VECTOR-BORNE AND ZOONOTIC DISEASES
Volume 8, Number 5, 2008

DOI: 10.1089/vbz.2007.0251

Borrelia bissettii Isolates Induce Pathology in a Murine Model of Disease

BRADLEY S. SCHNEIDER,[1,*] MARTIN E. SCHRIEFER,[1] GABRIELLE DIETRICH,[1] MARC C. DOLAN,[1] MUHAMMAD G. MORSHED,[2] and NORDIN S. ZEIDNER[1]

ABSTRACT

The spirochete *Borrelia burgdorferi* is a tick-borne pathogen that causes Lyme disease. Although *B. burgdorferi* sensu lato is a diverse group of bacteria, only three genospecies, *B. burgdorferi* sensu stricto, *Borrelia afzelii*, and *Borrelia garinii*, are known to be pathogenic and commonly recognized to cause human disease. To assess the potential of another common genospecies, *Borrelia bissettii*, to induce disease, a mouse model was employed. Two Colorado isolates of *B. bissettii* (CO-Bb) induced lesions of the bladder, heart, and femorotibial joint 8 weeks after inoculation into mice. In contrast, two British Columbia (BC-Bb) isolates, could not be cultured or amplified by PCR from target organs, and did not induce lesions. Consistent with pathology and culture results, the antibody response in mice to BC-Bb was minimal compared to CO-Bb, indicating either transient localized infection or rapid immune clearance of BC-Bb. Although sequence analysis of the *rrf* (5S)–*rrl* (23S) intergenic spacer region indicated 99% homology between CO-Bb and BC-Bb, polyacrylamide gel electrophoresis (PAGE) analysis indicated five distinct protein differences between these low-passage isolates. These studies support the prospect that *B. bissettii* may indeed be the causative agent of Lyme borreliosis cases in Eastern Europe, associated with the atypical *Borrelia* strain 25015, and in other regions. To our knowledge, this is the first evidence that *B. bissettii* can induce pathology in a vertebrate host. Key Words: Borrelia—Ixodes—Lyme disease—Tick(s)—Vector-borne

INTRODUCTION

LYME DISEASE IS THE MOST COMMON vector-borne disease in the United States (Centers for Disease Control and Prevention 2007). It causes a spectrum of clinical symptoms including erythema migrans, migratory joint and muscle pain, debilitating malaise, neurologic symptoms, and chronic arthritis (Steere 1987). Manifestations and severity of disease are highly variable, with different patterns of disease associated with distinct *Borrelia* spp. host populations and geographical locations. The disease is caused by the bacterium *Borrelia burgdorferi* sensu lato. Only three genospecies, *B. burgdorferi* sensu stricto, *Borrelia afzelii*, and *Borrelia garinii*, are known to be pathogenic and commonly recognized to cause human disease. At least two more species of the *B. burgdorferi* sensu lato complex, *Borrelia lusitaniae* and the recently identified *Borrelia spielmani* sp. nov., appear to have a pathogenic role in human disease in Europe (da Franca et al. 2005; Fingerle et al. 2007, Foldvari et al. 2005, Maraspin et al. 2002, Maraspin et al. 2006, Richter et al. 2004). At present, *B. burgdorferi* sensu stricto is the only member of the sensu lato complex known to be associated with Lyme disease in North America (Wang et al. 1999), although *Borrelia lonestari* is the putative agent of a clinical syn-

[1]Division of Vector-Borne Infectious Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, Colorado.
[2]Zoonotic Diseases and Emerging Pathogens Section, Laboratory Services, BC Centre for Disease Control, and Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC V5Z 4R4, Canada.
**Present address:* Institut Pasteur, Département de Parasitologie, Unités de Réponses précoces aux Parasites et Immunopathologie, 25 Rue du Docteur Roux, 75724 Paris Cedex 15, France.

drome southern tick associated rash illness (STARI), which resembles the skin lesions of Lyme disease (James et al. 2001).

In the United States *B. burgdorferi* is transmitted to humans by *Ixodes scapularis* ticks in the Northeastern and Midwestern portions of the country, and by *Ixodes pacificus* ticks on the West Coast (Bissett and Hill 1987; Brown and Lane 1992; Wilske 2005). Recently, *Borrelia bissettii* was determined to be a separate genospecies from *B. burgdorferi* sensu stricto (Postic et al. 1998). Previous work had determined that *B. bissettii* exists in similar enzootic cycles in the foothills of Colorado involving the vector *Ixodes spinipalpis* and rodent reservoir hosts (Norris et al. 1999; Schneider et al. 2000). *B. bissettii* survives in a comparable cycle along the West Coast, although *I. pacificus* is the typical vector (Brown and Lane 1992).

Isolates of *B. bissettii* have been discovered throughout North America and Europe (Anderson et al. 1990b; Banerjee et al. 2000; Lin et al. 2001; Mathiesen et al. 1997; Postic et al. 1998). Although *B. bissettii* has not been associated with disease in the United States, cerebrospinal fluid derived from neuroborreliosis patients in Slovenia yielded culture isolates that were genetically homologous to North American isolates later identified as *B. bissettii* (Strle et al. 1997). Despite this anomalous finding, only one study has been conducted with an isolate retrospectively identified as *B. bissettii*, to ascertain whether *B. bissettii* is pathogenic; these results indicated that strain 25015 isolated from larval ticks in New York was nonpathogenic (Anderson et al. 1990a). Given the known diversity of disease manifestations caused by isolates from different regions and the expanding list of human pathogens in the *B. burgdorferi* sensu lato complex in Europe, the studies reported here were conducted to more broadly assess the pathogenic potential of North American *B. bissettii* based on additional geographically divergent isolates.

MATERIALS AND METHODS

Isolation of Borrelia bissettii *strains*

British Columbian tick isolates of *B. bissettii* were recovered by culturing live *Ixodid* ticks. Isolate BC96T754 was derived from an *I. angustus* tick which was captured in the Squamish area outside Vancouver, BC; BC97T488 was isolated from an *I. pacificus* tick recovered from the Hope area, approximately 152 km northeast of Vancouver. *Borrelia* spp. isolates were recovered from ticks as described elsewhere (Barbour et al. 1982; Scott et al. 2004; Sinsky and Piesman 1989). Colorado rodent isolates of *B. bissettii* were isolated from *Neotoma mexicana* (CO-N275) and *Peromyscus maniculatus* (CO-P300) captured along the foothills of Colorado in Larimer County, as previously described (Maupin et al. 1994; Schneider et al. 2000). Spirochetes were grown to logarithmic phase and were mixed 1:1 with a 70% glycerol solution for storage at −80°C.

