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Evolution of the Serologic Response to *Borrelia burgdorferi* in Treated Patients with Culture-Confirmed Erythema Migrans

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We investigated the appearance and evolution of immunoglobulin M (IgM) and IgG antibodies to *Borrelia burgdorferi* in 46 patients with culture-proven erythema migrans (EM). All patients received antimicrobial treatment and were prospectively evaluated for up to 1 year. A total of 257 serially collected serum samples were tested by commercial IgG-IgM enzyme-linked immunosorbent assay and separate IgM and IgG immunoblots (IBs). At the baseline, 33% of the patients had a positive ELISA result and 43% of the patients had a positive IgM IB result by using the criteria of the Centers for Disease Control and Prevention-Association of State and Territorial Public Health Laboratory Directors for the interpretation of IB results. Positive serology at the baseline and the rate of seroconversion correlated directly with disease duration and/or evidence of dissemination prior to treatment. At days 8 to 14 after the baseline, 91% of patients had a positive ELISA result and/or IgM IB result. Peak IgM antibody levels were seen at this time in patients with localized or disseminated disease. The most frequent IgM bands at the baseline and the peak were of 24 kDa (OspC), 41 kDa, and 37 kDa. Although 89% of the patients developed IgG antibodies as determined at a follow-up examination, only 22% were positive by the IgG IB criteria of the Centers for Disease Control and Prevention-Association of State and Territorial Public Health Laboratory Directors. The persistence of antibodies was directly related to disease duration and/or dissemination prior to treatment. Since IgM antibodies to the 24- and 41-kDa antigens remained detectable for long periods, 38% of IgM IBs were still positive at 1 year postbaseline. IgM to antigens of 39, 58, 60, 66, or 93 kDa, conversely, were most often seen in sera obtained within 1 month postbaseline. Their presence may be of assistance in confirming a recent infection with *B. burgdorferi* in individuals living in areas where Lyme disease is endemic.

The most frequently used laboratory methods to support the clinical diagnosis of Lyme disease are those detecting the presence of antibodies to *Borrelia burgdorferi*. The limitations of serology include cross-reacting antibodies or the absence of antibodies in a high proportion of patients with very early Lyme disease and in a small number of patients with late Lyme disease (4, 9, 12, 21). False-positive reactions by enzyme-linked immunosorbent assay (ELISA) have been detected by the use of Western immunoblots (IBs) (10, 12, 15). Furthermore, we and others have found immunoglobulin M (IgM) IBs to be of greater sensitivity than polyvalent ELISAs in patients with early Lyme disease of short duration (1, 15, 17). Mitchell et al. (23) reported on the serology of patients with culture-confirmed erythema migrans (EM) in a comparison of four commercial immunoassays and found that an IgM immunofluorescence assay has the highest sensitivity, but the results of an evaluation of an IgM IB kit were not presented.

Most studies to date evaluating serologic methods have relied on clinically defined patients (1, 10, 11, 15–17, 20, 36) and have rarely considered the temporal appearance and evolution of the antibody response to the various *B. burgdorferi* antigens (8). We previously demonstrated that the presence of antibodies during the acute phase in patients with EM correlated directly with the duration of disease. In that study, regardless of antibiotic treatment, seroconversion was observed in the sera of about 70% of patients for sera collected at 30 days (1). In a treatment study of patients with EM, Massarotti et al. (22) performed serologic follow-up on 55 patients using capture IgM and IgG ELISA for up to 6 months. They found that the IgM and IgG responses were highest at 10 days postbaseline in patients with localized disease and at 30 days postbaseline in patients with disseminated disease. IgM antibodies were still present in about half of the patients at 6 months (22). Feder et al. (13) demonstrated the persistence of IgG and IgM antibodies for more than 2 years after treatment in certain patients with Lyme disease by using IgM and IgG ELISA and immunoblots. However, those investigators tested only two serum specimens from each patient: an acute-phase serum specimen and a convalescent-phase serum specimen collected between 2 and 36 months after the first sample was collected (13). Hammers-Berggren et al. (16) recently demonstrated the persistence of IgM antibodies for up to 17 months after treatment of EM as tested by a capture ELISA in which flagella are used as the antigen. They also tested relatively few sequential serum samples. More recently, Engstrom et al. (11) reported on the serology of 55 clinically defined patients with EM from whom sequential serum specimens were obtained. By using IgM and IgG ELISAs and IBs, they found that most patients (80%) seroconverted by 8 to 12 days into treatment.

In the present study we investigated the kinetics of the appearance and evolution of antibodies to *B. burgdorferi* antigens in a population of culture-positive patients with EM. Antibodies were assayed in a commercially available IgG-IgM ELISA and separate IgM and IgG IBs. To our knowledge this is the first report describing the serologic evolution of a large group

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TABLE 1. Evolution of ELISA positivity according to disease duration

EM duration (days)	No. (%) of positive serum specimens at[a]:						
	Baseline (n = 46)	8–14 days (*n* = 43)	20 days (*n* = 35)	30 days (*n* = 26)	3 mo (*n* = 39)	6 mo (*n* = 37)	1 yr (*n* = 31)
<7	3 (10)	24 (89)	19 (91)	14 (81)	6 (26)	3 (18)	0 (0)
7–14	7 (58)	8 (67)	8 (80)	6 (86)	8 (73)	6 (60)	2 (22)
>14	5/5[b]	4/4	4/4	3/3	5/5	4/4	3/4
Total	15 (33)	36 (84)	31 (89)	23 (88)	19 (49)	13 (35)	5 (16)

[a] $P < 0.05$ for comparisons of pairs: <7 days versus 7 to 14 days at the baseline and 3 months and <7 days versus ≥7 days at the baseline, 3 months, 6 months, and 1 year. All other comparisons were not statistically significant.
[b] Number of positive serum specimens/total number of serum specimens tested.

of patients whose Lyme disease was confirmed by the isolation of *B. burgdorferi* in culture.

