CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, June 2005, p. 746–751 Vol. 12, No. 6
1071-412X/05/$08.00+0 doi:10.1128/CDLI.12.6.746–751.2005

Borreliacidal OspC Antibodies Specific for a Highly Conserved Epitope Are Immunodominant in Human Lyme Disease and Do Not Occur in Mice or Hamsters

Steven D. Lovrich,[1] Dean A. Jobe,[1] Ronald F. Schell,[2] and Steven M. Callister[1,3]*

Microbiology Research Laboratory[1] and Section of Infectious Diseases,[3] Gundersen Lutheran Medical Center, La Crosse, Wisconsin 54601, and Wisconsin State Laboratory of Hygiene and Department of Medical Microbiology and Immunology, Madison, Wisconsin 53706[2]

Received 8 February 2005/Returned for modification 10 March 2005/Accepted 30 March 2005

Humans produce highly specific borreliacidal antibodies against outer surface protein C (OspC) shortly after infection with *Borrelia burgdorferi* sensu stricto. We previously demonstrated the epitope recognized by immunoglobulin M (IgM) and IgG OspC borreliacidal antibodies was located within the 50 amino acids nearest the carboxy (C) terminus. In this study, we show the immunodominant epitope is located in the highly conserved region within the seven C-terminal amino acids. Six early Lyme disease sera that contained borreliacidal activity and IgM and/or IgG OspC antibodies were chosen randomly and adsorbed with truncated OspC containing the 16 or 7 amino acids nearest the C terminus. Adsorptions with each truncated protein abrogated the borreliacidal activity completely. In addition, only small concentrations of OspC antibodies remained detectable by enzyme-linked immunosorbent assay and Western blotting. Moreover, borreliacidal OspC antibodies were not induced in laboratory mice or hamsters despite heavy infections with *B. burgdorferi* spirochetes. These findings confirm that borreliacidal antibodies comprise the majority of the IgM and IgG OspC antibody response in human Lyme disease and that the epitope is located in the highly conserved C terminus. In addition, rodent animal models appear to be inappropriate subjects for assessing the effectiveness of the epitope for serodiagnosis or as a human Lyme disease vaccine.

Lyme disease caused by infection with *Borrelia burgdorferi* sensu stricto is the most prevalent arthropod-borne human illness in the United States. The bacteria thrive in a variety of diverse niches as they cycle between small mammals, *Ixodes scapularis* ticks, and other alternative hosts including humans. Their ability to survive appears dependent on the differential expression or repression of genes selected in response to a variety of environmental cues. For example, the spirochetes express outer surface protein A (OspA) in the midgut of resting nymphal and adult ticks but downregulate OspA and upregulate OspC in response to temperature after the tick begins feeding (27). More recently, researchers have demonstrated the ability of the spirochetes to regulate a multitude of genes in the tick (13), at the tick-host interface (14), and in the mammalian host (4) in response to additional environmental signals that likely include components in blood, biochemical reactions, and the host immune response.

Despite the propensity for antigenic variation, however, the complement-dependent borreliacidal (killing) antibody response detected in a human infection is remarkably predictable. Several *B. burgdorferi* proteins, including OspA, OspB, OspC, DbpA, Oms66, and the 39-kDa periplasmic protein, induce borreliacidal antibodies (7, 9, 10, 17, 19, 26, 28, 31), but the response in early human Lyme disease is specific primarily for OspC (5, 6, 16, 17, 25). In addition, borreliacidal antibodies specific for OspA and OspB may be produced, but the response is almost exclusively seen in patients with Lyme arthritis (7, 10, 26). Moreover, traditional laboratory isolates that express OspA and OspB in vitro can be used to detect OspA and OspB borreliacidal antibodies, because the antibodies can attach to the outer surface proteins (Osps). However, OspC borreliacidal antibodies have not been detected by isolates that also express OspA and OspB, likely because the concurrent expression of these Osps hinders the attachment of OspC antibodies. Detecting OspC borreliacidal antibodies is therefore dependent on isolates such as *B. burgdorferi* 50772, because the spirochetes lack the plasmid containing *ospA* and *ospB* (2), and the absence of the Osps enables borreliacidal OspC antibodies to bind (6, 25). A borreliacidal antibody test that uses each type of isolate reliably provides sensitive and specific serodiagnostic confirmation of Lyme disease (5, 6, 8).

We previously exploited the susceptibility of *B. burgdorferi* 50772 to OspC borreliacidal antibodies (25) to demonstrate that the human antibody response was specific for the 50 amino acids (aa) nearest the carboxy (C) terminus of OspC (16). In this study, we examined smaller fragments of the OspC C terminus and again used borreliacidal activity specific for *B. burgdorferi* 50772 to more precisely identify the location of the borreliacidal epitope. We also determined whether the response was induced after the infection of laboratory mice or hamsters. The results confirm that the OspC borreliacidal antibody response is highly conserved in human Lyme disease and provide compelling evidence that the epitope responsible will be an extremely valuable serodiagnostic antigen and may provide the basis for an effective Lyme disease vaccine. Additionally, the results demonstrate that the production of OspC borreliacidal antibodies is unique to human infection.

