Persistence of Immunoglobulin M or Immunoglobulin G Antibody Responses to *Borrelia burgdorferi* 10–20 Years after Active Lyme Disease

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The interpretation of serological results for patients who had Lyme disease many years ago is not well defined. We studied the serological status of 79 patients who had had Lyme disease 10–20 years ago and did not currently have signs or symptoms of active Lyme disease. Of the 40 patients who had had early Lyme disease alone, 4 (10%) currently had IgM responses to *Borrelia burgdorferi*, and 10 (25%) still had IgG reactivity to the spirochete, as determined by a 2-test approach (enzyme-linked immunosorbent assay and Western blot). Of the 39 patients who had had Lyme arthritis, 6 (15%) currently had IgM responses and 24 (62%) still had IgG reactivity to the spirochete. IgM or IgG antibody responses to *B. burgdorferi* may persist for 10–20 years, but these responses are not indicative of active infection.

Lyme disease in the United States is caused by infection with the tickborne spirochete *Borrelia burgdorferi* sensu stricto [1]. Determining the antibody response to the spirochete is the most common laboratory test used to support the diagnosis [2–6]. The specific IgM response peaks during the first several weeks of disease and is generally highest among patients with early disseminated infection. The IgG response may require several additional weeks to develop and is usually highest months to years later in individuals with Lyme arthritis.

The Centers for Disease Control and Prevention (CDC) currently recommends a 2-test approach, using ELISA and Western blot, for the serodiagnosis of Lyme disease [7]. The ELISA gives information about the amount of antibody, whereas the Western blot shows the proteins of the spirochete to which the antibody is directed. The IgM assay should be used to support the diagnosis only during the first 1 month of illness, when most patients have erythema migrans. After that time, almost all patients with active disease have a positive IgG test result [2]. A major limitation of a true positive serological result is that it cannot accurately distinguish active from past infection. However, little information is available regarding the characteristics and duration of seroreactivity years after active infection.

The clinical features of Lyme disease were originally described in several hundred patients from the region of Lyme, Connecticut, who were evaluated at the Yale University School of Medicine from 1976 through 1984 [8–10]. Initially, patients were not treated with antibiotic therapy; later, antibiotic regimens were tested for each manifestation of the illness [11–13]. As a part of
the protocol, serum samples were frozen for subsequent determinations.

We recently completed a 10- to 20-year follow-up evaluation of 84 of these original study patients who were randomly selected from those seen with early or late manifestations of the infection [14]. As a part of the current evaluation, serum samples were obtained for the determination of antibody levels. We report here the serological status of the 79 patients for whom previous and current serum samples were available for testing.

**SUBJECTS AND METHODS**

*Study subjects.* The complete protocol for the long-term evaluation of the 84 randomly selected original study patients has been published previously [14]. All of the study patients were originally seen at the Yale University School of Medicine by one of us (A.C.S.); therefore, detailed clinical records and, in most instances, frozen serum samples were still available from the period of active infection. In the initial study years in the late 1970s, patients were not treated with antibiotic therapy; later, antibiotic regimens were tested for each manifestation of the illness [11–13]. Still later, most of the untreated patients were given antibiotics effective against *B. burgdorferi*. The long-term evaluations were done by another of us (R.A.K.) at a field site in East Lyme, Connecticut. All study patients met the CDC criteria for the diagnosis of this infection [15].

*Sero logical testing.* Paired serum samples, consisting of the first sample available from the period of active, early-, or late-stage infection and the sample obtained in follow-up 10–20 years later, were available for 79 of the 84 patients. Separate tests were used to detect IgM and IgG antibodies to *B. burgdorferi* by indirect ELISA with antigen from *B. burgdorferi* strain G39/40, as described elsewhere [2]. Paired samples from the same patient were always tested in adjoining wells on the same plate. The cutoff optical density readings for indeterminate or positive responses (at 405 nm) were 3 SD (for IgG) or 5 SD (for IgM) above the mean optical density of samples from 8 healthy control subjects included on the same plate (these 8 samples were representative of previously tested samples from 50 healthy control subjects). An antibody response was calculated by adjusting the value of each unknown sample with a standard curve made from the dilutions of known positive serum run on the same plate. For IgM, indeterminate responses were defined as 100–200 U and positive responses were >400 U. For IgG, indeterminate responses were 200–400 U and positive responses were >800 U.

