INFECTION AND IMMUNITY, Aug. 1997, p. 3352–3360 Vol. 65, No. 8
0019-9567/97/$04.00+0

Immunologic and Genetic Analyses of VmpA of a Neurotropic Strain of *Borrelia turicatae*

DIEGO CADAVID,[1]† PAMELA M. PENNINGTON,[1] TATIANA A. KERENTSEVA,[1,2] SVEN BERGSTRÖM,[3] AND ALAN G. BARBOUR[1,2]*

Departments of Microbiology and Medicine, University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284[1]; Departments of Microbiology & Molecular Genetics and Medicine, University of California Irvine, Irvine, California 92697[2]; and Department of Microbiology, University of Umeå, Umeå, Sweden[3]

Received 13 March 1997/Returned for modification 28 April 1997/Accepted 21 May 1997

In mice infected with serotype A but not serotype B of the relapsing fever spirochete *Borrelia turicatae*, early invasion of the brain occurs. Serotypes A and B are further distinguished by the abundant surface protein they produce: VmpA and VmpB, respectively. Western blotting with monoclonal antibodies, one-dimensional peptide mapping, and partial amino acid sequencing demonstrated regions of the VmpA protein that differed from VmpB. Oligonucleotide primers based on the partial amino acid sequences of unique regions were used to amplify a portion of the VmpA gene (*vmpA*) by PCR, and the product was used as a probe in Southern blot and Northern blot analyses. These experiments showed that (i) expression of the *vmpA* sequence was determined at the level of transcription and (ii) the *vmpA* sequence was in two locations in serotype A and one location in serotype B. The *vmpA* gene at the expression-linked locus of serotype A was cloned and sequenced. An open reading frame would encode a polypeptide of 214 amino acids. The polypeptide expressed by *Escherichia coli* was bound by VmA-specific but not VmpB-specific antibody. Primer extension analysis identified a consensus σ^{70}-type promoter for *vmpA* at the expression locus. Phylogenetic analysis revealed that VmpA is homologous to small Vmp (Vsp) proteins of *B. hermsii* and to OspC proteins of *B. burgdorferi*. These findings indicate that a function of the Vsp-OspC family of proteins of *Borrelia* spp. may be differential localization in organs, including the brain, during infection.

The term "neurotropism" commonly means preferential migration to and association with the nervous system. The concept of neurotropism is well established in virology (21, 41). Spirochetes are bacterial pathogens that have long been noted for invasion of the central nervous system (CNS), and "neurotropic" was frequently used in reference to the agents of syphilis and relapsing fever, two spirochetal diseases (reviewed in reference 29). Relapsing fever *Borrelia* species and strains were often distinguished by their ability to invade the CNS (reviewed in reference 8 and 13), but little is known about what makes these and other spirochetes neurotropic.

In our studies of CNS invasion by *Borrelia* spp., we initially used *Borrelia hermsii* but, like others previously (13), found that this species was less likely to persist in the brain of mice than was *B. turicatae* (14, 15); accordingly, we used the latter species for subsequent studies of the phenomenon of neurotropism in the mouse model (15). Although *B. turicatae* consistently invades the CNS of immunocompetent mice, identification of spirochetal components that might mediate invasion would be complicated by the extensive antigenic variation that the organisms undergo to evade the immune response. Consequently, immunodeficient animals, specifically *scid* mice, have been used. In these animals, there is no detectable specific immune response against the spirochetes, and any change in the population over time appears to represent an adaptation by the microorganisms to a selection other than antibodies or T cells (15).

Within a given strain of a relapsing fever *Borrelia* sp., the different antigenic variants are called serotypes (10, 39). In our studies of clonal populations of the Ozona strain of *B. turicatae*, we found that two of its serotypes differed in the expression of the disease they caused (15). Whereas serotype A produced the aforementioned invasion of the CNS, serotype B cells did not detectably pass into the brain parenchyma even when their numbers in the blood were large. Further evidence of the more invasive phenotype of serotype A was the greater penetration of endothelial cell monolayers in vitro by serotype A than serotype B cells (15). Serotypes A and B discernibly differed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis only in a single surface protein (15, 30). Serotype A had a protein with an apparent size of 23 kDa, and in serotype B the corresponding protein was 20 kDa. In keeping with the nomenclature for the polymorphic surface proteins of other relapsing fever species (2), these proteins were called VmpA and VmpB for serotypes A and B, respectively, although there was no evidence that these proteins of *B. turicatae* were in fact homologous to the Vmp proteins of *B. hermsii.*

These studies suggested that the neurotropic character of serotype A was conferred by expression of VmpA instead of VmpB. The fact that selection of serotype A occurred in a severly immunodeficient animal suggested that the Vmp structure is a determinant not only of variable antigenicity but also of tissue tropism during infection. Investigation of the structure of VmpA may provide a basis for understanding the invasion of the brain by *B. turicatae* and perhaps the neurotropism of other spirochetes as well. As an initial step toward this goal, we further characterized VmpA with polyclonal antisera and monoclonal antibodies (MAbs), peptide mapping, and

* Corresponding author. Mailing address: Department of Microbiology & Molecular Genetics, University of California Irvine, Irvine, CA 92697-4025. Phone: (714) 824-5626. Fax: (714) 824-8598. E-mail: abarbour@uci.edu.

† Present address: Department of Neurology, Georgetown University, Washington, DC.

partial amino acid sequencing. The structural gene encoding VmpA, designated *vmpA*, was cloned and sequenced and then expressed as an immunoreactive protein in *Escherichia coli.*

MATERIALS AND METHODS

Strains and culture conditions. The origins of serotypes A, B, and C of the Oz1 strain of *B. turicatae* have been described previously (15). These serotypes at the start of the study were distinguished by the apparent size of their Vmp proteins by polyacrylamide gel electrophoresis (PAGE) as described below. The populations of serotypes A and B for this study were started from a single cell (15). *B. hermsii* HS1 serotype 33 (ATCC 35209) has been described (16); it produces the serotype-specific protein Vmp33. A clonal population of strain B312 of *B. burgdorferi* (ATCC 35210) has the outer surface protein (Osp) phenotype of $OspA^+$ $OspB^+$ $OspC^+$ $OspD^-$ (37). All borrelias were grown in BSK II broth with 12% rabbit serum and harvested as previously described (5). Spirochetes were counted in a Petroff-Hauser chamber under phase-contrast microscopy. *E. coli* cells were grown in Luria-Bertani medium containing 100 μg of carbenicillin per ml.

Antibodies. The origin of the Vmp33-specific MAb H4825 was previously described (9). The OspC-specific MAb L22B8 was the gift of Bettina Wilske (University of Munich) (44). *B. turicatae* Vmp-specific MAbs were produced for this study by intravenously inoculating BALB/c mice with 10^5 live cells of serotype A or B on day 1, treating the infected mice at peak spirochetemia with tetracycline (11), and intravenously inoculating them with 10^7 live cells on day 28. The mice were sacrificed on day 31 for collection of sera and spleens. Spleen cells were fused with NS1 myeloma cells by a modification of the method of Oi and Herzenberg (28). Hybridoma supernatant fluids were screened by an enzyme-linked immunosorbent assay with whole, wet cells (36) and by Western blotting (see below). Hybridomas secreting antibodies that bound to either serotype A or serotype B by enzyme-linked immunosorbent assay and to either VmpA or VmpB by Western blotting were selected for further study. The hybridomas were cloned by limiting dilution, and ascites from the hybridomas was produced as described previously (10). The isotypes of antibodies were determined with a commercial kit (Immunotype; Sigma Chemical Co., St. Louis, Mo.). Serotype A-specific MAb 1H12 and serotype B-specific MAb 5F12, both of which were immunoglobulin G3 were used in the present study.

