Objective. In a murine model of antibiotic-refractory Lyme arthritis, the numbers of Treg cells are dramatically reduced. The aim of this study was to examine Treg cell numbers and function in patients with antibiotic-refractory Lyme arthritis.

Methods. CD4+ T cell subsets were enumerated in the peripheral blood (PB) and synovial fluid (SF) of 12 patients with antibiotic-refractory arthritis and 6 patients with antibiotic-responsive arthritis. Treg cell function was examined using Borrelia-specific and non-specific Treg cell proliferation assays.

Results. In both patient groups, interferon-γ-positive Th1 cells in SF were abundant and enriched (~50% of CD4+ T cells). In patients with antibiotic-refractory arthritis, the median percentages of FoxP3-positive Treg cells were significantly higher in SF than in PB (12% versus 6%; P = 0.03) or in SF from patients with antibiotic-responsive arthritis (12% versus 5%; P = 0.04). Moreover, in the antibiotic-refractory group, a higher percentage of Treg cells in SF correlated with a shorter duration until resolution of arthritis (r = −0.74, P = 0.006). In contrast, patients with fewer Treg cells had suboptimal responses to disease-modifying anti-rheumatic drugs and a longer duration of arthritis after antibiotic treatment, and they often required synovectomies for arthritis resolution. In each group, Treg cells in SF dampened Borrelia burgdorferi-specific proliferative responses, and in 2 patients with antibiotic-refractory arthritis, Treg cells were functional in non-specific suppression assays.

Conclusion. Treg cells were functional in patients with antibiotic-refractory arthritis, and in some patients, higher numbers of these cells in SF appeared to participate in arthritis resolution. However, as in the murine model, patients with antibiotic-refractory arthritis and lower numbers of Treg cells seemed unable to achieve resolution of synovial inflammation.
arthritides may persist even after total or nearly total eradication of spirochetes from the joint with antibiotic therapy.

The duration of antibiotic-refractory arthritis is variable. In a previous analysis of 67 patients, the median duration from the initiation of antibiotic treatment to the resolution of arthritis was 11 months (range 4–44 months) (2). During the post-antibiotic treatment period, we usually treat patients with a nonsteroidal antiinflammatory drug (NSAID) and a disease-modifying antirheumatic drug (DMARD) (2). If patients have only a minimal-to-moderate response after treatment for 12–18 months, we consider performing arthroscopic synovectomy.

Antibiotic-refractory Lyme arthritis shares certain pathogenetic themes with other forms of chronic inflammatory arthritis, particularly rheumatoid arthritis (RA). These include similar synovial histology (6,7), HLA–DR associations with DRB1*0401, 0101, and other related alleles (8–10), a dominant Th1 response in SF and synovial tissue (11–15), and high SF levels of pro-inflammatory cytokines and chemokines (16–18), especially CXCL9 and CXCL10, which are strong chemoattractants for CD4+ and CD8+ T effector cells. We have postulated that antibiotic-refractory arthritis may result from infection-induced, tissue-specific autoimmunity within affected synovia (19).

The autoimmunity hypothesis has been reinforced recently by the development of a murine model (20). In this model, both the presence of the human HLA–DR4 transgene, which is associated with antibiotic-refractory arthritis, and lack of the CD28 coreceptor, which leads to dramatically reduced numbers of Treg cells (21), are necessary for persistent synovitis after antibiotic therapy. In mice that lack only the CD28 coreceptor, without the HLA–DR4 transgene, persistent synovitis does not develop after treatment (22). Similarly, in mice that lack the CD28 coreceptor and have the human HLA–DR11 transgene, which is associated with antibiotic-responsive arthritis, posttreatment synovitis does not develop (23). These outcomes in mice support the HLA–DR findings in patients with Lyme arthritis (8), but Treg cell numbers and function have not yet been examined in human Lyme arthritis.

