

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

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The nucleotide sequences of the *ospC* gene from five Danish human *Borrelia burgdorferi* isolates representing all three *B. burgdorferi* genospecies (*B. burgdorferi* sensu stricto, *Borrelia garinii* sp. nov., and group VS461) and from the American type strain B31 were determined and compared with the published *ospC* sequence from the German *B. burgdorferi* isolate PKo (R. Fuchs, S. Jauris, F. Lottspeich, V. Preac-Mursic, B. Wilske, and E. Soutschek, Mol. Microbiol. 6:503-509, 1992). The *ospC* gene was present in all isolates, regardless of the presence or absence of its product, OspC. The deduced amino acid sequences of OspC from the seven isolates were aligned and revealed pairwise sequence identities ranging from 60.5 to 100%. Differences were scattered throughout the amino acid sequences. A phylogenetic tree was constructed and revealed three distinct phenotypic groups OspCI to OspCIII corresponding to the three delineated genospecies. Immunoblot analysis revealed that the seven OspC proteins tested have both common and specific epitopes. There is significant epitope diversity, since even polyclonal antisera showed serotype-restricted specificity. Therefore, a serodiagnostic assay for Lyme borreliosis utilizing OspC as a test antigen should include all three OspC phenotypes in order to obtain a species-wide sensitivity.

Borrelia burgdorferi is a tick-borne spirochete which causes a multisystemic disease known as Lyme borreliosis (6). Lyme borreliosis is now the most common vector-borne human disease in Europe and North America. Clinically it may involve the skin (1), nervous system (11), heart (23), and joints (22). Laboratory confirmation of the clinical diagnosis is achieved by measurement of a specific antibody response. The most widely used serological assays utilize whole-cell antigen preparations as test antigens (indirect fluorescent-antibody assay and sonic-extract enzyme-linked immunosorbent assay [ELISA]) and lack diagnostic specificity. Researchers have sought to improve serodiagnosis by utilizing purified immunodominant antigens. So far the only documented improvement has been seen when purified native *B. burgdorferi* flagella was used as the ELISA test antigen (10, 14). In early Lyme borreliosis, patients develop an antibody response primarily to the *B. burgdorferi* flagellum, and at least in the case of European patients, patients also frequently develop an antibody response to the 22-kDa outer surface protein C (OspC) (25, 27). Thus, the use of purified OspC in addition to the flagellum may improve early serodiagnosis of Lyme borreliosis.

The *ospC* gene sequences of three *B. burgdorferi* strains were recently reported (8, 13). While the flagellin protein is highly conserved with only minor and, from a serodiagnostic viewpoint, insignificant intraspecies sequence variation (20), the degree of heterogeneity of the *B. burgdorferi* OspC has been less well documented (13). This information is essential when OspC is considered as a test antigen.

Thus, the aims of this study were to determine the intraspecies diversity of the nucleotide sequence of the *ospC* gene and of the deduced amino acid sequence of OspC and

consequently to determine the implications for serodiagnosis, by considering all three genospecies of *B. burgdorferi* (2).

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. *Escherichia coli* JM105 (*endA1 thi rpsL sbcB15 hsdR4 Δlac-proAB*) [*F' traD36 proAB⁺ lacI^qZΔM15*] and the *B. burgdorferi* isolates used in the present study were from the laboratory collection. The genospecies delineated by Baranton et al. (2) of the seven *B. burgdorferi* isolates are given in Table 1. The genospecies was determined as described by Macroni et al. (16) and according to the OspA serotype classification determined by Wilske et al. (24). *E. coli* strains were grown in Luria broth (LB) medium (18), and *B. burgdorferi* isolates were grown in BSK medium (3). When required, LB medium was supplemented with ampicillin and kanamycin at 100 and 40 μg/ml, respectively.

DNA techniques and computer analysis. Restriction enzymes and T4 DNA ligase were used as recommended by the supplier (Bethesda Research Laboratories, Gaithersburg, Md.). DNA sequencing was accomplished by the chain termination method, essentially as described by Messing (17).

Sequence alignments (7) and construction of the phylogenetic tree was done on a VAX computer with the Pileup facility of the Genetics Computer Group Sequence Analysis Software Package (version 7, 1991).

PAGE and immunoblotting. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of *B. burgdorferi* proteins was performed by the Laemmli method (15). Approximately 10⁷ bacteria were applied to each lane. Transfer of proteins onto a nitrocellulose membrane was performed as recommended by the supplier (Bio-Rad, Rich-

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TABLE 1. *B. burgdorferi* isolates

Isolate	Source ^a	OspC		Genospecies ^d	Supplier
		Expression ^b	Phenotype ^c		
DK1	Skin, EM	—	OspCIII	Group VS461	Laboratory collection
DK6	CSF, LMR	++	OspCII	<i>B. garinii</i>	Laboratory collection
DK7	Skin, ACA	+	OspCI	<i>B. burgdorferi</i> sensu stricto	Laboratory collection
DK26	Skin, EM	++	OspCIII	Group VS461	Laboratory collection
DK27	Skin, EM	+	OspCII	<i>B. garinii</i>	Laboratory collection
PKo	Skin, EM	++	OspCIII	Group VS461	V. Preac-Mursic ^e
B31	Tick	—	OspCI	<i>B. burgdorferi</i> sensu stricto	A. G. Barbour ^f

^a EM, erythema migrans; ACA, acrodermatitis chronica atrophicans; CSF, cerebrospinal fluid; LMR, lymphocytic meningoradiculitis.

