Frequency and Specificity of Antibodies that Crossreact with *Borrelia burgdorferi* Antigens

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Abstract. **The frequency and specificity of antibodies that bind antigens of *Borrelia burgdorferi* in sera from 200 individuals with no evidence of past or current Lyme disease was determined. Sera were tested for both IgG and IgM antibodies to *B. burgdorferi* by Western blotting. The non-Lyme serum group included specimens from healthy adults and children in addition to specimens from patients with viral infection and rheumatic diseases. Crossreactive IgG antibodies occurred more frequently than IgM antibodies. The most frequently bound antigens corresponded to 41 kDa and 60 kDa Borrelial components. Of 200 specimens tested, 100 had antibodies that bound at least 1 antigen. Binding to multiple antigens occurred at much lower frequency. Our results indicate that determination of maximum crossreactivity of non-Lyme sera can be used to establish minimum criteria for determining a positive Western blot result for Lyme disease. (*J Rheumatol 1992;***19:582–7)**

Key Indexing Terms:
LYME DISEASE WESTERN BLOT CROSSREACTIVITY

Serologic tests are frequently used to aid in diagnosing Lyme disease[1,2]. They are particularly useful to identify patients in later stages of Lyme disease when clinical manifestations may resemble those of other diseases. The most widely used serologies are based on indirect immunofluorescence and enzyme linked immunosorbent assay (ELISA); however, Western blotting is increasingly used to confirm results of other assays[3-5]. Selection of Western blotting as a confirmatory test for Lyme disease is based on its sensitivity and ability to detect antibodies to multiple separate antigens of *Borrelia burgdorferi*. Like indirect immunofluorescence and ELISA, Western blotting analysis can yield indeterminate results. In uninfected individuals such results are presumably caused by the presence of antibodies that can crossreact with epitopes on antigens of *B. burgdorferi*. Studies have reported the detection of these nonspecific antibodies in sera from patients with other infectious and autoimmune diseases[6-9]. The frequency with which such nonspecific antibodies occur and the identity (blot location) of the major antigens to which they bind has not been adequately described[10].

Our goal was to determine frequency and antigen specificity of antibodies to *B. burgdorferi* in a population with diverse clinical histories, but no Lyme disease. We wished to establish minimum criteria for positivity (reactivity greater than the maximum observed in non-Lyme sera). Additionally, our results indicate which antigens are subject to frequent crossreactivity and thus may not be suitable for making specific purified antigen based tests.

For this study, sera from 200 individuals with no clinical evidence of Lyme disease were tested by ELISA for IgG and by Western blotting for IgG and IgM antibodies to antigens of *B. burgdorferi*. Our study population consisted of adult volunteers, presurgical pediatric orthopedic patients, patients with human immunodeficiency virus (HIV), patients with antibody positive high titer Epstein Barr virus (EBV), patients with juvenile rheumatoid arthritis (JRA), patients with systemic lupus erythematosus (SLE), and patients with other rheumatologic complaints. Before inclusion in this study, they were examined and their clinical histories evaluated by an adult rheumatologist, a pediatric rheumatologist, or an infectious disease specialist with expertise in Lyme disease.

Our findings demonstrate that specificity of Western blotting exceeds that of ELISA when stringent criteria are used regarding the number of bands required for defining a positive result.

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MATERIALS AND METHODS

Serum selection criteria. Specimens from 20 individuals with clinically diagnosed Lyme disease and 200 individuals with no clinical evidence of Lyme disease were used. Non-Lyme sera were obtained from individuals with no history of infection with *B. burgdorferi*. Medical records of individuals in the non-Lyme group were reviewed by a rheumatologist or infectious disease specialist participating in a Lyme disease specialty clinic. Six-month followup of this group gave no indication of Lyme disease after our study. Specimens were assigned to non-Lyme subgroups as follows: (1) adult volunteers (n=43); (2) presurgical pediatric orthopedic patients (n=43); (3) pediatric rheumatology patients (n=56); (4) patients with HIV (n=21); (5)