PAGE and Western blot analysis. Whole-cell lysates were subjected to PAGE (12.5% acrylamide) as described previously (10). For Western blot analysis, proteins were transferred to nitrocellulose membranes (Millipore), which were then blocked with 3% (wt/vol) dried nonfat milk in 10 mM Tris (pH 7.4)-150 mM NaCl (milk-TS) for 4 h (35). After the membranes were washed three times with 0.3% milk-TS, they were incubated with hybridoma supernatants diluted 1:10 in 0.3% milk-TS. Alkaline phosphatase-conjugated recombinant protein A/G (Immunopure; Pierce Chemical Co., Rockford, Ill.) served as the second ligand. The blots were developed with nitroblue tetrazolium chloride–5-bromo-4-chloro-3-indolylphosphatase *p*-toluidine salt as substrates (Pierce).

Protein extraction, digestion, and amino acid sequencing. A fraction enriched for Vmp proteins was obtained by differential solubilization in the detergent octyl-glocopyranoside as previously described for the Vmp proteins of *B. hermsii* (11). The enriched Vmp fractions were digested with 30 μg of endoproteinase Glu-C (Boehringer-Mannheim) per ml as described previously (6), with the modification that the samples were incubated at 65°C instead of 95°C before being loaded onto the gel. The resultant mixtures of peptides were separated by PAGE and either stained with Coomassie blue or transferred to polyvinyl difluoride membranes (Millipore Corp., Bedford, Mass.). N-terminal amino acid sequences were determined by Per-Ingvar Ohlsson of Umeå University with model 477A sequenators (Applied Biosystems, Foster City, Calif.).

Nucleic acid methods. DNA was isolated from *B. turicatae* by a previously described method (14). Total RNA from borrelias was obtained as described by Meier et al. (26). Plasmid DNA from *E. coli* was obtained with a plasmid kit (QIAGEN Inc., Chatsworth, Calif.). Nucleic acid concentrations were determined in a microfluorometer (model TKO-100; Hoefer Scientific). Restriction enzymes from Boehringer-Mannheim were used as described by the manufacturer. Southern and Northern blot analyses were performed, essentially as described previously (26), onto charged nylon membranes (Nytran; Schleicher & Schuell). Probe DNA was labeled with [α-^{32}P]dATP with a random prime kit (Boehringer-Mannheim) and purified by passage through nick translation columns (Pharmacia). Hybridization with nick-translated DNA probes was carried out as described previously (14). The blots were exposed to radiographic film with an intensifying screen at −80°C. Custom primers for sequencing and PCR were synthesized on an Applied Biosystems DNA synthesizer.

DNA cloning and sequencing. Enzymatic amplification of DNA was performed in 50-μl reaction volumes containing 1.25 U of *Taq* DNA polymerase (Boehringer Mannheim) and 50 pmol of each primer in a mixture containing 200 μM each deoxynucleoside triphosphate, 10 mM Tris (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, and 0.001% gelatin. The sequences of the primers are given in Results. Reactions were carried out in a thermal cycler (Perkin-Elmer) with an initial denaturation step of 94°C for 5 min followed by 40 cycles of 94°C for 1 min, 37°C for 1 min, and 72°C for 2 min and then by a final extension cycle of 72°C for 7 min. Amplified PCR products were ligated to the cloning vector pCRII and transformed into *E. coli* INVαF′ cells (Invitrogen Corp., San Diego, Calif.). DNA restriction fragments were recovered from agarose with Gene-Clean (BIO 101, La Jolla, Calif.) and blunt-end ligated into pUC19 vector that had been digested with *Sma*I and treated with alkaline phosphatase. The ligation mixture was used to transform *E. coli* JM109 (19). The DNA sequences of inserts of recombinant plasmids were determined by the dideoxy chain termination method on double-stranded templates with Sequenase (United States Biochemical, Cleveland, Ohio) with standard M13 forward and reverse primers and custom-synthesized primers. The transcriptional start site was determined by primer extension analysis of *B. turicatae* mRNA (16).

Recombinant Vmp expression. A modification of the method of Dunn et al. (17) was used to express VmpA in *E. coli*. Based on the nucleotide sequence of the cloned insert, primers were designed for PCR amplification of the complete *vmp* gene with the exception of the sequence encoding the presumed signal peptide of the Vmp. The cysteine residue of the presumed processed protein was replaced by methionine-alanine. For PCR amplification, the Expand Long Template PCR system (Boehringer Mannheim) was used as described by the manufacturer. To the forward and reverse primers were added *Nde*I and *Bam*HI sites, respectively; the sequences of the primers are given in Results. The amplified product was cloned into pCRII, and the insert was sequenced as described above to confirm its identity. The recombinant *vmpA* gene was excised from the pCRII vector with *Nde*I and *Bam*HI and then cloned into the plasmid vector pET-15b (Novagen, Madison, Wis.), which had been digested with those enzymes. The new plasmid construct was cloned into *E. coli* HMS174(DE3) and then into strain BL21(DE3) (Novagen). The recombinant *E. coli* isolates were grown at 37°C in Luria-Bertani broth medium containing 2% glucose until a cell density giving an absorbance of 0.550 at 600 nm was obtained. Isopropyl-β-D-thiogalactopyranoside (IPTG) at a final concentration of 1 mM was added, and incubation was continued for another 2 h. The cells were harvested by centrifugation at 6,000 × *g* for 20 min at 4°C, washed with phosphate-buffered saline containing 23 mg of phenylmethylsulfonyl fluoride per ml, and pelleted again by centrifugation. The pellets were frozen at −70°C, thawed, resuspended in 10 mM sodium phosphate (pH 7.4)–150 mM NaCl–10 mM EDTA–0.1% lysozyme, and sonicated on ice with a Branson Sonifier at 70% output for 40 s. The suspension was centrifuged at 12,000 × *g* for 10 min at 4°C, and the supernatant was subjected to PAGE and Western blot analysis as described above.

Sequence analysis. Deduced protein sequences were submitted as query sequences for BLASTP analysis with the nonredundant protein database at the National Center for Biotechnology Information (1). For comparison of protein sequences, the following *Borrelia* sequences (with accession numbers in parentheses) were used: *B. burgdorferi* sensu stricto Osp proteins A and B (X14407), D (M97452), E (L13924), and F (L13925); *B. burgdorferi* OspC proteins from strains B31 (X69596), 297 (U08284), DN127 (U04280), 25015 (U04282), 2591 (U01892), and CA11 (L25413); *B. anserina* OspC proteins from strains PKo (X62162) and ACA1 (L42892); *B. garinii* OspC proteins from strains PHei (X83553), Ip90 (L42886), SL14 (X84784), and HT37 (D49381); *B. hermsii* Vmp proteins 1 (L33870), 11 (L33900), 13 (L33901), 17 (L04788), 2 (L33897), 21 (M57256), 24 (L04786), 26 (L26497), 3 (L04789), 33 (L24911), 7 (X53926), and 8 (L33899); and *B. miyamotoi* sp. nov. Vmp protein (D78201). Pairwise comparisons of sequences were carried out with the GAP algorithm of the Wisconsin Package (version 8.1; Open VMS) of software programs from Genetics Computer Group, Inc. (Madison, Wis.) Multiple sequences were aligned with the aid of the Higgins-Sharp (20) routine, using default values of the MacDNASIS Pro suite (version 3.6) of programs from Hitachi Software (San Bruno, Calif.). Phylogenetic analyses of aligned sequences, which were in randomized data sets with 1,000 bootstrap trials, were carried out with the CLUSTAL W algorithm and expressed as unrooted trees, in which the computed evolutionary distance between proteins was proportional to the lengths of the branches between them (40) (Biology Workbench version 1.5; National Center for Supercomputing Applications, University of Illinois at Urbana-Champaign). Overall hydrophobicity vlues were determined by the Kyte-Doolittle procedure (24). Predictions of membrane-spanning or -associated regions of proteins were made with the TMpredict algorithm (Bioinformatics Group, Swiss Institute for Experimental Cancer Research).