PCR analysis and DNA sequencing

To confirm the genotype of these isolates, the small intergenic spacer (*rrf–rrl*) region was amplified and sequenced as described by others (Postic et al. 1998; Schneider et al. 2000). Resultant sequences of CO-Bb and BC-Bb strains were compared to reference sequences obtained from GenBank and were aligned with MegAlign (DNASTAR, Inc., Madison, WI) using the Clustal algorithm, and transferred to PAUP (Sinauer Associates, Inc. Sutherland, MA) for phylogenetic analysis.

Mouse inoculation studies and histopathology

In vivo studies were undertaken to determine the pathogenicity of both CO-Bb and BC-Bb. C3H/HeJ mice ($n = 5$/group) were infected by subcutaneous injection of 1×10^6 low-passage (fewer than 4 passages) spirochetes (numbers of spirochetes enumerated using a counter chamber, Hauser Scientific, Horsham, PA) in 0.10 mL of Barbour-Stoenner-Kelly (BSK) medium along the dorsal midline.

Spirochete viability in BSK-II media was noted before inoculation. Similarly, control groups of mice ($n = 5$) were injected with nonviable sonicates of 1×10^6 spirochetes or 0.10 mL of BSK medium alone. Serum samples were taken at 2, 4, and 8 weeks post inoculation. Four weeks after inoculation ear biopsies were taken and cultured as previously described (Sinsky and Piesman 1989). Individual cultures were

monitored weekly for spirochete growth by dark-field microscopy for 4 weeks. Eight weeks post inoculation, mice were euthanized, and the bladder, heart, and femorotibial joint were taken for histopathologic evaluation by light microscopy and polymerase chain reaction (PCR) analysis. For histopathology, tissues were placed in Streck's tissue fixative (Streck Laboratories, La Vista, NE) for 48 hours, embedded in paraffin, sectioned at 5.0 μM for evaluation by light microscopy, and stained with hemotoxylin and eosin (Zeidner et al. 2001).

DNA isolation and real-time PCR analysis of mouse tissues

DNA was isolated and real-time PCR analysis was conducted with a flagellin gene primer-probe set as described previously (Zeidner et al. 2001). Amplifications were performed and data were analyzed via the 7700 model sequence detection system (PerkinElmer, Foster City, CA) (Zeidner et al. 2001).

Protein analysis of B. bissettii strains and serology

Protein profiles of BC and CO *B. bissettii* isolates were compared by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Zeidner et al. 2002). The presence of anti-Borrelia antibodies was determined by Western blotting with a commercial kit (MarDx, Carlsbad, CA) and 100 μg of sonicated *B. bissettii* extract. Serum samples were diluted to 1:100 in MarDx buffer. A 1:2,500 dilution of goat anti-mouse alkaline phosphatase IgG and IgM (Kirkegaard & Perry Laboratories, Gaithersburg, MD) was used to develop blots. After three washes, strips were visualized with BCIP/NBT substrate (Kirkegaard & Perry Laboratories, Gaithersburg, MD).

RESULTS

Phylogenetic analysis confirmed that a single CO-Bb isolate (CO-N275) and both BC-Bb isolates clustered closely with the prototypical *B. bissettii* strain, DN127 (Fig. 1). The remaining CO-Bb isolate (CO-P300) fell within a clade associated with strain 25015 (Fig. 1). For this intergenic region, CO-N275 and BC96T754 matched exactly, and BC97T488 differed by only two base pairs and a gap of four base pairs. The remaining Colorado strain (CO-P300) diverged from the other isolates by four to six base pairs. BC-Bb and CO-Bb did not cluster separately from one another and were divergent from *B. burgdorferi* B31 (Fig. 1).

At 4 weeks post inoculation, all mice inoculated with Colorado isolates of *B. bissettii* were culture positive (CO-N275 isolate, 5/5 positive, CO-P300 isolate, 5/5 positive). In contrast, all mice injected with the British Columbian isolates were culture negative (BC96T754, 0/5 positive, BC97T488, 0/5 positive).

At 8 weeks post inoculation, tissues from mice inoculated with the CO-N275 isolate were examined and 80% (4/5) demonstrated heart lesions, 80% (4/5) had lesions of the bladder, while only one of five mice demonstrated a lesion of the femorotibial joint (Fig. 2). Likewise, 40% (3/5) of mice inoculated with the Colorado P300 isolate had lesions of the heart, 40% (3/5) had bladder lesions, and none (0/5) had joint lesions. In contrast, none (0/5) of the mice inoculated with BC *B. bissettii* demonstrated lesions within the heart, bladder, or joint tissues, and these tissues were culture negative. All mice inoculated with the control BSK-H medium were negative for lesions within the heart, bladder, and joint tissues.

Lesions of the bladder included perivascular accumulations of small lymphocytes and plasma cells within the interstitium (Fig. 2D, arrows, 10×). The infiltration of lymphocytes and plasma cells accumulated around small vessels (Fig. 2D, arrows). Infiltration of the epithelium or smooth muscle was not observed. Lesions in the heart consisted of perivascular, mononuclear cell infiltrates of the great vessels, both the pulmonary artery and the aorta. The cellular infiltrate was comprised of small lymphocytes, plasma cells, and macrophages that extended from the peripheral wall of the great vessels into the adjacent myocardium (Fig. 2E, arrows). Moreover, a severe inflammation of the atria was observed, with large accumulations of small lymphocytes, plasma cells, and macrophages infiltrating the superior borders of both atria with associated adjacent myocardial necrosis. Only

