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T Helper 1 Response Is Dominant and Localized to the Synovial Fluid in Patients with Lyme Arthritis

Dawn M. Gross,* Allen C. Steere†, and Brigitte T. Huber2*

Cytokines produced by subsets of CD4+ T helper cells responding to an infection influences the efficiency with which the host is able to mount a protective immune response. In an attempt to elucidate the population of active cells involved in the propagation of Lyme arthritis we have utilized intracellular cytokine staining to analyze the polyclonal immune response at the single cell level. We have determined the Th phenotype in the synovial fluid of patients with a variety of chronic inflammatory arthritides, including patients representative of the spectrum of Lyme arthritis. Th1 cells dominate the immune response in the synovial fluid of patients with Lyme as well as those with rheumatoid or other types of chronic inflammatory arthritis. In addition, the severity of Lyme arthritis directly correlates with the ratio of Th1 to Th2 cells in the synovial fluid, such that the larger the effusion, the higher the ratio (r = 0.67, p < 0.05). These results suggest that Th1 cells play a direct role in the pathogenesis of the inflammatory process seen in Lyme arthritis, and that Th2 cells modulate the pro-inflammatory response generated by Th1 cells in the joint. Finally, we identify Th1 cells specific for outer surface protein A of Borrelia burgdorferi, the agent of Lyme disease. These cells are restricted to patients with Lyme arthritis and are localized to the joint. Furthermore, they persist in patients with prolonged antibiotic treatment-resistant Lyme arthritis, suggesting the possibility of an autoimmune process. The Journal of Immunology, 1998, 160: 1022–1028.

Lyme disease, caused by the spirochete Borrelia burgdorferi (Bb), is a multisystem illness with dermatologic, neurologic, cardiac, and arthritic manifestations (1, 2). Months after disease onset about 60% of untreated patients experience intermittent episodes of oligoarticular arthritis, or chronic synovitis, particularly affecting the knee (3, 4). Although most patients with Lyme arthritis respond favorably to antibiotic therapy, approximately 10% have continuous joint inflammation for several months to years after the apparent eradication of the spirochete from the joint with antibiotic treatment (3–7). We have termed such disease persisting for 12 months or longer “treatment-resistant chronic Lyme arthritis.” The synovial lesion in chronic Lyme arthritis is similar to that of other forms of chronic inflammatory arthritis, including rheumatoid arthritis (RA) (8). Hence, Lyme arthritis is an important model for understanding the basic mechanism(s) underlying the development of chronic inflammatory arthritis and autoimmune diseases.

In patients with Lyme disease, the immune response to Bb develops over a period of months, resulting in reactivity to an increasing array of spirochetal Ags (9). In approximately 70% of patients with Lyme arthritis, the final point of immune response expansion is the development of IgG Ab to outer-surface proteins A and B (OspA and OspB) of the spirochete (7). The onset of this response occurs near the beginning of prolonged episodes of arthritis (7). Patients with HLA-DR4 specificity often have T and B cell reactivity toward OspA, which has been associated with lack of response to antibiotic treatment (7). Thus, reactivity to OspA is a significant correlate of prolonged Lyme arthritis.

The subset of CD4+ T helper cells activated during an infection determines the efficiency with which the host is able to mount a protective immune response. This is achieved by the production of characteristic cytokine profiles (10–16). Th1 cells, capable of secreting IL-2, lymphotoxin, and IFN-γ, elicit an inflammatory response, thereby regulating antiviral responses and immunity to intracellular pathogens (10–16). Alternatively, Th2 cells, which produce IL-4, IL-5, IL-10, and IL-13, mediate humoral immunity, but inhibit cell-mediated inflammatory responses (10–16). Therefore, the type of Th cells induced in response to invasion by a particular pathogen can have a significant effect on the host’s ability to successfully combat the infection (10–15).

