The T Helper Cell Response in Lyme Arthritis: Differential Recognition of Borrelia burgdorferi Outer Surface Protein A in Patients with Treatment-resistant or Treatment-responsive Lyme Arthritis

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Summary

The host response to Borrelia burgdorferi is likely to play a role in the pathogenesis of Lyme arthritis. Whereas most patients with Lyme arthritis can be cured with antibiotic therapy, ~10% of the patients have persistent arthritis for months or even several years after antibiotic treatment. In this study, we tested the hypothesis that the T cell response to one or more antigens of B. burgdorferi is different in patients with treatment-responsive or treatment-resistant Lyme arthritis. For this purpose, 313 B. burgdorferi-specific T cell lines were derived from the synovial fluid or peripheral blood of four patients with treatment-responsive Lyme arthritis and five patients with treatment-resistant arthritis. 87 T cell lines from treatment-responsive Lyme arthritis and 112 lines from the treatment-resistant group were examined for the recognition of five recombinant B. burgdorferi proteins: outer surface proteins A (OspA), B, C, p39, and p93. In both groups of patients, the T cell lines frequently recognized OspB, and only occasionally recognized OspC, p39, and p93. In contrast, OspA was preferentially recognized by T cell lines from patients with treatment-resistant arthritis, but only rarely recognized by T cell lines from patients with treatment-responsive arthritis (odds ratio 28.4, 95% confidence interval 9.2–87.8, p <0.005). These results are compatible with the hypothesis that the T cell response to B. burgdorferi OspA is involved in the pathogenesis of treatment-resistant Lyme arthritis.

Lyme borreliosis, a tick-borne infection caused by the spirochete Borrelia burgdorferi, is a multisystem disorder. A characteristic skin lesion, erythema migrans, accompanied by meningitis- or flu-like symptoms, is often the first sign of the disease (1, 2). The spirochetes frequently disseminate, and neurologic or cardiac symptoms or migratory joint pain may develop weeks to months after the infection. Months later, ~60% of untreated North American patients develop intermittent attacks of monoarticular or oligoarticular arthritis, primarily in large joints. Particularly during the second or third year of the illness, ~10% of patients with arthritis develop continuous joint inflammation for 1 yr or longer, which we have termed “chronic Lyme arthritis” (3, 4). Lyme arthritis coexists with specific and strong humoral and cellular immune responses to B. burgdorferi (5–10).

Patients with Lyme arthritis usually respond to antibiotics (11–13); however, in ~10% of patients with arthritis, the inflammation persists despite antibiotic therapy (11, 13, 14). Careful analysis of the treatment responses in a recent cohort of patients with Lyme arthritis led to the concept of dividing patients with Lyme arthritis into two groups: those with treatment-responsive Lyme arthritis, defined as resolution of arthritis within 3 mo after the initiation of appropriate antibiotic treatment; and those with treatment-resistant arthritis, which lasts for more than 3 mo after the initiation of treatment (13). Spirochetes have occasionally been seen in or isolated from the synovium of patients with Lyme arthritis (15, 16) and a few spirochete-like structures have also been detected in the synovium of some patients with chronic Lyme arthritis after antibiotic treatment (17). Recently, PCR has been shown to provide a sensitive and reliable method for the detection of B. burgdorferi DNA in synovial fluid (SF) in patients with Lyme arthritis (18, 19). Whereas Borrelia DNA can be amplified reliably from pretreatment samples of SF, it has been shown that most patients who have persistent Lyme arthritis despite multiple courses of antibiotic therapy yield consistently negative PCR test results in SF after antibiotic treatment (18, 19). Thus, although Lyme ar-

1 Abbreviations used in this paper: EBV-BCL, EBV-transformed B cell line; MBP, maltose binding protein; OspA, OspB, OspC, outer surface protein A, B, C; PB, peripheral blood; SF, synovial fluid; TCL, T cell line.
burstdorferi, the ongoing synovial inflammation in patients with treatment-resistant arthritis may be caused by factors other than persistent _B. burgdorferi_ infection.

Host factors have been linked to the pathogenesis of treatment-resistant Lyme arthritis. The presence of the HLA-DR4 specificity in patients with Lyme arthritis was found to be associated with a lack of response to antibiotic therapy (20). Among HLA-DR4+ patients, those with IgG antibodies against outer surface protein A (OspA) or OspB of _B. burgdorferi_ had arthritis for significantly longer after treatment than those who lacked reactivity with these proteins (10). Most recently, the HLA-DR4 specificity and the level of IgG reactivity with OspA were each reported to be a risk factor for treatment-resistant Lyme arthritis and the presence of both factors doubled the risk of this outcome (13). The synovial lesion in patients with chronic Lyme arthritis is similar to that seen in other forms of chronic inflammatory arthritis, including rheumatoid arthritis. Villous hypertrophy, vascular proliferation, and a lymphoplasmacellular infiltrate are present (15, 17). There is intense HLA-DR and 'DQ expression throughout the lesion, and the T cells are mostly CD4+ (17).

Lyme arthritis is one of the few forms of chronic inflammatory arthritis in which the cause is known with certainty. Therefore, the study of _B. burgdorferi_–specific T cells from patients with Lyme arthritis provides an opportunity to investigate the role of T cells in the pathogenesis of this form of inflammation. If there is a role for T cells in the pathogenesis of treatment-resistant Lyme arthritis, two competing hypotheses need to be considered. First, the patients with treatment-resistant arthritis might lack the particular T cells needed to eradicate _B. burgdorferi_ from the host because of a hole in the T cell repertoire. In such patients, _B. burgdorferi_ may persist, thereby maintaining chronic inflammation for a period of months after eradication of the spirochete.

We have started to test the hypothesis that in patients with treatment-resistant Lyme arthritis, a T helper cell response induced by one or more _B. burgdorferi_ antigens results in chronic synovial inflammation. This hypothesis leads to the testable prediction that the T helper cell response to _B. burgdorferi_ in patients with treatment-responsive Lyme arthritis differs from that in patients with treatment-resistant arthritis. Possible differences between the two patient populations include epitopes recognized or cytokines produced by _B. burgdorferi_–specific T cells. In this report, T cell lines (TCL) derived from the SF or peripheral blood (PB) of patients with treatment-responsive or treatment-resistant arthritis were examined for their recognition of _B. burgdorferi_ lysates and five of the major spirochetal antigens: p22 (OspC), p31 (OspA), p34 (OspB), p39, and p93.

