Comparison of Different Strains of *Borrelia burgdorferi* Sensu Lato Used as Antigens in Enzyme-Linked Immunosorbent Assays

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Eight strains of *Borrelia burgdorferi* sensu lato were tested with serum samples from persons who had Lyme borreliosis or syphilis in class-specific enzyme-linked immunosorbent assays (ELISAs). Antigens of *B. burgdorferi* sensu stricto, of *Borrelia garinii*, and of *Borrelia* spirochetes in group VS461 were prepared from cultured bacteria isolated from ticks, a white-footed mouse (*Peromyscus leucopus*), or human tissues in North America, the former Soviet Union, and Japan. Nearly all of the serum specimens that contained immunoglobulins to strain 2591, a Connecticut isolate, were also positive in antibody tests with the other seven strains. In general, all eight strains reacted similarly and were suitable as coating antigens in class-specific ELISAs. Assay sensitivities ranged from 82.6 to 100% in analyses for immunoglobulin M and G antibodies. Compared with reference antigen strain 2591, strains 231 (a tick isolate from Canada) and NCH-1 (a human skin isolate from Wisconsin) resulted in higher antibody titers in an ELISA. Syphilitic sera cross-reacted in all tests regardless of the antigen used. Key immunodominant proteins are shared among the closely related strains of *B. burgdorferi* sensu lato tested, but it is suspected that variations in antigen compositions among these spirochetes may sometimes affect assay performance for detecting serum antibodies.

The diagnosis of Lyme borreliosis depends on a physician’s assessment of clinical findings, epidemiological information, and laboratory results. When the clinical diagnosis is unclear, there is increased reliance on laboratory testing. Unfortunately, there are limitations in most conventional assays being used to detect serum antibodies to *Borrelia burgdorferi* sensu lato. An antibody assay may have low sensitivity when blood samples are collected from patients during early disease (29). False-positive test results also can occur (20, 23, 31). The use of purified recombinant proteins in enzyme-linked immunosorbent assays (ELISAs) and Western blot (immunoblot) analyses has helped improve specificity without loss of sensitivity (13, 21). The utility of immune electron microscopy and PCR techniques for detection of antigen or DNA in human tissues or fluids is being explored. These methods have high sensitivity and specificity (12, 15, 16, 24, 26), but they are still considered experimental as are other procedures for antibody detection in function-oriented immunoassays (9, 28). Compared with an ELISA, procedures for electron microscopy, PCR, and in vitro inhibition of *B. burgdorferi* growth require more expertise and are less cost effective. Consequently, these newly developed assays are not as widely used as an ELISA in clinical or research laboratories.

During the past decade, numerous test kits have been marketed in the United States and Europe. With increased knowledge of the global occurrence of Lyme borreliosis (30, 31), including Japan (14) and the former Soviet Union (11), these commercial products have had more extensive distribution and use. Personnel in clinical and research laboratories in Eurasia, like in the United States, also have developed their own ELISAs, immunoblotting assays, or indirect immunofluorescent antibody staining methods to detect immunoglobulins to *B. burgdorferi*. Because of its availability, the B31 Shelter Island, New York strain (ATCC 35210) of this spirochete is often included as an antigen in assays used commercially or in private laboratories. Related strains of *B. burgdorferi* have been selected by other clinical microbiologists for diagnostic testing.

The main objective of this study was to test various isolates of *B. burgdorferi* sensu lato from North America and Eurasia as antigens in ELISAs to determine whether a local strain is required for optimal assay performance.

MATERIALS AND METHODS

**Serum samples.** During the period 1985 to 1987, 53 blood samples were obtained from 30 persons. Of these, 17 patients in Connecticut and 4 in the lower Hudson River Valley Region of New York State had a diagnosis of expanding skin lesions (erythema migrans) characteristic of Lyme borreliosis. The remaining nine persons lived in the Hudson River Valley Region, had a history of tick bites, and had antibodies to *B. burgdorferi* sensu stricto, as determined by an ELISA. These persons were volunteers in a tick bite study at the New York Medical College and were asymptomatic after confirmed bites by *Ixodes scapularis*, formerly known as *Ixodes dammini*. Blood samples were obtained between 3 and 6 weeks after tick bites. Although erythema migrans and accompanying signs and symptoms of Lyme borreliosis were not observed by physicians for this group, the serologic and epidemiologic evidence for exposure to this spirochete was highly suggestive. In Connecticut, the serum specimens were submitted by physicians along with clinical data to the Connecticut State Department of Health as reported earlier (20, 22). Persons in New York State were seen by physicians at the Lyme Disease Diagnostic Center at the Westchester County Medical Center in Valhalla. Of those patients who had erythema migrans, multiple blood samples were obtained within a period of 3 months. Except for

