

## ASSOCIATION OF ANTIBIOTIC TREATMENT-RESISTANT LYME ARTHRITIS WITH T CELL RESPONSES TO DOMINANT EPITOPES OF OUTER SURFACE PROTEIN A OF *BORRELIA BURGENDORFERI*

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**Objective.** To explore further the association of antibiotic treatment-resistant Lyme arthritis and T cell reactivity with outer surface protein A (OspA) of *Borrelia burgdorferi*, including the identification of T cell epitopes associated with this treatment-resistant course.

**Methods.** The responses of peripheral blood and, if available, synovial fluid lymphocytes to *B burgdorferi* proteins, fragments, and synthetic peptides, as determined by proliferation assay and interferon- $\gamma$  production, were compared in 16 patients with treatment-responsive and 16 with treatment-resistant Lyme arthritis.

**Results.** The maximum severity of joint swelling correlated directly with the response to OspA. Moreover, the only significant difference between patients with treatment-resistant and treatment-responsive arthritis was in reactivity with N-terminal and C-terminal fragments of OspA, OspA1 (amino acids [aa] 16-106), and OspA3 (aa 168-273). Epitope mapping showed that 14 of the 16 patients with treatment-resistant arthritis had responses to OspA peptides (usually 4 or 5 epitopes), whereas only 5 of the 16 patients with treatment-responsive arthritis had reactivity with these peptides (usually 1 or 2 epitopes) ( $P = 0.003$ ). Patients with HLA-DRB1 alleles associated with treatment-resistant arthritis were more likely to react with peptide 15 (aa 154-173) and, to a lesser degree, with peptide 21 (aa

214-233) than patients with other alleles, whereas the responses to other epitopes were similar in both groups.

**Conclusion.** The maximum severity of joint swelling and the duration of Lyme arthritis after antibiotic treatment are associated with T cell responses to specific epitopes of OspA.

Lyme disease in the United States is caused by the tick-borne spirochete *Borrelia burgdorferi sensu stricto* (1). Months to years after disease onset, ~60% of untreated patients with this infection develop intermittent or chronic oligoarticular arthritis, primarily in the large joints, especially the knees (2). Lyme arthritis can usually be treated successfully with antibiotic therapy (3,4). However, ~10% of patients have persistent arthritis for months or even several years after antibiotic treatment (4). The synovial lesion in these patients, which shows synovial hypertrophy, vascular proliferation, and a lymphoplasmacellular infiltrate, is similar to that seen in other forms of chronic inflammatory arthritis, including rheumatoid arthritis (5,6).

Two hypotheses have been proposed to explain antibiotic treatment-resistant Lyme arthritis (7). First, persistent synovial inflammation may be due to persistent infection or retained spirochetal antigens, or alternatively, the spirochete may trigger tissue-specific autoimmunity within the joint. In untreated patients with Lyme arthritis, *B burgdorferi* DNA can be detected readily in both synovium and synovial fluid at any time in the illness (8,9). However, in our experience, patients with persistent arthritis have negative findings on polymerase chain reaction (PCR) testing of joint fluid after  $\geq 2$  months of oral antibiotic treatment or  $\geq 1$  month of intravenous antibiotic treatment (9). Thus, synovitis may sometimes persist after the apparent eradication of live spirochetes from joints with antibiotic therapy.

Host immunogenetic factors have been linked to the pathogenesis of chronic, treatment-resistant Lyme

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arthritis. In an initial study, patients with chronic Lyme arthritis had an increased frequency of the HLA-DR4 specificity, determined by serologic techniques (10). Recently, in a study of 105 patients with Lyme arthritis that employed contemporary molecular techniques, those who had arthritis for 1-4 years after the initiation of antibiotic treatment had an increased frequency of HLA-DRB1\*0401, 0101, or 0102 alleles; those who had arthritis for 4-11 months after therapy more often had DRB1\*0404 or 0701 alleles, and those in whom the arthritis resolved within 3 months after the start of treatment more frequently had DRB1\*11 or 0801 alleles (11). Thus, except for DRB1\*0701, the alleles associated with treatment-resistant Lyme arthritis also correlate with the severity of rheumatoid arthritis (12).

Since the cause of Lyme disease is known, efforts have been made to implicate specific spirochetal immune responses in the pathogenesis of chronic Lyme arthritis. In previous studies, the severity and duration of Lyme arthritis correlated with the IgG antibody responses to outer surface proteins A and B (OspA and OspB) of *B burgdorferi*, 2 highly homologous proteins (13), and among HLA-DR4 positive patients, those with antibodies to these proteins had arthritis for significantly longer after treatment than those who lacked such reactivity (14-16). However, the association of treatment-resistant Lyme arthritis with class II major histocompatibility complex (MHC) alleles suggests that T cell reactivity, rather than OspA antibody, are likely to be important in the persistence of synovial inflammation. In an initial study, OspA was preferentially recognized by T cell lines from 5 patients with treatment-resistant Lyme arthritis, but only rarely recognized by T cell lines from 4 patients with treatment-responsive arthritis (17).

In the first epitope-mapping studies, OspA-reactive T cell lines from a small number of patients with Lyme arthritis recognized multiple epitopes of OspA, including N-terminal and C-terminal epitopes of the protein (18,19). However, since only 3 patients were tested in each series, clinical correlations could not be made. Moreover, T cell lines and clones may not reflect the actual events within the joint, since certain antigen-activated cells may not grow in culture.

In the present study, our goal was to explore further the association of antibiotic treatment-resistant Lyme arthritis with OspA reactivity using fresh samples from large numbers of patients with Lyme arthritis and to identify T cell epitopes of OspA associated with this treatment-resistant course.

