Occurrence of Antibodies to *Borrelia burgdorferi* in Patients with Nonspirochetal Subacute Bacterial Endocarditis

Alan T. Kaell, MD; Patricia R. Redecha, BS; Keith B. Elkon, MD; Marc G. Golightly, PhD; Paul E. Schulman, MD; Raymond J. Dattwyler, MD; Diana L. Kaell, BS; Robert D. Inman, MD; Charles L. Christian, MD; and David J. Volkman, PhD, MD

■ *Objective:* To determine the prevalence and specificity of antibodies to *Borrelia burgdorferi* in patients with nonspirochetal subacute bacterial endocarditis and assess whether increased levels of antibodies to *B. burgdorferi* were attributable to rheumatoid factor.

■ *Design:* Retrospective case-control study.

■ *Setting:* Urban referral center in an area devoid of infected ticks as a source of endocarditis sera.

■ *Patients:* Sera from 30 consecutive patients with culture-proven subacute endocarditis between 1979 and 1981 were compared with 30 control sera collected between 1989 and 1990. In addition, sera from 20 consecutive patients with rheumatoid arthritis who were positive for rheumatoid factor were collected between 1991 and 1992. Sera were compared with a convenience sample from 15 patients who met the criteria for Lyme disease.

■ *Measurements:* Antibodies to *B. burgdorferi* were assessed by enzyme-linked immunosorbent assay (ELISA) and immunoblot analysis. IgM rheumatoid factor was quantified using solid-phase radioimmunoassay or latex agglutination techniques.

■ *Results:* Thirteen of 30 patients with endocarditis (43%) compared with 3 of 30 normal controls (10%) had increased levels of antibodies to *B. burgdorferi* ($P < 0.01$). Of these 13 patients, only 1 had an immunoblot consistent with previous infection. The others had nonspecific immunoblots: 5 showed isolated 60-kd reactivity; 1 patient had isolated 41-kd reactivity; and 6 had no bands of reactivity. Immunoblots of the 3 controls with increased antibodies showed only isolated 41-kd reactivity. Thus, the specificity of the *B. burgdorferi* antibody test in patients with endocarditis was only 60% (95% CI, 42% to 78%), compared with 90% (CI, 79% to 100%) in controls. No correlation was noted between IgM rheumatoid factor and antibodies to *B. burgdorferi* in patients with endocarditis ($r = 0.2$; $P > 0.2$). Only 1 of 20 patients with rheumatoid arthritis without known bacterial infections had antibodies to *B. burgdorferi*.

■ *Conclusions:* Although a positive ELISA test for *B. burgdorferi* may be a "true positive," a positive serologic test alone does not ensure that the clinical problem is due to Lyme borreliosis. Cross-reactive antibodies to shared epitopes between *B. burgdorferi* and the endocarditis organism may account for the high false-positive results.

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From the State University of New York at Stony Brook, Stony Brook, and Cornell University Medical Center, New York, New York; and Toronto Western Hospital, Toronto, Ontario, Canada.

Lyme disease, a consideration in the differential diagnosis of patients with fever and musculoskeletal symptoms (1), is often established serologically by detecting antibodies to the causative organism, *Borrelia burgdorferi* (2–12). False-positive Lyme test results are well recognized in patients with other spirochetal infections (for example, syphilis [6]), viral diseases (for example, Epstein-Barr virus mononucleosis and parvovirus [3, 5]), and sarcoidosis (3). We previously described (1) four patients with nonspecific musculoskeletal manifestations who were initially thought to have Lyme borreliosis based on positive results of serum tests for *B. burgdorferi* but in whom test results from blood cultures confirmed nonspirochetal subacute bacterial endocarditis. These patients all resided in an area where *B. burgdorferi* infection was endemic.

