ISOLATION AND EVALUATION OF *COXIELLA BURNETII* O-POLYSACCHARIDE ANTIGEN AS AN IMMUNODIAGNOSTIC REAGENT

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Summary. – A lipid A – deprived lipopolysaccharide (LPS) from *Coxiella burnetii* (*C b*) Priscilla strain in virulent phase I was separated by steric-exclusion chromatography and high performance liquid chromatography (HPLC). The isolated O-specific polysaccharide (PS) fractions were analyzed by different physico-chemical methods and showed noticeable differences in their overall composition The antigenic potential of the PS fractions was evaluated by ELISA with animal and human sera and in comparison with those of the native *C b.* LPSs and phase I and II *C b.* Nine Mile stain cells of which the latter are routinely used in diagnosis of Q fever The results indicate that the high molecular mass PS antigen SG501 could be used in ELISA for a sensitive and specific detection of anti-*C.b.* antibodies in the examined sera.

Key words: Coxiella burnetii, Chlamydophila psittaci, O-specific polysaccharide, ELISA, immunodiagnostic reagent

Introduction

C b., the etiological agent of Q-fever, is an obligate intraphagolysosomal bacterial pathogen of eukaryotic cells (Baca and Paretsky, 1983). Q fever is a zoonosis wide-spread throughout the world which can occur in acute or chronic form. Although the acute form of the disease is curable by a proper treatment with tetracycline (Stein and Raoult, 1995), there is a constant threat of valvular endocarditis, which is the most serious complication of chronic Q fever and is often fatal (Ellis *et al.*, 1983). The pathogenesis of Q fever endocarditis is unknown and the proposed mechanisms causing lesions in the cardiac valves are controversial.

Like other Gram-negative bacteria, *C b.* contains an LPS on the surface of its outer membrane. In virulent *C.b.* strains, the polysaccharide portion consists of an O-specific chain and a core oligosaccharide (Toman, 1996; Toman *et al.*, 1996). Low-virulent strains do not express the O-side chain but retain core oligosaccharides of varying length (Toman and Škultéty, 1996; Ftáček *et al.*, 2000). The transition of *C.b.* from virulent phase I to low-virulent phase II is accompanied by modification in both composition and structure of LPS (Ftáček *et al.*, 2000). Thus, the phase I cells express a smooth (S) LPS with O-polysaccharide chain and the phase II cells express a rough (R) LPS. Clinical bacteriologists need rapid and specific identification methods for the diagnosis of infectious diseases in order to investigate the epidemiology of a given bacterium. The LPS has been used successfully as

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Abbreviations: *C.b* = *Coxiella burnetii*, *Chl.p.* = *Chlamydophila psittaci*; GalA = D-galacturonic acid, GlcA = D-glucuronic acid; GalNAc = N-acetyl-D-galactosamine; GlcNAc = N-acetyl-D-glucosamine; GC = gas chromatography; GC-MS = GC-mass spectrometry, HPLC = high performance liquid chromatography, Kdo = 3-deoxy-D-manno-oct-2-ulosonic acid; LPS = lipopolysaccharide, MS = mass spectrometry; NM I = phase I cells of *C b* Nine Mile strain; NM II = phase II cells of *C b.* Nine Mile strain, PR I = phase I cells of *C b* Priscilla strain; PS = O-specific polysaccharide; R = rough; S = smooth; SDS-PAGE = polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate; Strep = dihydrohydroxystreptose TLC = thin layer chromatography; Vir = virenose

a chemotaxonomic and antigenic marker of many bacteria (Jann and Jann, 1984). In addition, serological specificity of an LPS is determined by epitopes present mainly in the O-polysaccharide chain (Sidorczyk *et al.*, 1995). For this reason, antigenic epitopes in LPS are potential candidates for immunodiagnostic tools. In order to prevent cross-reaction with other bacteria, diagnostic methods may utilize small oligosaccharides either as components of natural antigens or as synthetic compounds immobilized on a suitable surface to serve as capture agents in the detection of antibodies in patient sera (Kasper *et al.*, 1983).