In this study, we enumerated CD4+ T cell subsets, including Treg cells, in the peripheral blood (PB) and SF of 18 patients with antibiotic-responsive or antibiotic-refractory Lyme arthritis. In those with antibiotic-refractory arthritis, a higher percentage of Treg cells correlated with a shorter duration of time to the resolution of arthritis. However, as in the murine model, patients with antibiotic-refractory arthritis and lower numbers of Treg cells seemed unable to achieve resolution of synovial inflammation.

PATIENTS AND METHODS

Patients. During a 22-year period, from November 1987 through January 2009, we evaluated 192 patients with Lyme arthritis. The Human Investigations Committees at Tufts Medical Center (Boston, MA) (1987–2002) and Massachusetts General Hospital (2002–2009) approved the study, and all patients (or the parents of patients who were minors) provided written informed consent.

For this study, large numbers of concomitant PB mononuclear cells (PBMCs) and SF mononuclear cells (SFMCs) were available from 18 patients, 12 with antibiotic-refractory arthritis and 6 with antibiotic-responsive arthritis. All 18 patients met the Centers for Disease Control and Prevention criteria for the diagnosis of Lyme disease (24) and were entered into a study called Immunity in Lyme Arthritis. PCR testing for B burgdorferi DNA and serum antibody responses to B burgdorferi were determined as previously described (25,26). The patients received antibiotic therapy according to the guidelines of the Infectious Diseases Society of America (27). As in the past (2,4,5), antibiotic-responsive arthritis was defined as resolution of arthritis within 3 months after ≤4 weeks of treatment with IV antibiotics or 8 weeks of oral antibiotics, and antibiotic-refractory arthritis was defined as persistent joint swelling for >3 months after the start of ≥4 weeks of IV antibiotics or ≥8 weeks of oral antibiotics, or both.

Isolation and quantification of PBMCs and SFMCs. To collect PBMCs and SFMCs, heparinized PB and SF were centrifuged at 2,100 revolutions per minute in Lymphocyte Separation Medium (MP Biomedicals) for 30 minutes. The total number of mononuclear cells per milliliter of joint fluid was calculated by dividing the total number of cells recovered after Ficoll-Hyphaque separation by the volume of joint fluid. A fraction of cells in each PB or SF sample was stained with anti-CD3 and anti-CD4 monoclonal antibodies (mAb; BD Biosciences). The percentage of monocytes, CD4+ T cells, and non-CD4+ T cells was determined by flow cytometry (BD Biosciences), using CD3−CD4−, CD3+CD4+, and CD3+CD4+ as the markers.

Intracellular staining of T cell subsets in PBMCs or SFMCs. Intracellular staining of FoxP3 antibodies (eBioscience) or the cytokines interferon-γ (IFNγ), interleukin-4 (IL-4), or IL-17 (BD Biosciences) was done according to the manufacturer’s instructions. Frozen cells were quickly thawed and washed in phosphate buffered saline. For FoxP3 determinations, the cells were first stained for surface expression of CD3, CD4, and CD25 (BD Biosciences); they were then fixed with intracellular fixation buffer, washed with permeabilization buffer, and stained with anti-FoxP3 antibody. For cytokine staining, washed cells were first stimulated with phorbol 12-myristate 13-acetate (50 ng/ml; Sigma-Aldrich) and ionomycin (1 µg/ml; Sigma-Aldrich) in the presence of GolgiStop (BD Biosciences) for 5 hours at 37°C in 5% CO2. Stained cells were collected, washed with fluorescence-activated cell sorting buffer, and stained for surface expression with anti-CD4 and anti-CD3 or antibody isotypes (mouse IgG; BD Biosciences) as
a negative control. After fixation and staining, cells were stained intracellularly for expression of IFNγ, IL-17, and IL-4. The stained samples were analyzed by flow cytometry.

