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.

L yme 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 antibiotic therapy (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)[1] 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-

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[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.

thritis is clearly induced by infection with *B. burgdorferi*, 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 synovial inflammation. Alternatively, the patients with treatment-resistant Lyme arthritis might have autoreactive T cells that are activated during *B. burgdorferi* infection of the joint. These cells may then maintain 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 ar-

thritis, 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 pSPR33 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′GGGATCCTGTGCACA-AAAAGGTGCTGAG3′ and 5′CCGTCGACCTTCCTACACTAG-CTGATTGC3′ 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 contained 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 *malE* gene under the control of the inducible P$_{tac}$ 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 trans-

formed with pmalCRI containing the appropriate construct, induced, lysed, and the crude cell extracts purified over an amylose 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 p93, 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), 2×10^{-5} 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 marmoset 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). 2.5×10^4 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, MPB-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, [³H]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

(Top Count; Packard Instruments, Meridien, 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 10^6 cells/100-μl sample for 20 min at 4°C in PBS/1% BSA/0.1% NaN₃. The fluorescin-coupled mAbs recognizing CD3 (OKT3; Ortho Diagnostic Systems, Raritan, NJ), TCR-α/β (TCR-α/β-1, Becton Dickinson & Co., San Jose, CA), TCR-γ/δ (TCR-γ/δ-1, 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 FACScan® (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 testing; 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*	Before treatment	After treatment	IgG response to B. burgdorferi‡	PCR§
				mo			
FD	50	M	1	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	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

(The "Before treatment" and "After treatment" columns fall under a spanning header "Duration → Lyme arthritis".)

* 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*

	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 "From PBMC" and "From SFMC" columns fall under a spanning header "B. burgdorferi–specific/total".)

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
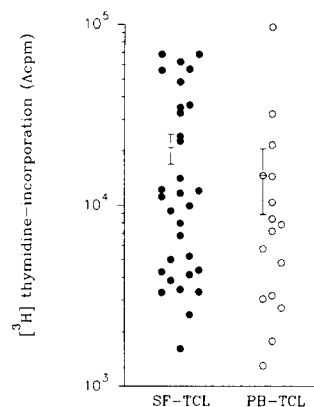

Figure 1. [³H]Thymidine incorporation of *B. burgdorferi*–specific SF- and PB-TCL. Proliferation assays were performed as described in Materials and Methods. [³H]Thymidine incorporation is shown for 31 SF-TCL (●) and 16 BP-TCL (○). The bars show the mean ± SE for SF- and PB-TCL, respectively. Data points are shown with background subtracted (Δcpm).

A

[³H] thymidine-incorporation (Δcpm)

35000
30000
25000
20000
15000
10000
5000
0

No Ag. MBP XLI   Bb   OspA OspB OspC p39 p93

B

[³H] thymidine-incorporation (Δcpm)

10^5
10^4
10^3

B.burg   OspA   OspB   OspC   p39   p93
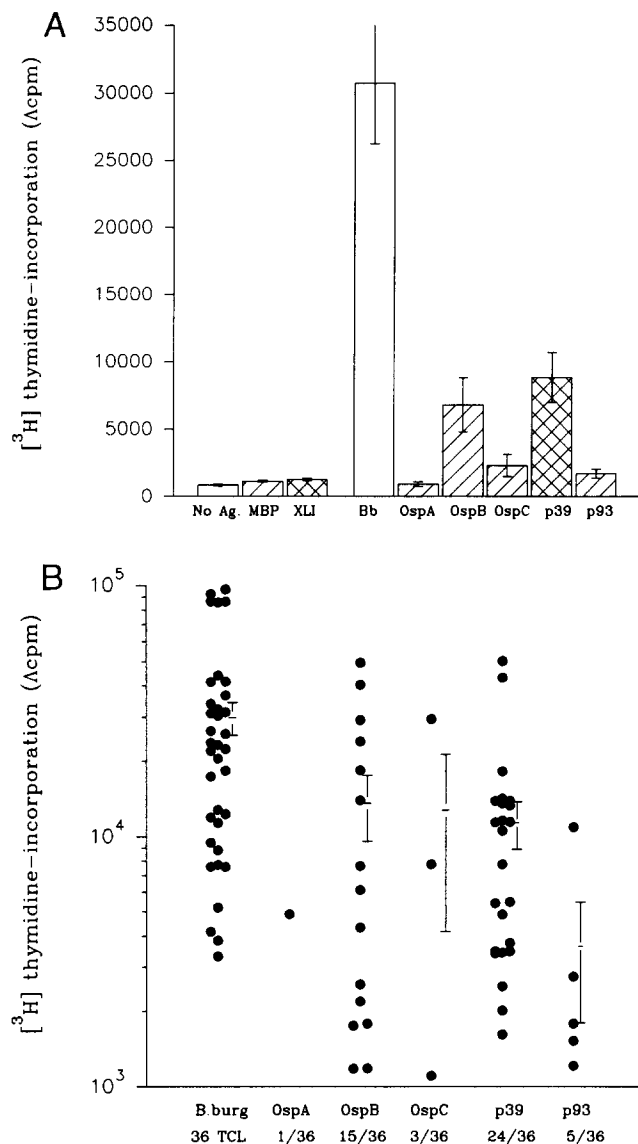36 TCL   1/36   15/36 3/36   24/36 5/36