patients with antibody positive EBV (n=18): (6) patients with JRA (n=14): and (7) patients with SLE (n=5). Serum specimens were aliquoted and stored at −70°C until needed for testing. Lyme disease positive control sera were classified according to disease stage as follows: Stage 1 sera (n=5) were obtained from patients with history of erythema migrans of ≤ 10 weeks duration; Stage 2 sera (n=5) were from patients having history of tick bite and/or erythema migrans who subsequently developed neurologic symptoms. Samples were obtained within 19 weeks following erythema migrans or suspected tick bite; Stage 3 sera (n=10) were obtained from patients with Lyme arthritis of at least 2 months duration. Only 2 of 10 Stage 3 patients recalled having an erythema migrans like rash and 5 recalled a tick attachment.

Western blot analysis. B. Burgdorferi (ATCC strain B31) was grown to late log phase in BSK media. The whole spirochetes were harvested by centrifugation (10,000 g) for 30 min at 4°C. The spirochetes were resuspended in phosphate buffered saline (pH 7.4) and the protein concentration determined by the Bradford method using a Bio-Rad protein assay kit (Richmond, CA). Electrophoresis was performed using a modification of the Laemmli method[11]. Spirochetes were diluted to a concentration of 1.1 mg/ml in sample buffer, containing dithiothreitol (0.1 M) as the reducing agent. The proteins were separated in a 10% polyacrylamide gel using an LKB multiphore-II electrophoresis unit. Separated antigens were transferred to nitrocellulose membrane (Schleicher & Schuell, Keene, NH) using a semi-dry Nova blot transfer system (LKB). Blotted membranes were blocked with 0.5% bovine serum albumin (BSA) in phosphate buffered saline solution (PBS) (pH 7.4) overnight at 4°C, cut into strips, dried and stored dessicated until needed. Sera was tested for IgG and IgM antibodies to *B. burgdorferi* by incubating strips with a 1:100 dilution of test serum in PBS (pH 7.4) with 5% newborn calf serum and 1% nonfat dry milk for 1 h at room temperature. Test strips were washed 3 times with PBS, then incubated with a 1:1000 dilution of biotinylated goat antihuman antisera (Kirkegaard & Perry Labs, Gaithersburg, MD) for 1 h at room temperature. Strips were washed as above, then incubated with a 1:1000 dilution of peroxidase conjugated streptavidin (KPL) for 1 h at room temperature. Strips were washed again and incubated for 15 min at room temperature with 4-chloro-1-napthol substrate (7.8 mM 4-chloro-1-napthol diluted 1:2 with 1:1500 30% H_2O_2 in citrate phosphate buffer, pH 4.0). Strips were washed with distilled water and air dried. Positive and negative control samples were tested simultaneously with patients' samples.

ELISA. The ELISA was performed as described[12]. Ninety-six well microtiter plates (Immulon-1, Dynatech, VA) were incubated with 5 μg/ml sonicate of *B. burgdorferi* (ATCC strain B31) for 2 h at 37°C. The wells were then fixed with 95% methanol for 10 min. Wells were blocked with 300 μl of 2% BSA in PBS (Sigma) for 1 h at 37°C, then dried and stored dessicated at −20°C.

Microtiter wells were rehydrated by incubation with 0.5% BSA in PBS (pH 7.4) for 15 min at room temperature before use. Patient samples, 100 μl diluted 1:80 with *E. coli* sonicate (0.7 mg/ml) in 0.5% BSA, were added to the wells and incubated for 1 h at 37°C. Wells were washed with PBS, then incubated with 100 μl of 1:1000 dilution of peroxidase conjugated goat antihuman IgG antisera (Cappel, West Chester, PA) for 30 min at 37°C. Wells were washed in PBS, then incubated with 100 μl 2,2′ Azinobis (3-ethylbenzthiazoline sulfonic acid) (Sigma) for 10 min at room temperature. The reaction was stopped with 50 μl of 250 mM oxalic acid. The optical density (OD) of each well was determined at 405 nm using a Titertek multiscan (Flow Laboratories).

