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Variability of *osp* Genes and Gene Products among Species of Lyme Disease Spirochetes

RICHARD T. MARCONI,* MICHAEL E. KONKEL, AND CLAUDE F. GARON

Laboratory of Vectors and Pathogens, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, Hamilton, Montana 59840

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A comparison of the *osp* operon in 24 Lyme disease isolates, including representatives from each of the three established species, *Borrelia burgdorferi*, *Borrelia garinii*, and group VS461, was conducted. Several properties were assessed to determine whether the variability observed in this operon was reflective of the species of the isolate. At the transcriptional level, start site and Northern (RNA) blot analyses were conducted. *B. garinii* and VS461 group isolates were found to possess an untranslated leader sequence 6 nucleotides longer than that observed in *B. burgdorferi* isolates. By Northern blot analyses all Lyme disease isolates, except the *B. garinii* isolate VS102, were found to produce a polycistronic full-length *ospAB* message. Isolate VS102 produced a truncated message lacking the *ospB* portion of the transcript. Southern blot analyses suggest that the deletion occurred at the DNA level and was not due to a posttranscriptional event. Analysis of the outer surface proteins by two-dimensional gel electrophoresis demonstrated that the OspB isoelectric points were variable, with the OspB of *B. garinii* isolates exhibiting a pronounced acidic shift. The reactivity of different isolates to OspA and -B monoclonal antibodies and to a hyperimmune anti-*ospAB* serum was also variable. The results presented here demonstrate genotypic and phenotypic heterogeneity in the *osp* operon at both the inter- and intraspecies levels. The results have implications concerning the use of the *osp* genes or their gene products in the development of a Lyme disease vaccine, as diagnostic markers of Lyme disease, and in subtyping of Lyme disease isolates.

Several studies have demonstrated that Lyme disease isolates are highly heterogeneous in their phenotypic and genotypic properties (1–3, 5, 13, 16, 17, 19–21, 23, 29, 31). Earlier studies revealed that a high degree of variability occurred in plasmid and protein profiles when different isolates were compared (3, 6, 12, 16, 33, 36). The major outer surface proteins (Osps), OspA and OspB, have been found to vary in molecular weight, in reactivity with various monoclonal antibodies (MAbs) (2, 3, 6, 40), and in gene sequence (4, 8, 11). Variability in the Osps was found to be most pronounced when North American and European isolates were compared.

It was demonstrated by Postic et al. (29), through DNA-DNA hybridization studies, that the species *Borrelia burgdorferi* could be divided into two species or subgroups. We demonstrated previously that Lyme disease spirochetes can be resolved into three distinct phylogenetic groups, using 16S rRNA sequence analysis, and that the evolutionary distance between these groups warranted separate species status for each (19, 20). Recent studies utilizing DNA-DNA hybridization (2), multilocus enzyme electrophoresis (5), and arbitrarily primed polymerase chain reaction (38) support the division of *B. burgdorferi* into three species. Baranton et al. (2) have proposed a revised nomenclature with the species designations *B. burgdorferi*, *B. garinii*, and VS461 group replacing the subspecies designation sensu stricto, *20047* (29), and group 3 (20), respectively. Herein we will utilize the revised species nomenclature put forth by Baranton et al. (2).

In this study, we sought to determine whether the variability in OspA and -B was reflective of the species status of an isolate and to define the potential molecular characteristics associated with this variability. We have compared various properties of the Osps, at both gene and protein levels, in 24 Lyme disease isolates. The isolates investigated included representatives from each of the three Lyme disease-associated species (2, 5, 19–21, 23, 38). We have analyzed properties of the *osp* operon, including transcriptional start sites and hybridization profiles, using several *osp*-directed oligonucleotides. At the protein level, we have compared the relative isoelectric points of the Osps and their reactivity with Osp-directed MAbs and with an anti-OspAB hyperimmune serum (AB serum). While some properties are conserved among isolates of a given species, others are not. In addition, we have identified a *B. garinii* isolate which does not produce OspB. We sought to identify the molecular mechanisms responsible for the lack of OspB production in this isolate. This isolate was found to be lacking a functional OspB gene. The results presented here have important implications for the use of OspA or OspB as a vaccine candidate, as an indicator of phylogeny, and as a diagnostic marker.

