Evasion of protective immunity by *Borrelia burgdorferi* by truncation of outer surface protein B

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ABSTRACT   We analyzed variability in outer surface protein B (OspB) from *Borrelia burgdorferi* (*Bb*), the causative agent of Lyme disease, to determine how *Bb* escapes immune destruction. We have shown that vaccination with OspB from *Bb* strain B31 protected mice from infection with *Bb* B31 but not against *Bb* N40. The present study demonstrates that *Bb* N40 spirochetes which evade vaccination immunity to OspB have a truncated form of OspB, due to a TAA stop codon at nucleotide 577. In contrast, *Bb* N40 spirochetes that express full-length OspB are unable to infect mice immunized with OspB, analogous to our previous studies with *Bb* B31. Mapping of the OspB antibody response shows that epitopes in the C terminus of OspB are surface-exposed and bind protective monoclonal and polyclonal antibodies. This suggests that the C terminus of OspB is important for eliciting a protective immune response to OspB. Truncation or modification of outer surface proteins that do not bind protective antibody may be a means by which *Bb* evades host defenses.

Lyme disease is the most common arthropod-borne infection in North America (1). The bite of an infected *Ixodes* tick transmits the causative spirochete, *Borrelia burgdorferi* (*Bb*), to humans and other animals (1). Infection can result in erythema migrans, arthritis, carditis, or neurological impairment (1). The *Bb* membrane antigens OspA and OspB have been shown to be candidates for a vaccine against Lyme disease (2–6). We showed that active immunization of C3H mice with recombinant OspA or OspB fusion proteins protected against challenge with *Bb* (2–4). Specifically, humoral immunity was important for protection, as passive immunization of mice with monoclonal or polyclonal antibodies to OspA prevented infection in the immunocompetent C3H mice (2). Likewise, others have passively protected immunodeficient CB.17 *scid* mice with polyclonal and monoclonal antibodies to OspA, and polyclonal anti-*Bb* serum protected hamsters from infection (5–7).

Antigenic heterogeneity of the outer membrane proteins may, however, allow *Bb* to survive in the presence of protective antibody or to infect otherwise immune hosts. Vaccination of mice with recombinant OspA from *Bb* N40 protected against challenge with several other strains of *Bb*, including B31, CD16, and 297, but protection did not extend to challenge with *Bb* 25015 (2–4). OspA, however, was still important in protection against *Bb* 25015, as vaccination of mice with recombinant OspA-25015 fusion protein protected against challenge with *Bb* 25015 (4).

We have determined the epitope(s) on OspA important in eliciting a protective immune response. The protein sequence of OspA-25015 differs from OspA-N40 by 40 aa, and most of the variability (28 aa) is within the C-terminal half of OspA, suggesting that epitopes in this region are important in binding protective antibody (3, 4). Moreover, we showed that

anti-OspA-N40 monoclonal antibodies (mAbs) that protect mice from infection in passive immunization experiments bind conformational epitope(s) within the C terminus of OspA-N40 (8). These protective mAbs did not bind to recombinant OspA-25015 on an immunoblot (4), demonstrating that sequence heterogeneity within the C terminus of OspA accounts, at least in part, for the lack of cross protection.

Active immunization of C3H mice with OspB also protected against challenge with spirochetes (3). As was the case with OspA, protection afforded by OspB vaccination was somewhat strain-specific. Vaccination of mice with recombinant OspB-B31 protected against challenge with a syringe inoculum of 10^2 *Bb* B31 cells, but not against 10^2 *Bb* N40 cells (3). This is somewhat surprising, as strain B31 and N40 are classified within similar taxonomic groups of *Bb* and the OspA sequences in these two strains are almost identical (2). The purpose of the current study was to explore the mechanism by which *Bb* N40 evades the host immune response to OspB.

MATERIALS AND METHODS

Mice. Female, virus antibody-free C3H/HeJ (C3H) mice were obtained from The Jackson Laboratory. They were shipped in filtered crates and housed in microisolator cages. Food and water were provided *ad libitum*. Mice were killed with carbon dioxide gas.