Analysis of T cell subsets involved in the development of Lyme arthritis in mice reveals a picture reminiscent of murine Leishmania or human leprosy in which the Th response determines the severity of the disease (17, 18). BALB/c (H-2b) mice, which show only a mild arthritis when infected with Bb, develop a predominant Th2 response. In contrast, C3H/HeJ (H-2k) mice, which mount severe arthritis with Bb infection, have a dominant Th1 response (19, 20). When these T cell responses are reversed by administration of anti-cytokine blocking Abs, the severity of the arthritis is exacerbated or ameliorated in the respective mouse strains (19). Interestingly, the resistant and susceptible phenotypes appear to be MHC linked, as mice with an H-2d haplotype show minimal or no sign of arthritis any time during infection; mice with an H-2d haplotype develop severe arthritis, and mice with H-2b or H-2k haplotypes demonstrate varying degrees of arthritis (21). A correlation with development of treatment-resistant chronic Lyme arthritis in human patients has been established with HLA-DR4 (5, 7), although the molecular basis of the susceptibility is not yet clear (22).
We have determined the Th phenotype in the synovial fluid of patients with Lyme arthritis, as well as those with rheumatoid arthritis and other forms of chronic inflammatory arthritis. In addition, we have identified Ag-specificity of T cells in the synovial fluid of patients representing the spectrum of Lyme arthritis. The novelty and power of our analysis lies in the determination of the polyclonal T cell response as an accurate representation of the repertoire present in the patient at the time the sample was obtained. We have achieved this unbiased picture by screening the total T cell population without in vitro selection for individual clones that are able to proliferate. This is particularly important, as it has been well established that Th2 responses cannot be detected by proliferation assays in vitro. Thus, we have designed an experimental approach that allows the analysis of individual cells, both in terms of Ag-specificity and lymphokine profile, while minimizing the number of patient cells required to obtain the clearest answer to our objective. No such investigations have been possible so far due to technical limitations. Our results yield three points: 1) IFN-γ-producing CD4+ cells dominate the T cell profile in the SF of patients with active arthritis. Furthermore, the severity of active arthritis, as determined by joint fluid effusion volume, directly correlates with the ratio of Th1:Th2 cells in the SF. 2) Elevated Th2 responses inversely correlate with severity of arthritis and duration of disease in Lyme arthritis patients; and 3) IFN-γ-producing T cells, which are Bb Ag-specific, are localized to the SF only in patients with Lyme arthritis. The identification of Ag-specific reactive Th1 cells in synovial fluid is of particular importance with regard to patients with treatment-resistant chronic Lyme arthritis, as such reactivity in the absence of Bb suggests the possibility of an autoimmune process.

Materials and Methods

Patients and controls

We studied 10 patients with Lyme arthritis and 12 control patients with rheumatoid arthritis or other forms of chronic inflammatory arthritis. The Lyme arthritis patients represent the spectrum of disease severity and duration. All Lyme patients met the CDC case definition for diagnosis of Lyme Disease. They had arthritis affecting the knee and serologic reactivity with Bb by ELISA and Western blotting: two patients had a prior history of erythema migrans. Patients with other chronic inflammatory arthritides were seronegative for Bb. The 10 Lyme arthritis patients and 7 of the control patients were evaluated in the Lyme Disease Clinic at New England Medical Center (Boston, MA). The remaining 5 RA patients' samples were a gift of Dr. S. Copper and Dr. R. Budd (Department of Medicine, University of Vermont Medical School, Burlington, VT). The protocol was approved by the Human Investigations Committee, and informed consent was obtained from each subject.

Duration of active arthritis in patients with Lyme arthritis has previously been categorized into three groups based upon months of active inflammation (5). We have expanded this categorization as follows: a score of 1+ was given for arthritis lasting between 1 wk and 3 mo; 2+ for 4 to 6 mo; 3+ for 7 to 12 mo; and, 4+ for longer than 12 mo. Severity of active arthritis can be assessed by a number of means including patient-reported pain evaluations, radiographic measurements of joint destruction, and inflammation as determined by SF effusion volume (23). We chose to measure disease severity based upon the volume of fluid drained from the knee joint: a score of 1+ for 1 to 10 ml; 2+ for 11 to 30 ml; 3+ for 31 to 50 ml and 4+ for greater than 50 ml. This measurement was the most reliable and least expensive means of acquiring this type of information for each patient. The duration and severity index were the sum of these two scores. The patients with Lyme arthritis were treated with oral doxycycline, 100 mg twice a day for 1 to 2 mo, or with i.v. ceftriaxone, 2 g once a day for 1 to 2 mo (Table I). Two of these patient samples were obtained before antibiotic treatment (TB and RA), whereas the remaining eight patient samples were obtained after initiation or completion of antibiotic therapy. The ten patients with Lyme arthritis were seen at 3- to 6-month intervals after therapy to determine duration of arthritis.

