JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1997, p. 774–776 Vol. 35, No. 3
0095-1137/97/$04.00+0

Temporal Study of Immunoglobulin M Seroreactivity to *Borrelia burgdorferi* in Patients Treated for Lyme Borreliosis

EILEEN HILTON,[1]* ANTHONY TRAMONTANO,[1] JAMES DEVOTI,[1] AND SUNIL K. SOOD[2]

Division of Clinical Research[1] and Department of Pediatrics,[2] Long Island Jewish Medical Center, the Long Island Campus for Albert Einstein College of Medicine, New Hyde Park, New York 11040

Received 10 June 1996/Returned for modification 20 August 1996/Accepted 12 December 1996

Forty-six patients with late Lyme disease who were considered improved or cured following treatment were monitored by immunoglobulin M (IgM) immunoblotting (mean monitoring period, 27.6 months). There was a persistent IgM response in 32 (97%) of 33 initially positive patients. All but three showed a consistent number, type, and intensity of IgM bands over the entire follow-up period. IgM immunoblotting may not be useful for monitoring the response to treatment of Lyme borreliosis.

Lyme borreliosis is a multisystem disease caused by the spirochete *Borrelia burgdorferi* (13). This is presently the most common arthropod-borne disease in the United States, with 11,144 cases reported in 1994 (2). The illness often begins with a localized infection of the skin (erythema migrans [EM]). This may be followed within days to weeks by dissemination of the spirochete to other organs, including the nervous system and the joints. Diagnosis relies on the recognition of a characteristic clinical picture along with serological confirmation (3, 10, 11).

Serodiagnosis of Lyme borreliosis has been complicated by the cross-reactivity of certain spirochetal polypeptides with other antigens, the delay in the development of the humoral response, the dampening effect of early antibiotic therapy on this response, and the variability of the response in different patients (3, 4, 6, 9–12). Recent recommendations from the Association of State and Territorial Public Health Laboratory Directors and the U.S. Centers for Disease Control and Prevention include a two-test protocol for the diagnosis of Lyme borreliosis. The first step is a sensitive but not very specific serological test, such as an enzyme-linked immunosorbent assay (ELISA), and the second is confirmatory immunoblotting. Both immunoglobulin G (IgG) and IgM responses may be assessed by either of these assays. Currently, IgM immunoblotting is recommended for use in the diagnosis of early Lyme borreliosis and IgG immunoblotting is recommended when more than 4 weeks have elapsed since the onset of infection.

In some infectious diseases, an IgM antibody response occurs during early infection, subsides during convalescence, and may reappear during reactivation. Thus, assessment of IgM responses may be helpful in determination of disease activity or response to therapy. The utility of following serial IgM responses in treated Lyme borreliosis patients is unknown. As patients may have recurrent complaints after treatment for Lyme borreliosis, a laboratory parameter by which disease activity could be monitored would be valuable. We investigated whether the IgM antibody response, as measured by immunoblotting, might be a useful parameter in the assessment of response to treatment of Lyme borreliosis. To answer this question, we analyzed serum samples collected from patients before and after treatment and while they were being monitored over time.

Patients and sera. A diagnosis of Lyme borreliosis by use of the Centers for Disease Control and Prevention criteria was required for inclusion of a patient in the study. Patients had to have late arthritic or neurological manifestations of Lyme borreliosis with or without EM with confirmation by a positive ELISA and IgG immunoblot. Patients were evaluated at the Lyme Diagnostic and Treatment Center at Long Island Jewish Medical Center in New Hyde Park, N.Y. For inclusion, patients were required to have been cured or to have improved in signs and symptoms of late Lyme disease following treatment and to be compliant in returning for follow-up blood tests and clinical evaluations. If a patient developed symptoms of recurrent or new infection at any time during the follow-up period, he or she was excluded from the analysis.

Serum samples were collected from patients with disseminated late Lyme disease just prior to treatment and then at 3 months, 6 months, 12 months, and thereafter every 6 months following treatment and stored at −70°C. At each visit, patients were assessed for signs and symptoms of Lyme borreliosis.