Bb. Low *in vitro* passage N40 and B31 tick isolates with proven infectivity and pathogenicity in C3H mice were utilized. *Bb* cells were grown to logarithmic phase in modified Barbour–Stoenner–Kelly (BSK II) medium and counted in a hemocytometer under darkfield microscopy (9). Tissue samples (blood, spleen, bladder, and skin) were collected, homogenized, placed in BSK II medium for 2 weeks, and examined by darkfield microscopy as described (2–4). Twenty high-power fields were scanned per culture. Positive cultures had between 1 and 100 *Bb* cells, whereas negative cultures had no organisms. Mice were considered infected if at least one tissue was culture-positive.

Bb cells were cloned twice by limiting dilution; 10^3 spirochetes were placed in 10 ml of BSK II medium, and six serial 1:10 dilutions in BSK II were performed. Cultures were incubated for 2 weeks at 32°C and examined for spirochetes. Two weeks is sufficient for 1 cell to grow to stationary phase. If the growth pattern conformed to a Poisson distribution, individual cultures from the serial dilutions were considered to contain clonal populations of *Bb*. To ensure clonality, the cells were then recloned by limiting dilution.

Amplification, Cloning, and Sequencing of OspB and Expression of the Recombinant Protein. Recombinant OspB-B31 was expressed and purified as a fusion protein with glutathione transferase (GT) as described (3). Recombinant OspB-N40 was prepared in a similar manner. Ten microliters

Abbreviations: *Bb*, *Borrelia burgdorferi*; GT, glutathione transferase; mAb, monoclonal antibody.

of *Bb* N40 passage 3 (10^7 cells per ml) in BSK II medium was used as a template for the PCR to amplify the *ospB*-N40 gene. The oligonucleotide primers used for amplification were based on known nucleotide sequences of *ospB*-B31. The oligonucleotides contained nt 1–20, or 865–888 of the *ospB*-B31 gene and were flanked by *Bam*HI and *Eco*RI restriction enzyme sites to facilitate cloning and expression. The amplified DNA was digested with *Eco*RI and *Bam*HI, and the *ospB*-N40 gene was ligated into plasmid pGEX-2T (Pharmacia), in frame with the GT gene (3). The recombinant plasmids were used to transform *Escherichia coli* DH5α.

For the epitope mapping studies, we constructed overlapping fragments of the *ospB*-N40 gene. The *ospB* gene fragments were amplified by PCR using oligonucleotide primers containing 18 nt of the ospB gene, beginning at the positions listed in Fig. 1. The primers were flanked by *Bam*HI and *Eco*RI sites for subcloning. The amplified gene fragments, digested with *Bam*HI and *Eco*RI, were subcloned into pGEX-2T and expressed in *E. coli*.

Recombinant fusion protein production was induced with 1 mM isopropyl β-D-thiogalactopyranoside for 3 hr (2–4). Cells were washed in phosphate-buffered saline (PBS), suspended in 1/100th the volume of PBS with 1% (vol/vol) Triton X-100 and 0.5 μM phenylmethanesulfonyl fluoride, and lysed by sonication. The lysate was centrifuged at 10,000 × *g* and the supernatant containing the recombinant fusion proteins was passed over a glutathione-Sepharose 4B column (Pharmacia). The OspB-B31 or OspB-N40 fusion protein was eluted with 5 mM glutathione.

The *ospB*-B31 and *ospB*-N40 genes were also ligated into plasmid p197-12 and expressed in *E. coli* as OspB proteins, without a fusion partner, by using the *Bb* ATG codon, as previously described for OspA (2). p197-12, obtained from Walter Fiers (University of Ghent) and Biogen, expresses recombinant antigens under control of the phage λ P_L promoter and cI857 thermolabile repressor. The *ospB* genes were sequenced, by the dideoxy method, from the amplified PCR products and after the construction of recombinant plasmids (10).

