Borreliacidal Antibody Production against Outer Surface Protein C of *Borrelia burgdorferi*

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Early Lyme borreliosis sera with significant titers of anti—outer surface protein C (OspC) borreliacidal antibodies were identified. Human anti-OspC borreliacidal antibodies could be either IgM or IgG. Significant concentrations of borreliacidal activity were detected after vaccination of mice with OspC. Detection of anti-OspC borreliacidal activity was dependent on surface expression of OspC by *Borrelia burgdorferi* isolate 50772. The ability of OspC to induce borreliacidal antibodies in vivo and after vaccination offers another possible explanation for the ability of vaccination with OspC to protect against infection with *B. burgdorferi*. Furthermore, detection of anti-OspC borreliacidal antibodies, especially IgM antibodies, in early Lyme borreliosis sera provides additional evidence that borreliacidal antibody detection may be useful for the serodiagnosis of early Lyme borreliosis.

Lyme borreliosis is a tick-associated zoonosis caused by *Borrelia burgdorferi* sensu lato. This multisystem disorder has become the most common tick-transmitted disease in the United States [1] and causes significant morbidity worldwide. Initially, spirochetes infect the skin and, in the majority of early cases, cause an erythema migrans lesion [2]. If left untreated, spirochetes can disseminate to various tissues of the body, including the heart, nervous system, or joints, and cause manifestations such as meningitis, facial palsy, atrioventricular heart blockage, encephalopathy, polyneuropathy, and chronic arthritis [3–6].

Shortly after the discovery of Lyme borreliosis, researchers determined that vaccination of experimental animals with whole *B. burgdorferi* provided protection against challenge [7, 8]. Additional studies established the role of antibody-mediated protection and confirmed the ability of vaccination with *B. burgdorferi* to induce borreliacidal antibodies that specifically killed *B. burgdorferi* [9–11]. To date, vaccination of animals with outer surface protein (Osp) components of *B. burgdorferi*, especially OspA [12–14], OspB [12, 14], and OspC [14–16], have provided protection against infection with the Lyme spirochete. Furthermore, OspA and OspB have been shown to induce borreliacidal antibodies [13, 14, 17–24].

OspA has received the most intense evaluation as a protective vaccinogen. We [23] and others [21] have demonstrated that anti-OspA borreliacidal antibodies are responsible for providing protection after vaccination. Interestingly, OspC has also been shown to provide protection. However, anti-OspC borreliacidal antibodies have not been detected after vaccination [14–16]. In this investigation, we investigated whether OspC induces borreliacidal antibodies in vivo, because the ability of OspC vaccination to provide protection may also be dependent on borreliacidal antibodies. Borreliacidal antibodies have also been shown to serve as the basis for a sensitive and highly specific serodiagnostic test [17, 25–27]. The ability to detect anti-OspC borreliacidal antibodies would be useful for improving the serodiagnosis of Lyme borreliosis.

**Materials and Methods**

**Organisms.** *B. burgdorferi* sensu stricto isolate 297 was isolated from human spinal fluid. *B. burgdorferi* sensu stricto isolate 50772, originally isolated from an *Ixodes scapularis* tick, was obtained from John F. Anderson (Connecticut Agricultural Experiment Station, New Haven). The spirochete lacks the *ospA/B* operon and hence does not express OspA or OspB [28]. The original suspensions of spirochetes were serially 10-fold diluted in Barbour-Stoenner-Kelly (BSK) medium capable of supporting growth from a single organism [29]. The resultant population of spirochetes was then passaged 10 times in fresh BSK medium at 30°C or 35°C, dispensed into 200-μL aliquots in 1.5-mL screw-cap tubes (Sarstedt, Newton, NC), and stored at −70°C until used. *Escherichia coli* JM109 (Promega, Madison, WI) was used in all cloning experiments.

**Animals.** Ten-week-old female C3H/HeJ mice (Jackson Laboratories, Bar Harbor, ME) were housed 3 per cage at ambient temperature. Food and water were available ad libitum.