The diagnosis of a *C.b.* infection involves various immunochemical tests, which detect or assay antibodies against phase II *C b.* (phase II antibodies) in case of acute Q fever and antibodies against phase I and II (phase I and II antibodies) in case of chronic Q fever, which is characterized by a specific increase in antibodies against phase I *C.b.* (phase I antibodies) (Peacock *et al.*, 1983; Embil *et al.*, 1990). Of these assays, ELISA is easy to perform, has a potential for automation, and is more sensitive than the complement fixation test (Fournier *et al.*, 1998). ELISA has been demonstrated to be even more sensitive than the immunofluoresence assay and is routinely used for the serodiagnosis of Q fever (Peter *et al.*, 1988; Cowley *et al.*, 1992). The *C.b.* antigens used in immunochemical assays are usually derived from whole cells in phase I or II (Fiset *et al.*,1969; Schramek *et al* , 1970; Schramek *et al.*, 1972).

The present work is focused on the isolation and characterization of the *C.b* PS antigens and the evaluation of their role in the diagnosis of Q fever. The applicability of these antigens to ELISA is evaluated here by testing their reactivities with animal and human sera and by comparing them with those of the native LPSs and whole cell (corpuscular) antigens derived from *C.b.* in both phase I and II.

Materials and Methods

Cultivation and purification of C.b cells. The strains Nine Mile and Priscilla, serologically in virulent phase I (yolk sac passage 3 in this laboratory), and Nine Mile, serologically in low-virulent phase II (yolk sac passage 162 in this laboratory), were propagated in embryonated hen eggs. After cultivation, the phase I cells were purified by centrifugation and extraction with ether as described by Škultéty *et al.* (1998). The phase II cells were purified by three subsequent treatments with trypsin (Úrvölgyi, 1976) The phase I cells of the strains Nine Mile and Priscilla and the phase II cells of the strain Nine Mile (NM I, PR I, and NM II cells, respectively), were used for the isolation of the corresponding LPSs and in immunochemical studies as the corpuscular antigens.

LPS isolation In a typical isolation procedure, NM I, NM II, and PR I cells (1 g each) were extracted with chloroform-methanol (2:1, v/v) at 20°C overnight to remove phospholipids. LPSs were then isolated from the respective cells and purified further as described earlier (Škultéty *et al* , 1998; Toman and Škultéty, 1996). The final yields of LPSs from NM I (LPS NM I), NM II (LPS NM II), and PR I (LPS PR I) cells were 9 4, 3 7, and 10.8%, respectively. In order to obtain higher amounts of the LPSs from the cells, the isolation procedure was repeated several times.

Preparation of PS antigens from LPS PR I. Lipid A was released from LPS PR I (360 mg) by mild hydrolysis using a sodium acetate buffer (20 mmol/l, pH 4 5) at 100°C for 4 hrs, and the suspension was centrifuged at 14,000 x g for 20 mins to pellet lipid A. The pellet was washed twice with distilled water followed by centrifugation at 14,000 x g for 20 mins to extract residual PS. The supernatants were pooled, concentrated by evaporation, and lyophilized The yield of PS was 203 mg The PS was dissolved in redistilled water (4 ml)

Steric-exclusion chromatography and HPLC The PS described above was fractionated on a column (25 x 900 mm) of Sephadex G-50 (Pharmacia), using deionized water as eluant at a flow rate of 10 ml/hr The separated PS fractions (SG501, SG502, SG503, and SG504) were monitored by an RIDK 102 RI detector (Laboratorní přístroje, Czech Republic) and by the phenol-sulphuric acid reaction (Dubois *et al.*, 1956) The yields were 64 5, 44.5, 45 5, and 32.5 mg, respectively The fraction SG504 was further purified by HPLC using a C-18 column (8 x 250 mm, Separon, Czech Republic) and deionized water as the eluant The purified fractions were designated HPLC1, HPLC2, and HPLC3. The yields were 11.3, 8.7, and 9.5 mg, respectively

Human sera. Sera from patients with acute Q fever, showing atypical pneumonia, were collected during an outbreak of Q fever in western Slovakia (Varga, 1997) Q fever was considered acute when there was seroconversion or a fourfold increase of specific antibody titers at later testing (Serbezov *et al* , 1999, Kováčová *et al.*, 1998) Ten sera were collected from patients with chlamydial infection.

