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# Phenotypic Analysis of Outer Surface Protein C (OspC) of *Borrelia burgdorferi* Sensu Lato by Monoclonal Antibodies: Relationship to Genospecies and OspA Serotype

B. WILSKE,[1]* S. JAURIS-HEIPKE,[1] R. LOBENTANZER,[1] I. PRADEL,[1] V. PREAC-MURSIC,[1] D. RÖSSLER,[1] E. SOUTSCHEK,[2] AND R. C. JOHNSON[3]

*Max von Pettenkofer Institut für Hygiene und Medizinische Mikrobiologie der Universität München, 80336 Munich 2,[1] and Mikrogen GmbH, 80339 Munich 2,[2] Germany, and Department of Microbiology, University of Minnesota Medical School, Minneapolis, Minnesota 55455*

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**Molecular analyses of the genes encoding OspC, a major immunodominant protein of *Borrelia burgdorferi* sensu lato, revealed a considerable degree of heterogeneity. In the present study, we investigated whether a similar heterogeneity of the OspC phenotype can be shown by analysis with monoclonal antibodies (MAbs). Thirteen OspC-specific MAbs (L22 MAbs) were produced by immunizing mice with either different combinations of whole-cell antigens or recombinantly expressed OspCs cloned from strains belonging to different *Borrelia* spp. Ten of them differed in their reactivities with various strains. Western blot (immunoblot) analyses of 38 *B. burgdorferi* sensu lato strains resulted in 13 different reactivity patterns. These 13 different patterns were observed among only six different OspA serotypes, indicating that OspC is more heterogeneous than OspA. Patterns 1 to 4 were present only in *B. burgdorferi* sensu stricto, patterns 5 to 7 were present only in *Borrelia afzelii*, and patterns 9 to 13 were present only in *Borrelia garinii*. Pattern 8 was observed among *B. afzelii* and *B. garinii* strains but not among *B. burgdorferi* sensu stricto strains. One L22 MAb (2B8) recognized a common OspC-specific epitope of all 38 *B. burgdorferi* sensu lato strains analyzed, and another one (22C11) recognized a common epitope of OspC from both *B. afzelii* and *B. garinii* and was not reactive with OspC from *B. burgdorferi* sensu stricto. Western blot and sequence analysis of truncated OspCs located the 22C11 epitope as well as a species-specific sequence motif between amino acids 20 and 35. Other broadly reactive L22 MAbs were 10D3, 1F8, and 7G5. Some L22 MAbs (1C3, 1C3, 12E5, 1B11, 1F10, and 6C8) bound to epitopes present only in a few strains. Relapsing fever borreliae (*Borrelia hermsii*, *Borrelia turicatae*, and *Borrelia duttoni*) were nonreactive, with the following exception: three L22 MAbs (2B8, 6C4, and 10C5) recognized an abundantly expressed 20-kDa-range protein of *B. turicatae*. Because OspC is an immunodominant protein during the early immune response in Lyme borreliosis and has been shown to be effective as a vaccine in an animal model, our findings have important implications for the development of diagnostic reagents as well as vaccine research.**

*Borrelia burgdorferi* sensu lato is the causative agent of Lyme borreliosis, the most common tick-borne human disease (7). It consists of three different species, *B. burgdorferi* sensu stricto, *Borrelia afzelii*, and *Borrelia garinii* (2). The Lyme disease spirochetes are able to express variable amounts of three outer surface proteins, OspA, OspB, and OspC, which are encoded by genes located on linear and circular plasmids (5, 6, 15, 18, 24, 35). OspA and OspC (11, 16, 22, 25) are major candidates for a vaccine against Lyme borreliosis.

Since most strains (about 90%) express significant amounts of OspA in culture, this protein was the favorite target for phenotypic analysis and grouping of strains (4, 28, 36). Consequently, an OspA serotyping system, which correlated well with species determination and OspA sequence analysis (30), was developed. In contrast to OspA, OspC is often not expressed as a major protein in culture-grown borreliae. However, OspC (and not OspA) is the immunodominant outer surface protein recognized during early disease (1, 9, 13a, 21, 37, 38), possibly because of differences in expression of the Osp proteins in vitro and in vivo. Phenotypic analysis using a small number of OspC-specific monoclonal antibodies (MAbs) revealed four different antibody patterns among *B. burgdorferi* sensu lato strains (35). However, molecular analysis showed a considerable sequence variability of the *ospC* gene (17, 29, 35). Thus, a higher degree of phenotypic heterogeneity would be expected.

To analyze strains for OspC phenotypes, we established a library of OspC-specific MAbs. By using this expanded panel of antibodies for Western blot (immunoblot) analysis, we found 13 different reactivity patterns among 38 *B. burgdorferi* sensu lato strains. The different OspC types were correlated to species and OspA serotype classification.

