Elevated Levels of IL-23 in a Subset of Patients With Post–Lyme Disease Symptoms Following Erythema Migrans

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Background. The causes of post-Lyme disease symptoms are unclear. Herein, we investigated whether specific immune responses were correlated with such symptoms.

Methods. The levels of 23 cytokines and chemokines, representative of innate and adaptive immune responses, were assessed in sera from 86 antibiotic-treated European patients with erythema migrans, 45 with post-Lyme symptoms and 41 without symptoms, who were evaluated prior to treatment and 2, 6, and 12 months thereafter.

Results. At study entry, significant differences between groups were observed for the type 1 helper T cell (TH1)-associated chemokines CXCL9 and CXCL10, which were associated with negative Borrelia cultures, and the type 17 helper T cell (TH17)-associated cytokine interleukin 23 (IL-23), which was associated with positive cultures and the development of post-Lyme symptoms (P ≤ .02). Moreover, of the 41 patients with detectable IL-23 levels, 25 (61%) developed post-Lyme symptoms, and all 7 with IL-23 levels ≥230 ng/mL had such symptoms. Furthermore, antibody responses to the ECGF autoantigen were more common in patients with post-Lyme symptoms (P = .07) and were correlated directly with IL-23 levels (P = .02). Despite the presence of post-Lyme symptoms, all posttreatment culture results were negative, antiborrelial antibody responses declined, and there were no objective signs of disseminated disease, suggesting that spirochetal eradication had occurred with treatment in all patients.

Conclusions. High TH1-associated responses correlated with more effective immune-mediated spirochetal killing, whereas high TH17-associated immune responses, often accompanied by autoantibodies, correlated with post-Lyme symptoms, providing a new paradigm for the study of postinfectious symptoms in a subset of patients with Lyme disease.

Keywords. Lyme disease; post–Lyme disease symptoms; inflammation; IL-23; TH17.

Lyme disease in the United States is caused by Borrelia burgdorferi sensu stricto (Bb), whereas in Europe, the infection is due primarily to Borrelia afzelii and Borrelia garinii [1]. The most common sign of the infection is an initial expanding skin lesion, erythema migrans (EM), which is sometimes accompanied by flulike symptoms [2]. EM typically resolves with 10–21 days of oral antibiotics, and the majority of patients recover completely. However, about 10% of patients in Europe and the United States have persistent or new subjective symptoms, such as headache, fatigue, malaise, arthralgias, or myalgias, in the months after treatment, termed post–Lyme disease symptoms [3].

The term “post-Lyme symptoms” probably consists of >1 syndrome. At one end of the spectrum, one or a few subjective symptoms, such as malaise and fatigue or minor joint symptoms, may persist for several months after antibiotic treatment of EM. At the far end of the spectrum, patients may develop disabling joint and muscle pain, neurocognitive difficulties, and incapacitating fatigue that persist for years after Lyme disease [4–9]. This is sometimes called post–Lyme disease syndrome [4]. This area is further confused by...
the fact that “chronic Lyme disease” has become a diagnosis for disabling, medically unexplained symptoms, even when there is little or no evidence of past or present Lyme disease [4].

Pathogenetic mechanisms that account for post–Lyme disease symptoms remain unclear and are not likely to be the same in all patients. Four double-blind, placebo-controlled antibiotic trials have focused on the hypothesis that these symptoms may result from persistent infection [7–9]. In 2 trials, no significant differences were found between the antibiotic and placebo groups [7]. In the third trial, significant differences were noted only in fatigue for 1–6 months after therapy [8]. In the fourth study, significant differences were initially observed in fatigue and pain, but beneficial effects were not sustained [9]. Moreover, in all 4 trials, microbiologic measures of infection were negative. Finally, posttreatment culture results from EM skin lesions have been negative in almost all patients, including those with post-Lyme symptoms [3, 10].