* Corresponding author. Mailing address: Microbiology Research Laboratory, Gundersen Lutheran Medical Center, 1300 Badger Street, La Crosse, WI 54601. Phone: (608) 775-2042. Fax: (608) 775-6602. E-mail: smcallis@gundluth.org.

MATERIALS AND METHODS

Organisms. *B. burgdorferi* sensu stricto 50772 is a noninfectious isolate that lacks the plasmid containing *ospA* and *ospB* (36). *B. burgdorferi* sensu stricto 297 was isolated originally from human cerebrospinal fluid, and the spirochete expresses OspA, OspB, and OspC. *Escherichia coli* JM109 (Promega, Madison, Wis.) was used for cloning.

Animals. Ten-week-old female C3H/Hej mice (Jackson Laboratories, Bar Harbor, Minn.) or 6-week-old LVG hamsters (Charles River Laboratories, Kingston, N.Y.) were housed three or four per cage at ambient temperature. Food and water were available ad libitum. Animal experiments were reviewed and approved by the Gundersen Lutheran Animal Care and Use Committee (Institutional Animal Care and Use Committees).

Sera. Serum samples that contained significant concentrations of immunoglobulin M (IgM) and/or IgG OspC antibodies were obtained from patients with early Lyme disease characterized by single or multiple erythema migrans lesions. In addition, immune sera specific for host-adapted (3) Lyme disease spirochetes were obtained from hamsters and mice. Groups of donor animals were infected by injecting 100-μl volumes of Barbour-Stoenner-Kelly (BSK) medium containing 10^6 *B. burgdorferi* 297 cells subcutaneously in the lower back. Five weeks postchallenge, 12 approximately 2- by 2-mm pieces of infected ear tissue were removed from each animal by punch biopsy and implanted subcutaneously into the lower back of a naïve recipient animal. After 15 weeks, the recipient animals were bled to exsanguination and killed by inhalation of CO_2, and the bladder, kidney, spleen, heart, and ear tissues were cultured in BSK medium. Cultures were then examined weekly for 3 weeks by dark-field microscopy.

Cloning of OspC fragments. Recombinant OspC and OspC-Dra (50 C-terminal amino acids) were recovered from the previously described (16) *E. coli* JM109 containing either pX3-22 (OspC) or pX2-Dra (OspC-Dra). A recombinant protein containing the fragment with the final 16 OspC C-terminal amino acids (C16) was constructed using forward primer 5′-AGTTAAAGAACTTAC AAGTCCTGTTGTGG-3′, reverse primer 5′-GGTTAAGGTTTTTTTGGACT TTCTG-3′, and pX3-22 (15) as templates. The amplified C16 fragment was precipitated using Pellet paint (Novagen, Madison, Wis.) and ligated (pXT16) into a Pinpoint Xa-1 T-vector (Promega, Madison, Wis.). A fragment containing the final seven C-terminal amino acids (C7) was similarly made by annealing primers 5′-GGCAGAAAGTCCAAAAAAACCTTAACCA-3′ and 5′-GGTTA AGGTTTTTTTGGACTTTCTTTCTTCTGCCAA-3′ and also ligated (pXT7) into a PinPoint Xa-1 T-vector (Promega). Both pX16 and pX7 were then transformed into *E. coli* JM109, and proper orientations were confirmed by DNA sequencing (BigDye ABI, Foster City, Calif.).

Recovery of OspC or OspC fragments. The recombinant OspC or OspC fragments were recovered by culturing the *E. coli* in a 100-ml volume of 2× tryptone-yeast extract (TY) broth containing 100 μg/ml of ampicillin (Sigma Chemical Co., St. Louis, Mo.) for 12 h at 37°C, diluting tenfold with additional 2× TY broth, and incubating for another hour. Isopropyl-β-D-thiogalactosidase (IPTG) (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 15 min at 4°C; resuspended in purification buffer containing 50 mM 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 20 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 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 proteins were eluted with purification buffer that also contained 5 mM biotin (Sigma). The proteins were then examined by using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blotting with mouse monoclonal antibodies specific for OspC to confirm their purity.

Adsorption with OspC or OspC fragments. One-milliliter volumes of Tetralink tetrameric avidin resin (Promega) were washed and suspended in 40 ml of phosphate-buffered saline (PBS; pH 7.2) and loaded into separate 10- by 70-mm polypropylene columns. Amounts (0.5 mg) of OspC, OspC-Dra (18), C16, or C7 were solubilized in 1-ml volumes of PBS and passed over separate columns. Complete binding was confirmed by protein assay (Bio-Rad). One-milliliter volumes of sera diluted 1:10 in PBS were then passed over the column four times, and the amounts of antibodies removed were confirmed by using OspC-Dra, C16, or C7 enzyme-linked immunosorbent assays (ELISAs).