## MATERIALS AND METHODS

***Borrelia* spp. strains and cultivation.** The designations and origins of *B. burgdorferi* sensu lato strains used in this study are given in Table 2. All strains, with the exception of three, have been described and classified previously (30, 35). These three strains were the OspC-expressing low-passage strains B31, 297, and Sh-2-82. Borreliae were cultivated on MKP medium as described previously (23). Strains of *Borrelia hermsii* (HS1 serotype C), *Borrelia turicatae*, and *Borrelia duttoni* were the same as those used previously (36).

**Recombinantly expressed OspC proteins.** Recombinantly expressed OspC was used to establish MAbs, and recombinant OspC fragments were constructed to determine antibody-binding regions. OspC was produced by transformed *Escherichia coli* clones containing the *ospC* genes of *B. burgdorferi* sensu stricto strain B31, *B. afzelii* PKo, and *B. garinii* PBi as described previously (17). To obtain overexpression of OspC, constructs lacking the leader peptide were used (32, 35). In short, the *ospC* genes were amplified by PCR, cloned in pUC8, and transformed in *E. coli*. An overnight culture of the recombinant cells was diluted 1:100 in 300 ml of L broth supplemented with 2% glycerin and ampicillin (50 μg/ml). After growth for 3 h, IPTG (isopropyl-β-D-thiogalactopyranoside) was

* Corresponding author. Mailing address: Max von Pettenkofer Institut für Hygiene und Medizinische Mikrobiologie der Universität München, Pettenkoferstrasse 9a, 80336 Munich 2, Germany. Phone: +49 89 5160 5231. Fax: +49 89 5160 4757.

TABLE 1. Immunoreactivity of OspC with L22 MAbs

| Strain | OspA serotype | Immunoreactivity with L22 MAb: | | | | | | | | | | OspC MAb pattern |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2B8 (IgG1)[a] | 6C4 (IgG1) and 10D3 (IgG2b) | 22C11 ND[b] | 1F8 (IgG1) | 7G5 (IgG1) | 10C5 (IgG1) | 2E3 (IgG1) | 1C3 (IgG2a), 12E5 (IgG2a), and 1B11 (IgG1) | 1F10 (IgG2b) | 6C8 (IgG1) | |
| PKa2 | 1 | + | + | − | + | + | + | + | + | − | − | 1 |
| B. pac. | 1 | + | + | − | + | + | − | − | − | − | − | 2 |
| 297 | 1 | + | − | − | + | − | − | − | − | − | − | 3 |
| T255 | 1 | + | + | − | − | + | − | − | − | − | − | 4 |
| PKo | 2 | + | + | + | + | + | − | − | − | + | − | 5 |
| PLud | 2 | + | + | + | + | + | + | − | − | − | − | 6 |
| PLe | 2 | + | + | + | + | + | − | + | − | − | − | 7 |
| PLj7 | 2 | + | + | + | + | + | − | − | − | − | − | 8 |
| PBi | 4 | + | + | + | + | + | − | − | − | − | − | 8 |
| WABSou | 5 | + | + | + | − | + | − | − | − | − | − | 9 |
| TN | 6 | + | + | + | − | + | − | − | − | − | + | 10 |
| N34 | 6 | + | + | + | + | − | − | − | − | − | − | 11 |
| TIs I | 6 | + | + | + | − | + | − | − | − | − | − | 12 |
| T25 | 7 | + | − | + | − | − | − | − | − | − | − | 13 |

[a] Immunoglobulin class and isotype of MAbs are shown in parentheses.
[b] ND, immunoglobulin class and isotype not determined.

added to a final concentration of 2 mM, and the cultures were incubated for another 4 h at 37°C. Cells were harvested by centrifugation. The cell pellet was lysed by the addition of lysozyme (0.4 mg/ml), and the OspC-containing supernatant was further purified by anion-exchange chromatography. The OspC-containing fractions were then subjected to cation-exchange chromatography on a fast protein liquid chromatography system (Pharmacia, Freiburg, Germany).

In addition, two constructs of the PKo *ospC* gene which expressed OspC fragments covering amino acids (aa) 35 to 212 and aa 55 to 212 deleted at the N terminus (13) were investigated.

**Partial sequencing of *ospC* genes.** *ospC* genes were amplified by PCR from genomic DNAs isolated from strains T255, PBo, PLj7, WABSou, and TIs I. The amplification products were cloned in pUC8, and the N-terminal amino acid sequence was determined as described previously (17).

**MAb generation, isolation, and characterization.** OspC-specific MAbs L22 1F8, L22 1C3, and L22 1F10 have been described previously and were derived from BALB/c mice immunized with whole washed cells from strains PKa2 (clone 9), PKo, PBi, and TN (35). Immunization with a mixture of whole cells from strains TN and PKa2 (clone 9) (live cells washed three times in phosphate-buffered saline containing 1 mM $MgCl_2$) resulted in the isolation of hybridoma cells producing MAb L22 6C8 (35). All other MAbs used in this study were obtained from a BALB/c mouse immunized with a mixture of recombinantly produced OspCs derived from strains B31, PKo, and PBi (see above).