Vaccination and Protection Studies with C3H Mice. Mice were vaccinated with recombinant OspB-B31 fusion protein as described (3, 4). Briefly, 3-week-old mice were immunized with 10 μg of OspB in complete Freund's adjuvant and given two booster injections with the same preparation in incom-

plete Freund's adjuvant. Mice were challenged with *Bb* 2 weeks after the last active immunization boost. Mice were passively immunized with a subcutaneous injection of 0.1 ml of mAb supernatant or rabbit anti-*Bb* serum 24 hr prior to challenge with *Bb*.

Mice were challenged with an intradermal inoculation of 10^2 B31 or N40 spirochetes in 0.1 ml of medium and sacrificed 14 days after inoculation, the time at which the disease manifestations are most pronounced. The joints and heart were formalin-fixed, paraffin-embedded, sectioned, and examined microscopically in a blind study for evidence of inflammation as described (2). An animal was considered to have arthritis if at least one joint showed evidence of inflammation. Blood, spleen, bladder, and skin specimens from experimental animals were cultured in BSK II medium.

Patient Sera. Sera from patients with chronic Lyme arthritis, marked by 6 months of chronic arthritis, following erythema migrans and positive serologic tests for Lyme disease, were used in the immunoblot assays. All sera were obtained from the Yale Lyme Disease Clinic.

Immunoblot Analysis. Protein extract (1 μg) of *Bb* N40 or *E. coli* expressing OspB from the p197-12 or pGEX-2T plasmid system was suspended in SDS sample buffer, heated at 95°C for 5 min, and subjected to SDS/12% PAGE (11). The transfer of the electrophoretically separated proteins to nitrocellulose and the incubation of the blots with antibody were performed as modifications of the protocols of Towbin *et al.* (12). The proteins were electrotransferred to nitrocellulose overnight at 4°C in 14.5 mM glycine/50 mM Tris/20% methanol/0.01% SDS. Anti-OspB mAbs or serum samples were diluted from 1:100 to 1:100,000 and incubated with the nitrocellulose strips. The strips were washed three times, incubated with a 1:5200 dilution of alkaline phosphatase-labeled goat anti-mouse, -human, or -rabbit IgG, and developed with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate (Stratagene). Antibody titers were determined by immunoblot, based on the final dilution at which binding was detectable.

Protein Sequencing. Thirty micrograms of a protein extract of *E. coli* expressing OspB from plasmid p197-12, or 30 μg of protein of cloned *Bb* N40, was separated by SDS/PAGE and transferred to a poly(vinylidene difluoride) membrane (Immobilon, Millipore). The amount of protein loaded on the gel was determined by the method of Bradford (13). The Immobilon membrane was probed with a 1:100 dilution of an OspB-N40 mAb, designated B10, produced at Yale, or stained with Coomassie brilliant blue. The bands which showed binding to the OspB mAb B10 were excised from the Coomassie blue-stained membrane for direct N-terminal sequence analysis, which was done at the Yale Protein Chemistry Laboratory. Additional sequence data were obtained by subjecting the proteins to digestion with trypsin and separating the proteolytic fragments by HPLC for direct sequence analysis.

