The Borrelial Fibronectin-Binding Protein RevA Is an Early Antigen of Human Lyme Disease

Catherine A. Brissette,† Evelyn Rossmann,‡ Amy Bowman, Anne E. Cooley,† Sean P. Riley,§ Klaus-Peter Hunfeld, Michael Bechtel, Peter Kraczy, and Brian Stevenson

Department of Microbiology, Immunology and Molecular Genetics, University of Kentucky College of Medicine, Lexington, Kentucky; Genzyme Virotech GmbH, Rüsselsheim, Germany; and Institute of Medical Microbiology and Infection Control, University Hospital of Frankfurt am Main, Frankfurt, Germany

Received 26 October 2009/Returned for modification 23 November 2009/Accepted 13 December 2009

Lyme disease, or Lyme borreliosis, is the most common arthropod-borne disease in the United States and throughout Europe (12, 31, 40). Lyme disease often presents as a nonspecific flu-like illness, with symptoms such as fever and body aches. In the absence of the characteristic erythema migrans (EM) bulls-eye rash (present in 60 to 90% of patients), serology is key to a proper diagnosis (8, 9, 44, 48). With the potential for serious sequelae, such as musculoskeletal, cardiovascular, and neurological damage, early diagnosis is important (6, 33). In the United States, Lyme disease is caused almost exclusively by Borrelia burgdorferi sensu stricto. In Europe, however, several Lyme borreliae are responsible for the disease, including Borrelia burgdorferi sensu stricto, B. afzelii, B. garinii, B. spielmanii, and others (4, 24, 36). Different genospecies of Borrelia are associated with different Lyme disease manifestations (1, 3, 37, 43, 45, 46, 49). The heterogeneity among these isolates complicates diagnosis, as interspecies amino acid identities for commonly used borrelial antigens, such as the decorin-binding protein DbpA, can be as low as 40% (47). A highly sensitive test in the United States involves the C6 invariant variable domain of the VlsE molecule; this test works well in Europe for B. burgdorferi sensu stricto and B. garinii, but differences in sensitivity for detection of antibodies induced by B. afzelii limit its usefulness (21).

Improved diagnostics for early Lyme disease are needed. Currently, serological tests for Lyme disease are used for confirmation of infection only. The utility of these tests is hindered by their propensity toward false negatives due to lack of antibodies early in infection (2, 17, 25, 29) and false positives due to cross-reactivity with Treponema pallidum, autoimmune, or mononucleosis antibodies (6). The relatively low sensitivity of the available Lyme diagnostics indicates the need to identify additional highly antigenic borrelial proteins.

RevA is a surface-exposed 17-kDa outer membrane protein of B. burgdorferi with no significant homology to any bacterial proteins outside Borrelia spp. (10, 11, 14, 34). RevA is expressed during mammalian infection and repressed in the tick vector (7, 15, 16, 39). Production of RevA can be regulated during mammalian infection and repressed in the tick vector. The function of RevA was previously unknown, yet its surface location and expression during mammalian infection suggested to us that this protein may be involved in interactions with the Lyme spirochete’s host. We recently discovered that RevA binds fibronectin, and its expression early in mammalian infection may be important for the bacterium to establish initial interactions with the host (7). Serum samples from human patients and experimentally infected animals identified the frequent presence of antibodies recognizing RevA (5, 15, 30). Laboratory-infected mice produced antibodies to RevA within 4 weeks postinfection (7), suggesting that RevA might be a useful serological marker of Lyme disease. In this report, we examine serum samples from human Lyme disease patients from the United States and Germany for the
presence of antibodies that recognize recombinant RevA proteins.

**MATERIALS AND METHODS**

**Bacteria.** *B. burgdorferi* B31 MI-16 is an infectious clone of the sequenced type strain (11, 14) which contains all parental plasmids (26). *B. garinii* PBi is a European isolate from human cerebrospinal fluid (35). All *B. burgdorferi* strains were grown at 34°C to cell densities of approximately 1 × 10^7 bacteria/ml in Barbour-Stoenner-Kelly (BSK-II) medium supplemented with 6% rabbit serum. Total DNA (genomic and plasmids) was isolated using a DNAeasy blood and tissue kit (Qiagen, Valencia, CA).