## PATIENTS AND METHODS

**Patients.** During a 3-year period, from January 1995 through December 1997, we evaluated cellular immune findings in 32 consecutive patients with Lyme arthritis. The study protocol was approved by the Human Investigations Committee at New England Medical Center. The patients with Lyme arthritis met Centers for Disease Control and Prevention (CDC) criteria for the diagnosis of Lyme disease (20). They had monarticular or oligoarticular arthritis, exposure in an area endemic for the disease, and markedly positive IgG antibody responses to *B burgdorferi* by enzyme-linked immunosorbent assay (ELISA) and Western blotting, interpreted according to the CDC/ASTPHLD (Association of State and Territorial Public Health Laboratory Directors) criteria (21). When possible, PCR testing was done on joint fluid samples using primer-probe sets that target plasmid DNA encoding OspA, as previously described (9).

The 32 patients were referred to our clinic at different times during the course of their arthritis; the number of followup visits varied according to the duration of active arthritis, and cells were sometimes available only from peripheral blood or joint fluid. Thirteen patients were referred prior to antibiotic treatment, 12 were first seen during the course of therapy, and 7 were referred to the clinic after antibiotic treatment. For analysis, the patients were divided into 2 groups according to their response to antibiotic therapy. Group 1 (the treatment-responsive group) consisted of 16 patients who had a complete resolution of arthritis within 3 months after the initiation of antibiotic treatment. Group 2 (the treatment-resistant group) consisted of 16 patients who required 4 months to 4 years after the initiation of recommended courses of antibiotic treatment (1,22) for the resolution of arthritis.

The 16 patients with treatment-responsive arthritis had 1 sample obtained during active arthritis, and 8 of them had an additional sample obtained at the conclusion of treatment. Among the 16 patients with treatment-resistant arthritis, 10 had 2-4 samples obtained during active arthritis, and 6 had 1 sample obtained during this period. Single or serial samples of both peripheral blood lymphocytes (PBL) and synovial fluid lymphocytes (SFL) were available in 23 of the 32 patients. PBL alone were available in 7, and SFL alone were available in 2. For comparison, single samples of PBL from 15 normal control subjects and single samples of SFL from 10 patients with other forms of chronic inflammatory arthritis, including rheumatoid, psoriatic, or spondylarthritis, were tested.

The severity of Lyme arthritis was assessed by the volume of synovial fluid drained from the knee joint during the maximal period of arthritis: score of 1+ = 1-10 ml, 2+ = 11-30 ml, 3+ = 31-50 ml, and 4+ = >50 ml.

**Antigens.** Sonicated lysates of *B burgdorferi* sensu stricto proteins were made from strain G 39/40. The spirochetes were grown in BSK medium, harvested by centrifugation, washed with phosphate buffered saline (PBS), sonicated, and filtered, as previously described (17). The protein content was estimated by spectrophotometry (optical density 280 nm). Full-length OspA, the fragments OspA1, OspA2, OspA3, and full-length OspB and OspC were generated as recombinant fusion proteins with *Escherichia coli* maltose binding protein (MaBP), as previously described (15,23). Restriction fragments containing these gene segments were inserted at the 3' end of

the *malE* gene of *E. coli*, which encodes MaBP. During the logarithmic growth phase, protein production was induced and the bacteria were lysed by passage through a French pressure cell. The supernatant was then passed over a crosslinked amylose column to purify the MaBP fusion proteins, and the protein content of the eluate was determined.

Recombinant P39, P41, and P93 were a kind gift from Dr. John M. Robinson at Abbott laboratories (Abbott Park, IL). *E. coli* carrying the appropriate plasmids encoding these *B. burgdorferi* proteins and the fusion partner CMP-2-keto-3-deoxyoctulosonic acid synthetase (CKS) were lysed by sonication, and the recombinant proteins were purified by washing and centrifugation, as previously described (24). A set of 25 OspA peptides, which were 20-mers, each overlapping by 10 amino acids (aa), were a kind gift of Dr. Geetha Bansel, formerly at MedImmune, Inc. (Gaithersburg, MD). The peptides were derived from the published sequence of *B. burgdorferi sensu stricto* ZS7 (GenBank accession number X16467). They were synthesized by conventional Merrifield solid-phase chemistry with *t*-Boc (*tert*-butoxy-carbonyl) or Fmoc (9-fluorenylmethoxycarbonyl) amino acids, as previously described (19). Lyophilized peptides were diluted in PBS, and aliquots appropriate for testing cells from 1 or 2 patients were stored at  $-70^{\circ}\text{C}$  until ready for use.

**T cell proliferation assay.** Mononuclear cells from peripheral blood or synovial fluid were separated by the Ficoll-Hypaque method (lymphocyte separation medium; Organon Teknika, Durham, NC). The cells were tested for responses to *B. burgdorferi* sonicate, 9 different recombinant *B. burgdorferi* antigens, or 25 synthetic OspA peptides in standard proliferation assays. Briefly, mononuclear cells were plated in round-bottomed, 96-well plates (Costar, Cambridge, MA) at a final concentration of  $2 \times 10^5$  cells in 200  $\mu\text{l}$  of complete medium containing RPMI 1640 (Gibco BRL, Gaithersburg, MD) with 10% human AB serum (Sigma, St. Louis, MO), 2

mM glutamine, penicillin (100 units/ml), streptomycin (100  $\mu\text{g/ml}$ ), and 9.5 mM HEPES buffer.

To identify optimal antigen concentrations, the cells from initial patients were stimulated in preliminary experiments with 3 dilutions of each antigen. However, because of the large number of cells required to test as many as 35 different antigens, assays were subsequently performed in duplicate, with only the optimal concentration of antigen. These concentrations were 50  $\mu\text{g/ml}$  for *B. burgdorferi* lysate, 10  $\mu\text{g/ml}$  for MaBP- or CMP-*B. burgdorferi* fusion proteins (except for P93, which was 5  $\mu\text{g/ml}$ ), and 2.5  $\mu\text{g/ml}$  for OspA peptides. The negative controls were medium alone (background control) and MaBP alone (fusion partner control) (5  $\mu\text{g/ml}$ ).