Indirect ELISA. An OspC ELISA and ELISAs containing the C16 or C7 OspC fragments were prepared as described previously (16). Serum from a person not exposed to *B. burgdorferi* was used as a normal control.

Detection of borreliacidal antibodies. Borreliacidal antibodies were detected by a flow cytometric procedure (6, 8). Viable *B. burgdorferi* 297 or 50772 organisms in logarithmic growth phase were enumerated with a Petroff-Hausser counting chamber and diluted with fresh BSK medium to a concentration of approximately 5×10^5 organisms/ml. Concomitantly, serum samples were diluted 1:20 with BSK and filter sterilized by passage through a 0.2-μm microfuge filter (Costar, Cambridge, Mass.). The filtered serum samples were then transferred to sterile 1.5-ml screw-cap microfuge tubes (Sarstedt, Newton, N.C.) and diluted serially (1:40 to 1:20,480) with BSK. Serum samples were heat inactivated at 56°C for 10 min, and a 100-μl aliquot of the spirochetes and 10 μl of sterile guinea pig serum (50% hemolytic component ≥ 200 units/ml) 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 polystyrene tube (Becton-Dickinson, Franklin Lakes, N.J.) containing 400 μl of PBS and 1 μg of acridine orange (Sigma) per ml. A FACScan flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA) 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 kill the spirochete by inducing a complement cascade that disrupts the outer membrane and causes the membrane to bleb. 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 (5, 6). The presence of blebbed, nonmotile *B. burgdorferi* was then confirmed by dark-field microscopy. The undiluted and adsorbed sera were assayed concurrently to eliminate the variability of titers caused by interassay variation in the concentrations of spirochetes.

Western blotting. Western blotting was performed using standard procedures. Briefly, 225 μg of *B. burgdorferi* 50772 protein was loaded into the preparative wells of 0.1% sodium dodecyl sulfate-12% polyacrylamide gels, and the proteins were separated by running in an electrophoresis unit (PROTEAN IIxi; Bio-Rad Laboratories, Hercules, Calif.) at 24 mA for 4 h. The proteins were transferred from the gels to polyvinylidene difluoride membrane (Perkin-Elmer Life Sciences Inc., Boston, Mass.) by electrophoresing overnight at 10 V. The polyvinylidene difluoride was cut into strips and blocked with 1% bovine serum albumin in PBS (pH 7.2)-0.1% Tween 20 for 1 h at 22°C. Strips were incubated for 1 h at 22°C with serum diluted to a ratio of 1:100 and washed four times with PBS-0.1% Tween 20. Horseradish peroxidase-labeled anti-human IgM or IgG heavy and light chains (Kirkegaard and Perry Laboratories, Gaithersburg, Md.) were added, and the strips were incubated for 1 h at 22°C. Strips were washed and developed using the TMB membrane peroxidase substrate system (Kirkegaard & Perry).

RESULTS

Ability of adsorptions to remove OspC antibodies. The early Lyme disease sera contained high concentrations of IgM OspC antibodies, and three sera also contained significant amounts of IgG OspC antibodies (Table 1). In agreement with previous findings (16), the OspC antibodies were removed almost completely by adsorption with OspC-Dra. More notably, binding to the C16 and C7 OspC fragments also almost completely removed the OspC antibodies detected by ELISA (≥4-fold reduction in titer) or by Western blotting (Fig. 1). In contrast, the reactivity on the Western blots for the other *B. burgdorferi* proteins appeared unaffected.

Effect of removing OspC fragment-specific antibodies on borreliacidal activity. We then determined whether removing the OspC antibodies by adsorption to the OspC-Dra, C16, or C7 OspC fragments also affected the borreliacidal activity. To confirm that the decreased activity was not due to nonspecific binding, we passed a Lyme disease serum that contained only OspA and OspB borreliacidal antibodies (7) over the columns prior to passing the Lyme disease sera that contained the OspC borreliacidal antibodies. In addition, we passed the Lyme disease sera over a column that contained just the fusion protein. As reported previously (16), adsorbing the early Lyme disease

TABLE 1. Removal of OspC antibodies from early Lyme disease sera by adsorption with OspC-Dra, C16, or C7

Serum sample	OspC ELISA reactivity[a] after adsorption with:									
	Untreated serum		OspC		OspC-Dra		C16		C7	
	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG
1	40,960	20,480	ND	ND	160	640	640	320	640	640
2	81,920	2,560	ND	ND	ND	160	160	160	ND	320
3	20,480	640	ND	ND	ND	80	160	160	80	80
4	40,960	10,240	ND	ND	2,560	320	2,560	320	2,560	640
5	5,120	320	ND	ND	160	ND	160	ND	320	ND

