragment of the rOmpA gene by polymerase chain reaction (PCR), restriction fragment length polymorphism and sequence analyses of the PCR products have become the method of choice for SFGR identification (Beati *et al.*, 1994; Yan and Uchiyama, 1994; Zhang *et al.*, 1994).

Abbreviations: PCR = polymerase chain reaction; SFGR = spotted fever group rickettsiae

In China, the SFGR have been extensively studied for the last 40 years. The existence of a few natural foci of North Asian tick-borne spotted fever (Fan *et al.*, 1992) and the occurence of this disease south of 26° north latitude (Bi *et al.*, 1994) have been reported. In addition, many SFGR isolates from *Haemaphysalis silvarum* (Lou *et al.*, 1985), *Dermacentor sinicus* (Yu *et al.*, 1991), *Hyalomma asiaticum* (Jin *et al.*, 1993) and *Haemaphysalis concinna* (Zhang *et al.*, 1996) differred genetically and/or antigenically from prototype strains of SFGR.

In this study, in order to determine the taxonomic status of the Chinese isolates and their relationship to prototype SFGR strains, we amplified the characteristic 533 bp fragment of rOmpA gene of three isolates by PCR using primers Rr 190.70p and Rr 190.602n. Besides the Chinese SFGR isolates BJ-90, Ha-91 and HLJ-054, also prototype strains *R. sibirica* and *R. parkeri* were subjected to PCR. The PCR products were cloned into pGEM-T vector and sequenced by the dideoxy method. The obtained nucleotide and putative amino acid sequences were compared with those of Chinese HL-93 isolate and additional prototype SFGR strains (*R. japonica, R. rickettsii,* and *R. conori*) published earlier.

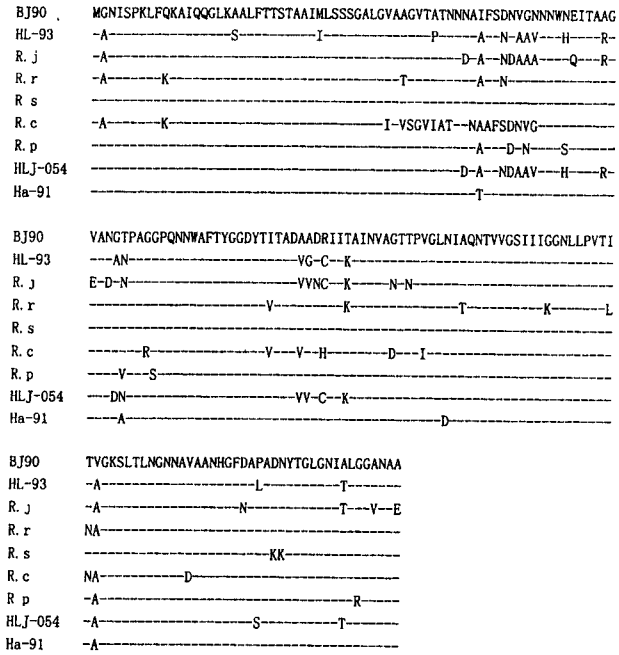

Fig. 1
Putative amino acid sequences of the rOmpA gene fragment of various SFGR

The putative amino acid sequence of the PCR fragment and the respective homology of the SFGR under comparison are shown in Fig. 1 and Tables 1 and 2, respectively.

The homology of the prototype strains ranged from 90.06% to 96.62% in nucleotide (Table 1) and from 83.05% to 94.35% in amino acid sequences (Table 2), respectively. The Chinese isolates BJ-90 and Ha-91 had a very high homology to each other (99.25% in nucleotide and 97.74% in amino acid sequences), and a relatively lower homology to the Chinese isolates HLJ-054 and HL-93 (94.75% and 94.56% in nucleotide and 89.83 and 88.70% in amino acid sequences, respectively). In relation to the prototype strains, the BJ-90 and HA-91 isolates had the highest homology to *R. sibirica* (99.06% and 98.31% in nucleotide and 98.87% and 96.61% in amino acid sequences, respectively).

As regards the Chinese isolates HLJ-054 and HL-93, isolated from different tick species in the same locality, their homology to each other was 98.12% in nucleotide and 94.92% in amino acid sequences. In relation to the prototype strains, they had the highest homology to *R. japonica*

Table 1. Nucleotide sequence homologies of the rOmpA gene fragment of various SFGR

	R.r	*R.s*	*R.c*	*R.p*	*R.j*	HL-93	HLJ-054	BJ-90	Ha-91
R.r	100	95.68	90.99	96.06	92.87	94.18	93.99	96.62	96.62
R.s		100	90 06	96.62	92.30	93.62	93.99	99.06	98.31
R.c			100	90.24	89.49	90.24	90.06	90.99	89 12
R.p				100	92.68	93.62	94.37	97.56	98.12
R.j					100	95.68	96.62	93.25	93 06
HL-93						100	98.12	94.56	94.56
HLJ-054							100	94.75	94.75
BJ-90								100	99 25
Ha-91									100

R.r = *R. riskettsii* (R strain), *R.s* = *R. sibirica* (246 strain), *R c* = *R. conori* (Malish 7 strain), *R.p* = *R parkeri*, *R.j* = *R japonica* (YH strain).

Table 2. Putative amino acid sequence homologies of the rOmpA gene fragment of various SFGR

	R.r	*R s*	*R c*	*R.p*	*R.j*	HL-93	HLJ-054	BJ-90	Ha-91
R.r	100	92.09	85.31	90.06	84.75	87.57	87.57	93.22	92.66
R.s		100	83.05	94.35	84.75	87.57	88.70	98.87	96 61
R.c			100	83.62	79 10	80.79	81.92	84.18	83.62
R.p				100	85.88	88.14	90.40	95.48	94.92
R.j					100	89.27	92 09	85.88	85 88
HL-93						100	94.92	88.70	88.70
HLJ-054							100	89.83	89 83
BJ-90								100	97.74
Ha-91									100

For the legend see Table 1.

(96.62% and 95.68% in nucleotide and 92.09% and 89.27% in amino acid sequences, respectively).

According to Yu *et al.* (1993), there are three types of SFGR in the northern China. Type 1 (representative TO-85 strain), isolated from *D. nuttalli* tick ova, genotypically and antigenically identical to *R. sibirica*. Type 2 (representative BJ-90 strain), genotypically identical and antigenically related to *R. sibirica*; however, its profile in polyacrylamide gel electrophoresis in the presence of sodium dodecylsulphate was different from that of *R. sibirica*. Type 3 (representative HL-93 strain), both genotypically and antigenically unique strain among SFGR.

The data obtained by us lead us to suggest that most of SFGR strains so far isolated from various sources in China are identical or closely related to *R. sibirica*. The isolates HL-93 and HLJ-054 may represent new, unique members of SFGR.

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