Immunofluorescence. Approximately 10^5 *Bb* spirochetes were placed on a glass slide and allowed to air dry. This procedure maintains an intact bacterial membrane, so that only surface antigens are exposed to antibody. Specimens were also fixed by incubation in acetone for 10 min, and then air dried. This procedure permeabilizes the outer membrane. Slides with unfixed or fixed *Bb* were then incubated with serum or mAbs (diluted 1:100) for 30 min at 24°C, washed three times with PBS, incubated with fluorescein-conjugated goat anti-mouse total immunoglobulin (diluted 1:100) for 30 min at 24°C, and again washed three times. The slides, with a drop of mounting oil, were examined by fluorescence microscopy in a blinded fashion.
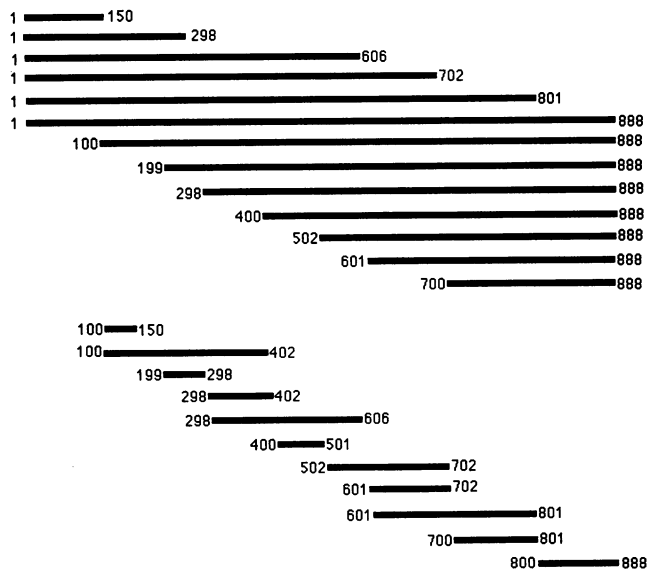

FIG. 1. Schematic diagram of the *ospB* gene fragments amplified by PCR. The encompassed nucleotides are listed 5′ (left) and 3′ (right) on the fragments.

RESULTS

***Bb* N40 Expresses Either a Full-Length or a Truncated Form of OspB.** We used PCR to amplify the *ospB* genes from *Bb* N40, to determine the heterogeneity of OspB within the strain. The amplified PCR products were cloned into pGEX-2T and p197-12. Nucleotide sequencing of *ospB* from N40, both directly from the PCR product and from the recombinant plasmids, revealed two types of spirochetes. One group, which represented 20% of the samples, had a full-length OspB (N40-B) of 296 aa. The other group of N40 spirochetes, comprising 80% of the population, had an *ospB* gene with an identical sequence, except for a TAA stop codon at position 577 (N40-ΔB), resulting in a truncated OspB protein of 192 aa. In addition, the *ospB*-N40 nucleotide sequence differed from the *ospB* sequence (14) at five nucleotide positions (nt 376, 382, 385, 526, and 758) that resulted in amino acid differences between OspB-N40 and OspB-B31: in OspB-N40, aa 126 is Thr; aa 128, Asn; aa 129, Thr; aa 176, Thr; and aa 253, Thr. The *ospA* sequences in each group were identical, as previously published (2).

Cloning of spirochetes by limiting dilution verified that N40 spirochetes separated into two groups, based on differences in OspB: one group expressed a full-length OspB and the other a truncated OspB. SDS/PAGE identified two groups of *Bb*, identical to the groups based on the PCR results. N40-B expressed a full-length OspB (34 kDa), whereas N40-ΔB lacked the full-length OspB and expressed a truncated form of OspB (22 kDa) (Fig. 2A, lanes 4 and 6). N-terminal sequencing of the 34- and 22-kDa bands from *E. coli* expressing recombinant OspB indicated that the N termini were identical, corresponding to the known OspB-B31 sequences of aa 1–36 and 1–23, respectively. The 22-kDa band was identical to the band previously described as 17 kDa (3). The recombinant truncated OspB expressed in *E. coli* was, however, slightly larger than the native truncated OspB (Fig. 2A, lanes 3 and 4). N-terminal protein sequencing of the corresponding bands in *Bb* N40 (Fig. 2A, lane 5) was unsuccessful, indicating that the sites were blocked. Protein sequencing of HPLC-separated tryptic peptides of the 34- and 22-kDa bands from the *Bb* extracts confirmed that each band was OspB (34 kDa, aa 49–55, 81–89, and 168–178; 22 kDa, aa 47–63, 81–89, and 142–151). Indeed, sequencing of the region distal to aa 192 in the 34-kDa band confirmed that the full-length OspB was translated in frame (34 kDa, aa 212–223, 229–235, and 236–251). Nucleotide sequencing of the amplified PCR products from the two limiting-dilution clones pGEX-2T verified the two *ospB* groupings: N40-ΔB had a stop codon at nt 577, resulting in the expression of a truncated *ospB* product, whereas N40-B had a full-length *ospB* product.