In vitro restimulation

Peripheral blood and synovial fluid were obtained simultaneously on each patient with Lyme arthritis. Lymphocytes were isolated via Ficoll-Hypaque (Sigma Chemical Co., St. Louis, MO) centrifugation. Cells were plated in 96-well flat-bottom plates (Costar, Cambridge, MA) at a density of 3 to 5 × 10^6 cells/200 ml in serum-free medium (AIM-V, GIBCO, Grand Island, NY). Restimulations consisted of a 6 h polyclonal stimulus or a 48 h Ag recall. For the 6 h pulse, cells were stimulated with PMA (50 ng/ml) plus ionomycin (1 mg/ml) in the presence of 2 μM monensin (Sigma, St. Louis, MO). Monensin acts as a Golgi transport inhibitor, resulting in retention of intracellular cytokines, which facilitates FACS analysis (24). A

### Table I. Lyme arthritis patient information

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>SF PCR Detection of Bb Before Therapy</th>
<th>Duration of Active Arthritis Before Antibiotic Therapy (mo)</th>
<th>Number of Months Therapy Received Before Sample</th>
<th>Antibiotic Therapy Received</th>
<th>Duration of Active Arthritis After Starting Antibiotic Therapy (mo)</th>
<th>SF PCR Detection of Bb After Antibiotic Therapy</th>
<th>Total Duration of Active Arthritis (mo)</th>
<th>Severity of Arthritis at Time of Sample</th>
<th>Duration and Severity Index</th>
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<tr>
<td>WA^b</td>
<td>47</td>
<td>M</td>
<td>ND</td>
<td>2.0</td>
<td>17</td>
<td>amox-2 wk* doxy-4 wk</td>
<td>cei-10 wk</td>
<td>34 (--)</td>
<td>36</td>
<td>4+</td>
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</tr>
<tr>
<td>MM</td>
<td>21</td>
<td>M</td>
<td>(+)</td>
<td>7</td>
<td>0.2</td>
<td>amox-8 wk doxy-4 wk</td>
<td>cei-4 wk</td>
<td>12 (--)</td>
<td>19</td>
<td>4+</td>
<td>7</td>
</tr>
<tr>
<td>LB</td>
<td>14</td>
<td>F</td>
<td>ND</td>
<td>1.0</td>
<td>15</td>
<td>amox-8 wk doxy-4 wk</td>
<td>cei-4 wk</td>
<td>23 (--)</td>
<td>24</td>
<td>3+</td>
<td>7</td>
</tr>
<tr>
<td>RA</td>
<td>52</td>
<td>M</td>
<td>(+)</td>
<td>4.5</td>
<td>0</td>
<td>amox-3 wk doxy-4 wk</td>
<td>cei-4 wk</td>
<td>4.0 (+) right knee left knee</td>
<td>8.5</td>
<td>4+</td>
<td>7</td>
</tr>
<tr>
<td>EM</td>
<td>15</td>
<td>F</td>
<td>(--)</td>
<td>1.0</td>
<td>1.0</td>
<td>amox-3 wk doxy-4 wk</td>
<td>cei-4 wk</td>
<td>13 (--)</td>
<td>14</td>
<td>3+</td>
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<tr>
<td>AM^b</td>
<td>49</td>
<td>M</td>
<td>(--)</td>
<td>4.0</td>
<td>3.0</td>
<td>amox-3 wk doxy-8 wk</td>
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<td>8.0</td>
<td>3+</td>
<td>6</td>
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<td>2.5</td>
<td>4+</td>
<td>6</td>
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<td>1.0</td>
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<td>3.5</td>
<td>2+</td>
<td>5</td>
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<td>17</td>
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<tr>
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<td>41</td>
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<td>3.0</td>
<td>0</td>
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<td>0.5 ND</td>
<td>3.5</td>
<td>2+</td>
<td>3</td>
<td></td>
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</table>

* amox = amoxicillin; doxy = doxycycline; cei = ceftriaxone; all control patients and 5 of the 10 Lyme patients were taking some form of non-steroidal anti-inflammatory drugs (NSAIDs).

** Patients had a prior history of erythema migrans.
48 h recall Ag response was induced with a delipidated form of purified Bb outer surface protein A, nL-OspA, (6.25 mg/ml). During the final 5 h of stimulation, the cells were pulsed with anti-hCD3 (OKT3 1:10,000 dilution of ascites) and anti-hCD28 (1 mg/ml), a generous gift from Dr. Gordon Freeman (Dana-Farber Cancer Institutes, Boston, MA), in the presence of monensin (2 nM). This restimulation protocol allows for enhanced cytokine accumulation in the active cells, which aids in detection by FACS. Coded cells pulsed with anti-hCD3 and anti-hCD28 alone for 5 h show minimal cytokine production at this time point. This restimulation protocol was adapted from PhamMingen (San Diego, CA).

