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Molecular Analysis of Genes Encoding Outer Surface Protein C (OspC) of *Borrelia burgdorferi* Sensu Lato: Relationship to *ospA* Genotype and Evidence of Lateral Gene Exchange of *ospC*

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It has been shown by analysis with monoclonal and polyclonal antibodies that outer surface protein C (OspC) of *Borrelia burgdorferi* sensu lato is highly heterogeneous. To determine if the heterogeneity has a genetic basis, the genes of 18 different *B. burgdorferi* sensu lato strains have been amplified by PCR, cloned, and sequenced. The *ospC* genes could be amplified from all strains tested, even from two strains which did not express OspC in detectable amounts. Among the 18 strains, 16 significantly different types of *ospC* sequences have been found. The sequence identities of the deduced amino acid sequences of different *ospC* genotypes range between 62 and 80% (determined without the leader peptide). The sequences correspond to one of the 13 OspC types distinguishable by analysis with monoclonal antibodies (B. Wilske, S. Jauris-Heipke, R. Lobentanzer, I. Pradel, V. Preac-Mursic, D. Roessler, E. Soutschek, and R. C. Johnson, J. Clin. Microbiol. 33:103–109, 1995) or represent additional types. Two completely new types were found, and OspC type 8 (which was found in *Borrelia afzelii* and *Borrelia garinii*) could be divided into two groups with different sequences but the same antibody pattern. Thus, strains belonging to different species or OspA serotypes were always significantly different in their *ospC* sequences. This was also confirmed by *ospA* sequence analysis. Interestingly, some strains of the same OspA serotype or genotype were very heterogeneous with respect to OspC, while others had nearly identical OspC proteins. Such groups of strains were found among *B. burgdorferi* sensu stricto, *B. afzelii*, and *B. garinii* strains. Cluster analysis of 5′-terminal and 3′-terminal stretches of *ospC* suggested recent intragenic recombination events in the *ospC* gene of at least one *B. afzelii* strain. In addition, other recombination events between ancestors of strains belonging to the same or different species were evidenced by this type of analysis.

Borrelia burgdorferi sensu lato is the causative agent of Lyme borreliosis, a disease transmitted throughout the world by ixodid ticks (8). Since the first description and cultivation of *B. burgdorferi* sensu lato (5, 8), many strains have been isolated from ticks, animals, and patient specimens (skin, cerebrospinal fluid [CSF], and blood). Analysis of some of these strains revealed significant differences among them. Thus, *B. burgdorferi* sensu lato has been divided into three species (3, 10): *B. burgdorferi* sensu stricto, *Borrelia afzelii*, and *Borrelia garinii*. The heterogeneity of several proteins and the respective genes has been analyzed. The greatest variability has been found between the outer surface proteins (Osp proteins) of different strains. The OspA (6, 7, 23, 53, 55, 60), OspC (22, 47, 52, 54), and OspD (28) proteins and genes have been studied in this respect. The genes encoding the Osp proteins are located on plasmids. OspA and OspB are encoded in one operon on a 49-kb linear plasmid (4, 20), the *ospC* gene is located on a 27-kb circular plasmid (27, 41), and the *ospD* gene is located on a 38-kb linear plasmid (32). Immunological analysis of OspA with monoclonal antibodies (MAbs) revealed at least seven different serotypes (53). The same analysis has been carried out for OspC (52), resulting in 13 different types. Thus, antigenic heterogeneity is much more pronounced in OspC than in OspA.

OspC is an immunodominant protein of the early humoral immune response in humans (1, 14, 56, 57). First studies using a recombinant OspC protein for serodiagnosis have been carried out and showed that OspC is a specific and sensitive marker for the early stage of Lyme borreliosis (33, 50, 51). However, cross-reactivity to relapsing-fever borreliae must be considered, since relapsing-fever borreliae possess OspC homologs recognized by polyclonal antibodies and MAbs (11, 29, 52, 54). Moreover, it has been shown that vaccination with recombinant OspC is effective in protecting gerbils against *B. burgdorferi* infection (35). This indicates that the analysis of OspC is important for serodiagnosis and vaccine development.

The aim of this study was to determine the diversity of OspC on a molecular level. Therefore, the genes encoding OspC of 18 *B. burgdorferi* sensu lato strains have been cloned. At least one of each of the 13 different immunologically defined OspC types was chosen. The complete coding sequences of these clones have been determined, and their gene products have been analyzed in Western blots (immunoblots) using MAbs. In addition, *ospC* sequences were compared with the respective *ospA* sequences determined in another study (49a). Our studies revealed a very high degree of sequence heterogeneity for *ospC*. The degree of sequence variability was considerably higher than for *ospA*. Tree construction for certain stretches of the *ospC* genes indicated recent intragenic recombination events.

MATERIALS AND METHODS

Bacterial strains and plasmids. *B. burgdorferi* strains listed in Table 1 were grown in MKP medium at 37°C for 4 or 5 days (36). For cloning and expression procedures, *Escherichia coli* JM109 (59) and plasmids pUC8 and pUC18 (49) were used. *E. coli* was grown at 37°C in Luria-Bertani (LB) medium with or without the addition of 50 μg of ampicillin per ml as described by Sambrook et al. (42).

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TABLE 1. *B. burgdorferi* sensu lato strains used in this study

Strain	Biological origin	Geographic origin[a]	Species	OspA serotype	OspA sequence	OspC type	OspC sequence	nt	aa
B31	Tick	USA	*B. burgdorferi* sensu stricto	1	1	NE[b]	a	633	210
PKa	CSF	G	*B. burgdorferi* sensu stricto	1	1	1	a	633	210
PBre	Skin	G	*B. burgdorferi* sensu stricto	1	1	NE	b	636	211
B. pac	Tick	USA	*B. burgdorferi* sensu stricto	1	ND[c]	2	c	630	209
297	CSF	USA	*B. burgdorferi* sensu stricto	1	1	3	d	636	211
T255	Tick	G	*B. burgdorferi* sensu stricto	1	1	4	e	633	210
PKo	Skin	G	*B. afzelii*	2	2	5	f	639	212
PWud I	Skin	G	*B. afzelii*	2	2	5	f	639	212
PBo	CSF	G	*B. afzelii*	NE	2	5	f	639	212
PLud	Skin	G	*B. afzelii*	2	2	6	g	645	214
PLe	Skin	G	*B. afzelii*	2	2	7	h	639	211
PLj7	Skin	S1	*B. afzelii*	2	2	8	i	639	212
PBr	CSF	G	*B. garinii*	3	3	NE	k	636	211
PTrob	Skin	G	*B. garinii*	4	4	8	l	624	207
PBi	CSF	G	*B. garinii*	4	4	8	l	624	207
PHei	CSF	G	*B. garinii*	5	5	NE	m	633	210
WABSou	Skin	A	*B. garinii*	5	5	9	m	633	210
TN	Tick	G	*B. garinii*	6	6	10	n	636	211
N34	Tick	G	*B. garinii*	6	ND	11	o	630	209
TIs1	Tick	G	*B. garinii*	6	6	12	p	624	207
T25	Tick	G	*B. garinii*	7	6	13	q	636	211

[a] USA, United States; G, Germany; Sl, Slovenia; A, Austria.
[b] NE, no expression.
[c] ND, not done.

