Clinical Relevance of Different IgG and IgM Serum Antibody Responses to *Borrelia burgdorferi* After Antibiotic Therapy for Erythema Migrans

Long-term Follow-up Study of 113 Patients

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**Objectives:** To investigate the kinetics of anti-*Borrelia burgdorferi* antibodies for a minimum of 1 year after antibiotic therapy in patients with erythema migrans (EM) and to correlate antibody titer kinetics with clinical variables.

**Design:** Retrospective study of serial anti-*B burgdorferi* antibodies in correlation to clinical variables.

**Setting:** University-based hospital.

**Patients:** One hundred thirteen patients with EM.

**Interventions:** Pretreatment and a median of 4 consecutive posttreatment serum samples from median follow-up of more than 400 days were simultaneously investigated for anti-*B burgdorferi* IgG and IgM antibodies. Semiquantitative titers were plotted to identify different groups of antibody kinetics. Individual patients were then stratified to those groups according to their antibody development. A statistical comparison of clinical and therapy-related characteristics among the serologic groups was performed.

**Results:** Anti-*B burgdorferi* IgG and IgM antibody titers developed in 3 distinct courses: persistent positivity across follow-up (IgG: 12 patients, 11%; IgM: 14, 12%), persistent negativity (IgG: 63, 56%; IgM: 47, 42%), and decrease of a positive pretreatment titer to a negative titer approximately 5 months after therapy (IgG: 34, 30%; IgM: 49, 43%). Statistics revealed significant correlations only between persistent positive IgG titers and long disease duration or large EM lesions before therapy.

**Conclusions:** Long duration or large size of EM before therapy correlates with persistence of a positive anti-*B burgdorferi* IgG antibody titer after therapy. Serologic profiles do not depend on the type or duration of therapy or the clinical course thereafter. Thus, antibody testing in the follow-up of patients with EM is inappropriate for the assessment of therapeutic response.

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**LYME DISEASE** is a multisystem infectious disease caused by arthropod-borne spirochetes of the *Borrelia burgdorferi* sensu lato complex.1 The annual incidence rate in endemic countries of the northern hemisphere ranges from more than 50 to more than 400 cases per 100,000 persons.2 It affects both sexes and all age groups. Approximately 70% to 80% of all cases represent erythema migrans (EM), the hallmark of early Lyme disease. Erythema migrans is defined as a round to oval, sharply demarcated, red to bluish red, expanding skin lesion at least 5 cm in diameter, with or without central clearing.1,4 It is accompanied by extracutaneous, nonspecific signs and symptoms in one third to two thirds of patients.3,5 If EM is not treated adequately with antibiotic agents, the infection may spread to various other organs, leading to an early disseminated stage in days to weeks or to a late persistent or progressive stage in months to years.1,7 Manifestations of these stages include, besides other skin diseases (disseminated EM, borrelial lymphocytoma, and acrodermatitis chronica atrophicans), neurologic, musculoskeletal, cardiac, and ocular disorders.1,7

Early antibiotic drug treatment is effective in resolving EM. The skin lesion generally clears within 1 to 4 weeks after initiation of therapy.8-11 Extracutaneous signs and symptoms, usually mild to moderate and intermittent, rarely continue beyond therapy but may sometimes resolve slowly.8-13 Subsequent objective manifestations of Lyme disease (neurologic, cardiac, or joint abnormalities) develop in only a few patients.9,12-14 Patients with true...
treatment failure (persistence or recurrence of EM, major sequelae, or survival of *B burgdorferi*) have to be treated again. On the other hand, 10% to 50% of patients still have some subjective symptoms (post-Lyme disease syndrome), including fatigue, musculoskeletal pain, and cognitive dysfunction, for 1 year or more after treatment of EM. These symptoms are not caused by the persistence of *B burgdorferi* and cannot be affected by repeated courses of antimicrobial drug therapy.