After 5 days in culture,  $^3\text{H}$ -thymidine (0.5  $\mu\text{Ci/well}$ ) in 50  $\mu\text{l}$  of complete medium was added to each well. The cells were then harvested 16–18 hours later with an automated harvester (Packard Instruments, Meriden, CT), and the incorporated thymidine was detected in a liquid scintillation counter (Top Counter; Packard Instruments). In an effort to adjust for day-to-day and patient-to-patient variations in test performance, the results are expressed throughout as a stimulation index, which represents the counts per minute obtained with antigen stimulation divided by the cpm in unstimulated control cultures.

**In vitro stimulation and cytokine measurement.** To measure in vitro cytokine production in response to *B. burgdorferi* sonicate and recombinant borrelial proteins, culture supernatants (60  $\mu\text{l}$ ) were harvested from each well of proliferation assay plates at 24 hours for the detection of interleukin-4 (IL-4) and at 5 days for the detection of interferon- $\gamma$  (IFN- $\gamma$ ). In preliminary experiments, these were shown to be the optimal times for detecting each cytokine. Because of the large number of synthetic peptides, cytokine measurements were not made in the epitope mapping studies.

**Table 1.** Demographic and clinical data in patients with treatment-responsive or treatment-resistant Lyme arthritis

	Treatment-responsive disease (n = 16)	Treatment-resistant disease (n = 16)
Age, median (range) years	46 (7–74)*	25 (11–51)
Sex, no. of males/females	14/2	10/6
Duration of arthritis, median (range) months		
Before treatment	0.75 (0.25–10)	1 (0.25–7)
After treatment	0	7 (1–48)
Severity of arthritis, median (range) score	3+ (1–4+)	4+ (2–4+)
Treatment, no. of patients		
Doxycycline		
30–45 days	11	4
$\geq 60$ days	3	11
IV ceftriaxone		
$\geq 30$ days	2	11†
PCR tests for <i>Borrelia burgdorferi</i> DNA in joint fluid, no. positive/no. tested		
Before or during treatment	3/6	6/6
After treatment	ND‡	0/14

\*  $P = 0.02$  versus treatment-resistant patients.

† Treatment-resistant patients often received both oral and intravenous (IV) antibiotic therapy.

‡ No polymerase chain reaction (PCR) tests were done after treatment in this group because their joint effusions had resolved. ND = not done.

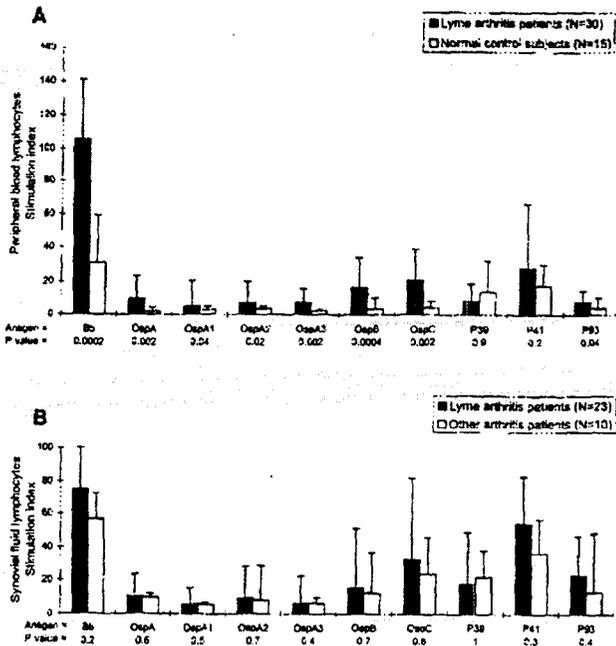


Figure 1. T cell responses to *Borrelia burgdorferi* (Bb) sonicate and 9 recombinant spirochetal proteins in A, peripheral blood lymphocytes (PBL) and B, synovial fluid lymphocytes (SFL) from patients with Lyme arthritis compared with those in normal control subjects. Bars show the median values for each group (half of the samples); I-bars represent the third quartile values (three-quarters of the samples). The T cell responses of PBL to each borrelial antigen were significantly higher in patients with Lyme arthritis than in normal control subjects, except for reactivity with P39 and P41. The responses of SFL to borrelial antigens tended to be higher in patients with Lyme arthritis than in those with other chronic inflammatory arthritides, but the differences were not statistically significant. Osp = outer surface protein.

Cytokine levels were determined by ELISA, with slight modifications of the manufacturer's instructions. The capture anti-human IL-4 or IFN $\gamma$  IgG monoclonal antibodies (PharMingen, San Diego, CA) were diluted to 1  $\mu$ g/ml in coating

buffer (0.1M NaHCO $_3$ , pH 8.2) and bound to 96-well ELISA plates (Corning, Corning, NY) overnight at 4°C. Following blocking with PBS with 10% fetal bovine serum for 2 hours at room temperature, 100- $\mu$ l samples of diluted supernatants (1:2) were added to duplicate wells and incubated overnight at 4°C. After washing in PBS-0.05% Tween 20, biotin-conjugated anti-IL-4 or anti-IFN $\gamma$  IgG antibodies (0.5  $\mu$ g/ml) (PharMingen) were added for 45 minutes at room temperature.

Following washing, avidin-peroxidase (1:400; Sigma) was added for 30 minutes at room temperature. After a final wash, the substrate, tetramethylbenzidine (TMB 100, at 100  $\mu$ l; Gibco) was added. The reaction was stopped in 0.5-1 minute for IL-4 and in 5 minutes for IFN $\gamma$  by the addition of 1N H $_2$ SO $_4$  (100  $\mu$ l). The absorbance was read at 450 nm, and the concentration of each cytokine was calculated from a standard curve.