Western blots were done with commercial kits (MarDx) made with *B. burgdorferi* strain B31. The blots were interpreted according to the CDC/Association of State and Territorial Public Health Laboratory Directors’ criteria [7]. The IgM blot was considered positive if at least 2 of 3 bands at 23, 39, or 41 kDa were present, and the IgG blot was considered positive if ≥5 of the 10 bands at 18, 23, 28, 30, 39, 41, 45, 58, 66, or 93 kDa were present. By use of the 2-test approach, a sample was considered positive only if the result was indeterminate or positive by ELISA and positive by Western blot.

*Statistical methods.* The numbers of patients with positive results during active infection and long-term follow-up were compared by Fisher’s exact test. The distribution of ELISA values between active infection and follow-up was compared by Wilcoxon signed rank test. Concordance among ELISA and Western blot results was calculated by κ values. All P values are 2-tailed.

**RESULTS**

*Clinical status of study subjects.* Of the 79 patients, 40 had had early, localized, or disseminated Lyme disease 10–20 years ago (the early disease group), and 39 had Lyme arthritis, which is a late manifestation of the illness (the late disease group). Of the 40 patients with early disease, 8 had localized erythema migrans and 32 had early disseminated infection; 15 of these 32 had facial palsy, meningitis, or radiculoneuritis. At the 10- to 20-year follow-up evaluation, the 79 patients were found to have good overall health [14]. Although some patients had sequelae, such as mild residual facial palsy, no patient had signs of active infection. In addition, no patient reported a clinical picture suggestive of reinfection during the intervening years, such as recurrent erythema migrans or tick bite followed by florulike illness.

*Sero logical results for patients who had had early Lyme disease.* The numbers of patients with positive IgM or IgG responses to *B. burgdorferi* are shown in table 1, as determined by ELISA, Western blot, or both. A positive result by the 2-test approach requires an indeterminate or positive result by ELISA and a positive result by Western blot.

By the 2-test approach, 33 (83%) of the 40 patients with early Lyme disease had a positive IgM response to *B. burgdorferi*, 19 (48%) had a positive IgG response, and 35 (88%) had a positive IgM or IgG response. The 5 patients who did not meet criteria for a positive IgM or IgG response by the 2-test approach had early localized infection of the skin. Three of these 5 patients had an ELISA with an IgM result in the indeterminate range, but they had a Western blot with a negative result. In comparison, in follow-up 10–20 years later, 4 patients (10%) still had a positive IgM response to the spirochete (P < .00001), and 10 other patients (25%) currently had a positive IgG response (P = .06). The 4 patients who still had positive IgM responses had had IgM reactivity with 4–7 spirochetal
Table 1. Positive and indeterminate antibody responses to *Borrelia burgdorferi*, as determined by ELISA, Western blot, or both, in patients who had early Lyme disease or Lyme arthritis.

<table>
<thead>
<tr>
<th>Test, antibody, result</th>
<th>No. (%) of patients</th>
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<tbody>
<tr>
<td></td>
<td>With early Lyme disease</td>
</tr>
<tr>
<td></td>
<td>(n = 40)</td>
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<tr>
<td></td>
<td>During active infection</td>
</tr>
<tr>
<td>ELISA</td>
<td></td>
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<tr>
<td>IgM</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>20 (50)</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>17 (43)</td>
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<tr>
<td>IgG</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>17 (43)</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>11 (28)</td>
</tr>
<tr>
<td>Western blot</td>
<td></td>
</tr>
<tr>
<td>IgM</td>
<td>35 (88)</td>
</tr>
<tr>
<td>IgG</td>
<td>20 (50)</td>
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<tr>
<td>ELISA and Western blot</td>
<td></td>
</tr>
<tr>
<td>IgM</td>
<td>33 (83)</td>
</tr>
<tr>
<td>IgG</td>
<td>19 (48)</td>
</tr>
<tr>
<td>IgM or IgG</td>
<td>35 (88)</td>
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proteins during active infection, including several bands not used in the diagnostic criteria. In the follow-up evaluation, the number of IgM and IgG bands had decreased, but at least 2 diagnostic IgM bands were still apparent. Altogether, 14 patients (35%) were still seropositive by the 2-test approach 10–20 years after having early Lyme disease.