**Materials and Methods**

**Patients.** For these studies, nine representative patients, four with treatment-responsive and five with treatment-resistant Lyme arthritis, were chosen from patients seen in the Lyme Disease Clinic at New England Medical Center. All nine patients, who came from the northeastern United States, met established clinical and serologic criteria for the diagnosis of Lyme arthritis (21). They had intermittent episodes of arthritis or chronic arthritis in one or both knees, sometimes preceded by erythema migrans, and they had markedly elevated IgG antibody responses to _B. burgdorferi_ (see Table 1). The protocol and consent forms for these studies were approved by the Human Investigation Review Committee of New England Medical Center. Blood and joint fluid were drawn on the same day. Samples from patients with treatment-responsive arthritis were obtained before antibiotic therapy. Samples from patients with treatment-resistant arthritis were obtained after antibiotic treatment. The treatment-resistant patients were referred to our clinic after treatment by their physicians with antibiotic therapy. During this study, it became possible to test for _B. burgdorferi_ DNA in SF by PCR (18). Before this time, joint fluid was obtained in the presence of heparin, a known inhibitor of PCR amplification (22), and therefore these samples could not be used for PCR. In the other five patients, an aliquot of SF was obtained without heparin for PCR testing.

_B. burgdorferi_ Lysates. The N40 strain of _B. burgdorferi_ was propagated in Barbour-Stoenner-Kelly (BSK) medium with 6% rabbit serum (both from Sigma Chemical Co., St. Louis, MO). Bacteria were grown for 5-7 d at 33°C, harvested by centrifugation at 15,000 g for 20 min, and washed three times in PBS, sonicated, and filtered (0.2 µm). The protein content was estimated by spectrophotometry (OD 280 nm), aliquots were made and stored at -70°C.

**Constructs for Recombinant _B. burgdorferi_ Proteins.** p22 (OspC), p31 (OspA), p34 (OspB), and p93 were produced as maltose binding protein (MBP) fusion proteins and p39 was used as lysates from transformed Escherichia coli. The construct into which the ospA gene from _B. burgdorferi_ strain B31 had been cloned, as described (10), was provided by Dr. R. Kalish (New England Medical Center). The constructs containing the ospC gene or the gene for the 93-kD antigen from _B. burgdorferi_ strain 297 have been described earlier (23) and were provided by Dr. B. Fung (New England Medical Center). The plasmid pSPR53 with a restriction fragment containing gene coding for the 39-kD protein of the _B. burgdorferi_ strain Sh-2-82 (24) was a gift from Dr. T. Schwan (Rocky Mountain Laboratories, Hamilton, MT).

To produce the MBP-OspB fusion protein, _B. burgdorferi_ strain G39/40 genomic DNA was isolated as described (25). 1–10 ng G39/40 genomic DNA was used as template in subsequent PCR amplifications. The oligonucleotides 5' GCCGATCCCTGTCAGCAAAAAGGTGCTAG GAG3' and 5'CCTCTGACCTTCTACACTGACTGATTGC3' were used as 5' and 3' primers, respectively. The primer sequences were deduced from the published nucleotide sequence of the closely related _B. burgdorferi_ strain B31 (26) and contain restriction enzyme digestion sites to allow for directional in-frame insertion. The purified PCR products were digested with SalI and BamHI, and ligated into the vector pmalCRI (New England Biolabs, Inc., Beverly, MA) which carries the maltE gene under the control of the inducible _p mal_ promoter. The construct obtained codes for OspB amino acids 16–296, the whole mature protein without the signal sequence (26).

**Sequencing of Constructs.** To confirm the correct insertion and orientation of the constructs before producing recombinant proteins, all plasmid DNAs were partially sequenced by the dideoxy chain termination method, using modified T7 polymerase in a commercially available kit (Sequenase®; United States Biochemical Corp., Cleveland, OH) according to the manufacturer's instructions.

**Expression of Recombinant Proteins.** _E. coli_ strain 71.18 was transplanted.
formed with pmalCRI containing the appropriate construct, induced, lysed, and the crude cell extracts purified over an amylase column as described before (10). mAbs H 5332 (27), H 6831 (28), L22 1F8 (29), and D7 (30) were used to verify the presence of OspA, OspB, OspC, and p39, respectively. E. coli strain XL1-blue was transformed with pSPR33, and lysates were obtained as described above. Lysates from untransformed XL1 blue were produced to serve as controls in our experiments. mAb p39, which was also provided to us by Dr. T. Schwann, was used to detect p39 in the lysates.

TCL. Mononuclear cells were obtained from heparinized PB or SF by the Ficoll-Hypaque method (lymphocyte separation medium [LSM]; Organon Teknika, Durham, NC). The mononuclear cells were plated in 96-well plates (Costar Corp., Cambridge, MA) in a final volume of 200 µl at a density of 10^6 cells/ml in complete medium containing RPMI 1640 (GIBCO BRL, Gaithersburg, MD) with 5% human AB serum (Sigma Chemical Co.), 10 mM glutamine, penicillin (100 U/ml), streptomycin (100 µg/ml), 2 x 10^{-3} M 2-ME, and 10 mM Hepes buffer. Sonicated B. burgdorferi lysate was added at a final concentration of 10–20 µg/ml. 4 d later, human recombinant IL-2, (final concentration 5 U/ml; Cetus, Emeryville, CA) and IL-4 (final concentration 5 U/ml; Schering-Ploegh, Bloomfield, NJ) were added to the plates. The cells were expanded in the same medium for 18–21 d. Subsequent additions of medium, IL-2, and IL-4 were necessary during this period. After this growth period, the viable T cells were retrieved through Ficoll-Hypaque separation. Aliquots of the TCL were used for assays and restimulation with antigen. Irradiated (3.5 Gy) autologous PBMC at a T cell/PBMC ratio of 1:2.5 were used as APC for the first restimulation. After the first restimulation, irradiated (8–10 Gy) autologous EBV-transformed B cell lines (EBV-BCL) were used as APC. The TCL were maintained through several rounds of antigen stimulation and rest. After the first or second antigen exposure, TCL were tested for recognition of B. burgdorferi in proliferation assays. The B. burgdorferi-specific TCL were then tested for recognition of the recombinant proteins after the second or third antigen exposure.