DNA techniques. *B. burgdorferi* strains were grown to a cell density of 10^9/ml. Cultures were harvested by centrifugation. Whole DNA was isolated without separating plasmid and chromosomal DNAs as described by Langenberg et al. (25) or on a small scale as described by Luft et al. (26).

Preparation of plasmid DNA from *E. coli* JM109, restriction enzyme digestion, DNA ligation, transformation of competent cells, agarose gel electrophoresis, oligonucleotide synthesis, and PCR were performed by standard techniques.

The recombinant plasmids were sequenced partly by the dideoxynucleotide chain termination method (43) using T7 polymerase and radioactively labelled dATP or by the cycle sequencing method using dye terminators on an ABI 373 DNA sequencer as suggested by the manufacturer (Applied Biosystems). The nucleotide sequences of at least one clone with the leader sequence and one without the leader sequence were determined in both directions. Sometimes the PCR product of the gene was sequenced directly. Standard primers for M13 derivatives or internal primers were used.

For analysis of sequences, the Genetics Computer Group software package, version 7.1 (13), and the PROTPARS and DNAPARS programs of the PHYLIP package (16) were used.

The accession numbers of *ospC* sequences from the indicated strains which were published previously or which are available through the EBI/GenBank database (18, 19, 22, 30, 34, 46, 47) are as follows: B31, X69596; PKo, X62162; PBi, X69595; 297, U08284; DK1, X73627; DK6, X73626; DK7, X73625; DK26, X73624; DK27, X73623; 2591, U01892; CA-11.2A, L25413; N40, U04240; DN127c19-2 (DN127), U04280; HB19, U04281; and 25015, U04282.

Protein techniques. Overnight cultures of *E. coli* clones containing the desired plasmid were diluted 1:100 in LB medium containing 50 μg of ampicillin per ml. After growth for 3 h, isopropyl-β-D-thiogalactoside (IPTG) was added to a final concentration of 1 mM and the cultures were incubated for another 2 h at 37°C. Cell pellets from 1.5 ml of culture were lysed in 150 μl of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) loading buffer (24) and boiled for 5 min, and 10 μl of the lysate was separated by SDS-PAGE. After separation, the proteins were either evaluated after being stained with Coomassie blue or transferred onto nitrocellulose as described elsewhere (58).

For detection of OspC of *B. burgdorferi* and recombinantly expressed OspC, the L22 panel of MAbs described by Wilske et al. (52) was used.

Nucleotide sequence accession numbers. The nucleotide sequences of the *ospC* genes of the following strains have been assigned the indicated EBI accession numbers (partial sequences of these genes are also referred to by Wilske et al. [52, 54]): PKa, X69589; PWud I, X69590; T25, X69592; TN, X69593; PBr, X69594; PLe, X80255; PBo, X81521; PBre, X81522; PLj7, X81523; T255, X81524; TIs1, X81525; WABSou, X81526; PLud, X83552; PHei, X83553; PTrob, X83554; *Borrelia pacificus* (B. pac.), X83555; and N34, X83556.

RESULTS

Cloning and sequencing. The cloning and sequencing of the *ospC* genes of strains B31, PKo, and PBi have been described previously (18, 22). The *ospC* genes of the other 18 strains listed in Table 1 have been amplified by PCR using two different 5′ primers in combination with one 3′ primer. This was done to obtain the coding sequences for the genes with and without leader sequences. The amplification products were cloned in pUC8 or pUC18 via *Bam*HI and *Pst*I sites in the PCR primers. The coding regions of all *ospC* genes were between 624 and 645 nucleotides (nt) long. There is no strict correlation between the lengths of the genes and the species they are derived from, but the longest *ospC* gene is found in a *B. afzelii* strain (PLud) and the shortest ones are derived from *B. garinii* strains (PTrob, PBi, and TIs1).

Analysis of *ospC* sequences and comparison with the immunologically defined OspC types. The identities of the different OspC sequences determined by analysis of the deduced amino acid sequences without leader peptides are shown in Table 2. The 13 OspC types previously described (52) could be confirmed on a molecular basis. OspC type 8, which has been found in *B. afzelii* PLj7 and in *B. garinii* PBi, could be divided into two genotypes with significantly different sequences (76% sequence identity [Table 2]). Two completely new genotypes were found in strains PBre and PBr, which do not express OspC in culture. Thus, 16 different *ospC* genotypes having amino acid sequence identities between 62 and 80% have been identified. Five different *ospC* genotypes were found among the six *B. burgdorferi* sensu stricto strains, four were found among the six *B. afzelii* strains, and seven were found among the nine *B. garinii* strains. Four *ospC* types (sequence identities of >98%) were detected several times.