Sera were tested for IgM antibodies to *B. burgdorferi* by ELISA. The IgM ELISA was performed with a commercially available kit (Lyme Stat M; BioWhittaker, Inc., Walkersville, Md.). Briefly, 10-μl volumes of control, calibrator, and patient sera were individually pipetted into tubes containing 150-μl volumes of pretreatment reagent; each specimen was then mixed and allowed to remain at room temperature for 30 min. The tubes were centrifuged to remove the IgG specific antibody. Twenty-five microliters of each supernatant was then added to 100 μl of serum diluent. One hundred microliters of each diluted serum specimen was added to the appropriate well of an antigen plate. These plates were incubated at room temperature with shaking and subsequently washed three times with phosphate-buffered saline containing Tween 20. Next, 100 μl of goat anti-human IgM alkaline phosphatase conjugate was added to each well, and the plates were then incubated for 30 min. After a final wash, 100 μl of phenolphthalein monophosphate substrate was added to each well of every plate, and color was allowed to develop. Sodium phosphate (tribasic) was added to stop the reaction, and the plates were read with a spectrophotometer. A positive result was determined by following a linear regression analysis program supplied by the kit manufacturer.

Immunoblotting was performed with a commercially avail-

* Corresponding author. Mailing address: Long Island Jewish Medical Center, 270-05 76th Ave., New Hyde Park, NY 11040. Phone: (718) 470-4411. Fax: (718) 470-9859.

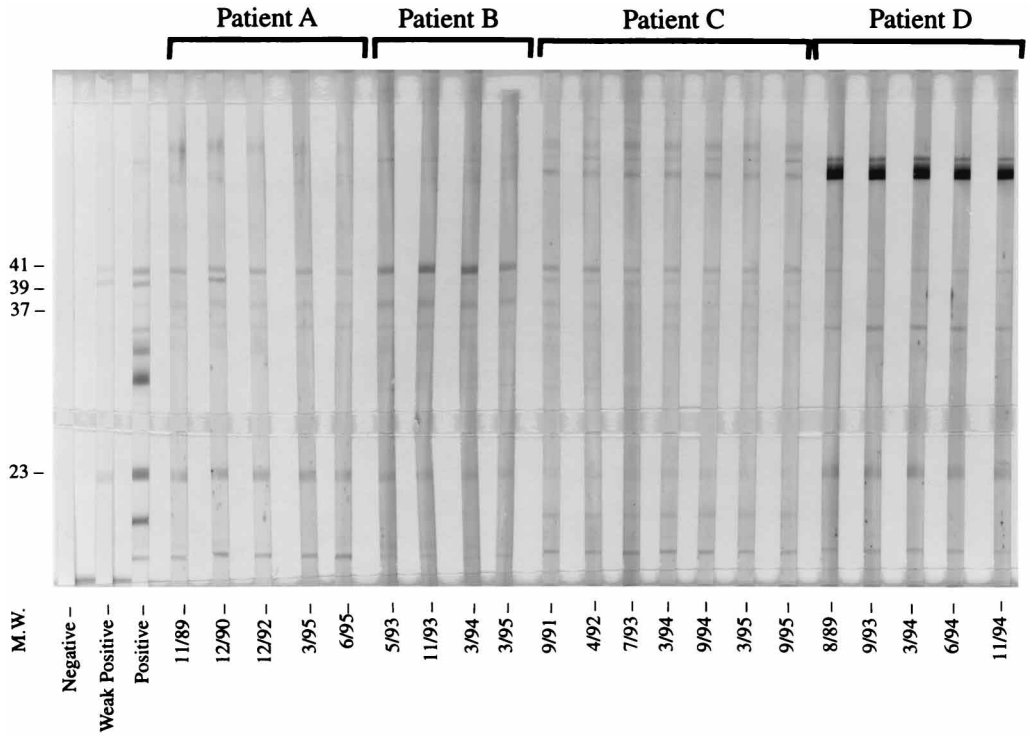

FIG. 1. IgM immunoblot of serum specimens from four different patients. The three lanes on the left are controls. Molecular size standard positions (M.W.; in kilodaltons) are shown to the left of the blot.

able kit (Lyme Disease IgM Marblot strip test system; Mardx Diagnostics, Inc., Carlsbad, Calif.). Briefly, strips were incubated with a 1:100 dilution of serum, washed with buffer (5% milk in phosphate-buffered saline) three times, incubated with a 1:100 dilution of goat anti-human IgM conjugated to alkaline phosphatase, and washed again. Substrate was added to each strip, and the strips were incubated until control strips were sufficiently developed. Positive, weak positive, and negative control samples supplied in the kit were tested simultaneously with patients' samples. The presence of any two of the following three bands—24 (OspC), 39, and 41 kDa—was indicative of a positive reaction. For both the ELISA and immunoblot determinations, all samples from each individual patient were run simultaneously on the same day by the same technician.