MATERIALS AND METHODS

Bacterial isolates and cultivation. All isolates and their in vitro passage numbers are described in Table 1. All *Borrelia* species and isolates were cultivated in BSKII media at 34°C (3).

Southern blot analyses of the *osp* operon. Total isolated DNA was digested with either *Eco*RV, *Eco*RI, *Hin*dIII, or *Bam*HI and separated in 0.8% agarose gels (16 h at 30 V, constant) in standard TAE buffer. The DNA was transferred, fixed onto GeneScreen membranes, and hybridized at 37°C as described previously (24). The following oligonucleotide probes were used in this study: ospA-5′ (5′-GGCT GCTAACATTTTGCTTACATGC) targets nucleotides 46 to

* Corresponding author.

TABLE 1. Lyme disease isolates

Isolate	Source	Passage[a]
B. burgdorferi		
Illinois 1	Mouse; Ill., USA	H
B31, Sh-2-82, 25015	*Ixodes dammini*; N.Y., USA	H, H, 6
1352	*Amblyoma americanum*; Tex., USA	H
21721	*Ixodes dammini*; Wis., USA	6
NY13-87	Skin, human; N.Y., USA	L
CA2-87, CA3-87	*Ixodes pacificus*; Calif., USA	11, 11
3028	Pus, human; Tex., USA	H
Veery	Veery bird; Calif., USA	5
27985	*Ixodes dammini*; Conn., USA	5
IP2A	Cerebrospinal fluid, human; France	H
VS219	*Ixodes ricinus*; Switzerland	11
20004	*Ixodes ricinus*; France	H
B. garinii sp. nov.		
G1, G2	Cerebrospinal fluid, human; Germany	H, H
VS102	*Ixodes ricinus*; Switzerland	9
G25	*Ixodes ricinus*; Sweden	H
R-IP90	*Ixodes persulcatus*; Russia	H
VS461 group isolates		
R-IP3, R-IP21	*Ixodes persulcatus*; Russia	H, H
VS461	*Ixodes ricinus*; Switzerland	9
J1	*Ixodes persulcatus*; Japan	H

[a] H, high passage, >15 in vitro passages; L, low passage, <15 passages when the precise number is not known. The passage histories are indicated respectively for each isolate on a given line.

70 of the coding sequence of the *ospA* gene, ospB-5′ (5′-AGCACCTTTTTGTGCACA) targets bases 46 to 63 of the coding sequence of the *ospB* gene, and ospB-3′ (5′-GTAC TGTAATTGTACCATCTG) targets bases 747 to 767 of the *ospB* gene. All numbering is in reference to that of the *osp* operon of *B. burgdorferi* isolate B31 (4). The oligonucleotide designations used describe the general region of the gene targeted.

Transcriptional start site mapping. The transcriptional start sites for the *osp* operon were determined through reverse transcriptase primer extension analysis. Primers were 5′-end-labeled with [γ-^{32}P]ATP (6,000 Ci/mmol), using polynucleotide kinase (Boehringer Mannheim), and gel purified by standard methods. Reagents were treated with diethylpyrocarbonate by standard methods. Transcriptional start site determination was accomplished by incubating 5×10^5 cpm of radiolabeled oligonucleotide primer with 15 μg of total cellular RNA, isolated as described previously (35), at 85°C for 10 min in annealing buffer (100 mM KCl, 5 mM Tris-HCl; pH 8.3) and then incubating at 30°C for 4 h. After incubation, 15 μl of reverse transcription mixture (0.9 mM dATP, dTTP, dCTP, and dGTP, 83 mM Tris-HCl [pH 8.3], 8 mM dithiothreitol, 8 mM $MgCl_2$, 83 μg of bovine serum albumin per ml) and 50 U of avian myeloblastosis virus reverse transcriptase were added, and reaction mixtures were incubated for 1.5 h (47°C). To digest the RNA, 1 μl of 0.5 M EDTA and 1 μl of RNase (0.5 μg/μl) were added, and the mixture was incubated for 0.5 h at 37°C. One hundred microliters of 2.5 M NH_4Ac was added to each tube, and extraction was with phenol-chloroform-isoamyl alcohol. The extension products were precipitated with ethanol, washed with 70% ethanol, vacuum dried, resuspended in 10 μl of formamide loading buffer (50% formamide, 10 mM EDTA, 0.025% bromophenol blue, 0.025% xylene cyanol), heated at 85°C for 5 min, and loaded onto a 7% polyacrylamide sequencing gel (8 M urea, 83 mM Tris-borate, 1 mM EDTA; pH 8.0). After electrophoresis the gel was transferred directly onto 3MM paper (Whatman) and exposed to Kodak XAR-5 film for 3 h.