RESULTS

Lyme disease and non-Lyme sera were tested for IgG antibodies to a sonicated preparation of *B. burgdorferi* antigens using ELISA before being assessed by Western blotting. Results of ELISA indicated that 19 of the 20 Lyme disease sera were considered positive (OD > 0.2 at 1:80 dilution) based on previously determined criteria. The specimen that was not positive by ELISA was indeterminate by IgG, but positive by IgM Western blotting, and was obtained from an individual with an erythema migrans of less than 6 weeks duration. Testing of non-Lyme sera by ELISA resulted in 4 positives if the ELISA was performed using a sample diluent containing *E. coli* antigens, which has been shown to reduce false positives[12], while 24 of the experimental sera were considered positive if samples were diluted in standard diluent PBS containing 2% BSA (data not shown).

Results of testing Lyme sera by Western blotting for IgG and IgM antibodies to *B. burgdorferi* showed that 19 of the 20 Lyme disease sera had IgG antibodies to 4 or more antigens, while only 4 sera had IgM antibodies to a similar number of antigens. The 4 Lyme sera that yielded 4 or more bands on IgM blots included 2 from patients with Stage 1 disease and 1 each classified as Stage 2 and Stage 3 Lyme disease. The specimen that had less than 4 bands by IgG Western blotting showed IgG binding to 2 blotted antigens, and was obtained from a patient classified as Stage 1 Lyme disease. That specimen showed 4 bands by IgM and Western blotting; both IgG and IgM blots had bands corresponding to 41 kDa and 60 kDa antigens. All Lyme sera tested had IgG antibodies to both a 41 kDa and a 60–69 kDa component of *B. burgdorferi*. Selected Western blotting strips from the Lyme disease serum group for IgG and IgM antibodies are shown in Figures 1a and 1b, respectively.

Data in Table 1 indicate the reactivity (average number of bands) determined for each Lyme disease stage when tested for IgG and IgM antibodies to *B. burgdorferi* by Western blotting. As expected, results indicate that the IgG antibody response increases with increased disease duration, while the IgM antibody response declines.

Selected Western blots from non-Lyme groups for IgG and IgM antibodies to *B. burgdorferi* are shown in Figures 2a and 2b. Analysis of these blots shows that reactivity of non-Lyme sera is, in general, both quantitatively and qualitatively less than that observed for Lyme disease sera.

A tabulation of IgG antibody reactivity observed for Lyme and non-Lyme groups is shown in Table 2. Results show that non-Lyme sera may have antibodies that bind to blotted Borrelial antigens in all ranges examined (0–100 kDa). The most frequently detected reactivity occurred between 41 and 60 kDa. Of 59 bands detected from experimental sera in the 41–50 kDa range, 57 corresponded to the 41 kDa flagellar antigen location, and all 20 bands in the 51–60 kDa range corresponded to a 60 kDa antigen location. Other bands on Western blots of non-Lyme sera were at locations corresponding to the following approximate kDa values: 14,18,22,24,28,30,32,34,38,45,61,67,69,70,72,73,80,85 and 94. With the exception of sera from patients with HIV and EBV, the majority of reactivity (about 75%) for each non-Lyme group occurred at locations corresponding to 41 and 60 kDa antigens. HIV and EBV sera showed predominant reactivity in the 61–70 kDa and 0–30 kDa range, respec-

