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C-Terminal Region of Outer Surface Protein C Binds Borreliacidal Antibodies in Sera from Patients with Lyme Disease

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Borreliacidal antibodies specific for outer surface protein C (OspC) are induced shortly after infection with *Borrelia burgdorferi*. In this study, we identified the region of OspC recognized by immunoglobulin M (IgM) and IgG borreliacidal antibodies. Sera from patients with early Lyme disease were screened for borreliacidal activity specific for *B. burgdorferi* 50772 and OspC antibodies. Seven sera that contained similarly high titers of each response were then chosen randomly and adsorbed with OspC or a truncated OspC (OspC-Dra) containing the 50 amino acids nearest the carboxy terminus. Adsorption with OspC or OspC-Dra completely eliminated the borreliacidal activity in six (86%) of seven sera and significantly decreased the activity in the remaining serum (titer of 10,240 to 1,280). Moreover, OspC antibodies were no longer detected by OspC enzyme-linked immunosorbent assay or in a Western blot that contained native OspC. The findings confirmed that sera from patients with early Lyme disease contain high concentrations of IgM or IgG borreliacidal antibodies that bind a conserved region of OspC.

Lyme disease in the United States is caused primarily by transmission of *Borrelia burgdorferi* sensu stricto from *Ixodes scapularis* ticks. Spirochetes localize in the skin initially and often cause an erythema migrans lesion (1). Untreated infections may spread to the nervous system or joints and cause additional complications, depending on the location and duration of infection, vulnerability of the immune system, and genetic factors that may predispose a patient to Lyme arthritis (19).

Antibodies produced after infection with *B. burgdorferi* have at least two distinct functions. The antibodies opsonize the spirochetes to facilitate ingestion by phagocytic cells (25) or kill the spirochetes with or without activation of complement (20, 21, 22, 28). The killing (borreliacidal) antibodies appear to be ineffective at eliminating an existing infection, presumably because the spirochetes undergo antigenic variation (32, 37), suppress the immune response (5), or localize in immune privileged sites (27). Lyme disease can be prevented, however, when high concentrations of borreliacidal antibodies are induced in vivo prior to challenging with the spirochetes. For example, vaccination with outer surface protein A (OspA) provides protection by inducing borreliacidal OspA antibodies that kill the spirochetes in the tick midgut as the infected tick ingests blood from the vaccinee (14, 24). In addition, vaccination with decorin binding protein A (DbpA) (33) or the 66-kDa outer membrane surface (Oms) protein (13) induce borreliacidal antibodies that eliminate spirochetes after transmission to the host.

Despite the inability of borreliacidal antibodies to eliminate an existing infection, detecting the response provides a highly specific serodiagnostic confirmation of Lyme disease (6–9). The strain of *B. burgdorferi*, however, is a critical determinant when evaluating serum for the presence of borreliacidal antibodies. For example, borreliacidal OspC antibodies are produced shortly after infection, but the antibodies are not detected when *B. burgdorferi* 297 spirochetes are used for testing. The 297 spirochetes express OspC, but the concurrent expression of OspA and OspB hinders the attachment of the OspC borreliacidal antibodies (30). In contrast, *B. burgdorferi* 50772 is highly susceptible to OspC borreliacidal antibodies (7, 30), because the spirochetes lack the plasmid containing *ospA* and *ospB* (2) and the absence of the Osps enables the OspC borreliacidal antibodies to bind. In this study, borreliacidal activity against *B. burgdorferi* 50772 was used to determine the region of OspC recognized by the immunoglobulin M (IgM) and IgG borreliacidal antibodies in sera from patients with early Lyme disease.

MATERIALS AND METHODS

Lyme disease sera. Serum samples from patients with early Lyme disease characterized by culture-positive or physician-documented single or multiple erythema migrans lesions were obtained from patients at the Gundersen Lutheran Medical Center in La Crosse, Wis. Serum from a person not exposed to *B. burgdorferi* was used as a normal control.