Mechanisms other than active infection, including the possibility of immune system abnormalities, have also been considered. Heightened antineuronal antibody levels were reported in patients with disabling pain or neurocognitive or fatigue symptoms for years after Lyme disease [11]. In addition, in MyD88−/− mice, retained spirochetal antigens were proposed as a reason for joint symptoms after Lyme disease [12]. However, the causes for postinfectious phenomena after Lyme disease remain poorly understood.

Control of Borrelia burgdorferi sensu lato (Bbsl) infection requires both innate and adaptive immune responses [13]. EM skin lesions often contain high levels of interleukin (IL) 6, IL-10, and interferon (IFN)–γ, and the IFN-γ–inducible chemokines CXCL9 and CXCL10 [14], which orchestrate type 1 helper T cell (TH1) responses [15]. In addition, spirochetes may stimulate the production of IL-23 [16, 17]. This cytokine promotes the proliferation and maintenance of type 17 helper T cells (TH17), which are important for the control of extracellular pathogens, and have also been implicated in autoimmune phenomena [18–20]. Herein we assessed cytokine and chemokine profiles representative of innate and adaptive immune responses in serum samples from Slovenian patients with EM who were followed for 12 months to assess their posttreatment status.

METHODS

Selection of Patients
Initially, 937 patients ≥15 years of age with putative EM were evaluated for participation in clinical trials: an efficacy study of 15-day treatment with doxycycline vs cefuroxime axetil [3] and a trial of 10- vs 15-day treatment with doxycycline [10]. The studies were approved by the Medical Ethics Committee of the Ministry of Health of Slovenia and each patient provided written consent. All patients were evaluated at the Lyme Borreliosis Outpatient Clinic at the University Medical Center Ljubljana, Slovenia. After exclusions, 510 patients with a single EM lesion, as defined using modified Centers for Disease Control and Prevention criteria [3, 10], participated in the study.

Patients were assessed by the study physicians at baseline, and at 2, 6, and 12 months of follow-up about health-related difficulties, including fatigue, arthralgias, myalgias, headache, dizziness, malaise, irritability, nausea, or paresthesias. Symptoms that developed or worsened after the onset of EM that did not have another known medical explanation were regarded as post-Lyme symptoms. The severity of individual symptoms was graded by the subject on an 8-cm visual analogue scale (8 = most severe). Of the 510 patients, 159 (31%) had EM with associated symptoms, 62 (12%) had post-Lyme symptoms at 2 months, and 18 (4%) had symptoms 12 months after the start of antibiotics (Figure 1).

For this study, a total of 86 patients were selected from both treatment trials [3, 10], including all 45 of the 62 patients who reported at least 1 post-Lyme symptom after antibiotic therapy.

![Screened 937 patients](http://cid.oxfordjournals.org/)

**Figure 1.** Selection of patients. Patients for this study were selected from 2 previous European studies [3, 10]. Of the 937 patients evaluated initially, 510 had erythema migrans and were treated with antibiotics and reevaluated over 12 months. Of the 510 patients, 62 had post-Lyme symptoms. For this study, sera were available from 45 of the 62 patients. For comparison, sera were randomly selected from 41 patients whose symptoms resolved with antibiotic therapy. Abbreviation: EM, erythema migrans.
RESULTS

Clinical Characteristics of Patients at Study Entry
For this study, 86 patients with EM were selected, 45 with at least 1 post-Lyme symptom after antibiotic therapy, and 41 without posttreatment symptoms (Table 1). In addition to EM, approximately half of the patients had at least 1 associated symptom, such as headache, myalgias, arthralgias, malaise, or fatigue. Of the 86 patients, 47 (55%) had a positive EM skin biopsy culture for *B. burgdorferi* (predominantly *B. afzelii*), and 55 (64%) had reactivity with the *B. burgdorferi* VlsE C6 peptide. Altogether, 71 (83%) had laboratory documentation of *B. burgdorferi* infection by culture or serology. When stratified according to subsequent development of post-Lyme symptoms, the 2 groups did not differ significantly in age, sex, duration of illness prior to enrollment, the number and intensity of EM-associated symptoms, or positive culture or serology result (Table 1). Thus, at the time of infection, patients who did or did not develop post-Lyme symptoms had similar clinical pictures.