An alignment of the deduced amino acid sequences (Fig. 1) shows a variable region from amino acids 75 to 192 (aa 75 to

TABLE 2. Amino acid identities of the 16 different OspC types distinguishable by sequence analysis[a]

Strain	% Identity with the indicated strain (OspA type, OspC sequence)															
	PKa (1, a)	PBre (1, b)	B. pac (1, c)	297 (1, d)	T255 (1, e)	PKo (2, f)	PLud (2, g)	PLe (2, h)	PLj7 (2, i)	PBr (3, k)	PBi (4, l)	WABsou (5, m)	TN (6, n)	N34 (6, o)	TIs1 (6, p)	T25 (7, q)
PKa (B31)	100	80	74	76	72	72	75	75	74	67	68	72	73	71	69	73
PBre		100	77	77	71	69	72	74	73	70	76	68	69	68	71	70
B. pac.			100	75	74	78	72	71	76	71	69	71	67	74	70	71
297				100	68	68	70	72	74	65	66	64	63	65	66	67
T255					100	71	66	70	67	63	67	68	62	67	69	65
PKo (PWud I, PBo)						100	78	80	80	73	73	75	74	78	70	76
PLud							100	76	79	78	73	75	76	80	69	76
PLe								100	80	75	77	72	69	76	74	75
PLj7									100	79	76	72	75	78	72	78
PBr										100	73	69	75	76	72	80
PBi (PTrob)											100	73	70	74	76	75
WABSou (PHei)												100	76	78	77	73
TN													100	74	68	71
N34														100	73	77
TIs1															100	76
T25																100

[a] Identities have been calculated without the leader peptide. Strains with identical OspC proteins (>98%) are listed in parentheses in the leftmost column. OspC proteins of strains belonging to one serotype are boxed.

192), with up to 7 different aa at one position. The more-conserved N- and C-terminal regions have maximally 4 aa in a certain position. Two hypervariable regions are located between aa 83 and 95 and between aa 143 and 162. In these portions of the protein, all 16 sequences are completely different; not a single pair of identical sequences could be found.

Expression and immunological characterization of recombinant proteins. The *ospC* genes were expressed in *E. coli*. Cells harboring the recombinant plasmids with the genes without leader peptide were induced with IPTG. Even if the pUC plasmids are not designed for a high level of expression of the introduced DNA fragment, the amount of gene product is sufficient for Western blot analysis with MAbs. We used the recombinants of the two nonexpressing strains, which represent an additional *ospC* genotype, and some others for comparison (Table 3). The recombinant OspC proteins of strains

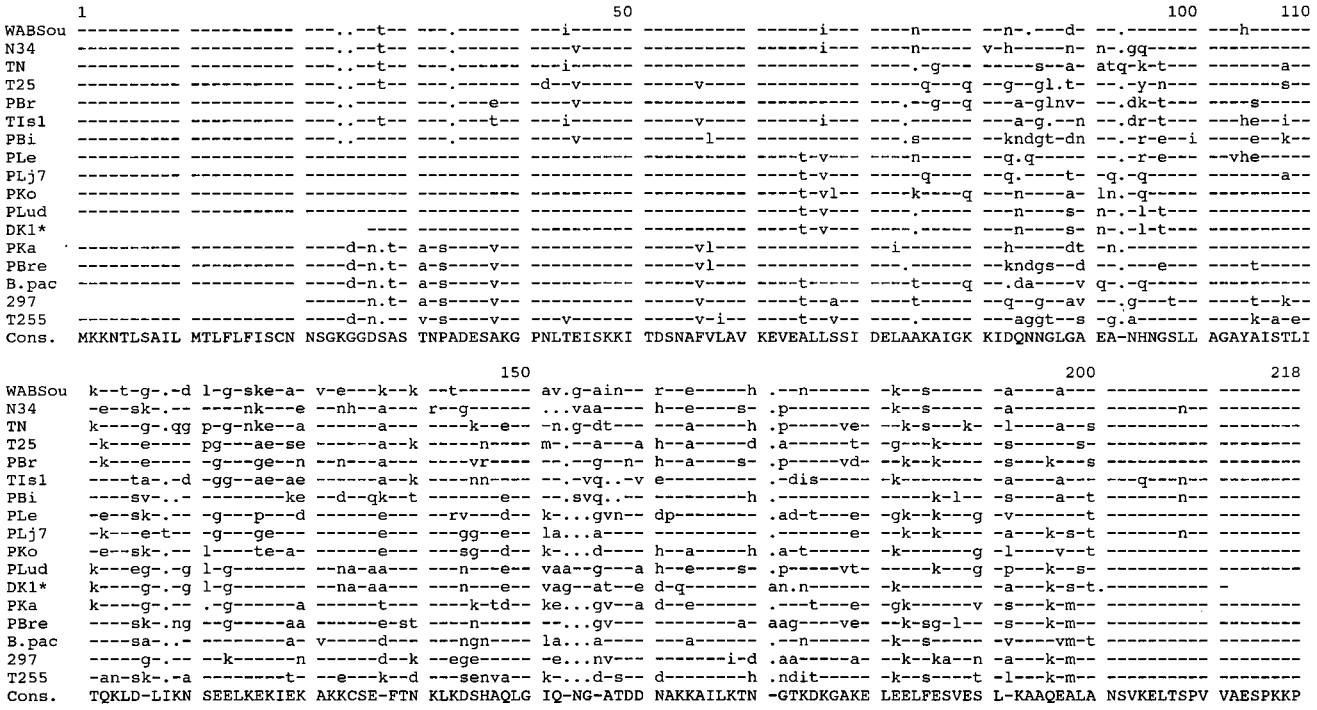

FIG. 1. Alignment of the deduced amino acid sequences of OspC. A consensus sequence (Cons.) is shown below the deduced sequences. *, sequence of DK1 determined by Theisen et al. (47). Dashes represent identical amino acids, while dots represent missing amino acids.

TABLE 3. Reactivity of recombinant OspC proteins with OspC-specific MAbs[a]

Strain	OspA type	OspC type	Reactivity with MAb:									OspC type of recombinant protein
			2B8	6C4	22C11	1F8	7G5	10C5	2E3	1F10	6C8	
PBre	1	NE[b]	+	+	−	+	+	−	+	−	−	New
PBr	3	NE	−	+	+	+	+	−	−	−	−	New
PLj7	2	8	+	+	+	+	+	−	−	−	−	8
B31	1	NE	+	+	−	+	+	+	+	−	−	1
PKo	2	5	+	+	+	+	+	−	−	+	−	5
PBi	4	8	+	+	+	+	+	−	−	−	−	8

[a] Determined by Western blotting with a panel of MAbs against OspC (52).
[b] NE, no expression.

B31, PKo, and PBi have been analyzed previously (52) and are included as references. To our surprise, the broadly reacting MAb L22 2B8 was not reactive with the recombinant OspC protein of strain PBr. All other OspC proteins, both native and recombinant, showed a positive signal with this antibody.

