INFECTION AND IMMUNITY, May 1993, p. 2182–2191
0019-9567/93/052182-10$02.00/0

Vol. 61, No. 5

Immunological and Molecular Polymorphisms of OspC, an Immunodominant Major Outer Surface Protein of *Borrelia burgdorferi*

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Received 13 November 1992/Accepted 9 February 1993

The gene of the immunodominant major protein pC of *Borrelia burgdorferi* was previously cloned and sequenced (R. Fuchs, S. Jauris, F. Lottspeich, V. Preac-Mursic, B. Wilske, and E. Soutschek, Mol. Microbiol. 6:503–509, 1992). pC is abundantly expressed on the outer surface of *B. burgdorferi*, as demonstrated by immunoelectron microscopy with monoclonal antibody L22 1F8. Accordingly, pC is renamed OspC, by analogy to the outer surface proteins OspA and OspB. Western immunoblot analysis of 45 *B. burgdorferi* isolates with monoclonal antibodies revealed that OspC is immunologically heterogeneous. Partial sequence analysis of the *ospC* gene confirmed the protein heterogeneity at the genetic level. We found that the degree of identity between the *ospC* partial sequences of five strains representing different OspA serotypes was only 63.3 to 85.4%. Immunological heterogeneity was also observed among representatives of the three newly designated genospecies of *B. burgdorferi* sensu lato, *B. burgdorferi* sensu stricto, *B. garinii*, and group VS461. Heterogeneity was confirmed for *B. garinii* at the genetic level. The *ospC* gene was also cloned from strains that did not express OspC, and antibody-reactive OspC was expressed in *Escherichia coli*. In addition, OspC-expressing variants were obtained from a nonexpressing strain by plating single colonies on solid medium. These findings confirm that the *ospC* gene is also present in nonexpressing strains. Because OspC is an immunodominant protein for the early immune response in Lyme borreliosis and was effective as a vaccine in an animal model, the immunological and molecular polymorphisms of *ospC* and OspC have important implications for the development of diagnostic reagents and vaccines.

The spirochete *Borrelia burgdorferi* is the causative agent of Lyme borreliosis (19), a multisystem disorder characterized by an initial skin lesion (erythema migrans) present in about 50% of those with the disease. In addition, neurological and cardiac manifestations may follow weeks to months later. If not treated, the infection may recur as chronic encephalomyelitis, chronic arthritis, and, in Europe, acrodermatitis chronica athrophicans (40). *B. burgdorferi* has been isolated from different biological materials, including skin and cerebrospinal fluid (CSF) of humans, wild and domestic animals, and ixodid ticks from different geographic areas (North America, Europe, and Asia) (for reviews, see references 2 and 41).

Considerable antigenic heterogeneity has been observed among *B. burgdorferi* isolates from Europe (7–9, 45, 47). Isolates from North America appeared to be more homogeneous except for variants described by Anderson et al., which have been predominantly isolated from *Ixodes dentatus* (3, 4). North American strains usually express two abundant outer surface proteins, OspA and OspB, of 31 and 34 kDa, respectively (9, 11). The Osp proteins of the European isolates are heterogeneous in size, and these strains often (about 40% of the strains) express an additional abundant protein of about 22 kDa which was designated pC (45, 47). Some North American *B. burgdorferi* strains expressing a major protein of about 20 kDa have been described (4, 14, 29, 30).

Very recently, Brown and Lane (16) reported that about one-third of 91 strains isolated from Californian ticks and wild animals express a major ca. 20-kDa protein which could be analogous to the pC protein expressed by European isolates. Despite the lesser abundance of pC than of OspA in cells in culture, pC elicits an intense and early humoral immune response in patients with Lyme borreliosis (46, 47). Antibodies to OspA are only rarely observed and appear, if ever, only late during disease (6, 44, 46, 47). The pC protein was recently cloned by our group (23). Sequence analysis revealed a leader peptide-encoding sequence, indicating processing of pC similar to that of lipoproteins (as previously shown for OspA and OspB, two other outer surface proteins of *B. burgdorferi* [13]).

In this article we give conclusive data that pC is a major outer membrane protein of *B. burgdorferi*, as revealed by immunoelectron microscopy with a monoclonal antibody (MAb). The protein is therefore renamed outer surface proteinC (OspC), by analogy to OspA and OspB. Like OspA (21, 37), OspC appears to be a major candidate for a *Borrelia* vaccine (33).

Consequently, it was considered important with regard to vaccines and diagnostic assays to determine the immunological and genetic heterogeneity of OspC, especially in relation to the OspA serotype (41, 47a) and genomic groups of *B. burgdorferi* (1, 5). In the present study, 45 *B. burgdorferi* isolates that expressed a major OspC and three strains that did not were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblot with polyclonal and monoclonal antibodies against OspC.

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OspC-expressing variants of nonexpressing isolates were also obtained by cultivation of the spirochetes on solid medium. Furthermore, we were able to amplify the *ospC* gene by polymerase chain reaction (PCR) from nonexpressing strains. The *ospC* gene of one of these strains was cloned and expressed in *Escherichia coli*. Thus, it was possible to characterize the OspCs not only of expressing strains but also of nonexpressing strains.

MATERIALS AND METHODS

***Borrelia* strains and culture.** The designations and origins of the *B. burgdorferi* strains investigated in this study are given in Table 1. The strains were analyzed for their OspA serotype by using a panel of eight MAbs as described previously (41, 47a). Culture was performed in modified Kelly medium (MKP) as described before (35). OspC-expressing clones (Table 1) were obtained from a nonexpressing isolate (strain PKa2) by culture on solid agar for 3 weeks (34). Randomly selected colonies were grown on liquid medium (MKP) for further immunochemical analysis. Two other OspC-negative strains (*Ixodes dammini* isolate B31 [ATCC 35210] [19] and German CSF isolate PBr [45]) and *B. hermsii* HS1 serotype C (ATCC 35209) (10) were also included in the study.