Northern (RNA) blot analysis. Samples were prepared and electrophoresed in 1.2% agarose–formaldehyde gels as described previously (24) and transferred onto GeneScreen membranes (DuPont) with the Vacugene vacuum blot system (Pharmacia), and the RNA was fixed to the membrane via UV cross-linking with a Stratalinker as described by the manufacturer (Stratagene). Hybridizations were performed at 37°C as described previously (24).

Western blot (immunoblot) and isoelectric point analysis. Proteins separated by one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis were electroblotted in Towbin buffer (0.25 M Tris base, 0.192 mM glycine, 20% methanol; pH 8.3) onto Immobilon-P membranes (Millipore Corp.) at 100 V for 1 h. After transfer, membranes were rinsed with 0.01% Tween in phosphate-buffered saline (PBS-Tween) and incubated with a 1:200 dilution of AB serum or MAb in 20% fetal bovine serum in PBS-Tween (15 to 18 h at 4°C). The AB serum, which recognizes specifically OspA and OspB, was provided by David Dorward (Rocky Mountain Laboratories) and has been described previously (7). MAbs H4610 (32) and H5332, which target OspB and OspA, respectively, were provided by Tom Schwan (Rocky Mountain Laboratories). The membranes were then rinsed three times with PBS-Tween and incubated for 1.5 h at room temperature with a 1:500 dilution of a goat anti-rabbit immunoglobulin G antibody coupled to horseradish peroxidase (Organon Teknika). After 10 1-min washes with PBS-Tween and 3 washes with H_2O, bound antibody was visualized with 4-chloro-1-naphthol–H_2O_2 by standard methods.

Two-dimensional gel electrophoresis was performed as described by O'Farrell (27). Samples were solubilized in sample buffer (9.5 M urea, 2.0% Triton X-100, 5% beta-mercaptoethanol, 1.6% 5/7 Bio-Lyte ampholyte, 0.4% 3/10 Bio-Lyte ampholyte [Bio-Rad Laboratories]), and cellular debris was removed by centrifugation at 100,000 × *g* for 2 h. First-dimension tube gels consisted of 9.2 M urea, 4% acrylamide, 20% Triton X-100, 1.6% 5/7 Bio-Lyte ampholyte, 0.4% 3/10 Bio-Lyte ampholyte (Bio-Rad), 0.01% ammonium persulfate, and 0.1% *N,N,N′,N′*-tetramethylethylenediamine (TEMED). The upper and lower reservoir buffers were 20 mM NaOH and 10 mM H_3PO_4, respectively. Isoelectric focusing was done at 500 V for 10 min and then at 750 V for 3.5 h. The gels were then equilibrated with Laemmli buffer (15) (15 min at room temperature), loaded onto sodium dodecyl sulfate–12.5% polyacrylamide gels, and electrophoresed in the second dimension.

RESULTS

Southern blot analysis of the *osp* operon. DNA digested with either *Hin*dIII, *Eco*RV, *Eco*RI, or *Bam*HI was probed with the ospA-5′, ospB-5′, and ospB-3′ probes. Consistent with the Northern analysis described below restricted DNA from *B. garinii* isolate VS102 and all group VS461 isolates did not hybridize with the ospB-5′ probe (Fig. 1). All hybridization results are summarized in Table 2. While all isolates were found to be hybridization positive with the ospA-5′ probe, the sizes of the fragments hybridizing with this as well as other probes varied among isolates (Fig. 2).