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one blood sample obtained 20 weeks after onset of illness, the first blood sample from each person was collected between 3 and 6 weeks after signs and symptoms of disease appeared. Seven additional serum samples from persons who had syphilis (23) and antibodies to *Treponema pallidum* but no history of Lyme borreliosis were included in analyses to assess specificity. All serum specimens were kept at −60°C until analyzed.

**Antigens.** Eight strains of Lyme borreliosis spirochetes were chosen for comparative analyses. Strain 2591 of *B. burgdorferi* was isolated from a white-footed mouse (*Peromyscus leucopus*) in East Haddam, Conn., during 1982 (3) and has been used extensively in antibody detection assays at the Connecticut Agricultural Experiment Station. Strain 25015 of *B. burgdorferi* was recovered from a fully fed *I. scapularis* removed from a white-footed mouse captured in Millbrook, N.Y. (Dutchess County) (5). This isolate, unlike many others characterized as *B. burgdorferi*, failed to cause arthritis in laboratory mice and rats (1). Another strain of *B. burgdorferi* (no. 231) was cultured from an *I. scapularis* larva that had parasitized a white-footed mouse in Long Point, Ontario, Canada. Two additional strains of this bacterium consisted of a human skin isolate (NCH-1) from Wisconsin and a spirochete (strain 33203) recovered from an *Ixodes pacificus* female in Placerville, Calif. *Borrelia* isolates IPF and IP90, designated by some authors (6, 27) as a strain in group VS461 and as *Borrelia garinii*, respectively, were cultured from *Ixodes persulcatus* ticks in Japan and the Khabarovsk Territory of the former Soviet Union. Three species have been delineated for *B. burgdorferi* sensu lato: *B. burgdorferi* sensu stricto, *B. garinii*, and *Borrelia afzelii* (6, 7). On the basis of pulsed-field gel electrophoresis characterization procedures, different isolates from *I. persulcatus* have been classified as *B. garinii* and *B. afzelii* (7). The B31 Shelter Island strain of *B. burgdorferi* sensu stricto and strain 2591 each had been subcultured extensively (>30 passages) and were included as reference antigens. All spirochetes were grown in fortified Barbour-Stoenner-Kelly II medium (2) before the antigens were prepared for coating to the solid phase of an ELISA. Methods of antigen preparation have been described (22). Low-passage isolates (i.e., fewer than 15 subpassages) of the remaining strains were selected for antigen preparation.

**ELISA.** Class-specific ELISAs for immunoglobulin M (IgM) and IgG antibodies were used to determine titration end points for serum specimens. Details of the ELISAs are provided elsewhere in this report (19). In brief, serial dilutions of human sera were screened against all eight strains of Lyme borreliosis spirochetes to determine antibody titers. Each antigen was prepared the same way from cultured spirochetes and was standardized for protein content (3 to 5 µg of protein per ml) by using a commercially available assay (Bio-Rad Laboratories, Richmond, Calif.). The following affinity-puriﬁed, peroxidase-conjugated antibodies were used: goat anti-human IgM (µ-chain speciﬁc; Kirkegaard & Perry Laboratories, Inc., Gaithersburg, Md.) or goat anti-human IgG (γ-chain speciﬁc; Kirkegaard & Perry). Analyses included 11 normal serum samples tested previously (19) plus appropriate controls for antigen, conjugates, buffer solutions, and diluents. Tests on each plate were performed with the same lot of each antigen and were conducted to assess the variability of results.

**Statistical analyses.** Geometric mean antibody titers were calculated for each series of serologic test results. The Wilcoxon signed rank test (25), a nonparametric statistical procedure, was used to determine whether the selected strains of Lyme borreliosis spirochetes used as antigen in assays resulted in significantly higher or lower antibody titers than those obtained with strain 2591. We based our test on the statistic $T$, the absolute value of the smaller of the two sums of ranks, and defined $n$ as the number of nonzero difference values. Statistical analyses for paired samples were conducted at the level of significance of $P > 0.01$.