[a] Reciprocal of last dilution with adsorbance value of ≥0.200 above that of normal serum control. ND, no ELISA reactivity detected.

sera with OspC-Dra also abrogated the borreliacidal activity completely (Table 2). In addition, the borreliacidal antibodies were removed completely by binding to either the C16 or the C7 OspC fragments. The collective results therefore confirmed that the IgM and IgG OspC borreliacidal antibodies were specific for an epitope contained within the C-terminal 16- and 7-aa fragments of OspC. Moreover, removing the borreliacidal antibodies also removed almost the entire concentration (≥4-fold reduction) of OspC antibodies.

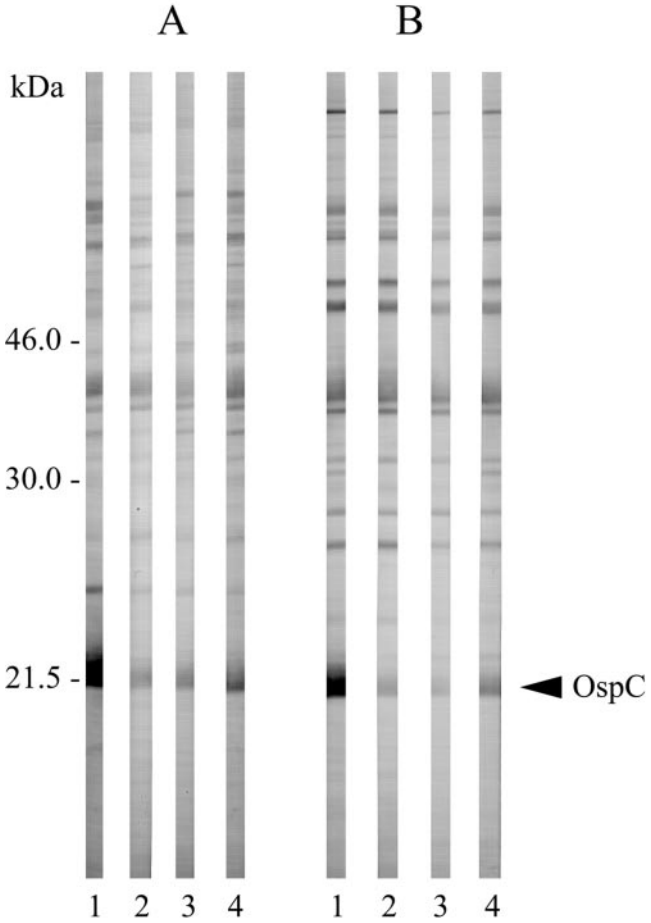

FIG. 1. Representative Western blots of antibodies in an early Lyme disease serum reactive against *B. burgdorferi* 50772 before adsorptions (lanes 1) and after adsorptions with OspC (lanes 2), C16 (lanes 3), or C7 (lanes 4). Panels A and B are IgM and IgG Western blots, respectively. Note that the effects of the adsorptions on the detection of OspC antibodies in the other four sera were identical.

OspC borreliacidal antibody response in mice and hamsters. We next determined whether the immune response in laboratory mice or hamsters would mimic the human antibody response after infection with Lyme disease spirochetes. To ensure that the antibodies were specific for proteins expressed in vivo (3), the animals were challenged with *B. burgdorferi* 297 spirochetes that were adapted to each animal by infecting donor animals with a needle challenge and harvesting spirochete-infected ear tissues to challenge additional naïve animals. The immune sera from the infected-tissue-challenged animals were then harvested, pooled ($n = 5$), and evaluated for borreliacidal activity and OspC antibodies. Both responses were detected, but the titers of all responses were significantly lower than the titers detected in human immune sera (Table 3), even though the animals were heavily infected (Table 4) and produced significant amounts of antibodies against many other proteins (Table 3). Furthermore, removing the OspC antibodies by adsorbing with the recombinant OspC had no effect on the borreliacidal activity (Table 3, Fig. 2).

DISCUSSION

Researchers showed previously (6, 16, 25) that the majority of IgM and IgG OspC antibodies produced after infection with *B. burgdorferi* sensu stricto were complement-dependent borreliacidal antibodies that killed the spirochetes without the necessity of scavenging by phagocytic cells. Moreover, the OspC borreliacidal antibodies were specific for an epitope(s) contained within the 50 C-terminal amino acids of the protein (16). In this study, we demonstrate that the OspC borreliacidal

TABLE 2. Removal of borreliacidal antibodies from Lyme disease sera after adsorption with OspC-Dra, C16, and C7

Serum sample	Borreliacidal activity[a] after adsorption with:			
	Untreated	OspC-Dra	C16	C7
Control[b]	5,120	5,120	5,120	5,120
1	10,240	ND	ND	ND
2	40,960	ND	ND	ND
3	10,240	ND	ND	ND
4	10,240	ND	ND	ND
5	2,560	ND	ND	ND

[a] Reciprocal of last dilution with significant borreliacidal activity. ND, no borreliacidal activity detected.
[b] Lyme disease serum containing OspA and OspB borreliacidal antibodies.

