Association of a Toll-like Receptor 1 Polymorphism With Heightened Th1 Inflammatory Responses and Antibiotic-Refractory Lyme Arthritis

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Objective. Single-nucleotide polymorphisms (SNPs) that alter immune function, inflammatory responses, and disease susceptibility have been identified in several genes encoding Toll-like receptors (TLRs). The TLR SNPs with the best evidence of an effect on immune function are those in TLR1 (1805GG), TLR2 (2258GA), and TLR5 (1174CT). This study was undertaken to assess the frequency and functional outcomes of these polymorphisms in patients with Lyme disease.

Methods. SNP frequencies and functional outcomes were assessed in 248 patients with Lyme disease. Cytokine and chemokine levels were determined using multiplex assays in the serum of patients with erythema migrans (EM), joint fluid of patients with Lyme arthritis, and supernatants of Borrelia burgdorferi–stimulated peripheral blood mononuclear cells (PBMCs) from patients with Lyme arthritis.

Results. The frequency of the TLR1-1805GG polymorphism was greater in patients with antibiotic-refractory arthritis compared with patients with EM or those with antibiotic-responsive arthritis. Early in the illness, patients with EM carrying 1805GG, primarily those infected with B burgdorferi 16S–23S ribosomal spacer RNA intergenic type 1 (RST1) strains, had higher serum levels of interferon-γ (IFNγ), CXCL9, and CXCL10 and had more severe infection than EM patients carrying the 1805TG/TT polymorphism. These inflammatory responses were amplified in patients with Lyme arthritis, and the highest responses were observed in patients with 1805GG in the antibiotic-refractory group who had been infected with RST1 strains. When PBMCs from patients with Lyme arthritis were stimulated with a B burgdorferi RST1 strain, the 1805GG group had a significantly larger fold increase in the levels of IFNγ, CCL2, CXCL9, and CXCL10 compared to the 1805TG/TT group. In contrast, the TLR2 and TLR5 polymorphisms did not vary in frequency or function among the groups.

Conclusion. The TLR1-1805GG polymorphism in B burgdorferi RST1–infected patients was associated with stronger Th1-like inflammatory responses, an environment that may set the stage for antibiotic-refractory arthritis.

Lyme borreliosis, which is caused by the tick-borne spirochete Borrelia burgdorferi (1), usually begins with an expanding skin lesion, erythema migrans (EM). Months later, untreated patients in the US often develop intermittent or persistent arthritis in a few large joints lasting for a period of several years (2). Lyme arthritis can usually be treated successfully with a 4–8-week course of oral antibiotic therapy (3) or a 2–4-week course of intravenous (IV) antibiotics (3,4), with resolution of the arthritis being a typical outcome. However, in a small percentage of cases, proliferative synovitis persists for months to several years after oral and IV antibiotic treatment (5); this is referred to as antibiotic-refractory Lyme arthritis.

Antibiotic-refractory Lyme arthritis is associated with infection with certain B burgdorferi genotypes, particularly 16S–23S ribosomal spacer RNA intergenic type 1 (RST1) strains (6). The RST1 strains, when com-
pared to the type 2 (RST2) or type 3 (RST3) strains, stimulate macrophages to secrete significantly greater amounts of macrophage-associated cytokines and chemokines (7). However, persistent synovitis after antibiotic therapy does not seem to result from persistent infection; results of polymerase chain reaction (PCR) analyses and cultures for *B. burgdorferi* have been uniformly negative in studies using synovial tissue obtained from patients at the time of synovectomy, within a few months after antibiotic treatment (8,9). Thus, we hypothesized that autoimmunity may play a role in antibiotic-refractory Lyme arthritis (10).