Organisms. *B. burgdorferi* 50772 (obtained from John F. Anderson, Connecticut Agricultural Experiment Station, New Haven) was incubated in Barbour-Stoenner-Kelly (BSK) medium to a concentration of approximately 10^7 spirochetes/ml. Two-hundred-microliter aliquots of the suspension were then dispensed into 1.5-ml screw-cap tubes (Sarstedt, Newton, N.C.) and stored at −70°C until used. Recombinant OspC was recovered from the previously described (30, 34) *Escherichia coli* JM109 containing *ospC* ligated into the expression vector pXa-3 (pX3-22). *E. coli* JM109 (Promega, Madison, Wis.) was also used for cloning.

Cloning of OspC-Dra. The pX3-22 plasmid was digested with *Bam*HI and *Sma*I (Gibco BRL, Gaithersburg, Md.). The *ospC* fragment was then redigested with *Dra*I (Gibco) to yield an approximately 0.2-kb *Dra*I-*Sma*I fragment, encoding the 50 amino acids nearest the carboxy (C) terminus (12), and a 0.4-kb *Bam*HI-*Dra*I fragment, coding for the remainder of *ospC*. The *Dra*I-*Sma*I frag-

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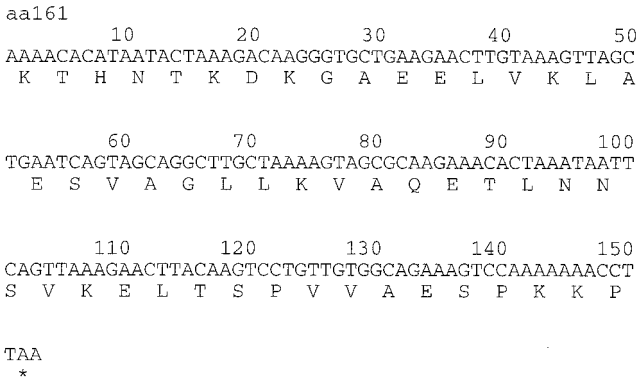

FIG. 1. DNA and predicted amino acid sequences of OspC-Dra. This region corresponds to amino acids (aa) 161 to 210 of OspC.

ment was ligated into *Sma*I-digested pXa-2 (pX2-Dra), and the *Bam*HI-DraI fragment was ligated into *Bam*HI- and *Sma*I-digested pXa-3. Both plasmids were then transformed into *E. coli*, but the fusion protein encoded by the *Bam*HI-*Dra*I fragment was insoluble and could not be investigated further. In contrast, the protein encoded by the *Dra*I-*Sma*I fragment, designated OspC-Dra, solubilized easily and could be used in the study. Proper ligation of the DNA encoding OspC-Dra was confirmed by restriction digestion. The DNA was sequenced by using primer 5′-AATTCCCGGGTTAAGGTTTTTTGGACTTTCTGC-3′ (22) and PinPoint sequencing primer (Promega). The DNA and predicted amino acid sequences of OspC-Dra are shown in Fig. 1.

Recovery of recombinant OspC or OspC-Dra. Expression of OspC or OspC-Dra was induced by culturing the *E. coli* in a 100-ml volume of 2xTY broth containing ampicillin (Sigma Chemical Co., St. Louis, Mo.) for 12 h at 37°C, diluting 10-fold with additional 2xTY broth, and incubating for another hour. Isopropyl-β-D-thiogalactopyranoside (Sigma) was then added to a final concentration of 0.1 μM, and the cultures were reincubated for 4 h at 37°C. The suspensions were pelleted by centrifugation at 10,000 × *g* for 30 min at 4°C; resuspended in purification buffer containing 50 μM Tris (pH 8.0), 50 mM NaCl, 2 mM EDTA, and 0.1% Triton X-100; and lysed with a sonicator (model W350; Branson Sonic Power, Danbury, Conn.). Sonicated *E. coli* cells were pelleted by centrifugation at 10,000 × *g* for 15 min, and the supernatants were passed over columns containing SoftLink resin (Promega) at a rate of 0.5 ml/min at 4°C. The fusion proteins contained a 16-kDa biotinylated purification tag on the amino (N) terminus of the protein that binds to SoftLink resin. Columns containing protein were then washed with 5 column volumes of purification buffer, and the bound OspC or OspC-Dra was eluted by using purification buffer containing 5 mM biotin (Sigma). The purity of the recovered proteins was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting with mouse polyclonal antibodies specific for OspC.