EBV-BCL. 10^7 PBMC were resuspended in complete FCS medium (same as complete medium except that 10% FCS [Sigma Chemical Co. or GIBCO BRL] was used instead of human AB serum). To this suspension supernatant from the chronically EBV-infected mammosed cell line B95-8 (gift from Dr. D. Thorley-Lawson, Tufts University) and cyclosporin A (Sandoz, East Hanover, NJ) were added. Transformed cells were then expanded in complete FCS medium.

T Cell Proliferation Assays. TCL were tested for responsiveness to antigen in a standard proliferation assay as described earlier (31). Negative controls consisted of no antigen and the appropriate control antigen (e.g., untransformed E. coli lysates for p39 or MBP-β-Gal-α alone for the MBP fusion proteins). Positive controls were stimulated with PHA (0.5–1 µg/ml, final concentration). 2.5 x 10^5 T cells and the same number of irradiated autologous EBV-BCL were plated in triplicate in 96-well plates (Costar Corp.) with complete medium alone or in the presence of antigen in a final volume of 100 µl/well. Antigen concentrations for the assays were titrated over a range that had been determined to be optimal in earlier experiments: B. burgdorferi lysate was used at 10–20 µg/ml, MBP-β-Gal-α and the MBP fusion proteins were used at 0.03–3 µM, and p39 and untransformed XL1 blue E. coli were used at dilutions of lysates ranging from 1:150 to 1:4050. To measure lymphocyte proliferation, [H]TDR (1 µCi/well) in 50 µl complete medium was added to each well after a 72-h incubation. The cells were harvested 18 h later with an automated harvester and incorporated thymidine was detected in a liquid scintillation counter plate (Top Count; Packard Instruments, Meriden, CT). Results, which are the mean values of triplicate cultures, are calculated as counts per minute with background subtracted (Δcpm). A result was considered positive if the cpm (test) was at least twice the cpm (control) and the Δcpm was >1,000.

Immunofluorescent Staining and Flow Cytometry. Cells were stained at 10^5 cells/100-μl sample for 20 min at 4°C in PBS/1% BSA/0.1% NaN3. The fluorescein-coupled mAbs recognizing CD3 (OKT3; Ortho Diagnostic Systems, Raritan, NJ), TCR-α/β (TCR-α/β, Becton Dickinson & Co., San Jose, CA), TCR-γ/δ (TCR-γ/δ, Becton Dickinson & Co.), CD4 (OKT4A, Ortho), and CD8 (CD8; Caltag Laboratories, South San Francisco) were used according to the manufacturers instructions. Isotype-matched FITC- or PE-coupled antibodies were used as negative controls. The cells were then analyzed on a FACS® (Becton Dickinson & Co.) flow cytometer.

Results

Clinical Characteristics of the Patients with Lyme Arthritis. The ages of the nine patients with Lyme arthritis ranged from 4 to 67 yr (mean 42 yr); seven were men and two were women. Of the nine patients, four responded to treatment with 1-mo courses of oral doxycycline or amoxicillin plus probenecid. They had the onset of symptoms of Lyme disease a mean duration of 7 mo before treatment; arthritis was evident for a mean of 2 mo during that period, and they had the resolution of arthritis within 1 to 3 mo after the initiation of treatment. In both patients in this group in whom PCR testing for B. burgdorferi DNA was done, the results were positive in SF before antibiotic treatment (Table 1). The other five patients had persistent arthritis, despite treatment with these antibiotic regimens and, in three instances, i.v. treatment with ceftriaxone for 3–6 wk. PCR testing for B. burgdorferi DNA was done in three patients in this group after antibiotic treatment; the results were negative. Similar to the patients with treatment-responsive arthritis, they had the onset of symptoms of Lyme disease a mean duration of 8 mo before testing. However, during that period they had arthritis for a mean duration of 5 mo and they had persistent arthritis for 6–24 mo (mean, 14 mo) after antibiotic treatment. The clinical characteristics of the nine patients are summarized in Table 1.

B. burgdorferi-specific T Cell Lines from PB and SF of Patients with Lyme Arthritis. We derived 375 TCL from the PB or SF of the nine patients. The 224 TCL derived from SF showed a very high frequency (97%) of specificity for B. burgdorferi, whereas 63% of the 152 TCL obtained from PB were B. burgdorferi–specific (Table 2). Thus, a total of 313 B. burgdorferi–specific TCL were derived from the SF and PB of these patients.

The proliferative responses of individual TCL obtained from SF were similar to the responses of TCL obtained from PB. As demonstrated in Fig. 1 for one representative patient (RB), the mean Δcpm ± SEM for the SF-derived TCL was 20,822 ± 3,925 and for the PB-derived TCL was 14,795 ± 5,842. Similar results were obtained for the other eight patients. Of note, B. burgdorferi–specific TCL were obtained from the PB and SF of patients RB, TB, and CD, in whose SF B. burgdorferi DNA was no longer detectable by PCR (RB, TB, and CD; see Tables 1 and 2).
Table 1. Clinical Characteristics of the Nine Patients with Lyme Arthritis

| Patient | Age  | Sex | Lyme disease | Duration | Before treatment | After treatment | IgG response to B. burgdorferi | PCR |
|---------|------|-----|--------------|----------|------------------|----------------|-------------------------------|-----|
| FD      | 50   | M   | 1            | mo       | 1                | 3              | 1: 25,600                     | +   |
| AL      | 67   | M   | 14           |          | 5                | 3              | 1: 25,600                     | +   |
| TP      | 49   | M   | 12           |          | 1                | 3              | 1: 6,400                      | ND  |
| MS      | 55   | F   | 1            |          | 1                | 1              | 1: 3,200                      | ND  |
| RB      | 52   | M   | 10           | mo       | 8                | 8              | 1: 800                        | -   |
| TB      | 4    | M   | 0.25         |          | 0.25             | 6              | 1: 3,200                      | -   |
| CD      | 36   | F   | 23           |          | 11               | 15             | 1: 3,200                      | -   |
| JP      | 35   | M   | 1            |          | 1                | >24            | 1: 25,600                     | ND  |
| DS      | 30   | M   | 6            |          | 6                | 15             | 1: 3,200                      | ND  |

* All manifestations of Lyme disease, including Lyme arthritis.