Comparison of the *ospC* groups with genospecies and OspA classification. Conserved OspC proteins were observed only among strains belonging to the same species (Table 1). However, OspC heterogeneity was present in strains of the same species. For example, the OspC proteins of *B. burgdorferi* B31 and PKa2 are completely identical, whereas OspC of *B. burgdorferi* T255 has about the same degree of identity (72% [Table 2]) to these strains as to *B. afzelii* or *B. garinii* strains. It is notable that these three strains belong to the same OspA serotype and have nearly identical OspA sequences (99.8% on the nucleotide level [49a]). OspC heterogeneity was observed in all three species among certain strains of the same OspA serotype or genotype. The amino acid identities of different OspC proteins from strains of the same OspA type are boxed in Table 2. They are in the range of 68 to 80% and thus are only slightly higher than the identities for of all strains.

Cluster analysis of the *ospC* genes suggests recombination events. In the EBI/GenBank database, 11 additional *ospC* sequences were found. This means that 32 sequences were available for analysis. Comparing our sequences with those available from the EBI/Genbank database, we found nucleotide sequences whose deduced amino acid sequences are more than 98% identical to ours or significantly different. The *ospC* sequences from all five Danish patient isolates studied by Theisen et al. (47) could also be found in our panel. But there were some interesting findings when these sequences were compared. The nucleotide sequences of *ospC* of strains WABSou and DK27 (both *B. garinii* strains) are almost identical, with a 6-bp insertion in DK27 between nt 65 and 66, two nucleotide exchanges at nt 203 and 204, an insertion of 1 base between nt 311 and 312, and a subsequent 1-bp deletion at nt 323 (numbering refers to the WABSou sequence), resulting in an overall identity of 97.6% on the nucleotide level. The 6-bp insertion between positions 65 and 66 is especially remarkable. These six bases are missing in all other *B. garinii* strains but can be found in *B. burgdorferi* and *B. afzelii* strains. Except for that insertion-deletion, the sequences of all *ospC* genes (with the exceptions of one nucleotide exchange in each of two sequences) are identical from nt 1 to 67. A surprising result was obtained when *ospC* genes of *B. afzelii* DK1 and PLud were compared. They share an overall identity of 94.1% but are almost identical at the 5′-terminal end. The region from nt 78 to 448 (PLud numbering) shows an identity of 99.7%; this means that only one nucleotide exchange occurred in the sequence. The region from nt 449 to 589 (also referring to PLud numbering), on the other hand, reveals a sequence identity of only 79.4%. No sequence with a high degree of identity to this region could be found in other known *ospC* genes. This suggests that either DK1 or PLud or both aquired part of their *ospC* genes from a hitherto-unknown *ospC* gene. To further investigate these results, trees were constructed by using the nucleotide sequences of the complete *ospC* gene (nt 1 to 645) and from nt 79 to 448 and nt 449 to 589 (referring to PLud numbering). The tree which was constructed from complete *ospC* sequences (Fig. 2) showed three main clusters corresponding to the three species with the exception of DN127, which was recently related to an additional group different from the species *B. burgdorferi* sensu stricto, *B. afzelii*, and *B. garinii* (2). The two trees which were obtained by analysis of parts of the genes (Fig. 3) did not exhibit this correlation. For example, strains B. pac and PKo fall into one group (Fig. 3b) but belong to different species. Strain 25015 forms a group with *B. burgdorferi* sensu stricto strains (Fig. 3a) but belongs to the same genospecies as strain DN127 (group DN127). Moreover, these trees showed a different order of branching. According to Dykhuizen et al. (15), this would indicate recombination events in the respective *ospC* gene.

DISCUSSION

We analyzed the *ospC* genes and proteins of 18 different *B. burgdorferi* sensu lato strains and compared the results with our previous findings with the *ospC* genes and proteins of strains B31, PKo, and PBi (18, 22). Since this 21-strain panel includes a broad variety of strains, including the three European species and the seven OspA serotypes described by us, and was characterized also in respect to the OspA genotype, our results give new insights into the degree of diversity of OspC and its relationship to other phenotypic and genotypic markers. In this study, we confirmed the high degree of diversity of the *ospC* gene which was suggested in previous studies performed with smaller numbers of strains (22, 47, 54). On the basis of this large number of strains, we were able to show some interesting features of OspC not previously described.

We found a close relationship between immunological and molecular heterogeneity. The 13 immunologically defined OspC variants (52) were confirmed by sequence analysis. Since OspC is an effective experimental vaccine (21, 35, 37), this is important for the design of cross-protection studies using recombinant OspC proteins for active immunization and OspC MAbs for passive immunization in animal models. In addition, we detected two new immunologically and genetically different OspC proteins by expressing PCR-amplified *ospC* genes from nonexpressing strains in *E. coli*. This type of analysis provides the possibility of immunological characterization of proteins whose expression is suppressed in the borrelial cell, which has already been shown for OspC cloned from strain B31 and

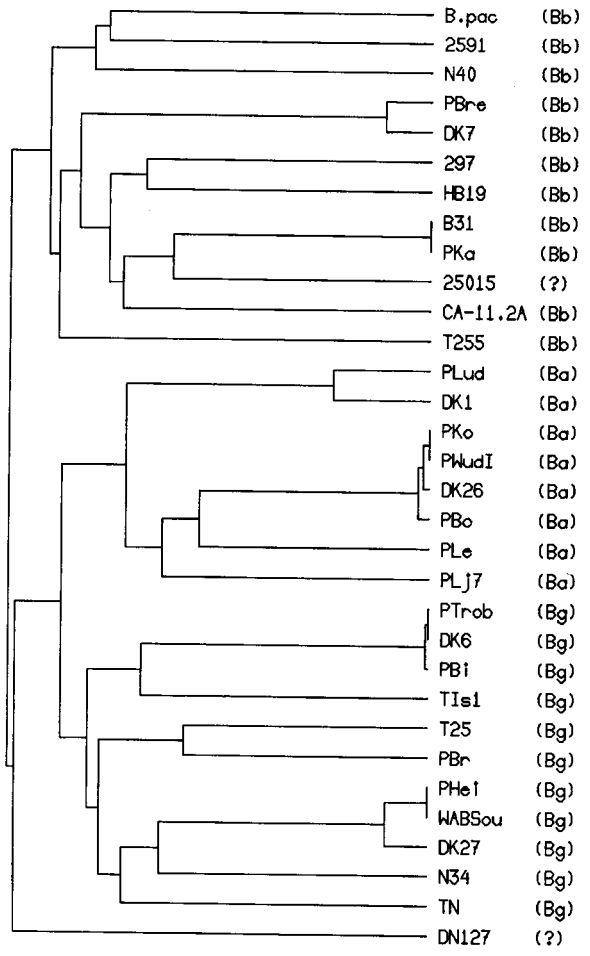

FIG. 2. Cluster analysis of all available *ospC* sequences on the nucleotide level from nt 1 to 645 (PLud numbering). Bb, *B. burgdorferi* sensu stricto; Ba, *B. afzelii*; Bg, *B. garinii.*

OspA cloned from strain PBo (53, 54). In contrast to the present findings, the two recombinantly expressed proteins represented already-known OspC phenotypes. In general, cloning and expression of genes not expressed by *B. burgdorferi* in culture are an important approach for development of serodiagnostic tests and vaccines.