^b Determined by Coomassie blue staining (Fig. 1A). Symbols: —, no expression; +, intermediate expression; ++, high expression of *ospC*.

^c According to the phylogenetic tree (Fig. 4).

^d Determined by the method described by Marconi and Garon (16) or by comparison with the *OspA* typing system of Wilske et al. (24).

^e Max von Pettenkofer-Institut, Munich, Germany.

^f Health Science Center, San Antonio, Texas.

mond, Calif.). Blots were incubated with rabbit or human sera diluted 1:200 with Tris-buffered saline (TBS) (10 mM Tris-Cl [pH 7.5], 140 mM NaCl)—1% bovine serum albumin (BSA) for 1 h. The antisera used were either rabbit sera against *OspC* or sera from patients with Lyme borreliosis. After three washes in TBS containing 0.05% Tween 20, antibody-reactive proteins were detected with swine anti-rabbit immunoglobulin G (IgG), rabbit anti-human IgG, or rabbit anti-human IgM, coupled to alkaline phosphatase (Dako, Copenhagen, Denmark) at 1:1,000 in TBS—1% BSA. Alkaline phosphatase activity was visualized with the nitro-blue tetrazolium—5-bromo-4-chloro-3-indolylphosphate toluidinium system as described by the supplier (Sigma Chemical Co., St. Louis, Mo.). Prestained or nonstained protein molecular weight standards were obtained from Bio-Rad.

Antisera. Sera from patients with Lyme borreliosis and high IgG or IgM titers against the native flagellum had been tested for antibody reactivity against *OspC* from DK6 or PKo in a Western blot (immunoblot) assay. Four serum samples were selected for this study. The antibody reactivity pattern of each serum sample is given in Table 2. Two monospecific rabbit serum samples were used, one raised against PAGE-purified *OspC* from DK6 and one directed against *OspC* from PKo (a gift from B. Wilske, Max von Pettenkofer-Institut, Munich, Germany).

Cloning of *ospC* from DK1, DK6, DK7, DK26, DK27, B31, and PKo. Genomic DNA was isolated from the *B. burgdorferi* isolates indicated in Table 1 as previously described (9).

TABLE 2. Lyme borreliosis patient sera

Serum sample	Source ^a	<i>B. burgdorferi</i> flagellum ELISA optical density ^b		Reactivity against <i>OspC</i> ^c	
		IgG	IgM	DK6	PKo
I	EM	0.240	2.800	+	—
II	EM	0.100	>2.800	+	+
III	ACA	2.300	0.250	+	—
IV	ACA	2.020	0.150	—	+

^a Sera were obtained from patients with erythema migrans (EM) or acrodermatitis chronica atrophicans (ACA).

^b Specific IgG and IgM antibody levels measured with an indirect and μ -capture ELISA using purified *B. burgdorferi* flagella (10, 12); the 98% specific diagnostic cutoff levels in these assays were optical densities of 0.160 for IgG detection and 0.5 for IgM detection.

^c Determined by IgG Western blotting of whole-cell lysates of either DK6 or PKo. Symbols: +, reactivity; —, no reactivity.

An *ospC* gene-specific primer set (primer 1 [5'-ATT TAC CAT GGG TAA TAA TTC AGG GAA AGG TGG] and primer 2 [5'-TCT TAC CAT GGT TTT TTT GGA CTT TCT GC]) was designed based on the published *ospC* sequence (8) and used to amplify the *ospC* gene from genomic DNA extracted from the *B. burgdorferi* isolates indicated in Table 1. Primer 1 was constructed to prime polymerase chain reaction (PCR) in the region encoding the lipoprotein box (28), and primer 2 is homologous to the carboxy-terminal portion of *ospC*. These sites were chosen because we assumed they would be highly conserved between different *B. burgdorferi* isolates. Primer 1 also turned out to be partially homologous to the nucleotide sequence of the *ospC* Shine-Dalgarno sequence. Both primers contain a nonhomologous *NcoI* site (underlined) to facilitate the cloning of the PCR products. PCRs were performed in a programmable heating block (Techn PH-C-1; Techn Ltd., Cambridge, United Kingdom) with 35 rounds of temperature cycling (93°C for 1 min, 45°C for 1 min, and 70°C for 1 min). In each reaction mixture, 10 pmol of both primers, 50 ng of template, 200 μ M each of the four deoxynucleotide triphosphates (dATP, dCTP, dGTP, and dTTP), 1 U of *Taq* polymerase, and the reaction buffer as recommended by the manufacturer (Perkin-Elmer Cetus, Norwalk, Conn.) were used in a total volume of 50 μ l. Amplified fragments were electrophoresed, purified with Gene-Clean according to the manufacturer's instructions (Bio 101, La Jolla, Ca.), digested with *NcoI*, precipitated, and ligated to the sequencing vector pAA480 (a gift from A. A. Potter, Veterinary Infectious Disease Organization, Saskatoon, Saskatchewan, Canada) cut at a unique *NcoI* site. Ligation mixtures were transformed into *E. coli* JM105, and four independent clones of each PCR product were kept for further analysis.