Immunoelectron microscopy. Cultures of strain PKo in late log phase were washed three times in phosphate-buffered saline (PBS)-Mg by the method of Barbour et al. (11). The cells from half the sample were fixed with 0.5% glutaraldehyde for 30 min at 22°C and with methanol for 3 min at 4°C; the cells from the other half of the sample were left unfixed. Both specimens were incubated with MAb L22 1F8 or MAb L41 1C11 for 2 h at 22°C. Cells were washed three times in PBS-Mg and reacted for 3 h with gold-labeled anti-mouse immunoglobulin conjugate (auroprobe EM GAM; immunoglobulin G [IgG] plus IgM; particle size, 10 nm; Amersham Buchler GmbH, Braunschweig, Germany). After three washings in PBS-Mg, the spirochetes were fixed with 2.5% glutaraldehyde in 75 mM sodium cacodylate–1 mM $MgCl_2$ (pH 7.2). After extensive rinsing with the same buffer, the cells were postfixed for 30 min in 1% osmium tetroxide in buffer. For en bloc staining, the cells were incubated with 1% uranyl acetate in 20% acetone for 30 min. Dehydration was performed with a graded series of acetone solutions. Cells were finally infiltrated and embedded in Spurr's low-viscosity resin, polymerized, cut with a diamond knife on a Reichert-Jung Ultracut E ultramicrotome, and mounted on uncoated copper grids. The sections were poststained with aqueous lead citrate (3%, pH 13). All pictures were taken with a Siemens Elmiskop 101 electron microscope.

Monoclonal and polyclonal antibodies. MAbs L22 1F8, L22 1F10, and L22 1C3 were produced by using BALB/c mice immunized intraperitoneally with whole washed *B. burgdorferi* cells containing about 100 μg of protein per dose. A mixture of strains PKo and PBi was used for production of MAbs L22 1F8 and L22 1F10. L22 1C3 was obtained from a mouse immunized with a mixture of strain TN and PKa2 clone 9 (OspC-expressing clone). The first immunization was performed with complete Freund's adjuvant, and the second (day 21) and the third (day 42) were done with incomplete Freund's adjuvant. Finally, the mice were boosted three times on days 56, 59, and 60 without Freund's adjuvant but with the amount of protein increased to 300 μg per dose.

Hybridomas were produced by standard protocols. Fusion was carried out 1 day after the last boost with HAT (hypoxanthine-aminopterinthymidine)-sensitive Ag8-653 mouse myeloma cells. Identification and cloning of antibody-producing hybridomas were accomplished as described previously (24, 42).

Polyclonal antibodies against OspC were obtained by immunizing a rabbit three times subcutaneously with 200 μg of purified recombinant OspC derived from strain PKo (produced in *E. coli* as described in references 23 and 43).

SDS-PAGE and Western immunoblots. SDS-PAGE and Western blots were performed as described previously (17). Whole-cell SDS lysates of washed *B. burgdorferi* cells (15 μg of protein for Coomassie blue staining or 7.5 μg of protein for Western blot) were separated by discontinuous SDS-PAGE (15% polyacrylamide gel). Transfer of the proteins to nitrocellulose was accomplished by the semidry technique. Transfer was controlled by staining with Ponceau-S solution (Serva, Heidelberg, Germany). After destaining and blocking, blots were reacted with hybridoma supernatants containing the different MAbs and with the polyclonal antibody against OspC. Antibody binding was detected with horseradish peroxidase conjugates specific for the respective antibodies (Dakopatts, Copenhagen, Denmark).

Cloning of *ospC* genes and DNA sequencing. Total genomic *B. burgdorferi* DNA was extracted from cultures of *B. burgdorferi* B31, PKa2, PWudI, PBr, PBi, TN, and T25, and the *ospC* genes were amplified from genomic DNA by PCR as described for amplification of the *ospA* gene (48). Cloning of the PCR products and sequencing were performed by standard protocols (23, 48).

Nucleotide sequence accession numbers. The *ospC* sequence data for strains PKa2, PWudI, T25, TN, PBr, PBi, and B31 will appear in the EMBL/GenBank/DDBJ nucleotide sequence data libraries under accession numbers X69589, X69590, X69592, X69593, X69594, X69595, and X69596, respectively.

RESULTS

Demonstration of the surface location of OspC by immunoelectron microscopy. Electron microscopic investigation of ultrathin sections of *B. burgdorferi* PKo labeled with MAb L22 1F8 shows that OspC is an outer surface protein (Fig. 1). The high density of the immunogold labeling, which is best seen in tangential section (Fig. 1A, arrowheads), documents that OspC is abundantly expressed on the outer surface and (as shown in Fig. 1B) is associated exclusively with the outer membrane. Some *B. burgdorferi* cells show disruption of the outer membrane. Despite the fact that the periplasmic space was accessible for antibody reaction, the immunogold-labeled antibody did not bind to flagella (Fig. 1A, arrows) or to the inner membrane (Fig. 1B). In contrast, in the control preparation, the flagellum-specific MAb L41 1C11 did not react with the outer membrane and bound only to the flagella (Fig. 1C). The late subculture of strain PKo used in this study did not express OspA. Using an OspA-expressing strain (PGau [47a]), we could also demonstrate that an OspA-specific MAb (L32 2E7 [44]) was bound to the outer surface (data not shown).