Cluster analysis of the hitherto-available *ospC* sequences revealed evidence for recent intragenic recombination of *ospC*, since we found inconsistencies between trees constructed from 5′ and 3′ stretches of the *ospC* gene. Ancestors of *B. afzelii* PLud or DK1 might have obtained parts of their *ospC* genes by recombination with other strains (Fig. 3). Evidence for intragenic recombination in the *ospA* gene of the CSF isolate PHei was previously described by Dykhuizen et al. (15) but appears to be a rare event. In contrast, Marconi et al. found evidence for frequent recombination events in the *ospD* gene encoding a protein associated with low-passage-number strains (28).

It is not clear whether recombination events play a major role in the evolution of the considerable heterogeneity of OspC in general. However, recent intragenic recombination, in combination with mechanisms to escape the immune system in vertebrate hosts, might be the reason for the considerable heterogeneity of OspC among some strains with highly conserved OspA proteins. Such strains have been also identified among *B. burgdorferi* sensu stricto strains by Stevenson and Barthold (45). Such mutants may have had an extraordinary advantage to survive in reservoir host populations immune to wild-type strains. In this respect, it is also of interest that despite the fact that OspA is conserved between most North American and European *B. burgdorferi* sensu stricto strains, OspC proteins on opposite sides of the Atlantic are different (with the exception of strains PKa and B31). Thus, OspC diverged probably after OspA and might be a better marker for geographic diversity than OspA. However, strains of the same OspC type have been isolated from different European countries, indicating that OspC may have diverged before distribution of the *Borrelia* strains in different geographic regions in Europe.

Intragenic recombination between different strains, as discussed above, requires double infection in the tick or host environment. An indication that double infection may occur even in the human host was the observation that a patient had immunoglobulin M antibodies against the OspA of a heterologous strain but not the OspA of the patient's own isolate (57). On the other hand, infections with two different strains appear to be rare events, and this might be also an explanation for the observation that recombination of *ospA* as well as *ospC* appears to play a minor role in persistence of the borrelia in chronically infected animals. Even if true antigenic variation and recombinations of Osp proteins could be produced in vitro (38, 39), experiments conducted by Stevenson et al. suggested that OspC is stable over long periods in chronically infected mice (46). However, other mechanisms, for example, immune escape mechanisms due to differences in expression of Osp proteins as well as molecular mimicry or invasion of immunologically privileged sites, might promote persistence in most infected individuals.

Recently, Margolis et al. described the location of *Borrelia* purine biosynthesis gene homologs *guaA* and *guaB* upstream from the *ospC* gene (31). They discuss a linkage of GuaA and GuaB expression with expression of OspC. Indeed, it appears that in *I. ricinus* and *I. scapularis*, in which purine levels are high, OspC is not efficiently expressed (17, 44). In contrast, OspC appears to be efficiently expressed in human tissue, since OspC is the immunodominant antigen recognized in early disease (1, 14, 56, 57). However, it is unknown in which human tissues or body fluids OspC is present, in other words, whether there are differences in OspC expression at different sites of infection. The findings of Cadavid et al. (9), who demonstrated differences in Vmp expression of *Borrelia turicatae* CSF or blood reisolates from scid mice having neural disease or arthritis, respectively, suggest that *Borrelia* outer membrane proteins might indeed be expressed differently in different tissues. In this respect, it is interesting that OspC proteins are conserved among the three OspA serotype 4 strains, as well as the two OspA serotype 5 strains, serotypes which are often isolated from CSF (53). In contrast, our data showed that the level of OspC diversity was often considerably high in OspA serotype 2 (or *B. afzelii*) strains, which are isolated predominantly from skin lesions (10, 48, 53, 55).

It is unknown whether the OspC molecule is directly involved in pathogenesis. OspA and OspB, for example, play a role in adhesion (12) and invasion processes. Sadziene et al. described differences in invasiveness between an *ospB* mutant and the wild-type strain (40). By analogy, mutations in the *ospC* gene might also cause changes in the virulence of a strain. However, even if OspC is not an adhesion or invasion molecule, expression of OspC instead of OspA could perhaps reduce adhesion and thus promote dissemination of the borreliae. In that case, the high level of OspC diversity might help the borreliae to escape host immune responses.