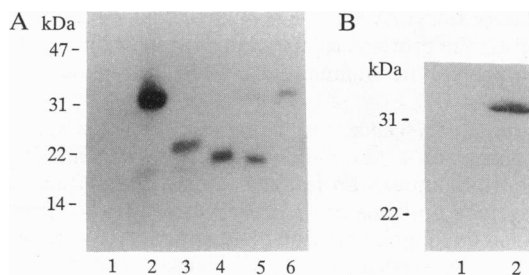Each clone of the spirochetes, N40-B, N40-ΔB, or the original N40 passage 3 (containing both clones), was then used to infect C3H mice, to determine whether each clone was infectious and pathogenic. Each mouse received 10⁴ spirochetes via an intradermal injection. Fourteen days after challenge the mice were sacrificed and examined for infection and disease. Mice challenged with either N40-B, N40-ΔB, or N40 passage 3 readily developed infection, arthritis, and carditis (>95%). Identical challenge experiments with an inoculum of 10² spirochetes yielded a 70% infection rate.

OspB Vaccination Protects Against N40-B but Not N40-ΔB. Vaccination experiments determined how the point mutation in OspB affected protection. Previous data showed that active immunization with OspB-B31 protected mice from infection with an inoculum of 10² B31 spirochetes but not against an inoculum of 10² N40 spirochetes (3). We performed immunization experiments using N40-B or N40-ΔB to infect mice. Mice vaccinated with OspB-B31 fusion protein developed high antibody titers to OspB, detected on immunoblot at a dilution of 1:5000, consistent with previous results (3). Active immunization of mice with the OspB-B31 fusion protein protected against challenge with an inoculum of 10² N40-B spirochetes but not against *Bb*-N40-ΔB (Table 1).

Molecular Mapping of OspB Epitopes That Bind Protective Antibody. Recombinant fragments of OspB were expressed as GT fusion proteins to determine the regions of OspB recognized by mAbs. The mAbs were then used in passive immunization studies to determine the epitopes of OspB that bind protective antibody. Twenty-four fragments of the *ospB* gene were amplified by PCR (Fig. 1). The fragments contained overlapping truncated and internal fragments, allowing for the precise identification of antibody binding sites. The fragments were cloned into pGEX-2T and expressed as GT fusion proteins in *E. coli* (Fig. 3).

The fusion proteins were probed on immunoblots with mAbs to OspB. mAbs 7E6C, B10, 27G, and 22J mapped to different regions within OspB (Table 2). mAb 7E6C bound a conformational epitope within aa 134–296 at the C terminus of OspB, and mAb B10 bound to an epitope within aa 1–50, near the N terminus (Fig. 4). Binding to the OspB epitopes depicted in Fig. 3B was not observed (data not shown). mAb 22J bound an epitope within aa 34–99, and mAb 27G bound a region within aa 134–167.

Table 1. Active immunization of mice with OspB-B31 fusion protein or passive immunization with rabbit anti-*Bb* N40 serum (Anti-N40), normal rabbit serum (NRS), OspB mAb 7E6C, or OspB mAb B10

| Challenge | Immunization | No. positive/no. inoculated | |
		Culture	Disease
N40-B	Active		
	OspB-B31	0/20	1/20
	Passive		
	Anti-N40	1/10	1/10
	mAb 7E6C	3/25	3/25
	mAb B10	15/25	13/25
	NRS	29/45	25/40
N40-ΔB	Active		
	OspB-B31	6/15	5/15
	Passive		
	Anti-N40	0/10	0/10
	mAb 7E6C	19/25	21/25
	mAb B10	18/25	16/24
	NRS	31/45	32/45