**Recombinant proteins.** Recombinant proteins contained amino-terminal polyhistidine tags, with the RevA or RevB segment beginning with that protein’s first amino acid following the cysteine lipidation site. revA genes were PCR amplified from total genomic DNA of *B. burgdorferi* B31 MI-16 and *B. garinii* PBi, and revB was amplified from *B. burgdorferi* B31 MI-16, using the oligonucleotides listed in Table 1. Amplicons were cloned into PET200 (Invitrogen, Carlsbad, CA). Resultant plasmid inserts were entirely sequenced on both strands to ensure that no undesired mutations had occurred during PCR or cloning procedures. Recombinant proteins were expressed in *Escherichia coli* Rosetta (DE3) pLysS (Novagen, Madison, WI) upon induction with isopropylthiogalactopyranoside. Induced *E. coli* cultures were harvested and lysed by sonication, and debris was cleared by centrifugation. Recombinant proteins were purified from cleared lysates using either MagHis nickel-conjugated magnetic beads (Promega, Madison, WI) or His-Trap HP columns and an AKTA fast-performance liquid chromatograph (FPLC) equipped with a UPC-900 UV absorbance monitor and a FPLC 920 fraction collector (GE Healthcare, Piscataway, NJ). Proteins were eluted from FPLC columns by a constantly increasing gradient between the lysis buffer (30 mM imidazole, 0.5 M NaCl, 20 mM NaPO4 [pH 7.4]) and the elution buffer (0.75 M imidazole, 5 M NaCl, 20 mM NaPO4 [pH 7.4]). All recombinant proteins were dialyzed at 4°C overnight against phosphate-buffered saline (PBS) using 3,500 Da filtration columns by a constantly increasing gradient between the lysis buffer (30 mM imidazole, 5 M NaCl, 20 mM NaPO4 [pH 7.4]) and the elution buffer (0.75 M imidazole, 5 M NaCl, 20 mM NaPO4 [pH 7.4]). All recombinant proteins were dialyzed at 4°C overnight against phosphate-buffered saline (PBS) using 3,500 Da filtration columns by a constantly increasing gradient between the lysis buffer (30 mM imidazole, 5 M NaCl, 20 mM NaPO4 [pH 7.4]) and the elution buffer (0.75 M imidazole, 5 M NaCl, 20 mM NaPO4 [pH 7.4]). All recombinant proteins were dialyzed at 4°C overnight against phosphate-buffered saline (PBS) using 3,500 Da filtration columns by a constantly increasing gradient between the lysis buffer (30 mM imidazole, 5 M NaCl, 20 mM NaPO4 [pH 7.4]) and the elution buffer (0.75 M imidazole, 5 M NaCl, 20 mM NaPO4 [pH 7.4]).

**Seralogical analyses.** Both enzyme-linked immunosorbent assay (ELISA) and immunoblotting methods were used, essentially as we previously described (20, 26, 27, 41). Preexisting human Lyme disease and control serum samples were kindly provided by Gary Wormser (New York Medical College, Valhalla, NY), the University Hospital of Frankfurt, or the blood bank of Frankfurt, Germany. U.S. serum samples were from clinically diagnosed patients and have been used in previous serological studies (26, 27). German serum samples obtained from Lyme disease patients and from the control groups were pretested for the presence of anti-borrelial IgG antibodies using a commercially available line immunoblot assay (Genzyme Virotech, Rüsselsheim, Germany) in which VSE, BmA, p83, BBA36, BBO323, BcCRASP-3 (ErpP), and pG (ErpG) are included as target antigens. Additional positive control sera were obtained 4 weeks postinfection from four mice that had been infected with *B. burgdorferi* B31 MI-16 by infestation with infected *Ixodes scapularis* ticks.