To determine the frequency and specificity of antibodies to *B. burgdorferi* (as measured by ELISA) in patients with endocarditis, we studied patients who resided in an area devoid of *B. burgdorferi*-infected ticks. We also assessed whether increased levels of antibodies to *B. burgdorferi* in patients with endocarditis were attributable to rheumatoid factor often present in patients with endocarditis.

Methods

All patients had nonspirochetal, subacute bacterial endocarditis (proven by blood culture) diagnosed at Cornell Medical Center between 1979 and 1981. All resided in areas not endemic for Lyme borreliosis. No patient had a history of deer tick bite or erythema migrans rash. These patients with endocarditis were diagnosed before the advent of Lyme serologic tests, and none had their illness attributed to Lyme disease. Sera obtained from these patients during their endocarditis illness were stored at −40 °C. Anti-*B. burgdorferi* antibodies were detected by ELISA using sonicated, whole B31 strain as previously described (13). A convenience sample of frozen sera collected between 1989 and 1990 from asymptomatic persons residing in Suffolk County, New York, were used as negative controls ($n = 30$); this baseline control value was 1.8 ± 0.6 units. Sera were rated positive if they were more than 4.0 units (>3 SD above the mean of the controls). In the figures, data are reported as the ratio of the sample optical density to the negative cut-off value of 4.0 units.

Immunoblotting was done with these sera using the laboratory-adapted B31 strain of *B. burgdorferi* as previously described (13). Spirochetes were sonicated in phosphate-buffered saline containing 1 μmol/L *p*-methyl sulfinylfluoride and were then concentrated and boiled in 2.5% sodium dodecyl sulfate/2.5% β-mercaptoethanol for 3 minutes. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transfer to nitrocellulose (0.2 pore size; Hoeffer, San Francisco, California) were done according to standard methods (14). Samples for specific IgG were incubated at a 1:200 dilution of sera, and

samples for specific IgM and IgA were incubated at a 1:50 dilution. Bound antibodies were detected with a 1:5000 dilution of goat anti-human gamma-, mu-, or alpha-specific IgG conjugated to alkaline phosphatase and were developed with BICIP-Substrate System (5-bromo-4-chloro-3-indole phosphate, nitroblue tetrazolium; Kirkegaard and Perry Laboratories, Gaithersburg, Maryland). Reactions were stopped by rinsing the blots in distilled water. Immunoblots were interpreted to be diagnostic of Lyme disease according to published criteria (15) (that is, positive immunoblots have distinct 41-kd bands and at least four other defined bands).

IgM rheumatoid factor was quantified by a solid-phase radioimmunoassay using iodine 125–labeled F $(Ab)_2$ anti-μ reagents to detect IgM rheumatoid factor bound to solid-phase, adsorbed, heat-aggregated human IgG, as previously described (16). A positive result was defined as more than 2.2% binding, a value 2 SD above the mean (10 normal controls had a mean = 1.8% ± 0.2 SD). Values are reported in the figures as the ratio of the sample percentage binding to the negative control cut-off binding of 2.2%.

Rheumatoid factor was also measured in two additional groups. IgM rheumatoid factor was measured by latex agglutination (Behring) in a convenience sample of frozen sera collected between 1991 and 1992 at the Stony Brook Lyme Center from 15 patients with seropositive Lyme disease who met criteria for Lyme disease established by the Centers for Disease Control and Prevention (17). In addition, consecutive sera were collected from 20 patients with rheumatoid arthritis who were positive for rheumatoid factor and were treated in a private rheumatology office practice in Suffolk County between August 1991 and January 1992. Sera were tested for antibodies to *B. burgdorferi* by ELISA. All patients with rheumatoid arthritis fulfilled at least four of seven disease criteria of the American College of Rheumatology (18) and were positive for IgM rheumatoid factor using a latex agglutination test. None had coincident bacterial infection. All resided in Suffolk County, New York, an area endemic for Lyme disease, but none had a history of an erythema migrans rash.