Nucleotide sequence accession numbers. The *ospC* nucleotide sequences of the six *B. burgdorferi* isolates have been assigned EMBL accession numbers X73622 (B31), X73623 (DK27), X73624 (DK26), X73625 (DK7), X73626 (DK6), and X73627 (DK1).

RESULTS

SDS-PAGE and immunoblot analysis. SDS-PAGE and Coomassie blue staining of whole-cell proteins from 17 Danish *B. burgdorferi* isolates revealed the presence of a major *OspC* protein in six isolates (data not shown). Four Danish isolates expressing *ospC*, one isolate from which *OspC* was absent, the American type strain B31 (*OspC*⁻),

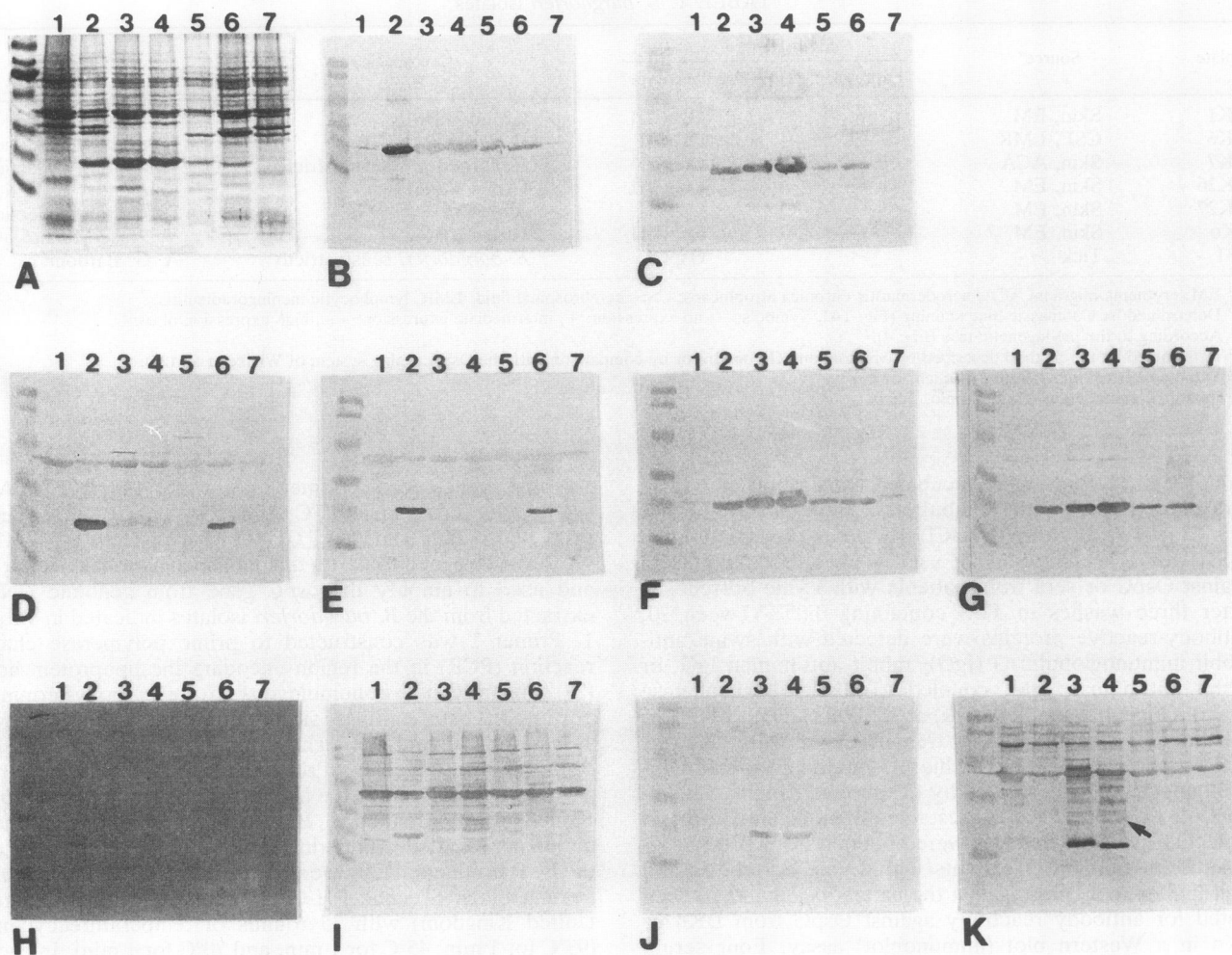


FIG. 1. SDS-PAGE and Western blot analysis of the indicated *B. burgdorferi* isolates. (A) Coomassie blue-stained 12.5% polyacrylamide-SDS gel of whole-cell proteins. Lane 1, DK1; lane 2, DK6; lane 3, PKo; lane 4, DK26; lane 5, DK7; lane 6, DK27; lane 7, B31. (B to K) Western blots of the gel in panel A incubated with the following sera: monospecific rabbit anti-OspC (from DK6) (B), monospecific rabbit anti-OspC (from PKo) (C), or one of four human immune serum samples, serum sample I (D and E), II (F and G), III (H and I), and IV (J and K). Panels D, F, H, and J are IgM Western blots, and panels E, G, I, and K are IgG Western blots. The arrow in panel K points at OspC. The molecular weight markers (shown in the leftmost lane of each panel) are proteins with molecular weights of 110,000, 84,000, 47,000, 33,000, 24,000, and 16,000.