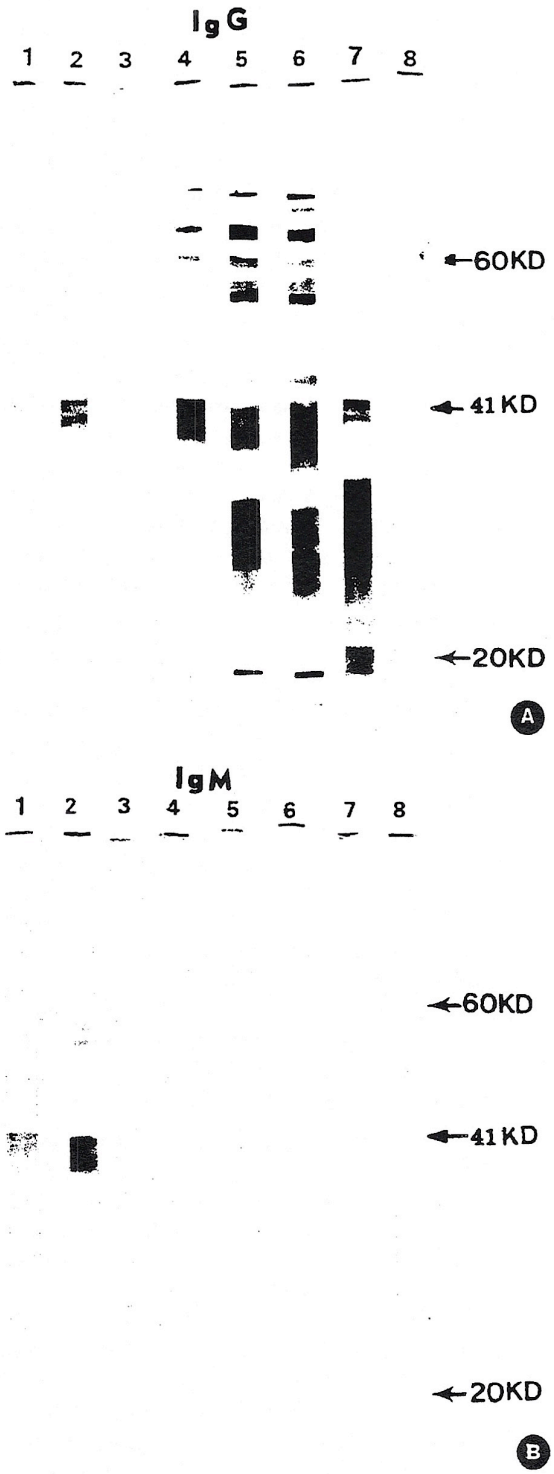

Fig. 1. Western blot results when serum from patients with clinically diagnosed Lyme disease were tested for (A) IgG, and (B) IgM antibodies to *B. burgdorferi*. Strips 1–7 in A show seroreactivity of individual patients of different disease stages, sequenced as follows: Stage 1, strips 1 and 2; Stage 2, strips 3 and 4; Stage 3, strips 5,6,7; strip 8 is a negative control. Strips shown in B follow the same sequence and are from the same patients.