MATERIALS AND METHODS

Patients. Forty-six patients presenting with EM to the Lyme Disease Diagnostic Center of the Westchester County Medical Center during 1991 and 1992 from whom *B. burgdorferi* was isolated from cultures of skin specimens were included in the study. These patients were clinically evaluated at the baseline, a skin biopsy sample and a blood sample were obtained for culture of *B. burgdorferi*, a serum specimen was collected, and antimicrobial treatment was prescribed. These individuals were periodically evaluated at days 8 to 14, 20, and 30, 3 to 4 months, 6 months, and 1 year postbaseline, and a serum specimen was collected at each visit. They were classified as having localized or disseminated disease by the criteria used by Massarotti et al. (22). Briefly, localized infection was defined as EM accompanied by no more than regional lymphadenopathy, fatigue, or minor headache; dissemination was defined by the presence of secondary annular skin lesions, arthritis or arthralgias, abdominal pain or tenderness, generalized lymphadenopathy, or signs or symptoms of central nervous system infection (headache and neck stiffness, facial palsy, or dysesthesias).

Sera. A total of 257 serum specimens were included in the study. Sera were kept frozen at −70°C until they were tested.

ELISA. All specimens were tested by Lyme Stat (Whittaker Bioproducts, Inc., Walkersville, Md.), an IgG-IgM ELISA, according to the manufacturer's instructions. Results were reported qualitatively, according to the Lyme index value (LIV), as negative (LIV, <0.8), equivocal (LIV, ≥0.8 but <1.09), and positive (LIV, ≥1.09).

IB. Separate IgM and IgG IB assays (kindly provided by MarDx Diagnostics, Inc., Carlsbad, Calif.) were used to test all specimens according to the manufacturer's instructions. All serum specimens from a single patient were run in parallel with the same IB kit lot to avoid variability because of differences between blot membranes. A panel of monoclonal antibodies made available through the Centers for Disease Control and Prevention (CDC) and kindly provided by Barbara Johnson were run in this blot system for band denomination. This panel included antibodies to p93 (181.1) (19), p62 (149) (18), p41 Fla (H9724) (2), p39 (H1141) (28), OspB (84C) (7), OspA (H5332) (3), OspD (H1C8) (26), OspC (4B8F4) (25), and p22 (CB625) (6). The IB results were interpreted according to the guidelines recently proposed by a CDC-Association of State and Territorial Public Health Laboratory Directors working group on standardization of immunoblotting for Lyme disease which uses the IgM criteria published by Engstrom et al. (11) and the IgG criteria of Dressler et al. (10). An IgM IB result was considered positive if it showed 2 of 3 bands (41, 39, or 24 kDa), and an IgG IB result was considered positive if 5 of the 10 following bands were present: 93, 66, 58, 45, 41, 39, 30, 28, 24, or 18 kDa. In this nomenclature, the 24-kDa band represents OspC, previously reported as a 21-kDa band by Dressler et al. (10), as a 23-kDa band by Padula et al. (25), and by us as a 25-kDa band (1). The 93-kDa antigen is the same as that previously reported by us as a 83-kDa antigen.

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

RESULTS

Patients. Of the 46 patients in the present study, 22 patients were seen during the spring-summer 1991 and 24 were seen during the spring-summer of 1992. Twenty-nine patients (63%) presented with EM of less than 7 days in duration, 12 (26%) presented with EM of 7 to 14 days in duration, and 5 (11%) presented with EM of >14 days in duration (range, 16 to 23 days). Evidence of dissemination was present in 29 patients (63%). One asymptomatic patient was classified as having disseminated infection because *B. burgdorferi* was isolated from the patient's blood. Although blood samples for culture were obtained from 42 of 46 patients, the blood cultures yielded *B. burgdorferi* for only 6 patients. Eighteen of 29 (62%) patients with EM of <7 days in duration, 6 of 12 (50%) patients with EM of 7 to 14 days in duration, and all 5 patients with disease of >14 days in duration had evidence of disseminated disease at the baseline. All patients were enrolled in antimicrobial treatment trials during their first visit. The majority of patients included in the study had an uneventful course after antimicrobial treatment; only three patients complained of arthralgias, myalgias, or fatigue after completing treatment, but none of these patients progressed to objective late disease.

ELISA. ELISA positivity according to disease duration at different time intervals is provided in Table 1. At the baseline, ELISA positivity was observed in 10% of individuals with EM of <7 days in duration, whereas it was observed 58 and 100% of individuals with EM of 7 to 14 days and >14 days in duration, respectively ($P < 0.005$ comparing those with EM for <7 days with those with EM for ≥7 days). The greatest number of positive ELISA results at all intervals tested was observed at 8 to 14 days postbaseline (36 of 43 results; 84%). The duration of ELISA positivity correlated directly with the duration of disease prior to treatment. ELISA results in the equivocal range at the baseline was only observed with sera from 3 patients in the group with EM for <7 days; specimens from all three patients tested positive at days 8 to 14.

Evolution of LIV by ELISA. As shown in Fig. 1, the highest mean LIV by ELISA was observed in specimens collected at days 8 to 14 from 20 of 22 (91%) patients with EM of <7 days in duration from whom sera were also collected at day 20 and/or day 30 postbaseline. Although the average LIV was also highest at days 8 to 14 days postbaseline in patients with EM of 7 to 14 days in duration, in two patients the highest ELISA LIV was found at the baseline, and in five patients the highest LIV was found at day 20. In all patients with EM of >14 days in duration the peak ELISA LIV was found at the baseline or days 8 to 14. The mean LIVs at the baseline and at the peak were higher for sera from patients with disease of >14 days in duration than for sera from the other two groups of patients ($P < 0.05$).