Intracellular cytokine staining for FACS analysis

Following the in vitro restimulation, cells were transferred to 96-well V-bottom plates (Costar, Cambridge, MA) and centrifuged at 1600 rpm for 4 min. Staining protocols were adapted from PhamMingen (San Diego, CA). Cells were incubated with anti-hCD4-PerCP (Becton Dickinson, San Jose, CA) in staining buffer (PBS–Mediatech, Cambridge, MA; 0.1% NaN₃ – Sigma Chemical Co., St. Louis, MO; 1% FCS–Intergen Co., Purchase, NY) for 30 min at 4°C in the dark. Cells were then washed in staining buffer and fixed in 4% paraformaldehyde for 20 min at 4°C in the dark. Cells were then washed in staining buffer and fixed in 4% parafomaldehyde for 20 min at 4°C in the dark. Cells were again washed with staining buffer, followed by an incubation with anti-hIL-4-PE and anti-hIFN-γ-FITC (PharMingen, San Diego, CA) in permeabilization buffer (PBS, 0.1% Na₂–Na, 1% FCS, 0.1% saponin–Sigma Chemical Co., St. Louis, MO) for 30 min at 4°C in the dark. Finally, cells were washed with permeabilization buffer and resuspended in staining buffer for FACS analysis.

FACS analysis

Cells were analyzed on a FACSscan flow cytometer (Becton Dickinson, San Jose, CA), equipped with a 480 nm Argon ion laser. 10,000 events were collected per sample. Data were analyzed with the LYSIS II program (Becton Dickinson, San Jose, CA).

PCR for detection of Bb DNA

An aliquot of SF was obtained without heparin for PCR testing, as previously described (6).

Statistical analysis

Individual variables were compared by using paired Student’s t-test analysis and are expressed as the mean ± one SD. Correlational analysis was performed on Th1:Th2 ratio and arthritis severity data and expressed as the Pearson coefficient (r).

Results

Study group

The 10 patients with Lyme arthritis are representative of the clinical spectrum of duration and severity of the disease (Table I). All Lyme patients experienced mild to severe arthritis, affecting primarily the knee for 1 wk to 4 mo before diagnosis and treatment with oral or i.v. antibiotics. At the mild end of the spectrum, patient TB’s arthritis resolved within 2 wk after initiation of antibiotic treatment. Similarly, patient RM resolved his arthritis within 6 wk after starting antibiotic therapy. Patients JH, SM, AM, and RA required between 2 to 4 mo for complete resolution of arthritis after the initiation of therapy. At the far end of the spectrum, patients WA and LB had arthritis that persisted for almost 2 years after multiple courses of oral and i.v. antibiotics, thereby categorizing their disease as treatment-resistant chronic Lyme arthritis. Finally, patients MM and EM are currently receiving antibiotic treatment. Twelve control patients were studied: 8 had rheumatoid arthritis, 2 had psoriatic arthritis, 1 had spondyloarthritis, and 1 had chronic monoarticular arthritis (Table II). Most of these patients had had active arthritis from 1 to 20 years at the time of testing.

Expression of IFN-γ in CD4⁺ T cells from SF

Peripheral blood and synovial fluid lymphocytes were brieﬂy pulsed in vitro for 6 h with the polyclonal stimulus of phorbol ester and calcium ionophore (PMA/ionomycin). This in vitro incubation allows for enhanced accumulation of cytokines only in cells that have previously been activated in vivo, but not in quiescent cells

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Type of Arthritis</th>
<th>Therapy Received</th>
<th>Total Duration of Active Arthritis (mo)</th>
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<tr>
<td>JW</td>
<td>63</td>
<td>M</td>
<td>chronic monoarticular</td>
<td>NSAIDs, MTX*</td>
<td>20</td>
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<td>SC</td>
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<td>NA</td>
<td>Spondyloarthritis</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>ML</td>
<td>NA</td>
<td>NA</td>
<td>RA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>HL</td>
<td>59</td>
<td>M</td>
<td>Psoriatic</td>
<td>NSAIDs, MTX</td>
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<tr>
<td>SJ</td>
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<td>NA</td>
<td>RA</td>
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<td>RA</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>NP</td>
<td>17</td>
<td>F</td>
<td>RA</td>
<td>NSAIDs, MTX</td>
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<tr>
<td>MS</td>
<td>59</td>
<td>F</td>
<td>RA</td>
<td>NSAIDs, MTX</td>
<td>120</td>
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<tr>
<td>JS</td>
<td>58</td>
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<td>RA</td>
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<td>192</td>
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<tr>
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<td>144</td>
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<tr>
<td>JE</td>
<td>44</td>
<td>F</td>
<td>RA</td>
<td>NSAIDs, MTX</td>
<td>240</td>
</tr>
</tbody>
</table>

* NSAIDs, non-steroidal anti-inflammatory drug; MTX = methotrexate.