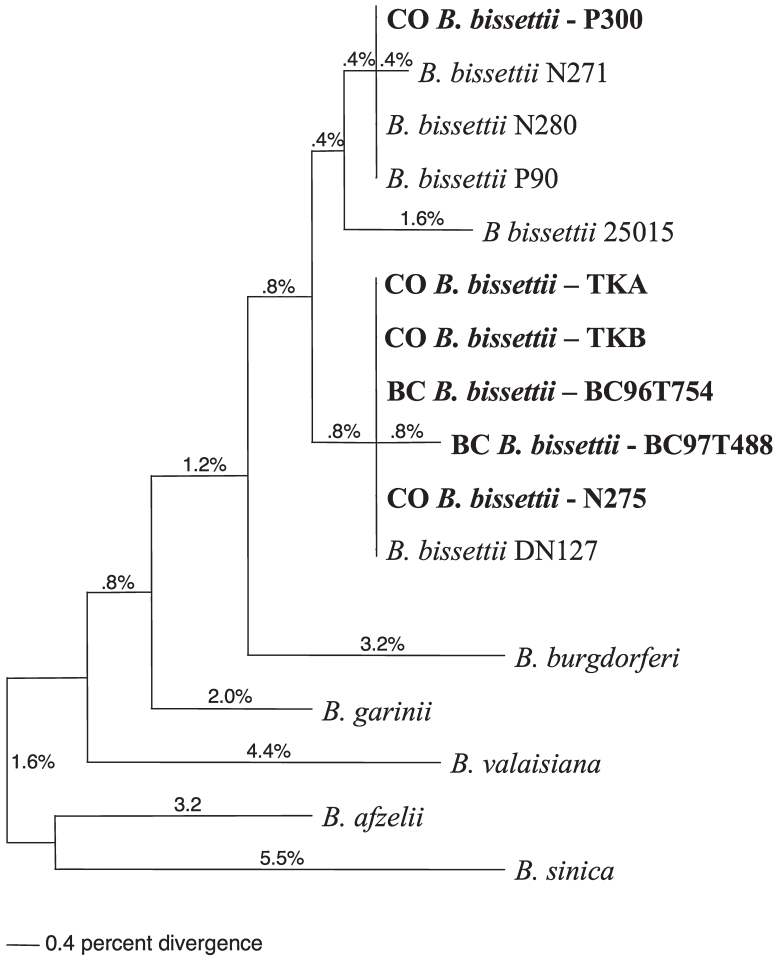

FIG. 1. Maximum-parsimony phylogenetic analysis comparing current isolates with representative sequences; the phylogenetic tree was generated using PAUP. Branches indicate the percent divergence of the *rrf–rrl* sequence. For phylogenetic analysis of 5S–23S spacer region, CO isolates AF230082 (*B. bissettii* N271), AF230084 (*B. bissettii* N275), AF230085 (*B. bissettii* N280), and AF230094 (*B. bissettii* P90) were used (Schneider *et al.* 2000), in addition to Z77177 (*B. garinii*), AY032911 (*B. burgdorferi*), AY032913 (*B. afzelii*), AB091795 (*B. valaisiana*), L30122 (*B. bissettii* 25015), L30126 (*B. bissettii* DN127), and AB100438 (*B. sinica*). CO = Colorado strain. BC = British Columbia strain.

one animal inoculated with CO-Bb developed a lesion associated with the femorotibial joint. That animal was inoculated with the CO-N275 isolate, and the lesion consisted of edema and a mild infiltration of mononuclear cells, predominantly macrophages and lymphocytes within the fascia separating the superior periosteum of the head of the tibia from the surrounding skeletal muscle (Fig. 2F, arrows). No infiltration of the associated synovial tissue or joint capsule was seen. None of the control animals inoculated with BSK-H medium developed lesions of the bladder, heart, or femorotibial joints (Fig. 2A, 2B, 2C).

Amplification of Bb DNA from paraffin-embedded heart and bladder tissue of all groups paralleled culture results for Colorado and British Colombia isolates. Bladder and heart from mice inoculated with either of the Colorado isolates were positive by real-time PCR, whereas mice inoculated with British Colombia isolates were uniformly negative (data not shown).

To better understand the phenotypic disparity, protein profiles of *B. bissettii* strains were compared to *B. burgdorferi* sensu stricto, evaluating similarities and differences in major proteins of these culture isolates. The SDS-PAGE profile indicated that at least five distinct differences were observed between the CO and BC isolates; CO-Bb (Fig. 3, lanes 1 and 2) contained three bands (23 kDa, 21.5 kDa, and 14.5 kDa) not