### Table 1. Clinical Characteristics of 86 Patients With Erythema Migrans at Study Entry

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All Patients (N = 86)</th>
<th>No Post-Lyme Symptoms (n = 41)</th>
<th>Post-Lyme Symptoms (n = 45)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>General characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>53 (17–75)</td>
<td>52 (18–75)</td>
<td>56 (17–74)</td>
</tr>
<tr>
<td>Sex, No. female/male</td>
<td>55/31</td>
<td>24/17</td>
<td>31/14</td>
</tr>
<tr>
<td>Time from tick bite to EM, da</td>
<td>16 (1–106)</td>
<td>16 (1–57)</td>
<td>15 (1–106)</td>
</tr>
<tr>
<td>Time from EM to study entry, da</td>
<td>7 (1–62)</td>
<td>6 (1–62)</td>
<td>11 (1–60)</td>
</tr>
<tr>
<td>EM diameter, cm²</td>
<td>13 (3–86)</td>
<td>12 (5–50)</td>
<td>13 (3–86)</td>
</tr>
<tr>
<td><strong>Symptoms at study entry</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of patients with symptoms</td>
<td>45 (51%)</td>
<td>20 (49%)</td>
<td>25 (56%)</td>
</tr>
<tr>
<td>No. of symptoms (≥ 1)</td>
<td>1 (0–6)</td>
<td>1 (0–5)</td>
<td>1 (0–6)</td>
</tr>
<tr>
<td>Intensity of symptoms (≥ 1)</td>
<td>4 (1–8)</td>
<td>4 (1–8)</td>
<td>5 (1–8)</td>
</tr>
<tr>
<td><strong>Laboratory findings at study entry</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of patients with</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive <em>Borrelia</em> skin biopsy culture</td>
<td>47 (55%)</td>
<td>21 (51%)</td>
<td>26 (57%)</td>
</tr>
<tr>
<td>Positive VlsE C6 antibody response</td>
<td>55 (64%)</td>
<td>25 (61%)</td>
<td>30 (67%)</td>
</tr>
<tr>
<td>Positive culture or VlsE result</td>
<td>71 (83%)</td>
<td>31 (76%)</td>
<td>40 (89%)</td>
</tr>
</tbody>
</table>

Abbreviation: EM, erythema migrans.

a Data are expressed as median (range).

b Scale of 0–8, with 8 being the highest intensity.
Cytokine and Chemokine Levels at Study Entry

Of the 15 cytokines and 11 chemokines tested, the only significant differences between groups were in the levels of CXCL9, CXCL10, and IL-23. Therefore, only the results of these mediators are presented here. Of the 86 study patients, 85 had detectable serum levels of CXCL9 and 84 of CXCL10, which are chemoattractants involved in Th1-like immune responses. In addition, 41 of the 86 patients had detectable levels of IL-23, a cytokine that is necessary for the proliferation and maintenance of Th17 cells. The levels of other Th17 mediators (IL-17, IL-21, IL-22, and IL-27) were below the limit of detection in most patients.

When cytokine and chemokine data in the 86 patients were stratified according to culture results at the initial visit, prior to the start of antibiotics, the 39 patients with negative *Borrelia* cultures tended to have higher levels of CXCL9 (1374 vs 847 pg/mL, *P* = .1), and they had significantly higher levels of CXCL10 (412 vs 229 pg/mL, *P* = .02) than the 47 culture-positive patients (Figure 2A). In contrast, the levels of IL-23 were significantly higher in the culture-positive group (217 vs 85 pg/mL, *P* = .05).