Clinical history was not available. In some instances, information regarding the duration and/or severity of arthritis at time of sample was not available, hence, calculation of a duration and severity index score was not possible.
A (OspA) of Bb was selected to restimulate SF cells and PBMC in vitro, as T and B cell responses to OspA have previously been shown to correlate with prolonged Lyme arthritis (25, 26). As shown by FACS analysis, the intracellular cytokine staining of OspA restimulated cells displayed a dominant Th1 profile in SF of all Lyme arthritis patients tested (7.1% vs 1.4% Th1 vs 2.3% Th2; p < 0.025; see Table I for severity scores). B, PMA/ionomycin pulsed CD4+ T cells from control arthritis patients show a dominant Th1 response localized to the SF (see Table II for description of arthritis). Unpulsed PBMC and SF CD4+ T cells show background intracellular cytokine staining levels of <2%. No significant differences were seen between the percentages of Th2 cells in SF compared with periphery. Since this Ag-specific response is relatively minor compared with the polyclonal response (7.1% vs 22.7%), it was not possible to make a quantitative statement regarding the ratio of OspA-reactive Th1 to Th2 cells and disease severity. However, the presence or absence of this response clearly indicates a qualitative difference between these patients.

To assure that the OspA recall response was specific for patients with Lyme arthritis, we tested SF cells from 12 patients with rheumatoid arthritis or other forms of chronic inflammatory arthritis. Upon in vitro restimulation with the Bb Ag OspA, no significant reactivity could be detected in any of the 12 control patients (Fig. 2B). Representative results are shown in Fig. 3A and 3B. Thus, the Th1 response seen in the SF of patients with Lyme arthritis represents a localized, Ag-specific response at the site of active joint inflammation.
Discussion

**Dominant Th1 response is localized to the synovial fluid in active arthritis**

The present study shows that freshly isolated, polyclonal, Ag-specific T cells from actively inflamed joints of patients with various forms of chronic inflammatory arthritis have a dominant Th1 phenotype. Previous analyzes have demonstrated Th1-like cytokine expression patterns in synovial tissue and T cell clones derived from a variety of chronic inflammatory arthritides (27–32). Synovial T cell clones derived from patients with *Chlamydia* or *Yersinia*-induced reactive arthritis have been reported to express Th1 cytokines (27–29). Similarly, T cell clones obtained from the SF of a patient with Lyme arthritis stimulated with *B. burgdorferi* Ags produced Th1-type cytokines (32). Comparable findings have been described for the inflammatory autoimmune disease rheumatoid arthritis (30, 33–35). Thus, the activation of cells able to produce Th1-type cytokines appears to be important in the pathogenesis of chronic inflammatory arthritis.

In an attempt to elucidate the population of active cells involved in the propagation of Lyme arthritis we utilized intracellular cytokine staining for FACS analysis, which allows a highly refined picture of the polyclonal Th1/Th2 profile to be analyzed at the single cell level with relative ease and accuracy. This technique quantifies individual cytokine-producing cells present in the SF, thereby obviating need for multiple in vitro restimulations that may result in outgrowth of selected populations. With this method we were able to demonstrate a higher prevalence of active Th1 cells in the SF compared with the periphery in patients with Lyme arthritis (Fig. 1A). We could detect no differences in the Th responses between patients who either had a history of erythema migrans or who had a positive PCR test for *B. burgdorferi* DNA in SF, as compared with patients who were negative for either of these parameters. Similarly, we showed that patients with rheumatoid arthritis or other forms of chronic inflammatory arthritis also have a dominance of Th1 cells in the SF. This is consistent with the expectation that Th1 cells predominate in a highly inflamed, localized environment such as the synovial space of the knee (Fig. 1B).

