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ANALYSIS OF PROTEINS AND LIPOPOLYSACCHARIDES FROM CHINESE ISOLATES OF *COXIELLA BURNETII* WITH MONOCLONAL ANTIBODIES

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Summary. - Four *Coxiella burnetii* isolates in China and two reference strains were compared by SDS-PAGE and immunoblotting. The SDS-PAGE profiles of whole cells and LPS of Chinese isolates Qiyi, Xinqiao, and YS-8 were found closely related to Henzerling strain, and different from the Grita strain. In immunoblot assay of LPS and proteinase K-digested whole rickettsiae minor differences were seen in polysaccharide structure among the Chinese isolates by phase I monoclonal antibody. The present results suggest that the strains reported here may be divided into three groups according to the polysaccharide structure: Xinqiao and Henzerling strains (1), YS-8 and Grita (2), and Qiyi (3).

Key words: Coxiella burnetii; LPS; SDS-PAGE; immunoblot; monoclonal antibody

Introduction

The distribution of Q fever in China is widespread. *Coxiella burnetii (C.b.)* was first isolated from a patient with chronic Q fever in Sichuan in 1962 (Yu *et al.*, 1964). Since then it has been isolated from patients, sheep placenta and ticks. *Coxiella burnetii* is unique as it undergoes a transition from a virulent (phase I) to avirulent (phase II) upon serial laboratory passages in embryonated eggs or in tissue culture. The total carbohydrate composition and carbohydrate and protein concentrations differ between phase I and phase II envelopes (Jerrels *et al.*, 1974), and there are distinct quantitative and qualitative differences between the LPSs in these two phases (Baca *et al.*, 1974; Schramek and Brezina, 1976; Schramek *et al.*, 1985). Study of the *C.b.* surface component variations and strain differences is necessary for identification of Coxiella virulence factors and immunogenic macromolecules, and is useful for preparation of Q fever subunit vaccine. In this report we analysed the whole cells and isolated LPS of different *C. burnetii* strains by SDS-PAGE and immunoblot-

ting. A heterogeneity in chemical structure was found among the Chinese isolates from various sources.

Materials and Methods

The C. burnetii isolates from China and the strains from foreign countries used in this study are listed in Table 1.

Monoclonal antibodies against C. burnetii 1-4B5, 3-2B8 to phase I (MoAb I) and 2-2E5 to phase II (MoAb II) were prepared as described (Yu *et al.*, 1986).

LPS isolation. LPS was extracted from purified *C. burnetii* by a modification of the hot phenol--water method of Westphal and Jann (1965).

SDS-PAGE. Rickettsiae were dissolved by boiling in Laemmli solubilizer (Laemmli, 1970), and their components were separated by SDS-PAGE. Stacking and separating gel consisted of 5 and 15 % acrylamide. The gels were subjected to electrophoresis at 20 mA for 2 hr and then at 30 mA for 5 hr. After separation, the gel was stained with Comassie blue for examination of the proteins, and silver staining for the LPS was as described by Tsai and Frasch (1982).

Immunoblotting. The separated rickettsial components were transferred to nitrocellulose paper using the technique described by Towin *et al.* (1979). Electroblotting was carried out in a Bio Rad transblot apparatus for 2 hr at 200 mA, and then for 3 hr at 100 mA. Specific antigens were then incubated with MoAb against *C. burnetii* or pooled mouse anti-*C. burnetii* antisera followed by enzyme-labelled pig anti-mouse Ig, and then substrate was added.

Chemical analysis. Total carbohydrate was determined by the phenol sulphuric acid method of Dubois *et al.* (1956) with glucose as a standard. Protein concentrations were determined according to Bradford (1976) with bovine serum albumin as standard.