and PKo (OspC⁺) were selected for further analysis. Figure 1 shows Coomassie blue staining (Fig. 1A) and immunoblots (Fig. 1B to K) of whole-cell proteins separated by SDS-PAGE. From Fig. 1A (summarized in Table 1), it appears that OspC is produced in variable amounts in the seven isolates. In DK1 and B31 (lanes 1 and 7), OspC is absent from whole-cell lysates. In DK7 and DK27 (lanes 5 and 6), OspC is produced in intermediate amounts, while it is synthesized in very large amounts in DK6, PKo, and DK26 (lanes 2, 3, and 4).

Figure 1B shows an immunoblot using a monospecific polyclonal rabbit antiserum raised against OspC from DK6. This serum recognized a 22-kDa protein in all isolates, with OspC from DK6 and DK27 showing the strongest reactions (lane 2 and 6). A monospecific serum against OspC from PKo (Fig. 1C) recognizes a 22-kDa protein in all isolates, although the strongest reactions were observed with OspC from DK6, PKo, and DK26 (lanes 2, 3, and 4). The distinct

pattern of reactivity even with polyclonal monospecific sera indicates significant antigenic differences.

To further study the antigenicity of the different OspC proteins, four human immune serum samples, previously selected for their reactivity with OspC from either DK6 or PKo (Table 2), were used as primary antisera in both IgM and IgG immunoblots against all seven *B. burgdorferi* isolates. Serum sample I (Fig. 1D and E) preferentially recognized OspC from DK6 and DK27 (IgG and IgM blots) which both belong to *Borrelia garinii* sp. nov. Serum sample II (Fig. 1F and G) recognized OspC from all isolates (IgG and IgM blots), whereas serum sample III contains IgG antibodies which recognize only OspC from DK6 (Fig. 1I, lane 2) and IgM antibodies which are weakly bound by all OspC variants (Fig. 1H, lanes 2, 3, 4, 5, and 6). Serum sample IV contains both IgM and IgG antibodies specifically recognizing OspC from the group VS461 isolates PKo and DK26 (Fig.

1J and K, lanes 3 and 4). Thus, the different OspC protein variants have both common and specific epitopes.

Comparison of the nucleotide sequences of the *ospC* genes. Figure 2 shows the nucleotide sequences of *ospC* from the five Danish isolates, DK1, DK6, DK7, DK26, DK27, and the American type strain B31, aligned and compared with the previously published *ospC* sequence from PKo (8). The sequence of each isolate was obtained from at least two independent clones of a PCR product, and no discrepancies were observed between any two clones from the same *B. burgdorferi* isolate. Thus, differences in the nucleotide sequences reflect true variability at the genetic level rather than errors introduced by PCR amplification. Furthermore, *ospC* from PKo was PCR amplified, cloned, and sequenced concomitantly with the other *ospC* genes. The nucleotide sequence thus determined was identical to the published sequence (8), further confirming the reliability of the sequence data (not shown). The nucleotide sequence alignment revealed 56% identities in the *ospC* genes of the seven isolates (Fig. 2). Of interest is the high degree of identity between PKo and DK26, since there was only one difference at position 599 in PKo (Fig. 2). This difference was confirmed by a second PCR amplification of *ospC* from DK26 and subsequent sequencing of the cloned product.

Nucleotide sequencing of the PCR products revealed that the upstream oligonucleotide had primed at two different positions within the *ospC* gene of all isolates, except for DK1 and B31. One position corresponds to the predicted primer site around the lipoprotein box and the other position corresponds to the region around the Shine-Dalgarno sequence (Fig. 2). Priming at the second site was unintended but in fact agrees well with the nucleotide sequence of *ospC* from PKo.

Comparison of the predicted amino acid sequences of OspC. The deduced amino acid sequences are aligned in Fig. 3 and compared with the published OspC sequence (8). Considerable variation is observed among the OspC amino acid sequences, with only 53% of the positions being the same in all OspC variants (Table 3). The amino-terminal portion of the protein is invariable, which agrees well with its suggested function as a signal peptide, and the putative signal peptidase II cleavage site has also been conserved in all isolates. Differences in the amino acid sequences appear to be scattered throughout the length of the polypeptide. A phylogenetic tree was constructed on the basis of the deduced amino acid sequences (Fig. 4) and revealed the presence of three OspC phenotypic groups named OspCI to OspCIII, which coincide with the recent delineation of *B. burgdorferi* into three genospecies (2).

DISCUSSION

In this article, we report the nucleotide sequences of six *ospC* genes originating from different *B. burgdorferi* isolates obtained from human Lyme borreliosis patients as well as serological data on the corresponding proteins. The isolates represent all three genospecies, *B. burgdorferi* sensu stricto, *B. garinii* sp. nov., and group VS461 (2).