† ELISA.

§ Detection of B. burgdorferi DNA in the patient's SF by PCR. Patients FD and AL were tested before antibiotic therapy. No later sample was available since treatment was effective. Patients RB, TB, and CD with therapy-resistant arthritis were tested after antibiotic treatment; pretreatment SF samples were not available from these patients.

FACS® analysis of 23 randomly selected B. burgdorferi–specific TCL derived from the SF or PB of two patients with treatment-responsive Lyme arthritis and three patients with treatment-resistant Lyme arthritis showed that all lines were CD3+, CD4+, TCR-α/β+, CD8−, and TCR-γ/δ− (data not shown).

Table 2. TCL from the Nine Patients with Lyme Arthritis

| B. burgdorferi–specific/total | Patient | From PBMC | From SFMC |
|-------------------------------|---------|-----------|-----------|
| Therapy-responsive arthritis  | FD      | 6/9       | 9/9       |
|                              | AL      | 12/14     | 13/13     |
|                              | TP      | 7/7       | 5/5       |
|                              | MS      | 4/5       | 96/96     |
| Therapy-resistant arthritis   | RB      | 16/21     | 29/30     |
|                              | TB      | 4/4       | 7/7       |
|                              | CD      | 11/13     | 20/20     |
|                              | JP      | 20/40     | 27/32     |
|                              | DS      | 15/38     | 12/12     |
| Total                         | 95/151  | 218/224   |

The number of B. burgdorferi–specific TCL and the number of TCL tested from each patient's PB and SF are shown.

T Cell Recognition of Recombinant B. burgdorferi Proteins in Patients with Treatment-responsive Arthritis. We analyzed 62 B. burgdorferi–specific TCL derived from the SF and 25 TCL derived from PB of four patients with treatment-responsive Lyme arthritis. Since these lines are polyclonal, they have the potential to recognize more than one B. burgdorferi antigen. Overall, OspB was the most frequently recognized antigen by both SF-TCL and PB-TCL. However, each patient had a different pattern of antigen recognition. For example, 67% of the 36 SF-TCL tested from patient MS recognized p39, and she was the only patient whose TCL recognized this antigen. All 11 SF- and PB-TCL from patient TP recognized OspC, and recognition of this antigen was unusual in other
by one patient each, and p93 and OspA were rarely recognized by all four patients (Table 3).

**T Cell Recognition of Recombinant *B. burgdorferi* Proteins in Patients with Treatment-resistant Lyme Arthritis.** We analyzed 74 TCL derived from the SF and 38 PB-TCL in the five patients with treatment-resistant arthritis. In striking contrast with the findings in treatment-responsive patients, OspA was the preferentially recognized antigen in those with treatment-resistant arthritis (Table 4). Of the 112 SF- and PB-TCL, 67 recognized OspA. OspB was also frequently recognized (59/112 SF- and PB-TCL). Patient JP was the exception: of the 22 SF- and PB-TCL from this patient, only two recognized OspA and two recognized OspB. TCL from this group of patients recognized OspC (15/112), p39 (10/112), and p93 (7/112) at a lower frequency than OspA and OspB. Fig. 3 shows that OspA and OspB were the most frequently recognized antigens for the 23 *B. burgdorferi*-specific SF-TCL from a typical patient, RB. Thus, in patients with treatment-resistant arthritis OspA and OspB were frequently recognized by T cells from both SF and PB, whereas OspC, p93, and p39 were seldomly recognized.

When the responses were compared in patients with treatment-responsive or treatment-resistant arthritis, OspB was a major antigen in both groups, whereas OspC, p39, and p93 were usually recognized with relatively low frequency. The major difference between the groups was the T cell recognition of OspA. Among the five recombinant proteins tested, OspA was the antigen most frequently recognized by TCL from patients with treatment-resistant Lyme arthritis (68/112), whereas this antigen was least frequently recognized by TCL from patients with treatment-responsive arthritis (5/87). Comparing SF-TCL from patients with treatment-resistant or treatment-responsive arthritis, the odds ratio for OspA recognition is 28.4 (95% confidence interval, 9.2-87.8). This difference is statistically significant ($p < 0.005$).

**Discussion**

Lyme arthritis is a suitable model to study chronic synovial inflammation for three reasons: The inciting agent, *B. burgdorferi*, is known (32), histopathologically Lyme arthritis is similar to other forms of chronic synovial inflammation, including rheumatoid arthritis (15, 17), and Lyme arthritis can be differentiated into two forms—one responding and the other resistant to antibiotic therapy (13). It has long been speculated that the host response to *B. burgdorferi* contributes to the pathogenesis of chronic Lyme arthritis (1, 2, 33). Therefore, it is of interest to compare the recognition of *B. burgdorferi* antigens by TCL derived from patients with the two forms of Lyme arthritis.