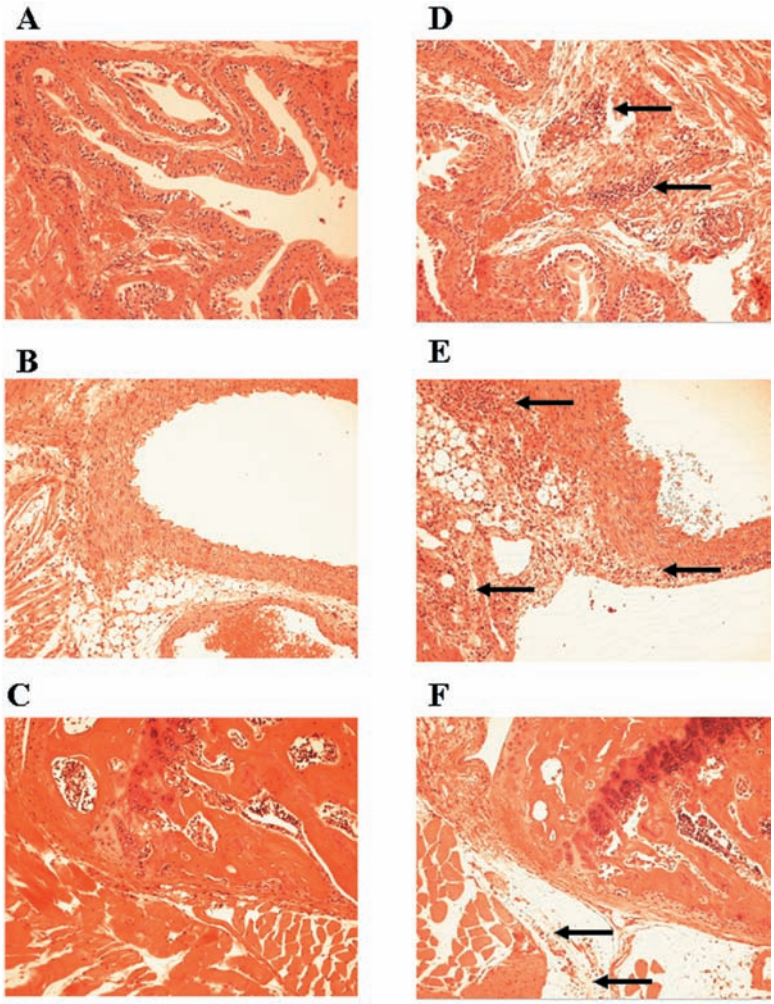

FIG. 2. Histopathology identified 8 weeks after *B. bissettii* challenge. **A.** Barbour-Stoenner-Kelly (BSK-H; 10×) bladder (arrows indicate perivascular inflammation). **B.** BSK-H (10×) heart. **C.** BSK-H (10×) femorotibial joint. **D.** N275 (10×). **E.** P300 (10×) heart (arrows indicate inflammation of the myocardium). **F.** N275 (10×) femorotibial joint (arrows indicate inflammation within the fascia adjacent to the head of the tibia).

evident in the BC-Bb isolates, whereas BC-Bb (Fig. 3, lanes 3 and 4) contained 2 bands (34.4 kDa and 31 kDa) not expressed by the CO isolates. Protein profiles also demonstrated that CO-Bb exhibited greater amounts of OspC, whereas BC-Bb strains displayed greater levels of both OspA and OspB (Fig. 3). Relative to the B31 laboratory strain of *B. burgdorferi* (Fig. 3, lane 5), Colorado isolates demonstrated similar 23 kDa and 21.5 kDa proteins compared to B31, whereas the BC isolates demonstrated the 31kDa molecule. A protein migrating to 33kDa, noted in both the rodent and tick isolates of *B. bissettii*, was absent in *B. burgdorferi* B31 (Fig. 3).

To clarify the identity of specific proteins and assess antigen conservation, immunoblots were performed, probing isolates with anti-*B. burgdorferi* monoclonal antibodies (Johnson et al. 1996). OspA and OspB were major constituent proteins in all Bb isolates and displayed strong immunreactivity with respective monoclonal antibodies (Fig. 4, panels 1–4). Both of these proteins were present as distinct doublets in BC isolates. Anti-OspC was strongly reactive against all *B. bissettii* isolates (Fig. 4, panels 1–4). In the CO-Bb isolates, OspC appeared as a doublet in both PAGE (Fig. 3, lanes 1–2) and Western blot profiles (Fig. 4, panels 1–2). Although p66 and p41 were major protein constituents of *B. bissettii* (PAGE profiles, Fig. 3), the anti-p66 and anti-p41 monoclonal antibodies were weakly reactive with these proteins (Fig. 4).

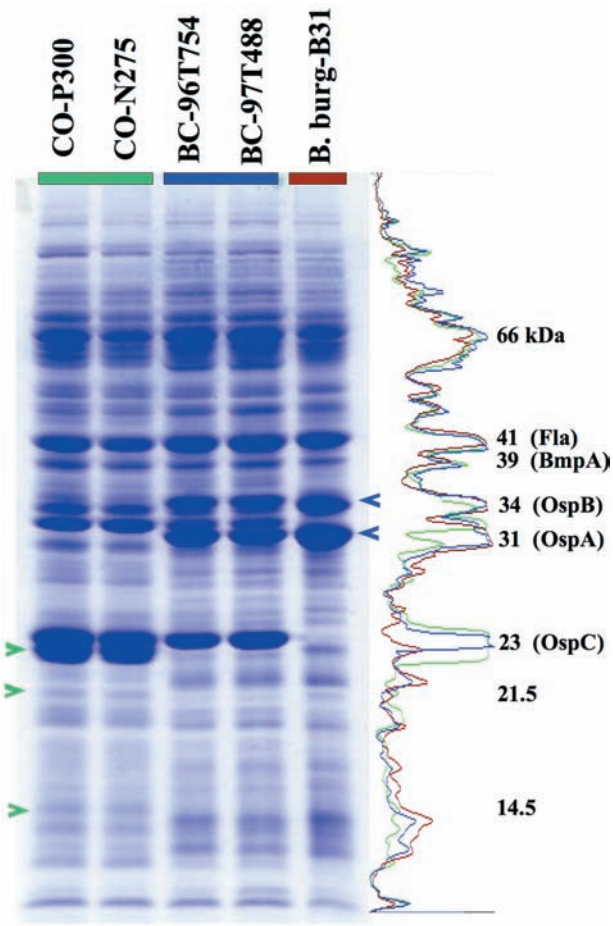

FIG. 3. Comparative sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of *B. bissettii* and *B. burgdorferi*. Lane 1: CO-P300. Lane 2: CO-N275. Lane 3: BC96T754. Lane 4: BC97T488. Lane 5: *B. burgdorferi* strain B31. Major protein differences between Colorado isolates of *B. bissetti* (CO-Bb) and British Columbia isolates of *B. bissetti* (BC-Bb) are indicated by arrows. Identity and molecular weights (kDa) of major *B. burgdorferi* proteins are shown in the right margin. The adjacent histogram reflects relative intensity of protein expression in CO (green) and BC (blue) isolates of *B. bissettii*, as compared to *B. burgdorferi* strain B31 (red).

Murine antibody responses against the CO and BC-Bb strains, 2, 4, and 8 weeks post inoculation, were also assessed by Western blot assay (Johnson et al. 1996). Weak IgM responses against OspB and OspA were observed in CO-Bb inoculated mice (Fig. 4, panels 1–2) and no IgM response was detected in BC-Bb inoculated mice (Fig. 4, panels 3–4). IgG responses against the CO-Bb strains expanded from 6 reactive proteins, including p66, p41, OspB, OspA, and OspC, 2 weeks post inoculation to over 10 reactive proteins at week 8 (Fig. 4, panels 1–2). In contrast, IgG reactivity to the BC-Bb strains were against a much smaller range of proteins and did not expand over time to the extent observed in CO inoculated mice (Fig. 4, panels 3–4). Over the 8-week observation period, mice inoculated with BC97T488 (Fig. 4, panel 3) produced only antibody against OspA. The antibody response in mice inoculated with the second BC isolate, BC96T754, was greater, but also limited (Fig. 4, panel 4). IgG against OspA, OspB, and OspC, detectable at 2 weeks post inoculation, increased in intensity throughout the 8-week period. In addition, low levels of antibody against p66, 39, and 37 were detected at 4 and 8 weeks (Fig. 4, panel 4). The modest antibody responses in mice inoculated with live BC-Bb isolates were mirrored by low-level antibody responses to the same antigens in mice inoculated with nonviable culture lysates (equivalent to 1×10^6 spirochetes) of the BC strains (data not shown). Combined with the inability to culture *Borrelia* from animals inoculated with live BC-Bb, these findings suggest minimal virulence and persistence of the BC-Bb isolates in this murine model.