To correlate the inflammatory responses with disease severity, CXCL9, CXCL10, and IL-23 levels were stratified by the presence or absence of associated symptoms at study entry, prior to antibiotic therapy (Figure 2B). The levels of CXCL9 were significantly higher (1499 vs 668 pg/mL, *P* < .0001) in patients with symptoms compared to those without and a similar trend was observed for CXCL10 (370 vs 255 pg/mL, *P* = .16). In addition, IL-23 levels were significantly higher in those with symptoms (226 vs 128 pg/mL, *P* = .003). Thus, higher levels of

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**Figure 2.** Cytokines and chemokines stratified according to *Borrelia* culture test result or symptoms at first visit. Protein levels of the Th1-associated chemokines CXCL9 and CXCL10, and of the Th17-associated cytokine IL-23, were assessed in 86 patients with erythema migrans using bead-based multiplex assays. A, Patients were first stratified according to *Borrelia* culture test result at first visit, prior to antibiotic therapy. B, Patients were stratified according to associated symptoms at first visit. The bars represent the mean values and I-bars represent the standard error of the mean. *P* values for comparison of culture-positive vs culture-negative patients, and for comparison of patients with or without associated symptoms, are indicated in the graph. Abbreviations: EM, erythema migrans; IL-23, interleukin 23.
Table 2. Post-Lyme Symptoms During 2–12 Months After Treatment

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Post-Lyme Patients (n = 45)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients with symptoms*</td>
<td></td>
</tr>
<tr>
<td>Arthralgia</td>
<td>23</td>
</tr>
<tr>
<td>Headache</td>
<td>18</td>
</tr>
<tr>
<td>Fatigue</td>
<td>18</td>
</tr>
<tr>
<td>Myalgia</td>
<td>11</td>
</tr>
<tr>
<td>Dizziness</td>
<td>5</td>
</tr>
<tr>
<td>Malaise</td>
<td>4</td>
</tr>
<tr>
<td>Irritability</td>
<td>2</td>
</tr>
<tr>
<td>Nausea</td>
<td>2</td>
</tr>
<tr>
<td>Paresthesias</td>
<td>2</td>
</tr>
<tr>
<td>No. of post-Lyme symptoms per patient, median (range)</td>
<td>2 (1–6)</td>
</tr>
<tr>
<td>No. of patients with symptoms at</td>
<td></td>
</tr>
<tr>
<td>2 mo</td>
<td>32</td>
</tr>
<tr>
<td>6 mo</td>
<td>22</td>
</tr>
<tr>
<td>12 mo</td>
<td>12</td>
</tr>
</tbody>
</table>

* The number of patients who reported a given symptom at any of the follow-up time points. If a symptom was reported at >1 time point, it was only counted once here.

CXCL9 and CXCL10, which are involved in Th1 immune responses, were associated with more symptomatic early infection and culture-negative results, whereas high levels of IL-23, a Th17 mediator, were associated with more symptomatic infection and culture-positive results.

Cytokine and Chemokine Levels Stratified by Post–Lyme Disease Symptoms

Among the 86 patients, 45 had symptoms that persisted after the 2-week course of antibiotic therapy for EM, or they developed new symptoms in the weeks after treatment. The most common symptoms were arthralgia, headache, or fatigue, with a median number of 2 symptoms per patient (Table 2). When the 45 patients with post-Lyme symptoms were subdivided according to IL-23 levels, the group with detectable IL-23 responses tended to have more symptoms (median, 4 vs 2 symptoms, P = .09). The number of patients with post-Lyme symptoms decreased over time. By 12 months, only 12 patients still reported symptoms.

At study entry, prior to antibiotic therapy, the 45 patients who subsequently developed post-Lyme symptoms had similar levels of CXCL9 and CXCL10 compared with the 41 patients without posttreatment symptoms (Figure 3A), and the levels remained similar at the follow-up time points (Figure 3B). In contrast, IL-23 levels were significantly higher in the post-Lyme group at study entry, and remained significantly higher at each posttreatment visit (P ≤ .04). Moreover, of the 41 patients with detectable IL-23 levels, 25 (61%) had post-Lyme symptoms, and all 7 patients with IL-23 levels ≥230 ng/mL had such symptoms.