Transcriptional analyses. The transcriptional start site of

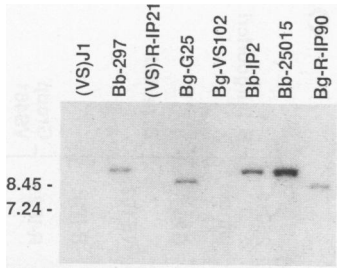

FIG. 1. Southern blot analysis of the *osp* operon, using the ospB-5′ probe. DNA from representative isolates was restricted with *Eco*RV. All methods were as described in the text. The species affiliations of the isolates are indicated by the following prefixes: Bb for *B. burgdorferi*; Bg for *B. garinii*; and (VS) for group VS461. Size standards (in kilobases) are indicated to the left.

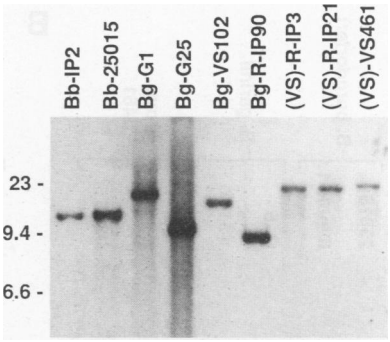

FIG. 2. Southern blot analysis of the *osp* operon, using the ospA-5′ probe. DNA from representative isolates was restricted with *Eco*RV. All methods were as described in the text. Size standards (in kilobases) are indicated to the left. See legend to Fig. 1 for species abbreviations.

the *osp* operon in all *B. burgdorferi* isolates was identified as a G residue 36 nucleotides upstream from the translational start codon, using the ospA-5′ probe as primer. A minor secondary band was also observed at 37 nucleotides from the start codon. The transcriptional start site analyses for representative isolates are presented in Fig. 3. The 5′ leader sequences for *B. garinii* and VS461 isolates differed in length, being 6 nucleotides longer. The locations of the transcriptional start sites were confirmed through the use of two additional extension primers (data not shown). Extension products were not observed for *Borrelia parkeri*, *Borrelia turicatae*, *Borrelia hermsii*, and *Borrelia anserina*, which do not carry the *ospAB* genes.

By Northern blot analysis, using the ospA-5′ probe, the *osp* transcript was found to be approximately 1,800 nucleotides in length in all isolates except the *B. garinii* isolate VS102, which had a transcript of approximately 875 nucleotides. This and subsequent Northern blot analyses, using the ospB-5′ and ospB-3′ probes, are presented in Fig. 4, and all hybridization results are summarized in Table 2.

Western blot and isoelectric point analyses. The reactivity of isolates with OspA- and OspB-directed MAbs was assessed via Western blot analysis. These and subsequent immunoblot analyses are summarized in Table 3. The OspA-directed MAb (H5332) recognized a protein in the 31-kDa range in all isolates except the group VS461 isolates, R-IP3 and R-IP21, and in *B. garinii* R-IP90. However, with the AB serum, OspA was detected in isolates R-IP3 and R-IP21. In isolate RIP90 an immunoreactive protein of 33.5 kDa was detected with the AB serum. Previously, Jonsson et al. reported that the OspA of R-IP90 was atypical, with an apparent molecular weight of 33,500 (13).

Isolates were also screened with an OspB-directed MAb (H4610) (Table 3). *B. burgdorferi* isolates 25015 and Illinois 1 and group VS461 isolates J1, R-IP3, and R-IP21 did not react with this antibody, but OspB was detected with the AB serum. The OspB of *B. garinii* isolates did not react with either H4610 or the AB serum. These observations are consistent with those reported by others who found that *B. garinii* OspB does not react with the OspB-directed MAb H6831 (2). While we have demonstrated in this report that *ospB* is transcriptionally expressed in *B. garinii* isolates, we refer to the *B. garinii* OspB as "putative OspB" since this protein does not react with the OspB MAbs H6831 (2) and H6410 or with the AB serum and has not been sequenced directly.