To date, one group has published reports on antigen recognition by *B. burgdorferi*-specific cloned TCL derived from patients with Lyme disease. They described T cell clones recognizing OspA (34, 35), OspB (35), flagellin (8), heat shock protein (HSP60) (8), and HSP70 (35). We analyzed the T cell response to five *B. burgdorferi* proteins, three of which...
Table 3. Recognition of Five Recombinant Proteins by B. burgdorferi-specific TCL from Four Patients with Treatment-responsive Lyme Arthritis

| Patient | Bb | OspA | OspB | OspC | p39 | p93 |
|---------|----|------|------|------|-----|-----|
|         | n  | n    | %    | n    | %   | n   |
| SF-TCL  |    |      |      |      |     |     |
| FD      | 9  | 2    | 22   | 8    | 89  | 2   |
| AL      | 13 | 0    | 0    | 0    | 0   | 0   |
| TP      | 4  | 1    | 25   | 2    | 50  | 4   |
| MS      | 36 | 1    | 3    | 15   | 42  | 3   |
| Total   | 62 | 4    | 7    | 25   | 40  | 9   |
| PB-TCL  |    |      |      |      |     |     |
| FD      | 6  | 0    | 0    | 6    | 100 | 0   |
| AL      | 8  | 1    | 13   | 2    | 25  | 1   |
| TP      | 7  | 0    | 0    | 0    | 7   | 100 |
| MS      | 4  | 0    | 0    | 2    | 50  | 1   |
| Total   | 25 | 1    | 4    | 10   | 40  | 9   |

The numbers (percentage) of B. burgdorferi (Bb)-specific SF-TCL and PB-TCL specific for each recombinant antigen are shown.

(OspC, p39, and p93) have not been studied before. The fact that B. burgdorferi-specific TCL could be derived from SF samples in which B. burgdorferi was no longer detectable by PCR, deserves comment. Although PCR detection of B. burgdorferi has been shown to be extremely sensitive (19, 36, 37), it remains possible that the number of spirochetes in PCR-negative SF samples was too small for detection or that B. burgdorferi organisms were still present in the synovial tissue or other sites although absent from the SF. In the absence of viable spirochetes, B. burgdorferi proteins might still be present in the synovium, similar to what has been found in Yersinia-induced reactive arthritis (38). Finally, T cell memory is likely to be long-lived, even in the absence of antigen (39). The pertinent point from our study is that a strong B. burgdorferi-specific T cell response could be detected whereas spirochetes were either present in very small numbers or absent altogether.

Three features emerge from our study comparing TCL from

Table 4. Recognition of Five Recombinant Proteins by B. burgdorferi-specific TCL from Five Patients with Therapy-resistant Lyme Arthritis

| Patient | Bb | OspA | OspB | OspC | p39 | p93 |
|---------|----|------|------|------|-----|-----|
|         | n  | n    | %    | n    | %   | n   |
| SF-TCL  |    |      |      |      |     |     |
| RB      | 23 | 21   | 91   | 18   | 78  | 7   |
| TB      | 7  | 7    | 100  | 7    | 100 | 0   |
| CD      | 17 | 8    | 47   | 7    | 41  | 4   |
| JP      | 15 | 1    | 7    | 1    | 7   | 0   |
| DS      | 12 | 1    | 100  | 11   | 92  | 2   |
| Total   | 74 | 49   | 66   | 44   | 60  | 13  |
| PB-TCL  |    |      |      |      |     |     |
| RB      | 8  | 4    | 50   | 3    | 38  | 0   |
| TB      | 3  | 3    | 100  | 2    | 67  | 0   |
| CD      | 15 | 5    | 33   | 4    | 27  | 2   |
| JP      | 7  | 1    | 14   | 1    | 14  | 0   |
| DS      | 5  | 5    | 100  | 5    | 100 | 0   |
| Total   | 38 | 18   | 47   | 15   | 40  | 2   |

The numbers (percentage) of B. burgdorferi (Bb)-specific SF-TCL and PB-TCL specific for each recombinant antigen are shown.
the two groups of patients. First, OspB was recognized at high frequency by T cells from patients with either form of Lyme arthritis. Second, OspC, p39, and p93 were recognized by TCL from both groups of patients but in most instances these antigens were recognized at low frequency. Finally, the most important finding of our study was the differential recognition of OspA: it was the most frequently recognized antigen by TCL from patients with treatment-resistant arthritis, whereas it was the least frequently recognized antigen by TCL from those with treatment-responsive arthritis (p < 0.005).

How should the differential recognition of OspA by TCL from patients with treatment-resistant or treatment-responsive Lyme arthritis be interpreted? Since the IgG response to OspA appears late, if at all, in patients with Lyme disease (5), a T cell response against OspA in treatment-resistant patients might simply be due to the duration of infection: the longer the disease duration, the more likely the development of an anti-OspA response. However, the period from the onset of Lyme disease to the onset of Lyme arthritis was similar in the two groups of patients: 7 mo for the treatment-responsive and 8 mo for the treatment-resistant group (Table 1). Furthermore, OspA was frequently recognized by TCL from patient TB (Tables 1 and 4). These TCL were derived only 3 mo after the onset of the disease. In addition, our findings fit with previous work, which showed a significant association between IgG antibody reactivity to OspA and treatment-resistant arthritis (10). Such an association was not found with late neurological manifestations of Lyme disease (10).

If the sole cause for treatment-resistant Lyme arthritis was persistent infection with B. burgdorferi, one could hypothesize that all patients initially have OspA-specific T cells, but this reactivity disappears in the patients with treatment-responsive arthritis after eradication of the spirochete. In contrast, in the patients with treatment-resistant arthritis, B. burgdorferi and OspA-specific T cells may persist. However, the fact that the samples in the patients with treatment-responsive arthritis were obtained before treatment argues against this interpretation.

Our data strongly indicate that a T cell response to OspA does not protect from Lyme arthritis. On the contrary, our finding that OspA is differentially recognized by TCL from the two groups of patients is compatible with the hypothesis that the T cell response to OspA is involved in the pathogenesis of the chronic synovial inflammation in these patients.

Two models, which are not mutually exclusive, could help explain an OspA-induced pathogenic immune response. Cross-reactivity ("molecular mimicry") (40), of a self-antigen with a T cell epitope on OspA could overcome T cell tolerance to a self-antigen present in the joint. A search of the GenBank, EMBL, and Swissprot databases did not reveal significant sequence homologies of OspA with human proteins. However, not all potential self-antigens are included in these databases. Furthermore, in experimental autoimmune encephalomyelitis in mice, it has recently been shown that peptides containing only four or five native residues of the encephalitogenic 11 amino acid peptide could induce the disease (41). Therefore, it is conceivable that an epitope with only limited homology to a self-antigen might induce cross-reactivity.