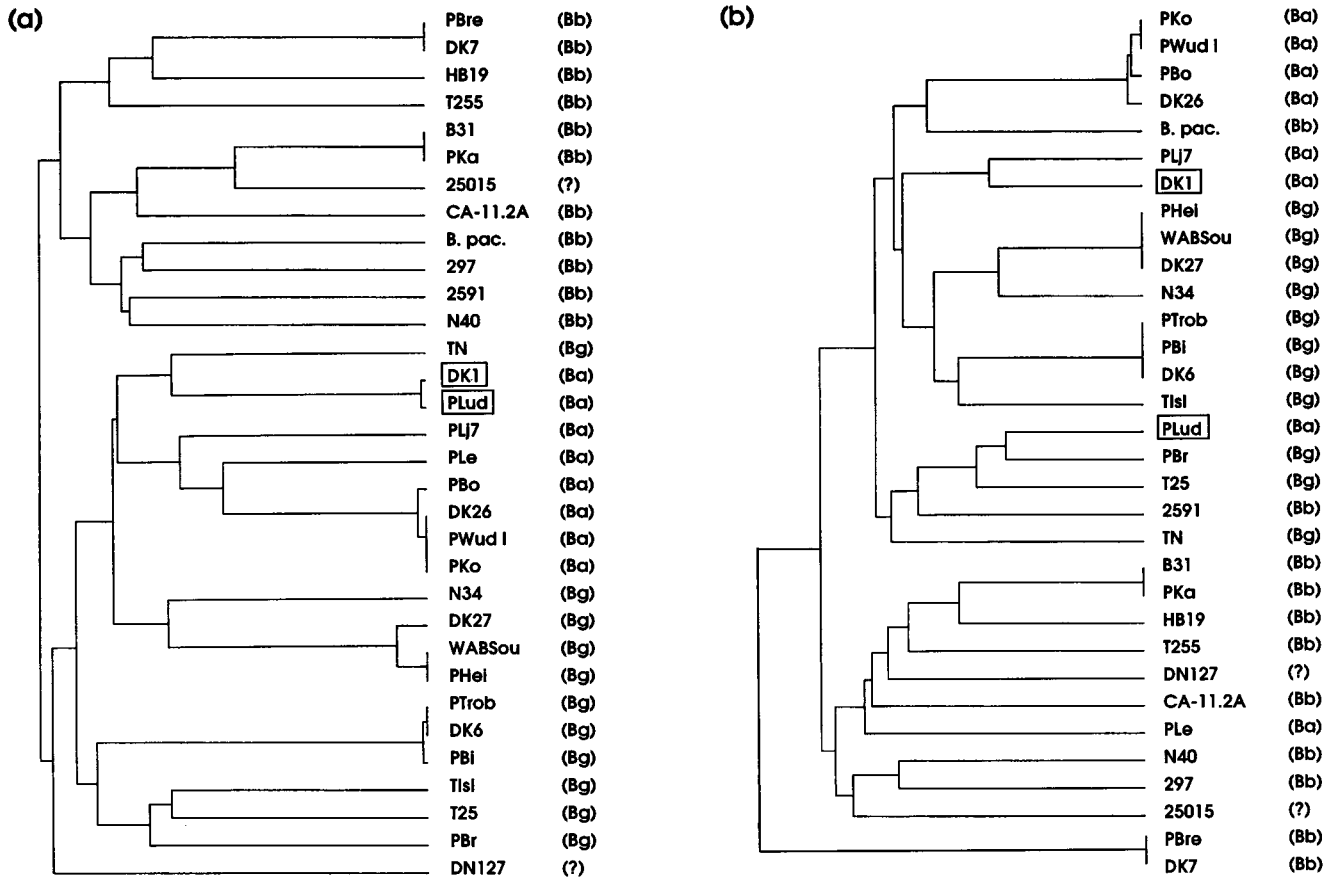

FIG. 3. Cluster analysis of all available *ospC* sequences on the nucleotide level, from nt 79 to 448 (a) and from nt 449 to 589 (b) (PLud numbering). PLud and DK1 (boxed) have different positions in the two trees. Bb, *B. burgdorferi* sensu stricto; Ba, *B. afzelii*; Bg, *B. garinii.*

In conclusion, we have described the molecular heterogeneity of OspC and its relationship to OspA and found evidence for intragenic recombination of the *ospC* gene. Our findings are important for analysis of strains, design of vaccine experiments, serological assays, and definition of the pathogenesis of Lyme borreliosis.

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REFERENCES

1. **Aguero-Rosenfeld, M. E., J. Nowakowski, D. F. McKenna, C. A. Carbonaro, and G. P. Wormser.** 1993. Serodiagnosis in early Lyme disease. J. Clin. Microbiol. **31:**3090–3095.
2. **Assous, M. V., D. Postic, G. Paul, P. Névot, and G. Baranton.** 1994. Individualisation of two new genomic groups among American *Borrelia burgdorferi* sensu lato strains. FEMS Microbiol. Lett. **121:**93–98.
3. **Baranton, G., D. Postic, I. Saint Girons, P. Boerlin, J.-C. Piffaretti, M. Assous, and P. A. D. Grimont.** 1992. Delineation of *Borrelia burgdorferi* sensu stricto, *Borrelia garinii* sp. nov., and group VS461 associated with Lyme borreliosis. Int. J. Syst. Bacteriol. **42:**378–383.
4. **Barbour, A. G.** 1988. Antigenic variations of surface proteins of *Borrelia* species. Rev. Infect. Dis. **10:**399–402.
5. **Barbour, A. G., W. Burgdorfer, S. F. Hayes, O. Peter, and A. Aeschlimann.** 1983. Isolation of a cultivable spirochete from *Ixodes ricinus* ticks of Switzerland. Curr. Microbiol. **8:**123–126.
6. **Barbour, A. G., R. A. Heiland, and T. R. Howe.** 1985. Heterogeneity of major proteins in Lyme disease borreliae: a molecular analysis of North American and European isolates. J. Infect. Dis. **152:**478–484.
7. **Barbour, A. G., and M. E. Schrumpf.** 1986. Polymorphisms of major surface proteins of *Borrelia burgdorferi*. Zentralbl. Bakteriol. Hyg. **263:**83–91.
8. **Burgdorfer, W., A. G. Barbour, S. F. Hayes, J. L. Benach, E. Grunwald, and J. P. Davis.** 1982. Lyme disease—a tick-borne spirochetosis? Science **216:** 1317–1319.
9. **Cadavid, D., D. D. Thomas, R. Crawley, and A. G. Barbour.** 1994. Variability of a bacterial surface protein and disease expression in a possible mouse model of systemic Lyme borreliosis. J. Exp. Med. **179:**631–642.
10. **Canica, M. M., F. Nato, L. Du Merle, J. C. Mazie, G. Baranton, and D. Postic.** 1993. Monoclonal antibodies for identification of *Borrelia afzelii* sp. nov. associated with late cutaneous manifestations of Lyme borreliosis. Scand. J. Infect. Dis. **25:**441–448.
11. **Carter, C. J., S. Bergström, S. J. Norris, and A. G. Barbour.** 1994. A family of surface-exposed proteins of 20 kilodaltons in the genus *Borrelia*. Infect. Immun. **62:**2792–2799.
12. **Comstock, L. E., E. Fikrig, R. J. Shoberg, R. A. Flavell, and D. D. Thomas.** 1993. A monoclonal antibody to OspA inhibits association of *Borrelia burgdorferi* with human endothelial cells. Infect. Immun. **61:**423–431.
13. **Devereux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12:**387–395.
14. **Dressler, F., J. A. Whalen, B. N. Reinhardt, and A. C. Steere.** 1993. Western blotting in the serodiagnosis of Lyme disease. J. Infect. Dis. **167:**392–400.
15. **Dykhuizen, D. E., D. S. Polin, J. J. Dunn, B. Wilske, V. Preac-Mursic, R. J. Dattwyler, and B. J. Luft.** 1993. *Borrelia burgdorferi* is clonal: implications for taxonomy and vaccine development. Proc. Natl. Acad. Sci. USA **90:**10163–10167.
16. **Felsenstein, J.** 1989. PHYLIP—phylogeny inference package (version 3.2). Cladistics **5:**164–166.
17. **Fingerle, V., U. Hauser, G. Liegl, B. Petko, V. Preac-Mursic, and B. Wilske.** 1994. Stage-, season- and region-dependent prevalence of *B. burgdorferi* in *Ixodes ricinus*, p. PO16W. *In* M. La Placa and R. Cevenini (ed.), VIth

