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Serodiagnosis in Early Lyme Disease

MARIA E. AGUERO-ROSENFELD,[1,2]* JOHN NOWAKOWSKI,[3] DONNA F. McKENNA,[4] CAROL A. CARBONARO,[2,5] AND GARY P. WORMSER[3]

Departments of Pathology,[1] Microbiology and Immunology,[2] and Medicine,[3] New York Medical College, and Department of Nursing[4] and Clinical Laboratories,[5] Westchester County Medical Center, Valhalla, New York 10595

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Using a commercially available enzyme-linked immunosorbent assay (ELISA) and an immunoblot assay (IB), we tested sera from 100 patients with erythema migrans (EM) seen in 1991 at the Westchester County Medical Center Lyme Disease Diagnostic Center. Convalescent-phase sera were available from 59 patients. Fifty-five patients had EM of <7 days' duration, 31 had EM of 7 to 14 days' duration, and 14 had EM of >14 days' duration. During the acute phase of infection, 35 patients had a positive ELISA result and 43 had a positive IB result by the recently published criteria of Dressler et al. (F. Dressler, J. A. Whalen, B. N. Reinhardt, and A. C. Steere, J. Infect. Dis. 167:392–400, 1993) for interpretation of IB in patients with Lyme disease. A greater sensitivity of IB was observed in patients with EM of <7 days' duration, as follows: 14 of 55 (25%) for IB versus 7 of 55 (13%) for ELISA ($P = 0.144$). Sera of all 14 patients with EM of >14 days' duration were reactive by both tests, as follows: 13 positive and 1 equivocal by ELISA and 12 positive and 2 indeterminate by the IB. The band reactivity most frequently observed in the IB was to the 41- and 25-kDa antigens, the latter being the most frequent band observed in immunoglobulin M blots. Seroconversion was observed in 74 and 64% of evaluable patients by ELISA and IB, respectively, despite the use of antibiotic therapy.

Lyme disease, which is caused by *Borrelia burgdorferi*, is the most common vector-borne disease in the United States (6). While the manifestations may be protean, diagnosis of early disease is frequently based on the presence of an expanding erythematous lesion, erythema migrans (EM). Although several laboratory tests can be used to assist in the diagnosis of early Lyme borreliosis, none has been completely satisfactory. The most direct approach is to attempt to culture the causative agent. Although cultures of skin biopsy samples from sites of EM may grow *B. burgdorferi* in up to 85% of cases (4, 29), this technique is slow, labor-intensive, and impractical for use in the clinical laboratory (24, 33). Other direct diagnostic methods such as tests for the presence of antigen or the use of the polymerase chain reaction to detect *B. burgdorferi* nucleic acid sequences are also currently limited to use at the investigational level (2, 21, 22). Consequently, laboratory methods that can be used to assist in clinical diagnosis have rested principally on indirect methods for detection of specific anti-*B. burgdorferi* antibodies. The enzyme-linked immunosorbent assay (ELISA) is the most widely used of these serological assays, but it may give both false-negative and false-positive results (9, 11, 20). Although not yet standardized for use in the diagnosis of Lyme borreliosis, the Western immunoblot assay (IB) should permit a clearer picture of which *B. burgdorferi* antigens elicit specific antibodies and define the temporal sequence in which antibodies appear. Reported results of IB testing of samples from patients with early disease have, however, involved only a limited number of patient sera (8, 10, 15, 19, 23, 36). Recently, Dressler et al. (12) proposed revised criteria for the interpretation of IB results on the basis of a retrospective analysis of sera from patients with different stages of Lyme disease and applied these criteria to the interpretation of IB of serum specimens from 57 patients with EM.

We describe the results of ELISA and IB analyses of sera from 100 patients presenting with EM at the Lyme Disease Diagnostic Center of the Westchester County Medical Center, Valhalla, N.Y. Data are also presented for 59 serum samples from the same group of patients during the convalescent phase of the disease.

(The present study was presented in part at the Fifth International Conference on Lyme Borreliosis, 30 May to 2 June 1992, Arlington, VA.)

MATERIALS AND METHODS

Patient population. One hundred patients presenting with EM to the Lyme Disease Diagnostic Center during the spring and summer of 1991 were included in the study. All cases of borreliosis satisfied the surveillance case definition for Lyme disease with EM of the Centers for Disease Control and Prevention (7). All patients received conventional antimicrobial therapy or were placed on an investigational antimicrobial protocol at the first visit.