Nucleotide sequence accession number. The sequence of *vmpA* has been assigned GenBank accession no. U85413.

RESULTS

Reactivities with antibodies. In the first description of isogenic serotypes A and B of *B. turicatae*, the abundant proteins VmpA and VmpB, respectively, were shown to differ in their apparent molecular weights (15). Inasmuch as these serotypes had been isolated from severely immunodeficient mice, it was possible that despite their dissimilar sizes the two proteins were antigenically similar. Conceivably, VmpB was a truncated form of the larger VmpA. To further characterize these different proteins, serotypes A and B were reacted with monoclonal antibodies. The antibodies were obtained from mice

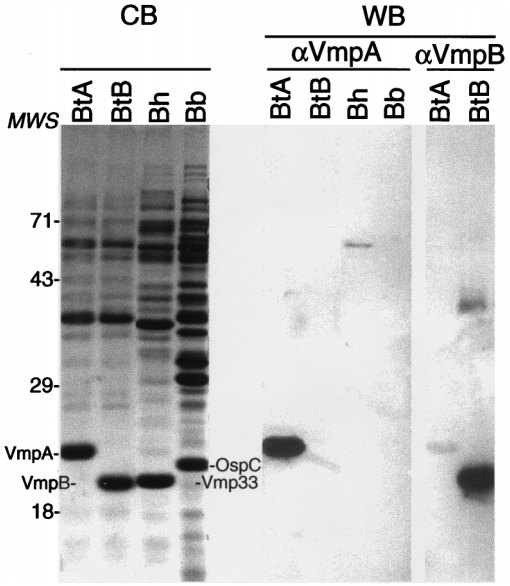

FIG. 1. Coomassie blue-stained 12.5% acrylamide gel (CB) and Western blot analysis (WB) of whole-cell lysates of *B. turicatae* Oz1 serotype A (BtA), *B. turicatae* serotype B (BtB), *B. hermsii* HS1 serotype 33 (Bh), and *B. burgdorferi* B312 (Bb) with MAb to serotype A (αVmpA) or MAb to serotype B (αVmpB). Molecular mass standards (MWS) in kilodaltons are shown on the left. The locations of VmpA, VmpB, Vmp33, and OspC in the gel are indicated.

infected with either serotype A or B. Also included in the analysis were *B. hermsii* serotype 33 and *B. burgdorferi* expressing OspA, OspB, and OspC. OspC of *B. burgdorferi* and Vmp33 of *B. hermsii* are homologous and are the same size range as VmpA and VmpB of *B. turicatae* (16).

The protein profiles of whole-cell lysates of these isolates are shown in the leftmost panel of Fig. 1. Serotypes A and B had the expected differences in the size of their most abundant cellular protein (15). The OspC protein of *B. burgdorferi* migrated at a rate between those of VmpA and VmpB. The MAb selected for their serotype specificity by whole-cell ELISA bound either to VmpA or VmpB but not to both. MAb to serotype B also produced faint bands with a larger polypeptide, which at approximately twice the size of VmpB may be a dimeric form of the protein. MAb to serotype A produced a faint band of approximately 60 kDa in *B. hermsii* but did not detectably bind to Vmp33 or OspC (Fig. 1). MAbs to Vmp33 of *B. hermsii* and OspC of *B. burgdorferi* did not detectably bind to VmpA or VmpB by Western blot analysis (data not shown).

Peptide mapping and amino acid sequencing. The experiments with antibodies indicated that there were Vmp-specific epitopes in VmpA and VmpB. To further characterize the primary structure of these proteins, one-dimensional peptide mapping of Vmp-enriched fractions was carried out. If VmpB was a truncated VmpA protein, we expected that the peptide maps would be the same except for one peptide, which would be smaller in VmpB than in VmpA.

The protein enrichment procedure yielded preparations of Vmp proteins which were substantially free of other proteins (Fig. 2). When these Vmp-enriched fractions were treated with endoproteinase GluC, which cleaves at the carboxy-terminal side of glutamate residues, partially proteolyzed products were obtained with both VmpA and VmpB. Digestion appeared to be more complete with VmpB than with VmpA, but with each protein the sum of the sizes of the proteolytic products was greater than that of the untreated Vmp, an indication that proteolysis was partial in both samples.

Three peptides that were subsequently sequenced from their amino termini for various extents were numbered I, II, and III. Peptide II, which had a size of 15 kDa, appeared to be the same in both VmpA and VmpB. Peptide I (13 kDa) and peptide III (10 kDa) were unique to VmpA and VmpB, respectively. The VmpA digest also had unique peptides of 9 and 7 kDa. VmpB had a unique peptide of 6 kDa. The dissimilarity of the peptide maps, together with the antibody experiments, indicated that VmpB was not a truncated version of VmpA.

No sequence was obtained when untreated VmpA protein was subjected to analysis, an indication that the amino terminus was blocked (11, 16, 27). The following sequences were obtained from the isolated proteolytic products: peptide I of VmpA, TDADKNAKLISGAYSVI; peptide II of both VmpA and VmpB, LAKAIGK; and peptide III of VmpA, NDAID ALKARTDLTQ. Comparison of these sequences with known Vmp and Osp proteins of *Borrelia* spp. revealed that peptide I was 64% identical to residues 90 to 106 of Vmp26 of *B. hermsii*; peptide II was 100% identical to residues 71 to 77 of Vmp3, Vmp24, and Vmp26 of *B. hermsii*; and peptide III was 56% identical to residues 181 to 196 of Vmp3 of *B. hermsii*. These results indicated that VmpA and VmpB were members of the "20-kDa" family of surface-exposed proteins of *Borrelia* spp. (16, 25) but also that VmpA was different from known Vmp and Osp proteins sequenced to date.

Expression of *vmpA* is associated with a unique restriction fragment. We concluded that the amino-terminal sequence peptide I of VmpA probably was located in the middle of the protein and designed the following reverse primer (Bt1) on the basis of its partial amino acid sequence and codon usage of other *Borrelia* spp. genes (4): 5′ATATGCCCTACTAATTAA TTTAGCATTTTTATCAGCATC3′. For the forward oligonu-

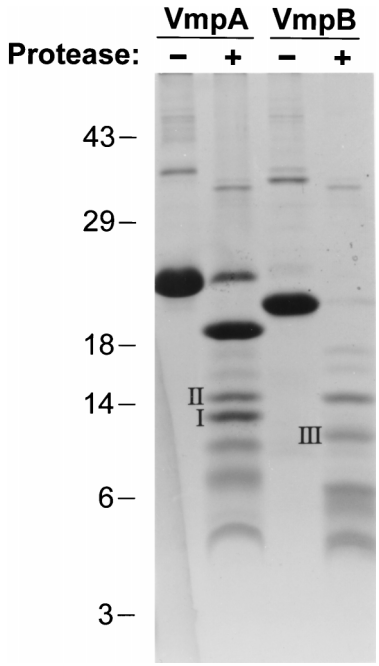

FIG. 2. Coomassie blue-stained 20% acrylamide gel of extracted VmpA or VmpB that had been incubated prior to electrophoresis with (+) or without (−) endoproteinase GluC (Protease). The locations of molecular mass standards in kilodaltons are shown on the left. I, II, and III indicate selected peptides that were subsequently subjected to amino acid sequence analysis (see the text).