We have compared the relative isoelectric points of the OspA and -B proteins of 10 isolates (B31, 25015, IP2A, R-IP90, G25, G1, VS102, R-IP3, R-IP21, and VS461) from each of the three species. The two-dimensional protein profiles of four representative isolates (*B. burgdorferi* B31,

TABLE 2. Summary of Northern and Southern blot hybridization results

Isolate	Northern blots			Southern blots		
	A-5′[a]	B-5′	B-3′	A-5′	B-5′	B-3′
B. burgdorferi isolates						
25015, Illinois 1	+	+	−	+	+	−
All other isolates	+	+	+	+	+	+
B. garinii isolates						
VS102	+	−	−	+	−	−
All other isolates	+	+	+	+	+	+
Group VS461 isolates						
All isolates	+	−	+	+	−	+

[a] Probes ospA-5′, ospB-5′, and ospB-3′ are abbreviated A-5′, B-5′, and B-3′, respectively.

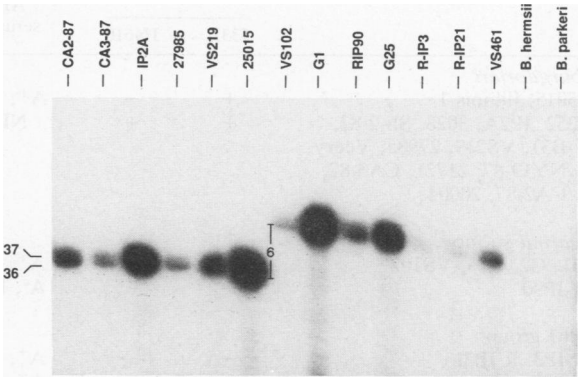

FIG. 3. Identification of *ospAB* transcriptional start sites. Primer extension and electrophoresis were performed as described in the text. The lengths of the extension products were determined through use of a sequencing ladder. Numbers on the left of the panel indicate the number of nucleotides 5′ of the translational start codon for each start site. The difference in length of the leader sequences among isolates (6 nucleotides) is indicated.

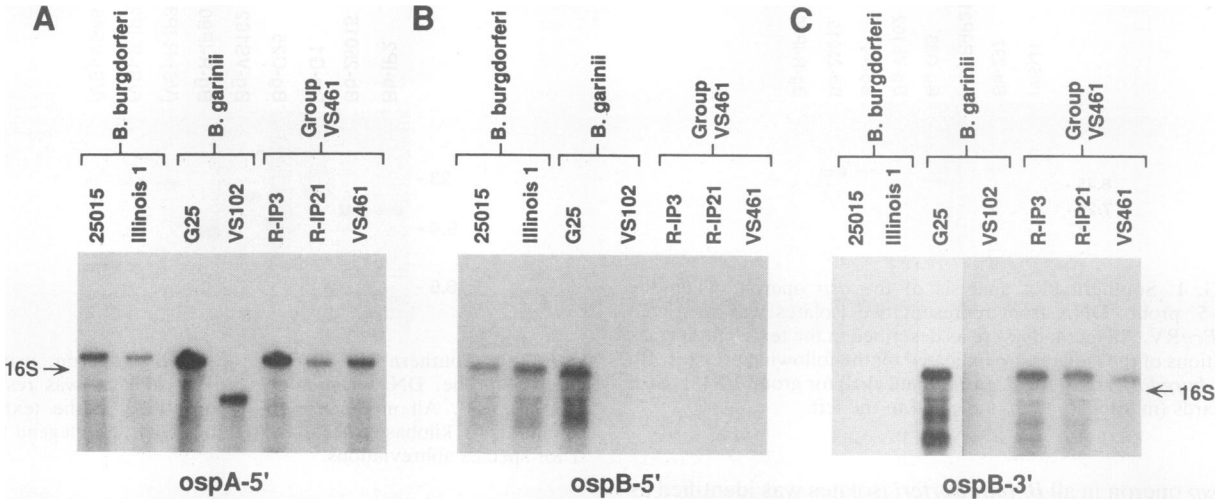

FIG. 4. Northern blot analyses of the *ospAB* operon. All methods were as described in the text. Fifteen micrograms of total cellular RNA was electrophoresed in 1.2% agarose–formaldehyde gels and vacuum blotted onto GeneScreen membranes. The blots in panels A, B, and C were probed with the oligonucleotides indicated below each panel. The sources of the RNA loaded are indicated above each lane.