Isolation of OspC-expressing clones of *B. burgdorferi* from a non-OspC-expressing strain. When the non-OspC-expressing isolate PKa2 was plated on solid agar, we obtained clones that expressed more or less OspC. Five of six randomly selected colonies subsequently grown in liquid medium and then analyzed by SDS-PAGE expressed a major OspC; OspA was present in all of them (Table 1, Fig. 2). The clones differed in the relative amounts of OspA and OspC. There seemed to be a negative correlation in expression between

TABLE 1. *B. burgdorferi* strains analyzed for OspC phenotype and comparison with OspA serotype

Expt no.	Strain	Source[a]	Geographic area	OspA serotype[b]	OspC MAb pattern	Immune reactivity of OspC			
						Anti-OspC	L22 1F8	L22 1F10	L22 1C3
1a	PKa2, clone 1	CSF	Germany	1	c	+	+	−	+
1b	PKa2, clone 5	CSF	Germany	1	OspC neg.	−	−	−	−
1c	PKa2, clone 9	CSF	Germany	1	c	+	+	−	+
1d	PKa2, clone 13	CSF	Germany	1	c	+	+	−	+
1e	PKa2, clone 17	CSF	Germany	1	c	+	+	−	+
1f	PKa2, clone 21	CSF	Germany	1	c	+	+	−	+
2	CA 9	*I. pacificus*	USA	1	c	+	+	−	+
3	CA 4	*I. pacificus*	USA	1	c	+	+	−	+
4	CA 7	*I. pacificus*	USA	1	b	+	+	−	−
5	CA 8	*I. pacificus*	USA	1	b	+	+	−	−
6	*B. pacificus*	*I. pacificus*	USA	1	b	+	+	−	−
7	T255	*I. ricinus*	Germany	1	d	+	−	−	−
8	PKo	Skin	Germany	2	a	+	+	+	−
9	PJu	Skin	Germany	2	a	+	+	+	−
10	PWudI	Skin	Germany	2	a	+	+	+	−
11	PLj13	Skin	Slovenia	2	a	+	+	+	−
12	PLj6	Skin	Slovenia	2	a	+	+	+	−
13	DK26	Skin	Denmark	2	ND[c]	+	+	+	ND
14	T245	*I. ricinus*	Germany	2	b	+	+	−	−
15	PLud	Skin	Germany	2	b	+	+	−	−
16	PLe	Skin	Germany	2	b	+	+	−	−
17	DK23	Skin	Denmark	2	ND	+	+	−	ND
18	WABTha	Skin	Austria	2	b	+	+	−	−
19	WABPlo	Skin	Austria	2	b	+	+	−	−
20	PLj3	Skin	Slovenia	2	b	+	+	−	−
21	PLj9	Skin	Slovenia	2	b	+	+	−	−
22	PLj11	Skin	Slovenia	2	b	+	+	−	−
23	PBi	CSF	Germany	4	b	+	+	−	−
24	PFei	CSF	Germany	4	b	+	+	−	−
25	PHoe	CSF	Germany	4	b	+	+	−	−
26	DK6	CSF	Denmark	4	b	+	+	−	−
27	PTrob	Skin	Germany	4	b	+	+	−	−
28	PSou	Skin	Austria	5	d	+	−	−	−
29	N34	*I. ricinus*	Germany	6	b	+	+	−	−
30	TN	*I. ricinus*	Germany	6	d	+	−	−	−
31	TIsI	*I. ricinus*	Germany	6	d	+	−	−	−
32	TIsII	*I. ricinus*	Germany	6	d	+	−	−	−
33	B29	*I. ricinus*	Germany	6	d	+	−	−	−
34	DK29	Skin	Denmark	6	ND	+	−	−	ND
35	G25	*I. ricinus*	Sweden	6	d	+	−	−	−
36	TNA7	*I. ricinus*	Switzerland	6	d	+	−	−	−
37	T25	*I. ricinus*	Germany	7	d	+	−	−	−
38	IP90	*I. persulcatus*	Russia	X	d	+	−	−	−
39	19950	*I. dentatus*	USA	X	d	+	−	−	−
40	PBo	CSF	Germany	Neg.	a	+	+	+	−
41	PBoj	CSF	Germany	Neg.	a	+	+	+	−
42	PPop	Skin	Germany	Neg.	a	+	+	+	−
43	FI	*I. ricinus*	Sweden	Neg.	b	+	+	−	−
44	20047	*I. ricinus*	France	Neg.	b	+	+	−	−
45	PLj2	Skin	Slovenia	Neg.	b	+	+	−	−
46	PLj10	Skin	Slovenia	Neg.	b	+	+	−	−

[a] Site of isolation from humans (skin or CSF) or tick species.
[b] Neg., no OspA produced.
[c] ND, not determined.

OspA and OspC. We were not able to detect any antigenic differences among the OspA and OspC proteins of the respective clones by reaction with MAbs against OspC (L22 1F8 and L22 1C3) (Table 1 and Fig. 2) and MAbs against OspA (H5332 and H3TS) (data not shown).

Partial sequencing of the *ospC* genes from different strains reveals OspC polymorphisms. The *ospC* genes were amplified from the DNAs of strains B31, PKa2, PWudI, PBr, PBi, TN, and T25, and the amplification products were cloned in pUC8. The plasmids were designated pUC8-B31/ospCL+, pUC8-PKa2/ospCL+, pUC8-PWudI/ospCL+, pUC8-PBr/ospCL+, pUC8-PBi/ospCL+, pUC8-TN/ospCL+, and pUC8-T25/ospCL+, respectively. In the case of B31, an *ospC* amplification product lacking coding sequence for the leader peptide was also cloned in pUC8 (pUC8-B31/ospCL−). Three of the strains investigated (B31, PKa2, and PBr) do not express OspC. Despite the fact that *ospC* is not expressed in strain B31 (Fig. 3b, lanes 3), we were able to express the *ospC* gene in *E. coli* (Fig. 3a). Deletion of the leader sequence prior to cloning apparently led to particularly high expression of the B31-derived OspC protein in *E. coli* (Fig. 3a). This had been observed for the cloned *ospC* gene of strain PKo as well. The full sequences of the *ospC* genes of strains B31 and PBi will be published elsewhere (26a).