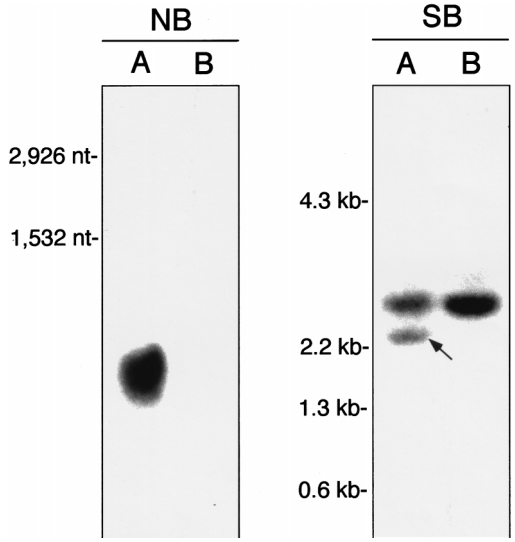

FIG. 3. Northern blot (NB) and Southern blot (SB) analyses of total RNA and DNA, respectively, from *B. turicatae* serotype A and serotype B cells with a probe for the *vmpA* gene (see the text). In the Northern blot (NB), the probe bound to RNA from serotype A but not serotype B. The sizes of 23S (2,926 nucleotides [nt]) and 16S (1,532 nucleotides) rRNAs of *Borrelia* are indicated to the left of the Northern blot (36). For the Southern blot, the DNA was digested with *Dra*I before electrophoresis and transfer to the membrane. The arrow indicates a hybridizing restriction fragment unique to serotype A. The migrations of size standards in kilobases are shown to the left of the Southern blot.

cleotide primer (Bt3; 5′AAGTGCGATAATAATGACTTTA TT3′), we assumed that the putative signal peptides of VmpA and VmpB would have the same or highly similar sequences to the sequence SAILMTLF found at the amino termini of other members of the 20-kDa family of *Borrelia* proteins (16, 25).

With primers Bt1 and Bt3 for PCR with *B. turicatae* serotype A DNA, the expected 280-bp product was obtained (data not shown). The PCR product was ligated into a plasmid vector, the resultant DNA was transformed into *E. coli*, and the cloned insert was sequenced. The deduced sequence of the cloned insert had an open reading frame encoding 93 amino acids, including the peptide II sequence LAKAIGK for amino acids 74 to 80 (data not shown).

This cloned fragment, having been shown to encode an amino acid sequence identified in VmpA, was used to determine whether the sequence was transcribed in serotype A by Northern blotting and, if so, to locate the *vmpA* gene by Southern blot analysis (Fig. 3). Total RNA was extracted from serotype A or B cells and probed with the labeled fragment after electrophoresis under denaturing conditions and transfer to a membrane (Fig. 3, left panel). The probe bound to a band in RNA from serotype A but not detectably to RNA from serotype B, an indication that the sequence was specific for expression of the *vmp* gene in serotype A.

We next prepared plasmid-enriched DNA samples from serotype A and B to identify restriction fragment length polymorphisms between the two serotypes. DNA samples were digested with the restriction enzyme *Dra*I and then probed in a Southern blot with the labled fragment for *vmpA* (Fig. 3, right panel). The probe bound to a 2.4-kb *Dra*I fragment that was present in serotype A but not serotype B DNA. Both serotypes A and B had a 3.0-kb *Dra*I fragment in common that was hybridized by the probe. These findings indicated that there was an expression-linked version of the *vmpA* gene sequence.

Characterization of the expression-linked copy of *vmpA*. The 2.4-kb *Dra*I fragment unique to serotype A was ligated into a plasmid vector and sequenced. The sequence was confirmed by direct sequencing of a PCR product that was obtained from serotype A but not serotype B DNA. The sequence revealed an open reading frame, beginning at position +20 and ending at position 661, that would encode a polypeptide of 214 amino acids (Fig. 4). The open reading frame was preceded by a consensus ribosomal binding sequence (GGAGG). The identity of this deduced protein with VmpA was confirmed by locating the nucleotides encoding two partial amino acid sequences within the open reading frame. A sequence encoding the consensus signal peptidase II site, LISC, was present at positions 16 to 19 of the open reading frame (43). Following the TAA stop codon at positions 662 to 664 were two other TAA stop codons in the same reading frame and two possible rho-independent terminators (positions 676 to 701 and 748 to 774).

```
  -40   "-35"          -20      "-10"         +1     RBS
AAAAAAAATTGAAAAATCGAATTTTTGAAATAAAATTTTGAGTTAATAAGGAGGCACGTA

  20                   40       >Bt3>        60
ATAATGAAAAGAATTACTTTAAGTGCGTTATTAATGACTTTATTTTTACTTATCTCTTGT
   M  K  R  I  T  L  S  A  L  L  M  T  L  F  L  L  I  S  C

  80                   100                  120
AATAATTCAGGAACTTCTCCTAAAGATGGGCAAGCAGCTAAATCTGATGGCACTGTTATT
N  N  S  G  T  S  P  K  D  G  Q  A  A  K  S  D  G  T  V  I

 140                   160                  180
GACCTAGCTACAATAACTAAAAACATTACCGATGCTGTTGCTTTTGCTAAGAGTGTTAAA
D  L  A  T  I  T  K  N  I  T  D  A  V  A  F  A  K  S  V  K

 200                   220                  240
GACGTTCATACTTTAGTTAAATCCATTGATGAGCTTGCTAAAGCTATCGGGAAAAAAATT
D  V  H  T  L  V  K  S  I  D  E  L  A  K  A  I  G  K  K  I

 260                   280        <Bt1<     300
GGTGCCAATGGTCTTGAAACTGATGCTGATAAGAATGCAAAATTAATTTCAGGAGCATAT
G  A  N  G  L  E  T  D  A  D  K  N  A  K  L  I  S  G  A  Y

 320                   340                  360
AGTGTAATATCAGCTGTAGATACTAAATTAGCATCATTAGAAAAAAAAAGTTGGAATTTCT
S  V  I  S  A  V  D  T  K  L  A  S  L  E  K  K  V  G  I  S

 380                   400                  420
GATGACTTAAAGGGAAAAATTACTACTGTTAAGAATGCAAGTACATCCTTTTTAACCAAG
D  D  L  K  G  K  I  T  T  V  K  N  A  S  T  S  F  L  T  K

 440                   460                  480
GCTAAATCAAAGACAGCTGATCTTCGTAAAGATGATGTTAAGGATGCTGATGCGAAGACA
A  K  S  K  T  A  D  L  R  K  D  D  V  K  D  A  D  A  K  T

 500                   520                  540
GCTATAGATATAGCAGATACTGGAGCCAAGGATAAAGGCGCGGAAGAGCTTATTAAACTC
A  I  D  I  A  D  T  G  A  K  D  K  G  A  E  E  L  I  K  L

 560                   580                  600
AATACAGCAATTGATGCTTTGTTAACTTCTGCTGAAGCTGCAGTAACAGCTGCAATAAAT
N  T  A  I  D  A  L  L  T  S  A  E  A  A  V  T  A  A  I  N

 620                   640                  660
GCGCTTTCAACTCCTGCTAAGTCAGCATCTACTGTTCAATCTAACTAAGGATAAACAAGT
A  L  S  T  P  A  K  S  A  S  T  V  Q  S  N  *     *     >>>

 680                   700                  720
TAATTTATTATTATAAGATTACTTTTTAATTAATCGTAATTATCTGATAAAATAAAGTCT
*> >>>>>    <<<<< < <<<
 740                   760                  780
ATAAATAATAAGCTAGGAGTTTTTTTCTCTTAGCTTTTTTTGTTTCTTTATTCTTTCTCT
         >>>>>>>>>>       <<<<<<<<<<
```

FIG. 4. DNA sequence and deduced amino acid sequence of the expressed *vmpA* gene of *B. turicatae*. Nucleotides are numbered relative to the transcription initiation site (+1). Putative −35 and −10 boxes, ribosomal binding sequence (RBS), termination codons (asterisks), and complementary regions consistent with hairpin loop formation (>>>>> and <<<<<) are indicated. The underlined nucleotide sequences correspond to oligonucleotide primers Bt3 (forward) and Bt1 (reverse). The underlined amino acids correspond to the sequences obtained from proteolytic cleavage fragments of the VmpA protein.