The innate immune response enables the host to differentiate self from pathogen, provides a rapid inflammatory response, and shapes the adaptive immune response (11). In recent years, single-nucleotide polymorphisms (SNPs) have been identified in several genes encoding Toll-like receptors (TLRs), and these SNPs have been found to lead to modified cellular immune responses, decreased cytokine production, and altered disease susceptibility (11,12). The TLR SNPs with the best evidence of effects on immune function are those in TLR1 (1174CT) (17–20), TLR5 (1805GG) (13,14), TLR2 (2258GA) (15,16), and TLR5 (1174CT) (17–20).

The TLR1/TLR-2 heterodimer recognizes triacylated lipids (palmitoyl-3-cysteine-serine-lysine-4 [Pam3CSK4]) found on lipopeptides in a diverse array of pathogens, whereas TLR-5 recognizes bacterial flagellar proteins. Of the 17 SNPs identified in the TLR1 gene sequence, a thymine-to-guanine transversion at position 1805 is among the most common (13). This SNP was found in 50% of European Caucasians, but in only 8% of Africans and in none of the Asian subjects evaluated (13). The 1805TT-to-1805GG transversion changes the amino acid at position 602 from an isoleucine (602II) to a serine (602SS). Compared with homozygous 1805TT or heterozygous TG, which are functionally similar, homozygous 1805GG leads to decreased numbers of TLR-1 on the cell surface (14), impaired downstream signaling, and reduced cytokine production (13,14). This less intense inflammatory response has been associated with protection against tuberculoid leprosy (14,21). The 1805GG polymorphism would be expected to have a substantial impact on function and disease expression because of its location in the transmembrane domain of the receptor and its high frequency in Caucasian populations.

TLR2-2258GA and TLR5-1174CT are each found in ~10% of the Caucasian population (12,15,20). The TLR2 polymorphism, which results in an arginine-to-glutamine transversion at amino acid position 753 in the intracellular domain of the receptor, was associated with reduced cytokine production in vitro and a decreased frequency of Lyme borreliosis in European subjects (15). The TLR5 polymorphism, which results in a transversion of arginine to a stop codon at amino acid position 392 in the extracellular domain, abolished flagellin signaling in a transfected cell line (11,20). This polymorphism was associated with down-regulated immune responses in Crohn’s disease and lupus (17,19), but did not seem to alter disease susceptibility in typhoid fever or chronic obstructive lung disease (18,22).

In this study, we determined the frequency of TLR1-1805GG, TLR2-2258GA, and TLR5-1174CT in 248 patients with EM, antibiotic-responsive Lyme arthritis, or antibiotic-refractory Lyme arthritis from the northeastern US. *B. burgdorferi* has a number of outer surface lipoproteins (OSPs) with the Pam3CSK4 modification (23–26), which stimulate the TLR-1/TLR-2 heterodimer on antigen-presenting cells, and the organism has a number of flagellar proteins (24,25), which presumably stimulate TLR-5. Whereas the TLR2 and TLR5 polymorphisms were not significantly different in frequency or function among the Lyme disease groups, the TLR1-1805GG polymorphism in *B. burgdorferi* RST1-infected patients was associated with stronger Th1-like inflammatory responses, more symptomatic infection, and antibiotic-refractory arthritis.

### PATIENTS AND METHODS

**Study patients.** TLR polymorphisms were determined in peripheral blood mononuclear cells (PBMCs) from 248 patients with EM, antibiotic-responsive Lyme arthritis, or antibiotic-refractory Lyme arthritis. All patients met the criteria of the Centers for Disease Control and Prevention for the diagnosis of Lyme disease (27). The Human Investigations Committees at Tufts Medical Center (1987–2002) and Massachusetts General Hospital (2002–2008) approved the studies, and all patients provided written informed consent.

From November 1987 through January 2008, 177 consecutive patients with Lyme arthritis (ages 12–79 years) were referred to our center before, during, or after antibiotic treatment according to the guidelines currently recommended by the Infectious Diseases Society of America (28). Of the 177 patients, 101 had antibiotic-refractory Lyme arthritis and 76 had antibiotic-responsive Lyme arthritis. This distribution of refractory and responsive cases is reflective of our role as a referral center. These patients had pain and swelling of the knees and positive IgG antibody responses to *B. burgdorferi*, determined by enzyme-linked immunosorbent assay and Western blotting (5). As part of the Immunity in Lyme Arthritis study, patients’ serum samples, PBMCs, and, if available, joint fluid samples were obtained and kept frozen for subsequent experiments.