Culture and Serology Results Stratified by Post–Lyme Disease Symptoms

Because patients with high IL-23 values were more often culture-positive (Figure 2), we used culture and serologic analyses to evaluate whether patients had persistent infection. All 47 patients who were culture-positive for B. burgdorferi at study entry had undergone rebiopsy close to the original biopsy site 2 months after the start of antibiotics [3, 10]. All repeat cultures in patients with or without post-Lyme symptoms were negative (Figure 4A). In addition, the anti-VlsE C6 peptide antibody responses were similar in patients who developed post-Lyme symptoms and in those who did not (Figure 4B), and these responses declined similarly in both groups over subsequent months (Figure 4C). Moreover, there were no objective signs of persistent infection or disseminated disease in either group. Thus, although high Th17-mediated immune responses seemed to be more effective in spirochetal killing than Th17-mediated responses, antibiotic therapy at the time of EM appeared to result in spirochetal eradication in all patients, including those with post-Lyme symptoms.

Antibody Responses to ECGF Stratified by Post–Lyme Disease Symptoms

An alternate hypothesis is that dysregulated Th17 responses may predispose patients to autoimmune phenomena. At study entry, 10 of the 45 patients (22%) with post-Lyme symptoms had antibody responses to ECGF, a recently identified autoantigen in Lyme disease [21], compared with 3 of the 41 patients (7%) who did not have post-Lyme symptoms (P = .07; Figure 5A), a difference of possible significance. Moreover, in patients with post-Lyme symptoms, IL-23 levels correlated directly with the magnitude of anti-ECGF antibody response (P = .02; Figure 5B). In contrast, there was no correlation between IL-23 and anti-ECGF antibodies in patients whose symptoms resolved with antibiotics. Furthermore, antibody responses to a B. burgdorferi-specific VlsE C6 peptide did not correlate with IL-23 levels in either patient group (Figure 5C). Thus, anti-ECGF antibody responses were more common in patients with post-Lyme symptoms, and they correlated directly with higher IL-23 levels, suggesting that post-Lyme symptoms could be associated with persistent Th17 responses in the absence of live spirochetes.

**DISCUSSION**

*B. burgdorferi* infection induces a complex immune response that drives the clinical signs and symptoms of disease. Our findings here suggest that Th1 and Th17 responses are differentially activated in patients with EM. A Th1 response, characterized by elevated
levels of CXCL9 and CXCL10, the chemokines for CD4+ and CD8+ T cells, appears to be the predominant response in clearing the infection. At the first visit, prior to antibiotics, CXCL9 and CXCL10 levels were detectable in serum of virtually all patients (>97%), and high levels of these chemokines were associated with negative Bb culture results in EM skin biopsies, presumably due to lower numbers or lack of viable spirochetes [22, 23]. The consequence of these elevated TH1 mediators was more symptomatic early infection.

In comparison, TH17 responses, characterized by IL-23 levels, were found in only a subset of patients (43%). These patients were more often culture positive and symptomatic, suggesting that TH17 responses were not as effective in spirochetal killing but were capable of causing disease associated with negative Bb culture results in EM skin biopsies, presumably due to lower numbers or lack of viable spirochetes [22, 23]. The consequence of these elevated TH17 responses was more symptomatic early infection.

The main function of IL-23 is to drive the proliferation and survival of TH17 cells, which are important in host defense against extracellular pathogens [18–20]. However, aberrant IL-23 responses have been implicated in several autoimmune diseases including rheumatoid arthritis, inflammatory bowel disease, lupus, and type 1 diabetes [18–20]. The under- and exaggerated responses of TH17 cells in Lyme disease are not well understood.

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neutralizing antibodies against IL-17 [25] or IL-23 [26], implying that TH17 responses were associated with more severe and prolonged disease.

In human patients, CD4+ T cell subsets, including TH17 cells, have been studied in patients with antibiotic-responsive or antibiotic-refractory Lyme arthritis. In both groups, IFN-γ-positive TH1 cells were the predominant population in joint fluid, but in some patients with antibiotic-refractory arthritis, up to 25% of synovial fluid T cells were TH17 cells [27]. Furthermore, in human cell culture models, the Borrelia protein NapA induced the secretion of several TH17-associated mediators, including IL-17 and IL-23 [16, 28]. Our findings here demonstrate that TH1 is the predominant response in this infection, but a subset of patients develops TH17 responses, which may be associated with more severe and prolonged symptoms.