During early disease, the median IgM response was 300 U and the median IgG response was 400 U by ELISA (figure 1, left); 10–20 years later, the median IgG response was still 400 U, and IgM reactivity was usually no longer detectable. During active disease, Western blot frequently detected IgM or IgG reactivity with the 23-kDa outer surface protein C (OspC) and the 41-kDa flagellar protein, sometimes with the 18-, 39-, 45-, 58-, 66-, or 93-kDa proteins, but rarely with the 28-, 30-, or 31-kDa (OspA) proteins or the 34-kDa (OspB) proteins (figure 2, left). At long-term follow-up, IgM reactivity was found almost exclusively with the 23- and 41-kDa proteins, whereas IgG responses to bands that had been present during active infection were often still apparent.

**Serological results for patients with late Lyme disease.** During active Lyme arthritis, 15 (38%) of the 39 patients had a positive IgM response to *B. burgdorferi*, and all had a positive IgG response by the 2-test approach (table 1). At long-term follow-up, 6 (15%) still had IgM reactivity with the spirochete (*P* = .04), and 24 (62%) had a positive IgG response (*P* < .0001). Altogether, 26 patients (67%) were still seropositive by the 2-test approach 10–20 years after having active Lyme arthritis. Although most patients who remained seropositive at long-term follow-up had a positive IgG antibody response, 2 patients in this group (5%) had a positive IgM response only. During active infection, both of these patients had IgM and IgG responses to multiple spirochetal proteins. In the follow-up evaluation, the number of IgM and IgG bands had decreased, but 2 diagnostic IgM bands were still apparent.

During active Lyme arthritis, the median IgM response by ELISA was 100 U, and the median IgG response was 12,800 U (figure 1, right); 10–20 years later, the median IgG response had declined 6-fold, to 1600 U, and only a few patients still had low-level IgM reactivity with the spirochete. During active arthritis, the patients often had IgM reactivity to the 23- and 41-kDa proteins and IgG responses to ≧10 spirochetal proteins, and about one-third had reactivity with the OspA and OspB proteins (figure 2, right). At long-term follow-up, IgM reactivity had faded considerably, but IgG reactivity was usually still present, with bands most often present at 18, 39, 41, 58, and 93 kDa.

**Concordance of ELISA and Western blot results at long-term follow-up.** The concordance of ELISA and Western blot results was similar for patients with early disease and those with Lyme arthritis, and therefore, data from both groups are presented together here. All 18 patients with a negative IgG response by ELISA had a negative Western blot result, but 27 (44%) of the 61 patients who had a positive or indeterminate IgG response by ELISA had fewer than the required minimum of 5 bands on Western blot (*κ* = 0.36; CI, 0.21–0.52). Of the 22 patients with a positive or indeterminate IgM response by
Figure 1. Antibody responses to *Borrelia burgdorferi* during active infection and at follow-up evaluation 10–20 years later in patients who had early Lyme disease (left) or Lyme arthritis (right), as determined by ELISA. Horizontal bar, median value; cross-hatching, range of negative values. At long-term follow-up, IgM reactivity with the spirochete had often decreased considerably. Although IgG responses had frequently declined, antibody levels were still found throughout the range that had been present during active infection.

ELISA, 12 (54%) had a negative result on Western blot, whereas 6 (11%) of the 57 patients with a negative IgM response by ELISA had a positive result on Western blot (κ = 0.38; CI, 0.15–0.62). Thus, all patients with a negative IgG result by ELISA had a negative Western blot result; similarly, most patients with a negative IgM result by ELISA had a negative Western blot result. However, for about half of the patients with positive or indeterminate IgM or IgG results by ELISA, Western blots no longer had the requisite number of bands.

**Effect of antibiotic treatment.** When the 40 patients in the early disease group were stratified according to whether they received antibiotic treatment for early disease, 5 (50%) of the 10 untreated patients had a positive IgG antibody response 10–20 years later, compared with only 5 (17%) of 30 treated patients (P = .09). In contrast, patients in the late disease group usually had much higher titers during active disease, and there were no differences in the frequency of IgM or IgG seropositivity 10–20 years after disease onset in the 15 of these patients who had received antibiotic therapy, usually parenteral penicillin, for active arthritis, compared with the 24 patients who did not receive antibiotics for arthritis. Therefore, early antibiotic treatment may dampen the antibody response and thereby diminish the longevity of the response. In contrast, the high antibody responses of patients with Lyme arthritis usually persist, albeit at lower levels, regardless of antibiotic treatment.