We have shown that IgM immunoblotting is a useful confirmatory test for *B. burgdorferi* infection in our patient population (8). In patients with acute facial palsy, the test was 100% specific in that no control patients with Bell's palsy were positive. It was at least 80% sensitive for the diagnosis of Lyme disease and possibly 94 to 100% sensitive if patients deemed clinically unlikely to have Lyme disease were excluded.

Of 51 patients evaluated for Lyme disease who had serial blood samples tested, 46 met the criteria for inclusion in the study. Of the five patients who were excluded, three had developed symptoms and signs of Lyme disease during the follow-up period and two had failed to keep regular appointments. There were 18 males and 28 females included in the study. The age range was 22 to 75 years (mean, 48 years). None of these patients was considered to have early Lyme disease (symptoms starting within 6 weeks of the first evaluation). The duration of symptoms prior to treatment ranged from 2 months to 8 years (mean, 2.5 years). The range of the follow-up period was 12 to 72 months (mean, 27.6 months) after the end of treatment. The case-defining signs and symptoms were primarily arthritic in 25 (54%) and primarily neurological in 14 (30%). The remaining seven (15%) had both arthritic and neurological symptoms. Patients were treated with either ceftriaxone (2 g per day parenterally) or doxycycline (100 mg twice a day orally) for a course ranging from 21 to 28 days. All of the patients showed marked and stable improvement in signs and symptoms for the duration of the follow-up period.

IgM seroreactivity results. Thirty-four of the 46 patients (74%) were positive by IgM immunoblotting at at least one time point during the study. Thirty-three of the 34 were positive prior to treatment. Of these 33, 32 (97%) remained positive over time, with 29 showing a consistent number, type, and intensity of bands over the follow-up period. Examples of the consistent nature of positive IgM blots over 2 to 5 years of follow-up are shown in Fig. 1.

Thirteen patients were negative when first tested; of these, only one became positive. This patient became positive 4 months into the follow-up period without any change in symptoms and remained positive throughout follow-up (16 months). The other 12 remained negative throughout the study.

An evaluation of the patterns of all positive IgM blots showed that the most frequently detected reactivity was with the 41-kDa (100%) and the 24-kDa (84%) bands, with all of the positive patients showing reactivity with the 41-kDa band and either the 24- or 39-kDa band (or both).

The results of IgM ELISAs performed on initial and all follow-up serum samples strongly supported the immunoblot determinations. Of the 34 patients who were immunoblot positive at some point during the study, 31 (91%) were also IgM ELISA positive. Of the 13 patients who were negative by IgM immunoblotting, only 2 were positive by the IgM ELISA.

Comments. IgM immunoblotting is an important confirmatory test in the diagnosis of early Lyme borreliosis. IgM antibody is usually detected after a patient's first exposure to an

infectious agent. In other infectious diseases, the identification of specific IgM antibodies may be helpful in early detection and has been used in the monitoring of disease activity.

Earlier studies have shown the persistence of IgG against *B. burgdorferi* in serum for years in patients with untreated Lyme borreliosis, including those who have spontaneously recovered from this disease, and in persons assessed in epidemiological screening surveys (3, 10, 11). Some studies of the serological response to Lyme borreliosis have included a few patients with early Lyme borreliosis in whom persistent IgM antibodies to *B. burgdorferi* were detected by sonicated *B. burgdorferi* ELISA (3), OspC ELISA (7), or IgM immunoblotting (6). Others have found persistently positive IgM immunoblots in treated EM patients (1, 5). Our study specifically assessed patients with late Lyme borreliosis who were stable and who improved after treatment in order to assess whether IgM could be used to monitor recurrences. We chose this population because its constituents had improved and were expected to have negative IgM serology results. We found that the majority of patients remain immunoblot positive after treatment. Moreover, the band patterns are remarkably consistent despite stable clinical improvement.