Table 1. Source and passage history of *C. burnetii* isolates

Strain	Material for isolate	Geographical source	Passage history	Phase
Qiyi	Marrow, human	Sichuan, China	*EP20/MP3/EP1 /MP49/EP2	I
Ya'an	Blood, human	Sichuan, China	EP10	I
Xingiao	Haemaphysalis campanulata	Sichuan, China	EP5/MP18/EP4	I
YS-8	Placenta, sheep	Yunnan, China	EP15	I
Henzerling	Blood, human	Italy	EP3-4/MP1 intervals**	I
Grita	Blood, human	Obtained from U.S.S.R.	EP84**	II

* EP: Passaged in chicken embryo, MP: Passaged in mouse
** Passage history in our laboratory

Results and Discussion

SDS-PAGE profile of whole cell lysates of C. burnetii

About 50 bands were seen in the migration profile of each strain. Seven major bands (with approximate M_r of 72.4, 58.9, 22.4, 20.4, 16.2, 15.1, and 11.7, kD) were found to be common to both strains, but a 95.5 kD band was unique and a 13.5 kD band was absent for Grita strain, and a 33.5 kD band was distinct for Henzerling strain compared with other strains (Fig. 1). The profile of four Chinese isolates were very similar to each other.

The components in the range M_r 30 to 94 were not detected in the proteinase K-digested lysates of YS-8 and Grita strains by silver staining; thus these probably represent protein antigens (Fig. 2).

Silver stained PAGE profile of isolated LPS of C. burnetii

The LPS profiles of Qiyi, Xinqiao, and YS-8 isolates in China were almost similar to that of Henzerling strain (phase I) and differed from the Grita strain (phase II). The bands above the 17.5 kD were absent for Grita strain (Fig. 3). Silver stained PAGE profile of proteinase K-digest of *C. burnetii* was similar to that of isolated LPS (Fig. 4). Minor differences were also found in the polysaccharide structure of Chinese isolates from various sources. The profile of Xinqiao tick isolates was same as that of Henzerling strain with the most

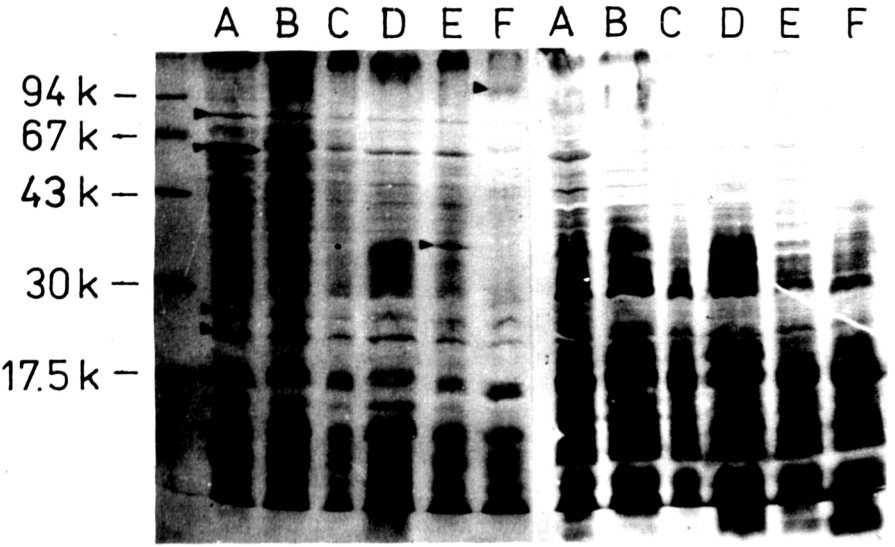


Fig. 1
SDS-PAGE profile of whole cell lysates of *C. burnetii*
Lanes: A, Qiyi; B, Ya' an; C, Xinqiao;
D, YS-8, E, Henzerling; F, Grita.
Left: Comassie blue staining; Right: Silver staining

complete profile of bands; the profiles of Qiyi human isolates and of sheep YS-8 isolates were distinct from that of Xinqiao isolate when using proteinase K-digests of whole cell for SDS-PAGE.

The results do coincide with chemical analysis. The LPS of Chinese isolates and Henzerling strain contained the most carbohydrate, and the protein concentration was generally low, while Grita LPS contained lower carbohydrates and higher protein. It is also shown that the LPS isolated from Qiyi and YS-8 isolates contained more protein than that from Xinqiao and Henzerling strain, and somewhat near to Grita strain (Table 2).