**Sera.** Lyme borreliosis sera were obtained from patients at the Gundersen Lutheran Medical Center (La Crosse, WI). Ten Lyme borreliosis sera were from persons with clinician-documented single or multiple erythema migrans lesions. Two of these sera were
from patients with dermal skin cultures positive for *B. burgdorferi*. Serum from a person not exposed to *B. burgdorferi* sensu lato was used as a normal serum control. This serum sample was tested by *B. burgdorferi* from patients with dermal skin cultures positive for 734 Rousselle et al. JID 1998;178 (September) 734 Rousselle et al. JID 1998;178 (September)

Colonies expressing OspC were detected by Western blot analysis passage through a 0.2-

**Plasmid enrichment.** DNA was isolated from *B. burgdorferi* sensu stricto isolate S-1-10 [12]. The DNA was used as a template for the amplification of the ospC gene (GeneAmp; Perkin Elmer Cetus, Norwalk, CT) [33]. Primers were used at a final concentration of 1.0 μM in a 1.5-μM concentration of MgCl₂. Thermal cycling parameters were 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 50°C for 30 s, and 72°C for 90 s. The final extension was done at 72°C for 7 min to fully extend any truncated DNA strands. The amino-terminal primer C1 (5'-CGTGGATCCATTAAACAAAGATCATAC-TAAGTCGCCATA-3') and the carboxy-terminal primer C2 (5'-AAATCCGGGTTAAAGTCTTTTGACTTTTCGC-3') were used for amplification. Underscores indicate regions recognized by the primers. Amplified DNA was purified with GeneClean (Bio101, La Jolla, CA). After digestion with Smal and BamHI (Gibco BRL, Gaithersburg, MD), purified DNA fragments were ligated into the PinPoint pXa-3 vector (Promega, Madison, WI) with T4 DNA ligase (Gibco BRL). The ligation mixture was used to transform competent *E. coli* JM109. Transformed *E. coli* were plated onto 2× tryptone-yeast (TY) medium containing ampicillin (100 μg/mL; Sigma, St. Louis) and incubated for 24 h at 37°C. Colonies expressing OspC were detected by Western blot analysis using a streptavidin–horseradish peroxidase conjugate (Gibco BRL) and an early Lyme disease serum containing anti-OspC antibodies.

The DNA sequence of the ospC gene was determined by double-stranded sequencing (TaqTrack; Promega). Analysis and BLAST searches were done with the GCG software system (GCG, Madison, WI). The ospC gene of *B. burgdorferi* sensu stricto S-1-10 was similar (78.5%) to *B. burgdorferi* sensu stricto B31 [34]. In addition, the S-1-10 ospC nucleotide sequence differed by 3 bases (98% homology) with *B. burgdorferi* sensu stricto DUNKIRK [35].

**Purification of recombinant OspC.** E. coli containing the ospC gene was grown in 100 mL of 2× TY broth containing ampicillin for 12 h at 37°C. The culture was diluted 1:10 with 2× TY broth and incubated for an additional 1 h. Isopropyl-β-d-thiogalactopyranoside (final concentration, 0.1 mM; Sigma) was added to the culture and incubated for an additional 4 h. The suspension was centrifuged at 10,000 g for 15 min at 4°C, resuspended in purification buffer (50 mM Tris [pH 8.0], 50 mM NaCl, 2 mM EDTA, 0.1% Triton X-100) and lysed with a sonicator (model W350; Branson Sonic Power, Danbury, CT). Sonicated *E. coli* were centrifuged at 10,000 g for 15 min, and the supernatant was passed over a column containing SoftLink resin (Promega) at a rate of 0.5 mL/min at 4°C. The column was then washed with 5 column vol of purification buffer. OspC was eluted with 5 mM biotin (Sigma), and the recovered fractions were analyzed by SDS-PAGE.