During the summers of 1998 through 2001, 115 patients with physician-identified EM were recruited at two field sites, one in Wakefield, Rhode Island (by N. Damle) and the
other in East Lyme, Connecticut (by V. J. Sikand), for a study of the diagnosis and pathogenesis of early Lyme disease. Of the 115 patients with EM, 93 had culture confirmation of _B burgdorferi_ infection from skin biopsy specimens, and 71 of the culture-confirmed cases had PBMCs available for typing of the _TLR_ polymorphisms.

**Genotyping.** PCR amplification–restriction fragment length polymorphism (RFLP) assays were used to determine the genotypes of _TLR1_ at position 1805 (13), _TLR2_ at position 2258 (15,16), and _TLR5_ at position 1174 (20) (Figure 1). Frozen PBMCs (5 × 10^6_ cells/ml) were thawed in a water bath at 37°C and washed with 50 ml of phosphate buffered saline (PBS). Total genomic DNA was isolated from the washed cells using the QIAamp DNA Mini kit (Qiagen). Isolated genomic DNA (~50 ng) from each patient was amplified in a 50-μl reaction containing 200 μM of dNTP (Fisher Scientific), 0.5 μM of forward and reverse PCR primers (Integrated DNA Technologies), and 2.5 units of HotStar DNA polymerase (Qiagen). PCR amplification conditions (Integrated DNA Technologies) are 94°C for 3 min, 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min, and finally 72°C for 5 min. PCR products were digested with _Pst_ I for _TLR1_, forward primer CTTGATCTTCCAAGAAGATT-AATAAAGAGCATT and reverse primer GGCCACTCCAGGTAGGTCTT (15); and for _TLR2_, forward primer GCCTACTGGGTGGAGAACCT and reverse primer GGCCATGATACACTAGAACACACATCACT (13); for _TLR5_, forward primer GAAGATGATACACTAGAACACACATCACT (13); and for _TLR5_, forward primer GCCTACTGGGTGGAGAACCT and reverse primer GGCCATGATACACTAGAACACACATCACT (13); and for _TLR5_, forward primer GCCTACTGGGTGGAGAACCT and reverse primer GGCCATGATACACTAGAACACACATCACT (13); and for _TLR5_, forward primer GCCTACTGGGTGGAGAACCT and reverse primer GGCCATGATACACTAGAACACACATCACT (13); and for _TLR5_, forward primer GCCTACTGGGTGGAGAACCT and reverse primer GGCCATGATACACTAGAACACACATCACT (13); and for _TLR5_, forward primer GCCTACTGGGTGGAGAACCT and reverse primer GGCCATGATACACTAGAACACACATCACT (13); and for _TLR5_, forward primer GCCTACTGGGTGGAGAACCT and reverse primer GGCCATGATACACTAGAACACACATCACT (13); and for _TLR5_, forward primer GCCTACTGGGTGGAGAACCT and reverse primer GGCCATGATACACTAGAACACACATCACT (13); and for _TLR5_, forward primer GCCTACTGGGTGGAGAACCT and reverse primer GGCCATGATACACTAGAACACACATCACT (13); and for _TLR5_, forward primer GCCTACTGGGTGGAGAACCT and reverse primer GGCCATGATACACTAGAACACACATCACT (13); and for _TLR5_, forward primer GCCTACTGGGTGGAGAACCT and reverse primer GGCCATGATACACTAGAACACACATCACT (13); and for _TLR5_, forward primer GCCTACTGGGTGGAGAACCT and reverse primer GGCCATGATACACTAGAACACACATCACT (13); and for _TLR5_, forward primer GCCTACTGGGTGGAGAACCT and reverse primer GGCCATGATACACTAGAACACACATCACT (13); and for _TLR5_, forward primer GCCTACTGGGTGGAGAACCT and reverse primer GGCCATGATACACTAGAACACACATCACT (13); and for _TLR5_, forward primer GCCTACTGGGTGGAGAACCT and reverse primer GGCCATGATACACTAGAACACACATCACT (13); and for _TLR5_, forward primer GCCTACTGGGTGGAGAACCT and reverse primer GGCCATGATACACTAGAACACACATCACT (13); and for _TLR5_, forward primer GCCTACTGGGTGGAGAACCT and reverse primer GGCCATGATACACTAGAACACACATCACT (13); and for _TLR5_, forward primer GCCTACTGGGTGGAGAACCT and reverse primer GGCCATGATACACTAGAACACACATCACT (13); and for _TLR5_, forward primer GCCTACTGGGTGGAGAACCT and reverse primer GGCCATGATACACTAGAACACACATCACT (13); and for _TLR5_, forward primer GCCTACTGGGTGGAGAACCT and reverse primer GGCCATGATACAC...
When all of the groups in our study were compared, we found that patients with antibiotic-refractory arthritis had a higher frequency of TRL1-1805GG than did patients with EM (51%) or patients with antibiotic-responsive arthritis (47%) (odds ratio [OR] 1.7, P = 0.1 and OR 1.9, P = 0.05, respectively) (Figure 1A).