Serum collection. Serum samples were obtained from all 100 patients during their first visit to the Lyme Disease Diagnostic Center. Additional serum samples were obtained from 59 of these patients at approximately 1 month after the initial visit. For IB analysis, a control group of 69 serum samples was collected from individuals with systemic lupus erythematosus ($n = 18$), rheumatoid arthritis ($n = 18$), and syphilis ($n = 10$) (all 10 samples from patients with syphilis tested positive by both reagin and treponemal antibody tests) and from a group of healthy donors ($n = 23$); 10 of these healthy individuals were from an area in which Lyme disease is not endemic. Samples were stored at 5°C for up to 1 week. Long-term storage was at −70°C.

ELISA. All serum samples were assayed for their seroreactivities in Lyme Stat (Whittaker Bioproducts, Inc., Walk-

* Corresponding author.

TABLE 1. Characteristics of 100 patients with EM

Characteristic	Value
No. of males/no. of females	59/41
Avg age (yr)	40.2
No. of patients with:	
Tick bite at site of EM	24
Multiple EM lesions	13
Associated symptoms	68
Avg no. of symptoms	4.4
Avg EM duration (days [range])	7.38 (1–60)
Avg EM size (cm [range])	13.75 (5–31)

ersville, Md.), a commercially available polyvalent ELISA kit for the detection of *B. burgdorferi* antibodies. Samples were tested according to the manufacturer's instructions. Results were reported qualitatively on the basis of the Lyme index value (LIV) in the following categories: positive (LIV, >1.09), equivocal (LIV, ≥0.8 to ≤1.09), and negative (LIV, <0.8).

IB. For the separate determination of immunoglobulin G (IgG) and IgM reactivities to *B. burgdorferi* antigens, each serum sample was applied to the Marblot Strip Test System (the generous gift of MarDx Diagnostics Inc., Carlsbad, Calif.) according to the manufacturer's instructions. IB reactivities were interpreted by the criteria of Dressler et al. (12). Briefly, a blot was considered positive if it showed at least two of the eight most common IgM bands (18, 21, 28, 37, 41, 45, 58, and 93 kDa) or at least 5 of the 10 most frequent IgG bands (18, 21, 28, 30, 39, 41, 45, 58, 66, and 93 kDa). Blots were considered indeterminate when a single band or a band combination not meeting the criteria presented above was present and negative when no bands were seen. The band with a molecular mass of 21 kDa in the criteria of Dressler et al. (12) was denominated 25 kDa in the present study (see Discussion).

Seroconversion. Seroconversion was defined as an increase in LIV or an increase in the number of bands that resulted in a category change by ELISA or IB, respectively, of samples obtained during the convalescent phase of disease.

Statistics. Fisher's exact test (two-tailed) and Student's *t* test (two-tailed) were used in the analysis of the data.

RESULTS

Patient population. The demographic and clinical features of the 100 patients included in the present study are presented in Table 1. The average duration of EM was 7.38 days (range, 1 to 60 days). Fifty-five patients had EM of less than 7 days' duration, 31 patients had EM for 7 to 14 days' duration, and 14 had EM of more than 14 days' duration. The average size of the EM lesions was 13.75 cm (range, 5 to 31 cm), and 13 patients had multiple EM lesions. Sixty-eight patients reported the presence of systemic symptoms at the time of diagnosis. Fatigue, headache, myalgia, neck stiffness, and arthralgia were the most frequently reported symptoms. One patient had an associated seventh cranial nerve palsy.

ELISA. Of the 100 serum samples tested, 49 had a reactive ELISA at the time of presentation (14 equivocal and 35 positive). The relationship between ELISA reactivity and the duration of EM is shown in Table 2. Sera from all 14 patients with EM for more than 14 days had a reactive ELISA (1 equivocal and 13 positive). A pairwise comparison of the ELISA reactivities among sera from the three groups of patients according to EM duration showed a statistically significant difference ($P < 0.05$).