Statistical Analyses

Values are presented as mean ±SE. Comparisons between dichotomous values were done using chi-square analysis with the Yates correction. For continuous measures, *t*-tests and Pearson correlations were used.

Results

Thirteen of 30 patients with subacute bacterial endocarditis (43%) had a positive ELISA result (> 4 units) for *B. burgdorferi*, whereas 3 of the 30 controls (10%) had ELISA values greater than 4.0 units ($P < 0.01$). The mean of the *B. burgdorferi* antibody-positive sera was 7.52 units in the patients with endocarditis (range, 4.2 to 19.8 units). The mean of the normal controls was 1.8 units (range, 0.1 to 10 units). The causative organisms in the 30 patients with endocarditis included a broad spectrum of streptococci in addition to five *Staphylococcus aureus* isolates and one isolate each of *Staphylococcus epidermis* and *Lactobacillus casei*. No preponderance of any one organism was noted in patients with endocarditis who were either positive or negative for *B. burgdorferi* antibody.

Immunoblot analysis of 22 of the 30 sera are shown in Figure 1. Positive Lyme sera are positioned in lanes 1 and 24 (lane 24 is serum from a previously reported patient with both endocarditis and Lyme disease [1]). The three normal control sera from patients with increased antibodies for *B. burgdorferi* (using immunoblot tests) showed only isolated 41-kd reactivity (data not shown). Of the 13 patients with endocarditis who had positive ELISA results for *B. burgdorferi* antibodies, 1 (*lane 19*) had high antibody levels (19.8 units), and the immunoblot of this serum showed multiple bands, including a strong 41-kd band consistent with, but not diagnostic of, previous exposure to *B. burgdorferi* (15). Five of the remaining 12 patients (42%) had 60- to 67-kd reactivity (Figure 1; *lanes 7, 18, 19, 22, and 23*). An additional dark 41-kd reactivity line, seen in almost all seropositive Lyme patients, was not seen. The weak anti–41-kd reactivity seen in many of the sera from patients with endocarditis is indistinguishable from the faint anti–41-kd bands seen in sera from normal patients (13). Seven of 12 patients had positive ELISA results (mean, 7.86 units), yet 3 (43%) of these had no strong bands on immunoblots. These three immunoblots, despite positive ELISA results, were indistinguishable from normal negative controls. Overall, 12 of 30 (40%) patients with endocarditis who had increased antibodies to *B. burgdorferi*, as measured by ELISA, were falsely positive. This represents a specificity of only 60% (95% CI, 42% to 78%). Of our 30 patients with endocarditis, sufficient sera were available from 13 patients to measure IgM rheumatoid factor. Eleven of the 13 patients (85%) tested positive for quantitative IgM rheumatoid factor, but the titers varied widely. Figure 2 shows the comparison between normalized levels of IgM rheumatoid factor in the endocarditis groups positive ($n = 5$) and negative ($n = 8$) for *B. burgdorferi* antibody. Although the mean levels of IgM rheumatoid factor were slightly higher in the group seropositive for antibody to