With regard to the TLR2-2258GA polymorphism, 88% of normal healthy subjects in a previously described Caucasian population (15) had this genotype, whereas 12% had the GA polymorphism at this position. In the present study, 10% of the patients with EM had TLR2-2258GA, whereas 3% of the patients with antibiotic-responsive arthritis and 8% of those with antibiotic-refractory arthritis had this polymorphism, but the differences in frequency were not statistically significant (Figure 1B).

In another normal healthy Caucasian population (20), ~90% of the healthy subjects had TLR5-1174CC, and ~10% had the 1174CT polymorphism. A similar distribution of the TLR5 genotypes was observed in each Lyme disease patient group in the present study (Figure 1C).

Serum levels of cytokines and chemokines in patients with EM. We next determined the functional consequences of these polymorphisms in patients with various manifestations of Lyme disease. Serum samples, obtained a median of 3 days after disease onset, were available from 66 of the 71 patients with EM in whom TLR polymorphisms were determined. Among the 66 patients with EM, those with TLR1-1805GG had significantly higher serum levels of the IFN-γ-inducible chemokines CXCL9 and CXCL10, and tended to have higher levels of IL-6 (Figure 2A).

Moreover, when patients were also stratified according to the B burgdorferi genotype of the infecting strain, those with TLR1-1805GG who were infected with the RST1 strains (also called OspC types A and B) had strikingly higher serum levels of CXCL9, CXCL10, and IL-6 when compared with patients infected with the other strains (Figure 2B). In addition, among the patients infected with the RST3 strains, which is the most diverse RST type (6,29,31), there was a trend toward higher levels of CXCL9 and CXCL10 within the group carrying the 1805GG polymorphism; this was attributable to infection with an RST3 subtype, OspC type I (7) (results not shown). In contrast, when chemokine and cytokine levels were stratified according to the presence or absence of the TLR2-2258GA polymorphism or TLR5-1174CT polymorphism, the chemokine and cytokine levels in the serum were similar between the groups (results not shown).