One mouse was immunized four times with whole cells from strains TN and PKa2 (clone 9) (200 μg of protein per dose on days 0, 21, 42, and 63) and received three booster injections (400 μg per dose on days 68, 69, and 70). The same time schedule was used to immunize another mouse with recombinant OspCs from B31, PKo, and PBi mixed in equal amounts. Four injections of 120 μg per dose and then three booster injections with 240 μg per dose were given. In both mice, the first immunization was performed with complete Freund's adjuvant. Immunizations on days 21, 42, and 63 were done with incomplete Freund's adjuvant. The booster injections on days 68, 69, and 70 were given without adjuvant. Hybridomas were produced by standard protocols (14).

Identification of antibody-producing hybridomas was accomplished by immunofluorescence and immunoblot (in the case of whole-cell antigen) or enzyme-linked immunosorbent assay and immunoblot (in the case of recombinant antigen). Cloning and determination of the immunoglobulin class and the isotype were performed as described previously (34).

**SDS-PAGE and Western blots.** Spirochetes were harvested and processed for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blots as described previously (30). By use of a mini-gel apparatus (Mini-Protean II electrophoresis cell; Bio-Rad Laboratories GmbH, Munich, Germany), samples (5 μg of protein per lane) were subjected to SDS-PAGE (12% polyacrylamide) on gels (8.5 by 5.5 cm). Transfer of the proteins to a nitrocellulose membrane (Schleicher & Schüll, Dassel, Germany) 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 supernatant fractions containing the different MAbs. Antibody binding was detected anti-mouse immunoglobulin-specific horseradish peroxidase conjugate (Dakopatts, Copenhagen, Denmark).

**Nucleotide sequence accession numbers.** The *ospC* sequence data of strains T255, PBo, PLj7, WABSou, and TIs I will appear in the EMBL, GenBank, and DDBJ nucleotide sequence data libraries under the following accession numbers: X81524, X81521, X81523, X81526, and X81525.

The accession numbers of *ospC* sequences of various strains published previously (13, 13a, 17, 35) are as follows: B31, X69596; PKa2, X69589; 297, U08284; PKo, X62162; PWud I, X69590; PBi, X69595; TN, X69593; T25, X69592.

## RESULTS

**L22 MAb isolation and characterization.** As shown in Table 1, we generated 13 MAbs against OspC (L22 MAb series; instead of the full designations of the MAbs, we subsequently use abbreviated designations without the preface L22). Four MAbs (1F8, 1C3, 1F10, and 6C8) were produced with whole-cell antigens derived from strains PKa2, PKo, PBi, and TN. Nine other MAbs (2B8, 6C4, 10D3, 22C11, 7G5, 10C5, 2E3, 12E5, and 1B11) were generated with recombinantly expressed OspC cloned from strains B31, PKo, and PBi.

As shown in Table 1, the L22 MAbs differed in their immunoreactivities with the OspCs from various *B. burgdorferi* sensu lato strains except for the following two groups of antibodies which had identical OspC reactivity patterns: (i) 1C3, 12E5, and 1B11 and (ii) 6C4 and 10D3. Thus, we found 10 different OspC reactivity patterns among the 13 MAbs.

Despite having the same OspC reactivity patterns, MAbs 6C4 and 10D3 differed in two respects: (i) 6C4 was reactive with the relapsing fever agent *B. turicatae* (see below), whereas 10D3 was not, and (ii) 6C4 was isotype immunoglobulin G1 (IgG1), and 10D3 was isotype IgG2b. Both antibodies bound to a to-date-uncharacterized peptide with a molecular mass of about 18 kDa of strain 297. This protein clearly differs in molecular weight from OspC (see reactivity of the strain 297 OspC with MAb 2B8 in Fig. 1), and thus these two MAbs do not recognize the OspC of strain 297.

The second group of antibodies (1C3, 12E5, and 1B11) having the same reactivity patterns belonged to isotypes IgG2a, IgG2a, and IgG1, respectively. Immunoglobulin class and IgG isotypes of the remaining MAbs, with the exception of 22C11, could be determined: MAbs 10D3 and 1F10 belonged to isotype IgG2b, and the other MAbs belonged to isotype IgG1.