**Statistical analysis.** The clinical findings among patient groups were compared in 3  $\times$  2 or 2  $\times$  2 tables by chi-square or, when appropriate, by Fisher's exact test. Because the responses in patients were not normally distributed, the reactivity of PBL or SFL with each borrelial antigen or synthetic peptide, as measured by proliferation assay or cytokine production, was compared in patients with treatment-resistant or treatment-responsive Lyme arthritis by the Kruskal-Wallis test, a nonparametric statistical method. Therefore, the data for each group are presented as median and third quartile values. In patients who were seen more than once during the period of active arthritis, values from each visit were averaged so that each patient had 1 value for reactivity of PBL or SFL with each borrelial antigen or synthetic peptide. The maximum severity of arthritis was correlated with PBL or SFL responses with each antigen, using Spearman's correlation coefficients. All P values are 2-tailed. P values above 0.1 were rounded to 1 decimal place; values above 0.01 were rounded to 2 decimal places, and values above 0.001 were rounded to 3 decimal places.

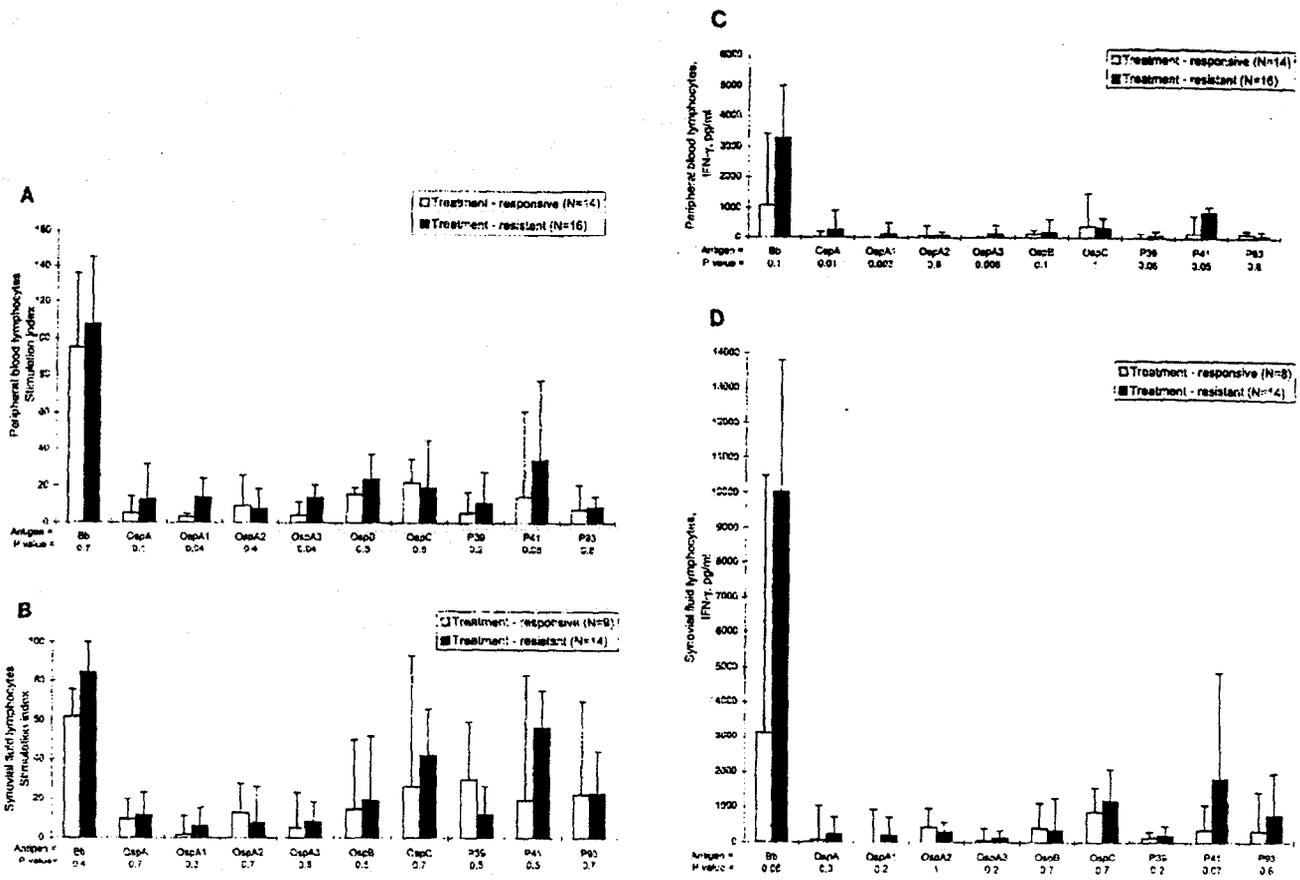
RESULTS

**Clinical characteristics.** Of the 32 patients with Lyme arthritis, 16 had complete resolution of arthritis within 3 months after the initiation of antibiotic treat-

Table 2. Correlation of arthritis swelling with proliferative and cytokine responses to *Borrelia* antigens\*

	Proliferative response				IFN $\gamma$ response			
	PBL		SFL		PBL		SFL	
	r	P	r	P	r	P	r	P
Bb	-0.08	0.6	0.12	0.6	0.06	0.7	0.22	0.4
OspA	0.47	0.008	0.59	0.005	0.44	0.01	0.39	0.1
A1	0.39	0.03	0.39	0.08	0.41	0.02	0.13	0.6
A2	0.03	0.9	0.50	0.02	-0.004	1.0	0.57	0.01
A3	0.41	0.02	0.58	0.006	0.34	0.07	0.53	0.02
OspB	0.09	0.6	0.45	0.04	0.28	0.1	0.50	0.03
OspC	-0.006	1.0	0.28	0.2	0.03	0.9	0.07	0.7
P39	-0.26	0.2	0.13	0.6	-0.04	0.8	0.30	0.2
P41	-0.30	0.1	-0.09	0.7	-0.06	0.7	-0.08	0.7
P93	-0.24	0.2	0.25	0.3	-0.20	0.3	0.12	0.6

\* r determined by Spearman's rank correlation coefficient. IFN $\gamma$  = interferon- $\gamma$ ; Bb = *Borrelia burgdorferi*; Osp = outer surface protein.