We find that variable amounts of OspC are synthesized by the seven isolates, with expression ranging from virtually zero (DK1 and B31) to very high levels, as judged by Coomassie blue staining of whole-cell proteins subjected to SDS-PAGE (Fig. 1A). DK1 and the American type strain B31 do, however, possess a 22-kDa protein which reacts with patient sera, suggesting that a low level of *ospC* is expressed in these two isolates. OspC is produced as an

abundant major protein in 35% of the Danish isolates, which agrees well with a previous observation by B. Wilske et al. (26), who found that OspC is present in 30 to 50% of the strains they tested.

The differences in the nucleotide sequences among the *ospC* genes of the seven isolates appear to be evenly distributed in all three codon positions. The deduced OspC amino acid sequences of the seven *B. burgdorferi* isolates showed amino acid sequence identities ranging from 60.5 to 100% (Table 3). Very recently, a comparable degree of heterogeneity in OspC was observed among three *B. burgdorferi* isolates (13). One isolate characterized by Jauris-Heipke et al. (13) was the American type strain B31; comparison of the *ospC* sequence with the B31 *ospC* sequence determined in the present work revealed differences at two positions. The stretch of six nucleotides, TCTTTT, from positions 480 to 485 (Fig. 2) has been replaced by CTT, giving rise to a change in the predicted amino acid sequence from Ser-Phe to Leu. The G in position 641 (Fig. 2) has been replaced by an A, leaving the encoded amino acid unaltered. The numbers of amino acids and the molecular weights of the seven proteins studied in the present work vary only slightly. A phylogenetic tree revealed the presence of three OspC phenotypic groups (Fig. 4), which interestingly coincide completely with the three genospecies of *B. burgdorferi*. The OspCI isolates are *B. burgdorferi* sensu stricto strains, the OspCII isolates are *B. garinii* sp. nov. strains, and the OspCIII isolates are group VS461 strains. According to the phylogenetic tree obtained, it seems that OspCIII (group VS461) evolved from OspCII (*B. garinii*).

OspC showed a significant phenotypic diversity based on the observation that polyclonal antisera of human and rabbit origin could differentiate between the isolates. The seroreactivities correlated well with the three OspC phenotypic groups and with the three genospecies, as serum sample I recognized exclusively *B. garinii* (OspCII) (Fig. 1D and E) and serum sample IV recognized only group VS461 (OspCIII) (Fig. 1J and K). Possibly because of the limited number of serum samples tested, we have not yet found a serum sample specific for *B. burgdorferi* sensu stricto; however, serum sample II recognized all three phenotypic groups (Fig. 1F and G). Of the sera recognizing *B. garinii* strains (serum samples I and III), serum sample III recognized only DK6 (Fig. 1H and I), indicating diversity even between the *B. garinii* strains, which is in accordance with two recent reports. Baranton et al. (2) showed by restriction fragment length polymorphism of the rRNA genes that *B. garinii* was significantly more diverse than *B. burgdorferi* sensu stricto and group VS461. Wilske et al. (24) studied OspA seroreactivity with monoclonal antibodies and found that all sensu stricto strains corresponded to OspA serotype 1, all group VS461 strains corresponded to serotype 2, and *B. garinii* strains corresponded to serotypes 3 to 7. In an earlier study, Wilske et al. (27) observed an immunological heterogeneity in OspC of five different *B. burgdorferi* isolates using patient and mouse immune sera. Three of these strains were later determined by Baranton et al. (2) to be *B. garinii*.

A similar serotype diversity recognized by polyclonal patient sera has not been shown for *B. burgdorferi* OspA and OspB. In *B. hermsii*, however, several serotypes have been demonstrated with the variable major lipoproteins (Vmp), which also are abundant outer surface proteins (4, 5). Interestingly, the amino acid sequences of Vmp3 and Vmp24 (sequenced by Restrepo et al.; EMBL accession numbers L04789 and L04786) showed from 41 to 46% identity to the

1
 PKo TGGACTATTAATTCATAAAAAGGAGGCACAAATTAATGAAAAAGAATACATTAAGTGCATATTAATGACTTTATTTTTATTATATCTTGTAATAATTC
 DK26 CACAAATTAATGAAAAAGAATACATTAAGTGCATATTAATGACTTTATTTTTATTATATCTTGTAATAATTC
 DK1
 DK27 CACAAATTAATGAAAAAGAATACATTAAGTGCATATTAATGACTTTATTTTTATTATATCTTGTAATAATTC
 DK6 CACAAATTAATGAAAAAGAATACATTAAGTGCATATTAATGACTTTATTTTTATTATATCTTGTAATAATTC
 DK7 CACAAATTAATGAAAAAGAATCTTAAAGTGAATATTAATGACTTTATTTTTATTATATCTTGTAATAATTC
 B31