According to the second model, non-B. burgdorferi-specific potentially autoreactive "bystander" cells could be activated within the joint. B. burgdorferi is known to be a strong inducer of IL-1 (42) and TNF-α (43) production by mononuclear cells. We and others have shown that certain substances such as bacterial toxins (31, 44), IL-1 (45), or IL-2 (46, 47) can interfere with T cell tolerance. According to this model, the acute synovial inflammation caused by infection with B. burgdorferi allows the influx of self-reactive T cells into the synovium where they could overcome self-tolerance.

However, our data do not provide experimental proof for a detrimental effect of a patient's T cell response against OspA. In fact, only 1/15 SF-TCL from one patient with treatment-resistant arthritis recognized OspA in a manner consistent with the molecular mimicry hypothesis. As bacterial toxins (31, 44), IL-1 (45), or IL-2 (46, 47) can interfere with T cell tolerance, it is conceivable that an epitope with only limited homology to a self-antigen might induce cross-reactivity.
resistant arthritis recognized OspA. Thus, other factors, persistent *B. burgdorferi* infection or host factors, must also be important in the pathogenesis of chronic synovial inflammation. The cytokine profile of *B. burgdorferi*-specific T cells is likely to be such a factor. Earlier studies on cloned PB-TCL from patients with Lyme disease (8) and cloned SF-TCL from patients with reactive arthritis recognized OspA. Thus, other factors, particularly in the pathogenesis of Lyme and reactive arthritis. Analysis of the cytokines produced by the TCL described here will allow us to compare cytokine patterns in patients with treatment-responsive and treatment-resistant arthritis.

In our study, OspB was recognized with high frequency by TCL from patients with either treatment-responsive or treatment-resistant Lyme arthritis. This is in agreement with earlier work in which 8 of 43 *B. burgdorferi*-specific T cell clones derived from the PB of a patient with acute Lyme arthritis recognized OspB (35). The amino acid sequences of OspA and OspB of the prototypical North American *B. burgdorferi* strain B31 are 56% identical with long stretches of sequence identity (up to 13 amino acids). T cell clones recognizing homologous peptide epitopes on OspA and OspB have been described (35). Thus, it is surprising to find no cross-reactivity with OspA among the many OspB-specific TCL that we derived from patients with treatment-resistant Lyme arthritis. Mapping of the OspB epitopes recognized by TCL from patients with treatment-responsive Lyme arthritis should provide an answer to this question.

In experimental models of Lyme disease, injection of mAbs against OspA (50) as well as active immunization with OspA, have been shown to protect mice from infection with *B. burgdorferi* (51–55). The protection seems to be mediated both by killing of the spirochete within the host at the time of inoculation and by elimination of the spirochete from the vector ticks feeding on immunized mice (56). These findings have led to the development of an OspA-based vaccine for human use which is currently undergoing clinical trials (33). This work forms part of the Ph.D. thesis of B. Lengl-Janßen.

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References

1. Steere, A.C. 1989. Lyme disease. N. Engl. J. Med. 321:586.

2. Szczepanski, A., and J.L. Benach. 1991. Lyme borreliosis: host responses to Borrelia burgdorferi. Microbiol. Rev. 55:21.

3. Steere, A.C., A. Gibofsky, M.E. Patarroyo, R.J. Winchester, J.A. Hardin, and S.E. Malawista. 1979. Chronic Lyme arthritis. Clinical and immunogenetic differentiation from rheumatoid arthritis. Ann. Intern. Med. 90:896.

4. Steere, A.C., R.T. Schoen, and E. Taylor. 1987. The clinical evolution of Lyme arthritis. Ann. Intern. Med. 107:725.

5. Craft, J.E., D.K. Fischer, G.T. Shimamoto, and A.C. Steere. 1986. Antigens of Borrelia burgdorferi recognized during Lyme disease. Appearance of a new immunoglobulin M response and expansion of the immunoglobulin G response late in the illness. J. Clin. Invest. 78:934.

6. Sigal, L.H., A.C. Steere, D.H. Freeman, and J.M. Dworak. 1986. Proliferative responses of mononuclear cells in Lyme disease. Reactivity to Borrelia burgdorferi antigens is greater in joint fluid than in blood. Arthritis Rheum. 29:761.

7. Neumann, A., M. Schlesier, H. Schneider, A. Vogt, and H.H. Peter. 1989. Frequencies of Borrelia burgdorferi reactive T lymphocytes in Lyme arthritis. Rheumatol. Int. 9:237.

8. Yssel, H., M.-C. Shanafelt, C. Soderberg, P.V. Schneider, J. Anzola, and G. Peltz. 1991. Borrelia burgdorferi activates a T helper type I-like T cell subset in Lyme arthritis. J. Exp. Med. 174:593.

9. Krause, A., G.-R. Burmester, A. Rensing, C. Schoerner, U.E. Schabib, M.M. Simon, P. Herzer, M.D. Kramer, and R. Walllich. 1992. Cellular immune reactivity to recombinant OspA and flagellin from Borrelia burgdorferi in patients with Lyme borreliosis. Complexity of humoral and cellular immune responses. J. Clin. Invest. 90:1077.

10. Kalish, R.A., J.L. Leong, and A.C. Steere. 1993. Association of treatment-resistant chronic Lyme arthritis with HLA-DR4 and HLA-DR2 alleles. Arthritis Rheum. 36:2077.

11. Steere, A.C., R.T. Schoen, and E. Taylor. 1987. Treatment of late Lyme borreliosis-randomised controlled trial. Lancet. i:1191.

12. Dattwyler, R.J., J.J. Halperin, D.J. Volkman, and B.J. Luft. 1988. Treatment of late Lyme borreliosis—randomised comparison of ceftriaxone and penicillin. Lancet. i:49.

