COXIELLA BURNETII PHASE I AND II PROTEINS STUDIED BY SDS-PAGE

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Summary. – *Coxiella burnetii* cells in both phase I and II reveal in sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) similar protein profiles with only small differences. *C. burnetii* protein profile in SDS-PAGE depended on the method of purification of *C. burnetii* cells from chick embryo yolk sacs. Immune mouse sera against *C. burnetii* phase I cells recognized in phase I and II cell protein profiles mainly the 61 K and 29 K proteins by the immunoblot method. Hyperimmune mouse and rabbit sera against phase I and II cells reacted in different way with phase I and II cells. Sera against phase I cells recognized in both phase I and II profiles more protein bands than sera against phase II cells. Thus phase I LPS present in phase I cells exerted adjuvant effect on the antibody response in animals immunized with phase I cells.

Key words: *Coxiella burnetii*; proteins; SDS-PAGE; immunoblot analysis

Introduction

C. burnetii, the etiologic agent of Q fever, has structure of its outer membrane similar to that of the other Gram-negative bacteria, with lipopolysaccharide (LPS), proteins and phospholipids. This bacterium is unique among obligatory intracellular parasites, because it undergoes a phase variation (Stoker and Fiset, 1956), similar to S – R transitions of LPS of Gram-negative bacteria (Schramek and Mayer, 1982; Lukáčová *et al.,* 1993).

Wild *C. burnetii* strains circulate in the nature in virulent phase I, whereas the relatively avirulent phase II is the result of laboratory passaging in immunologically incompetent hosts.

In response to the infection with phase I cells both phase I and II antibodies are produced, phase II preceding phase I antibodies (Fiset and Ormsbee,1968). Thus the phase II antigen is present in the *C. burnetii* phase I cells and masked by phase I antigen (Schramek *et al.*, 1978). According to Hackstadt (1988), for the phase II antigenic specifity are responsible both phase I and II surface proteins, whereas in the phase I they are sterically blocked by the phase I LPS. The LPS profiles of *C. burnetii* phase I strains in SDS-PAGE are easy to evaluate and according to the bands in the lower molecular mass region these strains can be divided into three groups (Hackstadt, 1986). On the other hand, the protein profiles of *C. burnetii* phase I strains in SDS-PAGE are hardly distinguishable and they do not show significant differences (Novák and Brezina, 1989; Kováčová *et al.*, 1994). Protein profiles of phase I and II cells in SDS-PAGE are very similar (Hackstadt *et al.*, 1985), however , till now, proteins of *C. burnetii* phase II have not been extensively studied by immunoblot analysis.

In the present work we compared protein and immunoblot profiles of *C. burnetii* phase I and II cells, which were isolated by the Verografin gradient method and reacted with immune mouse and rabbit sera. The effect of ether treatment on *C. burnetii* phase I and II protein profiles was also tested.

Materials and Methods

C. burnetii strain Nine Mile serologically in phase I (yolk sac passage No. 3) or phase II (yolk sac passage No. 163) was propagated in chick embryo yolk sacs. Phenol killed cells were purified by a Verografin (SPOFA, Prague) gradient procedure (Williams *et al.*, 1981). Part of Verografin purified phase I and II cells was treated with ether.

SDS-PAGE was carried out according to Laemmli (1970) with 12 and 5% acrylamide in separating and stacking gels, respectively. The gels were stained by Coomassie Brilliant Blue R 250

(CBB) for proteins. 50 μg of purified cells in one sample were applied on gels throughout.

Immunoblot analysis was performed according to Towbin *et al.* (1979). Blots were reacted with corresponding mouse or rabbit sera (1:200) in 10% non-fat dry milk. The reaction was visualized with horseradish peroxidase-conjugated secondary antibodies (1:500) and 4-chloro-1-naphtol. As control, purificate of chicken embryo yolk sac in PBS was used in SDS-PAGE or immunoblot analysis.

