

Borreliacidal OspC Antibody Response of Canines with Lyme Disease Differs Significantly from That of Humans with Lyme Disease[∇]

Steven D. Lovrich,¹ Rhonda L. La Fleur,³ Dean A. Jobe,¹ Jennifer C. Johnson,³ Krista E. Asp,¹
Ronald F. Schell,⁴ and Steven M. Callister^{1,2*}

Microbiology Research Laboratory¹ and Section of Infectious Diseases,² Gundersen Lutheran Medical Center,
La Crosse, Wisconsin 54601; Schering Plough Animal Health Corporation, Elkhorn, Nebraska 68022³;
and Wisconsin State Laboratory of Hygiene, Madison, Wisconsin 53706⁴

Received 16 November 2006/Returned for modification 6 February 2007/Accepted 22 February 2007

Humans reliably produce high concentrations of borreliacidal OspC antibodies specific for the seven C-terminal amino acids shortly after infection with *Borrelia burgdorferi*. We show that dogs also produce OspC borreliacidal antibodies but that their frequencies, intensities, and antigenicities differ significantly. The findings therefore confirm a major difference between the borreliacidal antibody responses of humans and canines with Lyme disease.

Lyme disease is a tick-associated zoonosis caused by *Borrelia burgdorferi* sensu lato. The bacterium induces a variety of clinical manifestations in humans, including skin lesions, pericarditis, inflammation of the nervous system, and arthritis (14). An immunodominant antibody response during human illness involves the production of outer surface protein C (OspC) borreliacidal antibodies (9, 12, 13) that activate complement to kill the spirochete (10). High titers of borreliacidal OspC antibodies are reliably produced shortly after infection, and the response can be detected by a flow cytometric test (2, 4). This method of detection also requires live organisms, such as *B. burgdorferi* 50772 spirochetes, because these unique spirochetes lack the ability to produce OspA and OspB but do express OspC. This characteristic is essential since the expression of OspA or OspB prevents OspC antibodies from binding (4). Not surprisingly, the complexity of this test has contributed to decreased use despite the high sensitivity and specificity (2, 4). Researchers recently reported (12), however, that human OspC borreliacidal antibodies appear to be entirely specific for a conserved epitope within the C-terminal seven amino acids (C7). A peptide-based enzyme-linked immunosorbent assay (ELISA) could therefore likely accurately detect the response. In addition, the epitope could be a valuable constituent of a human Lyme disease vaccine.

B. burgdorferi spirochetes also cause similar clinical abnormalities in dogs. Canine Lyme disease manifests most often as subclinical polyarthritis and/or periarteritis (15) but can progress to renal disease (7), cardiac disorders (11), or arthritis (16). In addition, infected dogs produce borreliacidal antibodies that are also detected by using unique *ospA*- and *ospB*-defective spirochetes such as *B. burgdorferi* 50772 (3). We therefore determined whether *B. burgdorferi* 50772-specific borreliacidal antibodies in immune sera from dogs with Lyme disease were also induced by the C-terminal epitope of OspC.

Challenge and confirmation of infection. Fifteen healthy 14-week-old laboratory-reared beagles were used. To ensure that the animals were not exposed to *B. burgdorferi* previously, serum samples were obtained prior to the study, and seronegativity was confirmed by using a *B. burgdorferi* 297 whole-cell ELISA (17). Ticks were also collected from a focus of endemicity near La Crosse, WI (8), and infection was confirmed by examining the midguts from 50 adult male ticks after staining with fluorescein isothiocyanate-labeled OspA monoclonal antibody H5332 (8). *B. burgdorferi* was detected in 15 (30%) ticks. Ten male and 10 female ticks were then selected randomly and placed into a rubber cup that was secured to the left dorsal-anterior region of each animal for 1 week. The dogs were isolated, fed commercial food and water ad libitum, and observed daily for lameness.

To confirm transmission of spirochetes by the tick challenge, 4-mm-punch skin biopsies were obtained from the tick bite sites at 34 and 90 days postchallenge, placed into separate tubes containing 9 ml of modified (1) Barbour-Stoenner-Kelly medium, incubated at 34°C, and examined weekly by dark-field microscopy for 4 weeks. *B. burgdorferi* was recovered from 12 (80%) and 11 (73%) dogs at 34 and 90 days postchallenge, respectively (Table 1). In addition, spirochetes were recovered from at least one skin biopsy from 14 (93%) dogs, and 4 (27%) dogs also developed lameness in one or more limbs that lasted for at least three observation periods.