SDS-PAGE and Western blotting. SDS-PAGE and Western blotting was performed by standard procedures. Briefly, 6 μg of protein was loaded into individual wells of 0.1% SDS–12% polyacrylamide gels, and the proteins were separated by running in an electrophoresis unit (SE600; Hoefer Scientific, San Francisco, Calif.) at 55 mA for 3 h. The gels were then stained with 0.125% Coomassie blue, or the proteins were transferred from the gels to nitrocellulose by electrophoresing for 3 h at 300 mA. The nitrocellulose was cut into strips and blocked with phosphate-buffered saline (PBS) (pH 7.2)–0.3% Tween 20 for 30 min at 22°C. The strips were incubated for 1 h at 22°C with serum diluted 1:100 and washed three times with PBS–0.5% Tween 20 (PBS-Tween). Horseradish peroxidase-labeled anti-human IgM or IgG heavy and light chains (Kirkegaard & Perry Laboratories, Gaithersburg, Md.) were added, and the strips were incubated for 30 min at 22°C. Strips were washed and developed by using the TMB membrane peroxidase substrate system (Kirkegaard & Perry).

Indirect enzyme-linked immunosorbent assay (ELISA). Protein was diluted to 1 μg/ml in coating buffer (0.015 M Na_2CO_3, 0.035 M $NaHCO_3$ [pH 9.6]), and 100-μl amounts were added to individual wells of flat-bottom amine-binding microtiter plates (Costar, Cambridge, Mass.). The plates were incubated overnight at 4°C, washed with PBS, and blocked by addition of PBS-Tween containing 1% bovine serum albumin (Sigma) and incubation for 1 h at room temperature. Plates were then washed with PBS-Tween, 100 μl of serum serially diluted twofold (1:80 to 1:81,920) in PBS-Tween was added to the individual wells, and the plates were incubated for 1 h at room temperature. Following incubation, the plates were washed with PBS-Tween, and 100 μl of anti-human IgM or IgG horseradish peroxidase conjugate (Organon Teknika Cappel, Durham, N.C.) diluted 1:3,000 in PBS-Tween was added. Plates were then reincubated at room temperature for 1 h and washed with PBS-Tween, and 100 μl of *o*-phenylenediamine phosphate (0.4 mg/ml; Sigma) was added. After a 30-min incubation at room temperature, reactions were stopped by adding 100 μl of 1 N H_2SO_4, and absorbances at 490 nm (model EL 311 instrument; Bio-Tek Inc., Winooski, Vt.) were immediately determined.

Detection of borreliacidal antibodies. Borreliacidal antibodies were detected by using a flow cytometric procedure (6, 7). A culture of *B. burgdorferi* 50772 in logarithmic growth phase was enumerated by using a Petroff-Hausser counting chamber and diluted with fresh BSK to a concentration of approximately 5×10^5 spirochetes/ml. Concomitantly, serum samples were diluted 1:40 with BSK and sterilized by passage through a 0.2-μm-pore-size microcentrifuge filter (Costar). A 200-μl aliquot was then transferred to a sterile 1.5-ml screw-cap microcentrifuge tube (Sarstedt) and diluted serially from 1:80 to 1:20,480 with BSK. Serum samples were heat inactivated at 56°C for 10 min, and a 100-μl aliquot of the *B. burgdorferi* 50772 (5×10^4 spirochetes) and 15 μl of sterile guinea pig complement (Sigma) were added. The assay mixtures were mixed thoroughly and incubated for 16 to 24 h at 35°C.