Immunoblots of whole cell lysates and isolates LPS of C. burnetii

In the immunoblot assay with MoAb I 3-2B8, the profile of whole cell among Qiyi, Ya'an, and Xinqiao isolates in Sichuan Province were very similar to each other, while the bands lower than 67 kD were absent for YS-8 isolate in Yunnan Province. In the immunoblot assay of LPS, although staining component below the 17.5 kD M_r markers were apparent for all strains which reacted with pooled mouse anti-*C. burnetii* antisera, yet some shared antigens were not detected by MoAb. A 14 kD fraction of Qiyi isolate, and a 6-17 kD fraction of YS-8 and Grita strains reacted specially with MoAb I 1-4B5, the LPS of Xinqiao and Henzerling strains did not react with this MoAb (Fig. 5). There-

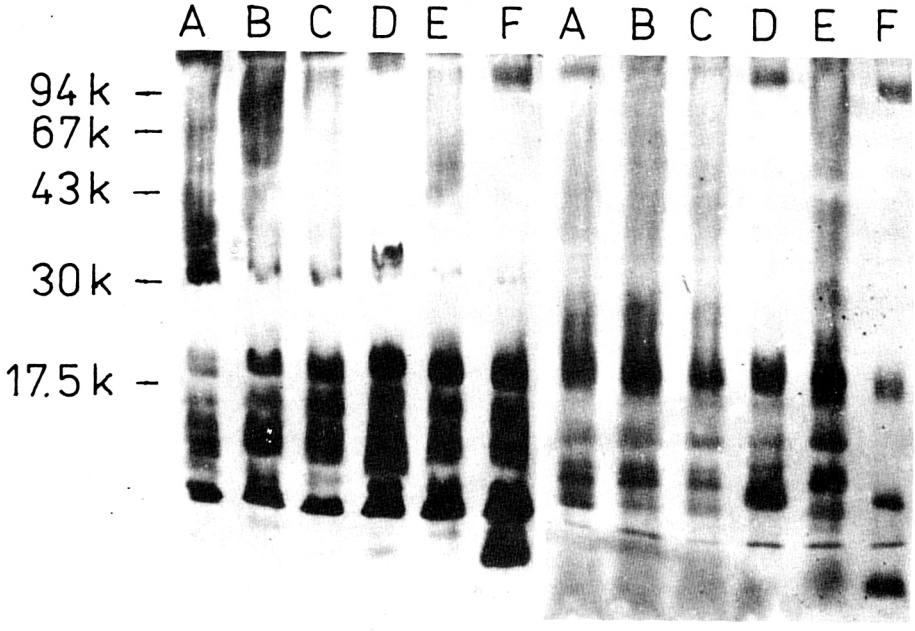
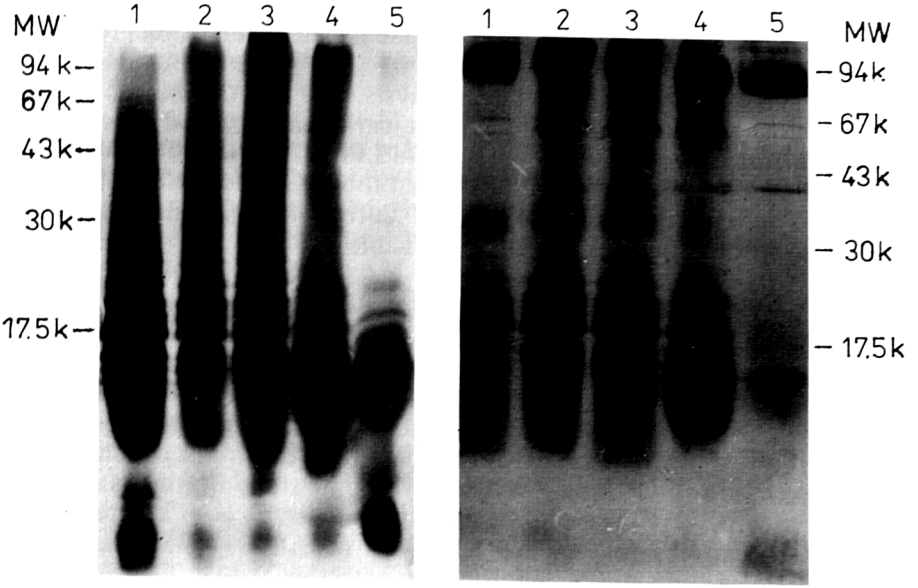
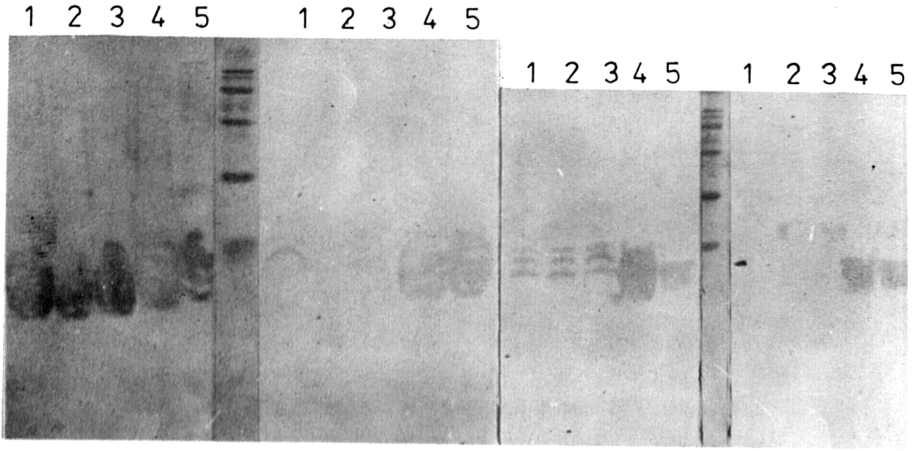