ELISA LIV of sera from patients with localized versus disseminated disease. As shown in Fig. 2, sera from patients with disseminated disease, regardless of the duration of EM prior to treatment, had significantly higher LIVs, with their peaks occurring at days 8 to 14 postbaseline. Sera from patients with disseminated disease remained positive by ELISA for a longer time than sera from individuals with localized disease.

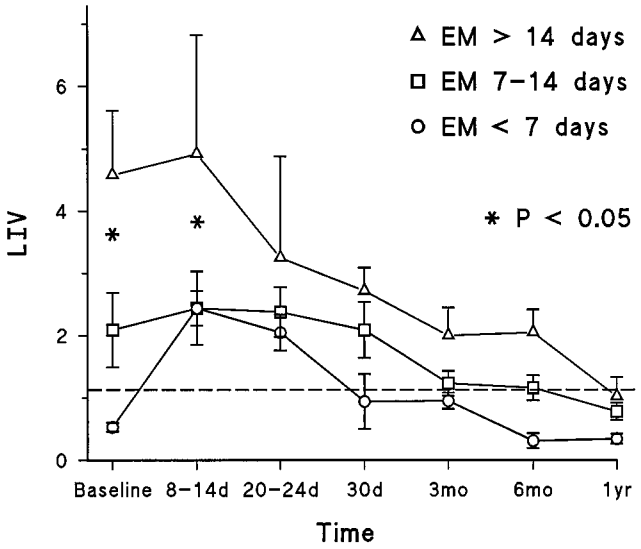

FIG. 1. Means ± standard errors of the means of the LIVs for sera from the three groups of subjects with EM of various durations at different intervals tested for up to 1 year. The dashed line at the LIV of 1.09 indicates the cutoff for ELISA positivity.

IB. As shown in Table 2, IgM IBs were positive for 20 of 46 (43%) of serum samples at the baseline, detecting 5 positive serum samples found to be negative by ELISA. The largest number of positive IgM blots was found at days 8 to 14 postbaseline. Like for the ELISA, the persistence of a positive IgM IB result was also related to disease duration prior to treatment. IgM IBs, however, remained positive for a longer period of time than the ELISA.

The IgG blot reactivity also correlated with the duration of disease at the baseline. Although 24 of 29 (83%) patients with EM of <7 days in duration had an average of three additional bands in IgG blots on follow-up, only one (3%) fulfilled the criteria for positivity for sera collected at day 8 and day 20 postbaseline. Sera from patients with EM of 7 to 14 days in duration prior to treatment also showed an average of three additional IgG bands on follow-up; three (25%) serum samples were positive at the baseline and two additional serum samples became positive at days 8 to 14. Three of five serum samples from patients with EM of >14 days in duration had a positive IgG blot at the baseline, and one more became positive at day 30.

The number of bands in IgM and IgG blots was also directly related to disease duration and to the presence of disease dissemination. Bands increased in number from the baseline up to days 8 to 20 and then declined. At the baseline, sera from patients with EM of <7 days in duration showed an average of 1.1 and 1.6 IgG and IgM bands, respectively, compared with 4.3 and 5.3 IgG and IgM bands, respectively, in the group with disease of 7 to 14 days in duration and 8.2 and 11.6 IgG and IgM bands, respectively, in the group with EM of >14 days in duration. The highest number of IgM bands in the group with disease of <7 days in duration was observed between day 8 (5.6 bands) and day 20 (5.7 bands). The highest number of IgM bands was observed at days 8 to 14 for those with disease of 7 to 14 days and >14 days in duration, with an average of 6.2 and 12.3 bands, respectively, in each group. The highest number of IgG bands was seen in sera collected at day 20 from patients with EM of <14 days in duration but at days 8 to 14 postbaseline in patients with disease of >14 days in duration. The number of IgG and IgM bands was higher in sera from patients with disseminated disease at most intervals tested, as shown in Fig. 3.

IB band evolution. As reported previously (1) and as shown in Table 3 and Fig. 4, IgM reactivity to the OspC (24-kDa) antigen was observed most frequently at the baseline in 48% of serum samples from individuals with disease of short duration and in 59% of all IgM blots at the baseline. Following in frequency, IgM antibody to the 41-kDa antigen was present in 31% of the serum samples from patients with EM of <7 days in duration and in 50% of serum samples from all patients at the baseline. These two IgM bands were present in 84 and 88% of all blots, respectively, at days 8 to 14 postbaseline. Next in frequency was IgM antibody to the 37-kDa antigen, which was present in 35% of all blots at the baseline and in 70% of all blots at days 8 to 14 postbaseline. Although it was present in only 14% of IgM blots of sera from patients with EM of <7 days in duration at the baseline, it was detected in 58% of blots of sera from patients with EM of 7 to 14 days in duration and in all IgM blots of sera from patients with EM of >14 days in duration. IgM antibodies to the 39-kDa antigen were observed in 15% of all blots at the baseline and in 35% of all blots at days 8 to 14 postbaseline. IgM antibodies to 66- and/or 60-kDa antigens were seen in about 20% of all blots at the baseline and up to half of the blots of sera from individuals with EM of ≥7 days in duration at the baseline or days 8 to 14 postbaseline.