Following incubation, 100 μl of each assay suspension was transferred to a 12- by 75-mm polypropylene tube (USA Scientific, Ocala, Fla.) containing 400 μl of PBS and 1 μg of acridine orange (Sigma) per ml. A FACScan flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, Calif.) was then used to detect borreliacidal activity. Spirochetes were isolated by gating (CellQuest software; Becton Dickinson) and analyzed for 1 to 2 min with the flow rate set at low. Borreliacidal antibodies were detected indirectly by monitoring the increased fluorescence intensity that occurs when the acridine orange intercalates into blebbed, nonviable spirochetes. A ≥13% shift in the mean fluorescence intensity compared to that of a normal serum control was considered positive (7). The presence of blebbed, nonmotile *B. burgdorferi* was then confirmed by dark-field microscopy. The undiluted and adsorbed sera from each patient were assayed concurrently to eliminate variability of titers caused by interassay variation in the concentration of spirochetes.

Adsorption with OspC or OspC-Dra. Tetralink tetrameric avidin resin (Promega) was washed with PBS, and a 1-ml volume was loaded into a 10- by 70-mm polypropylene column. Three milligrams of the OspC or OspC-Dra fusion protein was solubilized in a 1-ml volume of PBS and passed over the column. The fusion proteins bind to the avidin resin via the purification tag. Binding was confirmed by monitoring absorbance at 280 nm. A 1-ml volume of serum diluted 10-fold with PBS was then passed 10 times over the column at 4°C. Columns were regenerated between serum samples by eluting the captured antibodies with 5 column volumes of 0.1 M sodium citrate buffer (pH 3) and rinsing with 5 volumes of phosphate buffer (pH 7). In some instances, the fractions containing the captured antibodies were combined with an equal volume of Tris buffer (pH 9), reconcentrated to a 1-ml volume (Centricon; Millipore Corporation, Bedford, Mass.), and dialyzed in PBS.

Purification of IgM antibodies. IgM antibodies were recovered as previously described (4). A 16-mm by 90-cm column was packed with 200 ml of Sephacryl S-300 HR resin (Amersham Pharmacia Biotech, Piscataway, N.J.) at 4°C at a flow rate of 1 ml/min by using a positive pressure pump (Cole-Parmer, Chicago, Ill.), and PBS was run through the column for an additional hour at a flow rate of 2.3 ml/min. A 2-ml volume of Lyme disease serum diluted twofold with PBS was loaded onto the column, and the remaining headspace was filled with PBS. The suspension was then forced through the column at a flow rate of 1 ml/min, and 1-ml volumes were collected with a fraction collector (Frac-100; Amersham). Recovered antibodies were detected by monitoring the protein levels in the fractions (Bio-Rad, Hercules, Calif.). IgM antibodies were identified by applying 100 μl of each fraction diluted to a protein concentration of 1 μg/ml onto individual wells of a 96-well microtiter plate and analyzing by an IgM and IgG ELISA. There was a clear delineation between the fractions containing IgM or IgG antibodies. Fractions that contained IgM antibodies only were pooled, concentrated (Centriprep; Millipore), and analyzed by SDS-PAGE.

Purification of IgG antibodies. IgG antibodies were recovered by using affinity for protein G (3). A 19- by 105-mm polypropylene column was loaded with 5 ml of CNBr-activated Sepharose 4B fast-flow beads bound with protein G (Sigma). One milliliter of serum diluted twofold with 0.1 M sodium acetate (pH 5) was passed over the column, followed by several 20-ml volumes of sodium acetate. IgG antibodies were then eluted from the column with 20 ml of 0.1 M glycine HCl (pH 2.8) and collected in 1-ml amounts in tubes containing 50 μl of 1 M Tris (pH 9). The fractions containing the IgG antibodies were pooled, concentrated (Centriprep), dialyzed in PBS, and analyzed by SDS-PAGE.