**Figure 2.** T cell responses to *Borrelia burgdorferi* sonicate and 9 recombinant spirochetal proteins in A and C, PBL and B and D, SFL from patients with treatment-responsive or treatment-resistant Lyme arthritis. Bars show the median values for each group; I-bars represent the third quartile values. Compared with patients with treatment-responsive arthritis, the median responses of PBL in patients with treatment-resistant arthritis were significantly greater only in comparison with the OspA fragments A1 (amino acids 16–106) and A3 (amino acids 168–273), as measured both by proliferation assay (A) and by interferon- $\gamma$  (IFN- $\gamma$ ) production (C). A similar pattern was seen with SFL responses (B and D), but the differences were not statistically significant. Differences of possible significance were found between the 2 groups in reactivity with P41. See Figure 1 for definitions.

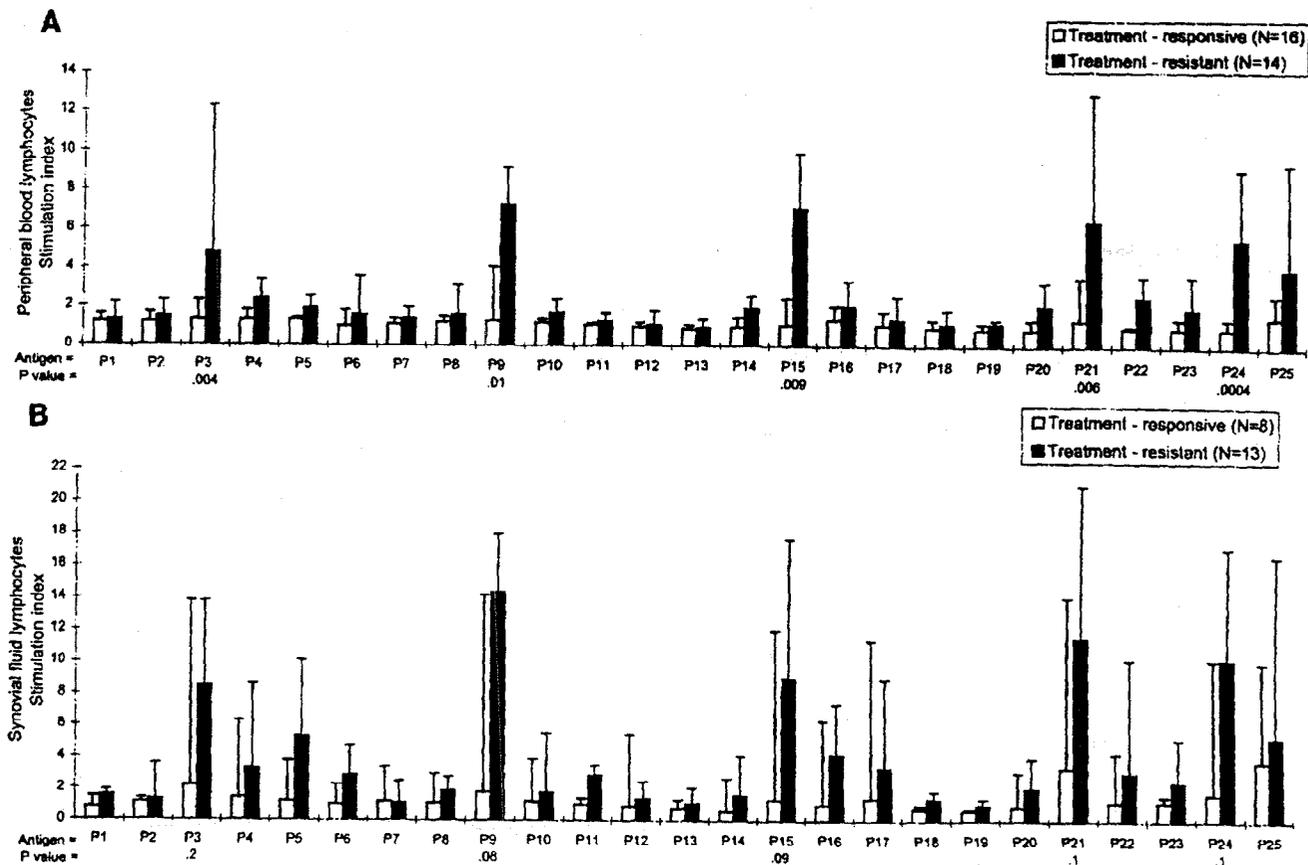
ment (the treatment-responsive group), whereas the other 16 patients had resolution of arthritis from 4 months to 4 years after the start of therapy (the treatment-resistant group) (Table 1). Most of the patients in the treatment-responsive group were treated with oral doxycycline for 1 or 2 months, whereas those in the treatment-resistant group usually received oral doxycycline for 2 months, followed by intravenous ceftriaxone for 1 month.

PCR tests for *B burgdorferi* DNA were positive in 9 of the 12 patients in whom joint fluid samples were available prior to or during antibiotic treatment. In the 14 treatment-resistant patients in whom samples were available after therapy, PCR results were negative.