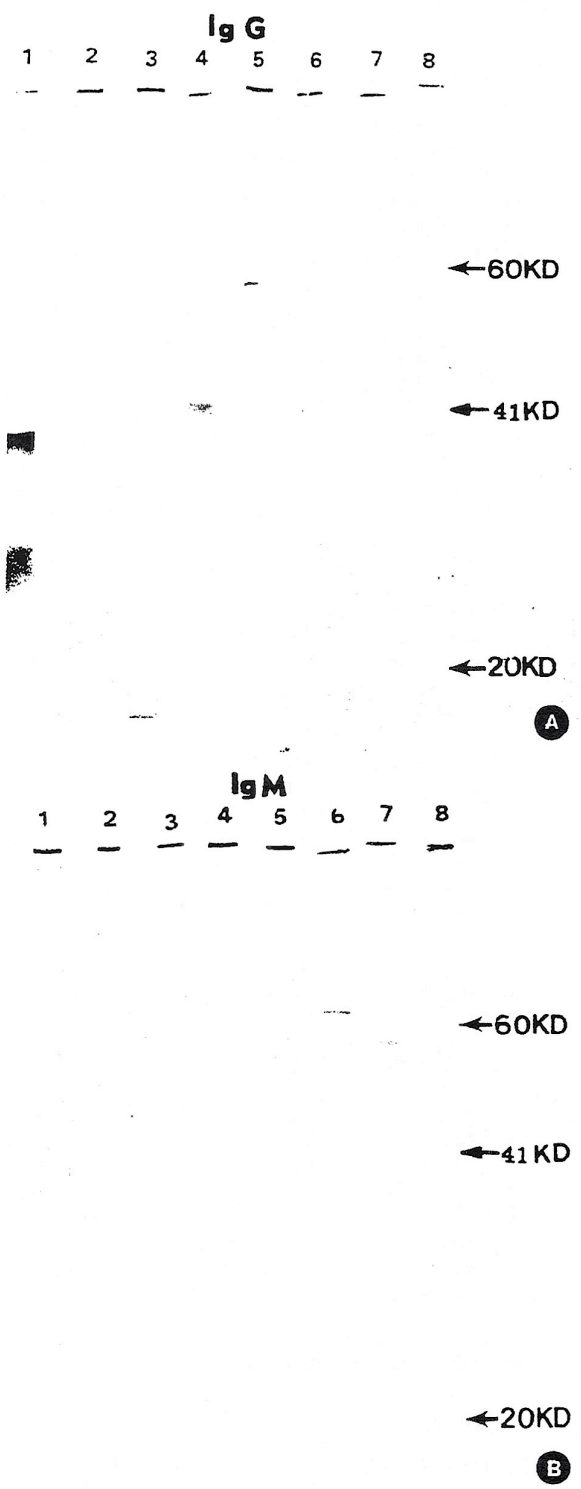

Fig. 2. Selected Western blotting results from the non-Lyme group for (A) IgG, and (B) IgM antibodies to *B. burgdorferi*. The same sample was run for IgG and IgM antibodies. Samples tested were: strip 1, EBV; strip 2, HIV; strip 3, MR; strip 4, JRA; strips 5 and 7, adult volunteers, strip 6, presurgical pediatric orthopedic patients; strip 8 is a negative control.

Table 1. *Western blot results for patients with clinically diagnosed Lyme disease*

	No. of Samples	IgG Average No. of Bands (Range)	IgM Average No. of Bands (Range)
Stage I	5	5.6 (2–10)*	3.2 (0–6)
Stage II	5	5.8 (4–9)	1.4 (0–4)
Stage III	10	12.3 (6–16)	1.6 (0.4)

Serum specimens from patients with Lyme disease were classed by disease stage based on clinical history.

* One sample was negative by ELISA and showed only 2 IgG bands, but showed 4 bands by IgM Western blotting results.

tively. Pediatric patient reactivity (SLE, JRA, presurgical patients and patients with other rheumatological complaints) was predominantly against a 41 kDa antigen, while adult volunteer sera were most reactive to a 60 kDa antigen. The reactivity of Lyme positive control sera changed with increasing disease duration or stage. Reactivity of IgG antibodies to Borrelial antigens between 0 and 70 kDa was relatively uniform when sera from patients with Stage 1 and Stage 2 Lyme disease were evaluated. In contrast, sera from patients with Stage 3 Lyme disease demonstrated increased binding to Borrelial antigens in the 0–40 kDa range.

Data in Table 3 show that the presence of IgM antibodies to Western blotting of *B. burgdorferi* from non-Lyme and Lyme disease sera is less than that observed for IgG antibodies. The Lyme disease group had a total reactivity count of 179 bands for IgG antibodies, compared to 38 bands for IgM antibodies, while non-Lyme sera yielded 116 IgG and 19 IgM bands. These results reflect a decrease in reactivity for IgM versus IgG Western blots of approximately 79% for Lyme disease and 84% for experimental sera.