TABLE 1. Removal of OspC antibodies from early Lyme disease sera by adsorption with OspC or OspC-Dra

Serum sample	OspC ELISA reactivity[a] after adsorption with:							
	Undiluted serum		Purification tag[b]		OspC		OspC-Dra	
	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG
1	20,480	10,240	20,480	10,240	160	ND[c]	ND	ND
2	81,920	1,280	163,840	1,280	ND	ND	80	ND
3	20,480	320	20,480	2,560	ND	ND	ND	ND
4	40,960	5,120	40,960	5,120	320	ND	80	ND
5	5,120	320	2,560	160	ND	ND	ND	ND
6	20,480	81,920	20,480	40,960	ND	80	80	ND
7	81,920	20,480	40,960	10,240	320	ND	ND	ND

[a] Reciprocal of last dilution with adsorbance value of ≥0.200 above that of the normal serum control. ELISA reactivity that remained after adsorption with OspC or OspC-Dra could be removed completely by additional passages over fresh columns.
[b] Serum passed through column containing purification tag only.
[c] ND, no ELISA reactivity detected.

RESULTS

Detection of OspC antibodies and borreliacidal activity. Sera from patients with early Lyme disease were screened initially for OspC and borreliacidal antibodies. Similar to previous findings (6, 30), both antibody responses were commonly present. We identified 29 sera from patients with early Lyme disease that contained borreliacidal antibodies specific for *B. burgdorferi* 50772 (titers of 320 to >10,240). Of these, 28 (97%) also contained significant concentrations of OspC antibodies detected by an OspC ELISA. The concentration of OspC antibodies was considered significant when the serum diluted 1:100 with PBS-Tween yielded an absorbance value of >0.200 U greater than the absorbance value of a normal serum control that did not contain OspC antibodies. Sera from seven patients with sufficient volumes for additional studies were chosen randomly. The patients had one or more culture-positive or physician-documented erythema migrans lesions and fever, headache, arthralgia, or myalgia for various lengths of time (mean, 19 days; range, 1 to 48 days). The sera contained various concentrations of borreliacidal antibodies (titers of 5,120 to ≥40,960) and IgM OspC antibodies (titers of 5,120 to 81,920) (Table 1). Four sera also contained IgG OspC antibodies (titers of 5,120 to 81,920).

Ability of adsorption with OspC or OspC-Dra to remove OspC antibodies. We first determined the ability of OspC or OspC-Dra to bind the OspC antibodies. The OspC antibodies were eliminated completely after adsorption with OspC. Low titers (≤320) of IgM or IgG OspC antibodies remained in four sera after the sera were passed over the OspC column 10 times (Table 1). The remaining antibodies were removed, however, by additional passages over a regenerated column. Similarly, the OspC antibodies were removed completely by adsorption with OspC-Dra. Relatively low titers (1:80) of IgM antibodies remained in three sera after 10 passages over the OspC-Dra column, but this activity could also be removed completely by additional passages over a fresh column. Moreover, OspC antibodies were no longer detected by Western blotting with *B. burgdorferi* 50772 antigen after the sera were passed over the columns containing either OspC or OspC-Dra (Fig. 2). Therefore, adsorption with the OspC-Dra fragment completely removed the IgM and IgG antibodies detected by the ELISA and, more significantly, also removed the reactivity detected by the Western blot that contained native OspC.