101
 PKo AGGGAAAGGTGGGGATTCTGCATCTACTAATCTGCTGACGAGTCTGCGAAAGGGCCTAATCTTACAGAAAATAGCAAAAAATACAGATTCTAATGCA
 DK26 AGGGAAAGGTGGGGATTCTGCATCTACTAATCTGCTGACGAGTCTGCGAAAGGGCCTAATCTTACAGAAAATAGCAAAAAATACAGATTCTAATGCA
 DK1 AGATTCTGCATCTACTAATCTGCTGACGAGTCTGCGAAAGGGCCTAATCTTACAGAAAATAGCAAAAAATACAGATTCTAATGCA
 DK27 AGGAAAAGGTGGGGATTCTGCATCTACTAATC...CTGATGAGTCTGCGAAAGGGCCTAATCTTACAGAAAATAGCAAAAAATACAGATTCTAATGCA
 DK6 AGG...TGGGGATTCTGCATCTACTAATC...CTGATGAGTCTGCGAAAGGGCCTAATCTTACAGAAAATAGCAAAAAATACAGATTCTAATGCA
 DK7 AGGAAAAGGTGGGGATTCTGCATCTACTAATCTG...CTGATGAGTCTGTTAAAGGGCCTAATCTTACAGAAAATAGCAAAAAATACAGATTCTAATGCG
 B31 GAATA...CATCTGCAAATCTGCTGATGAGTCTGTTAAAGGGCCTAATCTTACAGAAAATAGCAAAAAATACAGATTCTAATGCG

201
 PKo TTTGTACTTGCTGTTAAAGAAGTTGAGACTTTGGTTTTATCTATAGATGAACCTGCTAAGAAAGCTATTGGTCAAAAAATAGACAATAATAGTTTGTAG
 DK26 TTTGTACTTGCTGTTAAAGAAGTTGAGACTTTGGTTTTATCTATAGATGAACCTGCTAAGAAAGCTATTGGTCAAAAAATAGACAATAATAGTTTGTAG
 DK1 TTTGTACTGGCTGTTAAAGAAGTTGAGACTTTGGTTTTATCTATAGATGAACCTGCT...AAAGCTATTGGTCAAAAAATAGACAATAATAGTTTGTAA
 DK27 TTTGTACTGGCTGTTAAAGAAGTTGAGACTTTGGTTTTATCTATAGATGAACCTGCTAATAGAAAGCTATTGGTCAAAAAATA...AATCAAAATGGTTTGTAG
 DK6 TTTTACTGGCTGTTAAAGAAGTTGAGACTTTGGTTTTATCTATAGATGAACCTGCT...AAAGCTATTGGTCAAAAAATAGACAATAATAGTTTGTAG
 DK7 GTTTTACTTGCTGTTAAAGAAGTTGAGACTTTGGTTTTATCTATAGATGAACCTGCT...AAAGCTATTGGTCAAAAAATAGACAATAATAGTTTGTAG
 B31 GTTTTACTTGCTGTTAAAGAAGTTGAGACTTTGGTTTTATCTATAGATGAACCTGCTAAGAAAGCTATTGGTCAAAAAATAGACAATAATAGTTTGTAG

301
 PKo CTGCTTTAAATAATCAGAATGGATCGTTGTTAGCAGGAGCCTATGCAATATCAACCCTAATAACAGAAAAATGAGTAAATGAAAAATTTAGAAGAATT
 DK26 CTGCTTTAAATAATCAGAATGGATCGTTGTTAGCAGGAGCCTATGCAATATCAACCCTAATAACAGAAAAATGAGTAAATGAAAAATTTAGAAGAATT
 DK1 GTGCTAATGCGAATTTAAACACCTCGTTGTTAGCAGGAGCCTATGCAATATCAACCCTAATAAAACAAAAATTAGATGGATTGAAAGGTCTAGAAGGATT
 DK27 ATGCTGATGTAATCACACGGATCATTGTTAGCAGGAGCCTATGCAATATCAACTCTAATAAAACAAAAACAGATGGATTGAAAGATCTAGAAGGTT
 DK6 ATACCGAAGCAAAATCGAAAGGTAAGGAATGTTCCGATAAATTTACTAAAAAGCTAACAGATAGTCAATACACAAAAATTAAGTGTATTGAA...TTCAGAAGAATT
 DK7 GTGATGAAGCAAAATCACACGGATCATTGTTAGCAGGAGCCTATACAAATCAACCCTAATAACACAAAAATTAAGTAAATTAACCGGATCAGAAGGTTT
 B31 ATACCGAAAAATAATCACAAATGGATCATTGTTAGCGGGAGCTTATGCAATATCAACCCTAATAAAACAAAAATTAGATGGATTGAAAAAT...GAAGGATT

401
 PKo AAAGACAGAAATGCAAAGGCTAAGAAATGTTCCGAAGAATTTACTAATAAACTAAAAAGTGGTCATGCAGATCTT...GGCAAACAGGATGCT
 DK26 AAAGACAGAAATGCAAAGGCTAAGAAATGTTCCGAAGAATTTACTAATAAACTAAAAAGTGGTCATGCAGATCTT...GGCAAACAGGATGCT
 DK1 AAAGGAAAAAATGAAAAGGCTAAGAACGCTTCTGCAGCATTACTAATAAATAAAAATAGTCATGCAGAGCTTGGATGGCGGGGAATGGTCTACT
 DK27 AAGTAAAGAAATGCAAAGGTAAGGAATGTTCCGATAAATTTACTAAAAAGCTAACAGATAGTCAATACACAGCTTGGAGCAGTTGG...TGGTCTATT
 DK6 AAAGGAAAAAATTAAGAGGCTAAGGATTGTTCCGAAGAATTTACTACTAAGCTAAAAGATAGTCATGCAGAGCTT...GGTATACAAAGCGTT
 DK7 AAAGGAAAAAGATTGCCGAGCTAAGAAATGCTCTGAAGAGTTTACTAATAAACTAAAAAGATAATCATGCAGAGCTT...GGTATACAGGGCGTT
 B31 AAAGGAAAAAATGATGCGGCTAAGAAATGTTCTGAAACATTTACTAATAAATAAAAAGAAAAACACACAGATTCTTTT...GGTAAAGAAGGTGTT