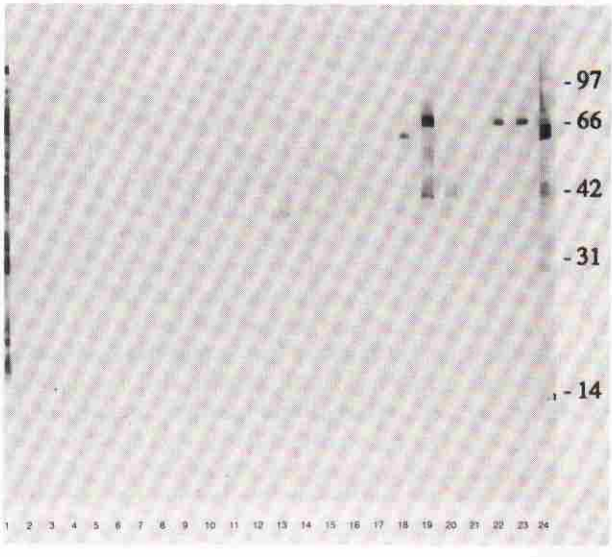

Figure 1. Immunoblot analysis of sera from 23 of 30 patients with nonspirochetal bacterial endocarditis who reside in nonendemic areas for Lyme disease. Sera was blotted against solubilized Borrelia antigen (*B. burgdorferi* strain 31) and was probed with anti-human IgG, gamma-chain–specific goat antibody conjugated to alkaline phosphatase. A positive control serum sample in lane 1 shows an expanded humoral response in a patient with newly diagnosed chronic Lyme disease. Thirteen of the sera from patients with carditis yielded positive ELISA results for *B. burgdorferi* (lanes 3, 4, 6, 7, 8, 12, 13, 16, 17, 18, 19, 22, and 23). Immunoblots from the remaining 17 patients with endocarditis, who had nonreactive ELISA results for *B. burgdorferi,* were indistinguishable from negative control results (data shown for nine patients).

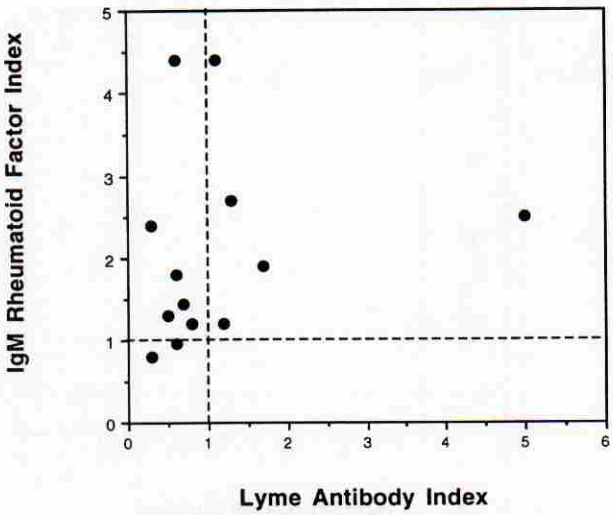

Figure 2. Relation between IgM rheumatoid factor and *B. burgdorferi* antibody levels in 13 patients with endocarditis. The values are shown for quantitative IgM rheumatoid factor and anti-borrelia ELISA indices that represent the ratio of the sample values to the negative cut-off value. No significant correlation was noted between IgM rheumatoid factor and *B. burgdorferi* antibody levels (Lyme antibody index) using linear regression analysis (R = 0.2; $P > 0.2$).

B. burgdorferi ($n = 5$; mean, 5.6 ± 1.15 units) than in the seronegative group ($n = 8$; mean, 3.96 ± 0.90 units), the differences were not statistically significant ($P > 0.2$). No correlation was found between the IgM rheumatoid factor level and the *B. burgdorferi* antibody level for both groups combined (R = 0.2; $P > 0.2$).

Both IgM rheumatoid factor and *B. burgdorferi* antibodies were also measured in two control groups (Figure 3). Of 15 patients who met criteria for Lyme disease established by the Centers for Disease Control and Prevention and had antibodies to *B. burgdorferi,* none had a positive rheumatoid factor (Figure 3, *left*). Conversely, in 20 patients with rheumatoid arthritis who were positive for rheumatoid factor, only one had increased levels of *B. burgdorferi* antibodies using ELISA (Figure 3, *right*). An immunoblot of this patient's sera showed reactivity to the 31-, 41-, 56-, 58-, and 66-kd bands of *B. burgdorferi* consistent with previous infection (data not shown).