Animal sera. Immune rabbit sera were prepared by inoculation of animals with the corpuscular antigens NM I, NM II, PR I, and with those of *Chlamydophila psittaci* (*Chl p*) strains 6BC and PK 5082. Rabbits were inoculated intraperitoneally with 100 μg of the respective corpuscular antigen. The second dose (500 μg) was applied two weeks later The animals were bled 10 days later.

Serological tests ELISA was carried out as described earlier (Kováčová *et al.*, 1987). Briefly, wells of microtiter plates were coated with 1.25 μg per well of the respective antigen in a carbonate buffer pH 9 6. Two-fold dilutions of sera, starting with 1:100, were prepared Peroxidase-conjugated anti-rabbit and anti-human IgG (1:4,000) were used (Sevac, Czech Republic) After addition of the substrate (O-phenylenediamine, Sigma) the reaction was stopped with 4 mol/l H_2SO_4 A_{492} values were read in a Multiscan MCC 340 ELISA reader (Labsystems, Finland). A serum dilution was considered positive when it gave A_{492} value equal to or greater than the mean A_{492} value of 10 negative sera plus 3 SD ELISA titers were expressed as inverted values of the highest dilutions still positive.

Analytical methods. 3-deoxy-D-manno-oct-2-ulosonic acid (Kdo), protein, phosphate, and hexosamine contents were determined as reported earlier (Toman and Škultéty, 1996). PS fractions were hydrolyzed with 2 mol/l trifluoroacetic acid at 100°C for 2 hrs and neutral sugars were analyzed as the corresponding

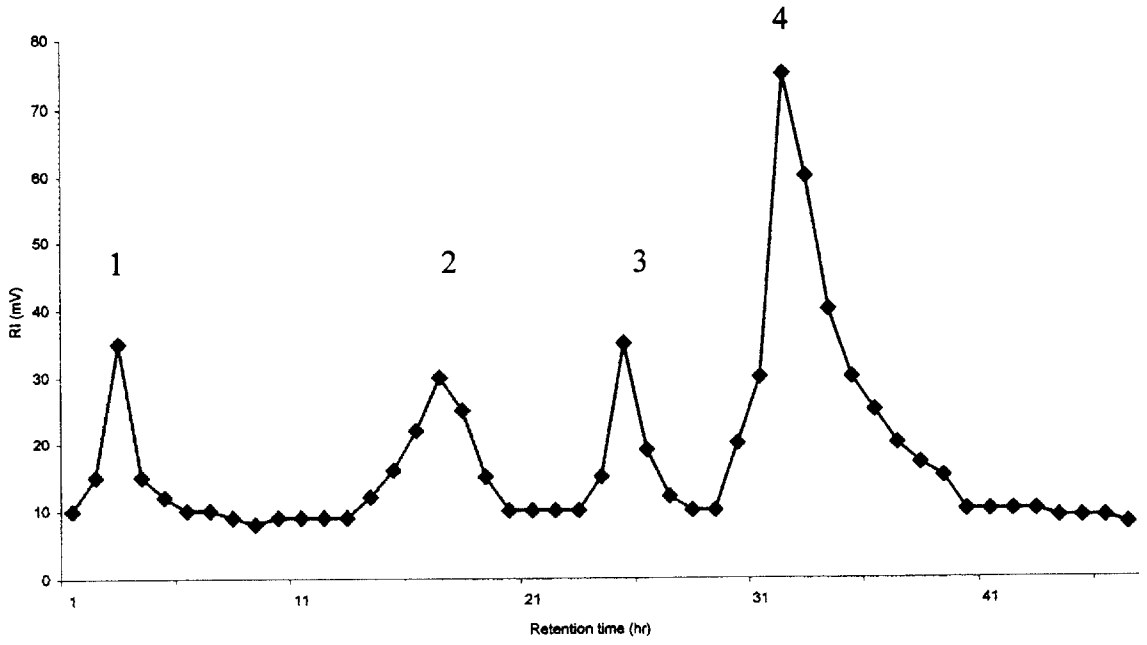

Fig. 1
Fractionation of PS obtained from LPS PR I by steric-exclusion chromatography
Peaks 1, 2, 3, and 4 represent PS fractions SG501, SG502, SG503, and SG504, respectively.

alditol acetates by gas chromatography (GC) and GC-mass spectrometry (GC-MS) (Škultéty *et al.*, 1998). In GC and GC-MS, amino sugars were determined as alditol acetates after hydrolysis of PS fractions with 3 mol/l HCl for 8 hrs.