B. garinii VS102 and G1, and VS461 group isolate R-IP3) are shown in Fig. 5. OspA and OspB were identified either by immunoblotting with the AB serum or MAbs or, in the case of the putative OspB of *B. garinii* isolates, by relative molecular weight and isoelectric point. *B. burgdorferi* and VS461 group isolates were found to have highly similar Osp isoelectric points. The isoelectric points for the putative Osps in *B. garinii* isolates (with the exception of VS102) were similar to each other but exhibited an acidic shift in OspB when compared with *B. burgdorferi* and VS461 isolates. This acidic shift is consistent with that predicted from the amino acid sequence of OspB from the *B. garinii* isolate G2 (30). In the *B. garinii* isolate VS102, OspB was not detected. In *B. burgdorferi* B31, only minor amounts of OspB are expressed. While the high-passage B31 used in this study produced only minor amounts of OspB, we did not observe a strict correlation in other isolates between passage number and OspB expression.

TABLE 3. Immunoreactivity of Lyme disease isolates with OspA- and -B-directed MAbs and AB serum

Isolate	Immunoreactivity with:		
	MAb[a]		AB serum[b]
	5332	H4610	
B. burgdorferi			
25015, Illinois 1	+	−	A^+, B^+
1352, IP2A, 3028, Sh-2-82, B31, VS219, 27985, Veery, NY13-87, 21721, CA3-87, CA2-87, 20004	+	+	ND
B. garinii sp. nov.			
G1, G2, G25, VS102	+	−	A^+, B^-
R-IP90	−	−	A^c, B^c
VS461 group			
R-IP3, R-IP21	−	−	A^+, B^+
VS461	+	+	A^+, B^+
J1	+	−	A^+, B^+

[a] MAbs H5332 and H4610 recognize OspA and OspB, respectively. Reactivity is scored as + for positive and − for negative.

[b] A positive or negative reaction of the AB serum with either OspA (A) or OspB (B) is indicated by a + or − superscript. ND, not done.

[c] An immunoreactive protein of approximately 33.5 kDa was detected, but its absolute identity could not be determined (see text).

DISCUSSION

While variability in OspA and -B has been reported by others, it is not known whether the variability reflects the species classification of an isolate. It has been suggested that Lyme disease isolates can be subtyped on the basis of the molecular weights of the Osps and/or by their reactivities with Osp-directed MAbs (39). Hence, it is important to determine whether subtypes based on the Osps are consistent with those obtained by other approaches (1, 2, 5, 19–21, 23, 38). Here we present a comparative study of 24 Lyme disease isolates which have been identified previously to the species level (19–21, 23).

Hybridization analyses utilizing both Northern and Southern blotting demonstrated sequence variability in the *ospAB* operon. In *B. garinii* VS102, a truncated *osp* message was detected which did not hybridize with *ospB*-directed probes. The *ospB* probes also did not hybridize with either uncut or restricted VS102 DNA, and we were unable to amplify the operon, by polymerase chain reaction, with primers targeting various segments of the *ospB* gene (22). These results suggest that a functional *ospB* gene is lacking. This isolate may prove useful in studying the potential role of OspB in the pathology of Lyme disease.

Two *B. burgdorferi* isolates, 25015 and Illinois 1, were unique among isolates of this species in that they did not hybridize with the ospB-3′ probe. In a previous 16S rRNA-based phylogenetic study, these isolates were found to reside peripherally to the main *B. burgdorferi* cluster (21). The hybridization profiles of isolates 25015 and Illinois 1 support their peripheral relationship to other *B. burgdorferi* isolates. In contrast to *B. burgdorferi* and *B. garinii*, group VS461 isolates did not hybridize with the ospB-5′ probe but did so with the ospB-3′ probe. However, both the operon and the resulting transcript were found to be full-length;