Discussion

The diagnosis of noncutaneous Lyme borreliosis is based on the serologic confirmation of previous exposure to *B. burgdorferi* plus clinical manifestations suggestive of Lyme borreliosis (1, 2, 19). Such manifestations are defined by the Centers for Disease Control and Prevention for the purpose of detecting patients with Lyme borreliosis (National Surveillance of Lyme borreliosis) (17). Lyme borreliosis shares with other illnesses nonspecific symptoms such as fatigue, malaise, arthralgia, and myalgia (19–22). In such patients who do not meet strict criteria for Lyme borreliosis, the use of serologic assays for antibodies to *B. burgdorferi* has been advocated (23). However, our data suggest that positive *B. burgdorferi* serologic test results must be interpreted with caution.

In our study, 43% of patients (CI, 25% to 61%) with nonspirochetal endocarditis produced antibodies reactive to *B. burgdorferi* that were detectable by ELISA using whole-sonicated *B. burgdorferi*. Positive serologic test results for *B. burgdorferi* appeared to be related to antibodies that were cross-reactive to shared epitopes between *B. burgdorferi* and the endocarditis organisms. Importantly, none of our 30 documented patients with endocarditis satisfied the immunoblot criteria for definitive *B. burgdorferi* exposure (15). Only one patient with endocarditis had an immunoblot compatible but not diagnostic of *B. burgdorferi* exposure.

The spectrum of antibodies reactive against *B. burgdorferi* as detected on immunoblot is diverse and includes antibodies to the 60-kd protein. Fifty-eight percent (8 of 13) of our patients with endocarditis who were positive for *B. burgdorferi* had antibodies reactive to the 60-kd protein on immunoblot. The 60-kd groEL antigen of *B. burgdorferi* is homologous to the 65-kd heat-shock protein of mycobacteria (24). Epitopes within this antigen are widely shared by many bacteria and eukaryotic cells (25). Therefore, antibodies to this antigen are nonspecific and do not confirm previous exposure to *B. burgdorferi*. Bacteria-specific regions of the *B. burgdorferi* 60-kd heat-shock protein have recently been reported and may form the basis of a more specific *B. burgdorferi* assay (26).

Only 1 of 13 patients with endocarditis who were seropositive for *B. burgdorferi* had antibodies strongly reactive to the 41-kd protein. This serum also had reactivity to other Borrelia antigens, perhaps suggesting true exposure to *B. burgdorferi* (19, 27). Epitopes within the 41-kd endoflagellar protein are conserved among other spirochetes including commensal mouth treponemes (28). Low-level reactivity to this protein is frequently seen in sera of normal, non-Lyme persons (13). This reactivity may represent polyclonal B-cell activation or anamnestic response or both to either the endoflagellar protein of *B. burgdorferi*, or related intraoral spirochetes (28).

Three of seven patients with positive ELISA test results to *B. burgdorferi* had negative results using immunoblot analysis (that is, they had patterns indistinguishable from normal controls). This phenomenon may be due to antibodies reactive to capsular lipopolysaccharides or peptidoglycans that are not detected using immunoblot analysis. Such reactivity has been shown for antibodies to streptococcal organisms (29). Attempts to detect lipopolysaccharides in *B. burgdorferi* have been unsuccessful (30). However, peptidoglycans are present in *B. burgdorferi* (31) and may account for positive ELISA and negative immunoblot test results.

Increased rheumatoid factor is often present in patients with endocarditis (32). Rheumatoid factor has been associated with false-positive serologic test results for *B. burgdorferi* measured by immunofluorescent assay (33, 34). However, one study of patients with rheumatoid arthritis did not find an association between rheumatoid factor and antibodies to *B. burgdorferi* (35). Similarly, we found no correlation between IgM rheumatoid factor levels and antibodies to *B. burgdorferi* in