To date, there is uncertainty about the application and interpretation of laboratory tests in the follow-up of patients with EM after antibiotic drug treatment. Analyses of skin biopsy samples for the presence of *B burgdorferi* (specific DNA) from the site of infection by cultivation or polymerase chain reaction are very specific, but their sensitivity is insufficient. Polymerase chain reaction is a quick method, but cultivation of *B burgdorferi* is laborious and does not yield timely results. Furthermore, both procedures are invasive and do not provide any evidence about possible (residual) *B burgdorferi* infection of compartments other than the skin. Culture or polymerase chain reaction testing of other clinical samples (eg, blood or urine) is even less valuable and currently is not recommended for routine diagnosis. In clinical practice, serum anti-*B burgdorferi* antibody analysis is most often applied because of its general availability and convenience and the general assumption of usefulness of serologic testing in the follow-up of patients with infectious diseases. However, owing to a lack of larger specific studies of antibody kinetics and their correlation to clinical variables in treated patients with EM, the value of anti-*B burgdorferi* antibody investigations for the evaluation of a patient after therapy is largely unclear and probably overestimated. For example, physicians tend to retreat patients whose antibody titers do not decline (soon) after therapy.

We, therefore, studied 113 patients with EM to investigate (1) the development of IgG and IgM anti-*B burgdorferi* antibody responses during a minimum of 1 year after standard antibiotic therapy and (2) the correlation of serologic responses with various clinical and therapy-related variables. This study, thus, sought to determine whether serologic follow-up is helpful for the evaluation of therapy efficiency in patients with EM.

### METHODS

#### PATIENTS

From December 1, 2002, through May 31, 2003, we performed a retrospective study of serum IgG and IgM antibody responses to *B burgdorferi* before and after antibiotic therapy in 113 patients with EM. These patients were selected from a series of 1014 consecutive patients with suspected EM seen between June 1, 1994, and December 31, 2000, at the Department of Dermatology, Medical University of Graz. Selection of these 113 patients was based on the following inclusion criteria (Figure 1): (1) a clinical diagnosis of definite EM, that is, a round to oval, sharply demarcated, red to bluish red, expanding erythema at least 5 cm in diameter with or without central clearing made by a dermatologist; (2) treatment with a standard oral antibiotic agent after the clinical diagnosis; (3) clinical follow-up for a minimum of 1 year after therapy;

#### Figure 1. Flow diagram for the selection of 113 study patients. EM indicates erythema migrans.

(4) at least 3 serum samples available for the analysis of anti-*B burgdorferi* antibodies, including a sample from directly before therapy and at least 2 posttreatment samples (the last sample had to be obtained ≥1 year after therapy); and (5) information on the following clinical variables was available: age and sex of the patient, duration of EM before therapy, size of EM, type of EM (solitary or multilocular), presence of associated extracutaneous signs and symptoms, type and duration of antibiotic therapy, and duration of EM and of associated extracutaneous signs and symptoms after initiation of therapy. Exclusion criteria for this study were (1) reinfection, that is, the patient had a previous EM episode or developed a second EM treatment failure (persistence or recurrence of EM, major sequelae, or survival of *B burgdorferi*) have to be treated again. On the other hand, 10% to 50% of patients still have some subjective symptoms (post-Lyme disease syndrome), including fatigue, musculoskeletal pain, and cognitive dysfunction, for 1 year or more after treatment of EM. These symptoms are not caused by the persistence of *B burgdorferi* and cannot be affected by repeated courses of antimicrobial drug therapy.

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#### ANTIBODY TESTING

Serum samples obtained directly before therapy from each of the 113 included patients, and a median of 4 posttreatment serum samples (range, 2-6 samples) obtained during median follow-up of 407.5 days (range, 360-2403 days) and stored at −70°C, were available for enzyme-linked immunosorbent assay (ELISA) testing of IgG and IgM antibodies to *B burgdorferi*. The test antigen of the ELISA kit (IDEIA; Dako, Glostrup, Denmark) is purified, native flagella of *Borrelia afzelii*, strain DK-1, isolated from a human EM lesion. The highly immunogenic, prototypically stable flagellum antigen elicits an early and strong immune response and shows no variation between strains of different geographic origin. All samples from each patient were tested together at the same time and on the same well microtitration plate to avoid intertest variability in the assessment of the serologic response in a given patient.