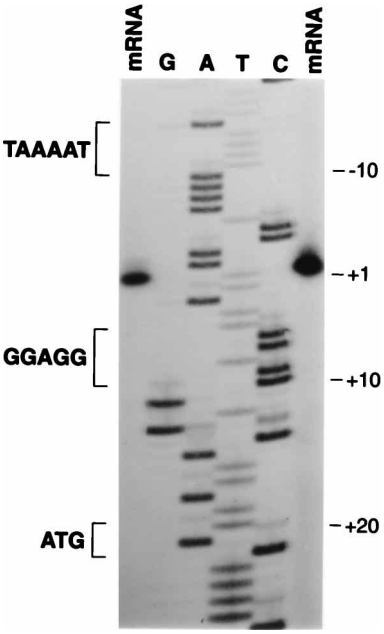

FIG. 5. Primer extension analysis of the 5′ terminus of the expressed *vmpA* gene of *B. turicatae* Oz1. The oligonucleotide used was Bt1 (see the text). The primer was annealed to the mRNA, and the DNA was extended with reverse transcriptase. The resulting primer extension product is in the leftmost and rightmost lanes (mRNA) and is indicated by +1. A dideoxynucleotide sequencing reaction was carried out in parallel with the primer extension reaction, yielding a sequence complementary to the coding strand. The locations of the −10 box (TAAAAT), a consensus ribosomal binding sequence (GGAGG), and the start codon (ATG) are indicated. For convenience, these designations refer to sequences that would be on the strand opposite that shown under lanes G, A, T, and C.

Figure 5 shows the results of the primer extension analysis. The probable transcriptional site is an A, designated position +1 in Fig. 4. At positions −13 to −7 was the likely −10 element (TAAAAT) of a σ^{70}-type promoter. This was preceded at positions −36 to −31 by the hexanucleotide TTGA AA, which is consistent in sequence and spacing with a −35 element of a promoter. Just upstream of this element and terminating in the *Dra*I recognition sequence of TTTAAA was a homopolymeric run of 8 A's.

Recombinant protein is bound by VmpA-specific antibody. Further confirmation of the identity of the cloned open reading frame was obtained by expression of the gene product in *E. coli*. For this study, PCR primers were designed to eliminate the presumed signal peptide, and the product was cloned into an expression vector under *lac* control. The forward and reverse primers were 5′TTTTTACTTCATATGGCCAATAAT TC3′ and 5′TAAATTAACTGGATCCTTATTAGTTAG3′, respectively. The forward primer spanned nucleotides +59 to 84 of the sequence in Fig. 4 but provided for a methionine-alanine in place of serine-cysteine in the encoded protein. The culture yielded an abundant protein with an estimated size of 27 kDa in the induced cells (Fig. 6). The greater size of the recombinant product is consistent with the histidine-rich leader peptide provided by the pET15b vector to the combined fusion protein. In Western blot analysis, this protein was bound by MAb to serotype A but not by MAb to serotype B (Fig. 6) or to Vmp33 or OspC (data not shown).

VmpA is a member of the small Vmp/OspC family. By BLAST analysis, the amino acid sequence of VmpA was highly similar (smallest sum probability of less than 10^{-20}) to that of small Vmp proteins of *B. hermsii*, a Vmp protein of *B. miyamotoi* sp. nov. (18), and OspC proteins of *B. burgdorferi* sensu lato. By pairwise comparisons with the GAP algorithm, VmpA was more than 40% identical to these proteins. On the other hand, by these two analyses there was no discernible homology (smallest sum probability of greater than 0.2) to OspA, OspB, OspD, OspE, or OspF of *B. burgdorferi*; to the large Vmp proteins, such as Vmp7, Vmp17, and Vmp21 of *B. hermsii*; or to any other protein in the database.

Small Vmp and OspC proteins are highly variable in the middle thirds of their sequences (16, 25); alignment of sequences is difficult because of the infrequency of common residues. Accordingly, phylogenetic analysis was carried out with the more highly conserved N termini and C termini of these proteins. For VmpA, residues 1 to 80 and 164 to 214 were compared to the corresponding regions of small Vmp and OspC proteins of other *Borrelia* spp. Alignments of these regions are shown in Fig. 7. Without gaps, the presumptive signal peptide of VmpA (MKRITLSALLMTLFLLLSC) was identical at 15 of 19 residues to those of Vmp33 and OspC of *B. burgdorferi*. Even with the introduction of gaps, only 9 of these positions were identical to the first 19 residues of the other small or large Vmps of *B. hermsii*. On the other hand, the C-terminal tripeptide of VmpA, QSN, was more like the QNN that terminates most small Vmps of *B. hermsii* than it was to the tripeptides KKP or VKP that terminate OspC and Vmp33, respectively.

Figure 8 shows that in unrooted trees of both the N-terminal and C-terminal regions, VmpA of *B. turicatae* occupied a middle position between clusters of OspC proteins of the Lyme disease agents *B. burgdorferi*, *B. anserina*, and *B. garinii* and several small Vmp proteins of *B. hermsii*. In these relationships, VmpA resembled Vmp33 of *B. hermsii* and the small Vmp protein of *B. miyamotoi.*

VmpA is more hydrophobic than other Vmp or OspC proteins. The association of VmpA with invasion of the brain

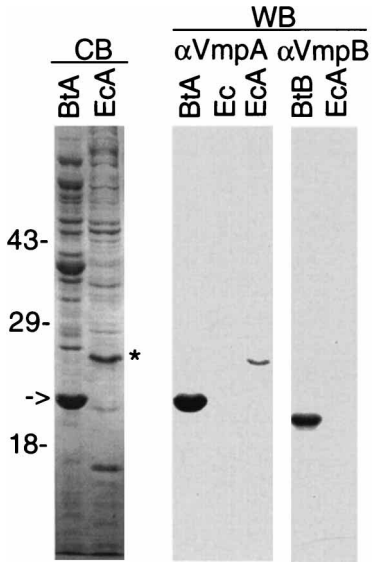

FIG. 6. Coomassie blue-stained 12.5% acrylamide gel (CB) and Western blot analysis (WB) of whole-cell lysates of *B. turicatae* serotype A (BtA), *B. turicatae* serotype B (BtB), and *E. coli* with (EcA) or without (Ec) the recombinant *vmpA* gene. The WB was incubated with MAbs to VmpA (αVmpA) or VmpB (αVmpB). The locations of molecular mass standards in kilodaltons are shown on the left. The arrow indicates the location of native VmpA of *B. turicatae*. The asterisk indicates the location of the fusion protein containing VmpA.