Gel electrophoresis and immunoblot analysis were carried out as described elsewhere (Škultéty and Toman, 1992; Willems *et al.*, 1992). The SDS Molecular Weight Markers kit from Sigma was used After blotting, the nitrocellulose strips were developed with the corresponding hyperimmune rabbit sera (1:100) in 10 % skimmed milk. The reaction was visualized by horseradish peroxidase-conjugated secondary antibodies (1:500, Sevac, Czech Republic). Color was developed by addition of diaminobenzidine (Sigma).

TLC was performed on precoated Silica Gel 60 plates (Merck) with a mixture (3:6, v/v) of isobutyric acid-1 mol/l ammonium hydroxide. Spots were revealed by charring.

GC and GC-MS. GC was performed with a Shimadzu Model 17A chromatograph equipped with a flame ionization detector using helium as carrier gas. Alditol acetates of amino sugars were analyzed on an HP-5 column (25 m x 0.32 mm, Hewlett Packard) for 3 mins at 160°C, the temperature increasing to 245°C at 2°C/min. Alditol acetates of neutral sugars were analyzed on an SP-2330 column (30 m x 0.25 mm, Supelco, USA) using a temperature program of 180°C for 2 mins, the temperature increasing to 245°C at 6°C/min. GC-MS was performed on a Finnigan MAT SSQ 710 mass spectrometer with helium as carrier gas. Electron impact mass spectra were recorded at 70 eV and an ion-source temperature of 150°C. GC-MS was run on the columns and with the temperature programs described above.

HPLC Semi-preparative HPLC was carried out on a Biospher SI120C18 column (5 µm, 8 x 250 mm, Labio, Czech Republic) using ultra-pure water as eluent at 20°C and a flow rate of 0.6 ml/min. The HPLC system consisted of a 2152 LC controller, a 2150 HPLC programmable pump (both from LKB), a 7125 injector valve (Rheodyne, USA) equipped with a 100 µl loop, an RIDK 102 differential refractometer(Laboratorni přístroje, Czech Republic), and a CSW v. 1.6 integrator (DataApex, Czech Republic). D-Glucose and its α (1→4)-linked oligomers (from dimer to heptamer) were used for column calibration.

Results and Discussion

Isolation and chemical composition of O-specific PS antigens

Steric exclusion chromatography of the PS obtained from LPS PR1 yielded four PS fractions, SG501, SG502, SG503, and SG504 (Fig. 1). The SG504 fraction, eluted as the last from the column, was further separated by HPLC giving three fractions, HPLC1, HPLC2, and HPLC3. The fractions were examined by TLC and each of them gave a single spot (Fig. 2). Chemical analyses of the fractions (Tables 1 and 2) revealed noticeable differences in Kdo, D-GlcNAc, D-GalNAc, and phosphate contents, and also in the composition of neutral sugars. Moreover, differences in size

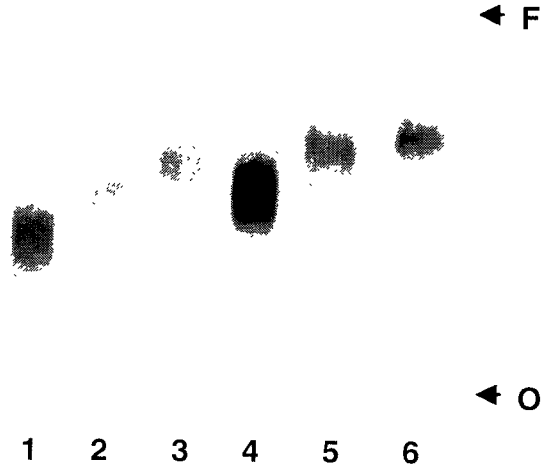

Fig. 2
Mobility of O-specific PS antigens assayed by TLC
SG501, SG502, SG503, HPLC1, HPLC2, and HPLC3 (lanes 1-6) A 10 to 20 μg amount of sample was deposited on each lane Origin (O) and front (F) are marked by arrows

and shape of PS molecules could be anticipated. Prior to subjecting the PS preparations to subsequent serological studies, the levels of antibodies against the NM I and NM II corpuscular antigens were detected in the examined rabbit sera (Table 3).