The percentage of non-Lyme sera showing any reactivity for IgG antibodies by Western blotting was 47%, while only

Table 2. *Location (kDa) of antigens to which IgG antibodies react on Western blots of B. burgdorferi*

Group	No. Tested	No. of Bands Located by kDa 0–30	31–40	41–50	51–60	61–70	71 or >
EBV	18	7	2	4	1	0	0
HIV	21	1	0	0	0	3	0
SLE	5	0	0	2	0	0	1
JRA	14	0	0	8	3	0	0
AV	43	2	0	3	12	0	4
PS	43	2	0	20	3	4	3
MR	56	3	1	22	1	3	1
Total	200	15	3	59	20	10	9
Lyme I	5	6	4	5	6	5	2
Lyme II	5	4	7	5	6	6	1
Lyme III	10	47	23	13	16	16	7
Total Lyme	20	57	23	23	28	27	10

Bands were listed by kDa range for ease of presentation. Where appropriate finite kDa assignments are provided in the text. AV = adult volunteer, PS = presurgical sera from pediatric orthopedic patients, MR = sera from patients with other rheumatic diseases or minor rheumatic complaints.

Table 3. *Location (kDa) of antigens to which IgM antibodies react on Western blots of B. burgdorferi*

Group	No. Tested	No. of Bands Located by kDa 0–30	31–40	41–50	51–60	61–70	71 or >
EBV	18	1	0	0	0	0	0
HIV	21	0	1	0	0	0	0
SLE	5	0	0	0	0	0	0
JRA	14	0	0	0	0	0	0
AV	43	0	0	0	2	0	4
PS	43	2	0	3	1	3	1
MR	56	0	0	0	0	1	0
Total	200	3	1	3	3	4	5
Lyme I	5	0	3	4	3	6	0
Lyme II	5	1	1	2	2	1	0
Lyme III	10	4	3	5	0	2	1
Total Lyme	20	5	7	11	5	9	1

Table 4. *Comparison of seroreactivity by Western blotting*

Group	No.	No. of Bands — IgG						No. of Bands — IgM					
		1	2	3	4	>4	% with at Least 1 Band	1	2	3	4	>4	% With at Least 1 Band
EBV	18	6	2	0	1	0	50	1	0	0	0	0	6
HIV	21	4	0	0	0	0	19	1	0	0	0	0	5
JRA	14	5	3	0	0	0	57	0	0	0	0	0	0
SLE	5	1	1	0	0	0	40	0	0	0	0	0	0
AV	43	17	2	0	0	0	44	3	0	1	0	0	9
PS	43	17	6	1	0	0	56	4	3	0	0	0	16
MR	56	27	2	0	0	0	52	1	0	0	0	0	<1
Total	200	77	16	1	1	0	47	10	3	1	0	0	7
Lyme disease controls	20	0	1	0	2	17	100	2	3	4	3	1	65

HIV sera was obtained from patients with advanced disease, which may account for the relatively low % of crossreactivity.

7% had at least 1 IgM band (Table 4). Sera from the group with HIV was the least reactive for IgG antibodies (19%) and JRA sera the most reactive (57%). Evaluation of IgM antibody Western blotting revealed no IgM antibodies to *B. burgdorferi* in the groups with JRA and SLE with highest levels occurring in the presurgical sera group.

The incidence of reactivity to multiple antigens on Western blotting is lower than occurrence of a single band in non-Lyme sera. Only 9.5% of the non-Lyme sera were found to have 2 bands, 1% 3 bands and 0.5% (1 sample) 4 bands. The specimen yielding 4 bands on an IgG Western blot was from the group with EBV and all 4 bands occurred at regions associated with Borrelial antigens < 40 kDa. In contrast, 100% of Lyme disease sera had more than 4 bands (counting IgG and IgM) and included bands corresponding to antigens of 41 kDa and 60–69 kDa.