Clinical correlations in patients with EM. In the northeastern US, EM is often accompanied by headache, neck stiffness, myalgias, arthralgias, fever, malaise,
and fatigue. When the presence or absence of these associated symptoms was assessed for a correlation with the chemokine and cytokine levels, the protein expression of IL-6, IFN\textsubscript{γ}/H9253, CXCL9, and CXCL10 was significantly higher in those patients with symptomatic illness (Figure 3A). Moreover, all 18 RST1-infected patients with the 1805GG polymorphism had symptoms, whereas 60–70% of the patients in all other groups had symptoms, including RST1-infected patients with 1805TG/TT and all RST2- or RST3-infected patients (Figure 3B). The difference between RST1-infected patients with 1805GG and each of the groups with 1805TG/TT was statistically significant (each \( P \leq 0.01 \)). However, the numbers of organisms in EM lesions were similar regardless of the TLR1 polymorphism or B burgdorferi genotype (Figure 3C). Moreover, the frequencies of disseminated infection, defined by a positive PCR result for B burgdorferi DNA in the blood or multiple EM lesions, did not differ significantly among these host and spirochetal genotypes (results not shown). Thus, early in the illness, RST1-infected patients with TLR1-1805GG,
which affects cells of the innate immune system, had greater adaptive Th1-like inflammatory responses, and they were more likely to have symptomatic infection despite having similar numbers of spirochetes in the EM skin lesions and a similar frequency of disseminated infection.

**Chemokine and cytokine levels in the joint fluid of patients with Lyme arthritis.** In a previous study (32), elevated levels of cytokines and chemokines in patients with Lyme arthritis were found only in the joint fluid, and not in serum samples. For this study, joint fluid samples were available from 49 of the 177 patients in whom TLR polymorphisms were determined. Of the 49 patients, 16 had antibiotic-responsive arthritis. Joint fluid samples from this group could only be obtained during the infectious period, prior to or soon after the start of antibiotic treatment, since joint effusions resolved soon thereafter. The remaining 33 patients had antibiotic-refractory arthritis, and these patients were referred to our clinic because they had not responded to treatment with at least 1 course of antibiotics. Thus, the joint fluid samples from these patients were usually obtained in the post–antibiotic treatment period, within a median of 4 months (range 0–23 months) after the start of antibiotics.

Despite these differences in the timing of sample retrieval, the levels of almost all of the chemokines and cytokines measured in the joint fluid were significantly higher in the 33 patients with antibiotic-refractory arthritis (22 [67%] of whom had TLR1-1805GG) compared to the 16 patients with antibiotic-responsive arthritis (8 [50%] of whom had 1805GG) (Figure 4A). The group with antibiotic-refractory arthritis had especially high joint fluid levels of CXCL9, CXCL10, and IL-6. The levels of CXCL9 and CXCL10 were 2-fold higher in patients with antibiotic-refractory arthritis compared to patients with antibiotic-responsive arthritis. Moreover, the joint fluid levels of these mediators were 15-fold higher in patients with antibiotic-refractory arthritis compared to patients with antibiotic-responsive arthritis. Furthermore, the joint fluid levels of these mediators were 2-fold higher in patients with antibiotic-refractory arthritis compared to patients with antibiotic-responsive arthritis (Figure 4A) compared to the serum levels in patients with EM (Figure 2A).

Since the differences in cytokine and chemokine levels in the serum from patients with EM were found primarily in those with TLR1-1805GG who were infected with the *B burgdorferi* RST1 strains, we sought to stratify the patients with Lyme arthritis according to both of these parameters. Because it has been nearly impossible to culture *B burgdorferi* from joint fluid, we were able to determine the *B burgdorferi* genotype in only 14 patients, 10 with antibiotic-refractory arthritis and 4 with antibiotic-responsive arthritis, in whom enough *B burgdorferi* DNA was present in the joint fluid to allow successful PCR amplification and RFLP analysis (6).