Use of the L22 MAb panel for Western blot analysis resulted in 13 different reactivity patterns among *B. burgdorferi* sensu lato strains. Strains with different reactivity patterns (patterns 1 to 13 in Table 1) were designated different OspC types. One group of MAbs (2B8, 6C4, 10D3, 22C11, 1F8, and 7G5) was

TABLE 2. *B. burgdorferi* sensu lato strains analyzed with OspC-specific MAbs and comparison with OspA serotype and species classification

| Species | Strain | Biological origin | Geographic origin | Reference or provider | OspA serotype | OspC MAb pattern |
|---|---|---|---|---|---|---|
| *B. burgdorferi* sensu stricto | B31 | *Ixodes dammini* | United States | S. Norris | 1 | 1 |
| | PKa2 | Human CSF[a] | Germany | 35 | 1 | 1 |
| | MUL | Human skin | United States | 30 | 1 | 1 |
| | Txgw | Human skin | United States | 30 | 1 | 1 |
| | CA9 | *Ixodes pacificus* | United States | 30 | 1 | 1 |
| | CA4 | *I. pacificus* | United States | 30 | 1 | 1 |
| | B. pac. | *I. pacificus* | United States | 30 | 1 | 2 |
| | CA7 | *I. pacificus* | United States | 30 | 1 | 2 |
| | CA8 | *I. pacificus* | United States | 30 | 1 | 2 |
| | 297 | Human CSF | United States | R. C. Johnson | 1 | 3 |
| | KIPP | Human skin | United States | 30 | 1 | 3 |
| | DUN | Human skin | United States | 30 | 1 | 3 |
| | Sh-2-82 | *I. dammini* | United States | S. Norris | 1 | 3 |
| | T255 | *Ixodes ricinus* | Germany | 35 | 1 | 4 |
| *B. afzelii* | PKo | Human skin | Germany | 30 | 2 | 5 |
| | PWud I | Human skin | Germany | 30 | 2 | 5 |
| | PJu | Human skin | Germany | 30 | 2 | 5 |
| | PBo | Human CSF | Germany | 30 | 2 | 5 |
| | PLud | Human skin | Germany | 30 | 2 | 6 |
| | PLe | Human skin | Slovenia | 30 | 2 | 7 |
| | PLj3 | Human skin | Slovenia | 35 | 2 | 7 |
| | PLj9 | Human skin | Slovenia | 35 | 2 | 7 |
| | PLj7 | Human skin | Slovenia | 30 | 2 | 8 |
| | PLj11 | Human skin | Slovenia | 30 | 2 | 8 |
| *B. garinii* | PBi | Human CSF | Germany | 30 | 4 | 8 |
| | DK6 | Human CSF | Denmark | 30 | 4 | 8 |
| | PFei | Human CSF | Germany | 30 | 4 | 8 |
| | PTrob | Human skin | Germany | 30 | 4 | 8 |
| | MABSou | Human skin | Austria | 30 | 5 | 9 |
| | TN | *I. ricinus* | Germany | 30 | 6 | 10 |
| | PMa | *I. ricinus* | Germany | 30 | 6 | 10 |
| | PWud II | *I. ricinus* | Germany | 30 | 6 | 10 |
| | VS 468 | *I. ricinus* | Switzerland | 30 | 6 | 10 |
| | N34 | *I. ricinus* | Germany | 30 | 6 | 11 |
| | TNA7 | *I. ricinus* | Switzerland | 30 | 6 | 12 |
| | TIs I | *I. ricinus* | Germany | 30 | 6 | 12 |
| | TIs II | *I. ricinus* | Germany | 30 | 6 | 12 |
| | T 25 | *I. ricinus* | Germany | 30 | 7 | 13 |

[a] CSF, cerebrospinal fluid.

broadly reactive, recognizing between 8 and 13 OspC types; other MAbs (10C5, 2E3, 1C3, 12E5, and 1B11) bound to only one or two OspC types.

**Grouping of strains.** Table 2 summarizes the results of typing 38 *B. burgdorferi* sensu lato strains with the L22 MAb panel by Western blot. Figure 1 shows representative blots. Each strain could be classified into one of the 13 groups defined by a specific MAb pattern according to Table 1. We found four different OspC types among 14 *B. burgdorferi* sensu stricto (or OspA serotype 1) strains and also four different OspC types among 10 *B. afzelii* (or OspA serotype 2) strains. Six different OspC types were present among 14 *B. garinii* strains (representing only four different OspA serotypes). However, OspC pattern 8 was observed among *B. afzelii* as well as *B. garinii* strains (e.g., strains PLj7 and PBi in Table 1).

MAb 2B8 identified an epitope common to all strains. MAb 22C11 recognized a common epitope of *B. afzelii* and *B. garinii* not present in *B. burgdorferi* sensu stricto. Five MAbs, i.e., 1C3, 12E5, 11B1, 1F10, and 6C8, were type specific and identified OspCs from subgroups of OspA serotypes 1 (1C3, 12E5, and 11B1), 2 (1F10), and 6 (6C8).

Three of the L22 MAbs (2B8, 6C4, and 10C5) also reacted with a major 20-kDa-range protein of the relapsing fever agent *B. turicatae* (Fig. 2). *B. hermsii* and *B. duttoni* were not reactive with any of the OspC-specific antibodies.

**Immunoreactivity of recombinantly expressed OspC and N-terminal sequence analysis.** The three recombinantly expressed OspCs derived from the three strains B31, PKo, and PBi showed the same reactivity patterns with the L22 MAbs as whole-cell SDS lysates from the respective strains (data not shown).