TABLE 3. Effect of removal of OspC antibodies on borreliacidal activity in mouse or hamster immune serum samples

Serum sample[c]	OspC IgG ELISA reactivity[a]		Borreliacidal activity[b]	
	Untreated	OspC adsorbed	Untreated	OspC adsorbed
Mouse	2,560	ND	160	160
Hamster	320	ND	320	320

[a] Reciprocal of last dilution with adsorbance value of ≥0.200 above that of normal serum control. ND, no ELISA reactivity detected.
[b] Reciprocal of last dilution with significant borreliacidal activity.
[c] Pooled serum samples (n = 5).

antibodies are specific for a highly conserved epitope within the seven C-terminal amino acid residues.

This finding is remarkable. As an infected tick begins feeding on a human, OspC is expressed (27) and becomes the major outer membrane protein expressed in early infection, regardless of the *Borrelia* genospecies infecting the patient (11, 22, 24, 35). Correspondingly, OspC antibodies quickly become the dominant humoral immune response (22, 35); however, OspC ELISAs have lacked accuracy for confirming Lyme disease (12, 20, 24, 30, 33). The shortcomings were explained most often by the heterogeneity of *ospC* (15, 18, 29, 30), even among isolates within the same genospecies (32). The antibody responses induced by the highly variable regions would have widely variable specificities. Additionally, antibodies induced by other infections may also react to these regions. However, previous studies that characterized the ability of OspC antibodies to kill the spirochetes independent of scavenging by phagocytic cells have provided compelling evidence that the borreliacidal antibodies are specific for a conserved region of the protein. For example, a flow cytometric test that detects borreliacidal OspC antibodies by monitoring the ability of the antibodies to kill only *B. burgdorferi* sensu stricto 50772 detected the response in >70% of early Lyme disease sera collected from throughout the United States (5).

In this study, we extend these findings by demonstrating that the dominant OspC borreliacidal antibody epitope is located within the surface-exposed (22) seven amino acid residues of the C-terminal end of the protein. More importantly, a BLAST search (1) confirmed the region is highly conserved among infectious *B. burgdorferi* sensu stricto, *B. garinii*, and *B. afzelii* isolates. The results therefore provide explanation for the increased accuracy (5, 6) of the flow cytometric borreliacidal antibody test. Moreover, the findings provide strong evidence that an ELISA comprised of the epitope would correlate directly with the highly specific borreliacidal antibody response. This should provide a sensitive and highly specific serodiagnostic confirmation of Lyme disease without the technical complexity and live organisms necessary to detect borreliacidal activity.

TABLE 4. Recovery of *Borrelia burgdorferi* from mice and hamsters challenged with spirochete-infected ear tissues

Animal	No. of positive samples/no. tested in:					Total no. positive/no. tested
	Spleen	Kidney	Bladder	Heart	Ear	
Mice	0/5	2/5	5/5	4/5	1/5	5/5
Hamsters	2/5	0/5	3/5	4/5	4/5	5/5

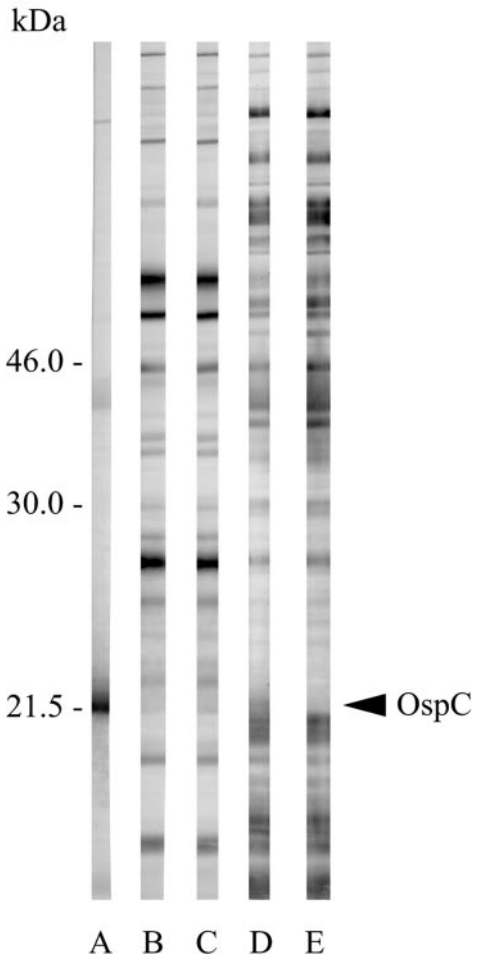

FIG. 2. Western blots of pooled (n = 5) hamster immune serum before (lane B) and after (lane C) adsorptions with OspC or pooled (n = 5) mouse immune serum before (lane D) and after (lane E) adsorptions with OspC. Lane A shows reactivity by polyclonal antibodies specific for OspC.