Effect of removing OspC or Osp-Dra antibodies on borreliacidal activity. We next determined whether removing the OspC antibodies affected the borreliacidal activity. After the OspC antibodies had been removed by binding to OspC or OspC-Dra, the borreliacidal activity was eliminated completely in six sera (86%) and decreased eightfold in the remaining serum (Table 2). The activity that remained was not specific for OspC or OspC-Dra, since additional passages over fresh columns had no effect. To further confirm that the borreliacidal antibodies were specific predominantly for a region within OspC-Dra, the antibodies captured in the columns were eluted and evaluated. The OspC ELISA and borreliacidal titers of the recovered antibodies were significantly lower than the titers detected in the undiluted serum despite attempts to reconcentrate the antibodies. This was likely due to degradation in the highly acidic (pH 3) citrate buffer or loss during concentration.

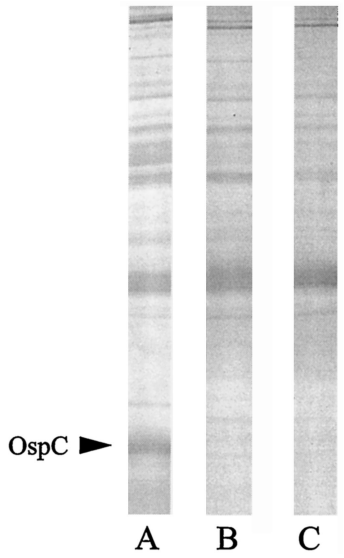

FIG. 2. Western blots of early Lyme disease serum before adsorption (lane A) and after adsorption with OspC (lane B) or OspC-Dra (lane C).

TABLE 2. Removal of borreliacidal antibodies from early Lyme disease sera after adsorption with OspC or OspC-Dra

Serum sample	Borreliacidal activity[a] after adsorption with:		
	Undiluted serum	OspC	OspC-Dra
1	5,120	ND[b]	ND
2	≥40,960	ND	ND
3	20,480	ND	ND
4	20,480	ND	ND
5	5,120	ND	ND
6	10,240	1,280	1,280
7	20,480	ND	ND

[a] Reciprocal of last dilution with significant borreliacidal activity.
[b] ND, no borreliacidal activity detected.

Sufficient levels of antibodies were recovered, however, to confirm borreliacidal activity and specificity for OspC. For example, antibodies recovered after the sera from patient 1 had been passed over the OspC or OspC-Dra columns bound OspC only (Fig. 3) and were borreliacidal to a titer of 640 or 1,280, respectively. Similar results were obtained when the antibodies adsorbed from the other sera were recovered and evaluated.

Recovery of IgM and IgG borreliacidal OspC antibodies. The OspC borreliacidal antibodies were probably IgM because all the sera contained high levels of IgM OspC antibodies (Table 1). Four sera (57%), however, contained IgG OspC antibodies that could also contribute to the borreliacidal activity (30). To confirm that the IgM and IgG OspC antibodies were borreliacidal, we recovered pure concentrations of each isotype from an early Lyme disease serum and evaluated them separately. The recovered IgM or IgG antibodies were specific primarily for OspC (Fig. 4, lanes A) and the isotypes were not contaminated (Fig. 4, lanes B). When the IgM and IgG antibodies were tested, borreliacidal antibodies were detected to titers of 1,280 and 640, respectively. The results, therefore, confirmed previous findings (30) that borreliacidal OspC antibodies were IgM and IgG.

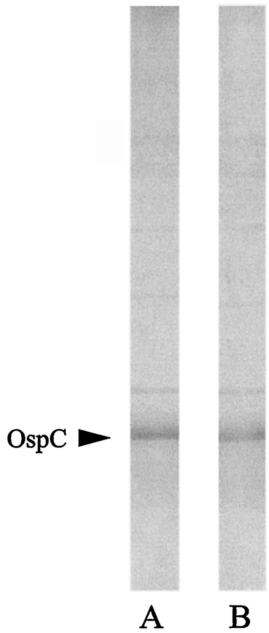

FIG. 3. Western blots of antibodies recovered from the OspC (lane A) and OspC-Dra (lane B) columns.