501
 PKo ACCGATGATCATGCAAAAAGCAGCTATTTTAAAAACACATGCAAC...TACCGATAAAGGTGCTAAAGAATTTAAAGATTTATTTGAATCAGTAGAAGGTT
 DK26 ACCGATGATCATGCAAAAAGCAGCTATTTTAAAAACACATGCAAC...TACCGATAAAGGTGCTAAAGAATTTAAAGATTTATTTGAATCAGTAGAAGGCT
 DK1 ACTGATGAAGATGCACAAAAGCTATTTTAAAAACAAATGCGAA...TAAAGATAAAGGTGCTAAAGAATTTAAAGATTTATTTGAGTCAAGTAGAAGGTT
 DK27 AATGATGATCGTGCAAAAAGGCTATTTTAAAAACACATGGGAC...TAAAGATAAAGGTGCTAAAGAATTTAAAGATTTATTTGAATCAGTAGAAGGCT
 DK6 CAGGATGATAATGCAAAAAGGCTATTTTAAAAACACATGCAAC...TAAAGATAAAGGTGCTAAAGAATTTAAAGATTTATTTGAGTCAAGTAGAAGGCT
 DK7 ACTGATGAAATGCAAAAAGGCTATTTTAAAGCAAAATGCAAGCGGTTAAAGATAAAGGCGTTGAAGAATTTGAAAGTTGCTCCGATCATTAGAAGGCT
 B31 ACTGATGCTGATGCAAAAAGGCTATTTTAAAAACAAATGGTAC...TAAAGATAAAGGTGCTAAAGAATTTAAAGATTTATTTGAATCAGTAGAGGCT

601
 PKo TGTTAAAAGCAGCTCAAGTAGCACTAACTAATTCAGTTAAAGAACTTACAAGTCTGTTGTAGCAGAAAGTCCAAAAAACCTTAATAGATCAATATTA
 DK26 TGTTAAAAGCAGCTCAAGTAGCACTAACTAATTCAGTTAAAGAACTTACAAGTCTGTTGTGTA
 DK1 TGGCAAAAGCAGCTAAAGAACTACTAACCAATTCAGTTAAAGAGCTTACAAGCCCTGTTGTGTA
 DK27 TGGCAAAAGCAGCTCAAGCAGCATTAGCTAATTCAGTTAAAGAGCTTACAAGTCTGTTGTGTA
 DK6 TGTCAAAAGCAGCGCAAGCAGCATTAACTAATTCAGTTAAAGAGCTTACAATCTGTTGTGTA
 DK7 TATCAAAAGCAGCTAAAGAGATGCTTGTCAATTCAGTTAAAGAGCTTACAAGTCTGTTGTGTA
 B31 TGTCAAAAGCAGCTAAAGAGATGCTTGTCAATTCAGTTAAAGAGCTTACAAGCCCTGTTGTGTA

FIG. 2. Nucleotide sequences of the *ospC* genes from the *B. burgdorferi* isolates. All sequences were aligned and compared with the previously published *ospC* sequence from PKo (8). The primer sites are underlined and the Shine-Dalgarno sequence (21) and the ATG codon are overlined in the PKo sequence (top row). Dots indicates gaps introduced to obtain maximum homology.

	1	50
PKo	MKKNTLSAILMTLFLFISCNNSGKGGDSASTNPADESAGPNLTEISKKI	
DK26	-----	
DK1	-----	
DK7	-----D-NT -A-S-----V-----	
B31	-----NT -A-S-----V-----	
DK27	-----T-----I-----	
DK6	-----V-----	
	51	100
PKo	TDSNAFVLAVKEVETLVLSIDELAKKAIGQKIDNNGLAALNNQNGSLLA	
DK26	-----S-----K-----S-NA-L-T-----	
DK1	-----S-----K-----S-NA-L-T-----	
DK7	-----VL-----A-LS-----A-----K--K-DGS-GDEA-H-E----	
B31	-----VL-----A-LS-----I-A-----K--HQ--DTE-H-----	
DK27	-----A-IS-V--N-----K--Q--D-DA-H-----	
DK6	-----L-----A-LS-----S-----K--K-DGT-DNEA-R-E--I--	
	101	150
PKo	GAYAISTLITEKLSKLNLEELKTEIAKAKKCSSEFTNKLKSGHADL	
DK26	-----	
DK1	-----KQ--DG--G--G--EK-E--NA-AA-----NS--E-GVA	
DK7	-----T-----Q-----NGS-G--EK-A-----ST--DN--Q L	
B31	-----KQ--DG--G--EK-DA-----T-----EK-T-SF	
DK27	-----H-----NKT-TDG--D--G--SK--V-E--DK--K--TDS--Q-GAV	
DK6	-----E--K--QL SVL-S--GK-KE--D--K--T--DS--E--	
	151	200
PKo	GKQDATDDHAKAAIILKTH ATTDKGAKEFKDLFESVEGLLKAAQVALTNS	
DK26	-----	
DK1	-----NGAT--ED-QK-----N -NN-----L-E-----S-A-----KES-----	
DK7	-----I-GV--EN--K-----ANA-GK--VE-LEK-SG-L-S-S-----KEM-A--	
B31	-----EGV--AD--E-----NGTK-----E-LGK-----V-S-----KEM-A--	
DK27	-----GAIN--R--E-----G-N-----L-E-S-----S-A--A--A--	
DK6	-----I-SVQ--N--K-----G-K-----LEE--K-L-S-S-----A-----	
	201	
PKo	VKELTSPVV	
DK26	-----	
DK1	-----	
DK7	--S-Q--	
B31	-----	
DK27	-----	
DK6	-----N--	