IgM antibodies to the 41- and 24-kDa antigens were detectable in about one-third of patients with disease of <7 days in duration at the 1-year follow-up, whereas among the patients with EM of ≥7 days in duration, 70% had IgM antibodies to

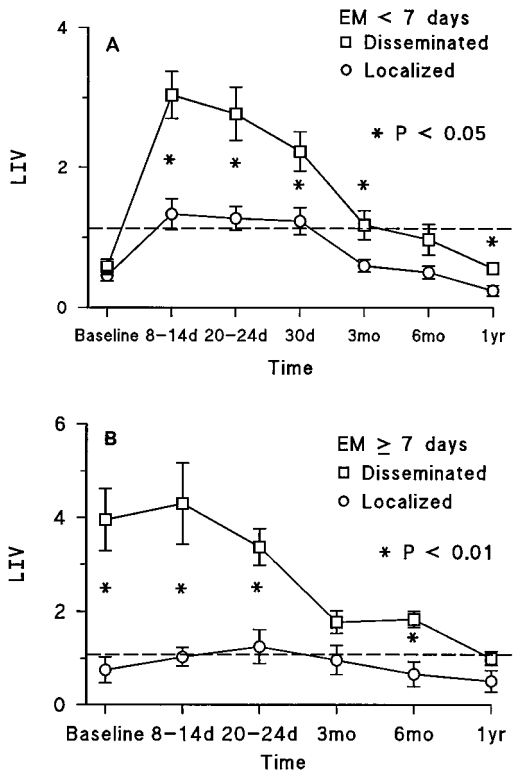

FIG. 2. Comparison of the evolution of ELISA by LIV (mean ± standard error of the mean) according to the presence of localized or disseminated disease in individuals with EM of <7 days in duration (A) or those with EM of ≥7 days in duration (B). The dashed line at the LIV of 1.09 indicates the cutoff for ELISA positivity.

TABLE 2. IgM blot evolution according to disease duration

EM duration (days)	No. (%) of positive blots at[a]:						
	Baseline (n = 46)	8–14 days (n = 43)	20 days (n = 35)	30 days (n = 26)	3 mo (n = 39)	6 mo (n = 37)	1 yr (n = 31)
<7	7 (24)	22 (81)	16 (73)	8 (53)	11 (52)	8 (36)	4 (25)
7–14	8 (67)	10 (83)	8 (80)	6 (86)	7 (64)	5 (50)	5 (71)
>14	5/5[b]	4/4	4/4	3/3	5/5	3/4	3/4
Total	20 (43)	36 (84)	28 (80)	17 (65)	23 (59)	16 (43)	12 (38)

[a] $P < 0.05$ for the comparisons of pairs: <7 days versus 7 to 14 days and <7 days versus ≥7 days at the baseline and 1 year. All other comparisons were not statistically significant.
[b] Number of positive serum specimens/total number of serum specimens tested.

the 41-kDa antigen and 80% had IgM antibodies to the 24-kDa antigen. In this latter group, IgM antibodies to the 37-kDa antigen were present at the 1-year follow-up in 70% of the patients, whereas they were present in 26% of the patients with disease of short duration. IgM antibodies to antigens of 93, 66, 60, 58, 39, and 21 kDa lasted for a shorter period than the antibodies mentioned above and were mostly observed in sera collected within 1 month of the baseline (Fig. 4 and 5).

IgG antibodies (Table 4) in patients with disease of <7 days in duration were less frequent and shorter lasting than those in individuals with disease of ≥7 days in duration: in the first group, IgG antibodies to the 41- and 24-kDa antigens were