Serological characterization

Suitability of the preparations for assaying antibodies in hyperimmune rabbit sera was examined by ELISA. As shown in Table 4, SG501 exhibited the highest binding activity (titer of 800) with the phase I and II antibodies against both corpuscular antigens NM I and PR I and could be of diagnostic value. It should be mentioned here that SG501 had the highest content of virenose (Vir) and a relatively high content of dihydroxydroxystreptose (Strep,

Table 1. Analysis of the PS fractions obtained from *C.b.*-derived LPS PR I

Fractions	Amount in nmol/mg PS			
	Kdo	Phosphate	GlcNAc	GalNAc
SG501	20 8	16 3	120 2	70 5
SG502	27.2	56 1	40 5	25 2
SG503	45 9	225 3	82 5	70 3
HPLC1	44.1	250 1	102 2	40 2
HPLC2	44 9	270 8	110.2	60 1
HPLC3	13.6	440 1	20 5	15.8

GlcNAc = N-acetyl-D-glucosamine, GalNAc = N-acetyl-D-galactosamine
For other abbreviations see the text

Table 2. Neutral sugar composition of the PS fractions obtained from *C.b.*-derived LPS PR I by steric-exclusion chromatography

Fractions	Composition (mole %)							
	Ara	Vir	Xyl	Man	Gal	Glc	Strep	Hep
SG501	0 6	44 5	1 6	14 2	0 6	3 2	15 1	20 2
SG502	0 4	23 3	1 3	33 3	1 6	3 9	11 1	25 1
SG503	1 2	8 8	–	29 3	1 8	2 8	25.3	30 8
HPLC1	1 4	5 8	0 8	25 5	0 5	13.9	23 9	28 2
HPLC2	2 4	12 8	0 9	20 6	1 5	12 1	24.2	25 5
HPLC3	3 6	10 7	5 4	29 9	11 4	5 4	13 1	20.5

Ara = L-arabinose, Gal = D-galactose, Glc = D-glucose, Man = D-mannose, Vir = virenose, Strep = dihydroxydroxystreptose, Hep = D-glycero-D-manno-heptose. For other abbreviations see the text.

Table 3. Assay of antibodies in hyperimmune rabbit sera by ELISA with *C.b.*-derived corpuscular antigens NM I and NM II

Antigens	Sera against/antibody titers ($\times 10^{-2}$)		
	NM I and NM II	PR I and PR II	NM II
NM I	32	64	4
NM II	128	512	512

For the abbreviations see their list on the front page.

Table 4. Assay of antibodies in hyperimmune rabbit sera by ELISA with the *C.b.*-derived PS fractions as antigens

Antigen/fraction	Sera against/antibody titers ($\times 10^{-2}$)			
	NM I and NM II	PR I and PR II	NM II	Normal serum
SG501	8	8	<1	<1
SG502	2	4	<1	<1
SG503	2	2	<1	<1
HPLC1	<1	1	<1	<1
HPLC2	2	4	<1	<1
HPLC3	1	2	<1	<1

For the abbreviations see their list on the front page or the text

Table 2) among the investigated fractions. Both sugars are considered (Škultéty *et al.*, 1998) important chemotaxonomic markers. Moreover, their release from the parental LPS by mild acidic treatment led to a considerable decrease (Schramek *et al.*, 1985) in the serological activity of the LPS in a passive hemolysis test. Thus, there is indication that the sugars are involved in binding to anti-*C.b.* antibodies and may be immunodominant.