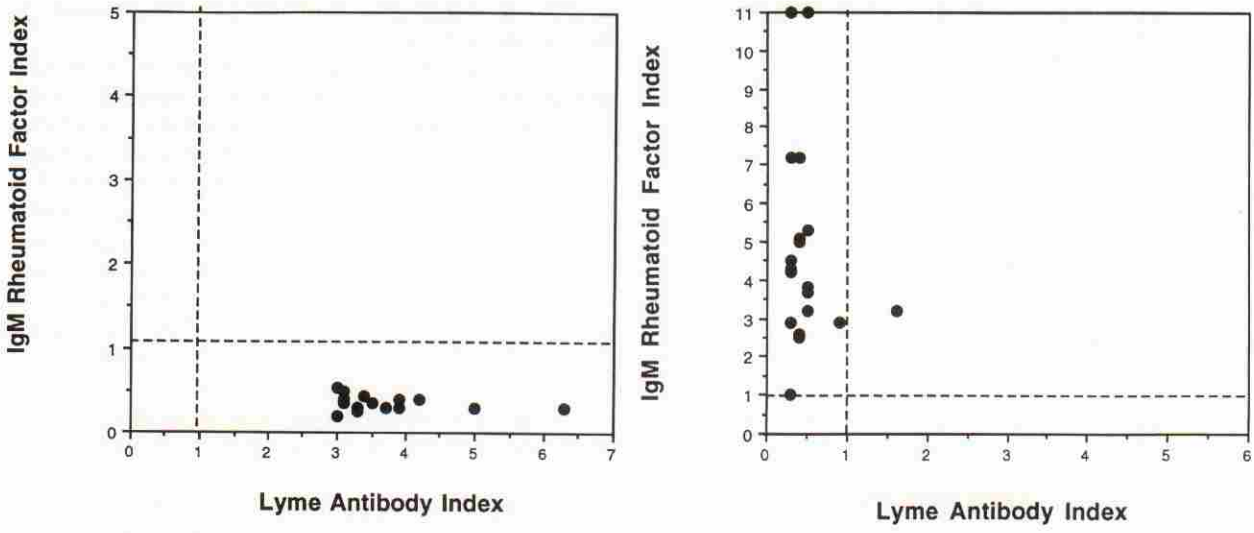

Figure 3. Relation between IgM rheumatoid factor and *B. burgdorferi* antibody levels. Left. In 15 patients from Suffolk County with seropositive Lyme disease. **Right.** In 20 patients with documented rheumatoid arthritis. Rheumatoid factor and Borrelia antibody levels are shown as in Figure 2.

that only 1 of our 20 patients with rheumatoid arthritis who were positive for rheumatoid factor also had a positive ELISA test result for *B. burgdorferi* (Figure 3, *right*). This patient had an immunoblot compatible with, but not diagnostic of, previous *B. burgdorferi* infection (26). Thus, a positive test result for IgM rheumatoid factor does not appear to yield a positive ELISA result for *B. burgdorferi* in the absence of true infection. Conversely, our patients with Lyme disease and high-titer antibodies to *B. burgdorferi* tested negative for rheumatoid factor (Figure 3, *left*). Although transient positive test results for rheumatoid factor have been reported in a small number of patients with Lyme arthritis (36, 37), our data suggest that this positive result does not persist. This is consistent with the absence of rheumatoid factor reported in 80 patients with frank Lyme arthritis (38).

The rate of false-positive test results for *B. burgdorferi* antibodies found in our patients with endocarditis highlights the hazards associated with assuming that the specificity of a test established in a healthy control population is applicable to persons with clinical illness that can be confused with Lyme disease (1). Although a patient with endocarditis who has a positive ELISA test result for *B. burgdorferi* may be a "true" positive if immunoblot analysis also confirms previous exposure to *B. burgdorferi*, a positive serologic result alone does not ensure that the current clinical problem is due to Lyme borreliosis (1, 39). The advent of immunoblot analysis (25), standardized ELISA procedures using selected pathogen-specific antigens of *B. burgdorferi* (40–42), and the application of polymerase chain reaction technology to detect *B. burgdorferi* DNA (43) do not circumvent the difficulty in interpreting the clinical significance of positive test results.

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