Antibody responses after infection. Blood samples were obtained 90 days after the tick challenge and examined by Western blotting and a borreliacidal antibody test. Western blotting was performed by using *B. burgdorferi* 297 and standard techniques. Briefly, the spirochetes were boiled in treatment buffer, and 150 µg of protein was loaded onto a 10 to 20% linear gradient polyacrylamide gel and electrophoresed. The proteins were then transferred to a polyvinylidene difluoride membrane, cut into strips, blocked with 1% bovine serum albumin in phosphate-buffered saline (PBS, pH 7.2)-0.1% Tween 20, and incubated sequentially at room temperature with dog serum diluted 1:100 and horseradish peroxidase-labeled anti-dog immunoglobulin G. Reactions were detected by development with the TMB Membrane

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

[∇] Published ahead of print on 7 March 2007.

TABLE 1. Recovery of *B. burgdorferi* from skin biopsies and development of lameness in tick-challenged dogs

Dog no.	Recovery of <i>B. burgdorferi</i> after:		Lameness ^a
	34 days	90 days	
1	+	+	-
2	+	-	+
3 ^b	-	-	-
4	+	+	+
5	+	+	-
6	-	+	-
7	+	-	-
8	-	+	-
9	+	+	-
10	+	+	+
11	+	+	-
12	+	+	+
13	+	+	-
14	+	+	-
15	+	-	-

^a Development of lameness in one or more joints lasting three or more days.

^b Data for dog 3 were removed from the subsequent table and Fig. 1.

peroxidase substrate system (Kirkegaard & Perry Laboratories, Gaithersburg, MD). Serum from a healthy dog was used as a negative control.

Borreliacidal antibodies were detected by flow cytometry as described previously (4, 5) by combining 5×10^4 *B. burgdorferi* 50772 spirochetes with serum and complement (guinea pig serum [50% hemolytic complement, ≥ 200 units/ml]) and incubating the suspension at 35°C. Following incubation, a 100- μ l aliquot of the assay suspension was combined with PBS and acridine orange, and the spirochetes were analyzed for killing by using a FACScan flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA). A $\geq 13\%$ increase in fluorescence intensity compared to a normal serum control was considered positive (2). In addition, the assays were performed in duplicate, and the presence of blebbed nonmotile *B. burgdorferi* organisms was confirmed by microscopy.

The animal with negative biopsy results (dog 3) was also seronegative by Western blotting and a whole-cell ELISA (data not shown), so the dog had likely not become infected and the serum was not evaluated further. In contrast, the 14 dogs with positive skin biopsies also produced immunoglobulin G antibodies against numerous *B. burgdorferi* proteins (Fig. 1). The specificities of the dog antibody responses were similar, but they differed significantly from the responses typically detected during human infection (6). As an example, the human Lyme disease serum control, collected from a patient who became infected in the same region as that where the ticks were collected, contained OspA, OspB, and OspC antibodies. In contrast, the Western blots of the immune dog sera did not detect OspA or OspB antibodies, and only rarely (dog 9) were antibodies specific for OspC detected. In addition, the immune dog sera typically contained high levels of antibodies specific for an uncharacterized, approximately 20-kDa protein that was not detected in the human serum. Additional studies to more completely characterize these differences should prove valuable for future efforts to develop effective canine Lyme disease tests or vaccines.

In this study, however, our efforts were focused on deter-

mining whether the OspC borreliacidal antibody response was also immunodominant during canine illness. We therefore quantitated the levels of OspC and C7 antibodies by using OspC and C7 ELISAs (17), respectively, and compared the responses to the titers of borreliacidal antibodies. Antibodies specific for C7 were not detected (data not shown). OspC antibodies were detected in 12 (86%) immune sera (Table 2), but as suggested by the Western blots, the levels were significantly lower than those detected routinely during human infection (13). In addition, nine (64%) sera contained borreliacidal antibodies at titers (titer range, 1:640 to 1:20,480) seen commonly during human illness (4, 9, 12, 13), but one OspC-positive serum did not contain borreliacidal activity and two OspC-negative sera contained borreliacidal antibodies. The results therefore confirmed previous findings (3) that high concentrations of *B. burgdorferi* 50772-specific borreliacidal antibodies were produced during canine Lyme disease, but in contrast to the case for human illness, the response did not correlate closely with the detection of OspC antibodies.