When the chemokine and cytokine levels in the 10 patients with antibiotic-refractory arthritis (all of whom had 1805GG) were stratified according to *B burgdorferi* genotype, the 5 patients who had been infected with the RST1 strains, when compared with the 5 patients who had been infected with either the RST2 or RST3 strain, had higher joint fluid levels of most of the chemokines and cytokines measured, particularly IFNγ, CCL2, and CXCL9 (Figure 4B). The levels of CXCL9 in patients with antibiotic-refractory arthritis who had the 1805GG polymorphism and who had been infected with the RST1 strain were the highest measured (median

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**Figure 4.** Cytokine and chemokine levels in joint fluid samples from patients with Lyme arthritis. Protein levels of 7 chemokines and 5 cytokines were measured in the joint fluid of 49 patients with Lyme arthritis, using bead-based multiplex assays, with stratification of patients according to the response to oral or intravenous antibiotic treatment (antibiotic-refractory, defined as persistence of proliferative synovitis for months to several years after antibiotic treatment, versus antibiotic-responsive) (A), or according to the *Borrelia burgdorferi* 16S–23S ribosomal spacer RNA intergenic type (RST) genotype of the infecting strain (type 1 versus types 2 and 3) among the 10 patients with antibiotic-refractory arthritis with 1805GG (B). Bars show median and third quartile values. 

* P ≤ 0.05; ** P ≤ 0.01; *** P ≤ 0.001 versus antibiotic-responsive. IL-6 = interleukin-6; TNF = tumor necrosis factor; IFNγ = interferon-γ.
value 263,000 pg/ml) in any group in this study. Moreover, the median duration from the start of antibiotics to resolution of arthritis among the RST1-infected patients was 15 months (range 4–30 months), compared with a median duration of 9 months (range 4–10 months) among those with RST2- or RST3-associated infection. However, because of the small numbers of patients, these differences in the levels of inflammatory mediators or in the duration of arthritis were not statistically significant. Because both the TLR2 and TLR5 polymorphisms were found in only a small percentage of patients, some of whom also had the TLR1 polymorphism, a meaningful comparison of cytokine and chemokine levels in these patients was not possible.

**Stimulation of PBMCs from patients with Lyme arthritis.** PBMCs were available from 41 of the 49 patients whose joint fluid samples were tested, to assess the results of stimulation with a *B. burgdorferi* RST1 strain. In these experiments, in which cells were stimulated with similar numbers of organisms of the same strain, the chemokine and cytokine expression in the cells was similar between patients with antibiotic-responsive arthritis and those with antibiotic-refractory arthritis, and therefore the results were combined for

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**Figure 5.** Effects of stimulation of peripheral blood mononuclear cells (PBMCs) from patients with Lyme arthritis on chemokine/cytokine expression. Protein levels of 6 chemokines and 6 cytokines in culture supernatants were assessed using bead-based multiplex assays in PBMCs from 43 patients with Lyme arthritis (25 with the 1805GG polymorphism and 18 with the TG/TT polymorphism). Cells were stimulated with palmitoyl-3-cysteine-serine-lysine-4 (300 ng/ml) (A) or with a 16S–23S ribosomal spacer RNA intergenic type 1 (OspC type A) isolate of *B. burgdorferi* at a multiplicity of infection of 25 spirochetes per cell (B), for 5 days. Data are calculated as the fold increase in the levels of cytokines and chemokines (values in stimulated cells divided by values in unstimulated cells). Bars show the median and third quartile values. In B, the double horizontal lines indicate that the third quartile values for the increase in CCL3 were beyond the scale (164,531 for patients with the 1805GG polymorphism and 56,050 for patients with the 1805TG/TT polymorphism). * = P ≤ 0.05; ** = P ≤ 0.001 versus TG/TT. IL-1β = interleukin-1β; TNF = tumor necrosis factor; IFNγ = interferon-γ.
further analysis. The PBMCs from 12 patients with EM showed little, if any, recall response to *B. burgdorferi* antigens, and therefore we did not test this group further.

Among the 41 patients with Lyme arthritis, unstimulated PBMCs secreted only basal amounts of each of the cytokines and chemokines tested, regardless of whether the *TLR1*-1805GG genotype was present. When the cells were stimulated with Pam$_3$CSK$_4$ (the specific agonist for TLR-1/TLR-2), PBMCs from patients with the 1805TG/TT polymorphism had a significantly greater fold increase in the levels of the innate immune response–associated mediators IL-1β, IL-10, and CCL3 when compared to PBMCs from patients with the 1805GG polymorphism (Figure 5A), and there was a trend in that direction for some of the other chemokines and cytokines measured.