**Immunofluorescence staining of T cells in synovial tissue.** Sections (8 μm) of synovium or tonsil were stained with hematoxylin and eosin. Adjacent slides were blocked with 5% donkey serum for 30 minutes at room temperature and stained with mouse anti-human anti-CD3 antibody (1:100; Dako) and goat anti-human anti-FoxP3 antibody (1:2,500) (Novus Biologicals) at 4°C overnight. The slides were then stained with Cy2-conjugated donkey anti-mouse IgG (1:100) and with Cy3-conjugated donkey anti-goat IgG (1:250) (Jackson Immunoresearch) at room temperature for 1 hour.

**Borrelia-specific proliferation assay.** SFMCs were stained with either fluorescein isothiocyanate [FITC]-conjugated anti-CD25 mAb (BD Biosciences) or allophycocyanin [APC]–conjugated anti-CD25 mAb (BD Biosciences) as a control, followed by anti-FITC microbeads (Miltenyi Biotech). Cells were then passed through a negative selection column (LD Column; Miltenyi Biotech) under a magnetic field (Miltenyi Biotech). The flow-through was collected as Treg cell–depleted SFMCs (cells stained with FITC-conjugated anti-CD25 antibody) or Treg cell–retained SFMCs (cells stained with APC-labeled anti-CD25 antibody). The depletion efficiency was >90%. Treg cell–depleted SFMCs or Treg cell–retained SFMCs were cultured at 1 × 10^5/well in a 96-well round-bottomed plate in complete RPMI 1640 medium containing 10% human AB serum (heat-inactivated; Mediatech), 1 mM sodium pyruvate, 2 mM l-glutamine, 0.1 mM nonessential amino acid, 100 units/ml penicillin, 50 μg/ml streptomycin (Gibco), and 5 × 10^{-5} M 2-mercaptoethanol (Sigma) at 37°C in a CO_2 incubator for 5 days. *B. burgdorferi* sonicate (strain G39/40) was added to each well at a final concentration of 25 μg/ml. After 5 days in culture, cells were pulsed with 0.5 μCi ^3^H-thymidine, and thymidine incorporation was measured 16 hours later with a TopCount instrument (Packard).

**Non-specific Treg cell suppression assay.** To isolate CD4 T cells, PBMCs or SFMCs were stained with a panel of antibodies, including APC-conjugated anti-CD8, anti-CD14, anti-CD19, and anti-CD56 (BD Biosciences) followed by anti-APC microbeads (Miltenyi Biotech). Cells were passed through a depletion LD Column attached to a magnetic field (Miltenyi Biotech). The flow-through had >90% purity of CD4 T cells. To isolate CD4 + CD25 + and CD4 + CD25 − cells, the CD4 + T cells obtained with negative selection were stained with FITC-conjugated anti-CD25 antibody (BD Biosciences) and anti-FITC microbeads (Miltenyi Biotech). Cells were then passed through a positive-selection column; the flow-through contained CD4 + CD25 − cells, and CD4 + CD25 + cells retained in the column were washed out. For the suppression assay, 1 × 10^4 CD4 + CD25 − cells were cultured in the presence or absence of 1 × 10^4 CD4 + CD25 + cells in 96-well, round-bottomed plates. Each well contained 2 × 10^6 irradiated (3,500 rad) PBMCs and 0.3 mg/ml of anti-CD3 antibody. After 5 days, cells were pulsed with 0.5 μCi ^3^H-thymidine, and thymidine incorporation was measured 16 hours later. For comparison, PBMCs from a healthy human donor, obtained from the Massachusetts General Hospital Blood-Component Laboratory, from which CD4 + CD25 + T cells were depleted or retained, were also stimulated with anti-CD3 antibody with irradiated PBMCs.

**Statistical analysis.** Categorical variables were compared in 2 × 2 contingency tables by chi-square analysis. The distribution of values between groups was compared by Mann-Whitney rank sum test. Correlations between the percentages of Treg cells and the duration of arthritis were sought by Pearson’s correlation test. All P values were 2-tailed. P values less than 0.05 were considered significant.