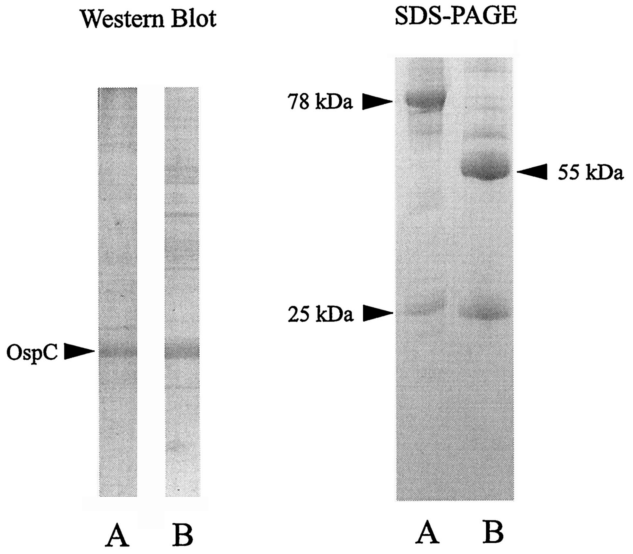

FIG. 4. SDS-PAGE and Western blotting of IgM (lanes A) or IgG (lanes B) antibodies purified from an early Lyme disease serum.

DISCUSSION

Several investigators have shown that borreliacidal OspC antibodies are formed shortly after infection with *B. burgdorferi* (17, 22, 30). Our results confirmed these findings and showed that OspC borreliacidal antibodies in early Lyme disease sera were predominantly IgM or IgG. Moreover, the borreliacidal OspC antibodies bound specifically to a region within the 50 amino acids nearest the C terminus of the protein. It was extraordinary that adsorbing the early Lyme disease sera with OspC-Dra removed the entire concentration of OspC antibodies detected by the ELISA or Western blot. The OspC-Dra contained only the α5 helix and a small region of the α4 helix (12), and high concentrations of antibodies specific for regions (α1 helix) near the N terminus have been observed in sera from patients with early Lyme disease (17). One would have expected these antibodies to remain detectable. Although the recombinant OspC in the ELISA may have undergone changes sufficient to affect binding by some OspC antibodies, the Western blot contained native lipidated OspC from *B. burgdorferi* 50772 that should have bound antibodies against any region of the protein.

The heterogeneity of OspC (34) may provide a partial explanation for this finding. A large portion of OspC is highly variable, especially the β-strands and loops connecting helix α2 with α3 and helix α3 with α4 (12, 23). The heterogeneity in these regions is extensive, even among isolates collected from a single endemic focus (34), and some OspC antibodies may not have recognized the OspC expressed by *B. burgdorferi* 50772. Other regions of the protein, however, are highly conserved. Small regions of homogeneity can be found throughout the α2, α3, and α4 helices, and more extensive regions are

located in the α1 or α5 helices near the N or C termini, respectively (12, 23). It is therefore unlikely that heterogeneity could have accounted entirely for the inability to detect any remaining OspC antibodies. In addition, antibodies specific for OspC have commonly been detected with ELISAs or Western blots that have used a wide variety of *B. burgdorferi* strains as antigens (10).

In this study, we detected OspC antibodies in 28 (97%) of 29 early Lyme disease sera that contained borreliacidal antibodies specific for *B. burgdorferi* 50772. We also showed that OspC borreliacidal antibodies were the predominant response in seven randomly chosen sera. Therefore, the *B. burgdorferi* 50772 borreliacidal antibodies in previous studies were likely also predominantly due to OspC antibodies. Borreliacidal antibodies specific for *B. burgdorferi* 50772 were commonly detected in serum samples from patients in the upper Midwest (6) and New York (7) and in reference sera supplied by the Centers for Disease Control and Prevention (6). More importantly, the OspC antibodies from the early Lyme disease sera in this study were almost entirely specific for a conserved region of OspC within OspC-Dra. These findings collectively provide strong evidence that the OspC antibody response is specific for epitopes that are highly conserved in a large number of *B. burgdorferi* isolates. The remarkably similar titers of borreliacidal activity and ELISA reactivity also suggest that the OspC antibodies in the early Lyme disease sera in this study consisted entirely of borreliacidal antibodies.