**DISCUSSION**

In the current study, most patients who had had Lyme arthritis and some who had had early Lyme disease 10–20 years before still had positive IgG antibody responses to *B. burgdorferi*, as determined by the 2-test approach (i.e., by both ELISA and Western blot). In follow-up evaluation, patients with Lyme arthritis often still had moderate-to-high IgG antibody responses by ELISA and reactivity with multiple spirochetal proteins on Western blot. In contrast, early Lyme disease patients who re-
Figure 2. Antibody responses to *Borrelia burgdorferi* during active infection and at follow-up evaluation 10–20 years later in patients who had early Lyme disease (left) or Lyme arthritis (right), as determined by Western blot. At long-term follow-up, IgM responses had often faded considerably, but IgG responses often still demonstrated the same bands that had been present during active infection.

Maintained seropositive usually had low-titer IgG antibody responses and a less-expanded Western blot profile. In a number of patients who were classified as having negative results by the 2-test approach, low-level IgG reactivity was still apparent by ELISA, but only 3 or 4 bands were present on Western blots rather than the requisite ≥5 bands.

Surprisingly, IgM reactivity was also seen in 10% of the patients in the early disease group and in 15% of those in the Lyme arthritis group. Did these IgM responses represent false-positive results or were they due to reinfection? None of the patients currently had signs of active infection, and none remembered clinical signs suggestive of reinfection, such as recurrent erythema migrans or tick bite followed by flulike illness. In addition, all patients who had positive IgM responses at the follow-up evaluation had had IgM reactivity with multiple spirochetal proteins during active infection. Although we cannot prove that IgM reactivity was continuously present, we favor the interpretation that these responses were due to persistence, albeit with some diminution, of the original IgM response. In a study of 43 patients with antibiotic-treated erythema migrans, 84% had IgM immunoblots with positive results 8–14 days after the initiation of treatment, and 38% still had positive IgM responses 1 year later [5].

The specific antibody response is first mounted in regional lymph nodes once *B. burgdorferi* antigens drain there or after the spirochete disseminates, a process that seems often to take several weeks. Because *B. burgdorferi* or spirochetal antigens probably do not persist in these nodes after antibiotic treatment, we postulate that the specific IgM and IgG antibody responses in the current patients, which were still apparent 10–20 years after infection, resulted from memory T and B cells. These antibody responses, particularly the expanded IgG responses associated with late infection, may offer protection from reinfection, which may explain why no patient had a clinical history of reinfection. Although persistence of specific IgG responses is well known after infectious diseases, persistent IgM responses are unusual. There is evidence, however, for a large compartment of IgM-expressing memory B cells in humans [16, 17].

There are few published data on the serological status of
patients who had Lyme disease many years ago. A study of 39 children with Lyme arthritis who had not received antibiotic therapy for the infection found that all still had a positive IgG antibody response to *B. burgdorferi* by ELISA 10–13 years later [18]. Similarly, 98% of our patients with Lyme arthritis, regardless of whether they had received antibiotic treatment, had positive or indeterminate IgG responses by ELISA at follow-up. In 2 other long-term follow-up studies, patients with Lyme disease, primarily those with early infection, were evaluated a mean of 6 years after the end of antibiotic treatment. Of 38 patients from Ipswich, Massachusetts, 47% still had IgG responses to *B. burgdorferi* by ELISA [19]. Of 186 patients from Nantucket, Massachusetts, 44% still had IgG reactivity by ELISA and 35% had a positive Western blot result [20]. In our study, 17% of antibiotic-treated patients who had had early Lyme disease were still seropositive by the 2-test approach 10–20 years after disease onset, which suggests that seroreactivity may continue to decline slowly with time. Because our patients with early infection generally had longer duration of symptoms before diagnosis and treatment than would be the case now, the frequency of long-term seroreactivity reported here may be higher than one would expect today.

In summary, more than half of patients with Lyme arthritis and one-third of those with early Lyme disease still had positive IgM or IgG responses to *B. burgdorferi* 10–20 years after having active infection. These results underscore an important limitation of serological testing: A positive IgG result, even in high titer many years after disease onset, does not distinguish active from past infection. Because a positive IgM response may also persist for years after active infection, this response cannot be interpreted as showing recent infection or reinfection in patients who have previously had Lyme disease unless the appropriate clinical picture is present (usually erythema migrans). If patients with past Lyme disease subsequently develop other illnesses, particularly with joint or neurological features, it is important that a borderline or positive serological test for Lyme disease not cause diagnostic confusion.

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**References**