The *ospC* genes had conserved 3′ and 5′ ends and were more variable in the internal part of the sequence. Sequence comparison of the 240 3′-terminal nucleotides of the *ospC* genes from the eight strains revealed considerable molecular variability of *ospC* (Fig. 4 and Table 2). This analysis showed that with the exception of the two *B. garinii* strains PBr and TN, which were OspA serotypes 3 and 6, respectively, strains belonging to different OspA serotypes have significantly different *ospC* genes. The degree of nucleotide identity ranged from 63.3 to 82.1% for all three genospecies of *B. burgdorferi*. A comparable degree of sequence differences was also observed among the *B. garinii* strains. The *ospC* sequences derived from *B. burgdorferi* group VS461 strains PKo and PWudI, which are both OspA serotype 2, were identical. The same was true for the *ospC* sequences of B31 and PKa2, which are both *B. burgdorferi* sensu stricto and OspA serotype 1 strains.

Immunochemical analysis of OspC. We used one polyclonal antibody and three MAbs for immunological analysis of OspC (Table 1). SDS-PAGE and Western blot analyses of representative strains of defined OspA serotypes are shown in Fig. 5, 6, and 7. Major proteins of about 20 kDa reacted with the polyclonal antibody against OspC. This was an indication that all these proteins are variants of the same borrelial protein. One interesting finding was the strong reactivity of the major ca. 20-kDa protein VMP_c of *B. hermsii* HS1 with the polyclonal OspC-specific antibody (Fig. 3c).

With the three MAbs, four different reactivity patterns were observed among the *B. burgdorferi* strains tested, which are designated a, b, c, and d in Table 1. All representatives of OspA serotype 2 strains were reactive with MAb L22 1F8; some were also reactive with L22 1F10 (pattern a) (see also Fig. 5). The latter antibody did not react with any of the other OspA serotypes, with the exception of some strains that do not express OspA and are thus of unknown OspA serotype. OspC proteins from pattern a strains have identical sizes, whereas pattern b strains of OspA serotype 2 may vary in the sizes of their OspCs. Three immunological variants of OspC (patterns b, c, and d) were observed among OspA serotype 1 strains and their OspC-expressing variants. Two immunological variants (patterns b and d) were observed among OspA serotype 6 strains (see also Fig. 5 and 6). The immunological OspC variants of a given OspA serotype differed in size; examples are shown in Fig. 5 and 6. We found that strains that did not express OspA also varied in the epitopes of their OspC proteins (Fig. 7). In contrast, five strains of OspA serotype 4 were identical in their OspC MAb patterns (Fig. 6). For OspA serotypes 5 and 7, only one OspC-expressing strain was available. The OspCs of these two strains could not be distinguished by MAbs, either from each other or from the OspCs of the majority of OspA serotype 6 strains (Fig. 6). The OspA serotype 3 strain was negative for OspC but, as shown above by PCR amplification, nevertheless contained an *ospC* gene.

DISCUSSION

This study has shown that OspC, a 22-kDa protein previously designated pC, is a major outer surface protein of *B. burgdorferi*. OspC was identified by reactivity with an OspC-specific polyclonal antibody, which was raised by immunization with recombinant OspC, and with OspC-specific MAbs. The location of OspC at the outer membrane was demonstrated by immunogold labeling of *B. burgdorferi* PKo with the OspC-specific MAb L22 1F8. OspC can therefore be regarded, with OspA and OspB, as the third major outer surface protein of *B. burgdorferi*.

Several authors have reported on different proteins of *B. burgdorferi* of about 20 kDa in size (14, 15, 17, 18, 29, 30, 31, 39). Surface labeling with ^{125}I or hydroxysuccinimide-biotin (31), extraction with Triton X-114 (15), and immunoelectron microscopy studies have suggested that some are outer membrane associated. Another protein of this approximate size, p22-A, appears to be located in the periplasmic space (39). The 25-kDa protein described by Bissett et al. (14) and Kurashige et al. (29) in a Californian strain also varied in expression among different subcultures of an isolate in the same manner as OspC (45, 47) and therefore might be identical to OspC. However, Brown and Lane showed that Californian strains may express more than one major ca. 20-kDa protein (16). Other minor proteins of about 20 kDa, some of which are especially reactive with immune sera against unrelated bacteria, can be mistaken for OspC in diagnostic Western blots (17). This is an indication that, for proper serodiagnosis, further investigations are necessary to delineate the different forms of 20-kDa-range proteins of *B. burgdorferi*.

Molecular analysis of OspC revealed that OspC (23) is different from OspA and OspB (13, 27, 48). However, the fact that different *B. burgdorferi* strains, as well as different clones derived from one strain, express small amounts of OspC when they express large amounts of OspA and vice versa suggests an interdependence in regulation of the expression of the two proteins. Identification of the *ospC* gene in the DNA of nonexpressing strains confirms that the *ospC* gene may also be present in OspC-negative strains. Although there may be a mechanism leading to suppression, regulation of OspC expression is not understood at this time. However, the finding of Šadžiene et al. (36a) that the *ospC* gene is located on a 27-kb circular plasmid and that OspC was expressed only in mutants lacking the 16-kb linear plasmid may be important in this respect. Šadžiene et al. (36a) reported the presence of OspC in a B31 mutant lacking the 49-kb plasmid. One interesting aspect of the immunological characterization of OspC was that the plasmidless mutant of Šadžiene et al., the recombinant OspC derived from strain