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To analyze IgG antibodies by indirect ELISA, each serum sample was diluted 1:200 with sample diluent (10 µL of serum added to 2 mL of sample diluent). Each sample was tested in duplicate by adding 100 µL of the diluted sample to adjacent wells of the antigen-coated 96-microwell plate. After incubation on a shaker at 500 rpm for 1 hour at room temperature, the microtiter plate was washed 4 times with washing buffer. Thereafter, 100 µL of peroxidase-conjugated anti-human IgG antibodies was added to the wells and incubated on a shaker at 500 rpm for 1 hour at room temperature, followed by a washing step. To visualize bound serum anti-\( B \) burgdorferi IgG antibodies, 100 µL of a chromogen (3,3′,5,5′-tetramethylbenzidine plus stabilized peroxide) was added and incubated for 10 minutes at room temperature. The reaction was stopped by the addition of 100 µL of 0.5M sulfuric acid. The optical density (OD) at 450 nm was determined spectrophotometrically (Bio-Rad Coda; Bio-Rad, Munich, Germany). Positive (100 µL of human patient serum in 2 adjacent wells), cutoff (100 µL of human patient serum in 3 adjacent wells), and negative (100 µL of sample diluent without serum in 1 well) control samples, supplied with the test kit, were included on every plate. According to the manufacturer’s instructions for a semiquantitative interpretation (arbitrary units [U]), the mean of the 2 OD values from the duplicate testing of each sample was calculated and adjusted to a standard curve, which results from a serial 2-fold dilution of the positive control. In the range of OD values between OD\(_{\text{IgG cutoff control}}\) and OD\(_{\text{IgG positive control}}\), the OD values of tested serum samples correspond directly to the logarithm of arbitrary units of specific antibodies in serum samples. The level of specific antibodies in the IgG cutoff control sample was defined as 1 U. The level of specific antibodies in the IgG positive control sample was set to 8 U. For calculation of the semiquantitative IgG antibody level in a tested serum sample, the following formula was applied:

\[
U_{\text{serum sample}} = 10^a; \quad a = \frac{(\text{OD serum sample} - \text{OD IgG cutoff control})}{0.9 \times \text{OD IgG positive control} - \text{OD IgG cutoff control}}
\]

The multiplier 0.9 results from the subtraction

\[
\log U_{\text{IgG positive control}} - \log U_{\text{IgG cutoff control}} = \log 8 - \log 1.
\]

The resulting anti-\( B \) burgdorferi IgG antibody level was reported as negative for U values less than 0.9, as cutoff for U values of 0.9 to 1.09, and as positive for U values greater than 1.09.

To analyze IgM antibodies in serum samples, a capture ELISA was applied. In this assay, the 96-well microtiter plate is coated with anti-human µ-chain antibodies that bind preferentially human IgM antibodies. For duplicate testing, 100 µL of each 1:200-diluted serum sample were added to 2 adjacent wells and incubated on a shaker at 500 rpm for 1 hour at room temperature. After washing, 100 µL of purified native peroxidase-conjugated flagellum of \( B \) burgdorferi was added to the wells and incubated on a shaker at 500 rpm for 1 hour at room temperature. To visualize specific IgM antigen-antibody reactions, 100 µL of a chromogen (3,3′,5,5′-tetramethylbenzidine plus stabilized peroxide) was added and incubated for 10 minutes at room temperature. The remaining IgM ELISA protocol was the same as for the IgG ELISA. For calculation of the semiquantitative IgM antibody level in a tested serum sample, the level of specific antibodies in the IgM cutoff control sample was defined as 1 U. The level of specific antibodies in the IgM positive control sample was set to 5 U. Thus, the multiplier in the formula for calculation of the semiquantitative IgM antibody level, resulting from \(\log U_{\text{IgM positive control}} - \log U_{\text{IgM cutoff control}}\), was 0.7. The anti-\( B \) burgdorferi IgM antibody levels were reported as negative for U values less than 0.9, as cutoff for U values of 0.9 to 1.09, and as positive for U values greater than 1.09.

For quality control, the mean OD value from duplicate testing of each positive or cutoff serum sample was calculated. Individual OD values differing more than 25% for IgG antibodies or 15% for IgM antibodies from that mean were retested. A test was also repeated if the difference between the OD value of the positive control and the cutoff control was less than 0.500 for the IgG ELISA and 0.800 for the IgM ELISA or if the OD value of the negative control was greater than 0.100 or less than 0.000.