Cross-reactivity with anti-chlamydial sera

In our further studies three different anti-*C b.* hyperimmune rabbit sera were tested against various *C b.*-derived antigens (Table 5). Sera against *Chl.p.* strains 6BC and PK 5082 were also included to examine possible cross-reactions. The serum against NM I and NM II bound strongly to LPS NM I and NM II (both with a titer of 12,800), followed by LPS PR I (6,400), NM I, PR I, and LPS NM II (all 3,200), and finally by SG501 (800). In contrast, the serum against PR I showed the highest sensitivity with NM II (51,200), followed by LPS NM I (12,800), PR I and NM I (6,400), LPS PR I (3,200), SG501 and LPS NM II (both 800). The serum against NM II reacted preferably with NM II (51,200) but less with LPS NM II (3200). SG501 did not react (100) with the antibodies against *C b* in phase II, which are currently produced in the case of acute Q fever. The antigens shown in Table 5 exhibited no or very slight cross-reactivity with the chlamydial sera tested. Only LPS NM I exhibited a moderate cross-reactivity with the anti-*Chl.p.* 6BC serum.

Table 5. Assay of antibodies in three anti-*C.b.* and two anti-*Chl.p.* hyperimmune rabbit sera by ELISA with various *C.b.*-derived antigens

Antigens	Sera against/antibody titers (x 10^{-2})					
	NM I and NM II	PR I and PR II	NM II	*Chl p* PK 5082	*Chl p* 6BC	Normal serum
NM I	32	64	4	2	2	<1
NM II	128	512	512	8	8	<1
LPS NM I	<128	128	4	8	64	<1
LPS NM II	32	8	32	4	4	<1
PR I	32	64	4	2	2	<1
LPS PR I	64	32	4	4	8	<1
SG501	8	8	<1	<1	<1	<1

For the abbreviations see their list on the front page or the text.

SDS-PAGE and immunoblot analysis

The SDS-PAGE profiles of LPS NM I and LPS NM II are given in Fig. 3. Remarkable differences between the both profiles were evident. The LPS NM I profile had two strong bands in the region of about 14.3–18.3 K and a group of unevenly distributed bands in the region of about 18.3–24.0

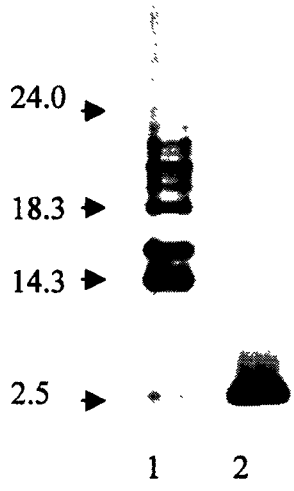

Fig. 3
SDS-PAGE of LPS NM I and LPS NM II from *C.b.*
LPS NM I (lane 1) and LPS NM II (lane 2). The amounts of LPS deposited on lanes 1 and 2 were 24 μg and 3 μg, respectively

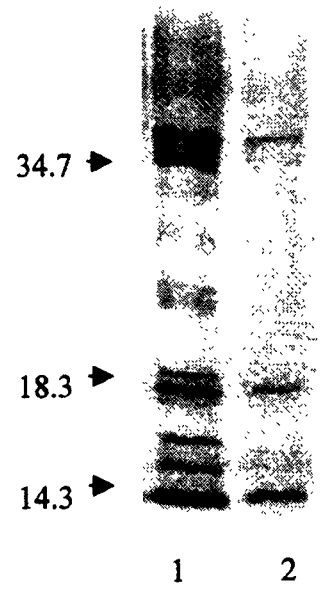

Fig. 4
Immunoblot analysis of the O-specific polysaccharide antigen SG501
Serum with antibodies against NM I and antigens LPS NM1 (10 μg, lane 1) and SG501 (10 μg, lane 2) were tested.

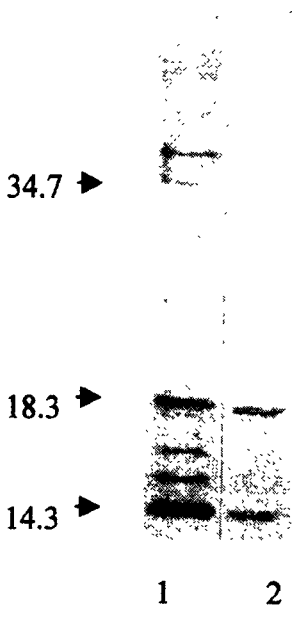

Fig. 5
Immunoblot analysis of the O-specific polysaccharide antigen SG501
Serum with antibodies against PR I and antigens LPS PR I (10 μg, lane 1) and SG501 (10 μg, lane 2) were tested