International Conference on Lyme Borreliosis, June 19–22. Program and Abstracts. Societa Editrice Esculapio, Bologna, Italy.
18. **Fuchs, R., S. Jauris, F. Lottspeich, V. Preac-Mursic, B. Wilske, and E. Soutschek.** 1992. Molecular analysis and expression of a *Borrelia burgdorferi* gene encoding a 22 kDa protein (pC) in *Escherichia coli*. Mol. Microbiol. **6:**503–509.
19. **Fung, B. P., G. L. McHugh, J. M. Leong, and A. C. Steere.** 1994. Humoral immune response to outer surface protein C of *Borrelia burgdorferi* in Lyme disease: role of the immunoglobulin M response in the serodiagnosis of early infection. Infect. Immun. **62:**3213–3221.
20. **Howe, T. R., L. W. Mayer, and A. G. Barbour.** 1985. A single recombinant plasmid expressing two major outer surface proteins of the Lyme disease spirochete. Science **227:**645–646.
21. **Hughes, C. A. N., S. M. Engstrom, L. A. Coleman, C. B. Kodner, and R. C. Johnson.** 1993. Protective immunity is induced by a *Borrelia burgdorferi* mutant that lacks OspA and OspB. Infect. Immun. **61:**5115–5122.
22. **Jauris-Heipke, S., R. Fuchs, M. Motz, V. Preac-Mursic, E. Schwab, E. Soutschek, G. Will, and B. Wilske.** 1993. Genetic heterogeneity of the genes coding for the outer surface protein C (OspC) and the flagellin of *Borrelia burgdorferi*. Med. Microbiol. Immunol. **182:**37–50.
23. **Jonsson, M., L. Noppa, A. G. Barbour, and S. Bergström.** 1992. Heterogeneity of outer membrane proteins in *Borrelia burgdorferi*: comparison of *osp* operons of three isolates of different geographic origins. Infect. Immun. **60:**1845–1853.
24. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
25. **Langenberg, W., E. A. J. Rauws, A. Widjojokusomo, G. N. J. Tytgat, and H. C. Zanen.** 1986. Identification of *Campylobacter pyloridis* isolate by restriction endonuclease DNA analysis. J. Clin. Microbiol. **24:**414–417.
26. **Luft, B. J., C. R. Steinman, H. C. Neimark, B. Muralidhar, T. Rush, M. Finkel, M. Kunkel, and R. J. Dattwyler.** 1992. Invasion of the central nervous system by *Borrelia burgdorferi* in acute disseminated infection. JAMA **267:** 1364–1367.
27. **Marconi, R. T., D. S. Samuels, and C. F. Garon.** 1993. Transcriptional analyses and mapping of the OspC gene in Lyme disease spirochetes. J. Bacteriol. **175:**926–932.
28. **Marconi, R. T., D. S. Samuels, R. K. Landry, and C. F. Garon.** 1994. Analysis of the distribution and molecular heterogeneity of the *ospD* gene among the Lyme disease spirochetes: evidence for lateral gene exchange. J. Bacteriol. **176:**4572–4582.
29. **Marconi, R. T., D. S. Samuels, T. G. Schwan, and C. F. Garon.** 1993. Identification of a protein in several *Borrelia* species which is related to OspC of the Lyme disease spirochetes. J. Clin. Microbiol. **31:**2577–2583.
30. **Margolis, N., D. Hogan, W. Cieplak, T. G. Schwan, and P. A. Rosa.** 1994. Homology between *Borrelia burgdorferi* OspC and members of the family of *Borrelia hermsii* variable major proteins. Gene **143:**105–110.
31. **Margolis, N., D. Hogan, K. Tilly, and P. A. Rosa.** 1994. Plasmid location of *Borrelia* purine biosynthesis gene homologs. J. Bacteriol. **176:**6427–6432.
32. **Norris, S. J., C. J. Carter, J. K. Howell, and A. G. Barbour.** 1992. Low-passage-associated proteins of *Borrelia burgdorferi* B31: characterization and molecular cloning of OspD, a surface-exposed, plasmid-encoded lipoprotein. Infect. Immun. **60:**4662–4672.
33. **Padula, S. J., F. Dias, A. Sampieri, R. B. Craven, and R. W. Ryan.** 1994. Use of recombinant OspC from *Borrelia burgdorferi* for serodiagnosis of early Lyme disease. J. Clin. Microbiol. **32:**1733–1738.
34. **Padula, S. J., A. Sampieri, F. Dias, A. Szczepanski, and R. W. Ryan.** 1993. Molecular characterization and expression of p23 (OspC) from a North American strain of *Borrelia burgdorferi*. Infect. Immun. **61:**5097–5105.
35. **Preac-Mursic, V., B. Wilske, E. Patsouris, S. Jauris, G. Will, E. Soutschek, S. Reinhardt, G. Lehnert, U. Klockmann, and P. Mehraein.** 1992. Active immunization with pC protein of *Borrelia burgdorferi* protects gerbils against *Borrelia burgdorferi* infection. Infection **20:**342–349.
36. **Preac-Mursic, V., B. Wilske, and G. Schierz.** 1986. European *Borrelia burgdorferi* isolated from humans and ticks: culture conditions and antibiotic susceptibility. Zentralbl. Bakteriol. Hyg. A **263:**112–118.
37. **Probert, W. S., and R. B. LeFebvre.** 1994. Protection of C3H/HeN mice from challenge with *Borrelia burgdorferi* through active immunization with OspA, OspB, or OspC but not with OspD or the 83-kilodalton antigen. Infect. Immun. **62:**1920–1926.
38. **Rosa, P. A., T. Schwan, and D. Hogan.** 1992. Recombination between genes encoding major outer surface proteins A and B of *Borrelia burgdorferi*. Mol. Microbiol. **6:**3031–3040.