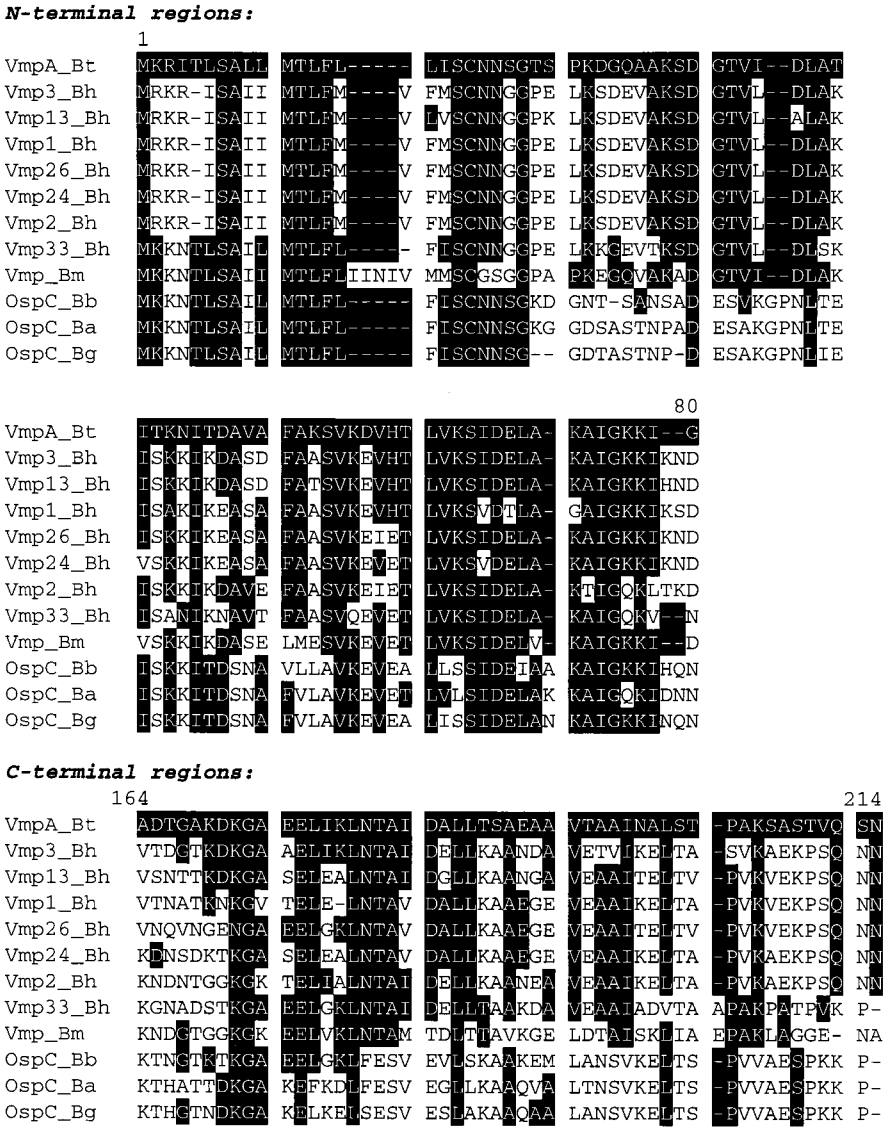

FIG. 7. Alignments of the N-terminal (top) and C-terminal (bottom) regions of selected small Vmp and OspC proteins of *B. turicatae* (Bt), *B. hermsii* (Bh), *B. miyamotoi* sp. nov. (Bm), *B. burgdorferi* (Bb), *B. afzelii* (Ba), and *B. garinii* (Bg). Sources of the sequences are given in the text. Numbers above the sequences refer to the amino acid positions of VmpA of *B. turicatae*. Dashes indicate gaps that have been introduced for alignments. Inverted characters are used for the VmpA sequence and for an identical residue or gap in another sequence at each position.

prompted consideration of possible characteristics of the protein that would facilitate passage across the blood-brain barrier. Hydrophobocity is one such characteristic (38). To measure overall hydrophobicity of the processed proteins (residues 19 to 214 for VmpA), Kyte-Doolittle values were calculated and compared with selected small Vmp and OspC proteins of other *Borrelia* species (Table 1). With a hydrophobicity value of −0.10, VmpA was more than 2 standard deviations more hydrophobic by this prediction than 22 small Vmp and OspC proteins representing different *Borrelia* spp. By further comparison, mature VmpA was also more hydrophobic than Vmp7 (−0.18) and Vmp17 (−0.18) of *B. hermsii* and OspA (−0.60), OspB (−0.72), and OspD (−0.72) of *B. burgdorferi*. The C terminus of VmpA was particularly hydrophobic. By using the TMpredict algorithm for the detection of membrane-associated regions of proteins, residues 176 to 201 of VmpA exceeded the threshold value of +0.0 for this prediction. None of the other 22 small Vmp and OspC proteins exceeded the threshold for this or any other part of the lengths of their mature protein sequences.

DISCUSSION

Unequivocal identification of a DNA sequence with a particular protein in relapsing fever *Borrelia* spp. can be difficult, largely because of the extensive repertoire of complete and incomplete *vmp* genes in the genome of these organisms (2). *B. hermsii* has at least 30 different *vmp* gene sequences, sharing various degrees of sequence identity with one another (12, 16, 32, 34). An oligonucleotide probe or MAb may bind to more than one *vmp* sequence or Vmp protein (3, 26). Furthermore, studies of *B. hermsii* have shown that the *vmp* gene at the expression site may change after a DNA rearrangement (22, 31). What was at the expression site at the start of an experi-

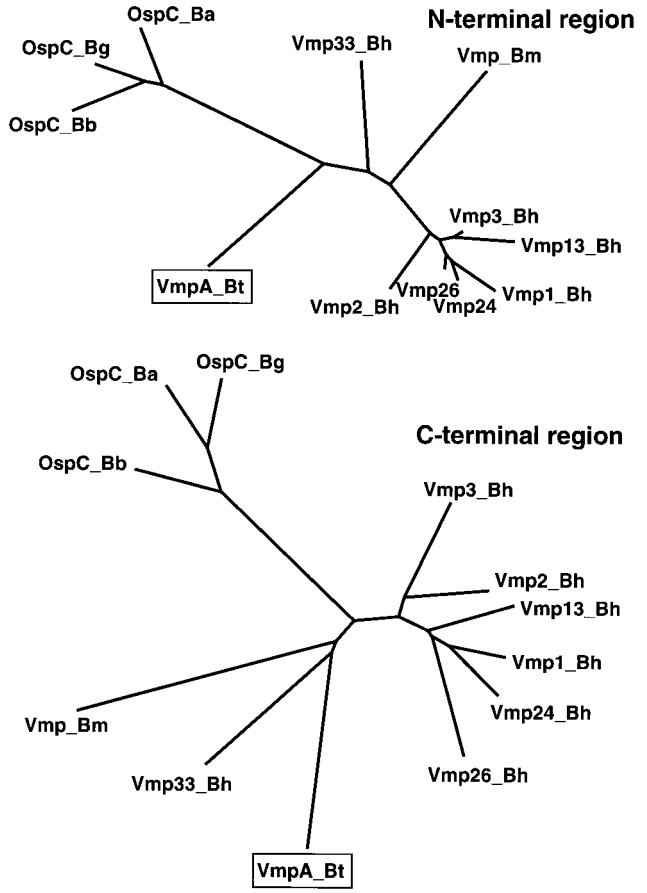

FIG. 8. Unrooted phylogenetic trees (CLUSTAL W algorithm) of the N-terminal and C-terminal regions of small Vmp and OspC proteins of *B. turicatae* Oz1 (Bt), *B. hermsii* HS1 (Bh), *B. miyamotoi* sp. nov. (Bm), *B. burgdorferi* B31 (Bb), *B. afzelii* ACA1 (Ba), and *B. garinii* PHei (Bg). In the case of VmpA of *B. turicatae*, residues 1 to 80 and 164 to 214 (Fig. 4) and aligned sequences of the other proteins were used for the N-terminal and C-terminal regions, respectively (Fig. 7). The lengths of the branches correspond to the predicted evolutionary distances between sequences.

ment may be replaced sooner or later by another *vmp* gene either in an animal or in the culture tube (39). We reduced the risk of this latter phenomenon through use of *scid* mice, thus removing the selective pressure of the immune system. But even in the absence of a specific immune response, there are shifts in the predominant Vmp protein expressed in a population (15). A second hindrance to Vmp studies is that expression of the Vmp protein in *E. coli* may be deleterious enough to host cells to be lethal (16, 26).