**T cell responses in case and control subjects.** The T cell responses of PBL to each borrelial antigen were significantly higher in patients with Lyme arthritis than in

normal control subjects, except for reactivity with P39 and P41, which were often found in both case and control subjects (Figure 1). Similarly, the responses of SFL to borrelial antigens tended to be higher among patients with Lyme arthritis than among those with other chronic inflammatory arthritides, but the differences were not statistically significant. Thus, as with antibody responses to *B burgdorferi* (25), the T cell responses to most borrelial antigens are not specific for *B burgdorferi* infection.

**T cell responses and severity of arthritis.** When the T cell responses to each borrelial antigen in patients with Lyme arthritis were correlated with the severity of arthritis, there was a significant direct correlation between the response to OspA and the severity of knee swelling for both PBL and SFL, as determined both by proliferation assay and IFN- $\gamma$  production (Table 2). The strongest association was with reactivity with the



**Figure 3.** T cell responses to overlapping synthetic 20-mers of OspA in A, PBL and B, SFL from patients with treatment-responsive or treatment-resistant Lyme arthritis. Bars show the median values; I-bars represent the third quartile values. Compared with patients with treatment-responsive arthritis, the median responses of PBL in those with treatment-resistant arthritis were greater with 5 OspA epitopes (A). A similar pattern was seen with SFL (B). See Figure 1 for definitions.

C-terminal OspA3 fragment (aa 168–273), although there were less consistent associations with PBL or SFL responses to the N-terminal OspA1 (aa 16–106) and middle OspA2 (aa 105–201) fragments and with the highly homologous OspB protein (13). None of the

responses to other *B burgdorferi* proteins correlated with the degree of joint swelling.

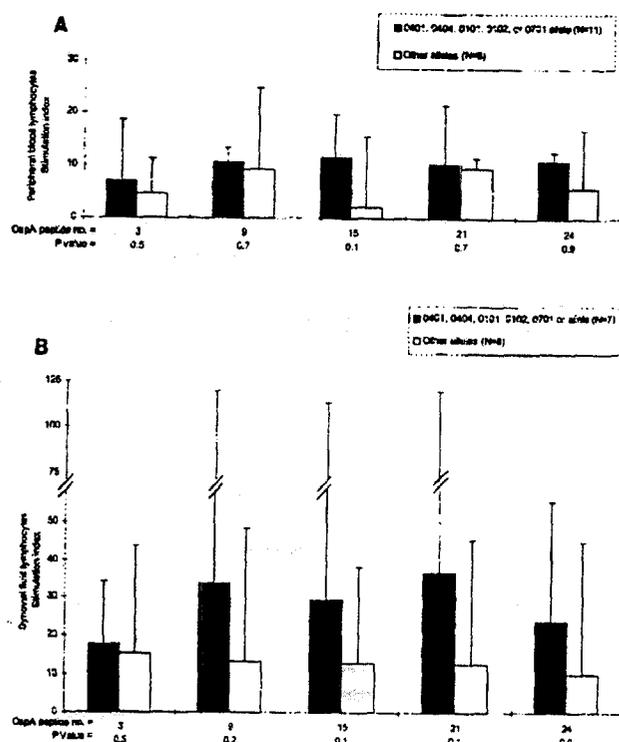
**T cell responses and the duration of arthritis after antibiotic treatment.** When the responses of PBL to each borrelial antigen were compared in patients with

**Table 3.** Frequency of HLA-DRB1 alleles in patients with Lyme arthritis, according to the duration of arthritis after antibiotic treatment

HLA-DRB1	Duration of arthritis after treatment*			P
	≤3 mos (n = 16)	4–11 mos (n = 11)	1–4 years (n = 5)	
Rheumatoid arthritis alleles 0401, 0404, 0101, or 0102	5 (31)	5 (45)	5 (100)	0.005
0701 allele†	1 (6)	3 (27)	0	0.2
0801 or 11 alleles†	4 (25)	1 (9)	0	0.3

\* Values are the number (%) of patients.

† In a larger study of 105 patients (11), the association of the 0701 allele with treatment-resistant arthritis of moderate duration and the association of the 0801 and 11 alleles with treatment-responsive arthritis were statistically significant.



**Figure 4.** T cell responses in A, PBL and B, SFL from the 19 patients who had responses to 1 or more of 5 OspA epitopes, analyzed according to HLA-DRB1 alleles associated with treatment-resistant Lyme arthritis. Bars show the median values; I-bars represent the third quartile values. With PBL (A), T cell reactivity with peptide 15 was greater in those with alleles associated with treatment-resistant arthritis than in those with other alleles. With SFL (B), reactivity with peptides 15 and 21 was greater in those with treatment-resistant arthritis alleles. See Figure 1 for definitions.

treatment-responsive or treatment-resistant arthritis, the only significant differences were that treatment-resistant patients had significantly greater reactivity with the OspA fragments OspA1 and OspA3, as determined both by proliferation assay and IFN $\gamma$  production (Figures 2A and C). A difference of possible significance was also found between the 2 groups in their responses to P41, and the strongest reactivity was often with this protein. A similar pattern of responses was seen with SFL, which often had greater reactivity than PBL. However, with SFL, the differences between the 2 groups were not statistically significant (Figures 2B and D). Some patients produced small amounts of IL-4, and the median values in the treatment-responsive group were often greater than those in the treatment-resistant group, especially with SFL. However, the differences between the groups were not statistically significant, and therefore, data on IL-4 responses are not shown.