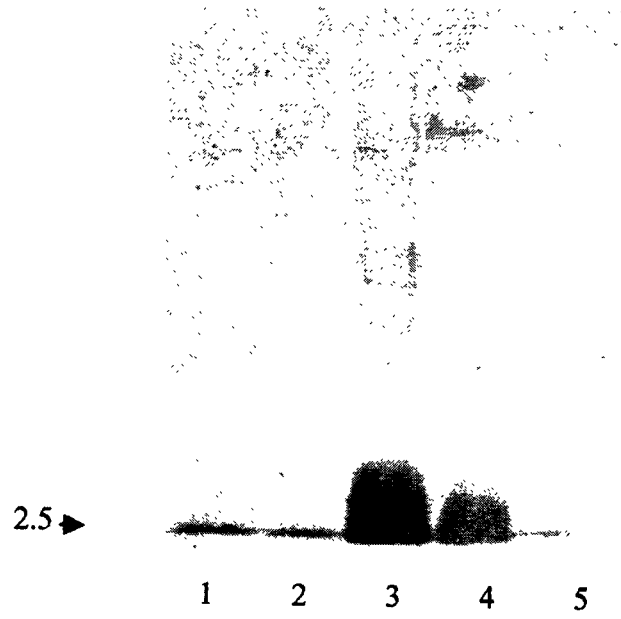

Fig. 6
Immunoblot analysis of *C.b.* antigens
Serum with antibodies against NM II and antigens LPS PR I (10 μg, lane 1), LPS NM I (10 μg, lane 2), LPS NM II (10 μg and 5 μg, lanes 3 and 4, respectively), and SG501 (10 μg, lane 5) were tested

K LPS species extending above the 24 K region were stained poorly or not at all, most probably due to the presence of a high content of hexosamine residues in the O-specific chain. In contrast, LPS NM II gave a single band characteristic for the rough (R) LPSs. Substantially different banding patterns of LPS NM I and LPS NM II clearly indicate deep changes in both composition and structure of LPS NM I which take place during serial laboratory passaging of *C.b.* in embryonated hen eggs and its transition from virulent phase I to low-virulent phase II. In immunoblot analysis, SG501 gave three bands at about 14.3, 18.3, and 34.7 K with the phase I and II serum against NM I, whereby the band at about 14.3 K was most intense (Fig. 4). The same bands were also seen with LPS NM I although the banding pattern was more complex. It is assumed that both SG501 and LPS NM I share the same immunoreactive epitopes for the serum applied. When the phase I and II serum against PR I was used, a similar banding pattern as that seen in Fig. 4 was obtained for SG501, although the band at about 34.7 K was not clearly seen (Fig. 5).

Likewise, LPS PR I exhibited similar banding pattern to that seen with LPS NM I. It appears that there is a remarkable cross-reactivity among the antigens and sera examined. The data may indicate structural similarity at least in some regions of the PS chains of LPS NM I and LPS PR I. Neither SG501 nor LPS NM I and LPS PR I were immunoreactive with the phase II serum against NM II (Fig. 6). In contrast, LPS NM II showed a strong immunoreactivity at about 2.5 K, indicating that the antibodies against the phase II *C b.* are directed against structural epitopes present in this LPS. Cross-reactive and cross-protective poly- and monoclonal antibodies against certain R mutant bacteria have been assumed (Lüderitz *et al.*, 1966) to be directed against common structures in the inner core region of an LPS molecule, which usually consists of hexose, heptose, and the Kdo residues. This is in agreement with our results, since it appears that the immune response to LPS NM II is directed against the heptasaccharide proximal to lipid A, which contains D-mannose, D-glycero-D-manno-heptose (D,D-Hep), and Kdo in a molar ratio of 2:2:3. (Toman and Škultéty, 1996). On the other hand, the phase I antibodies may be directed against the O-specific chains of the investigated LPSs, which contain mainly Vir, Strep, D-Man, D-Xyl, D-Glc, D-GlcA, D-GalA, D-GlcNAc, and D-GalNAc as constituent sugars (Toman *et al.*, unpublished results). From the results obtained one can assume that the biological activity of the O-polysaccharide antigens could be influenced by the content of Vir and Strep. However, an additional evidence is needed in this respect.