Fig. 2
Silver stained PAGE profile or whole cell lysates (left) and its proteinase K-digests



Figs 3-4



Figs 5-6

Table 2. Chemical comparisons of LPSs of Chinese isolates with reference strains

LPS	Yield (%)	Total carbohydrate (%)	Protein concentration (%)
Qiyi	17.0	25.2	6.0
Xinqiao	17.3	25.4	4.0
Henzerling	15.2	28.9	4.9
YS-8	13.6	29.4	8.7
Grita	7.1	11.0	10.0

fore, we supposed that the strains isolated from Sichuan and Yunnan are divided into three types in polysaccharide structure.

Immunoblotting of whole cell lysates of *C. burnetii* with MoAb I 1-4B5, three bands (12, 14, and 16 kD) were found to be common to Qiyi. Xinqiao, and Henzerling strains, and a 8.5-17.5 kD fraction was distinct for YS-8 isolate (Fig. 6). The immunoblots of proteinase K-digests of whole cell lysates with MoAb I 1-4B5 which were similar to that of isolated LPS.

We extracted LPS from *C. burnetii* strains isolated in China from various sources and compared the isolated LPSs and *C. burnetii* cells by SDS-PAGE and immunoblotting. The results showed that LPS is a prominent component that varies among isolates in association with the phase-related heterogeneity and antigenicity (Hackstadt *et al.*, 1985). It has become apparent from genetic analysis as well as LPS structure analysis of *C. burnetii* that strain variation does occur (Samuel *et al.*, 1985; Hackstadt, 1986). On the basis of the data presented here remains to be resolved whether phase variation in the laboratory or the different origin of isolation is the main course of the heterogeneity in chemical structure of the Chinese isolates of *C. burnetii.*

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Fig. 3. Silver stained PAGE profile of isolated LPS of *C. burnetii.* Lanes: 1, Qiyi; 2, Xinqiao; 3, Henzerling; 4, YS-8; 5, Grita.

Fig. 4. Silver stained PAGE profile of proteinase K-digests of *C. burnetii.*

Fig. 5. Immunoblots of *C. burnetii* LPS developed with pooled mouse anti-*C. burnetii* antisera (left) and MoAb I 1-4B5 (right).

Fig. 6. Immunoblotting of whole cell lysates of *C. burnetii* (left) and its proteinase K-digests with MoAb I 1-4B5 (right).

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