Mice were challenged with syringe inoculum of 10² N40-B or N40-ΔB spirochetes and sacrificed 14 days later. Mice were assayed for infection by culture and disease as described in *Materials and Methods.*

FIG. 2. (*A*) Immunoblot of *Bb* N40, or *E. coli* expressing recombinant OspB, probed with OspB mAb B10. Lane 1, *E. coli* DH5α; lane 2, *E. coli* transformed with p197-12 expressing full-length OspB; lane 3, *E. coli* transformed with p197-12 expressing truncated OspB; lane 4, *Bb* N40-ΔB; lane 5, *Bb* N40 (passage 3); lane 6, *Bb* N40-B. (*B*) Immunoblot of N40-ΔB (lane 1) and N40-B (lane 2) probed with OspB mAb 7E6C.
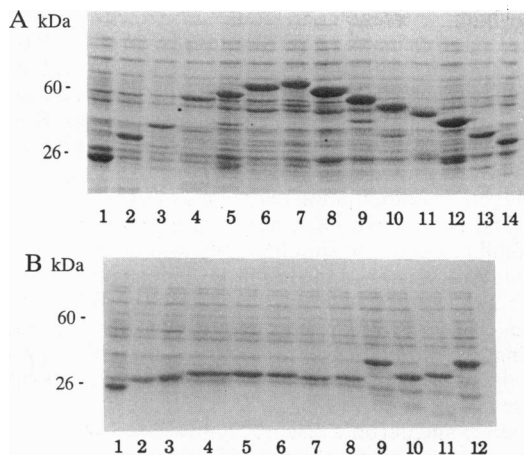
FIG. 3. SDS/PAGE of *E. coli* expressing fragments of OspB as GT fusion proteins. Nucleotides encoding the fragments are listed. (*A*) Lane 1, GT; lane 2, nt 1–150; lane 3, 1–298; lane 4, 1–606; lane 5, 1–702; lane 6, 1–801; lane 7, 1–888 (full-length OspB); lane 8, 100–888; lane 9, 199–888; lane 10, 298–888; lane 11, 400–888; lane 12, 502–888; lane 13, 601–888; lane 14, 700–888. (*B*) Lane 1, GT; lane 2, 800–888; lane 3, 100–150; lane 4, 199–298; lane 5, 298–402; lane 6, 400–501; lane 7, 601–702; lane 8, 700–801; lane 9, 298–606; lane 10, 502–702; lane 11, 601–801; lane 12, 100–402.

Epitopes of OspB were analyzed with sera from patients with chronic Lyme arthritis, to determine whether epitopes in the C terminus of OspB were recognized. The antibody response to OspB in patients with Lyme arthritis was polymorphic, varied among individuals, and included epitopes in both the N- and C-terminal regions of the protein (Table 2). Sera from patients with early Lyme disease did not have antibodies to OspB.

Immunofluorescence Identifies Epitopes of OspB on the *Bb* Surface. mAb B10, which recognizes the N terminus of OspB, bound to both populations of *Bb* N40 (N40-B and N40-ΔB), provided that they had been fixed with acetone. The B10 epitope is not exposed on the cell surface, because unfixed cells did not stain (Table 3). A conformational epitope in the C terminus of the protein is expressed on the cell surface and bound mAb 7E6C in immunofluorescence studies of both fixed and unfixed N40-B. As expected, no binding with mAb 76EC was seen with N40-ΔB, which lacks the C terminus of OspB. Both fixed and unfixed N40-B and N40-ΔB showed immunofluorescence when polyclonal mouse anti-OspB serum was used as the primary antibody, indicating that some OspB epitopes on the N40-ΔB spirochetes are surface-exposed. Indeed, mAbs 22J and 27G stained fixed and unfixed N40-B and N40-ΔB.
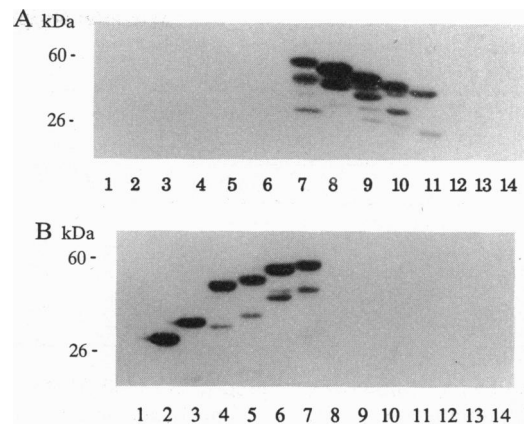