Effects of removing OspC or C-terminal fragment (C7) antibodies on borreliacidal activity. To confirm this significant divergence from the human serologic response, we used a procedure described previously (4, 12) to remove the OspC- or C7-specific antibodies and then determined the effects on the borreliacidal activity. Briefly, 1-ml volumes of Tetralink tetrameric avidin resin (Promega, Madison, WI) were loaded into separate 10- by 70-mm columns, and 0.5 mg of recombinant OspC or C7 was solubilized in PBS and passed over a column. A 1-ml volume of each immune serum diluted 10-fold with PBS was then passed over the column several times. The columns were regenerated between serum samples by using 0.1 M citric acid (pH 3), and a human serum control was passed over the columns at periodic intervals to confirm their ability to remove antibodies.

As reported previously (12), passage over the recombinant OspC or C7 column completely removed the antibodies and borreliacidal activity from the human Lyme disease serum control (Table 2). However, removing the C7 antibodies did not

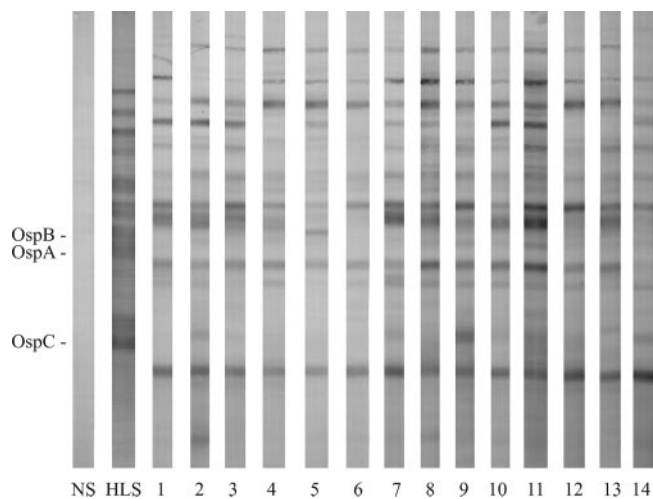


FIG. 1. Western blots of normal dog serum (NS), typical human Lyme disease serum (HLS), and immune sera (lanes 1 to 14) from dogs infected with *Borrelia burgdorferi* by tick challenge.

TABLE 2. ELISA titers and borreliacidal activities in immune sera before (untreated) and after removal of OspC or C7 antibodies

Serum	OspC ELISA titer ^{a,c}		Borreliacidal antibody titer ^{b,c}		
	Untreated	OspC removed	Untreated	OspC removed	C7 removed
Human	20,480	ND	10,240	ND	ND
1	160	—	ND	—	—
2	320	ND	5,120	160	5,120
3	80	ND	1,280	640	1,280
4	160	ND	2,560	640	5,120
5	640	—	ND	—	—
6	320	—	ND	—	—
7	ND	—	ND	—	—
8	160	ND	5,120	1,280	5,120
9	320	ND	640	640	1,280
10	640	ND	2,560	160	5,120
11	2,560	ND	20,480	1,280	20,480
12	160	ND	640	80	320
13	640	ND	5,120	640	5,120
14	ND	—	ND	—	—

^a Reciprocal of highest dilution with absorbance value >0.100 unit above normal serum control value.

^b Reciprocal of highest dilution with significant borreliacidal activity. Sera from each animal were evaluated concurrently to negate interassay variability.

^c ND, none detected; —, not tested.

affect the borreliacidal activity in the dog sera. In contrast, removal of the OspC antibodies reduced the borreliacidal activity significantly (≥4-fold reduction) in seven (78%) dog sera, but considerable borreliacidal activity remained even after additional passages over a rejuvenated column. In addition, the absence of OspC antibodies failed to affect two of the borreliacidal antibody-positive sera. The findings therefore confirmed that some canines with Lyme disease produce significant levels of borreliacidal antibodies specific for OspC but that the response does not always predominate and the region responsible is distinct from the epitope recognized by human OspC borreliacidal antibodies.

Conclusions. The immunodominant borreliacidal antibody response during human infection with *B. burgdorferi* is reliably specific for the C-terminal seven amino acids of OspC (12). Dogs with Lyme disease may also produce OspC borreliacidal antibodies, but the response may not predominate and the antigenicities of the antibodies differ significantly. This finding has important implications for future efforts to develop canine Lyme disease tests and vaccines.

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