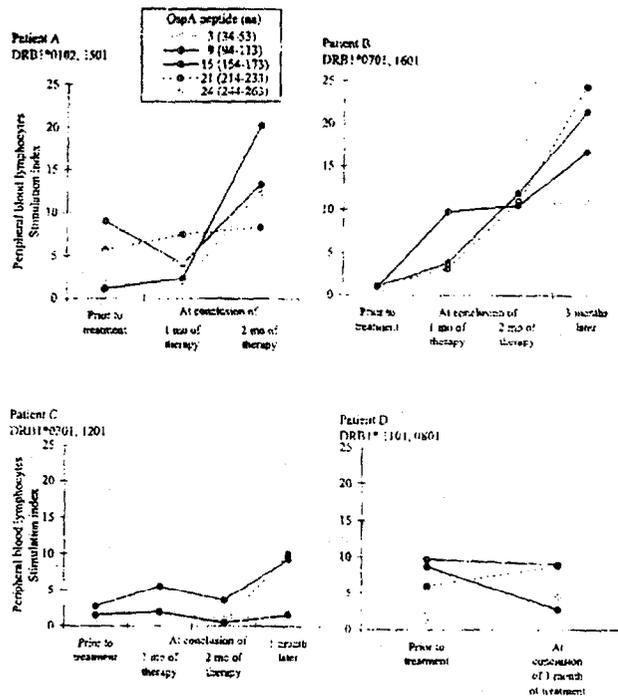
Thus, treatment-resistant and treatment-responsive patients differed only in their responses to certain regions of OspA.

**OspA epitope mapping.** When the reactivity of PBL were compared in treatment-resistant and treatment-responsive patients by use of synthetic OspA peptides, treatment-resistant patients had significantly greater responses to each of 5 T cell epitopes of OspA, including those contained in peptide 3 (aa 34–53), peptide 9 (aa 94–113), peptide 15 (aa 154–173), peptide 21 (aa 214–233), and peptide 24 (aa 244–263), as determined by proliferation assay (Figure 3A). A similar pattern was seen with SFL, and the magnitude of the reactivity was often greater than PBL, but the statistical significance of the comparisons was less (Figure 3B). Fourteen of the 16 patients with treatment-resistant arthritis had responses to OspA peptides (usually 4 or 5 epitopes), whereas only 5 of the 16 patients with treatment-responsive arthritis had reactivity with these peptides (usually 1 or 2 epitopes) ( $P = 0.003$ ).

**HLA alleles and OspA epitopes.** All 5 patients who had arthritis for 1–4 years after the initiation of antibiotic treatment (prolonged treatment-resistant arthritis) had HLA-DRB1\*0101, 0102, 0401, or 0404 alleles. DRB1\*0701 alleles tended to be increased in frequency in those who had arthritis for 4–11 months after treatment (treatment-resistant arthritis of moderate duration), and DRB1\*0801 or 11 alleles were found more often in those whose arthritis resolved within 3 months after the start of therapy (treatment-responsive arthritis) (Table 3).

Of the 19 patients with T cell responses to OspA in PBL, 11 had DR\*0401, 0404, 0101, 0102, or 0701 alleles, the alleles associated with treatment-resistant Lyme arthritis, and 10 of the 11 patients had a treatment-resistant course. In comparison, only 3 of the 8 patients who had other HLA-DRB1 alleles had such a course ( $P = 0.04$ ). With both PBL and SFL, the patients with alleles associated with treatment-resistant arthritis reacted more with peptide 15 than those with other alleles ( $P = 0.1$ ), a difference of possible significance (Figure 4). With SFL, a difference of possible significance was also seen with peptide 21. In contrast, the responses to the other epitopes were similar in both groups.

**Timing of OspA epitope development.** To delineate the timing of these responses, reactivity with the 5 epitopes was assessed in the 13 patients in whom serial samples of PBL were available prior to, during, and following antibiotic treatment. Of the 13 patients, 8 had treatment-responsive and 5 had treatment-resistant arthritis. In pretreatment samples, which were obtained a median of 1 month after the onset of arthritis, only 2 of the 13 patients, one with treatment-



**Figure 5.** Outer surface protein A (OspA) epitope development in 4 representative patients. Patient A, who had the DRB1\*0102 allele, had prolonged treatment-resistant arthritis that lasted for >12 months after the initiation of antibiotic treatment. In the initial sample, he had reactivity with several OspA peptides, but not with peptide 15. Two months later, he responded to 5 OspA epitopes, and the greatest reactivity was with peptide 15. Patient B, who had the DRB1\*0701 allele, had treatment-resistant arthritis of moderate duration that persisted for 8 months after therapy. She did not have reactivity with OspA peptides in the initial sample; 2 months later, she responded to the 5 peptides, including peptide 15. Although patient C, who had DRB1\*0301 and 1201 alleles, was categorized as having treatment-resistant arthritis, his joint swelling resolved 4 months after the start of treatment. During the course of treatment, he developed responses to 2 OspA peptides, but not with peptide 15. Patient D, who had DRB1\*1101 and 0801 alleles, had the rapid resolution of the third attack of arthritis during a 1-month course of oral doxycycline. He reacted with several OspA peptides in the sample obtained at the beginning of the third attack, but the response to peptide 15 disappeared as his arthritis resolved.

resistant and the other with treatment-responsive arthritis, had weak responses to 1 or 2 OspA epitopes. During the next 2 months, while receiving antibiotic treatment, reactivity developed to 4 or 5 of the OspA epitopes in 4 of 5 patients in the treatment-resistant group, but in only 1 of the 8 patients in the treatment-responsive group ( $P = 0.03$ ).

In samples obtained in the treatment-resistant patients after antibiotic treatment, when results of PCR tests for *B burgdorferi* DNA in joint fluid were negative, the

strength of the responses increased or remained high, but reactivity did not develop with additional OspA epitopes. Thus, several months of arthritis were usually required for the development of OspA responses, and reactivity persisted throughout the period of active arthritis.

**Courses of 4 representative patients.** To illustrate OspA epitope development, the courses of 4 representative patients are shown in Figure 5. Patient A, who had the DRB1\*0102 allele, had prolonged treatment-resistant arthritis that lasted for more than 12 months after antibiotic treatment. After 2 months of treatment, he responded to 5 OspA epitopes, and the greatest reactivity was with peptide 15.