Additionally, in this study, untoward TH17 responses were sometimes associated with autoimmune phenomena. Autoantibodies to ECGF, the first reported autoantigen in Lyme disease that is a target of T-cell and B-cell responses, often develop early in the illness in patients with EM [21]. However, as observed with murine models in which persistent autoantigen stimulation leads to more active T-regulatory cells and resolution of disease [29, 30], the autoimmune responses in our patients appear to subside, and postinfectious symptoms usually resolve.

In this study of European EM patients, most of whom were infected with B. afzelii, the most common post-Lyme symptoms were arthralgia, headache, and fatigue. These symptoms were not incapacitating, and typically resolved within months after antibiotic therapy. It will be important to determine whether some patients with post-Lyme symptoms in the United States,
including those with more debilitating symptoms, also have untoward T\textsubscript{H}17 responses associated with such symptoms.

Study limitations include possible bias in patient selection due to testing only a subset of the study population. However, we included serum samples from all available patients with post-Lyme symptoms (n = 45) and we randomly selected samples from 41 of the 296 patients without post-Lyme symptoms; this group was representative of the larger cohort [3, 10]. We also did not develop comparison groups of patients who had similar symptoms following other manifestations of Lyme

Figure 5. Antibody responses to a human autoantigen, endothelial cell growth factor (ECGF), in patients with erythema migrans at first visit stratified according to post-Lyme symptoms. A, Frequency of a positive antibody response to ECGF as defined by antibody levels >5 standard deviation above the mean of values in healthy control subject. Shaded region represents the values that were considered negative. B, Magnitude of the anti-ECGF antibody response was correlated with IL-23 levels in patients with post-Lyme symptoms (left panel) or no post-Lyme symptoms (right panel). C, Antibody responses against \textit{Borrelia} VlsE C6 peptide were correlated with IL-23 levels in patients with post-Lyme symptoms (left panel), or those without (right panel). The bars in panel A represent the mean values and the I-bars represent the standard error of the mean. P values and correlation coefficients are indicated in the graphs. Abbreviations: ECGF, endothelial cell growth factor; ELISA, enzyme-linked immunosorbent assay; EM, erythema migrans; IgG, Immunoglobulin G; IL-23, interleukin 23; OD\textsubscript{450}, optical density; Symp, symptoms.
disease or other illnesses. However, the goal of the current study was to test a uniquely well-characterized population of EM patients in whom cultures and long-term follow-up were conducted systematically in those who did or did not develop post-Lyme symptoms. This study design made possible the most cogent comparison group for patients with post-Lyme symptoms. Finally, coinfection with other tick-borne agents is a potential confounding variable in EM patients. In Slovenia, the other diseases transmitted by *Ixodes ricinus* ticks include tick-borne encephalitis, human granulocytic anaplasmosis, and, very rarely, tularemia. All patients in our study had a single EM lesion sometimes accompanied by nonspecific symptoms. They did not have the clinical characteristics of tick-borne encephalitis or human granulocytic anaplasmosis; they were rarely febrile, and they did not have thrombocytopenia or leukopenia. Thus, we do not think that the patients in this study had coinfection.

In summary, a subset of patients with EM may have immune dysregulation reflected by the persistently elevated levels of IL-23, resulting in post-Lyme disease symptoms. These observations offer a new paradigm for the study of patients with postinfectious symptoms following Lyme disease.

### Notes

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**Author contributions.** K. S., F. S., and A. C. S. designed the study. K. S. conducted the experiments and the data analyses for this study. F. S. and D. S. conducted the initial clinical trials [3, 9], and provided the patient samples and clinical information. E. E. D. helped with the design and interpretation of ECGF experiments. K. S., A. C. S., F. S., and E. E. D. helped with writing of the manuscript. All authors reviewed and approved the manuscript.

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