**STATISTICAL ANALYSIS**

Plotting of anti-\( B \) burgdorferi IgG and IgM antibody levels from before therapy and all follow-up dates of the 113 study patients identified 3 different courses of titer kinetics for each antibody class. In the next step, individual patients were stratified to those courses according to their IgG and IgM antibody response. A comparison of the clinical variables of the patients in the 3 groups was performed using the following methods. The Kruskal-Wallis test was applied to compare age, duration of EM before therapy, size of EM (largest diameter), duration of antibiotic therapy, and duration of EM and of extracutaneous signs and symptoms after initiation of therapy among the groups. The \(\chi^2\) test was used for comparisons of sex, EM type (solitary vs multilocular), presence of extracutaneous signs and symptoms, and type of antibiotic drug therapy among the groups. If these tests yielded a significant correlation for 1 of the variables, a subsequent pairwise comparison between any 2 of the 3 groups was performed using the Mann-Whitney test or the \(\chi^2\) test. A Bonferroni-adjusted critical value of \(P=0.05/3\) was used to determine statistical significance in these post hoc pairwise comparisons to provide an overall test \(\alpha\) level of .05. \(P\) values were 2-tailed.

**RESULTS**

**CLINICAL CHARACTERISTICS**

One hundred thirteen patients with EM (median age, 51 years; age range, 5-78 years) were investigated in this study. Sixty-one patients (54%) were females and 52 (46%) were males. The median duration of EM before therapy was 14 days (range, 1-180 days). The median size of the EM lesions (largest diameter) was 13 cm (range, 5-50 cm). Ninety-nine patients (88%) had a solitary EM lesion, and 14 (12%) had multiple EM lesions. Seventy-seven patients (68%) had EM alone with no associated signs and symptoms. The remaining 36 patients (32%) had EM with extracutaneous signs and symptoms, including headache, elevated temperature, arthralgias, myalgias, and fatigue. Seventy-seven patients (68%) were treated with tetracyclines (peroral doxycycline hyclate or minocycline hydrochloride), and 36 (32%) received \(\beta\)-lactam antibiotics (peroral penicillin V, amoxicillin trihydrate, or intravenous ceftriaxone sodium) in standard dosages. Fifty patients (44%) were treated for 2 weeks, and 63 (56%) were treated for 3 weeks. The EM cleared within a median of 18 days after initiation of therapy (range, 3-180 days). Extracutaneous signs and symptoms persisted for a median of 12 days after initiation of therapy (range, 1-350 days). These signs and symptoms cleared in 22 (61%) of 36 patients during antibiotic therapy. In 10 (28%) of the 36 patients they persisted for less than 1 month after therapy, and in 3 (8%) they persisted for 2 to 5 months. In 1 patient (3%), symp-
The analysis of anti–B. burgdorferi IgG and IgM antibodies before and after antibiotic therapy in the 113 study patients identified 3 distinct profiles of titer kinetics for each antibody class: persistence of a positive antibody titer throughout the observation period (PP), persistence of a negative antibody titer during their symptomatic period (PN), and decrease of a positive pretreatment antibody titer to a negative antibody titer at any time during follow-up (P-N) (Figure 2). A PP antibody profile was observed for the IgG class in 63 patients (56%). The median follow-up of these patients was 413 days (range, 363-1173 days). The antibody titer of these patients became negative a median of 137 days after the end of therapy (range, 1-686 days). The course of antibody titers of a few patients could not be attributed to any of the 3 distinct profiles. In 4 (4%) of 113 initially seronegative patients, a positive IgG antibody titer first occurred a median of 349 days (range, 287-908 days) after treatment. The same profile of antibody development was observed for the IgM class in 3 (3%) of 113 patients. The IgM antibody titer in these patients first became positive a median of 350 days (range, 301-386 days) after therapy. We assumed that the development of anti–B. burgdorferi antibodies at this late point in follow-up was not directly related to the original EM, and those patients were not included in the following statistical analyses.

Table 1. Clinical Characteristics and Therapy of 113 Patients With EM in 3 Distinct Anti–Borrelia burgdorferi Antibody Titer Profiles