**OspC ELISA.** Recombinant OspC was diluted to 750 ng/mL in coating buffer (0.015 M Na₂CO₃, 0.035 M NaHCO₃, pH 9.6), and 100-μL amounts were added to individual flat-bottom microtiter wells (Dynatech Laboratories, Chantilly, VA). Microtiter plates were incubated at 35°C for 4 h, followed by an overnight incubation at 4°C. After incubation, plates were washed three times with PBS, pH 7.2, containing 0.05% Tween 20, sealed, and stored at 4°C. Before use, plates were blocked with PBS–0.05% Tween 20 containing 1% bovine serum albumin for 30 min at 22°C and washed twice with PBS–0.05% Tween, and then 100 μL of serial 2-fold dilutions of normal or Lyme borreliosis serum in PBS–0.05% Tween were added to individual wells. Plates were incubated for 1 h at 22°C followed by three washes with PBS–0.05% Tween. Anti-human IgM–horseradish peroxidase conjugate (100 μL; Organon Teknika Cappel) diluted 1:3000 in PBS–0.05% Tween was added, and plates were incubated at 22°C for 1 h. After incubation, 100 μL of o-phenylenediamine phosphate (0.4 mg/mL; Sigma) was added to each well and incubated at 22°C for 30 min. Reactions were stopped by addition of 100 μL of 1 N H₂SO₄, and absorbances at 490 nm (model EL307; Bio-Tek Instruments, Winooski, VT) were immediately determined. An optical density >0.200 above that of the normal serum control was considered positive.

**Detection of borreliacidal antibodies.** The flow cytometric borreliacidal antibody test was done as previously described [26, 27]. Briefly, a frozen 200-μL aliquot of *B. burgdorferi* isolate 50772 or 297 was thawed and inoculated into 6 mL of fresh BSK medium, and cultures were incubated for 72 h at 35°C. After incubation, the concentration of spirochetes was determined by use of a Petroff-Hauser counting chamber and diluted in fresh BSK medium to a concentration of 10⁶ organisms/mL. Serum samples were diluted 1:20 in fresh BSK medium and sterilized by passage through a 0.2-μm microfuge filter (Costar, Cambridge, MA). A 100-μL aliquot was transferred to a 1.5-mL screw-cap microfuge tube (Sarstedt), and the diluted serum was heat-inactivated at 56°C for 10 min. After heat inactivation, a 100-μL aliquot of *B. burgdorferi* 50772 and 15 μL of sterile guinea pig serum (200 50% hemolytic complement units/mL; Sigma) were added to the diluted sera. After gentle agitation, the assay suspensions were incubated for 16–24 h at 35°C. After incubation, 100 μL of the assay suspension was diluted 1:5 with PBS (0.01 mol/L, pH 7.2) containing acridine orange...
Borreliacidal antibodies were determined as previously described \[17\]. Briefly, 125 µL of serum samples after removal of IgM or IgG antibodies was then passed over the column 10–15 times at 4°C to remove binding of OspC to the column. A 1-mL sample of each of 10 µm-µlter-sterilized PBS. These suspensions were then analyzed and assessed by FACScan software. Side scatter and fluorescence intensity parameters were used to distinguish B. burgdorferi from BSK and complement particles. Spirochetes were gated during data acquisition, and fluorescence signals were logarithmically amplified and converted to a linear scale. A 13% increase in fluorescence intensity compared with normal serum controls was considered positive \[27\]. All assays were done in duplicate or triplicate.

**Vaccination of mice and recovery of anti-OspC sera.** Mice were vaccinated intramuscularly with 75 µg of purified rOspC in 100 µL of Freund’s complete adjuvant (Sigma). Subsequently, mice were boosted with 75-µg amounts of rOspC in 100 µL of Freund’s incomplete adjuvant (Sigma) at 2 and 4 weeks after the primary vaccination. Two weeks after the second booster, blood was collected by intracardiac puncture. Blood was allowed to clot, and serum was separated by centrifugation at 3500 rpm for 5 min. Serum was removed and stored in 50-µL amounts at −70°C until used.