**RESULTS**

**Clinical characteristics and treatment of patients.** The clinical characteristics of the 18 study patients are shown in Table 1. Among the 6 patients with antibiotic-responsive arthritis who were treated with oral doxycycline for 1 or 2 months, joint fluid samples were available only prior to or soon after the start of antibiotic treatment because of the rapid resolution of joint inflammation. In contrast, the 12 patients with antibiotic-refractory arthritis were usually referred during or after a 2–4-month course of both oral doxycycline and IV ceftriaxone. In 6 of the 12 patients with antibiotic-refractory arthritis, the joints were aspirated during antibiotic therapy; in 5 of these 6 patients, the results of PCR testing for *B. burgdorferi* DNA in joint fluid were still positive. The remaining 6 patients were referred after treatment with antibiotics for 3–9 months, and in these patients, PCR results were negative. Among all 12 patients in the antibiotic-refractory group, the median duration from the start of antibiotic treatment to the sample date was 4 months (range 0.2–12 months), a date near or soon after the completion of antibiotic treatment.

During the post–antibiotic treatment period, 11 of the 12 patients with antibiotic-refractory arthritis were treated with NSAIDs and DMARDs, usually naproxen and hydroxychloroquine, which in 3 cases was changed to methotrexate. Because of incomplete responses, 4 patients, including the 3 who were treated with methotrexate, had arthroscopic synovectomies, with successful results. The final patient, who was referred after receiving antibiotic therapy for 9 months, elected to have a synovectomy, which also resulted in the resolution of arthritis.

**Total number of mononuclear cells in SF.** The concentration of mononuclear cells and the percentages of monocytes and T cells were similar in the SF of patients with antibiotic-responsive arthritis and those with antibiotic-refractory arthritis (Figure 1). CD3−CD4low monocytes comprised a median of 20–25% of the total mononuclear cells; CD3+CD4+ T cells made...
Table 1. Clinical and demographic characteristics and treatment regimens of the patients with Lyme arthritis*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Antibiotic-responsive (n = 6)</th>
<th>Antibiotic-refractory (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>26 (18–59)</td>
<td>29 (11–54)</td>
</tr>
<tr>
<td>No. of men/no. of women</td>
<td>3/3</td>
<td>8/4</td>
</tr>
<tr>
<td>Duration of arthritis prior to start of antibiotics</td>
<td>0.25 (0–2)</td>
<td>1 (0–5)</td>
</tr>
<tr>
<td>Duration of arthritis from start of antibiotics to sample date, months†</td>
<td>0 (0–0.25)</td>
<td>4 (0.2–12)</td>
</tr>
<tr>
<td>Findings on the sample date</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Size of joint effusion, ml</td>
<td>50 (30–70)</td>
<td>40 (10–70)</td>
</tr>
<tr>
<td>PCR results for Borrelia burgdorferi DNA in joint fluid, no. of positive samples/total no. tested‡</td>
<td>3/5</td>
<td>5/12</td>
</tr>
<tr>
<td>Duration of arthritis from start of antibiotics to resolution, months</td>
<td>2 (1–3)</td>
<td>14 (5–32)</td>
</tr>
<tr>
<td>Duration of arthritis from the sample date to resolution, months</td>
<td>2 (1–3)</td>
<td>5.5 (1–28)</td>
</tr>
</tbody>
</table>

* Except where indicated otherwise, values are the median (range). By definition, the 2 groups differed in the duration of arthritis after treatment. There were no other significant differences between the 2 groups.
† Patients with antibiotic-responsive arthritis were referred prior to or soon after the initiation of antibiotic therapy, whereas those with antibiotic-refractory arthritis were most commonly referred near or soon after the conclusion of a 2–4-month course of antibiotics.
‡ Among patients with positive results of polymerase chain reaction (PCR) analysis, those in the antibiotic-responsive group were seen prior to the initiation of antibiotic treatment, and those in the antibiotic-refractory group were seen during a 2–4-month course of antibiotic treatment. No patient had a positive PCR result during the post-antibiotic treatment period.