Recombinant RevA from *B. burgdorferi* B31 MI-16 (RevA_M16) or *B. garinii* PBi (RevAPBi) and RevB from *B. burgdorferi* B31 MI-16 were produced based on the sequence of *B. burgdorferi* B31 or *B. garinii* PBi (15). For ELISAs, Maxisorp 96-well plates (Nalg-Nunc, Rochester, NY) were coated overnight at 4°C with 10 μg/ml protein: bovine serum albumin (BSA; Millipore, Billerica, MA) or recombinant RevA or RevB protein in carbonate buffer, pH 9.6. Plates were brought to room temperature and washed once with PBS plus 0.5% (vol/vol) Tween 20 (PBS-T). Wells were blocked for 2 h at room temperature with 2% BSA in PBS-T, washed three times with PBS-T, and then incubated for 1 h at room temperature with a 1:100 dilution of human or murine serum. Plates were washed three times with PBS-T, then wells were incubated for 1 h at room temperature with horseradish peroxidase (HRP)-conjugated protein A or anti-mouse IgG, diluted 1:5,000 (GE Healthcare, Little Chalfont, United Kingdom). Wells were again washed three times with PBS-T. Aliquots (100 μl/well) of tetramethylbenzidine substrate (Pierce) were added, and then reactions were stopped by addition of 100 μl/well 2 N H2SO4. Absorbance was read at 450 nm using a Spectramax plate reader and SoftMax Pro software (Molecular Devices, Sunnyvale, CA).

For line blotting, recombinant proteins were transferred to nitrocellulose membranes by a microspreading method in amounts of 500, 250, 125, 62, 31, 16, 8, 4, and 2 ng per stripe. Individual membranes were incubated with human sera (German sera at 1:100 and U.S. sera at 1:200) or mouse sera (1:100). Serum dilutions were based upon serological studies with other borrelial antigens (see above). Binding of specific antibodies was detected by using alkaline phosphatase-conjugated goat anti-human IgG serum (1:100; Genzyme Virotech) or goat anti-mouse Ig antibodies (1:10,000; GE Healthcare), as appropriate. Immunoreactive bands were visualized by addition of 3 ml of diethanolamine buffer supplemented with 5-bromo-4-chloro-3-indolylphosphate (Sigma-Aldrich) at 165 mg/ml and nitroblue tetrazolium (Sigma-Aldrich) at 330 mg/ml as a substrate. Cutoffs were based on a standardized band intensity scale (Genzyme Virotech GmbH, version VI 0803093) of 0 to 5, with 5 being the most intense and 0 being no band visible. Line blots were considered positive if the band intensity was >2.

**RESULTS**

**Conservation of Rev sequences among Lyme disease borreliae.** Sequences of revA genes have been published from the Lyme disease spirochete strains B31 and 297 (15, 34). The two revA genes of *B. burgdorferi* strain B31 are located on its native circular prophages, cp32-1 and cp32-6. *B. burgdorferi* strain 297 has two copies of revA that share approximately 60 to 70% identity with each other and with the RevA protein of strain B31. *B. burgdorferi* strain B31 naturally contains a cp32 derivative, cp9-1, which contains a paralogous gene of revA that is designated revB (10, 11, 14). The revB gene shares 47.5% nucleic acid identity with the revA genes, and the RevB protein shares 28% amino acid sequence identity with the two cp32-encoded RevA proteins of strain B31.

Data mining of GenBank sequences (BLAST search of the translated nucleotide database using the RevA protein sequence from strain B31) indicated that many sequenced *B. burgdorferi* isolates contain genes homologous to revA and revB. Their predicted amino acid sequences were compared with those of strains B31 and 297. *B. burgdorferi* N40, 156a, 29805, ZS7, Bol26, 72a, 64a, and 64b and *B. garinii* PBi each carry one revA locus. Two separate revA alleles were identified in two different *B. spielmannii* strains, A14S and PC-Eq17 (Fig. 1). *B. burgdorferi* strains 94a and 118a each carry two revA alleles, while strain W1‐91-23 has three copies of revA on three different cp32 plasmids. Amino acid sequence identities among predicted RevA proteins were 64 to 100% compared to the sequenced strain B31. No revB loci were identified in other strains with a cp9 prophage (e.g., 94a, W193-23, 118a). How-ever...
ever, a revB-like locus was identified in *B. afzelii* strain PKo (Fig. 1). The sequenced *T. pallidum*, relapsing fever *Borrelia*, and pathogenic *Leptospira* genomes do not encode proteins with sequence similarities with RevA or RevB.