DISCUSSION

Current ideas about the pathogenicity of *B. bissettii* are based on experiments employing a single *B. burgdorferi* sensu lato complex isolate, 25015, retrospectively identified as *B. bissettii* (Anderson et al. 1990a). However, the genetic and pathogenic diversity among *Borrelia* species, and even among isolates within particular species (Baranton et al. 2001; Fingerle et al. 2002; Peavey and Lane 1996; Wilske et al. 1993), raised the question of whether this issue was adequately addressed. Such variability in phenotype could account for the seemingly incongruous report (Strle et al. 1997) linking European isolates of *B. bissettii* with human borreliosis. We therefore assessed the potential of North American isolates of *B. bissettii* to induce disease in a murine model. The isolates were geographically disparate and expressed distinctly different proteins based on molecular weight. The results of the present study indicate that *B. bissettii* is capable of inducing dis-

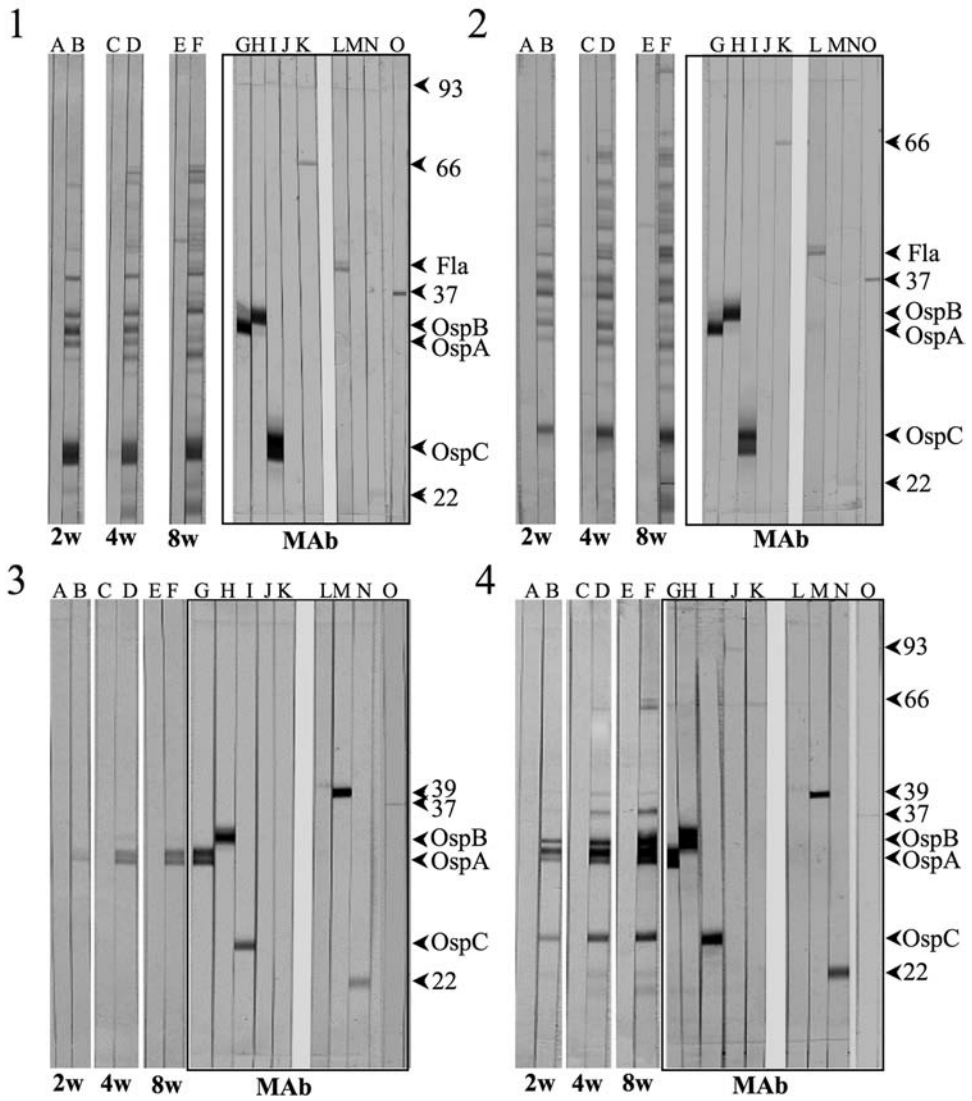

FIG. 4. Antibody reactivity to *B. bissettii* 2, 4, and 8 weeks after needle inoculation. Immunoblot of mouse serum reacted with their respective Bb antigens, Panel 1: CO P300. Panel 2: CO N275. Panel 3: BC 97T488. Panel 4: BC 96T754. Murine antibody response 2 (AB), 4 (CD), and 8 (EF) weeks post inoculation. IgM reactivity is displayed in columns A, C, and E, and IgG reactivity is shown in columns B, D, and F. Monoclonal antibody controls (boxed columns G–O) are indicated by arrows in the right margin: proteins are labeled by monoclonal antibodies corresponding to, G = OspA; H = OspB; I = OspC; J = p96; K = p66; L = flagellin; M = p39; N = p22; O = p37.

ease. Specifically, Colorado isolates of *B. bissettii* produce manifestations of disease in a murine model, causing pathology within the bladder, heart, and femorotibial joint.