IB. Sixty-five acute-phase serum samples had IB band reactivity; 43 (66%) were interpreted as positive by the criteria of Dressler et al. (12) (Table 2). Sera from 28 of 55 patients (51%) with EM of <7 days' duration had reactive blots. Thirty-six percent of the reactive blots had IgM bands only, and another 36% had IgG and IgM reactivities; the remaining 28% had IgG reactivity only. Fourteen of the 28 reactive blots were interpreted as positive; 12 of these 14 met Dressler's criteria for positivity solely on the basis of the IgM bands, 1 met the criteria for positivity on the basis of both IgG and IgM bands, and 1 met the criteria for positivity on the basis of IgG bands only. The one blot that was positive solely on the basis of the IgG criteria was obtained from a patient with a previous history of EM.

Sera from 23 of 31 patients (74%) with EM of 7 to 14 days' duration showed reactivity by IB. Seventy percent (16 of 23) of the reactive blots had IgG and IgM bands, 22% (5 of 23) had IgG bands only, and 8% (2 of 23) had IgM bands only. Seventeen of the reactive blots were interpreted as positive; 13 had positive IgM blots, 3 had positive IgG and IgM blots, and 1 had a positive IgG blot only. The one patient whose sera showed an isolated positive IgG blot had a past history of EM.

Sera from all 14 patients with EM of >14 days' duration had both IgG and IgM IB reactivities. Sera from 12 of these patients were interpreted as having a positive IB result; 5

TABLE 2. ELISA and IB reactivities of acute-phase sera from 100 patients with EM according to EM duration

EM duration (days)	No. of patients	No. (%) of serum specimens					
		ELISA			IB		
		Negative	Equivocal	Positive	Negative	Indeterminate	Positive
<7	55	38 (69)	10 (18)	7 (13)[a]	27 (49)	14 (25.5)	14 (25.5)[a]
7–14	31	13 (42)	3 (10)	15 (48)	8 (26)	6 (19)	17 (55)
>14	14	0	1 (7)	13 (93)	0	2 (14)	12 (86)
Total	100	51	14	35[b]	35	22	43[b]

[a] $P = 0.144$ for the frequency of positive ELISA versus IB in sera from patients with EM of <7 days' duration (7 of 55 versus 14 of 55 patients).
[b] $P = 0.310$ for the frequency of positive ELISA versus IB in sera from all patients (35 of 100 versus 43 of 100 patients).

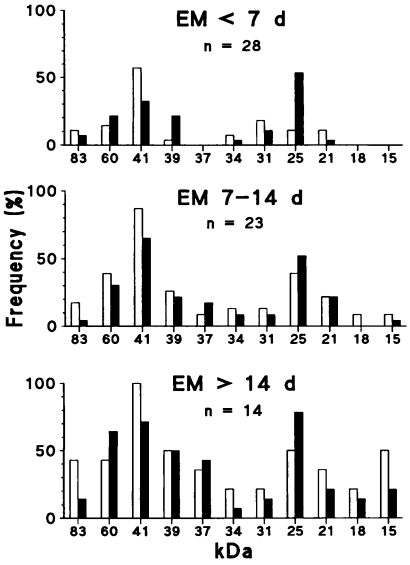

FIG. 1. Frequency of IB band reactivities according to EM duration in acute-phase sera from 65 patients with reactive blots. Open bars indicate IgG blots, and solid bars indicate IgM blots.

were positive on the basis of IgM blot criteria alone and 7 were positive on the basis of both IgG and IgM blot criteria.

As for the ELISA, IB reactivity correlated directly with the duration of EM. The average ± standard deviation number of bands for the group of patients with EM of <7 days' duration was 1.7 ± 1.7 and 2.0 ± 2.1 in the IgG- and IgM-reactive blots, respectively. For the group with EM of 7 to 14 days' duration, the average number of bands was 3.4 ± 2.8 in the IgG-reactive blots and 2.6 ± 2.2 in the IgM-reactive blots. All 14 patients with EM of >14 days' duration had reactive blots with an average of 5.6 ± 3.4 and 4.6 ± 2.3 bands in the IgG- and IgM-reactive blots. Pairwise comparison of the number of bands in IgG and IgM-reactive blots separately, according to EM duration, showed a significant difference for each pair ($P < 0.05$) except for the comparison of the number of bands in the IgM-reactive blots between the groups with EM of <7 days' and 7 to 14 days' duration ($P = 0.3$).