**Serological examination of human Lyme disease patient sera.** We previously established that *B. burgdorferi* produces elevated levels of *revA* transcript within 2 weeks of infecting mice and that such animals produce RevA-directed antibodies within 4 weeks of infection (7). ELISA formats are particularly well suited to serodiagnosis due to their adaptability and potential for automation. Therefore, we tested sera from Lyme disease patients and from infected mice for reactivity to *B. burgdorferi* strain B31 RevA (RevA B31) by ELISA. Antibodies to RevA B31 were readily detected at dilutions up to 1:1,000 for human sera (n = 1100/5) and 1:10,000 for mouse sera (n = 1100/4) in 100% of samples tested (Fig. 2).

To expand upon these results and ascertain if a majority of humans are exposed to RevA during the early stages of Lyme disease, we examined serum samples from patients with Lyme disease from Germany and the United States. The patients exhibited a wide range of symptoms, including erythema migrans and neuroborreliosis.

Human serum samples were examined by line immunoblot assays containing various amounts of recombinant RevA B31 or *B. garinii* strain PBi RevA (RevA PBi). A total of 35% of confirmed U.S. Lyme patient sera recognized RevA B31, while only 12% of German patient sera recognized RevA B31; however, 29% of the German patient sera recognized RevA PBi. Strikingly, 50% of German patients with EM yielded immunoblot signals to RevA B31. Mice infected with *B. burgdorferi* B31 MI-16 via tick bite or needle inoculation all produced antibodies directed to RevA B31 (Fig. 3), while uninfected mice did not produce anti-RevA antibodies (Fig. 2 and data not shown). The sensitivity of U.S. patient sera for RevA B31 was 61%, while the sensitivity of German patient sera for RevA B31 was only 88%.

Although RevB B31 shares limited amino acid identity with RevA B31, both proteins bind fibronectin (7). Nothing is yet known about revB expression during human or mouse infection. Therefore, human and mouse sera tested for antibodies to RevA B31 were also examined for reactivity to RevB B31 by line immunoblot assay. Sera from all mice infected with *B. burgdorferi* B31 MI-16 produced antibodies to RevB B31 (Fig. 3 and data not shown). However, very few sera obtained from Lyme disease patients recognized this antigen (Fig. 3 and Table 2).

**DISCUSSION**

The current study demonstrates that human Lyme disease patients are exposed to the borrelian fibronectin-binding protein RevA. A number of serum samples from early infection (e.g., patients manifesting the early-stage erythema migrans rash, which usually appears within days to weeks of initial infection) contained antibodies against RevA. Consistent with our previous studies with experimentally infected mice, the current results indicate that Lyme disease spirochetes produce RevA surface protein during early stages of human infection. Since RevA is an adhesin that promotes bacterial interactions with host tissue, this protein appears to be a good target for development of therapies to prevent and treat Lyme disease.