Figure 2. [³H]Thymidine incorporation of 36 *B. burgdorferi*-specific SF-TCL from patient MS (treatment-responsive Lyme arthritis). (*A*) Mean cpm ± SEM for all 36 SF-TCL. Background is not subtracted but is shown in the three leftmost columns for medium alone (*open bar*), MBP alone (*hatched bar*), and *E. coli* XLI-blue alone (*crosshatched bar*). (*B*) Individual representation of the SF-TCL recognizing the five recombinant *B. burgdorferi* proteins tested. Data points are shown with background subtracted (Δcpm).

patients. In patient AL, although two of the eight PB-TCL recognized OspB, none of the 13 SF-TCL recognized any of the five *B. burgdorferi* antigens tested. Fig. 2, *A* and *B* illustrates the range of observed reactivities for the 36 *B. burgdorferi*-specific SF-TCL derived from patient MS. These lines preferentially recognized OspB and p39, as shown by the mean ± SEM of the responses depicted in Fig. 2 *A* and by the individual responses of the 36 TCL demonstrated in Fig. 2 *B*. In summary, OspB was frequently recognized in three of the four patients, p39 and OspC were recognized primarily

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*	%	*n*	%	*n*	%
	FD	9	2	22	8	89	2	22	0	0	1	11
SF-TCL	AL	13	0	0	0	0	0	0	0	0	0	0
	TP	4	1	25	2	50	4	100	0	0	0	0
	MS	36	1	3	15	42	3	8	24	67	5	14
Total		62	4	7	25	40	9	15	24	39	6	10
	FD	6	0	0	6	100	0	0	0	0	0	0
PB-TCL	AL	8	1	13	2	25	1	13	0	0	0	0
	TP	7	0	0	0	0	7	100	0	0	0	0
	MS	4	0	0	2	50	1	25	0	0	0	0
Total		25	1	4	10	40	9	36	0	0	0	0

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*	%	*n*	%	*n*	%
	RB	23	21	91	18	78	7	30	0	0	2	9
	TB	7	7	100	7	100	0	0	0	0	0	0
SF-TCL	CD	17	8	47	7	41	4	33	2	16	0	0
	JP	15	1	7	1	7	0	0	1	7	0	43
	DS	12	12	100	11	92	2	17	2	17	3	43
Total		74	49	66	44	60	13	18	5	7	5	7
	RB	8	4	50	3	38	0	0	1	13	2	25
	TB	3	3	100	2	67	0	0	0	0	0	0
PB-TCL	CD	15	5	33	4	27	2	13	1	7	3	20
	JP	7	1	14	1	14	0	0	0	0	0	0
	DS	5	5	100	5	100	0	0	0	0	0	0
Total		38	18	47	15	40	2	5	2	5	5	13