In contrast, when PBMCs were stimulated with a *B. burgdorferi* RST1 isolate, most of the chemokines and cytokines, particularly the Th1-like adaptive immune response–associated mediators IFNγ and IFNγ-inducible chemokines CCL2, CXCL9, and CXCL10, were produced at a higher level in the 1805GG group compared to the TG/TT group (*P* = 0.001) (Figure 5B). In contrast, cells from patients with either 1805GG or TG/TT produced only basal levels of IFNα. Thus, consistent with the results in the joint fluid, *B. burgdorferi* RST1 stimulation of PBMCs from patients with *TLR1*-1805GG strongly induced the secretion of IFNγ and IFNγ-inducible chemokines, especially CXCL9, a process that seems to be important in setting the stage for antibiotic-refractory arthritis.

**DISCUSSION**

In this study, we assessed 248 patients, including 177 with Lyme arthritis, which represents the largest cohort of patients with this manifestation of the illness studied to date. Patients with antibiotic-refractory arthritis had an increased frequency of a *TLR1* polymorphism (1805GG) compared with patients with EM (OR 1.7, *P* = 0.1) or those with antibiotic-responsive arthritis (OR 1.9, *P* = 0.05). These differences are of borderline statistical significance, but the ORs are typical of what is found in rheumatoid arthritis or other autoimmune diseases in which multiple genetic factors (in this instance, both spirochetal and host genetic factors) influence the outcome of the disease (33). More importantly, we found a functional difference in immune response and disease expression associated with *TLR1*-1805GG. Early in the illness, patients with EM who had 1805GG, primarily those infected with *B. burgdorferi* RST1 strains, had strikingly higher serum levels of IL-6, CXCL9, and CXCL10 and had more symptoms than patients with the 1805TG/TT polymorphism. Although EM skin biopsy samples from these patients were not available for analysis, studies by our group have previously shown that these inflammatory mediators are also prominent in the skin lesions of patients with EM (34,35). Thus, early in the illness, RST1-infected patients with *TLR1*-1805GG, which affects cells of the innate immune system, had greater adaptive Th1 inflammatory responses, and they were more likely to have symptomatic infection. In contrast, we did not find significant differences in frequency or function according to the *TLR2*-2274GA or *TLR5*-1174CT polymorphism.

The chemokine/cytokine profile observed in patients with EM who had *TLR1*-1805GG was amplified in the joint fluid of patients with Lyme arthritis, particularly in those with 1805GG who had antibiotic-refractory arthritis. In these patients, the joint fluid levels of CXCL9 and CXCL10 were at least 15-fold higher than in the serum of patients with EM. Moreover, when PBMCs from patients with Lyme arthritis were stimulated with the same *B. burgdorferi* RST1 strain, most chemokines and cytokines, particularly IFNγ and the IFNγ-inducible chemokines, were produced at a higher level in the 1805GG group than in the TG/TT group, which demonstrated in cell culture the same chemokine/cytokine profile observed in these patients. We have previously demonstrated that synovial tissue from patients with antibiotic-refractory arthritis also had exceptionally high levels of CXCL9 and CXCL10 (32), which are chemoattractants for CD4+ and CD8+ effector T cells. These cells are the most numerous infiltrating cells in synovial lesions in antibiotic-refractory Lyme arthritis and in other forms of chronic inflammatory arthritis (36,37).