A comparison of ELISA versus IB positivity in acute-phase samples showed that, overall, IB was more sensitive than ELISA in detecting *B. burgdorferi* antibodies (43 versus 35%), but this difference was not statistically significant ($P = 0.310$) (Table 2). On the basis of the duration of EM, the difference in sensitivity was greatest for patients with EM of <7 days' duration; sera from 14 of 55 (25%) and 7 of 55 (13%) of patients in this group were positive by IB and ELISA, respectively ($P = 0.144$). Of the seven serum samples in this group positive by IB but with discordant ELISA results, 4 were equivocal and 3 were negative by ELISA. Of the sera from the group of patients with EM of 7 to 14 days' duration, 2 serum specimens that were equivocal by ELISA and one serum specimen that was negative by ELISA were interpreted as positive by IB; one serum specimen that was positive by ELISA was interpreted as indeterminate by IB. Sera from 12 of 13 patients with EM of >14 days' duration who had a positive ELISA result also tested positive by IB.

The band reactivities observed in IB of acute-phase serum samples according to the duration of EM are shown in Fig. 1. Overall, the 25-kDa band was the most frequently found band in IgM-reactive blots (75%) and the 41-kDa band the most frequent (94%) in IgG-reactive blots.

TABLE 3. Comparison of IB band reactivities of sera from individuals without clinical evidence of Lyme disease[a] with those of sera from patients with EM

Antigen (kDa)	% Reactive			
	IgM		IgG	
	Control, non-Lyme disease	EM	Control, non-Lyme disease	EM
83	0	5	4	13
75	0	4	1	9
66	0	14	4	7
60	0	22	9	19
41	0	34	28	50
39	1	18	0	14
34	0	4	1	8
31	1	7	4	11
25	3	38	1	19
21	0	9	1	13
18	0	2	0	5
15	0	4	1	9

[a] The control group consisted of sera from 69 individuals: systemic lupus erythematosus ($n = 18$), rheumatoid arthritis ($n = 18$), syphilis ($n = 10$), and healthy donors ($n = 23$).

The most frequently observed bands for sera from patients with EM of <7 days' duration were 25 kDa (54%) and 41 kDa (32%) in IgM-reactive blots and 41 kDa (57%) in IgG-reactive blots. Four of the 10 (40%) sera with only IgM reactivity in the blots reacted exclusively with the 25-kDa antigen. The most frequent immunoreactive antigens in sera from the group of patients with EM of 7 to 14 days' duration were 41 kDa (65%) and 25 kDa (52%) in IgM-reactive blots and 41 kDa (87%) in IgG-reactive blots. For the patients with EM of >14 days' duration, the bands most frequently seen were 25 kDa (79%), 41 kDa (71%), 60 kDa (64%), and 39 kDa (50%) in IgM-reactive blots and 41 kDa (100%), 39 kDa (50%), 25 kDa (50%), and 15 kDa (50%) in IgG-reactive blots.

The IB reactivities of sera from a control group in comparison with those of sera from patients with EM are shown in Table 3. When present in control sera, IgM reactivity was directed against single antigens. For example, IgM reactivity was observed in sera from one patient with rheumatoid arthritis (39 kDa), one patient with systemic lupus erythematosus (31 kDa), and two patients with syphilis (weak reactivity to the 25-kDa antigen). More immunoreactive antigens were observed in the IgG blots, with the 41- and 60-kDa antigens being the most frequent, but none was interpreted as positive. Eight of 10 (80%) serum specimens from patients with syphilis had IgG-reactive blots, with an average of 1.7 bands. Thirty-nine percent of serum specimens from patients with systemic lupus erythematosus and 56% of serum specimens from patients with rheumatoid arthritis had IgG-reactive blots, with an average of 1.2 and 1.3 bands, respectively. Ten of 23 (43%) serum specimens from healthy donors had IgG-reactive blots, with an average of 1.5 bands.

Seroconversion. Twenty-three of 31 (74%) patients with EM whose sera were initially seronegative by ELISA seroconverted in the convalescent phase; sera from 7 (23%) patients became equivocal and sera from 16 (51%) patients became positive (Table 4). Seroconversion to positivity was observed in five of the seven patients (71%) whose sera had an equivocal ELISA result in the acute phase of disease.