39. **Sadziene, A., P. A. Rosa, P. A. Thompson, D. M. Hogan, and A. G. Barbour.** 1992. Antibody-resistant mutants of *Borrelia burgdorferi*: in vitro selection and characterization. J. Exp. Med. **176:**799–809.
40. **Sadziene, A., P. A. Thompson, and A. G. Barbour.** 1993. In vitro inhibition of *Borrelia burgdorferi* growth by antibodies. J. Infect. Dis. **167:**165–172.
41. **Sadziene, A., B. Wilske, M. S. Ferdows, and A. G. Barbour.** 1993. The cryptic *ospC* gene of *Borrelia burgdorferi* B31 is located on a circular plasmid. Infect. Immun. **61:**2192–2195.
42. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
43. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
44. **Schwan, T.** 1994. Oral presentation at the VIth International Conference on Lyme Borreliosis, Bologna, Italy, 19 to 22 June 1994.
45. **Stevenson, B., and S. W. Barthold.** 1994. Expression and sequence of outer surface protein C among North American isolates of *Borrelia burgdorferi*. FEMS Microbiol. Lett. **124:**367–372.
46. **Stevenson, B., L. K. Bockenstedt, and S. W. Barthold.** 1994. Expression and gene sequence of outer surface protein C of *Borrelia burgdorferi* reisolated from chronically infected mice. Infect. Immun. **62:**3568–3571.
47. **Theisen, M., B. Frederiksen, A.-M. Lebech, J. Vuust, and K. Hansen.** 1993. Polymorphism in *ospC* gene of *Borrelia burgdorferi* and immunoreactivity of OspC protein: implications for taxonomy and for use of OspC protein as a diagnostic antigen. J. Clin. Microbiol. **31:**2570–2576.
48. **Van Dam, A. P., H. Kuiper, K. Vos, A. Widjojokusomo, B. M. De Jongh, L. Spanjaard, A. C. P. Ramselaar, M. D. Kramer, and J. Dankert.** 1993. Different genospecies of *Borrelia burgdorferi* are associated with distinct clinical manifestations of Lyme borreliosis. Clin. Infect. Dis. **17:**708–717.
49. **Vieira, J., and J. Messing.** 1982. The pUC plasmids, an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene **19:**259–268.
49a. **Will, G., S. Jauris-Heipke, E. Schwab, U. Busch, D. Rößler, E. Soutschek, B. Wilske, and V. Preac-Mursic.** Sequence analysis of *ospA* genes shows homogeneity with *Borrelia burgdorferi* sensu stricto and *Borrelia afzelii* strains but reveals major subgroups within the *Borrelia garinii* species. Med. Microbiol. Immunol., in press.
50. **Wilske, B., V. Fingerle, P. Herzer, A. Hofmann, G. Lehnert, H. Peters, H.-W. Pfister, V. Preac-Mursic, E. Soutschek, and K. Weber.** 1993. Recombinant immunoblot in the serodiagnosis of Lyme borreliosis. Med. Microbiol. Immunol. **182:**255–270.
51. **Wilske, B., V. Fingerle, V. Preac-Mursic, S. Jauris-Heipke, A. Hofmann, H. Loy, H.-W. Pfister, D. Rössler, and E. Soutschek.** 1994. Immunoblot using recombinant antigens derived from different genospecies of *Borrelia burgdorferi* sensu lato. Med. Microbiol. Immunol. **183:**43–59.
52. **Wilske, B., S. Jauris-Heipke, R. Lobentanzer, I. Pradel, V. Preac-Mursic, D. Roessler, E. Soutschek, and R. C. Johnson.** 1995. Phenotypic analysis of the outer surface protein C (OspC) of *Borrelia burgdorferi* sensu lato by monoclonal antibodies: relationship to genospecies and OspA serotype. J. Clin. Microbiol. **33:**103–109.
53. **Wilske, B., V. Preac-Mursic, U. B. Göbel, B. Graf, S. Jauris, E. Soutschek, E. Schwab, and G. Zumstein.** 1993. An OspA serotyping system for *Borrelia burgdorferi* based on reactivity with monoclonal antibodies and OspA sequence analysis. J. Clin. Microbiol. **31:**340–350.
54. **Wilske, B., V. Preac-Mursic, S. Jauris, A. Hofmann, I. Pradel, E. Soutschek, E. Schwab, G. Will, and G. Wanner.** 1993. Immunological and molecular polymorphisms of OspC, an immunodominant major outer surface protein of *Borrelia burgdorferi*. Infect. Immun. **61:**2182–2191.
55. **Wilske, B., V. Preac-Mursic, G. Schierz, R. Kühbeck, A. G. Barbour, and M. Kramer.** 1988. Antigenic variability of *Borrelia burgdorferi*. Ann. N. Y. Acad. Sci. **539:**126–143.
56. **Wilske, B., V. Preac-Mursic, G. Schierz, G. Liegl, and W. Gueye.** 1989. Detection of IgM and IgG antibodies to *Borrelia burgdorferi* using different strains as antigen. Proceedings of the Lyme borreliosis update Europe. Baden 2–4 June 1987. Zentralbl. Bakteriol. Suppl. **18:**299–309.
57. **Wilske, B., V. Preac-Mursic, G. Schierz, and K. von Busch.** 1986. Immunochemical and immunological analysis of European *Borrelia burgdorferi* strains. Zentralbl. Bakteriol. Hyg. A **263:**92–102.
58. **Wilske, B., G. Schierz, V. Preac-Mursic, K. von Busch, R. Kühbeck, H. W. Pfister, and K. Einhäupl.** 1986. Intrathecal production of specific antibodies against *Borrelia burgdorferi* in patients with lymphocytic meningoradiculitis (Bannwarth's syndrome). J. Infect. Dis. **153:**304–314.
59. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33:**103–119.
60. **Zumstein, G., R. Fuchs, A. Hofmann, V. Preac-Mursic, E. Soutschek, and B. Wilske.** 1992. Genetic polymorphism of the gene encoding the outer surface protein A (OspA) of *Borrelia burgdorferi*. Med. Microbiol. Immunol. **181:**57–70.