**Flow cytometric immunofluorescence assay.** Mouse serum samples containing anti-OspC antibodies were serially diluted in BSK medium (1:20 to 1:40,960). One hundred microliters of BSK medium containing 10^4 live B. burgdorferi 297 or 50772 organisms was added to each dilution. Suspensions were gently vortexed and incubated at 35°C for 30 min. After incubation, 10 µL of fluorescein isothiocyanate (FITC)–conjugated goat anti-mouse IgG (ICN/Cappel, Aurora, OH) antibody diluted 1:20 in sterile PBS (pH 7.2) was added to each assay. Assays were gently vortexed and incubated at 35°C for an additional 30 min. After incubation, 100-µL aliquots of each suspension were combined with 400 µL of 0.22-µm-filter–sterilized PBS. These suspensions were then analyzed by using a FACScan flow cytometer (Becton Dickinson Immunocytometry Systems).

**Neutralization of borreliacidal activity.** Borreliacidal activity of serum samples after removal of IgM or IgG antibodies was determined as previously described \[17\]. Briefly, 125 µL of dialyzed goat anti-human IgM or IgG (heavy and light chain; Kallestad Diagnostics, Chaska, MN) was added to 25 µL of normal or Lyme borreliosis serum, and the mixture was incubated for 2 h at 37°C. After centrifugation at 5000 g for 10 min (Surespin; Helena Laboratories, Beaumont, TX), the supernatant was diluted 2-fold with fresh BSK medium, sterilized by passage through a 0.2-µm microfuge filter (Costar), and assayed for borreliacidal activity. The borreliacidal activity of normal and Lyme borreliosis sera without treatment with anti-IgM or anti-IgG was determined after adding 125 µL of PBS (pH 7.2).

**Adsorption of Lyme borreliosis sera with rOspC.** Adsorption of anti-OspC antibodies from Lyme borreliosis sera was done by a modification of a previously described procedure \[17\]. Tetrameric Avidin Resin (Promega) was washed with PBS, and a 1-mL volume was loaded into a column. Three micrograms of dialyzed biotinylated rOspC in a 1-mL volume was passed over the column, and absorbance at 280 nm was monitored to confirm binding of OspC to the column. A 1-mL sample of each of 10 human Lyme borreliosis sera diluted 10-fold with PBS (pH 7.2) was then passed over the column 10–15 times at 4°C to remove anti-OspC antibodies. Removal of anti-OspC antibodies was confirmed by Western blotting.

**Results**

Temperature-regulated differential expression of B. burgdorferi OspC. Schwan and colleagues \[36, 37\] reported that B. burgdorferi did not express appreciable amounts of OspC when grown at 24°C but significantly increased expression of OspC when grown at 32–37°C. We performed several studies to optimize the growth of B. burgdorferi isolates 50772 and 297 for maximum production of OspC. Spirochetes were passaged 10 times at 30°C or 35°C and used as a source of antigen for Western blotting against an early Lyme borreliosis serum or mouse serum (figure 1) containing anti-OspC antibodies. Antibodies against OspC were readily detected by Western blotting, but only when B. burgdorferi 50772 or 297 was cultured at 35°C. Furthermore, borreliacidal antibodies were easily detected in the serum (titer <10) when B. burgdorferi 50772 or 297 were grown 30°C or 35°C, despite anti-OspC reactivity against isolate 297 grown at 35°C.

**Surface expression of OspC.** Abundant levels of OspC were readily apparent in SDS-PAGE profiles of B. burgdorferi isolates 50772 and 297 grown at 35°C (data not shown). Because anti-OspC borreliacidal antibodies were not detected using B. burgdorferi 297, we hypothesized that detection was dependent on the surface expression of OspC. As controls, we incubated B. burgdorferi isolates 50772 or 297