Patient B, who had the DRB1\*0701 allele, had treatment-resistant arthritis of moderate duration that persisted for 8 months after therapy. After 2 months of treatment, she responded to 5 OspA epitopes, including peptide 15.

Although patient C, who had DRB1\*0301 and 1201 alleles, was categorized as having treatment-resistant arthritis, his joint swelling resolved 4 months after the start of treatment. During treatment, he had responses to OspA peptides 9 and 21, but he did not develop reactivity with peptide 15.

Patient D, who had DRB1\*1101 and 0801 alleles, had previously had 2 short attacks of arthritis. During the third attack, he had the rapid resolution of joint swelling during a 1-month course of oral doxycycline. Although he reacted with OspA peptides 9, 15, and 21 in the sample taken at the beginning of the third attack, the response to peptide 15 disappeared as his arthritis resolved.

## DISCUSSION

Consistent with previous experience (26–29), T cell reactivity in the current study patients was directed against many spirochetal antigens, and the responses of SFL were generally greater than those of PBL, presumably because of the concentration of antigen-activated cells in the inflamed joint. Moreover, past studies have noted that T cells from peripheral blood or synovial fluid of patients with Lyme arthritis secrete primarily the proinflammatory Th1 cytokine IFN $\gamma$  (30–32), and a Th1 response is also dominant in the synovial tissue of such patients (33,34). In a previous study of 10 patients with Lyme arthritis, the severity of Lyme arthritis correlated directly with the ratio of Th1 to Th2 cells in synovial fluid, such that the larger the effusion, the higher the ratio (32). This study shows that it is Th1 responses primarily to OspA, and to a lesser degree to OspB, that correlate with the severity of arthritis. In a rat model,

intraarticular injections of OspA, but not OspB, were shown to be arthritogenic (35).

In an initial study, we compared T cell responses in 9 patients with treatment-resistant or treatment-responsive Lyme arthritis by generating T cell lines from their peripheral blood and joint fluid (17). In that study, OspA was preferentially recognized by T cell lines from 5 patients with treatment-resistant arthritis, but not all of them had OspA-reactive T cells and the number of patients tested was small. In the present study, T cells in bulk culture were tested for their reactivity with *B burgdorferi* proteins in 32 treatment-responsive or treatment-resistant patients. The only significant difference was the greater reactivity of treatment-resistant patients with the N-terminal and C-terminal fragments of OspA, as measured both by proliferative responses and IFN $\gamma$  production. This difference reached statistical significance with PBL, but not with SFL, presumably because SFL were unobtainable in 7 patients in whom the arthritis resolved rapidly. Data from these patients, who represented the mild end of the spectrum, were not available for the comparisons of SFL responses in treatment-responsive and treatment-resistant patients.

When OspA epitope mapping was done in patients with treatment-resistant and treatment-responsive arthritis, most patients with treatment-resistant courses had responses to 4 or 5 epitopes of OspA, whereas only a few of the treatment-responsive patients had reactivity with this protein, usually with 1 or 2 epitopes. Responses to OspA epitopes were rarely present in initial samples, and several months of arthritis were usually required for such reactivity to develop. In these patients, we were not able to demonstrate initial reactivity with a single dominant epitope of OspA followed by determinant spreading to other epitopes, perhaps because samples were only obtained at approximately monthly intervals.

Although the analysis of OspA epitope responses according to HLA-DRB1 alleles was limited by the small sizes of the groups, patients who had the alleles associated with treatment-resistant Lyme arthritis tended to have greater reactivity with peptide 15 (aa 154-173) and to a lesser degree with peptide 21 (aa 214-233) than those who had other DRB1 alleles. These results have now been confirmed by other methods. According to an algorithm generated by Hammer et al (36), the dominant epitope of OspA bound by the HLA-DRB1\*0401 molecule was predicted to be located in peptide 15, with a secondary epitope in peptide 21 (37). Moreover, this prediction was confirmed using transgenic mice that were deficient in their own class II molecules and expressed only a chimeric, human HLA-

DRB1\*0401 molecule (37). In a search of the Genetics Computer Group gene bank, only 1 human protein was identified, lymphocyte function associated antigen 1 (hLFA-1), that had sequence homology with OspA<sub>165-173</sub> and predicted binding in the DRB1\*0401 molecule. No human protein was identified that had homology with the secondary epitope and predicted binding in this MHC molecule.

We then showed that synovial fluid T cells from most patients with treatment-resistant arthritis responded to hLFA-1, whereas those from patients with other forms of chronic inflammatory arthritis did not (37). Molecular mimicry between the dominant T cell epitope of OspA and hLFA-1 would provide an explanation for persistent joint inflammation in genetically susceptible human patients after the apparent eradication of the spirochete from the joint with antibiotic therapy.

A few patients with treatment-resistant Lyme arthritis did not have T cell reactivity with OspA, and therefore, the mechanism of molecular mimicry between OspA and hLFA-1 would not explain all cases of treatment-resistant Lyme arthritis. In this study, a possible association was also found between treatment-resistant arthritis and reactivity with P41, the flagellar protein of the spirochete. An antibody epitope of this spirochetal protein, defined by the monoclonal anti-flagellin antibody H9724, has been shown to have molecular mimicry with a member of the 60-kd family of human heat-shock proteins (38). However, the possible mechanisms whereby T cell reactivity with P41 might be involved in the pathogenesis of treatment-resistant arthritis are not yet known.

In summary, this study demonstrates that both the severity and duration of Lyme arthritis after antibiotic treatment are associated with T cell responses to dominant epitopes of OspA. This response may be critical in the pathogenesis of antibiotic treatment-resistant Lyme arthritis.

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