Figure 1. Total concentration of mononuclear cells in synovial fluid (SF) and percentages of cell types in the peripheral blood (PB) and SF of patients with antibiotic-responsive (Resp) or antibiotic-refractory (Refr) Lyme arthritis. A, Concentration of mononuclear cells in SF and percentages of monocytes and CD4+ and non-CD4+ T cells. The number of mononuclear cells was not available for 1 of the 6 patients with antibiotic-responsive arthritis. B, Comparison of the percentages of each CD4+ T cell subset in the PB and SF of patients in the 2 groups. Horizontal bars represent the medians. IFNγ = interferon-γ; IL-17 = interleukin-17.
up a median of ~40% of the total cells, and CD3+CD4− T cells comprised a median of 15–20% of the total cells.

**Percentages of CD4+ T cell subsets in SF and PB.** In patients with antibiotic-responsive or antibiotic-refractory arthritis, proinflammatory IFNγ-positive CD4+ Th1 cells were the most abundant T cell subset in SF (50–55% of CD4+ T cells), and they were abundant and enriched in SF compared with PB (15–20%) (Figure 1). In contrast, in both patient groups, the median percentages of proinflammatory IL-17-positive CD4+ T cells were low in PB and SF (~5%), but a few patients in both groups had percentages in SF as high as 10–25%. Similarly, in both patient groups, the median percentages of IL-4–positive CD4+ Th2 cells were low in both PB and SF (~5%), but the range was again large (2–22%). Moreover, in the antibiotic-refractory group, the percentage of Th2 cells was significantly higher in SF than in PB or in the SF of patients in the antibiotic-responsive group (for both comparisons, P = 0.03). Finally, in patients with antibiotic-refractory arthritis, the median percentages of FoxP3+ Treg cells were significantly higher in SF than in PB (12% versus 6%; P = 0.03) or in the SF of patients in the antibiotic-responsive group (12% versus 5%; P = 0.04). This was the case whether Treg cells were identified with the transcriptional factor FoxP3 or the surface marker CD25. As expected, most of the CD4+CD25+ cells coexpressed the FoxP3 marker (data not shown).

**Correlation between CD4+ T cell subsets in SF and duration of arthritis.** In Figure 2A, the year at the onset of arthritis, the duration of joint swelling prior to, during, and after antibiotic treatment, and the results of PCR testing for *Borrelia burgdorferi* DNA on the sample date are shown for each of the 12 patients with antibiotic-refractory arthritis, arranged according to the percentage of Treg cells. On the sample date, which was usually near the completion of antibiotic treatment or early in the post–antibiotic treatment period, a higher percentage of Treg cells in SF correlated with a shorter duration of time to resolution of arthritis (r = −0.74, P = 0.006) (Figure 2B). In contrast, Treg cell percentages did not correlate with the duration of arthritis prior to the sample date (r = −0.20, P = 0.4), with the length of time during which the cells had been frozen prior to testing (r = −0.12, P = 0.6), or with positive or negative PCR results (median Treg cell percentage 12.2% in PCR-positive samples versus 8.5% in PCR-negative samples).

Among the 7 patients in whom arthritis resolved within 6 months after the sample date, the median percentage of Treg cells in SF was 12% (range 8–22%) (Figure 2B). Of these 7 patients, 6 experienced the
resolution of arthritis while being treated with NSAIDs and hydroxychloroquine; the seventh patient, who had the highest percentage of Treg cells (22%), chose to have a synovectomy after 9 months of antibiotic treatment. In contrast, the remaining 5 patients had lower percentages of Treg cells (median 5%, range 3–14%; \( P = 0.02 \)), incomplete responses to DMARDs (including methotrexate in 3 patients), and a longer duration of arthritis after antibiotic treatment (10–28 months); 4 of these 5 patients had synovectomies.

In the 6 patients with antibiotic-responsive arthritis, the percentage of Treg cells in SF did not correlate with either the prior or subsequent duration of arthritis (data not shown). Rather, arthritis resolution in this group occurred with spirochete killing during or near the end of antibiotic treatment.

