BRIEF REPORT

Characterization and protective effect of a 29 kDa protein isolated from Coxiella burnetii by detergent Empigen BB

M. Lukáčová, E. Gajdošová, L. Škultéty, E. Kováčová & J. Kazár
Institute of Virology, Slovak Academy of Sciences, Bratislava, Slovakia

Accepted in revised form 30 March 1994

Abstract. A 29 kDa protein, isolated from the outer membrane of Coxiella burnetii, strain Nine Mile phase I by detergent Empigen BB, was characterized. The failure in removing lipopolysaccharides (LPS) from preparations of the protein by the purification method used indicates a strong binding between proteins and LPS in the outer membrane of C. burnetii. The protein was immunogenic in mice and protected them against virulent C. burnetii challenge.

Key words: 29 kDa protein, Coxiella burnetii, Immunoreactivity, Protection ability

Coxiella burnetii, an obligate intracellular parasite of eucaryotic cells, is highly stable and resists elevated temperatures, chemical disinfectants and other unfavourable influences [1]. It can survive in the environment for long periods apparently due to its sporulation capability [2]. Nevertheless, for the stability and resistance of the agent the structure of its outer membrane is also important [3]. C. burnetii has an outer membrane typical of gram-negative bacteria with proteins, LPS and phospholipids. Apart from their structural function, the outer membrane proteins are also important immunodominant components of the C. burnetii cell wall. Williams et al. [4, 5] identified in C. burnetii phase I cells three antigenic and immunogenic proteins. Among them a 29.5 kDa protein was taken for a major surface antigen. A 27 kDa protein was extracted from C. burnetii phase II whole cells by detergents at 60 °C or above [6]. A similar 27 kDa immunoreactive protein was identified in C. burnetii phase I cells and cloned [7, 8]. Another dominant antigen of C. burnetii is a 62 kDa heat shock protein [9], which is localized on the surface of phase II but not phase I cells. All these proteins have been proposed as potential vaccine candidates for specific Q fever immunoprophylaxis.

Using the method of Lowell et al. [10], who extracted mainly peptides from Neisseria meningitidis using the detergent Empigen BB, we have attempted to obtain a protein-rich fraction from the outer membrane of phase I C. burnetii cells by similar treatment.

Empigen BB solubilised from C. burnetii outer membrane mainly two proteins, namely 29 and 40 kDa. Whereas the 29 kDa was precipitated with (NH₄)₂SO₄, the 40 kDa protein was not. SDS-PAGE showed that precipitated protein fraction (PP) contained mostly the 29 kDa protein and some protein impurities of higher molecular mass (Figure 1). The supernatant was composed of one strong band at 40 kDa and many less distinct bands of greater size. The extraction of C. burnetii with other detergents (SDS, sodium deoxycholate) resulted in obtaining a protein-LPS complex with a number of proteins of various size, which were difficult to separate (data not shown). We paid attention to the 29 kDa protein, because a protein of this size was already described by other authors as a major surface antigen of C. burnetii [4, 5, 11].

PP also contained, apart from proteins determined according to Bradford [12], 9.8% of neutral sugars assayed according to Dubois et al. [13]. After separation of PP on a Sephadex G-100 column into 6 fractions, various amounts of proteins were found in 4 of them and different proportions of neutral sugars in all of them. The lyophilized fractions were examined by SDS-PAGE for proteins and LPS (Figures 2 and 3). The first 4 fractions gave identical profiles and contained the 29 kDa protein, with the same LPS. It was impossible to completely remove LPS from the purified protein. The neutral sugars content varied from 2.0 to 3.75% (w/w), i.e., it was reduced by the chromatographic purification. The last two fractions contained neither proteins nor LPS. These fractions consisted of low molecular mass compounds, namely salts and saccharides.

The fractions obtained by chromatography purification were subjected to Western blot analysis. The first 4 fractions reacted intensively and bands were present in both LPS and protein part of the profile, fraction No. 5 reacted very weakly and fraction No. 6 did not react at all (Figure 4).

Fraction No. 3 (CPP) with the lowest neutral sugars content was used together with PP in the protective test in mice. BALB/c/Han mice were divided

Purified [21] whole cells of phase I C. burnetii, strain Nine Mile were extracted by 2% Empigen BB – N-dodecyl-N,N-dimethylglycine (Calbiochem, La Jolla, CA, USA) in 50 mM NaCl and 10 mM EDTA (pH 7.4), for 60 min. at 37 °C by shaking [10]. After centrifugation at 14,000 x g for 40 min. the supernatant was saved and the sediment was repeatedly extracted. Both supernatants were pooled and 3 times precipitated with (NH₄)₂SO₄ (500 g/l) at 0 °C. The precipitate was dissolved in water, dialysed and lyophilised. SDS-PAGE was carried out according to Laemmli [22], with 12 and 5% acrylamide in separating and stacking gel, respectively. Gels were stained by Coomassie Brilliant blue R 250 for proteins, and by silver for LPS after proteinase K treatment [23].

into two groups: the first was immunized with PP or CPP and challenged intraperitoneally with 10³EID₃₀ (egg infectious dose) of infections C. burnetii phase I (Nine Mile strain in the 3rd egg passage) [14]. Six days after challenge their spleen (20% suspension) was titrated in chick embryo yolk sacs. Mice were considered as resistant when the yield of C. burnetii was lower by at least 2 log EID₃₀ units than that of control mice (Table 1). The difference in the yield of C. burnetii from the spleen of the control group and that of the group immunized with 10, 50 and 100 µg PP, respectively, was 3.2 log units, i.e., there was a more than 1000-fold protection against C. burnetii phase I virulent challenge. A similar protection was observed with 50 µg CPP. Doses of 1 µg PP and 10 µg CPP had no protective effect, although 1 µg PP still induced LTT.

The second group was immunized with PP, bled, and spleens were used for assay of cellular immunity [15] by lymphocyte transformation test (LTT) (Table...