Mathiesen et al. (22) also reported that the OspC antibodies in sera from patients with neuroborreliosis caused by infection with *B. garinii* were specific predominantly for the identical sequence of 10 C-terminal amino acid residues of the protein. However, an ELISA with a synthetic peptide (21), while more reactive than the rOspC ELISA, detected OspC antibodies in European sera significantly less often (36%) than in sera from U.S. patients (11, 16, 20, 33). The authors noted the distinct absence of IgG OspC antibodies in sera from European patients (34) and suggested the failure of the response to reach maturity was because the epitope induced antibodies in a T-cell-independent fashion (22). However, this is clearly not the case after infection with *B. burgdorferi* sensu stricto. In this study, we detected high titers of borreliacidal IgG OspC antibodies, and numerous other investigators (5, 7, 11, 20, 25) have also confirmed that IgG OspC antibodies are commonly present in all stages of the illness. It seems unlikely the iden-

tical epitope would induce both a T-cell-independent and a T-cell-dependent response.

Rather, we suspect the failure of the OspC antibodies to switch classes in the European Lyme disease patients is more likely due to the predilection of the spirochetes to colonize the central nervous system, which may inhibit the maturation of the antibody response. In support, European patients are often infected with *B. garinii* or *B. afzelii*, which commonly cause neuroborreliosis (23). These genospecies are not found in the United States, where the patients are instead infected with *B. burgdorferi* sensu stricto, which most often colonizes the skin or joints. Additional studies to evaluate this hypothesis remain necessary, but the collective results still highlight the immunodominance of the OspC antibody response specific for the C terminus of the protein and provide support for the development of serodiagnostic procedures to exploit this finding.

In addition, the results provide compelling evidence that the C terminus of OspC is a good human vaccine candidate. However, we failed to detect borreliacidal OspC antibodies in sera from the laboratory mice or hamsters, despite vigorous antibody responses against many other *B. burgdorferi* proteins. The sera instead contained only small concentrations of OspC antibodies detected by ELISA or Western blotting. More significantly, the OspC antibodies were not borreliacidal antibodies. The findings therefore confirmed a significant difference in the antigenic reactivities of *B. burgdorferi* sensu stricto spirochetes that infect human or rodent hosts. More importantly, the results cast considerable doubt on the ability of rodent animal models to provide experimental data that accurately predict the ability of vaccination with OspC to provide protection against human infection.

In conclusion, the immunodominant IgM and IgG OspC antibody response in human Lyme disease is specific for the highly conserved seven C-terminal amino acid residues. This finding provides valuable information for future efforts to develop effective serodiagnostic tests and vaccines, but an accurate assessment of their effectiveness cannot be obtained by using laboratory mice or hamsters.

ACKNOWLEDGMENTS

We thank Jamie Griffin for expert technical assistance.

Financial support was provided by the Gundersen Lutheran Medical Foundation, La Crosse, Wisconsin.