The three recombinantly expressed OspC fragments derived from strain PKo (aa 20 to 212, aa 35 to 212, and aa 55 to 212) differed in reactivity with MAbs 6C4 (or 2B8), 1F8, and 22C11 (Fig. 3). MAb 22C11, specific for *B. afzelii* and *B. garinii*, reacts only with the aa 20 to 212 fragment, indicating that a possible epitope lies between aa 20 and aa 35. Indeed, there is a region (aa 25 to 33) which is highly conserved among *B. afzelii* and *B. garinii* strains but is significantly different compared with that of *B. burgdorferi* sensu stricto strains (Fig. 4). Interestingly, *B. garinii* strains have one gap upstream and another gap downstream from the region of aa 25 to 33 not seen in *B. afzelii* strains. Alignment with the N-terminal sequences of Vmp3 and Vmp33 of *B. hermsii* HS1 recently published by Carter et al.

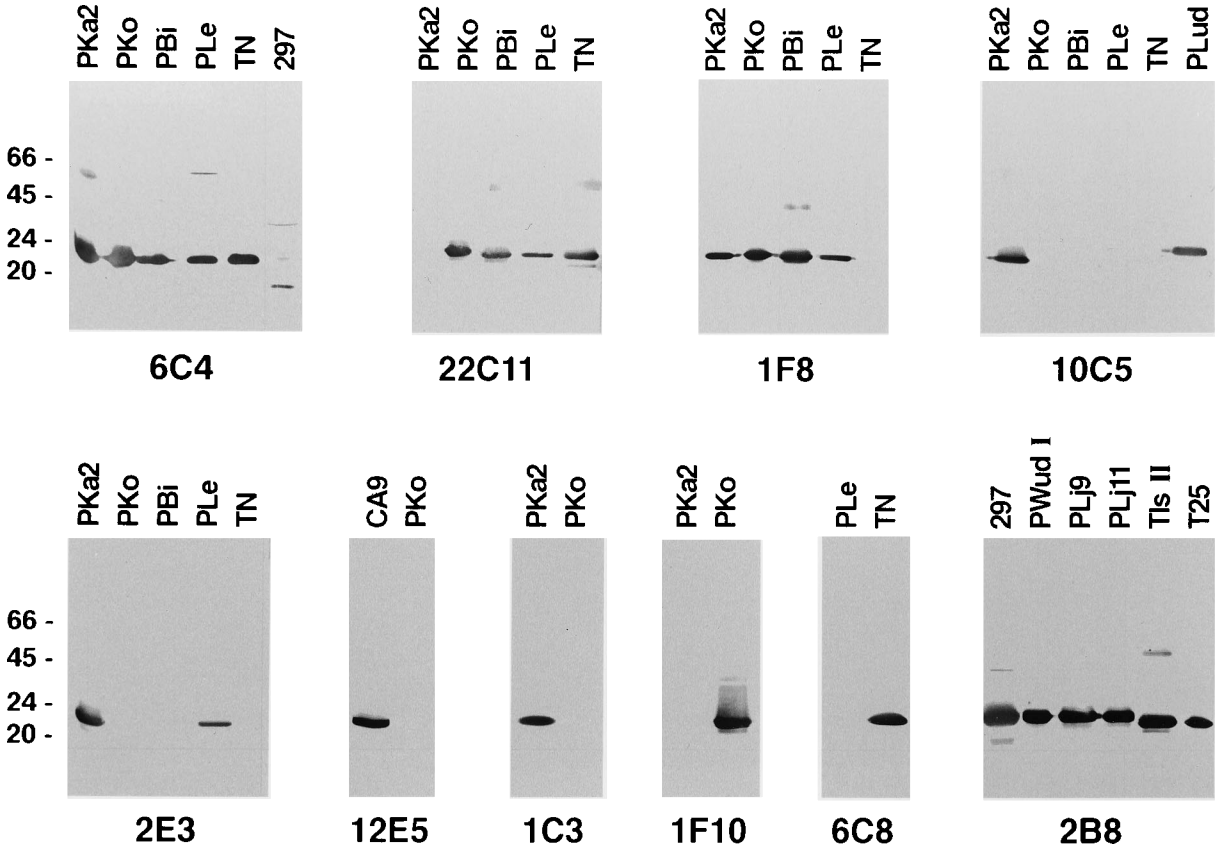


FIG. 1. Immunological characterization (Western blot) of *B. burgdorferi* sensu lato strains with OspC-specific L22 MAbs. Size standards (shown on the left of the figure) were bovine serum albumin (66 kDa), ovalbumin (45 kDa), trypsinogen (24 kDa), and trypsin (20 kDa). MAb 2B8 recognizes a common epitope of the OspC of *B. burgdorferi* sensu lato; MAbs 6C4, 22C11, and 1F8 are reactive with a broad panel of strains. In contrast, MAbs 10C5 and 2E3 recognize only a few strains, and MAbs 12E5, 1C3, 1F10, and 6C8 recognize only type-specific epitopes.