FIG. 4. Immunoblots of the OspB fragments expressed in *E. coli* and probed with mAb 7E6C (*A*) and B10 (*B*). Lanes in *A* and *B* correspond to the lanes in Fig. 3*A*.

Immunization with mAb 7E6C Protects Mice Against Infection with N40-B but Not N40-ΔB. Passive immunization with mAb 7E6C protected mice from infection with N40-B but not against N40-ΔB (Table 1). Passive immunization with mAb B10 did not protect against infection with either N40-B or N40-ΔB. On an immunoblot, mAb B10 bound to both N40-B and N40-ΔB OspB bands, whereas mAb 7E6C bound only N40-B OspB (Fig. 2). This suggested that protective antibodies bind to the C terminus of OspB. Both 7E6C and B10 are IgG2a antibodies, indicating that the immunization studies were controlled for IgG subclass. As expected, passive immunization of mice with rabbit anti-*Bb* serum protected against challenge with both N40-B and N40-ΔB, showing that antibodies to other *Bb* antigens, in particular OspA, are important in protection.

DISCUSSION

This study defines a molecular mechanism that *Bb* may use to evade immune destruction in the immunized host. Previous studies showed that mice immunized with OspB-B31 were protected from challenge with *Bb* B31 but not *Bb* N40. This report shows that two populations of *Bb* N40 exist, which are identical in the sequences of the *ospA* and *ospB* genes with the exception of a TAA stop codon at position 577 in *ospB* which results in a truncated OspB protein. Active immunization of mice with OspB-B31 fusion protein, or passive immunization with mAb 7E6C, which binds a conformational epitope within the C terminus of OspB, protects against challenge with *Bb* N40-B. This is similar to our previous report showing that vaccination with OspB-B31 protected mice against challenge with B31 spirochetes. Pro-

Table 2. Epitopes of OspB bound by polyclonal and monoclonal antibodies to OspB

Antibody	1 to 150	1 to 296	1 to 606	1 to 702	1 to 801	1 to 888	100 to 888	199 to 888	298 to 888	400 to 888	502 to 888	601 to 888	700 to 888	800 to 888	100 to 150	199 to 292	298 to 402	400 to 501	601 to 702	700 to 801	298 to 606	502 to 702	601 to 801	100 to 402
mAb 7E6C						+	+	+	+	+														
mAb B10	+	+	+	+	+	+																		
mAb 22J		+	+	+	+	+	+																	+
mAb 27G			+	+	+	+	+	+	+	+								+		+				
Patient 1			+	+	+	+	+	+	+	+	+								+			+	*	+
Patient 2			+	+	+	+	+	+	+	+	+	+				+				+				+
Patient 3				+	+	+	+	+	+	+	+			*		+								+
Patient 4		+	+	+	+	+	+	+	+	*						*								+
Patient 5		+				+	+	+	+	+	+	+		*							*	*	+

OspB epitopes are numbered according to nucleotides in the *ospB* sequence and correspond to the fragments in Figs. 1 and 3. +, Moderate to strong reactivity; *, weak reactivity (visible only on an overexposed immunoblot).