In the present study, we sought further information about VmpA of *B. turicatae* and presumed that this protein was also a member of a polymorphic group of proteins. Mindful of the possibilities for error and failure when cloning *vmp* genes, we required the following for correct identification of the gene: (i) that the deduced protein sequence contain the partial amino acid sequences found in the native VmpA protein and (ii) that the recombinant gene product be bound by antibody to VmpA. These criteria were met in the present study.

Northern and Southern blot analyses provided additional evidence that the deduced protein sequence from the cloned DNA was in fact VmpA. A DNA probe representing the cloned sequence bound to RNA of serotype A but not serotype B. The same probe also bound to a restriction fragment that was unique to serotype A. By analogy with *B. hermsii*, this fragment contained the expression-linked sequence (22, 26, 31). This was further indicated by demonstration of a σ^{70}-type promoter in the sequence upstream of the VmpA-encoding region and by primer extension analysis of mRNA from serotype A. In *B. turicatae*, as in *B. hermsii* (7), succession of a new expressed *vmp* gene may occur through a recombination that places a previously silent gene downstream of a promoter at a unique expression site. Confirmation of this model in *B. turicatae* awaits characterization of the silent site for *vmpA* and both the expression and silent versions of another *vmp* gene.

Our studies of *B. turicatae* have focused on differences in disease expression and, in particular for this study, the neurotropism of serotype A cells. The finding that serotypes detectably differing in a single protein also differ in their ability to invade the brain prompted this analysis of VmpA. We have been concerned about the function of VmpA more in terms of its interaction with host cells and tissues than with respect to VmpA as a variable antigen. After all, serotypes A and B were originally isolated from immunodeficient mice. Nevertheless, the findings from this study indicate that VmpA and VmpB are antigenically distinct from each other and from homologous proteins of other *Borrelia* spp. Neither antibody bound to Vmp33 of *B. hermsii* or OspC of *B. burgdorferi.*

Sequencing of its gene confirmed the differences between VmpA and other *Borrelia* surface proteins but also revealed similarities, notably to what have been called the small Vmp proteins of *B. hermsii* and the OspC proteins of *B. burgdorferi*

TABLE 1. Kyte and Doolittle hydrophobicity values for processed small Vmp and OspC proteins of *Borrelia* species

Protein[a]	Hydrophobicity value[b]
Vmp1[c]	−0.16
Vmp2[c]	−0.34
Vmp3[c]	−0.27
Vmp8[c]	−0.41
Vmp11[c]	−0.31
Vmp13[c]	−0.15
Vmp24[c]	−0.42
Vmp26[c]	−0.21
Vmp33[c]	−0.18
Vmp[d]	−0.22
OspC (B31)[e]	−0.47
OspC (297)[e]	−0.36
OspC (2591)[e]	−0.37
OspC (CA11)[e]	−0.31
OspC (DN127)[e]	−0.37
OspC (25015)[e]	−0.56
OspC (PKo)[f]	−0.38
OspC (ACA1)[f]	−0.50
OspC (PHei)[g]	−0.38
OspC (Ip90)[g]	−0.39
OspC (HT37)[g]	−0.38
OspC (SL14)[g]	−0.43
Mean (± 2 SD)	−0.34 (−0.12–0.56)
VmpA[h]	−0.10

[a] Strain names are in parentheses.
[b] Hydrophobic amino acids in the calculation have individual values of ≥+1.80; hydrophilic amino acids have values of ≤−0.70 (27).
[c] *B. hermsii.*
[d] *B. miyamotoi.*
[e] *B. burgdorferi.*
[f] *B. afzelii.*
[g] *B. garinii.*
[h] *B. turicatae.*

sensu lato (16, 25). Beyond its presumptive signal peptide, VmpA was most similar, by the criteria of identical residues and gaps, to a Vmp protein of unknown function of *B. miyamotoi* sp. nov., a *Borrelia* species that has been isolated from ixodid ticks in Japan (18). The grouping of VmpA of *B. turicatae* with small Vmp proteins of both *B. hermsii* and *B. miyamotoi* sp. nov. and not with the large Vmp proteins of *B. hermsii* leads us to question the terminology for the different variable proteins of *Borrelia* spp. The first descriptions of Vmp proteins included within that group proteins of about 20 kDa as well as those of about 35 to 40 kDa (6, 10, 11). The first sequence determinations of *vmp* genes indicated that small and large unprocessed Vmps had nearly identical N-termini (12, 34). More recently, however, we have found that the shared N termini may be the result of an intragenic recombination (23, 33). Some unprocessed small Vmps, such as Vmp33 (16) and VmpA in the present study, have N-terminal sequences that are unlike those of large Vmps (Fig. 7). Inasmuch as sequences of the processed small and large Vmp proteins are not discernibly homologous, we propose that the small Vmps of relapsing fever *Borrelia* spp. and their close relatives, such as *B. miyamotoi*, be termed Vsps (for variable small proteins). Correspondingly, the large Vmps would be called Vlps (for variable large proteins), and, thus, one would speak of the Vsp-OspC family of proteins and the Vlp family of proteins.

Finally, we ask whether the further characterization of VmpA provides insight into the association of expression of this protein with invasion of the nervous system. Comparison of short regions of VmpA with the protein database did not reveal the presence of motifs for specific cell or molecular binding, such as the tripeptide RGD and fibronectin binding. One feature of VmpA that distinguished it from other members of the family was its more hydrophobic nature, particularly near its C terminus. However, the significance of this structural feature for pathogen-host interactions remains to be determined.

The present study, by placing VmpA, and presumably VmpB as well, into a group of proteins, indicates that one function of this protein family is a specific host cell association or tissue localization. In the case of serotype A, that function is penetration into the brain (15). In the case of serotype B, it is achieving high numbers of microorganisms in the blood (30). Extrapolating, we also suggest that the small Vmp proteins of *B. hermsii* and the OspC proteins of *B. burgdorferi* may be distinguishable not only on the basis of their reactivities with antibodies but also on the basis of their host cell associations. For instance, the apparent differences in clinical manifestations of Lyme disease depending on the genospecies of the infecting agent may be attributable to differences in OspC proteins (42, 44).

ACKNOWLEDGMENTS

We thank Jill Schurr for expert technical assistance and Carol Carter for advice.

This work was supported by NIH grant AI24424 to A.G.B. and a Swedish Medical Research grant to S.B.