(8a) revealed a very high degree of nonidentity between *B. hermsii* Vmps and the various *B. burgdorferi* sensu lato OspCs.

MAb 1F8 recognized epitopes on the aa 35 to 212 fragment but not on the aa 55 to 212 fragment, indicating this antibody-binding site is located between aa 35 and 55. MAbs 2B8 and 6C4 bound to all three fragments. Therefore, the antibody-binding sites for these antibodies are located on the aa 55 to 212 fragment.

## DISCUSSION

Many aspects of the OspC protein of *B. burgdorferi* sensu lato have been investigated. This protein has many similarities to OspA, another major outer surface protein of *B. burgdorferi* sensu lato. OspC is also plasmid encoded. However, in contrast to OspA, the *ospC* gene is located on a circular plasmid (18, 24). The *ospA* and *ospC* genes are also present in nonexpressing strains and could be cloned and expressed in immunoreactive form in *E. coli* from such strains (17, 21, 30, 35). Apparently, OspC is also a lipoprotein (13).

In contrast to OspA, OspC is expressed by a smaller number of strains in culture. There is an apparent reverse relationship between the two proteins: strains with stronger expression of OspA have lower or no expression of OspC and vice versa (20, 35). Wild-type strains express at least one of the two proteins (36). Therefore, phenotypic characterization of strains should include OspA as well as OspC.

While immunological heterogeneity of OspA has been investigated intensively and an OspA serotyping system which correlates well with species determination (30) has been established, little was known about the immunological diversity of OspC. However, the higher degree of molecular heterogeneity and the fact that OspC is often not expressed in culture makes development of an OspC serotyping system more difficult. The strategy of using a mixture of three recombinant OspCs for immunization was very successful and probably was the key to establishing MAb 2B8, which recognizes a common epitope of the OspC protein. This antibody contributes to the list of MAbs binding to conserved epitopes of *B. burgdorferi* sensu lato antigens and had been systematically tested with

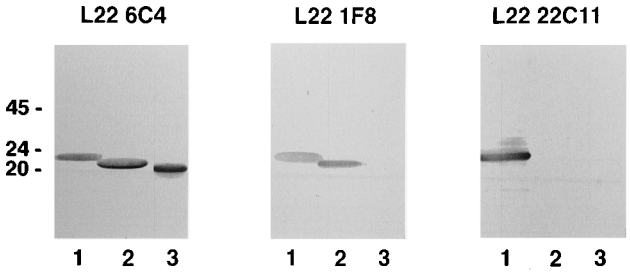


FIG. 2. Immunoreactivity of truncated OspCs (lanes 1, aa 20 to 212; lanes 2, aa 38 to 212; lanes 3, aa 55 to 212) with L22 MAbs (6C4, 1F8, and 22C11). MAb 2B8 had the same reactivity pattern with the truncated OspCs as MAb 6C4. Size standards (shown on the left of the figure) are given in the legend to Fig. 1.

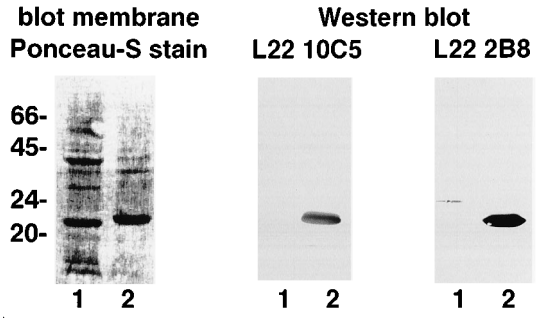


FIG. 3. Immunoreactivity of relapsing fever borreliae (*B. hermsii* [lanes 1] and *B. turicatae* [lanes 2]) with OspC-specific MAbs 10C5 and 2B8. Abundant 20-kDa-range proteins (VMPs) are visible in the Ponceau S stain. Size standards are given in the legend to Fig. 1.

large panels of strains. Examples of these MAbs are L32 1F11 for OspA (30), 84C for OspB (27), and H9724 or L41 1C11 for flagellin (3, 31).

The immunological diversity of OspC (13 different groups) appears to be significantly greater than that of OspA (7 serotypes) (30). It is notable that we found 13 OspC types among a group of only 6 OspA serotypes. However, there is a correlation between OspC type and OspA serotype: with the exception of OspC type 8, identical OspC MAb patterns were seen only in strains with the same OspA serotype. OspA serotype 4 strains and certain OspA serotype 2 strains (PLj7 and PLj11) representing OspC type 8 cannot be distinguished with our OspC-specific MAbs. However, molecular heterogeneity and differences in immunoreactivity with polyclonal sera (17a) suggest antigenic diversity not recognized by the MAbs.

Strains of a given OspA serotype may differ in their OspC types: there is a notable antigenic heterogeneity of OspC among OspA serotypes 1, 2, and 6. It is an open question whether the considerable heterogeneity of OspC is caused by horizontal gene transfer. Phylogenetic trees of the plasmid-encoded OspA and two chromosome-encoded proteins suggest that horizontal transfer of *ospA* genes occurs but is a rare event (10). A similar phylogenetic analysis for OspC remains to be performed.