RevA sensitivity for patient sera varied, likely due to antigenic differences between RevA from different genospecies.
FIG. 2. ELISA analyses of human Lyme disease patient sera (A) or murine sera (B) for antibodies recognizing RevA. Plates were coated with recombinant \textit{B. burgdorferi} RevA_{B31} (10 \mu g/ml). Sera were diluted serially 10-fold. Antibodies to RevA were detected using HRP-conjugated protein A (A) or HRP-conjugated anti-mouse IgG (B). (A) NY, patient serum from New York; WI, patient serum from Wisconsin; Neg, confirmed Lyme disease-negative human serum. (B) Tick, serum from mouse infected via tick infestation; Needle, serum from mouse infected via needle inoculation; Neg, uninfected mouse serum.
FIG. 3. Line blot analyses of human patient sera for antibodies recognizing RevA<sub>B31</sub> or RevB<sub>B31</sub>. Nitrocellulose membranes contained stripes of recombinant RevA<sub>B31</sub> and RevB<sub>B31</sub> in amounts of 500, 250, 125, 62, 31, 16, 8, 4, or 2 ng. (A) Acute-phase serum samples from patients diagnosed with EM at various locations in the United States. (B) Serum samples from German patients diagnosed with EM with positive Lyme disease serology. (C) Serum samples from German patients diagnosed with acrodermatitis chronica atrophicans (ACA). (D) Serum samples from German patients diagnosed with facial palsy, meningitis, or Bannwarth’s syndrome (neuroborreliosis [NB], stage II). (E) Serum samples from German patients diagnosed with Lyme arthritis. (F) Sera collected from mice 4 weeks postinfestation with ticks infected with <i>B. burgdorferi</i> B31 MI-16. (G) Serum samples from blood donors provided by the blood bank in Frankfurt, Germany. (H) Serum samples from German patients demonstrating a positive syphilis serology. (I) Serum samples from German patients diagnosed with leptospirosis. (J) Serum samples from German patients diagnosed with rheumatoid arthritis (RA). (K) Serum samples from German patients exhibiting antinuclear antibodies (ANA). (L) Serum samples from German patients diagnosed with HIV.
and strains of Lyme disease spirochetes. RevA from *B. garinii*, for example, shares 64% amino acid identity with the RevA from *B. burgdorferi* B31, while the two alleles of revA encoded by strain 297 share only 72% amino acid identity with each other. The diversity of Lyme disease borreliae in Europe prompted us to examine the reactivity of patient sera to RevA from different borrelia genospecies. Surveys of *Isodes ricinus* ticks in Germany suggest that either *B. garinii* (13) or *B. afzelii* (32) is the most common *B. burgdorferi* sensu lato species detected. However, we were unable to identify or amplify a *revB* paralogous gene in the sequenced strain of *B. afzelii* PKo, so we examined patient sera responses to RevA from *B. burgdorferi* sensu stricto and *B. garinii*. In general, serum samples from German patients were more likely to contain antibodies that reacted with *B. garinii* RevA than with *B. burgdorferi* RevA. These data suggest that serological assays may need to be adapted to account for regional and continent-specific strain differences.

Some of the serum samples examined from syphilis and leptospirosis patients contained antibodies that bound recombinant RevA. The sequenced *T. pallidum*, relapsing fever *Borrelia*, and pathogenic *Leptospira* genomes do not encode proteins with sequence similarities to RevA. Likely, *T. pallidum* and *Leptospira* species may possess proteins with conformational similarities to RevA and/or RevB. We are currently examining this possibility by Western blot assays of spirochetal lysates with anti-RevA antisera.

The related protein RevB was not a good serological indicator of infection. Although mice infected by tick bite produced antibodies against RevB, very few human sera reacted to it. RevB shares only 28% amino acid identity with RevA, but it retains the ability to bind fibronectin (7). Many *B. afzelii* strains lack the plasmid cp9, which in strain B31 carries the revB allele. Of the other genospecies examined, only *B. afzelii* appears to have a revB homolog.

*B. burgdorferi* has another fibronectin-binding protein, BBK32, that has also been investigated as a diagnostic marker. Differences in antibody levels due to sequence heterogeneity among BBK32 proteins from different species suggest that variant antigens are needed to cover all areas, as is the case with RevA (22). In addition, studies with BBK32 indicate that protein fragments may be better suited for early Lyme disease serology. We are currently investigating that approach for RevA, using various protein fragments to eliminate possible cross-reactive epitopes and increase the specificity of this serological test (23).

In conclusion, RevA is a surface-associated fibronectin adhesin that is expressed early in mammalian infection and is recognized by both mouse and human sera. Our studies indicated that while animals infected with *B. burgdorferi* strain B31 produced significant levels of antibodies directed against RevA, Lyme disease patients produced antibodies at varying levels, possibly due to differences in RevA sequences among the infectious borrelial genospecies. Other early antigens of *B. burgdorferi*, such as OspC, OspE proteins, BBK32, and DbpAB, also exhibit extensive sequence variation (18, 19, 38, 42). RevA is a widely distributed antigen among Lyme disease borreliae, but its interspecies heterogeneity may preclude RevA from being broadly applicable for serodiagnosis of human Lyme disease. Its expression early in infection, however, suggests that RevA may still be a useful target for preventative or curative therapies.