**Severity of Lyme arthritis correlates with the ratio of Th1:Th2 cells**

In this study, the magnitude of the Th1:Th2 ratio in SF cells correlated directly with the severity of joint swelling, suggesting that Th1 cells play an important role in the pathogenesis of Lyme arthritis. (Fig. 1A). Additionally, there was a trend between higher levels of Th2 activity in the synovial fluid and lower duration and severity index scores (Fig. 1A and Table I). Previous studies have demonstrated the ability of the Th2 cytokine, IL-4, to inhibit Th1-mediated inflammation (35–42). These observations are consistent with the findings in murine borreliosis where severity of joint disease correlates with the type of Th response. Thus, our data support the hypothesis that accumulation of Th2 cells in this highly activated environment allows for down-regulation of the pro-inflammatory Th1 cells, ultimately leading to resolution of the arthritis.

We predict that all patients who resolve their disease are able to do so by mounting a prominent Th2 response at the initiation of their disease resolution. This is in contrast to patients with chronic, treatment-resistant Lyme arthritis (WA and LB). FACS analysis of serial samples of SF cells from patient WA, taken at various time points over a two year period, never revealed the presence of Th2 cells (data not shown). Examination of serial samples may determine the critical period for development of a Th2 cell response capable of modulating the pro-inflammatory Th1 response. The exact ratio of Th1:Th2 cells necessary for down-regulation of the inflammatory response is unclear at this time. Based upon these initial observations, it may not be necessary to generate a response that is dominated by Th2 cells to resolve disease, but rather, potentiate a “yet to be determined” threshold of Th2 cell activity that is capable of modulating active Th1 cells. The developmental pathway leading to a Th1 or Th2 response is under intense study, and it is clear that cytokines play a role in precursor development. Nevertheless, it is critical to define Ag-specificity of the Th2 cells present in the SF of the rapidly treatment-responsive patient, as they do not appear to be OspA reactive (Fig. 2A). This may offer new insight into protective epitopes of *B. burgdorferi* (43–45).

In the patients who did not have significant levels of Th2 cells localized in the SF (Fig. 1A), the unopposed, highly inflammatory Th1-dominant immune response in the joint space was associated with ongoing inflammation, lasting for several months. This pattern was particularly evident in patients WA and LB, who were unable to resolve their arthritis 2 years after antibiotic therapy. Such Th1 cytokine production of IFN-γ has previously been associated with an increase in production of IL-1 and TNF-α, both of which have been shown to induce joint damage (35, 46).

**Treatment-resistant chronic Lyme arthritis patients contain Bb Ag-specific Th1 cells localized to the synovial fluid**

We provide evidence that the Th1 cells in the SF of patients with Lyme arthritis contain cells that are Ag-specific for OspA of *B. burgdorferi* (Fig. 2A and 3B), while the Th1 cells in the SF of control patients with other chronic inflammatory arthritides are unreactive to OspA (Fig. 2B and 3B). Thus, we have demonstrated Ag-specificity in
the arthritic lesion. The question that now arises is, what mechanism is responsible for continued inflammation in treatment-resistant chronic Lyme arthritis patients? C3H mice infected with Bb resolve their arthritis despite continued persistence of the spirochete (47). Chronic treatment-resistant Lyme arthritis patients seem to be the reverse. PCR testing of joint fluid for detection of spirochetes in treatment-resistant chronic Lyme arthritis patients has yielded negative results, despite the patients’ continuous joint inflammation (Table I). Yet, we show in this study that these patients retain Th1 cell reactivity to OspA (Fig. 2A). This suggests that the chronic Th1 response seen in the treatment-resistant chronic Lyme arthritis patients (WA and LB) may not require the presence of the spirochete for continued propagation of joint inflammation, but in fact, may represent an autoimmune process. On the other hand, spirochetes may have shed antigenic surface proteins into the joint space. Such a scenario would result in negative PCR data, but would not support our hypothesis of autoimmunity, as foreign Ag would still be present. It is important to note, however, that all immunocompetent mice, regardless of H-2 haplotype that determines the severity of the initial arthritic reaction to Bb infection, resolve the arthritic lesions (19, 21). Since this occurs despite continuous low level of Bb infection (47) and the presence of the HLA.DR*0401 transgene (48), it is likely that a factor other than spirochete Ag induces a chronic arthritic response. The simplest explanation is that mice do not have the cross-reactive autoantigen that elicits the chronic response seen in treatment-resistant Lyme arthritis patients. The search for such an autoantigen in these patients is under investigation. Understanding the genetic basis for the regulation of Th1 and Th2 cell differentiation in response to infection with Borrelia burgdorferi will further illuminate the process of protective, destructive and autoimmune response development, respectively.

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