In the 12 patients with antibiotic-refractory arthritis, higher percentages of Th2 cells in SF tended to correlate with faster resolution of arthritis. Among the 7 patients in whom arthritis resolved within 6 months after the sample date, the median percentage of Th2 cells in SF was 9% (range 4–22%), whereas among the 5 patients with longer courses, the median percentage was 6% (range 3–9%; \( P = 0.04 \)). The percentages of Th1 or Th17 cells in SF did not correlate with the duration of arthritis prior to or after the sample date. Patients with

Figure 3. Correlation of clinical and laboratory results in 2 patients (patient 1 and patient 4) with prolonged antibiotic-refractory arthritis who underwent arthroscopic synovectomy (Syn). A, Size of joint effusions and type of treatment regimens. B, Serum antibody titers to *Borrelia burgdorferi* (*Bb*). C, Percentages of interferon-\( \gamma \) (IFN-\( \gamma \))-positive and FoxP3-positive T cells in synovial fluid. All medications were stopped prior to arthroscopic synovectomy. Each open and closed circle indicates the time point at which antibiotic therapy was administered. Dox = doxycycline; Cef = intravenous ceftriaxone; DMARD = disease-modifying antirheumatic drug (hydroxychloroquine or methotrexate).
a higher percentage of Th1 cells tended to have a lower percentage of Treg cells, but this inverse correlation was not statistically significant (r = −0.4, P = 0.16). Positive results of PCR analysis did not correlate with the percentages of any of the CD4+ T cell subsets.

**Serial determinations of CD4+ T cell subsets in SF.** In 2 of the 4 patients (patients 1 and 4 in Figure 2A) who had synovectomies because of incomplete responses to DMARDs, 4 serial joint fluid samples were obtained during the course of the illness (Figure 3A). In patient 1, the first and second samples were obtained during treatment with oral doxycycline and then IV ceftriaxone when the results of PCR tests for *B burgdorferi* DNA in SF were positive. In patient 4, all 4 samples were obtained in the post–antibiotic treatment period when PCR results were negative. During methotrexate therapy, when the third sample was obtained, both patients had a >4-fold decline in IgG antibody responses to *B burgdorferi* (Figure 3B). Because of incomplete responses to methotrexate, both patients underwent arthroscopic synovectomies. When methotrexate was stopped prior to the procedure, both patients had increases in the size of joint effusions and small rebounds in antibody responses to *B burgdorferi*, but PCR results remained negative in synovial fluid and synovial tissue.

In both patient 1 and patient 4, the percentages of IFNγ-positive Th1 cells in the first 1 or 2 samples were exceedingly high in SF (~70%), and the percentages of FoxP3-positive Treg cells were very low (2–5%) (Figure 3C). During methotrexate therapy, the percentages of IFNγ-positive Th1 cells were lower, and the percentages of FoxP3-positive Treg cells were slightly higher. When all medications were stopped prior to synovectomy, the percentages of IFNγ-positive Th1 cells were almost as high, and the percentages of FoxP3-positive Treg cells were only slightly higher than those in

![Image](image_url)
the initial samples. In both patients, synovial tissue samples obtained during synovectomy contained scattered clusters of infiltrating mononuclear cells. These clusters consisted primarily of CD3+ T cells, whereas very few FoxP3-positive Treg cells were seen (Figure 4).

**Findings on Borrelia-specific proliferation assay.**

Eight patients had sufficiently large numbers of SFMCs for *B. burgdorferi*-specific proliferation assays in which Treg cells were depleted or retained. Of the 8 patients, 3 had antibiotic-responsive arthritis, 3 had antibiotic-refractory arthritis that resolved within months, and 2 had antibiotic-refractory arthritis with prolonged courses ending in synovectomies. Because it was not possible to sort cells using the intracellular FoxP3 marker, Treg cells were depleted using the surface marker CD25; the percentages of Treg cells among the 8 patients varied from 3% to 13%. T effector cells were defined as those that responded to either an antigen-specific or nonspecific stimulus, as measured by proliferation assay. Although *B. burgdorferi*-specific T effector cells were not enumerated, the percentages of these cells were surely variable as well.