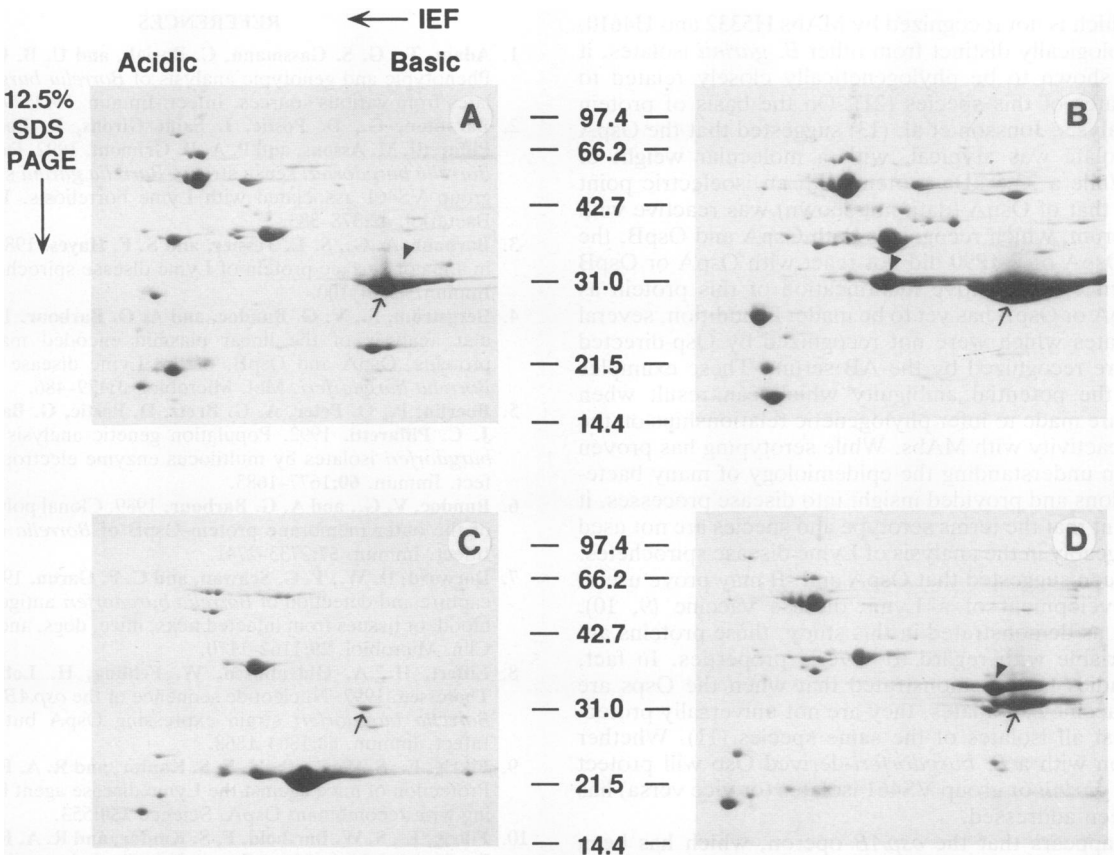

FIG. 5. Two-dimensional gel electrophoresis of Lyme disease isolates. Two-dimensional gel electrophoresis was performed as described in the text, and the gels were stained with Coomassie blue. (A) *B. burgdorferi* B31; (B) *B. garinii* G1; (C) *B. garinii* VS102; (D) group VS461 isolate R-IP3. The position of OspA is indicated with arrows, and that of OspB is shown by arrowheads. The identity of the Osps was confirmed by Western blotting, using MAbs. The putative OspB in *B. garinii* isolate G1 was identified on the basis of its apparent molecular weight since it was not reactive with the OspB MAb or AB serum. Molecular mass standards (kilodaltons) are listed between panels.

hence, the lack of hybridization with the ospB-5′ probe suggests sequence divergence in the 5′ end of the gene. Sequence variability in and around this operon, even within a species, was also evident from Southern blotting of restricted DNA in that variability in the size of the hybridizing fragments was observed. In contrast to that observed for the rRNA genes (2, 19, 21, 29), the restriction fragment length polymorphism patterns of the *osp* operon are not suitable for use in the differentiation of spirochete species associated with Lyme disease.

