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COMPARISON OF PROTEIN AND LIPOPOLYSACCHARIDE PATTERNS OF SEVERAL *COXIELLA BURNETII* STRAINS IN PHASE I

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Summary. — Electrophoresis in polyacrylamide gel gradients in the presence of sodium dodecyl sulphate revealed at least 18 identical protein bands in eight strains of *Coxiella burnetii*. By staining with Coomassie Brilliant Blue R-250 it was possible to visualize clearly at least 40 proteins. The protein pattern showed the greatest variability in the region from 27 to 18.5 kD. Strains S and Priscilla differed from the other strains also in proteins smaller than 18.5 kD. The lipopolysaccharide pattern obtained by the same technique consisted of from 6 to 12 various bands in the region from 23.5 to 11.8 kD. The protein and lipopolysaccharide patterns of four strains isolated from ticks did not significantly differ from those of strains Henzerling and L-35 isolated from men.

Key words: *Coxiella burnetii*; *proteins*; *lipopolysaccharides*; *polyacrylamide gel electrophoresis*

Introduction

Coxiella burnetii, the aetiological agent of Q fever, due to its composition and properties, has a unique position among rickettsiae. As a result of excellent adaptability, it has a broad range of vectors and hosts.

In recent years, the phase variation of *C. burnetii*, similar to the S ⇆ R variation of Gram-negative enteric bacteria, has been studied in detail (Fiset, 1957; Brezina, 1968, 1978) and in this connection also changes in various properties like virulence (Kazár *et al.*, 1974); antigenicity and immunogenicity (Višacká *et al.*, 1984); and structure, role and properties of lipopolysaccharides (LPS) of phase I and II (Baca and Paretsky, 1974; Schramek and Brezina, 1974, 1975; Schramek and Mayer, 1982; Hackstadt *et al.*, 1985; Hackstadt, 1986). Genetic studies were carried out on various *C. burnetii* strains and its plasmids (O'Rourke *et al.*, 1985; Samuel *et al.*, 1985). With the use of restriction endonucleases and agarose gel electrophoresis, differences in the genome of certain *C. burnetii* strains were established (Mallavia *et al.*, 1985; Vodkin *et al.*, 1986).

So far, little attention has been paid to the variability of the protein and LPS pattern of *C. burnetii* strains in phase I. Williams (1981) used polyacryl-

amide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS-PAGE) of iodinated proteins of the outer membrane of *C. burnetii* and found at least 35 components with molecular masses from 150 to 11.8 kD. Hackstadt *et al.* (1985) observed slight differences in the protein pattern and considerable ones in LPS between phases I and II of the Nine Mile strain of *C. burnetii*. Subsequently, Hackstadt (1986) reported differences in the LPS patterns of 14 *C. burnetii* strains which, based on certain criteria, he separated in three groups.

In the present study we compared protein and LPS patterns of eight *C. burnetii* strains in phase I.

Materials and Methods

The eight *C. burnetii* strains in phase I examined are listed in Table 1. They were purified by the method of Ormsbee (1962).

SDS-PAGE was carried out on 10—20 % polyacrylamide gel gradients according to Laemmli (1970).

Preparation of samples. One milligram of purified *C. burnetii* suspended in 1 ml was centrifuged for 5 min at 14 000 × g and the pellet was resuspended in 50 µl of distilled water. After adding 50 µl of sample buffer at pH 6.8 (0.125 M Tris, 4 % SDS, 10 % 2-mercaptoethanol, 20 % glycerol and 0.004 % bromophenol blue), the suspension was boiled for 5 min, then centrifuged (14 000 × g, 5 min) and the supernatant subjected to SDS-PAGE.

Gels stained with Coomassie Brilliant Blue R-250 (CBB) were loaded with 20 µl of the lysate (corresponding to 200 µg of *C. burnetii*), silver-stained gels with 5 µl of the lysate (50 µg of *C. burnetii*) and gels silver-stained for LPS and subsequently with CBB were loaded with 12 µl of the lysate (120 µg of *C. burnetii*).

Silver staining for LPS was carried out according to Tsai and Frasch (1982).

Results

CBB staining of the gels (Fig. 1) revealed at least 18 distinct proteins identical in all eight *C. burnetii* strains examined. They occurred in the zones of 77, 60, 57, 55 (double line), 52 (double line), 49, 46, 44, 27, 20.3 — 18.5

Table 1. Source and passage history of C. burnetii strains examined

Strain	Biological source	Geographic origin	Passage history*
S	Human liver	Montana, U.S.A.	3 EP
Priscilla	Goat placenta	Montana, U.S.A.	3 EP
Henzerling	Human blood	Italy	3 EP**
L-35	Human blood	Czechoslovakia	3 EP
27	Tick	Czechoslovakia	3 EP
48	Tick	Czechoslovakia	3 EP
1/IIA	Tick	Czechoslovakia	3 EP
Nine Mile	Tick	Montana, U.S.A.	306 GP(1 TC) 3 EP

* Number of passages in hen's eggs (EP). o tissue cultures (TC) and embryonated

** Unknown number of EP as phase II. Converted to phase I in 5 passages in mice and then subjected to 3 EP.

(probably double line), 17.3, 16.2, 15.7, 13 (heavy line) and 12.5 kD. In the region from 44 to 27 kD there occurred numerous proteins (e.g., approximately 12 clear-cut lines in strain L-35), practically the same in all strains with the exception of S and Henzerling, in which these proteins were hardly discernible. The same applies to proteins with molecular masses of 70 and 68 kD (as well as 71 kD in strain Priscilla) and those with molecular masses greater than 77 kD. In strain 27 we found at least 10 proteins of such a high molecular mass.