There are also strains of a given OspA serotype with identical OspC types: identical patterns were observed among OspA serotype 4 strains and certain groups of OspA serotype 1, 2, and 6 strains. These findings are in accordance with sequence analysis showing nearly identical OspC sequences between OspA serotype 1 strains B31 and PKa2 and OspA serotype 2 strains PKo and PWud I. In contrast, strains PKo and PLj7 as well as strains PKa2 and 297, belonging to the same OspA serotype but to different OspC types, were also different in their OspC sequences (13, 13a, 16a, 17). However, the relationship between OspC types and sequences needs to be investigated further.

Despite the great variability observed among OspC sequences, the N-terminal end of the OspC protein shows a sequence motif (aa 23 to 35) which is highly conserved within the species. Alignment with other published OspC sequences showed that species specificity of this motif also holds true for OspC sequences not shown in Fig. 4 (*B. burgdorferi* sensu stricto DK7 [29] and N40 [28a] and *B. afzelii* DK1 and DK26 [29]). Partial sequence analysis of OspC, therefore, might be a useful tool for species determination. These partial sequences are also highly different from those of *B. hermsii* Vmp3 and Vmp33 (8a). The comparably high conservation among N-terminal sequences within the species is an argument against explaining the OspC variability by transfer of the *ospC* gene between borreliae belonging to different species but does not exclude gene transfer within the species. Another explanation for the high OspC variability (which does not, however,

| *Borrelia* | strain | amino acid sequence of OspC (21 31 41 51) | OspC type | OspA serotype |
|---|---|---|---|---|
| *B. burgdorferi* sensu stricto | B31 | NSGK**D**G**NT**-S **A**N**S**A-DES**V**K GPNLTEISKKI | 1 | 1 |
| | PKa2 | NSGK**D**G**NT**-S **A**N**S**A-DES**V**K GPNLTEISKKI | 1 | 1 |
| | 297 | NSGKGG**NT**-S **A**N**S**A-DES**V**K GPNLTEISKKI | 3 | 1 |
| | T255 | NSGK**D**G**NA**-S **V**N**S**A-DES**V**K GPNL**V**EISKKI | 4 | 1 |
| *B. afzelii* | PKo | NSGKGGDSAS TNPA-DESAK GPNLTEISKKI | 5 | 2 |
| | PWudI | NSGKGGDSAS TNPA-DESAK GPNLTEISKKI | 5 | 2 |
| | PBo | NSGKGGDSAS T**D**PA-DESAK GPNLTEISKKI | 5 | 2 |
| | PLj7 | NSGKGGDSAS TNPA-DESAK GPNLTEISKKI | 8 | 2 |
| *B. garinii* | PBi | NS--GGDSAS TNP--DESAK GPNLT**V**ISKKI | 8 | 4 |
| | DK6 | NS--GGDSAS TNP--DESAK GPNLT**V**ISKKI | 8 | 4 |
| | WABSou | NS--GGD**T**AS TNP--DESAK GPNL**I**EISKKI | 9 | 5 |
| | TN | NS--GGD**T**AS TNP--DESAK GPNL**I**EISKKI | 10 | 6 |
| | TIs1 | NS--GGD**T**AS TNP--DES**T**K GPNL**I**EISKKI | 12 | 6 |
| | T25 | NS--GGD**T**AS TNP--DESAK GP**D**LT**V**ISKKI | 13 | 7 |
| | **strain/Vmp** | **amino acid sequence of Vmp** | | |
| *B. hermsii* | HS1/Vmp33 | N**G**G**PELKK**-**G** **EVTKS**D**GTVL** **DLSK**--ISA**N**I | | |
| | HS1/Vmp3 | N**G**G**PELK**S-**D** **EVAKS**D**GTVL** **DLAK**--ISKKI | | |

FIG. 4. Partial deduced amino acid sequence of OspC. A region of aa 25 to 33 is highly conserved among *B. afzelii* and *B. garinii* strains but is significantly different compared with that of *B. burgdorferi* sensu stricto strains. *B. afzelii* and *B. afzelii* strains differ in amino acids flanking this region. Compared with *B. afzelii*, *B. garinii* has two gaps at aa 23 and 24 and one gap at aa 34. The respective partial sequences from two Vmps of *B. hermsii* are highly different from OspC sequences. Amino acids differing from those in the PKo sequences are indicated by bold letters.

exclude the former) might be immune selection of OspC mutants in the vertebrate host. Humans, for example, have a strong OspC immunoresponse in contrast to a weak OspA response, indicating that OspC is better expressed in the vertebrate host than OspA and thus more exposed to immune selection. However, using a mouse infection model, Stevenson et al. found "that variation of OspC is not necessary for evasion from the host immune system" (28a).