Among the 8 patients with antibiotic-responsive or antibiotic-refractory arthritis, *B. burgdorferi*-specific responses were significantly lower when CD25+ Treg cells were retained than when they were depleted (*P* = 0.01) (Figure 5A). The median percentage of suppression was similar in patients with antibiotic-responsive arthritis and those with antibiotic-refractory arthritis (32% versus 38%) and in those with positive PCR results and those with negative PCR results (35% versus 34%). Thus, Treg cells in SF were functional in suppressing *B. burgdorferi*-specific proliferation responses. However, the percentage of Treg cells did not correlate with the degree of Borrelia-specific suppression. For example, in patient 3, a Treg cell frequency of 5% suppressed proliferation by 59%, whereas Treg cell frequencies of 12% in patients 7 and 8 suppressed proliferation by only 35% and 32%, respectively. This was presumably due to variation in the numbers of Borrelia-specific T effector cells in the patient samples.

**Findings on nonspecific Treg cell suppression assay with stimulation with anti-CD3 mAb.**

To control for variability in the numbers of Borrelia-specific T effector cells and Treg cells in different patients, we conducted crossover suppression assays with PB and SF in which equal numbers of T effector cells and Treg cells (1:1 ratio) were stimulated with irradiated syngeneic PBMCs and soluble anti-CD3 mAb, which activate all T cells. Sufficiently large numbers of SFMCs and PBMCs were available in only 2 patients with antibiotic-refractory arthritis (patients 4 and 10 in Figure 2A);
otrectate, and underwent a synovectomy 13 months after the sample date.

In both patients, Treg cells from either PB or SF suppressed T effector cells from PB by \( \sim 80\% \) (Figure 5b). Although T effector cells from SF were less susceptible to suppression compared with their PB counterparts (\( \sim 50\% \) versus \( \sim 80\% \)), Treg cells from PB and SF appeared similarly functional. Moreover, Treg cells from the PB of a normal human donor also suppressed autologous T effector cells from PB by \( 80\% \) (data not shown).

**DISCUSSION**

We characterized the CD4\(^+\) T cell subsets in PB and SF from 18 patients with antibiotic-responsive arthritis or antibiotic-refractory Lyme arthritis. Even though the samples in the antibiotic-responsive group were obtained prior to or soon after the start of antibiotic therapy, and those in the antibiotic-refractory group were usually obtained near or soon after the beginning of the post–antibiotic treatment period, the most abundant CD4\(^+\) T cell subset in SF in both groups was IFN\(\gamma\)-positive Th1 cells. The prominence of IFN\(\gamma\)-positive *Borrelia*-specific Th1 cells has been noted in the PB of patients with erythema migrans early in the infection (28) and in the PB and SF of patients with Lyme arthritis (13–15). *Borrelia*-specific Th1 cell responses decline with spirochete killing prior to the resolution of arthritis (4). However, as shown previously (15) and again in this study, Th1 cell responses in these patients persist at high levels in the post–antibiotic treatment period, and the antigen specificity of these cells may include T cells that react with currently unidentified autoantigens.

In contrast, the percentage of Th17 cells was low in each patient group, although in several patients the percentages were as high as 10–25%. Th17 cells were originally identified in the SF of a patient with Lyme arthritis (29), and neutrophil-activating protein A of *B burgdorferi* induced T cell lines derived from the SF of patients with Lyme arthritis to secrete IL-17 in culture (30). Thus, these cells may also play a role in control of the spirochete in Lyme arthritis, at least in some patients. However, high levels of IFN\(\gamma\), as seen in the SF of patients with Lyme arthritis (18), may often serve as a negative regulator of Th17 cell differentiation (31).