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>IgG Titer (n = 12)</th>
<th>IgM Titer (n = 14)</th>
<th>IgG Titer (n = 63)</th>
<th>IgM Titer (n = 47)</th>
<th>IgG Titer (n = 34)</th>
<th>IgM Titer (n = 49)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (range), y</td>
<td>53 (7-66)</td>
<td>47 (5-70)</td>
<td>49 (5-73)</td>
<td>55 (12-78)</td>
<td>49 (12-78)</td>
<td>46 (9-73)</td>
</tr>
<tr>
<td>Sex, M/F. No.</td>
<td>8/4</td>
<td>8/6</td>
<td>31/32</td>
<td>23/24</td>
<td>12/22</td>
<td>20/29</td>
</tr>
<tr>
<td>EM duration before therapy, median (range), d</td>
<td>28 (2-70)</td>
<td>14 (1-170)</td>
<td>10 (1-150)</td>
<td>14 (2-150)</td>
<td>21 (3-180)</td>
<td>14 (1-180)</td>
</tr>
<tr>
<td>EM size, median (range), cm</td>
<td>16.5 (5-50)</td>
<td>14.5 (7-40)</td>
<td>12 (5-40)</td>
<td>12 (5-40)</td>
<td>15 (5-40)</td>
<td>12 (5-40)</td>
</tr>
<tr>
<td>EM type, solitary/multilocular, No.</td>
<td>11/1</td>
<td>12/2</td>
<td>57/6</td>
<td>45/2</td>
<td>27/7</td>
<td>39/10</td>
</tr>
<tr>
<td>Extracutaneous signs and symptoms, No. (%)</td>
<td>6 (50)</td>
<td>4 (29)</td>
<td>16 (25)</td>
<td>21 (45)</td>
<td>14 (41)</td>
<td>11 (22)</td>
</tr>
<tr>
<td>Therapy, No.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minocycline hydrochloride, 200 mg/d</td>
<td>6</td>
<td>7</td>
<td>35</td>
<td>28</td>
<td>20</td>
<td>26</td>
</tr>
<tr>
<td>Doxycycline hydrochlorate, 200 mg/d</td>
<td>1</td>
<td>0</td>
<td>8</td>
<td>8</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Penicillin V, 4 500 000 IU/d</td>
<td>4</td>
<td>6</td>
<td>14</td>
<td>9</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>Amoxicillin trihydrate, 1500 mg/d</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Ceftriaxone sodium, 2 g/d</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Duration of therapy, 2 wk/3 wk, No.</td>
<td>5/7</td>
<td>5/9</td>
<td>26/37</td>
<td>21/26</td>
<td>15/19</td>
<td>22/27</td>
</tr>
<tr>
<td>EM duration after therapy, median (range), d</td>
<td>23 (3-71)</td>
<td>14 (4-70)</td>
<td>17 (3-80)</td>
<td>27 (3-126)</td>
<td>14 (3-126)</td>
<td>17 (3-180)</td>
</tr>
<tr>
<td>Duration of extracutaneous signs and symptoms after therapy, median (range), d</td>
<td>6 (5-45)</td>
<td>8 (5-42)</td>
<td>12 (4-350)</td>
<td>14 (4-350)</td>
<td>17.5 (1-90)</td>
<td>9.5 (1-150)</td>
</tr>
</tbody>
</table>

Abbreviations: EM, erythema migrans; P-N, declining antibody titer; PN, persistent negative antibody titer; PP, persistent positive antibody titer.

*The total number of patients in the IgG groups is 109 and in the IgM groups is 110 because of the 113 investigated patients, 4 and 3, respectively, were excluded owing to the missing association of their antibody profile with the original EM.
Correlation of Antibody Titer Kinetics with Clinical Variables

Statistical analyses of correlations among the 3 distinct profiles of IgG or IgM antibody titer kinetics and clinical variables revealed the following significant results. Kruskal-Wallis test calculations showed that IgG antibody responses were significantly correlated with the duration of EM before therapy and the size of EM. Subsequent pairwise comparisons among IgG antibody profiles using the Mann-Whitney test with the Bonferroni-adjusted critical value of \( P = .02 \) revealed that the duration of EM before therapy was significantly longer in the PP group than in the P-N group \( (P = .001) \) and in the PN group \( (P = .005) \) (Figure 3). Thus, EM duration before therapy was longest in the PP group and shortest in the PN group. The analogous pairwise comparisons between IgG antibody profiles and the size of EM yielded that EM lesions in the PP group were significantly larger than those in the P-N group \( (P = .001) \) (Figure 4). Moreover, there was a trend for EM lesions in the PP group to be larger than in the PN group \( (P = .02) \). It thus seems that patients in the PP group had the largest EM lesions. Regarding the IgM antibody class, Kruskal-Wallis or \( \chi^2 \) test calculations gave significant correlations between antibody responses and age of the patients, EM type, or extracutaneous signs and symptoms. However, in the post hoc pairwise comparisons with Bonferroni adjustment, those correlations were no longer significant but remained as trends only (correlation between older age and PN antibody profile: \( P = .03 \) vs P-N antibody profile; correlation between multiple EM lesions and P-N antibody profile: \( P = .04 \) vs PN antibody profile; correlation between extracutaneous symptoms and PN antibody profile: \( P = .03 \) vs P-N antibody profile).