B. bissettii infection causes accumulations of small lymphocytes and plasma cells within the bladder interstitium; perivascular mononuclear cell infiltrates of the great vessels of the heart breaching the peripheral vessel wall into the adjacent myocardium, severe inflammation of the atria, with associated adjacent myocardial necrosis; and a mild infiltration of mononuclear cells within the joint. This is similar to the findings observed during *B. burgdorferi* sensu stricto infection, which leads to a lympocytic cystitis, subacute myocarditis with a heterogeneous inflammatory response including neutrophils, lymphocytes, plasma cells, and macrophages within the superior portions of the heart as well as the great vessels, and evidence of a mild arthritis and periostitis in the femorotibial joints (Zeidner et al. 2001). As previously mentioned, these results are in contrast to a report of experimental infection of mice

with *B. bissettii* strain 25015, where no pathology was noted although it was infectious to mice (Anderson et al. 1990a). It is possible that experimental design played a role, as the present studies analyzed mice 8 weeks after inoculation, whereas the previous study examined tissues 21 days after inoculation (Anderson et al. 1990a). This relatively early time point may limit time for appreciable lesion development (Zeidner et al. 2001). It is likely that phenotypic variability between isolates, recognized in *Borrelia* isolates particularly in the far western United States, accounts for the disparity between studies (Peavey and Lane 1996). This possibility is supported by the significant differences in virulence between genetically similar isolates that were evaluated here, namely the inability of the BC isolates to cause a sustainable infection.

In the case of BC-Bb isolates, immunoblot data indicated that mice became, at best, only transiently infected with the BC tick isolates 96T754 and 97T488, and PCR, histopathology, and culture data indicated that these isolates did not disseminate or induce pathology to heart, bladder, or joint. Isolates of *B. burgdorferi* from the western coast of North America have previously been shown to have limited ability to infect laboratory mice (Barthold et al. 1990; Schwan et al. 1993). Inoculation with large numbers of *Borrelia* (10^7–10^8 spirochetes) failed to produce detectable infections in rodents with 16/19 isolates from California (Schwan et al. 1993). It is possible that BC strains exhibit very different tissue tropisms and are not found in tissues traditionally infected by spirochetes, but it is more probable that these spirochetes simply cannot successfully colonize the mammalian host and are rapidly cleared by the immune system. It is noteworthy that *B. bissettii* strain 25015, similar to the BC strains, was isolated from ticks (Anderson et al. 1990a). Thus, it could be concluded that BC isolates and 25105 may be comprised of spirochetes adapted to the tick midgut environment, whereas the CO strains, isolated from rodents, may be more suited to a mammalian tissue environment.

A supplemental experiment, conducted with a Colorado *B. bissettii* tick (*Ix. spinipalpis*) isolate, caused infection and pathology in mice (8/8; data not shown), suggesting that disparity between Colorado and BC isolates is not due to a mammalian or tick source of the isolate, but rather to inherent differences between the isolates. An earlier study (de Silva et al. 1998) supports this assertion as BSK II-, murine-, and tick-adapted *B. burgdorferi* were equally able to infect mice, although cultured spirochetes were more vulnerable to the protective effects of immune sera. In our study the inverse experiment with a vertebrate isolate of BC *B. bissettii* was not conducted, as regional prevalence in mammals is very low and the identity of reservoir host(s) remains unclear. Inoculation via tick feeding may alter the pathogenesis of *B. bissettii*, although previous research suggests that tick inoculation would lead to enhanced disease instead of reduced disease (Zeidner et al. 2002). Nonetheless, studies employing the natural vector are valuable and may provide additional information.

Possible explanations as to why isolates from Colorado caused pathology not observed in mice infected with BC strains are numerous. Variation in infection may be caused by a differential protein expression in BC isolates as compared to CO isolates. With *B. burgdorferi*, the pattern of protein expression is significantly altered during the transmission from tick to rodent, as OspA is downregulated concurrently with an upregulation of OspC in the mammalian host (Schwan and Piesman 2000), allowing for efficient transmission and dissemination of spirochetes (Schwan 2003). Distinct differences in protein expression could lead BC strains to be maladapted to the murine host environment, decreasing their early viability during initial contact with mammalian tissue and a host immune response. Indeed, PAGE results demonstrated distinct protein differences between CO and BC strains. Tick-derived BC strains demonstrated increased OspA and OspB compared to CO strains, and murine antibody responses elicited by inoculated tick-derived BC strains were limited, predominantly directed toward the immunodominant proteins OspA and OspB. The inoculation of spirochetes expressing OspA does not appear to be artificial, as data suggest that natural tick inoculation of *Borrelia* includes a significant proportion expressing OspA (Ohnishi et al.

2001). Colorado isolates expressed OspC as a doublet and at higher levels. Some evidence suggests that for *B. burgdorferi* OspC is required for early mammalian infection (Grimm et al. 2004), and its genetic sequence may dictate whether a spirochete causes no disease, local infection at the tick bite site, or systemic disease (Seinost et al. 1999), as well as influencing the host range (Brisson and Dykhuizen 2004). Thus, OspC heterogeneity could account for the differences observed between CO and BC isolates.

The humoral response elicited by differential outer surface protein expression could make the BC strains less able to evade the immune response. It is apparent that mice infected with BC isolates developed a more robust immune response to OspA. Data suggest that *Borrelia* is vulnerable to a strong anti-OspA immune response. This may account for the detection of OspA expression only in immunocompromised mice (Hodzic et al. 2003), and for the fact that spirochetes that continually express this surface protein are deficient in their ability to infect mice (Strother et al. 2007). Unlike this mutated strain, BC isolates have the ability to adapt to the host environment by downregulating OspA; indeed, in vitro–cultured spirochetes drastically alter their gene expression upon introduction to the host (Liang et al. 2002). Specific protein differences, and hence the isolates infective dissimilarity, could be due to simple geographic variation in the spirochetes genetically, leading to differences in dissemination, tissue tropism, or viability. Although not the focus of this work, the difference in infectiousness and pathological presentation between *B. bissettii* isolates is interesting and warrants further comparative study.

Although *B. burgdorferi* sensu stricto remains the most significant organism in terms of public health, the potential for *B. bissettii* to cause disease is of considerable importance given the extensive geographic range in which it has been recognized (Burkot et al. 2000; Schneider et al. 2000; Vredevoe et al. 2004). Additionally, greater attention may need to be devoted to *B. bissettii* tick vectors less commonly associated with *B. burgdorferi* sensu stricto, such as *I. spinipalpis*, because humans are increasingly encroaching into undeveloped land and ticks previously assumed to be nidicolous have demonstrated questing behavior (Burkot et al. 2001). Given the limited number of human Lyme borreliosis cases associated with *B. bissettii*, this spirochete likely plays a minor role in human disease. However, expectation of *B. burgdorferi* as the causative agent—and infrequent assaying for *B. bissettii*, in particular—may play a role in the rare recognition of *B. bissettii* in connection with disease.