**Figure 1.** Detection of anti-OspC antibodies by Western blotting using B. burgdorferi 50772 (lane A) or B. burgdorferi 297 (lane B) grown at 35°C and mouse serum containing anti-OspC antibodies.
with serum obtained from rOspC-vaccinated mice before treatment with FITC-conjugated goat anti-mouse IgG and then determined the fluorescence intensity of the live spirochetes by flow cytometry. When *B. burgdorferi* 297 or 50772 was incubated with FITC-conjugated goat anti-mouse IgG, the mean channel fluorescence intensity (MCF) of these spirochetes was 275 and 290 units, respectively (figure 2A, 2C). Similarly, when *B. burgdorferi* 297 organisms were incubated with a 1:40 dilution of mouse anti-OspC serum and FITC-labeled anti-mouse IgG, the MCF was 346 units (figure 2B). Since the organisms were viable, these results indicated that little or no OspC was expressed on the surface of *B. burgdorferi* 297. In contrast, the MCF of *B. burgdorferi* 50772 was 2677 units when these spirochetes were incubated with mouse anti-OspC serum (1:40) and FITC-labeled anti-mouse IgG (figure 2D). In addition, significantly increased fluorescence intensity was detectable, even when mouse anti-OspC serum was diluted 1:40,960 (figure 3). These results support our hypothesis that the surface of *B. burgdorferi* 50772 contains a significant concentration of OspC.

**Figure 2.** Histograms of mean channel fluorescence intensities of *B. burgdorferi* 297 (A) or 50772 (C) after incubation with fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG and after incubation of *B. burgdorferi* 297 (B) or 50772 (D) with FITC-labeled anti-mouse IgG and 1:40 dilution of mouse anti-OspC serum.
Effect of removal of anti-OspC antibodies on borreliacidal activity. Early Lyme borreliosis sera were screened for anti-OspC antibodies by an OspC ELISA, and 10 serum samples containing anti-OspC antibodies were selected. The borreliacidal activity of these sera was determined with *B. burgdorferi* 50772 grown at 35°C. High titers of borreliacidal antibodies (range, 640–40,960) were detected with the sera (table 1). Subsequently, each of the 10 early Lyme borreliosis sera was passed 10–15 times over a column containing rOspC. The removal of anti-OspC antibodies was considered complete because the eluted sera failed to demonstrate anti–22-kDa antibodies by Western blotting (figure 4). In addition, the anti-OspC ELISA reactivity decreased to levels similar to that of the normal serum control. Concomitantly, there was a significant reduction in the titers of borreliacidal antibodies (table 1). Borreliacidal activity decreased in 8 (80%) of the serum samples to <40 (normal background). The borreliacidal antibody titers decreased from 10,240 to 320 in the remaining 2 serum samples.

To affirm that the borreliacidal antibodies were OspC-specific, the captured antibodies were eluted from the OspC column and tested for anti-OspC antibodies and borreliacidal ac-

**Table 1.** Effect of removal of anti-OspC antibodies on OspC ELISA reactivity and borreliacidal activity.

<table>
<thead>
<tr>
<th>Early Lyme borreliosis</th>
<th>Titer* of OspC ELISA reactivity</th>
<th>Titer² of borreliacidal activity</th>
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<tr>
<td>Normal serum</td>
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<td>5120</td>
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<td>2</td>
<td>1280</td>
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<td>10</td>
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* Reciprocal of last dilution with optical density >0.200 compared with normal serum control.

¹ Reciprocal of last dilution with significant borreliacidal activity.
Immunoblots of *B. burgdorferi* 50772 probed with 10 early Lyme disease sera before (lanes A) and after (lanes B) passage over OspC column. Antibodies in sera 1–9 were reacted with anti-human IgM. Serum 10 antibodies were labeled with anti-human IgG.

Anti-OspC antibodies were readily detected in the fractions by Western blotting. Similarly, significant borreliacidal activity with titers as high as 640 was detected (data not shown). Collectively, these results demonstrate that high titers of anti-OspC borreliacidal antibodies were produced in sera shortly after natural infection with the Lyme borreliosis spirochete.

**Classes of anti-OspC borreliacidal antibodies.** Borreliacidal activity was decreased in 9 (90%) of the early Lyme borreliosis sera after treatment with anti-human IgM serum, but not after treatment with anti-human IgG antibodies (table 2). When the remaining serum was treated with anti-human IgG antibodies, the borreliacidal titer decreased 8-fold. Normal human serum was also treated with anti-human IgM or IgG antibodies.