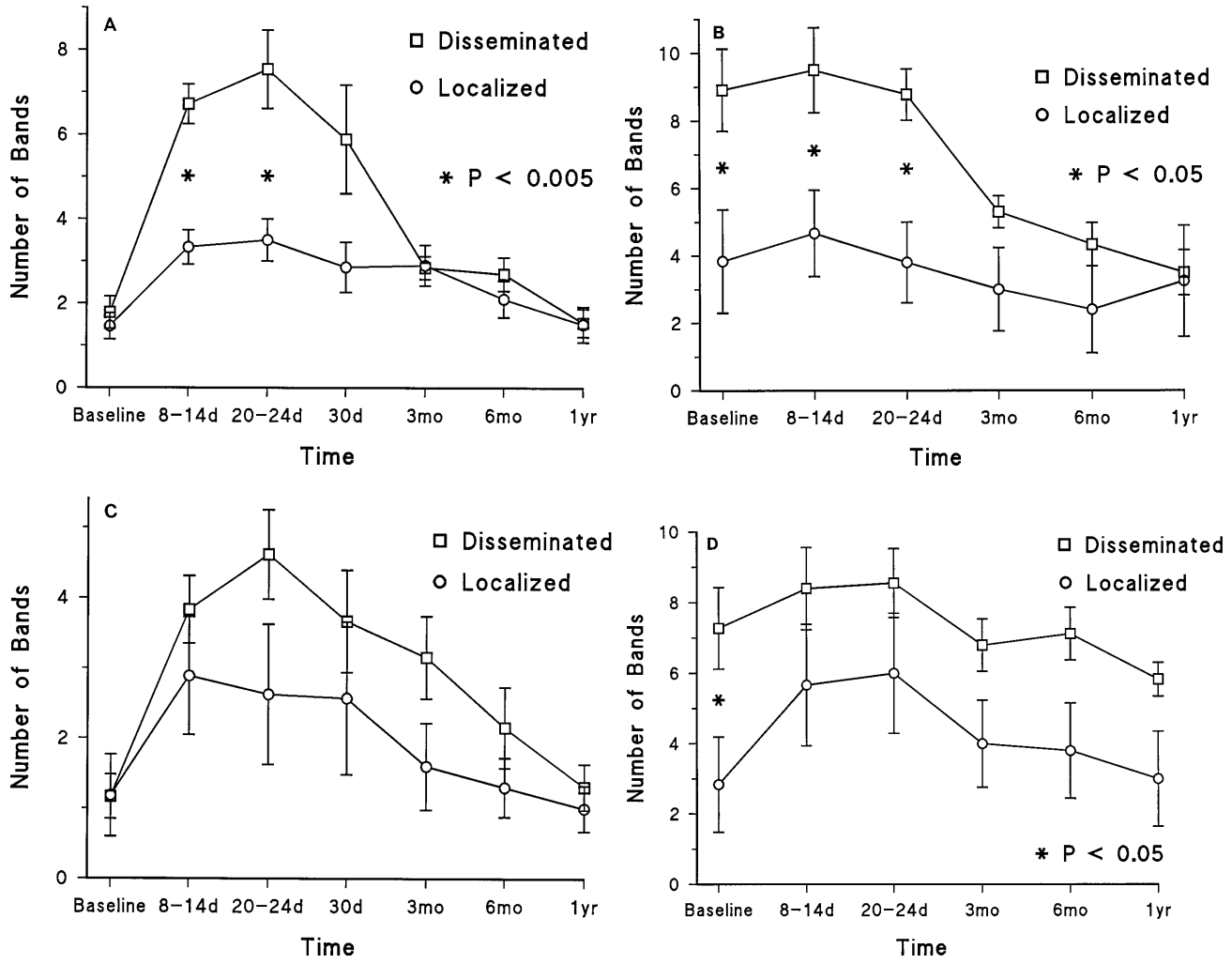

FIG. 3. Comparison of the mean ± standard error of the mean number of bands at different intervals in IgM and IgG blots of sera from individuals with localized or disseminated early Lyme disease. IgM bands in sera from patients with EM of <7 days (A) or ≥7 days (B) in duration and IgG bands in sera from patients with EM of <7 days (C) and ≥7 days (D) in duration are shown.

TABLE 3. Frequency of selected IgM IB bands at baseline and peak[a]

Band (kDa)	% IgM bands			
	EM of <7 days		EM of ≥7 days	
	Baseline	Days 8–14	Baseline	Days 8–14
93	3	11	35	25
66	10	30	41	38
60	3	37	47	44
58	7	15	35	38
41	31	85	82	94
39	3	37	35	31
37	14	63	71	81
24	48	81	76	88
21	3	15	35	44
Avg no. of bands	1.6	5.5	7.3	7.7

most frequently observed at the baseline and the peak. On the other hand, patients with EM of ≥7 days in duration developed IgG antibodies to a larger number of antigens, and these antibodies were detected for a longer period of time. Besides IgG antibodies to the 41- and 24-kDa antigens, IgG antibodies to the 39- and 21-kDa antigens were seen most frequently in sera from patients with disease of ≥7 days in duration, with their highest frequency (64% of blots) occurring at day 20 postbaseline. IgG antibodies to the 18-kDa antigen, which were infrequently seen in blots of sera from patients with disease of short duration, were very commonly seen in IgG blots of sera from patients with EM of ≥7 days in duration. In this group of patients, they were found in 53% of IgG blots of sera collected at the baseline and 75 to 80% of blots of sera collected between day 8 and 3 months postbaseline.

IgM blot positivity of sera from patients with localized versus disseminated disease. In the group of patients with EM of <7 days in duration, sera from 33% of patients with disseminated disease had a positive IgM blot at the baseline, whereas 9% of those with localized disease had a positive IgM blot at the baseline ($P = 0.20$) (Fig. 6 and Table 5). Sera from all 18 patients with disseminated disease had a positive IgM blot result at 8 to 14 days postbaseline, whereas 44% of serum samples from patients with localized EM had positive IgM blots ($P = 0.001$). IgM blots of sera from patients with early

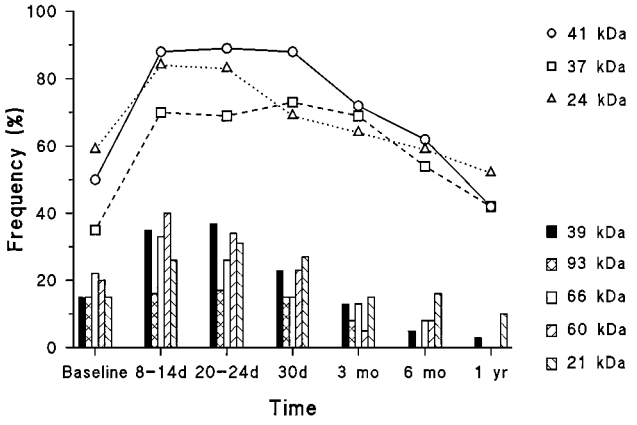

FIG. 4. Frequencies of selected IgM bands in sera from all patients at different time intervals. Those IgM bands seen at higher frequencies are presented with curves, while those seen less frequently are depicted with bars.

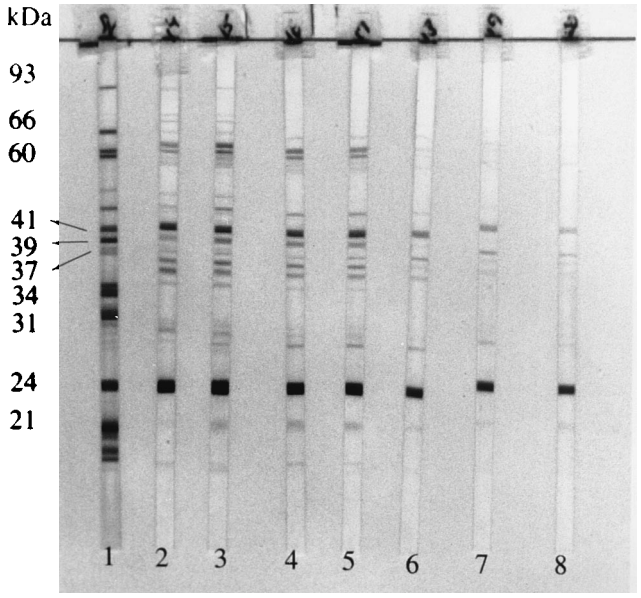

FIG. 5. IgM immunoblots of sera from a patient with early disseminated disease of 7 days in duration prior to treatment. Sera were collected at the baseline (lane 2) and at 8 days (lane 3), 20 days (lane 4), 30 days (lane 5), 3 months (lane 6), 6 months (lane 7), and 1 year (lane 8) postbaseline. Lane 1, positive control.