Application of PS antigen as immunodiagnostic reagent

The utility of SG501 in the Q fever diagnosis was further examined by testing its reactivity in ELISA with 6 human

sera collected during the last Q fever outbreak in Slovakia in 1993 (Varga, 1997) and by comparing it with the reactivities of other antigens used in this study (Table 6). Of them, NM II, which is routinely being used in Q fever diagnosis, showed the highest reactivity. Although SG501 had a lower immunoreactivity as compared to NM II, it was among the best of the antigens used in this study. Moreover, SG501 showed no cross-reactivity (titer of 100) with chlamydial antibodies in 10 human sera associated with chlamydial infections (data not shown). In contrast, NM II exhibited a noticeable cross-reactivity (800) with samples No. 8 and 9. Thus, we can conclude that SG501 could alternatively be used as a specific antigen in diagnosis of acute Q fever despite a higher sensitivity of NM II which is routinely used for this purpose, since the latter exhibits a significant cross-reactivity with chlamydial antibodies (Lukáčová *et al.*, 1999).

When isolated from humans or animals, *C b* expresses the phase I antigen and is very infectious. The phase I LPS is considered a major determinant of virulence and infectivity of *C b.* (Baca and Paretsky, 1983; Hackstadt, 1986). This LPS with its bulky PS chain sterically blocks access of antibodies to surface *C.b.* proteins (Hackstadt, 1988). This may explain at least partially the well known fact that the bacterium can persist at unknown sites after recovery of the patient from acute form of Q fever, accompanied by lifelong seropositivity. After serial passage of *C.b* in embryonated hen eggs or tissue cultures, changes in both LPS composition and structure result in an antigenic shift to the phase II form, which is less infectious. So far, LPS seems to be the only antigen and immunogen differing between the phases I and II of *C.b.* (Toman and Škultéty, 1996; Ftáček *et al.*, 2000). This antigenic peculiarity is extremely valuable for the serological differentiation between acute and chronic Q fever (Fournier *et al.*, 1998). ELISA has routinely been used in Q fever diagnosis. Its diagnostic utility resides in estimation of the ratio of phase II to phase I reactivity so that the seroepidemiological studies can distinguish the acute and chronic forms of the disease (Peacock *et al.*, 1983; Williams *et al.*, 1986). Our results indicate (Table 7) that SG501 could be used as a suitable antigen in the serological diagnosis of chronic Q fever. Thus, the clinical transition state from acute to chronic form can be detected as early as possible to prevent, *inter alia*, a Q fever-induced endocarditis by a suitable antibiotic treatment. However, further studies are needed in this respect.

Table 6. Assay of antibodies in human acute sera collected during a Q fever outbreak in western Slovakia in 1993 by various *C.b.*-derived antigens

Antigens	Sera No./antibody titers (x 10^{-2})					
	1	2	3	4	5	6
NM I	20	10	< 5	< 10	10	10
NM II	80	40	40	64	40	40
LPS NM I	40	10	10	16	< 5	< 5
LPS NM II	40	10	<5	10	< 5	<5
PR I	20	20	< 5	40	10	10
LPS PR I	40	10	10	32	10	10
SG501	40	10	10	40	40	80

For the abbreviations see their list on the front page or the text

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Table 7. Assay of antibodies in human convalescence sera collected during a Q fever outbreak in western Slovakia in 1993

Antigens[a]	Human convalescence sera No.[b]/antibody titers (x 10^{-2})											
	1	2	3	4	5	6	7	8	9	10	11[c]	12[d]
NM I	8	16	8	8	8	8	4	32	8	16	64	1
NM II	16	32	16	16	16	8	8	16	8	32	8	1
SG501	8	16	16	8	16	16	8	32	16	16	64	1

[a]2.5 μg antigen/well

[b]300 days onset.

[c]Chronic serum (with high phase I antibodies detected by different serological methods).

[d]Negative control

For the abbreviations see their list on the front page or the text.

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