13. Steere, A.C., D.H. Freeman, D.C. Shimamoto, and A.C. Steere. 1986. Antigens of Borrelia burgdorferi recognized during Lyme disease. Appearance of a new immunoglobulin M response and expansion of the immunoglobulin G response late in the illness. J. Clin. Invest. 78:934.

14. Dattwyler, K.J., and J.J. Halperin. 1987. Failure of tetracycline therapy in early Lyme disease. Arthritis. Rheum. 30:448.

15. Johnston, Y.E., P.H. Duray, A.C. Steere, M. Khashgarian, J. Buza, S.E. Malawista, and W.P. Askencase. 1985. Lyme arthritis: Spirochetes found in synovial microangiopathic lesions. Am. J. Pathol. 118:26.

16. Syndman, D.R., D.P. Schenkein, V.P. Berardi, C.C. Lastavica, and K.M. Paris. 1986. Borrelia burgdorferi in joint fluid in chronic Lyme arthritis. Ann. Intern. Med. 104:796.

17. Steere, A.C., P.H. Duray, and E.C. Butcher. 1988. Spirochetal antigens and lymphoid cell surface markers in Lyme synovitis. Comparison with rheumatoid synovium and tonsillar lymphoid tissue. Arthritis Rheum. 31:487.

18. Nocton, J.J., F. Dressler, B.J. Rutledge, P.N. Rys, D.H. Penning, and A.C. Steere. 1994. Detection of Borrelia burgdorferi DNA by polymerase chain reaction in synovial fluid in Lyme arthritis. N. Engl. J. Med. 330:229.

19. Bradley, J.F., R.C. Johnson, and J.L. Goodman. 1994. The persistence of spirochetal nucleic acids in active Lyme arthritis. Ann. Intern. Med. 120:487.

20. Steere, A.C., E.D. Dworak, and R. Winchester. 1990. Association of chronic Lyme arthritis with HLA-DR4 and HLA-DR2 alleles. N. Engl. J. Med. 323:219.

21. Centers for Disease Control. 1990. Clinical criteria for Lyme disease. MMWR (Morb. Mortal. Wkly. Rep.). 39:19.

22. Holodníny, M., S. Kim, D. Katzenstein, M. Evans, G. Groves, and T.C. Merigan. 1991. Inhibition of human immunodeficiency virus gene amplification by heparin. J. Clin. Microbiol. 29:676.

23. Fung, B.P., G.L. McHugh, J.M. Leong, and A.C. Steere. Humoral immune response to outer-surface protein C (OspC) of Borrelia burgdorferi in Lyme disease: role of the IgM response in the serodiagnosis of early infection. Infect. Immun. 62:3213.

24. Simpson, W.J., M.E. Schrumpf, and T.G. Schwan. 1990. Reactivity of human Lyme borreliosis sera with a 39-kilodalton antigen specific to Borrelia burgdorferi. J. Clin. Microbiol. 28:1329.

25. Sadziene, A., D.D. Thomas, V.G. Bundoc, S.C. Holt, and A.G. Babour. 1991. A flagella-less mutant of Borrelia burgdorferi. Structural, molecular, and in vitro functional characterization. J. Clin. Invest. 88:82.

26. Bergström, S., V.G. Bundoc, and A.G. Babour. 1989. Molecular analysis of linear plasmid-encoded major surface proteins Ospa and OspB of the Lyme disease spirochete Borrelia burgdorferi. Mol. Microbiol. 3:479.

27. Babour, A.G., S.L. Tessier, and W.J. Todd. 1983. Lyme disease spirochetes and ixodid tick spirochetes share a common surface antigenic determinant defined by a monoclonal antibody. Infect. Immun. 41:795.

28. Babour, A.G., S.L. Tessier, and S.F. Hayes. 1984. Variation in major surface protein of Lyme disease spirochetes. Infect. Immun. 54:94.

29. Wilke, B., V. Preac-Mursic, S. Jauris, A. Hofmann, I. Pradel, E. Schwab, G. Will, and G. Wanner. 1993. Immunological and molecular polymorphisms of OspC, an immunodominant major outer surface protein of Borrelia burgdorferi. Infect. Immun. 61:2182.

30. Volkman, D.J., B.J. Luft, P.D. Gorevic, J. Schultz, and L. Padovan. 1991. Characterization of an immunoreactive 93-kDa core protein of Borrelia burgdorferi with a human IgG monoclonal antibody. J. Immunol. 146:3177.

31. Kamradt, T., P.D. Soloway, D.L. Perkins, and M.L. Gefter. 1991. Pertussis toxin prevents the induction of peripheral T cell anergy and enhances the T cell response to an encephalitogenic peptide of myelin basic protein. J. Immunol. 147:3296.

32. Burgdorfer, W., A.G. Babour, S.F. Hayes, J.L. Benach, E. Grunwaldt, and J.P. Davis. 1982. Lyme disease—a tick-borne infection.
spirochetosis? Science (Wash. DC). 216:1317.

33. Barbour, A.G., and D. Fish. 1993. The biological and social phenomenon of Lyme disease. Science (Wash. DC). 260:1610.

34. Shanafelt, M.C., J. Anzola, C. Soderberg, H. Yssel, C.W. Tuck, and G. Peltz. 1992. Epitopes on the outer surface protein A of Borrelia burgdorferi recognized by antibodies and T cells of patients with Lyme disease. J. Immunol. 148:218.

35. Lahesmaa, R., M.-C. Shanafelt, A. Allsup, C. Soderberg, J. Anzola, V. Freitas, C. Tuck, L. Steinman, and G. Peltz. 1993. Preferential usage of T cell antigen receptor V region segment Vβ5.1 by Borrelia burgdorferi antigen-reactive T cell clones isolated from a patient with Lyme disease. J. Immunol. 150:4125.

36. Yang, L., J.H. Weis, E. Eichwald, C.P. Kolbert, D.H. Persing, and J.W. Weis. 1994. Heritable susceptibility to severe Borrelia burgdorferi-induced arthritis is dominant and is associated with persistence of large numbers of spirochetes in tissues. Infect. Immun. 62:492.