All patients in this study had late Lyme borreliosis and had not been treated until well after the initial infection. Thus, it appears that IgM antibodies can persist after treatment of late Lyme borreliosis. In contrast, the positive IgM immunoblots of most patients treated for early Lyme disease revert to negative. Six of seven patients with Lyme facial palsy were immunoblot negative by 3 months posttreatment (8). Only about one-third of treated EM patients had persistently positive IgM immunoblots after 1 year (1, 5). The clinical significance of this difference in IgM antibody persistence is unclear. It is also possible that persistent subclinical or indolent infection with *B. burgdorferi* accounts for the persistence of IgM antibodies. However, given the clinical improvement and stability of the course of infection in these patients, if persistent antigenic stimulation is present, its clinical relevance is questionable.

Our results show that IgM antiborrelial antibodies may persist for months to years after therapy. Although IgM immunoblotting is generally accepted as a reliable aid for the diagnosis of early Lyme borreliosis, it may be of limited or no use in monitoring the progression of disease or response to therapy.

We thank Carol Singer for her critical review of the manuscript and Elyse Bellefond for her excellent secretarial assistance.

REFERENCES

1. **Aguero-Rosenfeld, M. E., J. Nowakowski, J. S. Bittker, D. Cooper, R. B. Nadelman, and G. P. Wormser.** 1996. Evolution of the serologic response to *Borrelia burgdorferi* in treated patients with culture-confirmed erythema migrans. J. Clin. Microbiol. **34:**1–9.
2. **Centers for Disease Control and Prevention.** 1995. Lyme disease—United States. Morbid. Mortal. Weekly Rep. **44:**590–591.
3. **Craft, J. E., R. L. Grodzicki, and A. C. Steere.** 1984. The antibody response in Lyme disease: evaluation of diagnostic tests. J. Infect. Dis. **149:**789–795.
4. **Dressler, F., J. A. Whalen, B. N. Reinhart, and A. C. Steere.** 1993. Western blotting in the serodiagnosis of Lyme disease. J. Infect. Dis. **167:**392–400.
5. **Engstrom, S. M., E. Shoop, and R. C. Johnson.** 1995. Immunoblot interpretation criteria for serodiagnosis of early Lyme disease. J. Clin. Microbiol. **33:**419–427.
6. **Feder, H. D., M. A. Gerber, W. W. Luger, and R. W. Ryan.** 1992. Persistence of serum antibodies to *Borrelia burgdorferi* in patients treated for Lyme disease. Clin. Infect. Dis. **15:**788–793.
7. **Fung, B. P., G. L. McHugh, J. M. Leong, and A. C. Steere.** 1994. Humoral immune response to outer surface protein C of *Borrelia burgdorferi* in Lyme disease: role of the immunoglobulin M response in the serodiagnosis of early infection. Infect. Immun. **62:**3213–3221.
8. **Jain, V. K., E. Hilton, J. Maytal, G. Dorante, N. T. Ilowite, and S. K. Sood.** 1996. Immunoglobulin M immunoblot for diagnosis of *Borrelia burgdorferi* infection in patients with acute facial palsy. J. Clin. Microbiol. **34:**2033–2035.
9. **Ma, B., B. Christen, D. Leung, and C. Vigo-Pelfrey.** 1992. Serodiagnosis of Lyme borreliosis by Western immunoblot: reactivity of various significant antibodies against *Borrelia burgdorferi*. J. Clin. Microbiol. **30:**370–376.
10. **Sood, S. K., L. S. Zemel, and N. T. Ilowite.** 1995. Interpretation of immunoblot in pediatric Lyme arthritis. J. Rheumatol. **22:**758–761.
11. **Steere, A. C., E. Taylor, M. L. Wilson, J. F. Levine, and A. Spielman.** 1986. Longitudinal assessment of the clinical and epidemiological features of Lyme disease in a defined population. J. Infect. Dis. **154:**295–300.
12. **Steere, A. C., N. H. Bartenhagen, J. E. Craft, G. J. Hutchinson, J. H. Newman, D. W. Rahn, L. H. Sigal, P. N. Spieler, K. S. Stenn, and S. E. Malawista.** 1983. The early clinical manifestations of Lyme disease. Ann. Intern. Med. **99:**76–82.
13. **Steere, A. C., R. L. Grodzicki, A. N. Kornblatt, J. E. Craft, A. G. Barbour, W. Burgdorfer, G. P. Schmid, E. Johnson, and S. E. Malawista.** 1983. The spirochetal etiology of Lyme disease. N. Engl. J. Med. **308:**733–740.