This study is important because it presents the first evidence in an animal model that *B. bissettii* can produce pathology in vertebrates, and it reveals considerable variability in the infectious and pathogenic potential between strains, as previously noted in *B. burgdorferi*, suggesting that pathogenic attributes of a single isolate cannot be assumed to apply to other isolates. In addition to identifying the pathogenic potential of *B. bissettii*, this study presents a valuable comparative model with which to study the pathogenesis and infectious/pathogenic determinants of *B. bissettii*, and it adds credibility to the possibility that *B. bissettii* may be implicated in some cases of Lyme borreliosis.

ACKNOWLEDGMENTS

The authors thank Keerthi Fernando, BCCDC, and Craig Sampson and Darla Severin, CDC, for expert laboratory assistance; Robert Mann, BCCDC, for his support in the field; and Quantine Wong, BCCDC, for assistance in tick identification. The Pasteur Foundation, New York, NY, currently funds B. S. Schneider.

REFERENCES

Centers for Disease Control and Prevention. Lyme disease—United States, 2003–2005. MMWR Morb Mortal Wkly Rep 2007; 56:573–576.

Anderson, JF, Barthold, SW, Magnarelli, LA. Infectious but nonpathogenic isolate of Borrelia burgdorferi. J Clin Microbiol 1990a; 28:2693–2699.

Anderson, JF, Magnarelli, LA, Stafford, KC 3rd. Bird-feeding ticks transstadially transmit Borrelia burgdorferi that infect Syrian hamsters. J Wildl Dis 1990b; 26:1–10.

Banerjee, SN, Banerjee, M, Fernando, K, Scott, JD, et al. Presence of spirochete causing Lyme disease, *Borrelia*

burgdorferi, in the blacklegged tick, *Ixodes scapularis*, in southern Ontario. Can Med Assoc J 2000; 162: 1567–1569.

Baranton, G, Seinost, G, Theodore, G, Postic, D, et al. Distinct levels of genetic diversity of *Borrelia burgdorferi* are associated with different aspects of pathogenicity. Res Microbiol 2001; 152:149–156.

Barbour, AG, Todd, WJ, Stoenner, HG. Action of penicillin on Borrelia hermsii. Antimicrob Agents Chemother 1982; 21:823–829.

Barthold, SW, Moody, KD, Beck, DS. Susceptibility of laboratory rats to isolates of *Borrelia burgdorferi* from different geographic areas. Am J Trop Med Hyg 1990; 42:596–600.

Bissett, ML, Hill, W. Characterization of Borrelia burgdorferi strains isolated from Ixodes pacificus ticks in California. J Clin Microbiol 1987; 25:2296–2301.

Brisson, D, Dykhuizen, DE. *ospC* diversity in *Borrelia burgdorferi*: different hosts are different niches. Genetics 2004; 168:713–722.

Brown, RN, Lane, RS. Lyme disease in California: a novel enzootic transmission cycle of Borrelia burgdorferi. Science 1992; 256:1439–1442.

Burkot, TR, Maupin, GO, Schneider, BS, Denatale, C, et al. Use of a sentinel host system to study the questing behavior of *Ixodes spinipalpis* and its role in the transmission of *Borrelia bissettii*, human granulocytic ehrlichiosis, and *Babesia microti.* Am J Trop Med Hyg 2001; 65:293–299.

Burkot, TR, Schneider, BS, Pieniazek, NJ, Happ, CM, et al. *Babesia microti* and *Borrelia bissettii* transmission by *Ixodes spinipalpis* ticks among prairie voles, *Microtus ochrogaster*, in Colorado. Parasitology 2000; 121(Pt 6):595–599.

da Franca, I, Santos, L, Mesquita, T, Collares-Pereira, M, et al. Lyme borreliosis in Portugal caused by *Borrelia lusitaniae*? Clinical report on the first patient with a positive skin isolate. Wien Klin Wochenschr 2005; 117:429–432.

de Silva, AM, Fikrig, E, Hodzic, E, Kantor, FS, et al. Immune evasion by tickborne and host-adapted *Borrelia burgdorferi.* J Infect Dis 1998; 177:395–400.

Fingerle, V, Rauser, S, Hammer, B, Kahl, O, et al. Dynamics of dissemination and outer surface protein expression of different European Borrelia burgdorferi sensu lato strains in artificially infected Ixodes ricinus nymphs. J Clin Microbiol 2002; 40:1456–1463.

Fingerle, V, Schulte-Spechtel, UC, Ruzic-Sabljic, E, Leonhard, S, et al. Epidemiological aspects and molecular characterization of *Borrelia burgdorferi* s.l. from southern Germany with special respect to the new species *Borrelia spielmanii* sp. nov. Int J Med Microbiol. 2008; 298:279–290.

Foldvari, G, Farkas, R, Lakos, A. *Borrelia spielmanii* erythema migrans, Hungary. Emerg Infect Dis 2005; 11:1794–1795.

Grimm, D, Tilly, K, Byram, R, Stewart, PE, et al. Outer-surface protein C of the Lyme disease spirochete: a protein induced in ticks for infection of mammals. Proc Natl Acad Sci USA 2004; 101:3142–3147.

Hodzic, E, Feng, S, Freet, KJ, Barthold, SW. *Borrelia burgdorferi* population dynamics and prototype gene expression during infection of immunocompetent and immunodeficient mice. Infect Immun 2003; 71:5042–5055.

James, AM, Liveris, D, Wormser, GP, Schwartz, I, et al. Borrelia lonestari infection after a bite by an Amblyomma americanum tick. J Infect Dis 2001; 183: 1810–1814.

Johnson, BJ, Robbins, KE, Bailey, RE, Cao, BL, et al. Serodiagnosis of Lyme disease: accuracy of a two-step approach using a flagella-based ELISA and immunoblotting. J Infect Dis 1996; 174:346–353.