TABLE 4. ELISA and IB results for 59 patients with EM during acute and convalescent phases

Test and acute-phase result	No. of patients	No. (%) of serum specimens in convalescent phase		
		Negative	ELISA equivocal or IB indeterminate	Positive
ELISA				
Negative	31	8 (26)	7 (23)	16 (51)
Equivocal	7	0	2 (29)	5 (71)
Positive	21	0	0	21 (100)
IB				
Negative	22	7 (32)	6 (27)	9 (41)
Indeterminate	11	1 (9)	4 (36)	6 (55)
Positive	26	0	6 (23)	20 (77)

Convalescent-phase sera were available from 33 patients whose sera were either negative ($n = 22$) or indeterminate ($n = 11$) by IB in the acute phase of the disease. Twenty-one patients seroconverted (64%); of the 22 whose sera were initially negative, 6 (27%) became indeterminate and 9 (41%) became positive by IB; in addition, 6 of 11 (55%) serum specimens that were indeterminate by IB in the acute phase became positive in the convalescent phase. Overall, seroconversion was observed in 24 of 31 patients (77%) by ELISA or IB. Sera from all 21 patients that were positive by ELISA in the acute phase remained positive in the convalescent phase. Convalescent-phase sera were available from 26 patients whose sera were positive by IB during the acute phase; of these serum specimens, 20 (77%) remained positive and the other 6 became indeterminate (all 6 serum specimens tested positive by ELISA). Overall, 51 of 59 (86%) convalescent-phase serum specimens were reactive by IB, 35 of which were interpreted as positive: 26 based on IgM criteria, 8 based on both IgG and IgM criteria, and 1 based on IgG criteria. As for the acute-phase sera, the most frequent immunoreactive antigens during the convalescent phase were 41 and 25 kDa. The 41-kDa band was found in 88 and 78% of IgG- and IgM-reactive blots, respectively, and the 25-kDa band was found in 41 and 63% of the IgG- and IgM-reactive blots, respectively. Reactivity to the 39-kDa band was found in 37 and 33% of IgG- and IgM-reactive blots, respectively.

DISCUSSION

Until better diagnostic modalities are developed to confirm the diagnosis of Lyme borreliosis, serological assays will continue to be used in clinical laboratories. In this report, we described the ELISA and IB reactivities of sera from 100 clinically well-characterized patients presenting with EM in an area of New York State where Lyme disease is epidemic. To our knowledge, the present study included the largest number of patients with EM described to date. Furthermore, the immunological response to *B. burgdorferi* infection was found to be correlated with the duration of EM, which has not been reported previously. In our patient population, the frequency of prior tick bite (24%), constitutional symptoms (68%), or multiple skin lesions (13%) was similar to those reported elsewhere (3, 32).

Although the overall percentage of ELISA reactivity appeared to be similar to those published in other reports, the probability of seroreactivity directly correlated with the duration of EM; 31% of sera were reactive by ELISA when the skin lesion was of less than 7 days' duration compared with 58 and 100% reactive sera by ELISA when EM was present for 7 to 14 days and greater than 14 days, respectively ($P < 0.05$ for each pairwise comparison). Whether seropositivity is as frequent in patients without EM but with Lyme borreliosis of the same duration is unknown.

Similar to ELISA, IB reactivity was found to be directly related to the duration of EM. The number of bands increased from an average of 1.6 and 2.0 for IgG- and IgM-reactive blots, respectively, when patients sought medical attention within 7 days of the appearance of EM lesions to 5.6 and 4.6 for IgG- and IgM-reactive blots, respectively, for those patients with EM of greater than 14 days' duration ($P < 0.05$). Overall, sera from 65 patients had band reactivities by IB, 43 (66%) of which were considered positive by the criteria used in the interpretation of the results. Although sera from the majority of patients had both IgG and IgM reactivities (62%), isolated IgM reactivity was found in up to 36% of sera from patients with EM of short duration. Furthermore, 41 of the 43 (95%) positive blots met the IgM criteria for positivity. The two patients with positive blots based solely on IgG reactivity criteria had had EM in previous years. It is possible that in these two patients the antibodies detected were induced by prior infection with *B. burgdorferi*; nonetheless, the possibility of a booster effect of the current infection cannot be excluded.