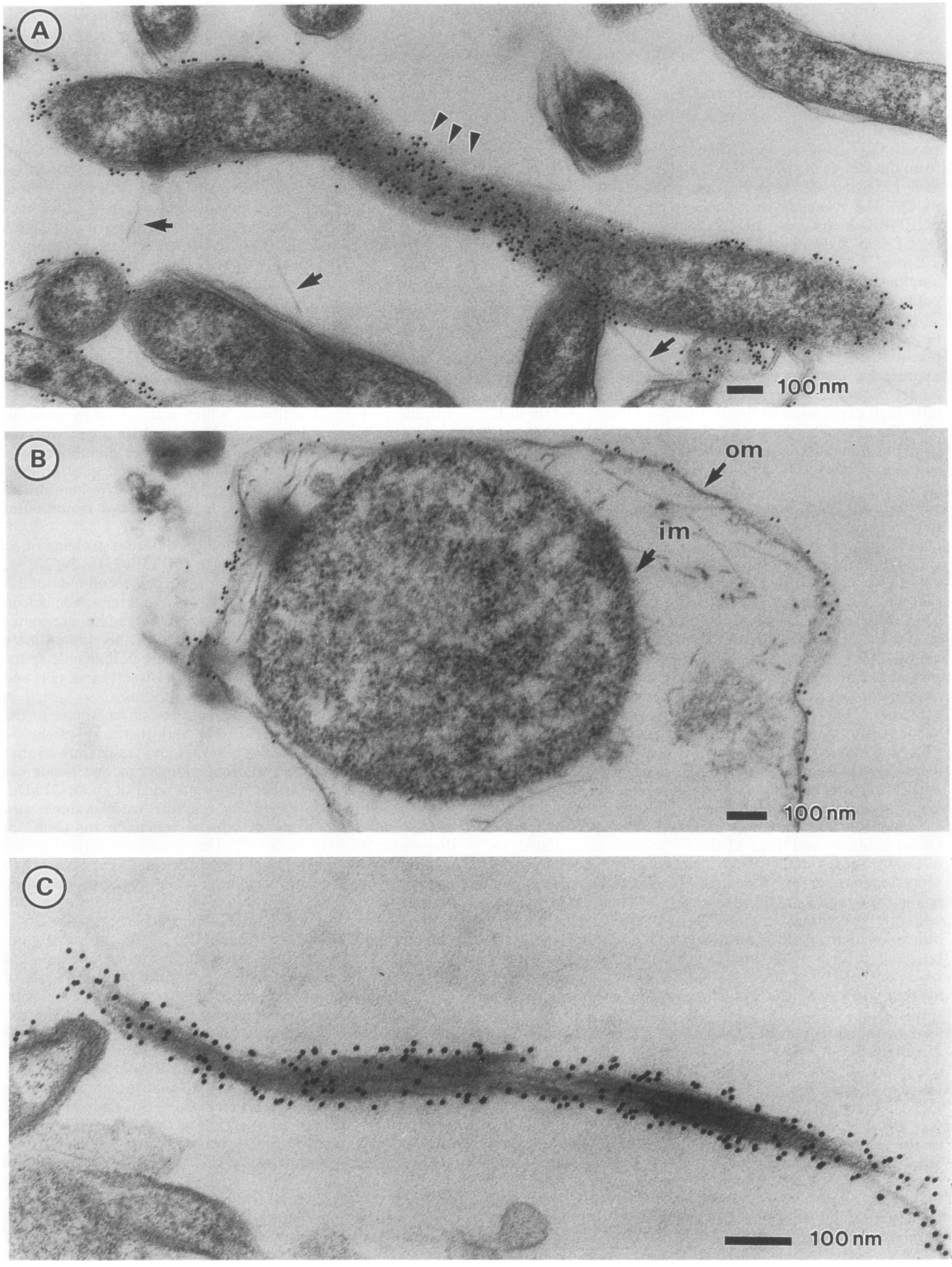
A
100 nm
B
om
im
100 nm
C
100 nm

FIG. 1. Electron micrographs of ultrathin sections of *B. burgdorferi* PKo labeled with MAbs L22 1F8 and L41 1C11, detected with 10-nm gold–anti-mouse IgG conjugate. OspC is a surface-associated protein; high-density labeling is best seen in tangential section (A, arrowheads). (B) *B. burgdorferi* cell with disrupted outer membrane is shown. The immunogold is bound exclusively to the outer membrane (om) and not to flagella or to the inner membrane (im). The flagellum-specific MAb L41 1C11 was not reactive with the outer membrane and bound exclusively to flagella (C).

B31, and OspC-positive clones derived from strain PKa2 had the same OspC MAb reactivity pattern. This is in agreement with the partial sequence analysis of the *ospC* gene and shows that the OspCs of B31 and PKa2 are immunologically closely related.

Another interesting finding was that the VMP_c of *B. hermsii* HS1 serotype C was reactive with the polyclonal antibody against recombinant OspC. Thus, the OspC of *B. burgdorferi* may be homologous to the VMP_c of *B. hermsii*. However, molecular analysis of VMP_c is necessary to prove this hypothesis. As described by Barbour et al. (10) and Barstad et al. (12), the relapsing-fever strain *B. hermsii* is characterized by alternating expression of outer surface proteins (VMPs). Therefore, it appears promising to investigate whether *B. burgdorferi* uses strategies of antigenic variation similar to those of *B. hermsii*.

OspC is, besides the flagellin protein, the immunodominant antigen of the early immune response in patients with Lyme borreliosis (25, 28, 46, 47). This was first recognized in European patients. Recently, however, Dressler et al. (20) also detected an OspC-specific immune response in American patients with erythema migrans by using an OspC-expressing variant of an American tick isolate as the antigen. This was confirmed in our laboratory by using recombinant OspC as the antigen (unpublished results).

Whereas patients with Lyme borreliosis show a good immune response against OspC, the immune response against OspA is rather weak. In contrast to this finding is the observation that more than 90% of *B. burgdorferi* culture isolates express OspA but only 40% express OspC (6, 46, 47). This may indicate that, in vivo, OspA expression is low (32) and OspC expression is high. Evidence of this is the finding of Schwan et al. (38) that during infection of mice with a *B. burgdorferi* strain that does not express a major ca. 20-kDa protein in vitro, the immune response shifted from a major OspA-specific response to a response against a 24-kDa protein. Other in vitro and in vivo studies have also confirmed variation of OspA versus OspC expression in *B. burgdorferi*. Such variations occurred among the progeny of individual isolates (45, 47).