Immune and hyperimmune sera. Female BALB/c Han mice weighing 10 – 12 g were immunized with one ip dose (100 μg) of Verografin-purified *C. burnetii* phase I and II cells. The blood was collected and pooled 1 month after immunization (immune sera). Two groups of mice were immunized after one month with second dose of 100 μg of phase I and II cells, respectively, the blood was collected and pooled 10 days after the second dose (hyperimmune sera).

Rabbits were immunized with two ip doses of 1 mg of phase I and phase II cells, respectively, with complete Freund's adjuvant at interval of 20 days. The blood was collected 14 days after the second dose (hyperimmune sera).

Results

The protein profiles of *C. burnetii* phase I and II cells in SDS-PAGE were similar (Fig. 1), though some difference was observed in the lower molecular mass region (10 – 18 K) and a 116 K protein was more marked in the phase I cells. The *C. burnetii* protein profiles exhibited three kinds of bands of various intensity: distinct and clear bands at the upper part corresponding to high molecular mass proteins up to 45 K, the middle part with hazy bands at 40 – 18 K, and the lower part with some intensive bands at 17 – 10 K. The 29 K protein was present in both phase I and II profiles as a weak band.

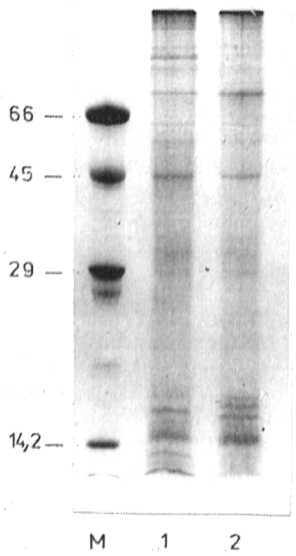

Fig. 1
Protein profile of C. burnetii cells in SDS-PAGE
Lanes: M - molecular mass marker (K values), 1 - phase I cells, 2 - phase II cells.

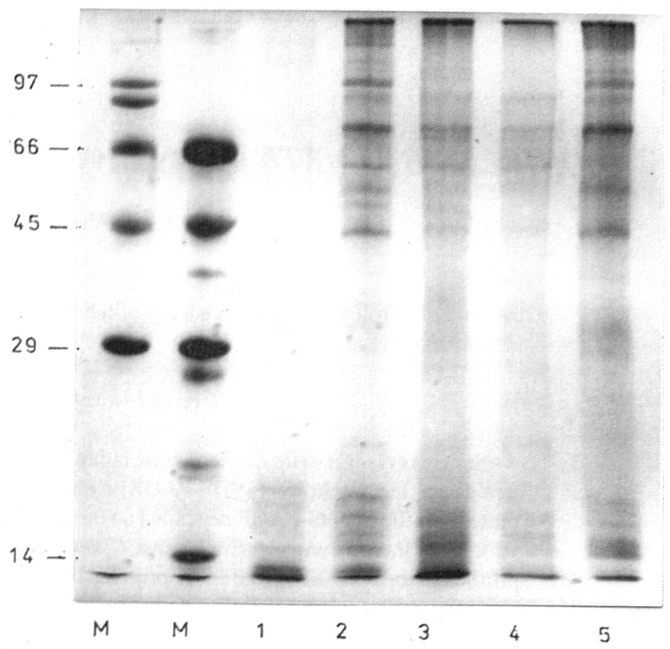

Fig. 2
SDS-PAGE protein profile of C. burnetii cells extracted after Verografin purification with ether
Lanes: M - molecular mass markers (K values), 1 - phase I cells extracted 2x with ether, 2 - phase I cells without extraction, 3 - phase II cells extracted 1x with ether, 4 - phase II cells extracted 2x with ether, 5 - phase II cells without extraction .

The influence of ether treatment on Verografin-purified *C. burnetii* protein profile in SDS-PAGE was also studied (Fig. 2). Ether extraction of *C. burnetii* whole cells resulted in hazy and unclear protein profiles of both phase I and II cells and disappearence of a 45 K protein band corresponding to ovalbumin from chick embryo yolk sac which remained as impurity in ether-untreated *C. burnetii* cell preparations.