**RESULTS**

The majority of serum specimens containing antibodies to strain 2591 of *B. burgdorferi* also were positive when tested against whole cells of other related strains of this spirochete in class-specific ELISAs. Assay sensitivities in IgM antibody analyses varied from 83.3 to 100% (Table 1). An ELISA containing the B31 strain was the least sensitive, while assays with strains 2591, IPF, and 33203 were the most sensitive. Similarly, comparable assay sensitivities were recorded when human sera were screened against the same series of whole-cell antigens in IgG antibody analyses. Seropositivity exceeded 91% for all strains except strain 33203 (which was 82.6% seropositive). In tests for specificity, the syphilitic sera reacted to each strain of Lyme borreliosis spirochete. All seven serum specimens were positive to strains 2591, B31, 25015, and NCH-1. Six of seven serum specimens contained cross-reactive antibodies to the remaining antigen strains. The syphilitic serum tested positive to strains 231, 33203, IPF, and IP90 had a low antibody titer to *T. pallidum* (1:64) in a fluorescent treponemal antibody absorption test (20).

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<tr>
<th>Serum samples tested for( \text{IgM antibodies} )</th>
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<tr>
<td>B. burgdorferi strain</td>
<td>Total no. tested</td>
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<tr>
<td>2591</td>
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<td>B31</td>
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* There were two study groups with different serum specimens for analyses of IgM or IgG antibodies.

TABLE 1. Reactivity of human sera to various strains of whole-cell *B. burgdorferi* sensu lato in an ELISA for IgM and IgG antibodies and comparisons of assay sensitivities.
strains 33203 and 231, respectively (Table 2). In general, the geometric mean IgG antibody titers were at least twofold greater than those calculated with the same standardized antigens in tests for IgM antibodies, regardless of the strain used. The mean values for an ELISA with antigen strains B31 and IP90 were about 10-fold higher than those calculated in parallel tests for IgM antibodies. Despite the higher geometric mean values for IgG titration end points, statistical analyses revealed no significant differences when antibody titers for three strains of antigens (B31, IPF, and IP90) were compared with those recorded for strain 2591. In other analyses of ranks for paired data, antibody titers for strain 2591 were significantly greater than those computed for strains 25015 (T = 1, n = 13) and 33203 (T = 0, n = 11). Similar to the results of the IgM analyses, titration end points for sera analyzed against strains 231 (T = 17.5, n = 21) and NCH-1 (T = 6, n = 13) were significantly greater than those computed in tests with strain 2591.

Serologic analyses were conducted with eight strains of spirochetes to determine antibody titers for paired serum specimens from 15 persons who had erythema migrans. In IgM analyses of the first and last serum specimens obtained from nine patients, representing a time interval of 14 to 90 days, there were twofold or no changes in titration end points (Table 3) in 57 (79.2%) of the 72 total trials (nine paired serum samples in tests with eight antigens). Fourfold (n = 14 trials) and eightfold differences (n = 1 trial) were noted for the remainder. Similar results were recorded when paired sera from six other persons, who had erythema migrans, were screened against the eight antigen strains in IgG antibody analyses. Forty-three (89.6%) of 48 trials had twofold or no changes in antibody titers, while fourfold (n = 4) or eightfold changes (n = 1) in titers were noted for the remaining trials.

Tests on reproducibility of titers were conducted to determine the variability of assay results (Table 4). In IgM analyses, there were no changes or twofold differences in antibody titers for 55 (85.9%) of 64 trials. Fourfold or eightfold differences were noted in nine trials. Assay results for antigen strains 2591, B31, and 25015 were the least variable. In IgG analyses of nine serum samples, 63 (87.5%) of 72 trials had no changes or twofold differences in titration end points. A fourfold (n = 9) variation in antibody titers was noted for the remaining trials. As in IgM analyses, results for an ELISA with antigen strain 2591 were the least variable.

**DISCUSSION**

All eight strains of whole-cell *B. burgdorferi* sensu lato performed equally well when used as coating antigens in an ELISA. Comparable assay sensitivities and specificity test results indicate that key immunodominant antigens are shared among these closely related strains and treponemes. These findings parallel those reported earlier (4). Flagellar components of *Borrelia* and *Treponema* spirochetes, in particular, have multiple shared epitopes (10, 13, 18, 21). The minor differences in assay sensitivity noted in both class-specific ELISAs are probably due, in part, to normal test variability.
Tests on reproducibility of titration end points with the same and different lots of antigens revealed no changes or twofold differences in results for IgM and IgG antibodies. Twofold changes are acceptable in antibody tests, but greater variability can occasionally occur with certain sera and must be considered when interpreting test results. The observed cross-reactivity with treponemes is consistent with previously published results (20, 31) but, in practice, should not confuse laboratory diagnosis because syphilitic cases can be clinically or serologically separated from Lyme borreliosis infections.