REFERENCES

1. **Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman.** 1990. Basic local alignment search tool. J. Mol. Biol. **215:**403–410.
2. **Anderson, J. F., R. A. Flavell, L. A. Magnarelli, S. W. Barthold, F. S. Kantor, R. Wallich, D. H. Persing, D. Mathiesen, and E. Fikrig.** 1996. Novel *Borrelia burgdorferi* from *Ixodes scapularis* ticks feeding on humans. J. Clin. Microbiol. **34:**524–529.
3. **Barthold, S. W., E. Fikrig, L. K. Bockenstedt, and D. H. Persing.** 1995. Circumvention of outer surface protein A immunity by host-adapted *Borrelia burgdorferi*. Infect. Immun. **63:**2255–2261.
4. **Brooks, C. S., P. Hefty, S. E. Jolliff, and D. R. Akins.** 2003. Global analysis of *Borrelia burgdorferi* genes regulated by mammalian host-specific signals. Infect. Immun. **71:**3371–3383.
5. **Callister, S. M., D. A. Jobe, W. A. Agger, R. F. Schell, T. J. Kowalski, S. D. Lovrich, and J. A. Marks.** 2002. Ability of the borreliacidal antibody test to confirm Lyme disease in clinical practice. Clin. Diagn. Lab. Immunol. **9:**908–912.
6. **Callister, S. M., D. A. Jobe, R. F. Schell, C. S. Pavia, and S. D. Lovrich.** 1996. Sensitivity and specificity of the borreliacidal antibody test during early Lyme disease: a "gold standard"? Clin. Diagn. Lab. Immunol. **3:**399–402.
7. **Callister, S. M., R. F. Schell, K. L. Case, S. D. Lovrich, and S. P. Day.** 1993. Characterization of the borreliacidal antibody response to *Borrelia burgdorferi* in humans: a serodiagnostic test. J. Infect. Dis. **167:**158–164.
8. **Callister, S. M., R. F. Schell, L. C. L. Lim, D. A. Jobe, K. L. Case, G. L. Bryant, and P. E. Molling.** 1994. Detection of borreliacidal antibody by flow cytometry: an accurate, highly specific serodiagnostic test for Lyme disease. Arch. Intern. Med. **154:**1625–1632.
9. **Exner, M. M., X. Wu, D. R. Blanco, J. N. Miller, and M. A. Lovett.** 2000. Protection elicited by native outer membrane protein Oms66 (p66) against host-adapted *Borrelia burgdorferi*: conformational nature of bactericidal epitopes. Infect. Immun. **68:**2647–2654.
10. **Fikrig, E., L. K. Bockenstedt, S. W. Barthold, M. Chen, H. Tao, P. Ali-Salaam, S. R. Telford, and R. A. Flavell.** 1994. Sera from patients with chronic Lyme disease protect mice from Lyme borreliosis. J. Infect. Dis. **169:**568–574.
11. **Fung, B. P., G. L. McHugh, J. M. Leong, and A. C. Steere.** 1994. Humoral immune response to outer surface protein C of *Borrelia burgdorferi* in Lyme disease: role of immunoglobulin M response in the serodiagnosis of early infection. Infect. Immun. **62:**3213–3221.
12. **Gerber, M. A., E. D. Shapiro, G. L. Bell, A. Sampieri, and S. Padula.** 1995. Recombinant outer surface protein C ELISA for the diagnosis of early Lyme disease. J. Infect. Dis. **171:**724–727.
13. **Gilmore, R. D., M. L. Mbow, and B. Stevenson.** 2001. Analysis of *Borrelia burgdorferi* gene repression during life cycle phases of the tick vector *Ixodes scapularis*. Microbes Infect. **3:**799–808.
14. **Hodzic, E., S. Feng, K. J. Freet, D. L. Borjesson, and S. W. Barthold.** 2002. *Borrelia burgdorferi* population kinetics and selected gene expression at the host-vector interface. Infect. Immun. **70:**3382–3388.
15. **Jauris-Heipke, S., G. Liegl, V. Preac-Mursic, D. Rossler, E. Schwab, E. Soutschek, G. Will, and B. Wilske.** 1995. Molecular analysis of genes encoding outer surface protein C (OspC) of *Borrelia burgdorferi* sensu lato: relationship to *ospA* genotype and evidence of lateral gene exchange of *ospC*. J. Clin. Microbiol. **33:**1860–1866.
16. **Jobe, D. A., S. D. Lovrich, R. F. Schell, and S. M. Callister.** 2003. C-terminal region of outer surface protein C binds borreliacidal antibodies in sera from patients with Lyme disease. Clin. Diagn. Lab. Immunol. **10:**573–578.
17. **Kraiczy, P., K. P. Hunfeld, S. Peters, R. Wurzner, G. Ackert, B. Wilske, and V. Brade.** 2000. Borreliacidal activity of early Lyme disease sera against complement-resistant *Borrelia afzelii* FEM1 wild-type and an OspC-lacking FEM1 variant. J. Med. Microbiol. **49:**917–928.
18. **Livey, I., C. P. Gibbs, R. Schuster, and F. Dorner.** 1995. Evidence for lateral transfer and recombination in OspC variation in Lyme disease *Borrelia*. Mol. Microbiol. **18:**257–269.
19. **Luke, C. J., M. A. Marshall, J. M. Zahradnik, M. Bybel, B. E. Menefee, and A. G. Barbour.** 2000. Growth-inhibiting antibody responses of humans vaccinated with recombinant outer surface protein A or infected with *Borrelia burgdorferi* or both. J. Infect. Dis. **181:**1062–1068.
20. **Magnarelli, L. A., E. Fikrig, S. J. Padula, J. F. Anderson, and R. A. Flavell.** 1996. Use of recombinant antigens of *Borrelia burgdorferi* in serologic tests for diagnosis of Lyme borreliosis. J. Clin. Microbiol. **34:**237–240.
21. **Mathiesen, M. J., M. Christiansen, K. Hansen, A. Holm, E. Asbrink, and M. Theisen.** 1998. Peptide-based OspC enzyme-linked immunosorbent assay for serodiagnosis of Lyme borreliosis. J. Clin. Microbiol. **36:**3474–3479.
22. **Mathiesen, M. J., A. Holm, M. Christiansen, J. Blom, K. Hansen, S. Ostergaard, and M. Theisen.** 1998. The dominant epitope of *Borrelia garinii* outer surface protein C recognized by sera from patients with neuroborreliosis has a surface-exposed conserved structural motif. Infect. Immun. **66:**4073–4079.
23. **Ornstein, K., J. Berglund, I. Nilsson, R. Norrby, and S. Bergstrom.** 2001. Characterization of Lyme borreliosis isolates from patients with erythema migrans and neuroborreliosis in southern Sweden. J. Clin. Microbiol. **39:**1294–1298.
24. **Padula, S. J., F. Dias, A. Sampieri, R. B. Craven, and R. W. Ryan.** 1994. Use of recombinant OspC of *Borrelia burgdorferi* for serodiagnosis of early Lyme disease. J. Clin. Microbiol. **32:**1733–1738.
25. **Rousselle, J. C., S. M. Callister, R. F. Schell, S. D. Lovrich, D. A. Jobe, J. A. Marks, and C. A. Wieneke.** 1998. Borreliacidal antibody production against outer surface protein C of *Borrelia burgdorferi*. J. Infect. Dis. **178:**733–741.
26. **Sadziene, A., P. A. Thompson, and A. G. Barbour.** 1993. In vitro inhibition of *Borrelia burgdorferi* growth by antibodies. J. Infect. Dis. **167:**165–172.
27. **Schwan, T. G., J. Piesman, W. T. Golde, M. C. Dolan, and P. A. Rosa.** 1995. Induction of an outer surface protein in *Borrelia burgdorferi* during tick feeding. Proc. Natl. Acad. Sci. USA **92:**2909–2913.
28. **Scriba, M., J. S. Ebrahim, T. Schlott, and H. Eiffert.** 1993. The 39-kilodalton protein of *Borrelia burgdorferi*: a target for bactericidal human monoclonal antibodies. Infect. Immun. **61:**4523–4526.
29. **Stevenson, B., and S. W. Barthold.** 1994. Expression and sequence of outer surface protein C among North American isolates of *Borrelia burgdorferi*. FEMS Microbiol. Lett. **124:**367–372.
30. **Theisen, M., B. Frederiksen, A. M. Lebech, J. Vuust, and K. Hansen.** 1993. Polymorphism in *ospC* gene of *Borrelia burgdorferi* and immunoreactivity of OspC protein: implications for taxonomy and for use of OspC protein as a diagnostic antigen. J. Clin. Microbiol. **31:**2570–2576.
31. **Ulbrandt, N. D., D. R. Cassell, N. K. Patel, W. C. Roberts, C. M. Bachy, C. A. Fazenbaker, and M. S. Hanson.** 2001. Conformational nature of the *Borrelia*