disseminated disease remained positive longer than those from patients with localized disease. Fifty percent of IgM blots of sera from patients with localized disease of 7 to 14 days in duration prior to treatment were positive at the baseline, whereas 83% of those from patients with disseminated disease of similar duration were positive ($P = 0.54$).

The sensitivities of the ELISA and the IBs at the baseline and days 8 to 14 postbaseline according to disease status and duration are provided in Table 5. Sera from patients with disseminated disease of ≥7 days in duration were found to be positive by ELISA, IgM IB, and IgG IB at the baseline significantly more often than sera from patients with disease of shorter duration or sera from those with localized EM ($P < 0.05$). A total of 67 and 44% of serum samples from patients with localized disease of <7 days in duration prior to treatment had a positive ELISA or IgM IB result at days 8 to 14, respectively, whereas 100% of serum samples from patients with

TABLE 4. Frequency of selected IgG IB bands at baseline and peak[a]

Band (kDa)	% IgG bands					
	EM of <7 days			EM of ≥7 days		
	Baseline	Day 8	Day 20	Baseline	Day 8	Day 20
93	0	4	5	12	25	14
66	0	7	5	12	19	21
60	7	15	24	41	63	64
45	7	30	38	59	75	64
41	28	70	67	76	88	100
39	3	15	33	35	56	64
24	10	52	38	53	63	57
21	7	11	14	29	50	64
18	3	11	19	53	75	79
Avg. no. of bands	1.1	3.5	3.9	5.6	9.8	7.1

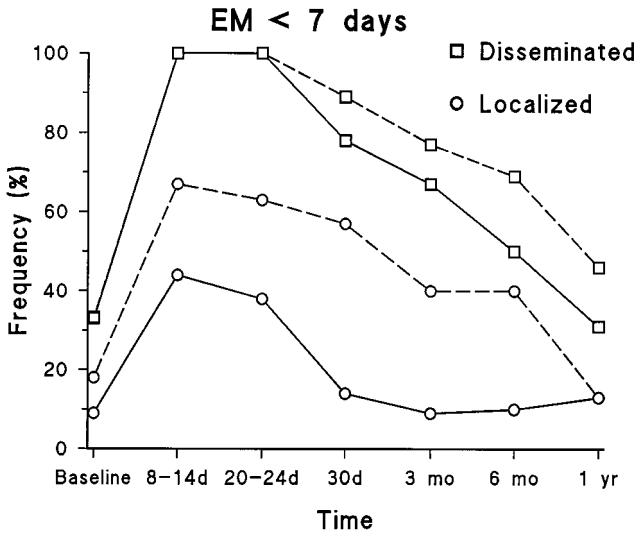

FIG. 6. Comparison of the percentage of positive IgM blots at different time intervals by using the criteria of Engstrom et al. (11) or the criteria that include the 37-kDa antigen among the significant bands in patients with localized or disseminated EM of <7 days in duration. The criteria of Engstrom et al. (11) for IgM positivity are represented by a solid line, while the criteria for IgM positivity that include the 37-kDa antigen are represented by a dashed line.

disseminated disease of short duration were positive by both tests ($P = 0.01$ for ELISA and $P < 0.001$ for IB).

Since IgM antibodies to the 37-kDa antigen were observed more often than antibodies to the 39-kDa antigen at the baseline and the peak, we analyzed the performance of a criterion requiring two of four bands (bands of 41, 39, and 37 kDa and the OspC band) for IgM positivity. Overall, a higher number of IgM blots would have been found to be positive at most time points tested, but the greatest increment in sensitivity was observed in individuals with localized disease of <7 days in duration, as shown in Fig. 6. Had the 37-kDa antigen been included, sera from 8 of 11 of these patients (73%) would have had a positive IgM blot at one or more of the follow-up visits, whereas sera from 36% of the patients would have been positive by using the criteria of Engstrom et al. (11) ($P = 0.19$). Even including the 37-kDa antigen in the IgM criteria for positivity, sera from three patients with localized disease (two with EM of 1 day in duration and one with EM of 8 days in duration) were never found to be positive by the ELISA or the IBs. Sera from two of the patients developed new IgM reactivities to single antigens (24 and 41 kDa) which lasted for a few weeks, and serum from the third patient had two IgM bands that were not considered to be significant.

DISCUSSION

We report here the serologic evolution of patients with culture-confirmed EM. In this very well defined group of patients, we sought to identify the optimal time to the detection of seroconversion and to try to characterize the pattern of immunoreactivity present at the peak of antibody production during the early stages of Lyme disease treated with antibiotics. We previously reported seroconversion in 74 and 64% of a group of clinically characterized patients with EM by ELISA and IB, respectively, by using a single convalescent phase serum specimen obtained 1 month after the baseline (1). In that study, however, we used the criteria of Dressler et al. (10) for blot interpretation. The seroconversion rate observed in the current study was higher (91%), but the peak response was observed in sera obtained 8 to 14 days into treatment. The difference between these results may be attributed to the time at which the convalescent-phase serum specimen was obtained or, possibly, the clinical misdiagnosis of EM. In agreement with Engstrom et al. (11), we recommend that serologic testing for seroconversion be carried out between 8 and 14 days after the baseline.