In the present study and in a previous study (34), we have shown that clones obtained from CSF isolates on solid agar

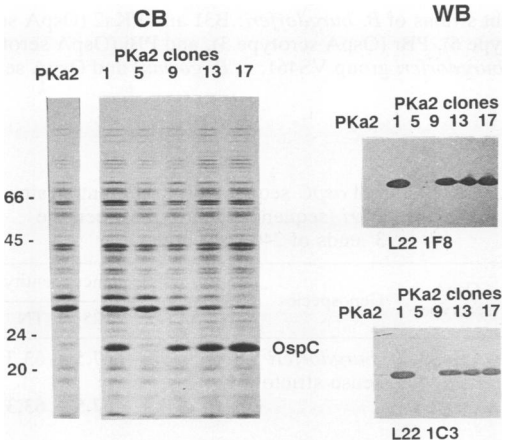

FIG. 2. Immunochemical characterization of five different clones derived from strain PKa2. SDS-PAGE and Coomassie blue staining (CB) shows variable expression of OspA and OspB and of OspC among clones 1, 5, 9, 13, and 17. OspC is not expressed by strain PKa2. The reactivity patterns of OspCs with MAbs (WB) are identical among OspC-expressing clones. Size standards were bovine serum albumin (66 kDa), OVA (45 kDa), trypsinogen (24 kDa), and trypsin (20 kDa).

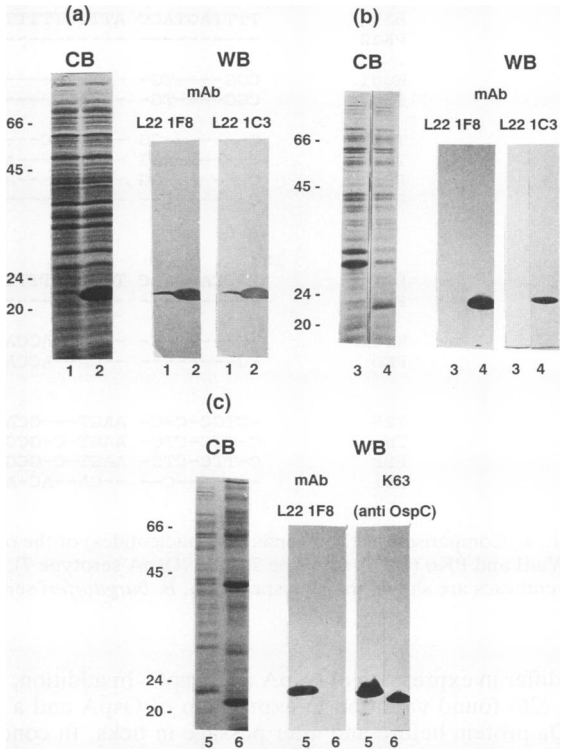

FIG. 3. (a) Immunochemical characterization of recombinant OspC derived from the OspC-negative strain B31 (see panel b, lanes 3). Expression of OspC is low in the recombinant *E. coli* containing *ospC* with the leader peptide-encoding sequence (CB, lane 1) and high in the recombinant *E. coli* containing *ospC* lacking the leader peptide-encoding sequence (CB, lane 2). OspC of both recombinants is reactive with MAbs L22 1F8 and L22 1C3 in the Western blot (WB). (b) Strain B31 (lanes 3) does not express OspC, as shown by SDS-PAGE and Coomassie blue staining (CB) and negative reaction with OspC-specific MAbs L22 1F8 and L22 1C3 in the Western blot (WB). The OspC-expressing variant PKa2 clone 17 (lanes 4) served as a control. (c) Immunochemical characterization of *B. hermsii* HS1 serotype C (lanes 6); strain PKa clone 17 (lanes 5) served as a control. The major ca. 20-kDa protein VMP_c of strain HS1 serotype C (CB) is reactive with polyclonal antibodies against recombinant OspC but not reactive with the OspC-specific MAb L22 1F8. Size standards are as in Fig. 2.