In the immunoblot analysis immune mouse sera against *C. burnetii* phase I whole cells reacted similarly with phase I and II whole cell antigens. They recognized, however, only some proteins, mainly 61 K and 29 K, present in SDS-PAGE protein profiles (Fig. 3). Moreover, in phase II cells, some proteins above 60 K were visible.

Interesting was also the reaction of hyperimmune mouse sera against phase I whole cells (Fig. 4): in phase II cells they recognized mainly the 45 K ovalbumin from yolk sac. In both phase I and II cells, there was a distinct LPS pattern: in phase I cells as background in whole profile, in phase II cells as one band at 10 K only (Lukáčová *et al.*, 1991).

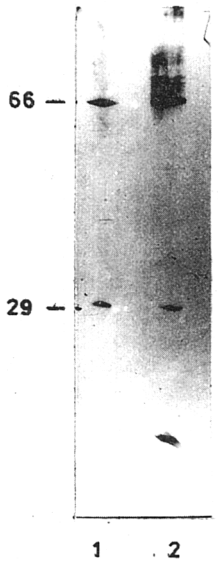

Fig. 3
Immunoblot analysis of C. burnetii cells with immune mouse sera against phase I cells
Lanes: 1 - phase I cells, 2 - phase II cells.

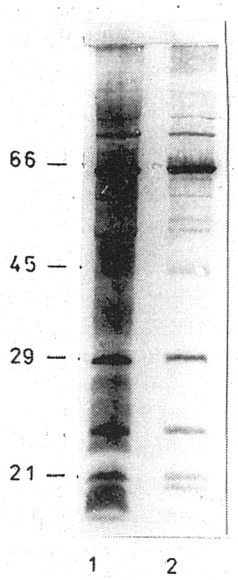

Fig. 5
Immunoblot analysis of C. burnetii cells with hyperimmune rabbit serum against phase I cells
For legend see Fig. 3.

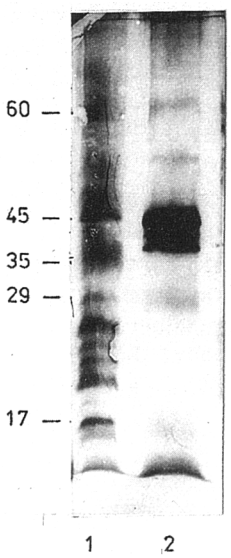

Fig. 4
Immunoblot analysis of C. burnetii cells with hyperimmune mouse sera against phase I cells
For legend see Fig. 3.

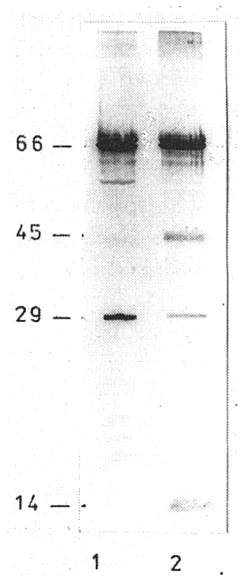

Fig. 6
Immunoblot analysis of C. burnetii cells with hyperimmune rabbit serum against phase II cells
For legend see Fig. 3.

Hyperimmune rabbit sera reacted differently, though the most distinct bands were again the 61 K and 29 K proteins in either *C. burnetii* phase (Fig. 5). Rabbit serum against phase I cells recognized more bands in phase I than in phase II cells with LPS background in whole profile. On the other hand, rabbit serum against phase II cells (Fig. 6) showed similar profiles with both phase I and II cells, but the 45 K band was more distinct in the phase II cells profile. These findings corresponded to the profile of the control chick embryo yolk sac purificate in SDS-PAGE and immunoblot analysis, in which only one band of 45 K protein was found (data not shown).