REFERENCES

1. **Altschul, S., W. Gish, W. Miller, E. Myers, and D. Lipman.** 1990. Basic local alignment search tool. J. Mol. Biol. **215:**403–410.
2. **Barbour, A.** 1990. Antigenic variation of a relapsing fever *Borrelia* species. Annu. Rev. Microbiol. **44:**155–171.
3. **Barbour, A.** 1985. Clonal polymorphisms of surface antigens in a relapsing fever Borrelia spp., p. 235–245. *In* G. Jackson (ed.), Pathogenesis of bacterial infection. Springer-Verlag KG, Heidelberg, Germany.
4. **Barbour, A., C. Carter, V. Bundoc, and J. Hinnebusch.** 1996. The nucleotide sequence of a linear plasmid of *Borrelia burgdorferi* reveals similarities to those of circular plasmids of other prokaryotes. J. Bacteriol. **178:**6635–6639.
5. **Barbour, A. G.** 1984. Isolation and cultivation of Lyme disease spirochetes. Yale J. Biol. Med. **57:**521–525.
6. **Barbour, A. G., O. Barrera, and R. C. Judd.** 1983. Structural analysis of the variable major proteins of *Borrelia hermsii*. J. Exp. Med. **158:**2127–2140.
7. **Barbour, A. G., N. Burman, C. J. Carter, T. Kitten, and S. Bergström.** 1991. Variable antigen genes of the relapsing fever agent *Borrelia hermsii* are activated by promoter addition. Mol. Microbiol. **5:**489–493.
8. **Barbour, A. G., and S. F. Hayes.** 1986. Biology of *Borrelia* species. Microbiol. Rev. **50:**381–400.
9. **Barbour, A. G., S. L. Tessier, and S. F. Hayes.** 1984. Variation in a major surface protein of Lyme disease spirochetes. Infect. Immun. **45:**94–100.
10. **Barbour, A. G., S. L. Tessier, and H. G. Stoenner.** 1982. Variable major proteins of *Borrelia hermsii*. J. Exp. Med. **156:**1312–1324.
11. **Barstad, P. A., J. E. Coligan, M. G. Raum, and A. G. Barbour.** 1985. Variable major proteins of Borrelia hermsii. Epitope mapping and partial sequence analysis of CNBr peptides. J. Exp. Med. **161:**1302–1314.
12. **Burman, N., S. Bergström, B. I. Restrepo, and A. G. Barbour.** 1990. The variable antigens Vmp7 and Vmp21 of the relapsing fever Borrelia hermsii are structurally analogous to the VSG proteins of the African trypanosome. Mol. Microbiol. **4:**1715–1726.
13. **Cadavid, D., and A. G. Barbour.** Neurologic manifestations of relapsing fever: comparative, clinical, and pathologic aspects. Clin. Infect. Dis., in press.
14. **Cadavid, D., V. Bundoc, and A. Barbour.** 1993. Experimental infection of the mouse brain by a relapsing fever Borrelia species: a molecular analysis. J. Infect. Dis. **168:**143–151.
15. **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.
16. **Carter, C. J., S. Bergstrom, 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.
17. **Dunn, J. J., B. N. Lade, and A. G. Barbour.** 1990. Outer surface protein (OspA) from the Lyme disease spirochete, *Borrelia burgdorferi*: high level of expression and purification of a soluble recombinant form of OspA. Protein Expression Purif. **1:**159–168.
18. **Fukunaga, M., Y. Takahashi, Y. Tsurata, O. Matsushita, D. Ralph, M. McClelland, and M. Nakao.** 1995. Genetic and phenotypic analysis of *Borrelia miyamotoi* sp. nov., isolated from the ixodid tick *Ixodes persulcatus*, the vector of Lyme disease in Japan. Int. J. Syst. Bacteriol. **45:**804–810.
19. **Hanahan, D.** 1983. Studies of transformation of *Escherichia coli* with plasmids. J. Mol. Biol. **166:**557–580.
20. **Higgins, D., and P. Sharp.** 1988. CLUSTAL: a package for performing multiple sequence alignment on a microcomputer. Gene **73:**237–244.
21. **Kennedy, P.** 1992. Molecular studies of viral pathogenesis in the central nervous system. J. R. Coll. Physicians London **26:**204–214.
22. **Kitten, T., and A. G. Barbour.** 1990. Juxtaposition of expressed variable antigen genes with a conserved telomere in the bacterium *Borrelia hermsii*. Proc. Natl. Acad. Sci. USA **87:**6077–6081.
23. **Kitten, T., A. V. Barrera, and A. G. Barbour.** 1993. Intragenic recombination and a chimeric outer membrane protein in the relapsing fever agent *Borrelia hermsii*. J. Bacteriol. **175:**2516–2522.
24. **Kyte, J., and R. F. Doolittle.** 1982. A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. **157:**105–132.
25. **Margolis, N., D. Hogan, W. Cieplak, T. Schwan, and P. Rosa.** 1994. Homology between *Borrelia burgdorferi* OspC and members of the family of *Borrelia hermsii* variable major proteins. Gene **143:**105–110.
26. **Meier, J., M. Simon, and A. G. Barbour.** 1985. Antigenic variation is associated with DNA rearrangement in a relapsing fever borrelia. Cell **41:**403–407.
27. **Norris, S. J., C. J. Carter, J. K. Howell, and A. G. Barbour.** 1992. Low-passage-associated proteins of *Borrelia burgdorferi*: characterization and molecular cloning of OspD, a surface-exposed, plasmid-encoded lipoprotein. Infect Immun. **60:**4662–4672.
28. **Oi, V. T., and L. A. Herzenberg.** 1980. Immunoglobulin-producing hybrid cell lines, p. 351–372. *In* S. M. Shiigis (ed.), Selected methods in cellular immunology. W. H. Freeman & Co., San Francisco, Calif.
29. **Pachner, A.** 1986. Spirochetal diseases of the CNS. Neurol. Clin. North Am. **4:**207–222.
30. **Pennington, P., C. D. Allred, D. Cadavid, S. Norris, and A. G. Barbour.** 1997. Arthritis severity and spirochete burden are determined by serotype in the *Borrelia turicatae*-mouse model of Lyme disease. Infect Immun. **65:**285–292.
31. **Plasterk, R. H., M. I. Simon, and A. G. Barbour.** 1985. Transposition of structural genes to an expression sequence on a linear plasmid causes antigenic variation in the bacterium *Borrelia hermsii*. Nature **318:**257–263.
32. **Restrepo, B. I., and A. G. Barbour.** 1994. Antigen diversity in the bacterium Borrelia hermsii through "somatic" mutations in rearranged *vmp* genes. Cell **78:**867–876.
33. **Restrepo, B. I., C. J. Carter, and A. G. Barbour.** 1994. Activation of a *vmp* pseudogene in *Borrelia hermsii*: an alternate mechanism of antigenic variation during relapsing fever. Mol. Microbiol. **13:**287–299.

34. **Restrepo, B. I., C. J. Carter, T. Kitten, and A. G. Barbour.** 1992. Subtelomeric expression regions of *Borrelia hermsii* linear plasmids are highly polymorphic. Mol. Microbiol. **6:**3299–3311.
35. **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.
36. **Sadziene, A., D. D. Thomas, V. G. Bundoc, S. H. Holt, and A. G. Barbour.** 1991. A flagella-less mutant of *Borrelia burgdorferi*. J. Clin. Invest. **88:**82–92.
37. **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.
38. **Seelig, A., R. Gottschlich, and R. M. Devant.** 1994. A method to determine the ability of drugs to diffuse through the blood-brain barrier. Proc. Natl. Acad. Sci. USA **91:**68–72.
39. **Stoenner, H. G., T. Dodd, and C. Larsen.** 1982. Antigenic variation of *Borrelia hermsii*. J. Exp. Med. **156:**1297–1311.
40. **Thompson, J. D., D. G. Higgins, and T. J. Gibson.** 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties, and weight matrix choice. Nucleic Acids Res. **22:**4673–4680.
41. **Tyler, K. L., R. T. Bronson, K. B. Byers, and B. Fields.** 1985. Molecular basis of viral neurotropism: experimental reovirus infection. Neurology **35:**88–92.
42. **van Dam, A., H. Kuiper, K. Vos, A. Widjojokusumo, B. de Jongh, L. Spanjaard, A. Ramselaar, M. 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.
43. **von Heijne, G.** 1983. Patterns of amino acids near signal sequence cleavage sites. Eur. J. Biochem. **133:**17–21.
44. **Wilske, B., V. Preac-Mursic, S. Jauris, I. Pradel, E. Soutschek, E. Schwab, and G. Wanner.** 1993. Immunological and molecular polymorphism of OspC: an immunodominant major outer surface protein of *Borrelia burgdorferi*. Infect. Immun. **61:**2182–2191.

Editor: J. G. Cannon