Previous studies of the development of serum anti-B. burgdorferi antibodies after antibiotic drug therapy yielded inconsistent results (Table 2). Decline of IgG and particularly IgM antibody titers was found in many patients, but time to decline in titers was variable and unpredictable.26-30,34,35 Second, IgG and IgM titers may persist after adequate therapy.26-33,35 Other patients remain seronegative throughout follow-up.10,14,29,34,36 Finally, it was described that antibody titers may develop for the first time or even rise during or after therapy.8,10,32,35 These studies had several shortcomings, such as small numbers of patients, short follow-up or testing of nonconsecutive samples, or examination of nonseronegative patients. None of these studies were primarily designed to analyze correlations of serologic follow-up data and clinical characteristics.

We investigated anti-B. burgdorferi antibody development for at least 1 year after antibiotic drug therapy in consecutive serum samples from a large series of patients with EM and correlated serologic results statistically with clinical data, including therapy-related variables. Our results are valid for European and American patients because the ELISA used is based on the flagellum antigen that is phenotypically stable between B. burgdorferi strains of different geographic origin.32,34 We found that IgG and IgM antibody responses develop in 3 different profiles. The most common profile was PN. It was found in approximately half of the patients for the IgG and IgM antibody class. Because of the absence of a specific immune response it could be questioned whether the diagnosis of EM was correct in these patients. However, it is generally agreed that the clinical diagnosis of EM is reliable, and international guidelines do not require laboratory confirmation.4,37 In the present study, the clinical diagnosis was made in accordance with defined criteria2,3,38 by dermatologists with recognition rates of EM greater than 90%.39 Furthermore, the clinical diagnosis was substantiated by a typical histopathologic picture, and a positive polymerase chain reaction for B. burgdorferi-specific DNA, or cultivation of B. burgdorferi in 48% and 57% of these patients for IgG and IgM, respectively. Before therapy, IgG or IgM antibodies to B. burgdorferi are lacking in approximately 50% of all patients with EM, particularly in those with shorter disease duration8,10,12,50 because seroconversion does not occur before 2 to 4 weeks of infection. In European patients with EM, infection with B. burgdorferi is often limited to the
skin, which could be another explanation for the absence of a systemic antibody response. After therapy, the lack of antibody development may be due to abrogation of the infection by adequate antimicrobial treatment. A number of treated, initially nonreactive patients may, therefore, remain seronegative. In uncontrolled earlier studies of a total of only 50 patients, 40% to 100% of EM cases were found to remain seronegative during follow-up. The second profile, PP, was found in 11% of patients for the IgG class. In previous German studies, only 3% of patients remained seropositive on follow-up examinations, whereas others reported this phenomenon in 21% to 60% of their patients. The latter studies may have been skewed, however, by the inclusion of only seropositive patients or patients with extracutaneous symptoms. Persistence of IgM antibodies was detected in 12% of our patients, which compares well with an IgM persistence rate of 6% for more than 12 months in a pooled collective of 151 patients in 2 German therapy studies and with a rate of 10% for up to 20 years in 40 US patients with early (disseminated) Lyme disease. It could be speculated that persistent seropositivity indicates a continuing specific immune stimulation by noneradicated spirochetes with the potential consequence of ongoing symptoms or sequelae of Lyme disease. However, molecular and cultural proof of elimination from the skin after antibiotic therapy make survival of in antibiotically treated patients with EM unlikely. In the present study, PP was not correlated with a worse disease outcome, which contradicts such earlier theories. It may instead be assumed that sustained positive titers indicate a long-term serologic memory that results from an antigen-independent polyclonal activation and differentiation of memory B cells. The third profile was P-N an average of 5 months after treatment in approximately one third of the patients (30%) for IgG titers and less than half (43%) for IgM titers. Earlier studies found a decline in IgG antibodies in 10% to 43% and in IgM antibodies in 24% to 100% of investigated patients. However, direct comparison with our study is difficult owing to examination of only a few (partly) preselected patients across variable follow-up periods of 5 months to 10 years.