In both patient groups, the percentage of IL-4–positive Th2 cells was usually low in both PB and SF. However, compared with patients with antibiotic-responsive arthritis, those with antibiotic-refractory arthritis had significantly higher levels of Th2 cells in SF, and there was a trend toward higher percentages of Th2 cells and faster resolution of arthritis. Thus, Th2 cells may have a role in the resolution of the postinfectious phase of antibiotic-refractory arthritis. Although Th1 cell and Th2 cell responses are more polarized in mice than in humans, the switch from Th1 cell responses to Th2 cell responses in *B burgdorferi*-infected BALB/c mice is accompanied by arthritis resolution (32).

In this study, the major difference in CD4\(^+\) T cell subsets was the higher median percentage of FoxP3-positive Treg cells in the SF of patients with antibiotic-refractory arthritis than in those with antibiotic-responsive arthritis. However, the Treg cell percentage in SF varied greatly, from 3% to 22%. Moreover, among patients with antibiotic-refractory arthritis, there appeared to be 2 subgroups. One consisted of patients with a higher percentage of Treg cells in whom arthritis resolved within several months after the completion of antibiotic treatment, either because of the natural history of the illness or hydroxychloroquine therapy, or both. The second subgroup had persistently lower percentages of Treg cells in the post–antibiotic treatment period, as in the murine model (20), less response to DMARDs, and longer courses of arthritis, which often ended with synovectomy. In patients with antibiotic-responsive arthritis, it was not possible to determine whether the percentages of Treg cells increased prior to arthritis resolution, but in these patients, synovial inflammation was clearly down-regulated along with spirochete killing.

Although the number of patients in whom enough cells were available for functional assays was small, Treg cell suppression seemed similar in the various patient groups. In patients with antibiotic-responsive arthritis or antibiotic-refractory arthritis of short or long duration, Treg cells from SF repressed T effector cells similarly in *B burgdorferi*-specific assays. In addition, in 1 patient each with antibiotic-refractory arthritis of short or long duration, nonspecific crossover suppression assays showed that Treg cells from either PB or SF were equally effective in suppressing T effector cells, although the level of suppression exerted on T effector cells from SF (50%) was lower than that exerted on those from PB (80%). The difference between Treg cell suppression of SF and PB may be explained simply by differences in cell composition in the 2 compartments, because SF presumably contained more activated T cells than PB, and activated cells are relatively more resistant to Treg cells than are naive T cells (33,34). However, it is also possible that T effector cells were functionally altered by the local inflammatory environment in the joint (35). Although these factors need to be determined in larger numbers of patients, current observations sug-
gest that patients with antibiotic-refractory arthritis are more likely to have functional differences in T effector cells than intrinsic defects in Treg cells.

As in antibiotic-refractory Lyme arthritis, a dominant Th1 response (13–15) and increased numbers of Treg cells have been observed in SF in several types of chronic inflammatory arthritis, including RA (35–41) and juvenile idiopathic arthritis (42). Moreover, as was seen here, DMARD therapy increases the numbers of Treg cells in patients with RA (41,43,44). In one study of untreated patients with RA, Treg cells from PB appeared to be defective due to their inability to suppress T effector cell inflammatory cytokine production (43). However, in another study in which most patients were treated with methotrexate, Treg cells from PB were as functional as cells from normal donors (35), as seen here. Furthermore, Treg cells from SF had enhanced suppressive abilities, but this enhanced ability was offset by heightened T effector cell function. Thus, even in RA, the relative contribution of T effector cells or Treg cells to persistent synovial inflammation is not yet clear and may not be the same in all patients.

In antibiotic-refractory Lyme arthritis, the correlation between higher percentages of Treg cells and faster resolution of arthritis suggests that the determination of Treg cell numbers in the post–antibiotic treatment period may be a useful prognostic marker. It will be important to learn prospectively whether a high percentage of Treg cells, as determined at the completion of antibiotic treatment, predicts a favorable course, whereas a low percentage indicates a longer and more difficult course. Such information may be valuable when making treatment decisions.

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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. Steere 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. Shen, Shin, Strle, Glickstein, Steere.

Acquisition of data. Shen, Shin, McHugh, Steere.

Analysis and interpretation of data. Shen, Li, Glickstein, Drouin, Steere.

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