**ACKNOWLEDGMENTS**

This work was supported by exploratory funds provided by the University of Kentucky College of Medicine to B. Stevenson, by Deutsche Forschungsgemeinschaft grant KR383/1-2 to P. Kraezy, and by NIH Ruth L. Kirschstein Individual National Research Service Award F32 AI018480 to C. A. Brissette.

We thank Logan Burns, Brandon Jutras, and Ashutosh Verma for helpful comments and technical advice.

**REFERENCES**

1. Anthonissen, F. M., M. De Kesel, P. P. Hitot, and G. H. Bigaignon. 1994. Evidence for the involvement of different genospecies of *Borrelia* in the clinical outcome of Lyme disease in Belgium. Res. Microbiol. 145:327–331.

2. Bacon, R. M., B. J. Biggerstaff, M. E. Schrieffe, R. D. Gilmore, Jr., M. T. Philipp, A. C. Steere, G. P. Wormser, A. R. Marques, and B. J. Johnson. 2003. Serodiagnosis of Lyme disease by kinetic enzyme-linked immunosorbent assay using recombinant VlsE1 or peptide antigens of *Borrelia burgdorferi* compared with 2-tiered testing using whole-cell lysates. J. Infect. Dis. 187:1187–1199.

3. Balmelli, T., and J. C. Piffaretti. 1995. Association between different clinical manifestations of Lyme disease and different species of *Borrelia burgdorferi* sensu lato. Res. Microbiol. 146:329–340.

4. Baranton, G., D. Postic, I. Saint Girons, P. Boerlin, J.-C. Piffaretti, M. Assous, and P. A. D. Grimont. 1992. Delineation of *Borrelia burgdorferi* sensu stricto, *Borrelia garinii* sp. nov., and group VS461 associated with Lyme borreliosis. Int. J. Syst. Bacteriol. 42:378–383.

5. Barbour, A. G., A. Jasinskas, M. A. Kayala, D. H. Davies, A. C. Steere, P. Baldi, and P. L. Felgner. 2008. A genome-wide proteome array reveals a limited set of immunogens in natural infections of humans and white-footed mice with *Borrelia burgdorferi*. Infect. Immun. 76:3374–3389.

6. Bratton, R. L., J. W. Whiteside, M. J. Hovan, R. L. Engle, and F. D. Edwards. 2008. Diagnosis and treatment of Lyme disease. Mayo Clin. Proc. 83:566–571.

7. Brissette, C. A., T. Bykowski, A. E. Cooley, A. Bowman, and B. Stevenson. 2009. *Borrelia burgdorferi* RevA antigen binds host fibronectin. Infect. Immun. 77:2802–2812.

8. Brown, S. L., S. L. Hansen, and J. J. Langone. 1999. Role of serology in the diagnosis of Lyme disease. JAMA 282:62–66.

9. Brown, S. L., S. L. Hansen, J. J. Langone, N. Lowe, and N. Pressly. 1999. Lyme disease test kits: potential for misdiagnosis. FDA Med. Bull., Summer 1999. http://www.fda.gov/medbull/summer99/Lyme.html.

10. Carroll, J. A., N. El-Hage, J. C. Miller, K. Babb, and B. Stevenson. 2001. *Borrelia burgdorferi* RevA antigen is a surface-exposed outer membrane protein...
protein whose expression is regulated in response to environmental temperature and pH. Infect. Immun. 69:5286–5293.

11. Casjens, S., N. Palmer, R. van Vugt, W. M. Huang, B. Stevenson, P. Rosa, R. Lathigra, G. Sutton, J. Peterson, R. J. Dodson, D. Haft, E. Hickey, M. Gwinn, O. White, and O. Fraser. 2000. A bacterial genome in flux: the twelve linear and nine circular extrachromosomal DNAs of an infectious isolate of the Lyme disease spirochete Borrelia burgdorferi. Mol. Microbiol. 35:890–516.