Analysis of the *osp* operon transcriptional start sites demonstrated heterogeneity in the upstream control region of the *ospAB* operon. Alignment of the *B. garinii* R-IP90 *ospAB* sequence (13) with the *B. burgdorferi* B31 sequence revealed a 6-base insertion in the R-IP90 sequence downstream of the transcriptional start site but upstream of the translational start codon. A 6-base gap is consistent with the difference in length between the untranslated leader sequences and, on the basis of primer extension analyses, appears conserved in *B. garinii* and group VS461 isolates. This difference in the upstream noncoding region of the *osp* operon can serve as an additional marker to differentiate *B. burgdorferi* from *B. garinii* and group VS461 isolates. It has been suggested that a second transcriptional start site within the *ospA* gene may be used for transcription of the *ospB* gene (6). However, by primer extension analysis with various ospA and -B primers, additional start sites were not detected (data not shown). Hence, variability in OspB expression and molecular weight is not a consequence of alternative promoter selection.

An analysis of the relative isoelectric points of OspA and -B in Lyme disease isolates revealed that in some isolates the OspB isoelectric point differed significantly. *B. garinii* isolates (with the exception of isolate VS102, which does not produce OspB) were found to exhibit a pronounced acidic shift in their putative OspB isoelectric point. This finding is consistent with a theoretical OspB pI of 5.7 for the *B. garinii* isolate G2 versus a pI of 8.8 for the OspB of *B. burgdorferi* B31 (30). It has been suggested either that only OspA is expressed in *B. garinii* isolates or that both OspA and OspB are expressed and comigrate in polyacrylamide gels (32). While the identity of the putative OspB in *B. garinii* could not be immunologically confirmed with reagents currently available, the analyses conducted here demonstrate that a major protein with an isoelectric point consistent with that expected for the OspB of *B. garinii* is expressed and comigrates with OspA in one-dimensional gels.

Subtyping of Lyme disease isolates on the basis of the immunological reactivity of OspA and -B has been proposed (39). However, as demonstrated here, variable reactivity patterns were observed with Osp-directed MAbs even within a species (Table 3). For example, while the *B. garinii* R-IP90

isolate, which is not recognized by MAbs H5332 and H4610, is immunologically distinct from other *B. garinii* isolates, it has been shown to be phylogenetically closely related to other isolates of this species (21). On the basis of protein profile analysis, Jonsson et al. (13) suggested that the OspA of this isolate was atypical, with a molecular weight of 33,500. While a 33.5-kDa protein with an isoelectric point similar to that of OspA (data not shown) was reactive with the AB serum, which recognizes both OspA and OspB, the putative OspA of R-IP90 did not react with OspA or OspB MAbs; hence, a definitive identification of this protein as either OspA or OspB has yet to be made. In addition, several other isolates which were not recognized by Osp-directed MAbs were recognized by the AB serum. These examples illustrate the potential ambiguity which can result when attempts are made to infer phylogenetic relationships on the basis of reactivity with MAbs. While serotyping has proven valuable in understanding the epidemiology of many bacterial infections and provided insight into disease processes, it is important that the terms serotype and species are not used interchangeably in the analysis of Lyme disease spirochetes.

It has been suggested that OspA and -B may prove useful in the development of a Lyme disease vaccine (9, 10). However, as demonstrated in this study, these proteins are highly variable with regard to several properties. In fact, recent studies have demonstrated that when the Osps are used as vaccine candidates, they are not universally protective against all isolates of the same species (11). Whether vaccination with a *B. burgdorferi*-derived Osp will protect against *B. garinii* or group VS461 isolates (or vice versa) has not yet been addressed.

It also appears that the *ospAB* operon, which has been proposed as a polymerase chain reaction diagnostic target (18, 25, 26, 28), may not be sufficiently conserved to serve as a diagnostic marker. The data presented here demonstrate that significant sequence variability exists in this operon. On the basis of an alignment of *osp* operon sequences, it is evident that the target sequences of several previously described *osp* primers (14, 28) are characterized by, in some cases, as much as 29% hybridization mismatches which in many cases occur at or near the 3′ end of the primer. Thus, it is likely that these primer sets would be unreliable in some cases.

The results of this study demonstrate that significant variability occurs in the *ospAB* operon. The variability is evident both among the Lyme disease spirochete species and within each species. Lyme disease poses a unique challenge to researchers who are seeking to develop diagnostic tools and vaccines because of the phylogenetic diversity of the organisms which cause this disease. In conclusion, while Osps A and B initially seemed attractive molecules for these goals because of their high expression levels, the heterogeneity in this gene both within and between the Lyme disease-associated spirochete species may render it an unreliable marker.

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