If this is the case, the inability to detect any OspC antibodies after the sera were adsorbed with OspC-Dra may be because OspC borreliacidal antibodies are specific for a conformational epitope comprised of regions near both termini. We did not investigate this hypothesis directly, because the fusion protein containing the region near the N terminus was insoluble. The results from several other investigations, however, provide strong support for this hypothesis. The crystal structure of OspC indicated that the N and C termini of OspC are adjacently exposed near the surface of the spirochete (12, 23). In addition, Gilmore and Mbow (15) showed that a protective monoclonal antibody was specific for a conformational epitope comprised of amino acids located near both termini of OspC. The monoclonal antibody was likely borreliacidal since the isotype was mouse IgG2a and this isotype fixes complement. Protective antibodies induced by vaccination with other *B. burgdorferi* Osps have also been borreliacidal (14, 16, 24, 26). More compelling is that Ikushima et al. (17) found a direct correlation between the levels of borreliacidal antibodies induced by vaccinating mice with a peptide derived from the N terminus of OspC and the level of protection against subsequent challenges with *Borrelia burgdorferi*, *Borrelia afzelii*, or *Borrelia garinii*. To investigate this possibility further, we are localizing the borreliacidal epitope within OspC-Dra and determining whether a similar epitope exists near the N terminus. As a prelude, we compared the DNA sequence corresponding to OspC-Dra with the DNA sequence corresponding to near the N terminus. A Clustal alignment (MacVector) identified a region of OspC-Dra similar to the protective epitope near the N terminus identified by Gilmore and Mbow (15).

The heterogeneity of OspC has caused considerable speculation that the protein would be ineffective as a vaccine or serodiagnostic antigen. However, the results in this study confirmed that early Lyme disease sera commonly contained OspC borreliacidal antibodies against a conserved region within OspC-Dra. While the results were obtained with a limited number of sera from patients in a relatively small geographic area, the findings suggest that vaccinations to induce OspC borreliacidal antibodies may provide more comprehensive protection than has been postulated. In addition, the results suggest the serodiagnosis of Lyme disease might be improved. Conventional indirect immunofluorescence assays (IFA) and ELISAs using *B. burgdorferi* are reasonably sensitive but lack specificity. To compensate, the Centers for Disease Control and Prevention recommends that serum be screened with a whole-cell IFA or ELISA and that equivocal or positive results be confirmed by Western blotting. This protocol, however, adds considerable cost and greatly increases the time necessary for testing. Western blots also require subjective interpretations, and some lack specificity (11, 36) or are less sensitive than IFA or ELISA (6, 18, 35). This can cause confusion, especially during early Lyme disease or when patients have less specific symptoms or little risk of a tick bite (29, 31). Tests that confirm Lyme disease by detecting borreliacidal antibodies can be more accurate than the two-test system (6), but the technical complexity and requirement for live spirochetes remain problematic. The accuracy of an OspC ELISA has also been investigated extensively, but the procedure has lacked specificity (35), possibly because antibodies formed during other illnesses bind the highly variable loop regions (12, 23). However, an ELISA with OspC-Dra should correlate more closely with the borreliacidal antibody response and therefore remain sensitive and provide greater specificity.

In conclusion, IgM and IgG borreliacidal antibodies in sera from patients with early Lyme disease are specific for a region within the 50 amino acids nearest the C terminus of OspC. The results also suggested indirectly that the entire OspC antibody response consisted of borreliacidal antibodies formed against an epitope of amino acids near the C terminus and possibly the N terminus. This finding provides valuable information for future efforts to develop more effective Lyme disease vaccines and serodiagnostic tests.

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