No borreliacidal activity was detected in either treated or untreated normal serum. These results affirm our previous observations [17] that human borreliacidal antibodies can be either IgM or IgG.

**Induction of anti-OspC borreliacidal antibodies.** We next determined whether vaccination of mice with rOspC induced anti-OspC borreliacidal antibodies. No borreliacidal activity was detected when *B. burgdorferi* isolate 297 was used. However, significant borreliacidal activity (titer ≥ 640) was detected with *B. burgdorferi* isolate 50772. These results show that vaccination with OspC induces borreliacidal antibodies. These results also confirm our finding with naturally infected human sera that detection of OspC borreliacidal antibody is dependent on surface expression of OspC by *B. burgdorferi* isolate 50772.

**Discussion**

Several *B. burgdorferi* Osps have been shown to induce a protective immune response in laboratory animals [12–16, 38]. Most of the efforts to date have focused on developing an OspA vaccine, primarily because of the large amounts of OspA expressed on the surface of many *B. burgdorferi* laboratory isolates. OspA vaccines have been shown to be effective against needle and tick challenges with *B. burgdorferi*. However, protection against tick challenge appears dependent on the presence of high levels of anti-OspA borreliacidal antibodies to destroy the spirochetes in the midguts of infected ticks [28]. Thus, the duration of high titers of anti-OspA borreliacidal antibodies is a critical determinant of the long-term efficacy of an OspA vaccine.

The use of borreliacidal assays to monitor the levels of anti-OspA borreliacidal antibodies has provided important insight into the efficacy of current Lyme borreliosis vaccines [13, 21,
organisms appear to up-regulate OspC and con-spirochetes to evade the host protective immune response. B. burgdorferi activity will expedite these investigations.

When vaccine-induced immunity. Identi®cation of isolate 297, which expresses OspA, OspB, and OspC, failed late Lyme borreliosis sera [17, 26, 27]. However, high levels of OspC to protect laboratory animals against needle challenge [14, 15] and natural infection [16]. Interestingly, anti-OspC borreliacidal antibodies have not been detected in immune serum [14–16], leading to speculation that additional mechanisms are responsible for OspC-mediated protection [16].

In this investigation, we demonstrate the ability of B. burgdorferi OspC to induce high levels of borreliacidal antibodies shortly after infection. Detection was dependent on the presence of high levels of OspC on the surface of B. burgdorferi. We also showed that vaccination of mice with OspC induced significant levels of anti-OspC borreliacidal antibodies. These antibodies, however, were detected only when B. burgdorferi isolate 50772, which expresses high levels of OspC on the surface, was used. In support, we showed that B. burgdorferi isolate 297, which expresses OspA, OspB, and OspC, failed to detect borreliacidal antibodies in sera from patients with early Lyme borreliosis and mice after vaccination with OspC because of the absence of OspC on the surface of the spirochetes. Additional studies are currently being done to determine whether anti-OspC borreliacidal antibodies are responsible for vaccine-induced immunity. Identification of B. burgdorferi isolate 50772 as an excellent monitor of anti-OspC borreliacidal activity will expedite these investigations.

The detection of anti-OspC borreliacidal antibodies shortly after infection with B. burgdorferi also provides insight into a progression of antigenic changes that may allow infecting spirochetes to evade the host protective immune response. B. burgdorferi organisms appear to up-regulate OspC and concomitantly down-regulate OspA shortly before tick inoculation of the host with the spirochete [36, 37]. Consequently, anti-OspC antibodies are among the first antibody responses detected in patients with early Lyme borreliosis, while detection of anti-OspA antibodies is frequently delayed [44]. Our findings indicate that the immune system responds to early, localized infection with the production of high concentrations of anti-OspC borreliacidal antibodies. If left untreated, however, B. burgdorferi organisms are able to disseminate, even in the presence of high concentrations of anti-OspC borreliacidal antibodies. We postulate that this occurs because B. burgdorferi organisms down-regulate OspC during the dissemination phase of infection. The down-regulation of OspC could be expected to coincide with up-regulation of other Osps, including OspA. Thus, anti-OspC borreliacidal antibodies are unable to kill B. burgdorferi because recognition and attachment may be hindered by production of other Osps. As B. burgdorferi spirochetes disseminate, the host immune system begins producing borreliacidal antibodies against these up-regulated Osps. Unfortunately, B. burgdorferi organisms become sequestered in immunologically privileged sites before high concentrations of these borreliacidal antibody levels develop. This may explain why borreliacidal and other functional anti-OspA and anti-OspB antibodies are most often detected in sera from patients with Lyme arthritis [17–19]. Additional studies are ongoing to test this hypothesis. However, our results provide evidence that OspC will be a crucial component for inclusion in a successful Lyme borreliosis vaccine.