It has frequently been stated that early antibiotic therapy may prevent the development of specific antibodies (30, 34). We found this to be less common than anticipated since 28 of 38 (74%) of our evaluable patients whose sera were initially seronegative or equivocal by ELISA and 21 of 33 (64%) patients whose sera were initially negative or indeterminate as determined by IB seroconverted with a single serum sample obtained approximately 1 month after the first visit. Seroconversion was not affected by the choice of antibiotic therapy (data not shown). For the eight patients who did not seroconvert, as determined by ELISA, it could be postulated that the clinical diagnosis of EM was incorrect. However, in two of these patients, *B. burgdorferi* was isolated from the skin lesion, and one of these two patients seroconverted, as determined by IB. Of the remaining five patients, skin cultures were not available for three and were nonevaluable for the other two patients (because of overgrowth by other bacteria). All of these five patients had EM lesions of 2 to 7 days' duration.

Feder et al. (13) recently reported that antibodies in the sera of patients with EM may persist for as long as 3 years following therapy. Although sera from our patients were only tested at 1 month after the initial visit, we also noted the absence of reversion to negativity in the convalescent-phase serum samples from patients who were initially seropositive by ELISA or IB. The general pattern observed was an expansion of the IB band reactivity in convalescent-phase serum samples. However, sera from 6 of 26 (23%) patients positive by IB in the acute phase had decreased blot reactivity in the convalescent phase and were classified as indeterminate, and serum from one patient indeterminate by IB in the acute phase became negative in the convalescent phase. Of interest, the majority (97%) of the positive blots in the convalescent phase still met the IgM reactivity criteria for positivity, whereas only 25% fulfilled the IgG reactivity criteria. If convalescent-phase sera had been collected later than 1 month into the convalescence, the disparity in favor of IgM would likely have been less or reversed in favor of IgG positivity.

As described by others (15, 16), IB is more sensitive than ELISA for the detection of specific *B. burgdorferi* antibodies. This difference, although not statistically significant, was greatest in those patients with EM of less than 7 days' duration at the initial visit. Of interest, sera from all patients in this group that were positive by IB but negative by ELISA had an ELISA LIV greater than or equal to 0.6 (the threshold for the equivocal range was an ELISA LIV of ≥0.8). In addition, sera from five of the six patients with isolated IgM reactivity to the 25-kDa antigen band had a negative ELISA result; 2 of these serum specimens also had an ELISA LIV of ≥0.6. It is possible that the difference in sensitivities of the two methodologies in the present study is due to the use of a polyvalent ELISA rather than an IgM ELISA, which is our standard practice to screen for antibodies to *B. burgdorferi*. It may also be attributed to differences in antigen composition and/or concentration between the test formats. On the basis of our findings, if serological tests are being used to confirm the clinical diagnosis, it seems prudent to use IB as a primary serologic test in patients with EM of short duration with a negative ELISA result, especially for those whose sera have ELISA reactivities approaching the equivocal range.

The band reactivities most frequently observed by IB in the acute phase were to the 41-kDa antigen (94% in IgG-reactive blots and 67% in IgM-reactive blots) and the 25-kDa antigen (36% in IgG-reactive blots and 75% in IgM-reactive blots). Moreover, IgM reactivity to the 25-kDa antigen was the band most frequently found in sera from patients with EM of less than 7 days' duration, and in most of the IgM blots, the 25-kDa antigen was the strongest immunoreactive antigen. Similarly, during 1992, we observed IgM reactivity to the 25-kDa antigen in sera from 55 of 88 (59%) patients with EM tested at the Lyme Disease Diagnostic Center (data not shown). In the present study, convalescent-phase sera were available from three of the six patients whose sera had IgM antibodies that reacted only with the 25-kDa antigen during the acute phase; all three patients seroconverted, and in two of these patients, *B. burgdorferi* was isolated from the skin. Weak IgM reactivity to the 25-kDa antigen was found in 3% of serum specimens from the control group, but sera from none of the controls was considered to have a positive IB result by the criteria of Dressler et al. (12).