37. Persing, D.H., B.J. Rutledge, P.N. Rys, D.S. Podzorski, P.D. Mitchell, K.D. Reed, B. Liu, E. Fikrig, and S.E. Malawista. 1994. Target imbalance: disparity of Borrelia burgdorferi genetic material in synovial fluid from Lyme arthritis patients. J. Infect. Dis. 169:668.

38. Granfors, S., S. Jalkanen, R. von Essen, R. Lahesmaa-Rantalta, O. Isomäki, K. Pekkola-Heino, R. Meeriilahi-Palo, R. Saario, H. Isomäki, and A. Toivanen. 1989. Yersinia antigens in the synovial-fluid from patients with reactive arthritis. N. Engl. J. Med. 320:216.

39. Müllbacher, A. 1994. The long-term maintenance of cytotoxic T cell memory does not require persistence of antigen. J. Exp. Med. 179:317.

40. Oldstone, M.B.A. 1987. Molecular mimicry and autoimmune disease. Cell. 50:819.

41. Gautam, A.M., and J.H. Weis. 1994. Heritable susceptibility to severe Borrelia burgdorferi-induced arthritis is dominant and is associated with persistence of large numbers of spirochetes in tissues. Infect. Immun. 62:492.

42. Fikrig, E., S.W. Barthold, F.S. Kantor, and R.A. Flavell. 1990. Protection of mice against Lyme disease agent by immunizing with recombinant OspA. Science (Wash. DC). 250:553.

43. Fikrig, E., S.W. Barthold, N. Marcantonio, K. Deponte, F.S. Kantor, and R.A. Flavell. 1992. Roles of OspA, OspB, and flagellin in protective immunity to Lyme borreliosis in laboratory mice. Infect. Immun. 60:657.

44. Simon, M.M., U.E. Schaible, M.D. Kramer, C. Eckerskorn, C. Musetanu, H.K. Müller-Hermelink, and R. Wällich. 1991. Recombinant outer surface protein A from Borrelia burgdorferi induces antibodies protective against spirochetal infection in mice. J. Infect. Dis. 164:123.

45. Telford, S.R. III., E. Fikrig, S.W. Barthold, L.R.T. Brunet, A. Spielman, and R.A. Flavell. 1993. Protection against antigenically variable Borrelia burgdorferi conferred by recombinant vaccine. J. Exp. Med. 178:755.

46. Stover, C.K., G.P. Bansal, M.S. Hanson, J.E. Burleme, S.R. Palazynsky, J.P. Young, S. Koenig, D.B. Young, A. Sadzine, and A.G. Barbour. 1993. Protective immunity elicited by recombinant Bacille Calmette-Guerin (BCG) expressing outer surface protein A (OspA) lipoprotein: a candidate Lyme disease vaccine. J. Exp. Med. 178:197.

47. McIntosh, K., and J.M. Fishaut. 1980. Immunopathologic mechanisms in lower respiratory tract disease of infants due to respiratory syncytial virus. Prog. Med. Virol. 26:94.

48. Zinkernagel, R.M., and P.C. Doherty. 1979. MHC-restricted cytotoxic T-cells: studies on the biological role of polymorphic major transplantation antigens determining T-cell restriction specificity, function, and responsiveness. Adv. Immunol. 27:51.

49. Simon, A.K., E. Seipel, P. Wu, B. Wenzel, J. Braun, and J. Seiper. 1993. Analysis of cytokine profiles in synovial T cell clones from chlamydial reactive arthritis patients: predominance of the Th1 subset. Clin. Exp. Immunol. 94:122.

50. Schaible, U.E., M.D. Kramer, K. Eichmann, M. Mochol, C. Musetanu, and M.M. Simon. 1990. Monoclonal antibodies specific for the outer surface protein A (OspA) of Borrelia burgdorferi prevent Lyme borreliosis in severe combined immunodeficiency (scid) mice. Proc. Natl. Acad. Sci. USA. 87:3768.

51. Goverman, J., A. Woods, L. Larson, L.P. Weiner, L. Hood, and D.M. Zaller. 1993. Transgenic mice that express a myelin basic protein-specific T cell receptor develop spontaneous autoimmune. Cell. 72:551.

52. Habicht, G.S., G. Beck, J.L. Benach, J.L. Coleman, and K.D. Leichtling. 1983. Lyme disease spirochetes induce human and murine T cells production. J. Immunol. 134:3147.

53. Radolf, J.D., M.V. Norgard, M.E. Brandt, J.D. Isaacs, P.A. Thompson, and B. Beutler. 1991. Lipoproteins of Borrelia burgdorferi and Treponema pallidum activate c reactive protein necrosis factor synthesis. Analysis using a CAT reporter construct. J. Immunol. 147:1968.

54. Goverman, J., A. Woods, L. Larson, L.P. Weiner, L. Hood, and D.M. Zaller. 1993. Transgenic mice that express a myelin basic protein-specific T cell receptor develop spontaneous autoimmune. Cell. 72:551.

55. Gahring, L.C., and W.O. Weigle. 1990. The regulatory effects of cytokines on the induction of peripheral immunologic tolerance in mice. J. Immunol. 145:1318.

56. Essery, G., M. Feldmann, and J.R. Lamb. 1988. Interleukin-2 can prevent and reverse antigen-induced unresponsiveness in chronic human T lymphocytes. Immunology. 64:413.

57. Gutierrez-Ramos, J.C., I. Moreno de Alboran, and A. Martinez. 1992. In vivo administration of interleukin-2 turns on anergic self-reactive T cells and leads to autoimmune disease. Eur. J. Immunol. 22:2867.

58. Lahesmaa, R., H. Yssel, S. Batsford, R. Luukkainen, T. Mottonen, L. Steinman, and G. Peltz. 1992. Yersinia enterocolitica activates a T helper type 1-like subset in reactive arthritis. J. Immunol. 148:3079.

59. Simon, A.K., E. Seipel, P. Wu, B. Wenzel, J. Braun, and J. Seiper. 1993. Analysis of cytokine profiles in synovial T cell clones from chlamydial reactive arthritis patients: predominance of the Th1 subset. Clin. Exp. Immunol. 94:122.