Liang, FT, Nelson, FK, Fikrig, E. Molecular adaptation of *Borrelia burgdorferi* in the murine host. J Exp Med 2002; 196:275–280.

Lin, T, Oliver, JH Jr, Gao, L, Kollars, TM Jr, et al. Genetic heterogeneity of Borrelia burgdorferi sensu lato in the southern United States based on restriction fragment length polymorphism and sequence analysis. J Clin Microbiol 2001; 39:2500–2507.

Maraspin, V, Ruzic-Sabljic, E, Strle, F. Lyme borreliosis and *Borrelia spielmanii.* Emerg Infect Dis 2006; 12:1177.

Maraspin, V, Cimperman, J, Lotric-Furlan, S, Ruzic-Sabljic, et al. Solitary borrelial lymphocytoma in adult patients. Wien Klin Wochenschr 2002; 114:515–523.

Mathiesen, DA, Oliver, JH Jr, Kolbert, CP, Tullson, ED, et al. Genetic heterogeneity of Borrelia burgdorferi in the United States. J Infect Dis 1997; 175:98–107.

Maupin, GO, Gage, KL, Piesman, J, Montenieri, J, et al. Discovery of an enzootic cycle of Borrelia burgdorferi in Neotoma mexicana and Ixodes spinipalpis from northern Colorado, an area where Lyme disease is nonendemic. J Infect Dis 1994; 170:636–643.

Norris, DE, Johnson, BJ, Piesman, J, Maupin, GO, et al. Population genetics and phylogenetic analysis of Colorado *Borrelia burgdorferi.* Am J Trop Med Hyg 1999; 60:699–707.

Ohnishi, J, Piesman, J, de Silva, AM. Antigenic and genetic heterogeneity of *Borrelia burgdorferi* populations transmitted by ticks. Proc Natl Acad Sci USA 2001; 98:670–675.

Peavey, CA, Lane, RS. Comparison of infectivities of six tick-derived isolates of Borrelia burgdorferi for rodents and ticks. J Clin Microbiol 1996; 34:71–75.

Postic, D, Ras, NM, Lane, RS, Hendson, M, et al. Expanded diversity among Californian borrelia isolates and description of Borrelia bissettii sp. nov. (formerly Borrelia group DN127). J Clin Microbiol 1998; 36:3497–3504.

Richter, D, Schlee, DB, Allgower, R, Matuschka, FR. Relationships of a novel Lyme disease spirochete, *Borrelia spielmani* sp. nov., with its hosts in Central Europe. Appl Environ Microbiol 2004; 70:6414–6419.

Schneider, BS, Zeidner, NS, Burkot, TR, Maupin, GO, et al. Borrelia isolates in Northern Colorado identified as Borrelia bissettii. J Clin Microbiol 2000; 38:3103–3105.

Schwan, TG. Temporal regulation of outer surface proteins of the Lyme-disease spirochaete *Borrelia burgdorferi.* Biochem Soc Trans 2003; 31:108–112.

Schwan, TG, Piesman, J. Temporal changes in outer surface proteins A and C of the Lyme disease-associated spirochete, Borrelia burgdorferi, during the chain of infection in ticks and mice. J Clin Microbiol 2000; 38:382–388.

Schwan, TG, Schrumpf, ME, Karstens, RH, Clover, JR, et al. Distribution and molecular analysis of Lyme disease spirochetes, Borrelia burgdorferi, isolated from ticks throughout California. J Clin Microbiol 1993; 31:3096–3108.

Scott, JD, Fernando, K, Durden, LA, Morshed, MG. Lyme disease spirochete, *Borrelia burgdorferi*, endemic in epicenter at Turkey Point, Ontario. J Med Entomol 2004; 41:226–230.

Seinost, G, Dykhuizen, DE, Dattwyler, RJ, Golde, WT, et al. Four clones of *Borrelia burgdorferi* sensu stricto cause invasive infection in humans. Infect Immun 1999; 67:3518–3524.

Sinsky, RJ, Piesman J. Ear punch biopsy method for detection and isolation of Borrelia burgdorferi from rodents. J Clin Microbiol 1989; 27:1723–1727.

Steere, AC. [Lyme disease—a tick-transmitted spirochete infection]. Ter Arkh 1997; 59:32–34.

Strle, F, Picken, RN, Cheng, Y, Cimperman, J, et al. Clinical findings for patients with Lyme borreliosis caused by *Borrelia burgdorferi* sensu lato with genotypic and phenotypic similarities to strain 25015. Clin Infect Dis 1997; 25:273–280.

Strother, KO, Hodzic, E, Barthold, SW, de Silva, AM. Infection of mice with Lyme disease spirochetes constitutively producing outer surface proteins A and B. Infect Immun 2007; 75:2786–2794.

Vredevoe LK, Stevens, JR, Schneider, BS. Detection and characterization of *Borrelia bissettii* in rodents from the central California coast. J Med Entomol 2004; 41:736–745.

Wang, G, van Dam, AP, Schwartz, I, Dankert, J. Molecular typing of *Borrelia burgdorferi* sensu lato: taxonomic, epidemiological, and clinical implications. Clin Microbiol Rev 1999; 12:633–653.

Wilske, B. Epidemiology and diagnosis of Lyme borreliosis. Ann Med 2005; 37:568–579.

Wilske, B, Preac-Mursic, V, Jauris, S, Hofmann, A, et al. Immunological and molecular polymorphisms of OspC, an immunodominant major outer surface protein of Borrelia burgdorferi. Infect Immun 1993; 61:2182–2191.

Zeidner, NS, Schneider, BS, Dolan, MC, Piesman, J. An analysis of spirochete load, strain, and pathology in a model of tick-transmitted Lyme borreliosis. Vector-Borne Zoonotic Dis 2001; 1:35–44.

Zeidner, NS, Schneider, BS, Nuncio, MS, Gern, L, et al. Coinoculation of *Borrelia* spp. with tick salivary gland lysate enhances spirochete load in mice and is tick species-specific. J Parasitol 2002; 88:1276–1278.

Address reprint requests to:
Bradley Schneider
Institut Pasteur
Département de Parasitologie
Unités de Réponses précoces aux Parasites et Immunopathologie
25 Rue du Docteur Roux
75724 Paris Cedex 15
France

E-mail: bradley.schneider@pasteur.fr