The commercial ELISA (IgG-IgM) used in the present study was less sensitive than the IgM IB for patients with EM of <7 days in duration (10 and 24% sensitivities, respectively), but the two methods had similar sensitivities for patients with EM of longer duration. Furthermore, the ELISA detected seroconversion in four patients with disease of short duration. Although the IgM IB reactivities of the sera from these patients increased from the baseline, they did not meet the CDC-ASTPHLD criteria for positivity. These four patients, however, had IgM blot reactivities which fulfilled the criteria of positivity according to Dressler et al. (10) or criteria that include the 37-kDa antigen. Since the recommendations of CDC-AST-PHLD state that testing of sera reactive by a first-stage method such as ELISA should then be tested by IB, the proposed criteria for the interpretation of IgM IB results would fail to confirm infection in these patients.

The ELISA LIV correlated directly with the intensity and number of immunoreactive bands observed in the IBs. The highest LIVs were obtained in baseline sera from individuals with EM of more than 1 week in duration and especially in those with EM of more than 14 days in duration. The reactivity of the ELISA declined before that of IB, and as expected, those who had the highest LIVs remained positive the longest during our period of observation. Besides duration of disease,

TABLE 5. Sensitivities of ELISA and IBs at baseline and days 8 to 14 according disease status and duration[a]

EM duration (days)	Localized disease					Disseminated disease			
	Visit	No. of patients	% of patients positive			No. of patients	% of patients positive		
			ELISA	IgM IB	IgG IB		ELISA	IgM IB	IgG IB
<7	Baseline	11	0	9	0	18	17	33	0
	8–14 days	9	67	44	0	18	100	100	6
≥7	Baseline	6	33	50	17	11	91	91	45
	8–14 days	6	50	83	33	10	90	90	60

[a] $P < 0.05$ for comparison of localized versus disseminated disease ELISA and IgM IB at days 8 to 14 in patients with disease of <7 days in duration. $P < 0.05$ for comparison of ELISA, IgM IB, and IgG IB at the baseline for patients with disseminated disease of ≥7 days in duration versus each of the other three groups at the baseline.

the presence of signs or symptoms of dissemination had a direct correlation with the presence and intensity of antibodies to *B. burgdorferi*. Patients with early disseminated Lyme disease had a greater likelihood of having a positive baseline ELISA, and they had higher LIVs at their peak than those with localized disease. Thirteen of the 15 (87%) serum specimens with positive ELISA results at the baseline were from patients with disseminated disease. Other investigators have also demonstrated that patients with early disseminated disease are more likely than those with localized disease to have positive serology at the baseline (17, 22). It has previously been reported that IgM and IgG antibodies, as determined by ELISA, were at their peak at about day 30 in patients with disseminated EM, whereas they were at their peak at day 10 in patients with localized disease (22). Our findings seem to indicate that the peak antibody response, as measured by ELISA, occurred at days 8 to 14 postbaseline in patients with localized or disseminated disease. It is important to keep in mind that ELISA LIVs may vary with different lots of reagents; therefore, when one is following Lyme serology it is necessary to run paired serum specimens by using the same kit lot. In this manner, the relative increase or decrease in antibodies can be determined.

As noted by Dressler et al. (10), we found that IgM antibodies to the 39-kDa antigen are less frequently observed than antibodies to the 37-kDa antigen. It could be postulated that the difference between the higher level of IgM reactivity to the 39-kDa antigen reported by Engstrom et al. (11) compared with our findings is due to the different source of antigens used (strains 297 versus strain B31) and/or a longer duration of disease among the patients in their study. Others have also reported that the 39-kDa antigen is a sensitive and specific marker for Lyme disease (20, 31). The disease duration among the patients included in those studies was unknown, however. In our experience the likelihood that the IgM antibody to the 39-kDa antigen is present increases directly with disease duration, and it is a significant immunoreactive antigen in IgG blots of sera from individuals with EM of long duration.

Undoubtedly, IgM antibodies to the 24- and 41-kDa antigens are observed the most frequently in blots of sera collected at the baseline or during convalescence and are the bands that are present for the longest periods of time. Several reports have indicated that IgM antibody to the 41-kDa antigen is one of the antibodies observed the most frequently in sera from individuals infected with *B. burgdorferi* (5, 8, 15, 20, 36). Wilske et al. (35) first described that antibodies to the 24-kDa antigen (OspC) appeared early in patients in Europe with Lyme disease, and subsequently, her group cloned and expressed this protein (14). Although this antigen has shown some antigenic heterogeneity among European isolates and among isolates from the western United States, it does not seem to limit its immunogenicity (24, 28, 33, 34). Recombinant OspC antigen from strain 2591 has been used to detect IgM antibodies by ELISA in sera from culture-positive patients with EM from the northeastern and midwestern United States and has shown a sensitivity similar to that of IgM IB (24). The expression of OspC is affected in *B. burgdorferi* strains highly passaged in culture (27, 29), which would explain the lack of reactivity to the 24-kDa antigen in earlier reports of Lyme serology which used as a source of antigen strains with high passage numbers (5, 8). The 41- and 24-kDa antigens are strong immunogens during the early stage of disease, which may explain why these antibodies were detected in more than half of our patients at the 1-year follow-up. IgM antibodies to other antigens were observed in sera from patients with EM of more than 1 week in duration at the time of presentation. IgM antibodies to antigens of 93, 66, 60, 58, and 21 kDa were observed in about one-third to two-thirds of sera collected within 1 month of the patient's initial visit. Since these bands usually disappear within a few months after treatment, their presence may signify a relatively recent infection with *B. burgdorferi.*