These results are important for an additional reason. The borreliacidal antibody test has been advocated for the laboratory diagnosis of Lyme borreliosis [17, 25–27]. We [26] and others [19, 20] have confirmed the high speci®city of the borreliacidal antibody test using B. burgdorferi isolate 297. For example, we did not detect borreliacidal activity in 104 serum samples from patients with syphilis, mononucleosis, systemic lupus erythematosus, rheumatoid factor, or antinuclear antibody positivity [26]. In contrast, 47 (45%) of these sera yielded positive results when tested by ELISA. Highly specific IgM or IgG borreliacidal antibodies against B. burgdorferi isolate 297, which expresses OspA and OspB, were detected in early or late Lyme borreliosis sera [17, 26, 27]. However, high levels of borreliacidal antibodies against isolate 297 were detected primarily in sera from patients with late stages of the disease [17–19] or in convalescent sera collected weeks to months after diagnosing patients with early Lyme borreliosis [20].

When early Lyme borreliosis sera were tested, we [27] and Agger and Case [45] detected anti–B. burgdorferi 297 borreliacidal antibodies in only 15% and 12.5% of patients, respectively. When B. burgdorferi isolate 50772 was used, we [27] detected highly specific borreliacidal antibodies in 34 (72%) of 47 culture- or case-defined early Lyme borreliosis sera from patients from Westchester County. We also confirmed the high specificity of borreliacidal activity against B. burgdorferi 50772. No borreliacidal antibodies were detected in normal sera or sera from patients with syphilis, mononucleosis, or rheumatoid factor [27]. In this investigation, we extended these findings by demonstrating that IgM and IgG borreliacidal antibodie...
Unlike OspA and OspB, anti-OspC antibodies are among the earliest antibody responses detectable by conventional serodiagnostic tests [44]. In this investigation, all 10 early Lyme disease sera containing ELISA-detectable OspC antibodies had significant titers of anti-OspC borreliacidal activity. Further studies are necessary to determine the percentage of early Lyme borreliosis sera that contain anti-OspC borreliacidal antibodies. 

*B. burgdorferi* 50772 is also susceptible to anti-OspC borreliacidal activity in serum from patients from a wide geographic area, even though distinct *ospC* variants among North American *B. burgdorferi* isolates have been described [43]. We recently received Lyme borreliosis sera from 25 patients in the eastern United States with early Lyme disease (erythema migrans) from Allen Steere (Tufts University School of Medicine, New England Medical Center, Boston). These serum samples were not identified until after we reported the results of borreliacidal antibody testing. Using *B. burgdorferi* 50772, we detected borreliacidal antibodies in 16 (60%) of 25 acute sera and an additional 5 convalescent sera (20%) (overall sensitivity, 80%). The majority of borreliacidal activity in these sera and sera from patients in Westchester County, New York, was anti-OspC (data not shown). This ability to detect anti-OspC borreliacidal antibodies in serum from patients in the midwestern and eastern United States suggests that the OspC borreliacidal epitope may be conserved. Additional studies to identify the *ospC* borreliacidal epitope are ongoing. However, confirmation of the ability of OspC to induce borreliacidal antibodies in vivo and the ability to detect these highly specific antibodies with *B. burgdorferi* 50772 provides additional evidence that borreliacidal assays could eliminate the specificity concerns that continue to hinder serodiagnosis of Lyme borreliosis.

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References