The numbers (percentage) of *B. burgdorferi (Bb)*-specific SF-TCL and PB-TCL specific for each recombinant antigen are shown.
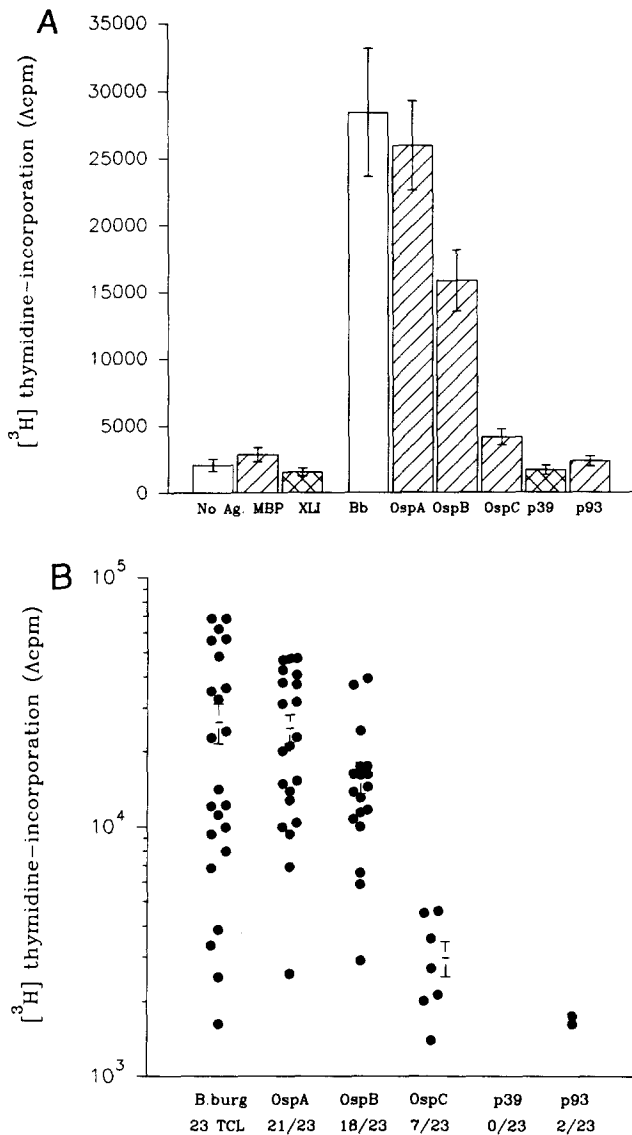
Figure 3. [³H]Thymidine incorporation of 23 *B. burgdorferi*–specific SF-TCL from patient RB (treatment-resistant Lyme arthritis). (*A*) Mean cpm ± SEM for all 23 SF-TCL. Background is not subtracted but shown in the three leftmost columns for medium alone (*open bar*), MBP alone (*hatched bar*), and *E. coli* XLI-blue alone (*crosshatched bar*). (*B*) Individual representation of the SF-TCL recognizing the five recombinant *B. burgdorferi* proteins tested. Data points are shown with background subtracted (*Acpm*).

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. 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 (48, 49) found a predominance of T cells producing Il-2 and IFN-γ, but not IL-4 and IL-5 (Th1 cells). This has led to the idea that a Th1-like pattern of cytokine production may be important 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). In healthy individuals, in whom the spirochete is not present, we would not predict any immunopathological side effects

of the vaccination of humans with OspA. However, there is the potential for concern if a vaccinated person is exposed later to *B. burgdorferi*. If the patient's antibodies do not kill the spirochete in the feeding tick, exposure to *B. burgdorferi* might induce immunopathological T cell responses.

Precedence for such a possibility comes from the experience with a vaccine against respiratory syncytial virus that was used in the 1960s. Although the vaccine induced strong serological responses, the vaccinees were not protected against disease. Moreover, they were at markedly increased risk of developing severe lower respiratory tract disease when infected with the virus, as compared with unimmunized controls (57). In addition, animal models have shown that protective T cell responses can also cause immunopathology (58). For example, adoptive transfer of pathogen-specific TCL potentiated clinical disease in recipient mice upon infection with the pathogen. However, the same TCL did not cause pathology in uninfected mice (59, 60). Similar evidence comes from experiments with transgenic mice expressing lymphocytic choriomeningitis virus proteins in pancreatic islet cells. T cells specific for the transgenic proteins were unresponsive in these mice. Upon infection with lymphocytic choriomeningitis, however, T cells infiltrated and destroyed the pancreatic islets, and the animals developed diabetes (61, 62). Thus, the presence of potentially immunopathogenic T cells alone is not sufficient to induce tissue damage. Only if the host is subsequently infected with the microorganism carrying the specific epitope are all the necessary signals delivered for T cell activation (for a review see reference 63). According to these findings, the possibility exists that immunopathological effects of an OspA-based vaccine might not be seen unless the vaccinees were later exposed to *B. burgdorferi*.

In summary, our data show that OspA is differentially recognized by TCL from patients with treatment-responsive or treatment-resistant Lyme arthritis. Although we cannot rule out that treatment-resistant Lyme arthritis is due to persistent infection with *B. burgdorferi*, and more work is needed to further characterize the differential response to OspA, our findings are compatible with a role for OspA-specific T cells in the immunopathogenesis of therapy-resistant Lyme arthritis.

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