Statistical analyses revealed only a few significant correlations between clinical characteristics and antibody titer profiles. A persistent positive IgG titer was found most often in patients with long duration or large size of EM before therapy. A correlation between longer duration of EM before therapy and persistence of antibody titers thereafter was already observed earlier in several patients. It thus seems that a persistent anti- antibody immune response develops preferentially in case of a longer pretreatment interaction between the host's immune system and the spirochete. In contrast, early antibiotic therapy may suppress immunologic reactions that lead to a sustained production of anti- antibodies. No significant correlations were found between a given IgM antibody titer profile and any clinical variable, a fact that has not been addressed specifically so far. Neither duration of EM or of extracutaneous signs and symptoms after therapy nor type or duration of therapy correlated with a particular serologic profile. Accordingly, smaller previous studies found no correlation between antibody responses and disease course after therapy. Thus, posttreatment antibody titers do not allow for the assessment of treatment efficiency. Persistent positive IgG or IgM antibody titers after therapy should not lead to further treatment as long as a patient does not have attributable clinical signs and symptoms.

In conclusion, the IgG and IgM immune response to in patients with EM after therapy develops

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**Table 2. Previous Studies on the Serologic Follow-up of Patients With Erythema Migrans**

<table>
<thead>
<tr>
<th>Source</th>
<th>Patients, No.</th>
<th>Follow-up, mo</th>
<th>Development of Anti-<em>Borrelia burgdorferi</em> Antibodies, %</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Åsbrink et al, 1985</td>
<td>5</td>
<td>6</td>
<td>60 PP, 100 P-N</td>
<td>Only patients with extracutaneous symptoms</td>
</tr>
<tr>
<td>Feder et al, 1992</td>
<td>21</td>
<td>2-36</td>
<td>43 PP, 66 PN, 10 P-N, 24 P-N</td>
<td>Uncontrolled therapy studies</td>
</tr>
<tr>
<td>Shadick et al, 1994</td>
<td>21</td>
<td>12-132</td>
<td>47 PP, ND</td>
<td>None</td>
</tr>
<tr>
<td>Aguero-Rosenfeld et al, 1996</td>
<td>46</td>
<td>≤12</td>
<td>16 PP</td>
<td>No differentiation between IgG and IgM antibodies</td>
</tr>
<tr>
<td>Hulshof et al, 1997</td>
<td>10</td>
<td>31-161</td>
<td>80 PN, 100 PN</td>
<td>None</td>
</tr>
<tr>
<td>Stanek et al, 1999</td>
<td>99</td>
<td>3-15</td>
<td>21 PP, 0 PP, 41 PN, 48 PN, 22 P-N</td>
<td>Follow-up of 3 mo only in 50% of patients</td>
</tr>
<tr>
<td>Lombhol et al, 2000</td>
<td>23</td>
<td>9-37</td>
<td>52 PP, 73 P-N, 25 P-P, 35 P-N</td>
<td>None</td>
</tr>
<tr>
<td>Kalish et al, 2001</td>
<td>40</td>
<td>120-240</td>
<td>79 PN, 45 PN</td>
<td>All patients were OspA vaccinated; only immunoblot performed</td>
</tr>
<tr>
<td>Smith et al, 2002</td>
<td>116</td>
<td>0.5-1.0</td>
<td>48 PP</td>
<td></td>
</tr>
</tbody>
</table>

(following table data)

Abbreviations: ND, not done; OspA, outer surface protein A; P-N, decline from positive to negative antibody titer; PN, persistent negative antibody titer; PP, persistent positive antibody titer.
in 3 distinct profiles: persistence of a positive titer, persistence of a negative titer, or decline of a positive to a negative titer. Except for long duration or large size of EM before therapy, which predisposes a patient to persistence of a positive IgG antibody titer after therapy, no correlations exist among serologic profiles and clinical variables. In particular, the serologic profile seems to depend on neither the type nor duration of antibiotic therapy nor the clinical course after therapy. Therefore, antibody testing during follow-up of patients with EM is unsuitable and unnecessary for the assessment of treatment response.

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7. Stanek G, Glatz and Golestani. Critical review of the manuscript for important intellectual content: Kerl and Mullegger. Statistical analysis: Glatz and Mullegger. Administrative, technical, and material support: Glatz, Golestani, and Kerl. Study supervision: Mullegger.


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