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

13. Fingerle, V., U. C. Schulte-Spechtel, E. Ruzic-Sabljic, S. Leonhard, H. Hofmann, K. Weber, K. Pfister, F. Strle, and B. Wilske. 2008. Epidemiological aspects and molecular characterization of Borrelia burgdorferi s.l. from southern Germany with special respect to the new species Borrelia spielmannii sp. nov. Int. J. Med. Microbiol. 298:279–290.

14. Fraser, C. M., S. Casjens, W. M. Huang, G. G. Sutton, R. Clayton, R. Laubera, O. White, K. A. Ketchum, R. Dodson, E. K. Hickey, M. Gwinn, B. Dougherty, J.-F. Tomb, R. D. Fleischmann, D. Richardson, J. Peterson, A. R. Kerlavage, J. Quackenbush, S. Salzberg, M. Hanson, R. van Vugt, N. Palmer, M. D. Adams, J. Gocayne, J. A. Mesecar, R. A. Marques, B. J. B. Fuji, M. D. Cotton, K. Horst, K. Roberts, B. Hatch, H. O. Smith, and J. C. Venter. 1997. Genomic sequence of a Lyme disease spirochete, Borrelia burgdorferi. Nature 390:580–586.

15. Fingerle, V., R. D. Jr., and M. L. Mbow. 1998. A monoclonal antibody generated by antigen inoculation via tick bite is reactive to the Borrelia burgdorferi Rev protein, a member of the 2,9 gene family locus. Infect. Immun. 66:980–986.

16. Gilmore, R. D., Jr., M. L. Mbow, and B. Stevenson. 2001. Analysis of Borrelia burgdorferi gene expression during life cycle phases of the tick vector Ixodes scapularis. Microbes Infect. 3:799–808.

17. Gomes-Solecki, M. J., G. P. Wormser, D. H. Persing, B. W. Berger, J. D. Glass, X. Yang, and R. J. Dattwyler. 2001. A first-tier rapid assay for the serodiagnosis of Borrelia burgdorferi infection. Arch. Intern. Med. 161:2015–2020.

18. Hekkila, T., I. Seppala, H. Saxen, J. Panelius, M. Peltomaa, T. Julin, S. A. Carlsson, and P. Lahdenne. 2002. Recombinant BBK32 protein in serodiagnosis of Lyme borreliosis. J. Med. Microbiol. 51:1174–1180.

19. Ivana, L., I. Christova, V. Neves, M. Aroso, L. Meirelles, D. Brisson, and M. Gomes-Solecki. 2009. Comprehensive seroprophiling of sixteen B. burgdorferi ospC glycoforms: implications for Lyme disease diagnostics design. Clin. Immunol. 132:368–380.

20. Kaczyinski, P., A. Seling, C. A. Brissette, E. Rossom, K. P. Hunfeld, T. Rykowski, L. H. Burns, M. J. Troese, A. E. Cooley, J. C. Miller, V. Brade, R. Hoboken, NJ.

21. M. Gomes-Solecki. 2008: Diagnosis of Lyme borreliosis in Europe. Vector Borne Zoonotic Dis. 8:315–227.

22. Wormser, G. P., M. E. Aguero-Rosenfeld, and R. B. Nadelman. 1999. Lyme disease serology problems and opportunities. JAMA 282:79–80.

23. Nowak, G. P., D. Liveris, J. Nowakowski, R. B. Nadelman, L. F. Cavaliere, D. McKenna, D. Holmgren, and I. Schwartz. 1999. Association of specific subtypes of Borrelia burgdorferi with hematogenous dissemination in early Lyme disease. J. Infect. Dis. 180:720–725.

24. Zickert, W. R. 2007. Laboratory maintenance of Borrelia burgdorferi, p. 12C.1.1–12C.1.10. In R. T. Colby, T. F. Kowalski, J. Quarch, B. Stevenson, and R. Taylor (ed.), Current protocols in microbiology. J. Wiley & Sons, Hoboken, NJ.