In parallel tests with strain 2591 being used as the reference antigen, antibody titers were consistently higher when other strains, such as 231 and NCH-1, were used as antigens. Elevated concentrations of antibodies to these strains were probably not entirely due to normal test variability. Furthermore, antigens and other reagents were standardized to minimize variability. Antigens of strains 231 and NCH-1 were prepared from low-passage isolates of *B. burgdorferi* and, consequently, might be more reactive than those prepared from strain 2591. Aside from subculturing differences, there may be important antigens associated with some strains that are lacking in others. Antigenic variability has been reported for *B. burgdorferi* sensu lato; striking differences in protein profiles of some strains of this bacterium have been observed in polyacrylamide gel electrophoresis or DNA analyses (4–6, 14, 34). Moreover, lipooligosaccharides from *B. burgdorferi* have been shown to be seroreactive (33). It seems appropriate, therefore, to consider nonprotein antigenic components, to identify complete antigen compositions, and to determine whether immunologic recognition of lipooligosaccharides, in particular, is as prevalent as antibody responses to proteins are. If antigen compositions vary considerably among strains of *B. burgdorferi* sensu lato over a broad geographical region, then the selection of a particular strain for antibody analyses should receive special consideration.

The analyses of multiple serum specimens from Lyme borreliosis patients revealed little or no change in antibody titers, regardless of the strain of *B. burgdorferi* used in the ELISA. During the early weeks of infection, IgM antibody is directed primarily to flagellin (10, 16, 18). Concentrations of IgM antibodies rise slowly and are usually detectable at about 4 to 6 weeks after the onset of illness (32). In the absence of antibiotic therapy, IgG antibodies are usually present and detectable at times when concentrations of IgM antibodies have peaked or have begun to decline. This normally occurs at about 6 weeks after the onset of illness. Subsequently, concentrations of IgG antibodies continue to rise and may be relatively high (titters, ≥1:10,240). For diagnostic purposes, there appears to be little or no advantage in serial testing if the first serum specimen was found to have antibodies to *B. burgdorferi*. If initial test results are negative, however, further testing of sequential serum specimens is warranted. Documentation of seroconversion (i.e., changes from negative to positive status) is supportive evidence of Lyme borreliosis, particularly if paired serum samples are tested together on the same day and on the same immunoplate.

Whether testing single or paired serum samples, the use of local strains of *B. burgdorferi* sensu lato in ELISAs did not appear to be required for optimal assay sensitivity. However, local strains are preferred because of availability. Many epitopes of antigens are immunologically recognized in humans when Lyme borreliosis progresses (10, 34). This expanding and broad host immune response to several polypeptides and possibly to lipooligosaccharides shared among strains increases the chances of serologically confirming *B. burgdorferi* infections. In terms of overall sensitivity or specificity, our selection of a particular whole-cell strain of Lyme borreliosis spirochete for analyses did not seem to be a critical factor. This agrees with results of earlier studies (8, 17), which included different sets of antigens and human sera. Our studies included strains of more genetically variable *B. burgdorferi* sensu lato obtained from widely separated locales. In other work, whole cells and a recombinant protein (p41G) of *B. burgdorferi* were compared in an ELISA. The use of the latter resulted in greater specificity without loss of sensitivity (13, 21). There was also minor improvement in assay sensitivity for sera collected during the early weeks of infection, a time when conventional antibody assays with whole-cell antigens usually fail to detect antibodies to *B. burgdorferi*. With variable host immune responses to multiple antigens, the use of a mixture of highly specific and purified subunit antigens, such as recombinant proteins of flagellin and outer surface proteins A, B, and perhaps p39, therefore, seems to be an appropriate alternative for a more sensitive and specific ELISA. To find the most suitable whole-cell strain or best mixture of specific antigens, particularly in view of recent findings (33), additional work is needed to identify further lipooligosaccharide as well as protein components of *B. burgdorferi* sensu lato that are immunologically recognized during early and late Lyme borreliosis.

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