Wilske et al. (35) first described an immunodominant antigen (pC) of 22 to 25 kDa in patients with early Lyme disease in Europe. Despite the frequency of antibodies to this antigen in their study, only about 50% of European *B. burgdorferi* strains expressed this protein when grown in culture. A protein in the range of 25 kDa was also described in *B. burgdorferi* isolates from *Ixodes pacificus* in California (5). This protein was subsequently characterized and was found to be loosely attached to the surface of the organism (17). A monoclonal antibody directed against this surface protein also reacted with *B. burgdorferi* 2591, which was isolated in Connecticut by Anderson et al. (1) and which is known to express a 22-kDa antigen. Other groups have also described isolates from the United States that express antigens in the 22- to 25-kDa range (18, 31), but whether they represent the 22-kDa antigen (pC) described by Wilske et al. (35) is unknown. Recently, Padula et al. (26) reported the cloning of the gene encoding the 25-kDa protein of *B. burgdorferi* 2591 and found there to be an 83% identity with pC by nucleotide sequence analysis and a 75% identity with pC by amino acid sequence analysis (14). Consistent with these results, we have observed that a monoclonal antibody, L221F8, directed against pC (kindly provided by B. Wilske) reacts with the 25-kDa protein present in the blots used in our study (data not shown). Specific antibodies to a 22- to 25-kDa antigen have been described by others among U.S. patients with early Lyme disease, but at a frequency lower than that reported here (15, 19). It seems likely that this is due to a difference in the antigen preparation used in the IB, since it has been shown that several antigens of *B. burgdorferi*, including a 25-kDa antigen, are expressed in variable amounts, depending on the in vitro conditions of growth of the organism (27, 28). Furthermore, several *B. burgdorferi* proteins are known to be lost or underexpressed in strains that have undergone high numbers of passages (25). Interestingly, in previous years, reactivity to the 25-kDa antigen was not observed in a similar patient population when immunoblots from the same manufacturer were used. It was only when the manufacturer began to use a low-passage B31 strain that this reactivity became evident. In their recent study, Dressler et al. (12) found that reactivity to a 21-kDa antigen was the most frequently present band in IgM IBs of sera from a group of patients with EM. In that report, *B. burgdorferi* G39/40 was used to prepare the blots, and it was demonstrated that this 21-kDa antigen also reacted with a monoclonal antibody to pC (L321F8) of B. Wilske. The discrepancies in the molecular sizes of these apparently similar or identical immunoreactive proteins can most likely be attributed to the different antigen preparations used or to technical differences in the preparation of the blots. Similar discrepancies in molecular size are likely to exist for other antigens. Since Dressler et al. (12) are proposing the use of specific antigens as significant immunoreactive bands, it is imperative that a consensus on nomenclature be established.

Aside from the results of Dressler et al. (12), most investigators to date who use IB on sera from patients with early Lyme disease have found that the first detectable antibodies are directed to the 41-kDa protein corresponding to the flagellar antigen (8, 10, 15, 19, 23, 36). We also found reactivity to this protein to be common but less frequent than reactivity to the 25-kDa protein in IgM blots of acute-phase sera. The high frequency of reactivity to the 41-kDa protein in IgG blots may be partially explained by the background prevalence of these antibodies in sera from patients without Lyme disease (12, 15, 19), as was observed in the sera of our control group of patients. Antibodies against the 39-kDa antigen were observed in 26% of the IgG-reactive blots and 35% of the IgM-reactive blots during the acute phase of disease and were more commonly found in patients with EM of greater than 14 days' duration (50%). This parallels the findings of Ma et al. (19), who reported IgM reactivity to the 39-kDa antigen in one-half of patients with early Lyme disease.

Since the earliest immunological response to infection with *B. burgdorferi* appears to be directed to the 25- and 41-kDa antigens, it may be important to include or perhaps enrich serological assays with these antigens for detection of antibodies in the early stage of disease. In our study, we found that in patients with EM of short duration, IgM reactivity to the 25-kDa antigen appears to be detectable the earliest. If we would have included this reactivity as specific for *B. burgdorferi* infection, six additional patients in the acute phase of disease would have been interpreted as having a positive IB result.

Until a consensus is established for the interpretation of IB results for *B. burgdorferi* antibodies, we suggest that a distinct IgM reactivity to the 25-kDa antigen be considered strongly indicative of an early, specific antibody response to *B. burgdorferi* infection.

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