DISCUSSION

Results of our study revealed a 50% prevalence rate for detection of IgG or IgM antibodies to *B. burgdorferi* antigens by Western blotting in non-Lyme sera. It is noteworthy that sera from individuals without known infectious, inflammatory, or autoimmune disorders that were obtained from both adults and presurgical pediatric patients (adult volunteers and presurgical groups) had a similar frequency of antibodies to Borrelial antigens as observed when sera from patients with such disorders were tested. Unlike some studies, all sera in our study, regardless of reactivity in screening tests, were analyzed by Western blotting. Specimens included sera from individuals with no clinical indications of active infectious or inflammatory diseases. When screened by a standard ELISA, 24 of the 200 non-Lyme specimens were reactive, and only 4 were reactive when tested using a soluble *E. coli* antigen adsorbed ELISA[12]. Comparison of ELISA and Western blotting reactivity in the non-Lyme group did not suggest any correlation between test methodologies, as only 9 of 24 ELISA positive sera were reactive by Western blotting. This finding probably reflects differences in the target antigens used for the tests (sonicated *B. burgdorferi* for ELISA and polyacrylamide gel electrophoresis separated antigens for Western blotting), as well as differences in sensitivity between the methods. Furthermore, for a specimen to be considered reactive by ELISA it had to meet or exceed a level of reactivity (OD $\geq$ 0.2) corresponding to 3 standard deviations above a previously determined mean for normal sera. In contrast, for the purpose of our study any bands detected by Western blotting were scored as a positive reaction (though not a positive serology for Lyme disease).

The majority of antibody reactivity in the non-Lyme group detected by Western blotting consisted of IgG antibodies to 41 kDa flagellar and 60 kDa antigens. These antigens have been shown to be among the first Borrelial components to which detectable levels of antibody are made[13]. They are also believed to have determinants common to similar antigens of other bacteria and proteins that may account for crossreactivity observed in this and other studies[14-17]. More than half (12/20) the specimens reacting in the 51–60 kDa range came from the adult volunteer group. All of these reacted with an antigen corresponding to a 60 kDa component. The difference in reactivity profiles between adults and the pediatric presurgical specimens (shown by predominant reactivity to 60 and 41 kDa antigens, respectively) may reflect age related differences in immune response and patterns of exposure to different infectious agents.

Crossreactivity of IgG antibodies with other Borrelial antigens occurred at lower frequencies than with the 41 kDa antigen. Specimens from the group with EBV showed the highest level of crossreaction to low molecular weight ($\leq$ 30 kDa) antigens. The least crossreactivity was seen in the 31–40 kDa range. Only 3 specimens reacted in this range; 2 were from the group with EBV both yielding bands at approximately 34 kDa in the third, from the group with other rheumatology complaints, with a band at 38 kDa.

The frequency of crossreactive binding of IgM antibodies to *B. burgdorferi* antigens in the non-Lyme group was lower than observed for IgG antibodies. Only 14 of 200 specimens from the non-Lyme group were found reactive for IgM antibodies. Of those specimens, 10 produced single bands, 3 two bands, and 1 three bands. Only 4 of 20 Lyme disease sera

showed 4 or more bands on IgM Western blotting, while 19 of 20 Lyme disease sera showed 4 or more bands on IgG. Thus while IgM Western blotting testing may have utility for distinguishing healthy individuals (and detecting early Lyme disease), it clearly lacks sufficient sensitivity for routine diagnostic screening.

The high incidence of crossreactive antibodies to Western blotting of *B. burgdorferi* demonstrated in our paper indicates the need for minimum criteria for positivity by this test methodology. Western blotting is used increasingly as a confirmatory test for Lyme disease, as well as a more sensitive and specific test on sera from individuals with vague nonspecific symptoms. Results of our study demonstrate that crossreactive antibodies are associated with infection and inflammatory diseases, and also frequently occur in healthy sera. Based on this and previous studies, we currently require the presence of 4 or more bands on an IgG Western blot, of which 1 must correspond with the 41 kDa antigen and 1 with a 60–69 kDa antigen, to be considered positive serologic evidence of infection with *B. burgdorferi*[18]. Because the spirochete strains, preparation, separation and blotting methods vary among laboratories, minimum criteria must be established for each laboratory using this test. Our findings suggest that basing criteria on maximal reactivity observed in non-Lyme sera rather than determining the minimum observed in sera from individuals with clinically diagnosed Lyme disease may be advisable.