burgdorferi decorin binding protein A epitopes that elicit protective antibodies. Infect. Immun. **69:**4799–4807.

32. **Wang, I. N., D. E. Dykhuizen, W. Qiu, J. J. Dunn, E. M. Bosler, and B. J. Luft.** 1999. Genetic diversity of *ospC* in a local population of *Borrelia burgdorferi* sensu stricto. Genetics **151:**15–30.
33. **Wieneke, C. A., S. D. Lovrich, S. M. Callister, D. A. Jobe, J. A. Marks, and R. F. Schell.** 2000. Evaluation of whole-cell and OspC enzyme-linked immunosorbent assays for discrimination of early Lyme borreliosis from OspA vaccination. J. Clin. Microbiol. **38:**313–317.
34. **Wilske, B., V. Fingerle, P. Herzer, A. Hofmann, G. Lehnert, H. Peters, H. W. Pfister, V. Preac-Mursic, E. Soutschek, and K. Weber.** 1993. Recombinant immunoblot in the serodiagnosis of Lyme borreliosis. Comparison with indirect immunofluorescence and enzyme-linked immunosorbent assay. Med. Microbiol. Immunol. **182:**255–270.
35. **Wilske, B., V. Fingerle, V. Preac-Mursic, S. Jouris-Heipke, A. Hofmann, H. Loy, H.-W. Pfister, D. Rossler, and E. Soutschek.** 1994. Immunoblot using recombinant antigens derived from different genospecies of *Borrelia burgdorferi* sensu lato. Med. Microbiol. Immunol. **183:**43–59.
36. **Zhang, Y. Q., D. Mathiesen, C. P. Kolbert, J. Anderson, R. T. Schoen, E. Fikrig, and D. H. Persing.** 1997. *Borrelia burgdorferi* enzyme-linked immunosorbent assay for discrimination of OspA vaccination from spirochete infection. J. Clin. Microbiol. **35:**233–238.