This scenario is reminiscent of findings in *B. burgdorferi*–infected, TLR-2–deficient C57BL/6 mice (38,39). Compared with their wild-type counterparts, these mice developed more severe arthritis (the sole manifestation of the disease in mice), and their joint tissue had greater numbers of T cells, high levels of CXCL9 and CXCL10, and increased transcripts for a prototypical IFNγ-inducible gene, GTPase (*igp*) (39). The authors concluded that TLR-2 deficiency in *B. burgdorferi*–infected mice leads to increased numbers of T cells in the joints, high levels of IFNγ, and overproduction of the T cell–attracting chemokines CXCL9 and CXCL10, suggesting that the TLR-2 pathway ordinarily modulates this IFNγ response. We did not see this effect
in patients with the TLR2-2274GA polymorphism, because it was present in only a low proportion of the patients, it affected only 1 allele, and, in contrast to observations in the knockout mice, it did not abolish TLR-2 function. The TLR-1/TLR-2 heterodimer recognizes lipopeptides both intracellularly and on the cell surface (40). Activation of these receptors in different compartments may result in distinct cytokine and chemokine profiles (40). However, it is not yet clear how B burgdorferi infection in humans with the 1805GG polymorphism or in mice with TLR-2 deficiency leads to heightened Th1-like inflammatory responses. In culture or murine models, the TLR-1/TLR-2 receptor complex has been shown to regulate cytokine production mediated by TLR-1/TLR-2 as well as other receptors. For example, in a dendritic cell culture system, TLR-2 has been shown to regulate TLR-4– and TLR-7/TLR-8–mediated cytokine production by inhibiting the type I interferon amplification loop (41). Moreover, in B burgdorferi–stimulated murine macrophages, a deficiency in part of the TLR-1/TLR-2 complex resulted in persistent, elevated expression of inflammatory cytokines and chemokines due to decreased activation of p38 and reduced expression of the downstream suppressor of cytokine signaling (SOCS) proteins (42). Similarly, in our preliminary experiments, B burgdorferi–stimulated PBMCs from Lyme arthritis patients with 1805GG had significantly reduced p38 phosphorylation compared with cells from patients with TG/TT (results not shown). Therefore, a deficiency in TLR-1 in human patients with 1805GG may cause decreased expression of p38 and SOCS, resulting in a loss of a balancing antiinflammatory signal. Moreover, because B burgdorferi stimulates multiple pathogen-recognition receptors on innate immune cells (43), activation of other TLRs may cause a compensatory increase in a proinflammatory signal. In either case, this could result in higher levels of IFNγ and IFNγ-inducible chemokines, as seen in patients with the 1805GG polymorphism in the present study. We speculate that antibiotic-refractory arthritis is associated with TLR1-1805GG because this polymorphism is one of several factors that may lead to exceptionally high levels of IFNγ and the IFNγ-inducible chemokines CXCL9 and CXCL10 in the joint fluid and synovial tissue (32). We postulate that this heightened Th1-like response leads to the recruitment of large numbers of CD4+ and CD8+ effector T cells, some of which may be autoreactive and not appropriately controlled by persistently low numbers of Treg cells (44). Since multiple factors may lead to exuberant Th1 responses (45), not all patients with antibiotic-refractory arthritis have the TLR1-1805GG polymorphism.

In conclusion, individuals in the northeastern US have become increasingly affected by B burgdorferi infection (46,47), and highly inflammatory RSTI strains (OspC types A or B) cause 30–50% of the infections in this region (29,31). This B burgdorferi genotype has a high transmission frequency among ticks (48), it may be increasing in frequency in nature, and it may be an important factor in the emergence of Lyme disease in epidemic form in the northeastern US in the late 20th century (49,50). Our results herein show that a TLR1 polymorphism (1805GG), which is present in one-half of the Caucasian population, causes heightened Th1 inflammatory responses, primarily in B burgdorferi RST1–infected patients. Thus, it is the combination of spirochetal and host genetic factors that leads to this host immune response in a subset of patients with Lyme disease. Rather than limiting the pathologic processes induced by infection, this heightened Th1-like inflammatory immune response is associated with more symptomatic early infection, putative autoimmune phenomena, and antibiotic-refractory arthritis.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Strle had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study conception and design. Strle, Shin, Glickstein, Steere. Acquisition of data. Strle, Shin. Analysis and interpretation of data. Strle, Shin, Glickstein, Steere.

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