Table 3. Indirect immunofluorescence of fixed or unfixed *Bb* N40-B and N40-ΔB with monoclonal and polyclonal OspB antibodies

	Staining			
	N40-B		N40-ΔB	
Antibody	Fixed	Unfixed	Fixed	Unfixed
mAb B10	+	−	+	−
mAb 7E6C	+	+	−	−
mAb 22J	+	+	+	+
mAb 27G	+	+	+	+
Anti-OspB serum	+	+	+	+

tection does not, however, extend to *Bb* N40-ΔB for either passive immunization with OspB antibodies or active immunization with OspB. Previous work has shown that OspB, like OspA, is a lipoprotein, and that lipidation is likely to be important in anchoring OspB in the *Bb* membrane (15). Our immunofluorescence studies suggest that the C terminus of OspB is accessible to antibodies and surface-exposed, whereas the N terminus of OspB is within the cell membrane or, less likely, inaccessible to antibody because of an association with other cell surface components.

The data indicate that *Bb* spirochetes lacking the C termini of the Osp proteins can evade immune destruction in the immunized host. Our previous studies have shown that antigenic heterogeneity within the C terminus of OspA accounts in part for a lack of cross protection due to vaccination with a single recombinant OspA antigen (3, 4), and we here show that truncations of OspB may make the spirochetes inaccessible to protective antibody. Indeed, our previous work showed that an OspA mAb (CIII.78) that binds a conformation-dependent epitope in the C-terminal half of OspA (aa 133–273) protected mice from infection in passive immunization studies (2, 8). Similarly, OspB mAb 7E6C binds a conformational epitope within the C-terminal half of OspB (aa 134–296) and is protective. This suggests that the C termini of both OspA and OspB are exposed on the surface of the spirochete and that their tertiary structures define the protective epitope(s). This has implications for the pathogenesis of Lyme disease and the development of an effective vaccine. For example, *Bb* spirochetes in a chronically infected host may be able to alter the conformation of their Osp proteins to evade host defenses or, as in this case, spirochetes that lack the C terminus of OspB can survive within a vaccinated host.

Bb may persist in the host for long times, even in the presence of high antibody titers to OspA and OspB (16–19). The mechanism used for survival within the host is not known and may involve numerous pathways. *Bb* has been shown to bind to host cells, including endothelial cells, and some reports indicate that the spirochete may invade cells (20–22). If such a phenomenon occurred *in vivo*, intracellular sequestration could contribute to spirochete persistence. The spirochete can be recovered from selected sites, including the joints, dermis, and bladder serosa, in chronic infection of humans and experimental animals (18, 23, 24). While the organism is mostly found in the extracellular matrix in these tissues, specific localization may allow *Bb* to establish persistent infection. The Osp proteins can bind the heavy chains of non-*Bb*-specific IgM (25). If such a phenomenon occurred *in vivo*, such binding of host proteins would perhaps mask OspA and OspB epitopes from specific antibody.

This study shows that *Bb* spirochetes lacking the C terminus of OspB may escape immune destruction in the immunized host. The selection of antibody-resistant organisms may be a general method that the spirochete uses to perpetuate infection and cause chronic disease. Indeed, the variable OspB antibody response in sera from patients with chronic

arthritis may reflect the persistence of *Bb* with variant OspB proteins. Unfortunately, spirochetes are rarely isolated from chronically infected patients and, when available, have been cultured and amplified *in vitro* so that their direct relevance to the organism *in vivo* is uncertain. Nevertheless, Rosa *et al.* (26) have observed point mutations in *ospB* that prematurely truncated the protein, as well as Osp variations generated by homologous recombination between *ospA* and *ospB* genes, *in vitro*. The latter have been detected in multiple *Bb* isolates and could result in the simultaneous loss of OspA and OspB epitopes and evasion of the immune response to both proteins (26). This suggests that the truncated OspB-N40 is not a unique event and that *Bb* may evade host defenses by masking or eliminating Osp epitopes that bind protective antibody. This bacterial response to the host's humoral defenses may contribute, at least in part, to spirochete persistence and chronic disease. Knowledge of the incidence of mutant spirochetes, the rate of spirochete mutation, and the pathogenicity of these different *Bb* strains will enhance our understanding of protective immunity against *Bb*.

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