REFERENCES

1. Duffy J, Mertz LE, Wobig GH, Katzmann JA: Diagnosing Lyme disease: The contribution of serologic testing. *Mayo Clin Proc 1988;*63:1116–21.
2. Schwartz BS, Goldstein MD, Ribeiro Jose MC, Schulze TL, Shahied SI: Antibody testing in Lyme disease: a comparison of results in four laboratories. *JAMA 1989;*262:3431–4.
3. Grodzicki RL, Steere AC: Comparison of immunoblotting and indirect enzyme-linked immunosorbent assay using different antigen preparations for diagnosing early Lyme disease. *J Infect Dis 1988;*157:790–7.
4. Petersen LR, Sweeney AH, Checko PJ, *et al:* Epidemiological and clinical features of 1,149 persons with Lyme disease identified by laboratory-based surveillance in Connecticut. *Yale J Biol Med 1989;*62:253–62.
5. Lane RS, Lennette ET, Madigan JE: Interlaboratory and intralaboratory comparisons of indirect immunofluorescence assays for serodiagnosis of Lyme disease. *J Clin Microbiol 1990;*28:1774–9.
6. Fawcett PT, O'Brien AE, Doughty RA: An adsorption procedure to increase the specificity of enzyme-linked immunosorbent assays for Lyme disease without decreasing sensitivity. *Arthritis Rheum 1989;*32:1041–4.
7. Raoult D, Hechemy KE, Baranton G: Cross-reaction with *Borrelia burgdorferi* antigen of sera from patients with human immunodeficiency virus infection, syphilis, and leptospirosis. *J Clin Microbiol 1989;*27:2151–5.
8. Magnarelli LA, Miller JN, Anderson JF, Riviere GR: Cross-reactivity of nonspecific treponemal antibody in serologic tests for Lyme disease. *J Clin Microbiol 1990;*28:1276–9.
9. Saulsbury FT, Katzmann JA: Prevalence of antibody to *Borrelia burgdorferi* in children with juvenile rheumatoid arthritis. *J Rheumatol 1990;*17:1193–4.
10. Zoller L, Burkard S, Schafer H: Validity of Western immunoblot band patterns in the serodiagnosis of Lyme borreliosis. *J Clin Microbiol 1991;*29:174–82.
11. Laemmli UK, Fayre M: Maturation of the head of bacteriophage T4. *J Mol Biol 1973;*80:575–99.
12. Fawcett PT, Gibney KM, Rose CD, Klein JD, Doughty RA: Adsorption with a soluble *E. coli* antigen fraction improves the specificity of ELISA tests for Lyme disease. *J Rheumatol 1991;*18:705–8.
13. Coleman JL, Benach JL: Isolation of antigenic components from the Lyme disease spirochete: their role in early diagnosis. *J Infect Dis 1987;*155:756–65.
14. Aberer E, Brunner C, Suchanek G, *et al:* Molecular mimicry and Lyme borreliosis: a shared antigenic determinant between *Borrelia burgdorferi* and human tissue. *Ann Neurol 1989;*26:732–7.
15. Carreiro MM, Laux DC, Nelson DR: Characterization of the heat shock response and identification of heat shock protein antigens of Borrelia burgdorferi. *Infect Immun 1990;*58:2186–91.
16. Sigal LH: Molecular mimicry and Lyme borreliosis. *Ann Neurol 1990;*28:195.
17. Collins C, Peltz G: Immunoreactive epitopes on an expressed recombinant flagellar protein of Borrelia burgdorferi. *Infect Immun 1991;*59:514–20.
18. Rose CD, Fawcett PT, Singsen BH, Dubbs SB, Doughty RA: Use of Western blot and enzyme-linked immunosorbent assays to assist in the diagnosis of Lyme disease. *Pediatrics 1991;*88:465–70.