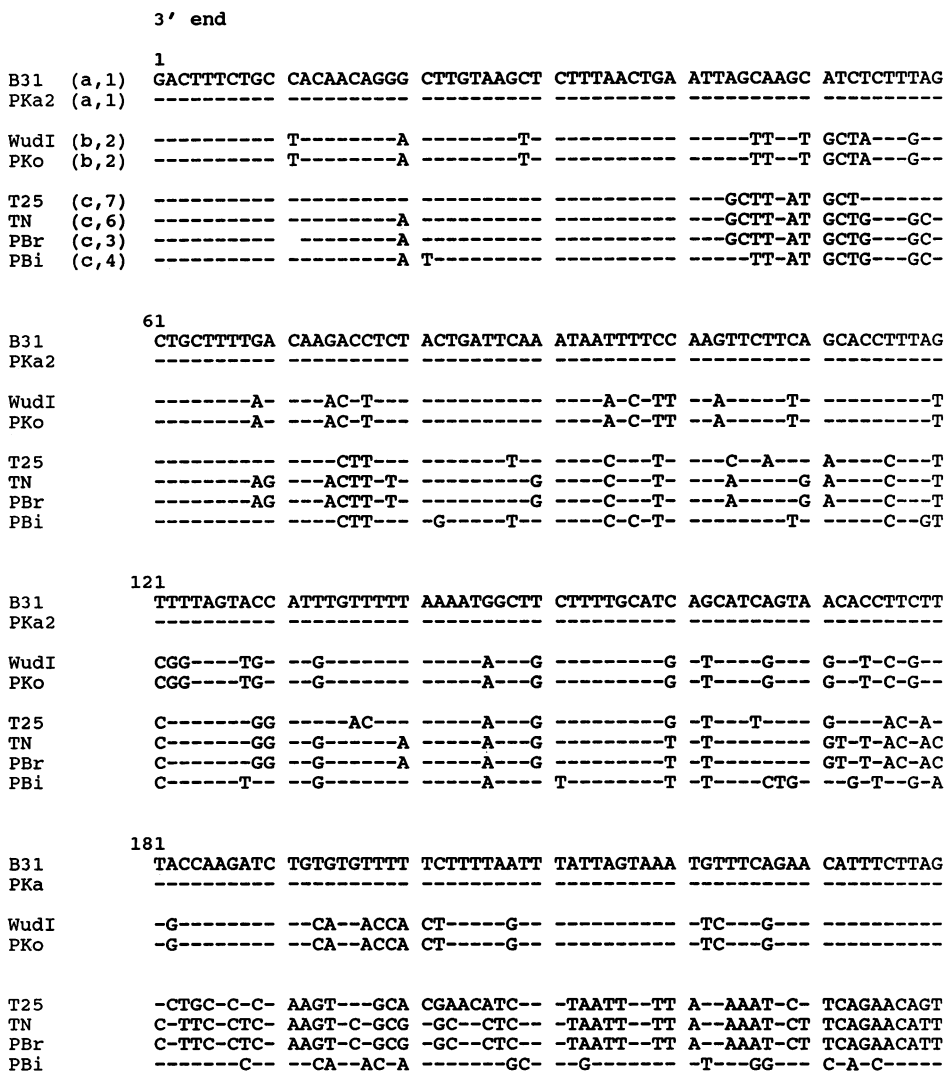

FIG. 4. Comparison of the 3′ ends (240 nucleotides) of the *ospC* genes of eight strains of *B. burgdorferi*: B31 and PKa2 (OspA serotype 1), PWudI and PKo (OspA serotype 2), T25 (OspA serotype 7), TN (OspA serotype 6), PBr (OspA serotype 3), and PBi (OspA serotype 4). In parentheses are shown the genospecies (a, *B. burgdorferi* sensu stricto; b, *B. burgdorferi* group VS461; c, *B. garinii*) and OspA serotype.

may differ in expression of OspA and OspC. In addition, Hu et al. (26) found variation in expression of OspA and a ca. 20-kDa protein before and after passage in ticks. In conclusion, these observations suggest that *B. burgdorferi* has the potential to vary expression of both OspA and OspC. This phenomenon may account for its evasion of the immune response in the host and may be one reason for persistence of infection.

Immunological analysis of OspC-expressing variants to date has not revealed true antigenic variation of this protein, although the number of variants examined in this study was small. From the present data, it appears that variation in the OspC proteins themselves is probably less frequent than variation of expression. Nevertheless, during infections, the immune response of the host may allow selection of antibody-resistent escape mutants. Recently, Šadžiene et al. (36) described various antibody-induced *B. burgdorferi* mutants that represent different classes of OspA and OspB variation. In view of these findings, it would be important to investigate

TABLE 2. Partial *ospC* sequences of different strains of *B. burgdorferi* (sequence identity between the 3′ ends of 240 nucleotides)

Strain	OspA serotype	Genospecies	% Sequence identity				
			B31	PKo	T25	TN	PBi
B31	1	*B. burgdorferi* sensu stricto	100	79.2	67.5	63.3	77.9
PKa2			100	79.2	67.5	63.3	77.9
PKo	2	*B. burgdorferi* group VS461		100	67.1	66.3	82.1
PWudI				100	67.1	66.3	82.1
T25	7	*B. garinii*			100	85.4	65.4
TN	6	*B. garinii*				100	66.3
PBr	6	*B. garinii*				100	66.3
PBi	4	*B. garinii*					100

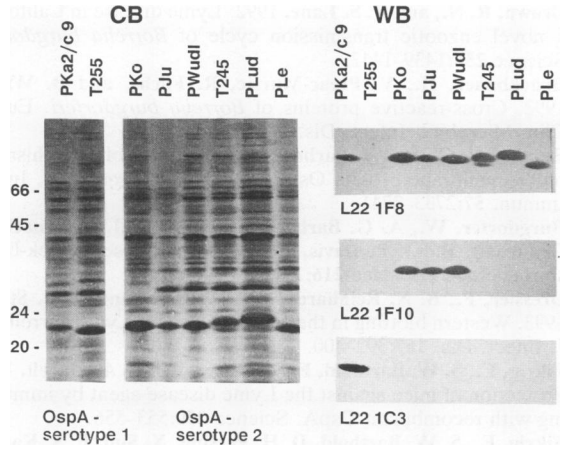

FIG. 5. Immunochemical characterization of *B. burgdorferi* strains of OspA serotypes 1 and 2. SDS-PAGE and Coomassie blue staining (CB) show that the OspCs of OspA serotype 1 as well as those of OspA serotype 2 strains vary in size. The Western blot (WB) with MAbs L22 1F8, L22 1F10, and L22 1C3 shows different reactivity patterns among OspA serotype 1 and 2 strains but also among strains of the same OspA serotype. Size standards are as in Fig. 2. PKa2/c9, PKa2 clone 9.