The protein patterns showed the greatest variability in the molecular mass region from 27 to 18.5 kD, as concerns the number of protein bands, intensity of staining and especially different molecular masses of the individual bands. This variability is clearly visible in Fig. 2, illustrating a silver-stained gel.

Proteins in the molecular mass region from 20.3 to 18.5 kD appeared as double lines: in strain Priscilla at 20.3 kD, in strains Henzerling, L-35, 27, 48 and 1/IIA at 19.5 kD and in strain Nine Mile at 18.5 kD.

In the region from 17.3 to 12.5 kD, strains S and Priscilla differed greatly from the other strains. As shown in Fig. 1, a heavily stained protein with a molecular mass of 17.3 kD was missing from these two strains and was replaced by a pair of proteins.

Strain S contained a heavily stained protein of 14.7 kD which did not occur in any of the other strains. Three distinct proteins (15.7, 15 and 14.4 kD) occurred in this region in strains Henzerling, L-35, 27, 48, 1/IIA and Nine Mile; of these, only the 15.7 kD protein occurred also in strains S and Priscilla.

Two more proteins with molecular masses lower than 10 kD were observed in strains Priscilla, Henzerling, 27 and 1/IIA. They were hardly detectable by CBB staining (Fig. 1) but clearly discernible after silver staining (Fig. 2).

The LPS patterns are illustrated in Figs. 3 and 4. The LPSs occurred in the region from 23.5 to 11.8 kD. The greatest variability was shown by strains Henzerling (12 zones) and L-35 (11 zones). Eight zones were obtained in strains 27, 1/IIA and Nine Mile, seven zones in strain 48 and six zones in strains S and Priscilla. Two zones that were identical in all eight strains examined corresponded to molecular masses of 18.5 and 14.3 kD respectively.

The protein and LPS patterns of strains isolated from ticks (27, 48, 1/IIA and Nine Mile) did not differ from those of the human strains Henzerling and L-35.

Discussion

Approximately 40 and 60 protein bands were visualized in gels stained with CBB and silver, respectively. Williams (1981) used iodination of tyrosine and histidine by the lactoperoxidase technique and detected 35 clearly distinct proteins which, however, originated only from the outer membrane of *C. burnetii*. We used lysates of whole rickettsiae which certainly contained more proteins than the outer membrane alone; this was also demonstrated by Moos and Hackstadt (1987).

We confirmed the results obtained by Hackstadt (1986) who separated *C. burnetii* strains into three groups based on differences in LPS patterns.

Those of the first, second and third group resembled the LPS pattern of the strains Nine Mile, Priscilla and S, respectively. Based on their LPS patterns, the five additional strains examined in the present study could be included into the first group. The separation into three groups was supported by the protein patterns, which in the region of molecular masses below 20 kD were mutually distinct in strains S, Priscilla and the five others. This applies especially to three proteins with molecular masses of 15.7, 15.0 and 14.4 kD, occurring in *C. burnetii* of the first group, but absent from strains S and Priscilla. A similar situation was found with the 17.3 kD protein.

As distinct from Hackstadt (1986) who reported LPSs occurring in a region of molecular masses from 14 to 18 kD, we found from 6 to 12 LPS bands on silver-stained gels in a region from 11.8 to 23.5 kD. The difference can be explained so that we used polyacrylamide gel gradients and subjected greater samples to SDS-PAGE.

Our results partially correspond to those obtained by Amano *et al.* (1987) as the LPS patterns of the strains Henzerling and Nine Mile concerns.

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Legend to Figures (Plates XXV—XXVIII):

Fig. 1. SDS-PAGE of eight *C. burnetii* strains (a — S, b — Priscilla, c — Henzerling, d — L-35, e — 27, f — 48, g — 1/IIA, h — Nine Mile). Gels stained with CBB. Molecular mass markers (M): bovine albumin — 66 kD, egg albumin — 45 kD, glyceraldehyde-3-phosphate dehydrogenase — 36 kD, carbonic anhydrase — 29 kD, trypsin inhibitor — 20.1 kD, alpha-lactalbumin — 14.2 kD.

Fig. 2. Like Fig. 1, but gel stained with silver.

Fig. 3. Like Fig. 1, but gel subjected to silver staining specific for LPS.

Fig. 4. The same gel as Fig. 3, but additionally stained with CBB.