Discussion

Isolation of *C. burnetii* phase I cells from yolk sac tissues is possible either by ether treatment and differential centrifugation (Ormsbee, 1962) or by the Verografin (Renografin) method (Williams *et al.*, 1981). However, purification of phase II cells by ether extraction and centrifugation only is difficult and a trypsin step is necessary to release the *C. burnetii* cells from yolk sac tissue. The trypsin purification, however, results in the damage of *C. burnetii* outer membrane proteins, so that the purificate does not yield a complete protein profile in SDS-PAGE (Lukáčová *et al.*, 1991). *C. burnetii* LPS profile is not influenced by the trypsinization step, because trypsin does not react with LPS (Seltmann, 1982).

Thus, a comparison of protein profiles of *C. burnetii* phase I and II cells is possible only when the cells are purified by the Verografin method. This method enables to purify phase I and II cells equally well, but the purity of cells is not guaranteed and impurities from the yolk sac may be present. We found that in Verografin-purified *C. burnetii* cells only one contaminating protein was visible, i.e. with its exception, no impurities from the yolk sac could influence the phase I and II protein profiles in SDS-PAGE and immunoblots.

When comparing the two *C. burnetii* phases in SDS-PAGE there were no significant differences between their protein profiles as similarly to Hackstadt *et al.* (1985), except for a more distinct band of phase I at about 116 K and a slight variation at the lower part of molecular masses. It supports, therefore, the Hackstadt's hypothesis (1988) about surface proteins shared by both phase I and II *C. burnetii* cells.

Of interest is the observation of the effect of ether treatment on the phase I and II cell protein profiles, resulting in fever unclear protein bands. The ether treatment not only removed impurities from the yolk sac, namely the 45 K ovalbumin, but it also affected a part of *C. burnetii* outer membrane protein structure, probably that with hydrophobic groups. The 45 K protein is not considered as a part of phase I and II profiles, because its band was visible also with sera of mice immunized with *C. burnetii*-uninfected chick embryo yolk sac (data not shown) and the yolk sac impurities in Verografin-purified *C. burnetii* cell preparations were more marked in immunoblots with mouse and rabbit hyperimmune sera than in those with non-hyperimmune sera, (Figs. 5, 6). Differences between the reactivity of mouse hyperimmune sera, recognizing more impurities from the yolk sac, and that of than rabbit hyperimmune sera can be attributed to the variability of immune response in living organisms.

Immune mouse sera against phase I cells obtained after immunization with one dose only recognized in phase I and II cells profiles the 61 K and 29 K bands. Similarly reacted also mouse sera against phase II cells (data not shown). These bands probably represented the first antibodies raised against *C. burnetii* both phase I and II cells in host organism. Similar results were showed also by Novák *et al.* (1992), in analyzing the antibody response in mice infected with *C. burnetii* phase I by immunoblot. Considering LPS as phase I antigen, and proteins as phase II antigen (Hackstadt, 1988), in response to phase I and II cells inoculation, mice produced in the first place antibodies against proteins, i.e. against *C. burnetii* phase II antigen. This result is in accordance with previous reports (Stoker and Fiset, 1956; Brezina, 1977).

The hyperimmune mouse and rabbit sera against phase I cells detected more bands, including phase I LPS pattern representing phase I antigen. The hyperimmune mouse sera against phase I cells reacted with phase I and II cells in different way. Whereas phase I cells showed more bands with phase I LPS background, phase II cells exhibited mainly strong band of ovalbumin (45 K).

The hyperimmune rabbit serum against phase I cells not only reacted clearly with phase I LPS in phase I profile, but also revealed more protein bands in both phase I and II profiles, than the hyperimmune rabbit serum against phase II cells. With the latter, the protein bands in both phase I and II profiles were also less distinct and in the phase II profile was visible, moreover, a 10 K band corresponding to the phase II LPS.

Adjuvant activity of LPS of bacterial cell walls, *C. burnetii* including, was demonstrated previously (Brezina, 1977; Gustafson and Rhodes, 1992; Verma *et al.*, 1992). Based on our observation of more intensive protein bands in blots with hyperimmune mouse and rabbit sera against phase I cells it is possible to conclude, that phase I LPS present in phase I cells only exerts a better adjuvant effect than does the truncated phase II LPS typical of phase II cells.

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