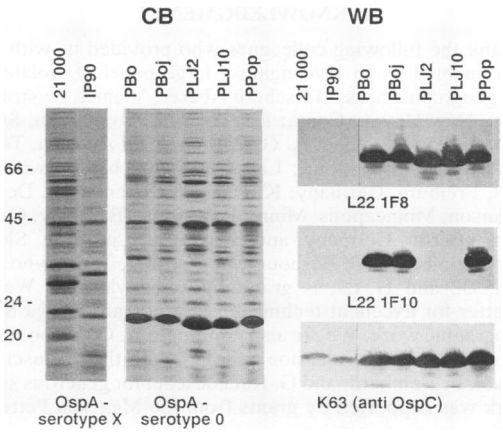

FIG. 7. Immunochemical characterization of *B. burgdorferi* strains of OspA serotype X and OspA-negative strains (OspA serotype 0). SDS-PAGE and Coomassie blue staining (CB) show that the OspCs of OspA serotype X as well as those of OspA-negative strains vary in size. The Western blot (WB) with MAbs L22 1F8 and L22 1F10 shows different reactivity patterns among OspA-negative strains. The OspC of OspA serotype X strains not reacting with the OspC-specific MAb is reactive with a polyclonal antibody against recombinant OspC (K63). Size standards are as in Fig. 2.

whether escape mutants with antigenic variation in OspC may also be selected with antibodies.

Previous reports have shown that OspA and OspB are rather polymorphic proteins. This study demonstrated a similar degree of heterogeneity for OspC by genetic analysis. It appears that strains belonging to different genospecies of *B. burgdorferi* sensu lato (B31, PKo, and PBi) (1, 5) do not show higher genetic variability than certain strains of different OspA serotypes within the *B. garinii* group (T25, TN, and PBi). This, however, needs to be confirmed by complete sequencing of the respective *ospC* genes. Of the eight strains investigated, two pairs of strains with the same OspA serotype were identical in the *ospC* partial sequence investigated. These pairs of strains reacted with specific MAbs. It is tempting to speculate that MAbs L22 1F10 and L22 1C3 recognize serotype-specific epitopes.

Whereas OspA serotype designation is correlated with certain genospecies (47a), the relationship between a defined OspC type and a certain genospecies is at this point less clear. Immunological analysis has shown that strains of the same OspA serotype may have different OspC phenotypes. Molecular analysis of the *ospC* genes and OspC proteins from such strains needs to be performed. On the other hand, more MAbs are needed to distinguish between OspCs of apparently different sequence. At present, we can assume that OspC is probably at least as heterogeneous as OspA.

Our findings also have important implications for the development of diagnostic tests, such as serodiagnostic antigens, DNA probes and primers for PCR, and vaccines. Inasmuch as OspC is a major target of the early immune response in patients with Lyme borreliosis, the use of recombinant OspCs derived from different strains as the antigen could improve early detection of *B. burgdorferi*-specific antibodies.

At present, OspA is regarded as the most promising candidate for a *Borrelia* vaccine (21, 37). However, OspA-negative strains constitute about 10% of European and Californian culture isolates (16, 45). All OspA-negative strains express a major OspC (45). In addition, *B. burgdorferi* has the potential to alternate expression of OspA and OspC. We have recently shown in the gerbil model that, in addition to OspA, OspC is a candidate for an effective vaccine (33). The group of Fikrig et al. found that mice were not cross-protected against challenge with two different strains when the animals were vaccinated with recombinant OspA derived from the two strains (22). Therefore, for development of OspC vaccines, the heterogeneity of the protein needs to be considered. The findings of this study may facilitate a rational design for further studies on the development of diagnostic reagents and vaccines.

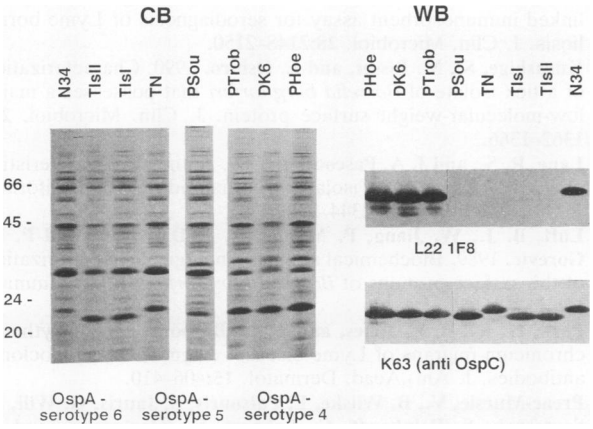

FIG. 6. Immunochemical characterization of *B. burgdorferi* strains of OspA serotypes 4, 5, and 6. SDS-PAGE and Coomassie blue staining (CB) show that the OspCs of OspA serotype 6 but not the OspCs of OspA serotype 4 strains vary in size. The Western blot (WB) with MAb L22 1F8 shows different reactivity patterns among OspA serotype 6 strains but not among OspA serotype 4 strains. OspC not reacting with the OspC-specific MAb is reactive with a polyclonal antibody against recombinant OspC (K63). Size standards are as in Fig. 2.

ACKNOWLEDGMENTS

We thank the following colleagues who provided us with strains and thus enabled us to investigate a large panel of isolates from different geographic areas: Elisabeth Aberer, Vienna, Austria; J. F. Anderson, New Haven, Conn.; Eva Asbrink, Stockholm, Sweden; G. Baranton, Paris, France; A. G. Barbour, San Antonio, Tex.; W. Burgdorfer, Hamilton, Mont.; Lise Gern, Neuchatel, Switzerland; U. Göbel, Freiburg, Germany; K. Hansen, Copenhagen, Denmark; R. C. Johnson, Minneapolis, Minn.; R. S. Lane, Berkeley, Calif.; A. Schönberg, Berlin, Germany; and Eva Rusic, Ljubliana, Slovenia. The cooperation of A. G. Barbour (San Antonio, Tex.), who sent us MAbs H5332 and H3TS, is gratefully acknowledged. We thank Birgit Vetter for excellent technical assistance and Helga Schmidt for photographic work. We are also grateful to A. G. Barbour and H. Nitschko for valuable discussion and help with the manuscript.

We thank F. Deinhardt and G. Ruckdeschel for generous support. This work was supported by grants from the Max von Pettenkofer Institute.

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