Although most patients (89%) developed IgG antibodies to various *B. burgdorferi* antigens, only 22% of the patients could fulfill the criteria used for positivity. The duration of EM prior to treatment correlated directly with a positive IgG blot of sera collected within 1 month of the baseline: sera from 1 of 29 (3%) patients with EM of <7 days in duration had a positive IgG blot, whereas sera from 53% (9 of 17) of patients with disease of ≥ 7 days in duration had a positive IgG blot ($P = 0.0001$). Since three of five patients with EM of >14 days in duration had a positive IgG blot when they first sought medical attention, it can be postulated that antimicrobial treatment may preclude the full development of the IgG antibody response but is less likely to do so for the IgM antibody response. The criteria of Dressler et al. (10) for IgG positivity best apply to serology of patients with disease of long duration or those with late Lyme disease. These criteria do not consider the dynamics of the specific IgG reactivity developed early in disease. Analysis of the sequential appearance of IgG bands in our study showed that the appearance of IgG antibody to the 41-kDa antigen is followed by the appearance of IgG antibody to the 24-, 45-, and 39-kDa antigens in individuals with disease of short duration. More immunoreactive bands were observed in individuals with EM of ≥ 7 days in duration, and these immunoreactive bands very frequently included a band of 18 kDa which was seen more frequently than the 21-kDa band. The exact location of the 18-kDa antigen described by Dressler et al. (10) is unclear and may include reactivity to the 21-kDa antigen described by us (which we frequently observe in sera from patients with late Lyme disease) as well as to the 18-kDa antigen. In the blot system used in our study, there are at least three frequently reacting antigens below OspC (24 kDa); the one described in this report as 21 kDa is located at or close to the antigen recognized by the monoclonal antibody to the p22 antigen, and the other two antigens were 18 and 15 kDa. Although the IgG criteria proposed by Engstrom et al. (11) depend on the intensities of certain bands, they appear to be less stringent than the criteria of Dressler et al. (10) for early disease but are difficult to apply because of the uncertain locations of some of the immunoreactive antigens described by Engstrom et al. (11), such as the 35- and 20-kDa antigens. Before these criteria are considered for interpretation of IB results, it is necessary to resolve nomenclature issues and to determine the exact locations of immunoreactive antigens, perhaps by use of monoclonal antibodies. Of interest, most of the patients in the study of Engstrom et al. (11) had an average of 6.7 IgG bands at the baseline, implying that the patients had a long duration of disease before they sought medical attention. In our study an average of 1.1 bands was seen at the baseline in IgG blots of sera from individuals with disease of less than 7 days in duration, in comparison with an average of 5.6 IgG bands in baseline blots of sera from individuals with EM of ≥ 7 days in duration. In 41 of 46 (89%) patients who developed IgG antibodies to *B. burgdorferi* antigens, an average of three additional IgG bands were observed in their follow-up sera. More studies with paired serum specimens from patients with well-defined infectious diseases other than Lyme borreliosis are needed to determine the specificity of IgG blot criteria for Lyme disease requiring fewer bands than the criteria of Dressler et al. (10).

Our study of the serology of treated patients with culture-confirmed early Lyme disease demonstrated that most individ-

uals infected with *B. burgdorferi* who present with EM develop antibodies to the organism. The immunological response correlates directly with the duration of the disease: IgM antibodies begin to appear within few days of the onset of EM and are found in most individuals with disease of at least 2 weeks in duration. The development of antibodies also correlated directly with the presence of signs or symptoms considered by some to be an indication of bacterial dissemination (30, 32). However, it could also be postulated that this subset of individuals is clinically hyperresponsive to the inflammatory mediators initiated by a localized infection with *B. burgdorferi* or the individuals are hyperimmune responders to bacterial antigens. Additionally, the presence of different virulence factors in the *B. burgdorferi* strains, leading to different clinical manifestations early in the course of the disease, cannot be excluded. Studies addressing these issues are needed to provide a better understanding of the pathogenesis of Lyme borreliosis. Whatever the clinical classification of early disseminated Lyme disease may signify, in our study 28 of 29 (97%) patients in this category seroconverted, developed higher titers of antibodies, and remained positive longer than individuals with localized disease. Patients with disease of short duration and particularly those with localized disease with negative serology at the baseline have their peak antibody responses 8 to 14 days into treatment. In our cohort of 46 patients, seroconversion of 5 patients (11%) would have been missed had a convalescent-phase serum specimen only been collected 1 month after the baseline. In concordance with the recommendation of Engstrom et al. (11), we recommend that a convalescent-phase serum specimen be obtained 8 to 14 days into treatment for patients with negative serology at the baseline. Nevertheless, six patients with localized disease, five of whom had disease of <7 days in duration, did not seroconvert according to the guidelines recently proposed by CDC. Three seroconverted by ELISA, but the IgM IB did not meet the criteria for positivity, although two would have been considered positive by IgM if the 37-kDa antigen had been included in the criteria for IgM blot positivity. This indicates that some patients with localized disease who are treated shortly after the onset of disease may not develop a serologic response considered to be diagnostic by the two-step approach for Lyme serology recommended at the Second National Conference on Serologic Diagnosis of Lyme disease.

Since a large proportion of patients in our cohort were still positive by IgM IB at the 1-year follow-up, caution should be exercised when interpreting serology with a single serum specimen from individuals living in areas where Lyme disease is endemic.

It should be further noted that this was a prospective study of patients with well-defined early Lyme disease associated with EM. No inferences as to the specificity of the reactivity observed or the diagnostic utility of similar serologic findings in patients with presentations less clinically consistent with Lyme disease are warranted.

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