FIG. 3. Comparison of the deduced amino acid sequences of the *B. burgdorferi* OspC proteins of the isolates. All sequences are compared and aligned with the *B. burgdorferi* PKo sequence (8). Dashes indicate identical amino acids at that position, capital letters indicate different amino acids in the strains, and gaps indicate missing amino acids.

OspC protein of all three phenotypes, whereas there was no significant homology with OspA and OspB. The fact that sequences of at least two different clones from every PCR product per isolate did not reveal different sequences excludes the possibility that *B. burgdorferi* contains different serotype-specific copies of the *ospC* gene sequence, as is true for the *vmp* genes in *B. hermsii*. The true significance of these observations is still unknown, but with the available data on OspA and OspC, antigenic variation found in *B. hermsii* has not yet been recognized in *B. burgdorferi*.

TABLE 3. Amino acid identity between OspC proteins from *B. burgdorferi* isolates^a

Isolate	% Amino acid identity between OspC from isolate:						
	DK1	DK6	DK7	DK26	DK27	PKo	B31
DK1		60.5	61.5	70.0	68.0	70.0	74.0
DK6			71.8	70.5	69.2	70.5	67.6
DK7				68.0	64.7	68.0	76.7
DK26					74.0	100	72.8
DK27						74.0	69.9
PKo							72.8

^a The FASTA programme ALIGN (19) was used to determine the alignments.

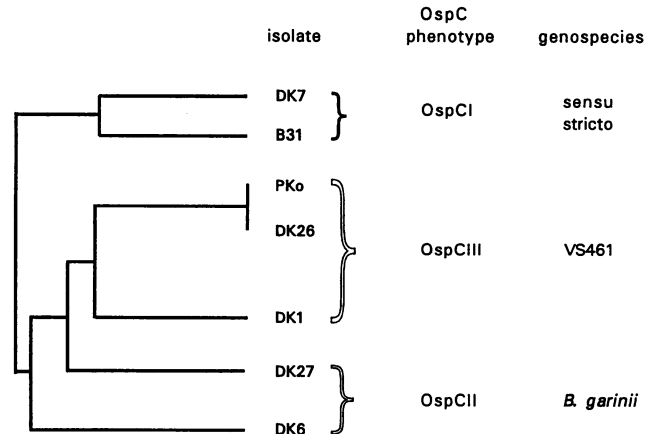


FIG. 4. Phylogenetic tree for the deduced OspC amino acid sequences derived from the seven *B. burgdorferi* isolates. The OspC phenotypic groups and corresponding genospecies are shown.

There appears to be no correlation between OspC phenotype and *ospC* expression levels. Major amounts of OspC are synthesized in only 30 to 50% of in vitro-grown *B. burgdorferi* strains. Compared with this frequency, the incidence of anti-OspC antibody reactivity in patients with Lyme borreliosis seems higher (27). An explanation might be that the expression level of *ospC* is different during natural infection and in vitro.

Western blotting revealed, in accordance with the results of Wilske et al. (27), that sera from patients with early disease may react strongly with OspC. OspC may in addition to the flagellum be a useful antigen for serodiagnosis of early Lyme borreliosis (26). The precise frequencies of anti-OspC IgG and IgM antibody reactivities in patients with Lyme borreliosis and as the significance of an anti-OspC immune response in late disease are areas that need investigation.

The seemingly low and in the case of serum II nonexistent Western blotting antibody reactivity to the 41-kDa *B. burgdorferi* flagellin band (Fig. 1F and G) is puzzling, considering the strong reactivity obtained in the flagellum ELISA (Table 2). This discrepancy may be a consequence of important conformational epitopes that are mainly present on the native flagellum and lost after denaturation of the protein by SDS-PAGE.

Our observation that OspC falls into at least three phenotypic groups which are serologically different may have important implications for the development of an improved serodiagnostic assay. Since sera from patients with Lyme borreliosis may have antibodies against only one of the three phenotypic groups, it appears that OspC from all three groups must be included in a serodiagnostic assay utilizing OspC as a test antigen.

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