Also important besides strain analysis are the implications of OspC heterogeneity for diagnostic tests and immunoprophylaxis. OspC has been recognized as an immunodominant antigen for the IgM immunoresponse in European (37) as well as North American (9) patients and is the earliest antigen detected by Western blot in sera from patients with erythema migrans (1).

The majority of IgM-binding epitopes of OspC appear to be conserved among strains as shown by using recombinant OspC derived from the three species as antigen (33). In this study, three of the nine OspC-reactive serum samples, however, reacted only with OspC derived from whole-cell lysate. Two of them recognized restricted epitopes of the OspA serotype 4 strain PBi; another reacted only with OspA serotype 1 strain PKa2 and OspA serotype 2 strain PLe (but not with strains PKo, PBi, and TN). This is interesting in the light of our findings with the MAbs. First, OspA serotype 4 strains appear to have restricted OspC-specific epitopes not recognized by our MAbs. Second, our MAb 2E3 also recognizes common epitopes of OspC from strains PKa2 and PLe.

Thus, it appears that B-cell epitopes recognized by humans and mice might be identical. However, more sera need to be analyzed with larger panels of OspC-expressing strains. Inhibition tests with L22 MAbs may be used for determination of OspC-specific B-cell epitopes recognized by patients. Such analysis could help to design an optimal combination of OspCs for early antibody detection. However, certainly more MAbs are needed to determine all immunorelevant epitopes of OspC. Another application of OspC-specific MAbs could be antigen detection. The OspC-specific MAb 2B8, in combination with the OspA-specific MAb L31 1F11, could provide a sensitive and specific tool for antigen detection independent from the serotype. Combining the two antibodies should overcome the problems caused by variable expression of OspA and OspC since at least one of these proteins is usually expressed in high amounts. One limitation concerning specificity could be the use of MAb 2B8 in areas endemic for *B. turicatae*. However, instead of 2B8, a combination of other broadly reacting antibodies not reacting with *B. turicatae* could be used for specific detection of *B. burgdorferi* sensu lato.

The reactivity of a major 20-kDa-range protein of *B. turicatae* with some of our MAbs appears interesting in light of a recent publication of Cadavid et al. (8), who described a mouse model for neuroborreliosis. The authors induced typical symptoms of neuroborreliosis by infection with *B. turicatae*. They found variation in the expression of three surface proteins of 20 to 23 kDa (VmpA, VmpB, and VmpC). There appears to be a correlation between neurotropism and the type of Vmp expressed by the borreliae. The 20-kDa-range protein of *B. turicatae* recognized by our MAbs might be identical to one of these Vmps, suggesting that *B. turicatae* might have virulence-associated proteins related to OspC.

An immunological relationship between OspC and the VmpC of *B. hermsii* serotype C has been observed previously by us, using a polyclonal antibody against recombinant OspC (35). In addition, Marconi et al. (19) reported reactivity of relapsing fever borreliae with a polyclonal antibody against OspC purified from *B. burgdorferi* cells. Very recently, Carter et al. (8a) published the sequence of VmpC, now renamed Vmp33, and showed that Vmp33 is more closely related to OspC of *B. burgdorferi* than the two *B. burgdorferi* outer surface proteins OspA and OspC are to each other. Thus, it appears that OspC belongs to a family of surface-exposed 20-kDa proteins expressed by various species of the genus *Borrelia* (8a, 19, 35).

OspA (11, 25) as well as OspC (16, 22) protected animals from challenge with *B. burgdorferi* strains expressing the respective protein as a major protein. It has been shown for OspA that recombinant OspA derived from one strain did not protect against the challenge with an unrelated strain (12). The same is true for OspC (21a). MAbs have been used to identify protective epitopes of OspA (25, 26). One protective antibody, LA2 (25), turned out to be serotype specific (34). To our knowledge, other MAbs have not been investigated in this respect. The OspC-specific MAb panel established here could be used for passive protection studies. It could be investigated whether OspC has protective epitopes conserved among various strains of *B. burgdorferi* sensu lato or whether cross protection is low, as in the case of OspA.

Immunoblot analysis located the epitope of MAb 2B8 (which appears to be conserved among *B. burgdorferi* sensu lato) on the aa 55 to 212 fragment. The 2B8-specific epitope is apparently closer to the C terminus (and thus more likely to be located on a site distant from the lipid moiety anchored within the borrelial membrane) than epitopes recognized by MAbs 22C11 and 1F8. Since even 1F8 binds to the outer surface of the borrelial cell (35), it seems likely that 2B8 may also recognize surface-exposed epitopes and (like 1F8) might also be a candidate for a borrelicidal antibody.

The immunological diversity of OspC detected by analysis with MAbs is notable. However, more MAbs are necessary, and in addition, the immunological differences need to be confirmed by sequence analysis to establish an OspC serotyping system similar to the OspA serotyping system (30). The MAbs described here, however, will provide valuable tools for research on pathogenesis, vaccine development, and improvement of diagnostic tests.

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