Antibody Response to IR_6, a Conserved Immunodominant Region of the VlsE Lipoprotein, Wanes Rapidly after Antibiotic Treatment of *Borrelia burgdorferi* Infection in Experimental Animals and in Humans

Mario T. Philipp,[1] Lisa C. Bowers,[1] Paul T. Fawcett,[2] Mary B. Jacobs,[1] Fang Ting Liang,[1] Adriana R. Marques,[3] Paul D. Mitchell,[4] Jeanette E. Purcell,[1] Marion S. Ratterree,[1] and Reinhard K. Straubinger[5]

[1]*Tulane Regional Primate Research Center, Tulane University Health Sciences Center, Covington, Louisiana;* [2]*Alfred I. duPont Hospital for Children, Wilmington, Delaware;* [3]*Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland;* [4]*Clinical Microbiology Section, Marshfield Laboratories, Marshfield, Wisconsin;* [5]*Institut für Immunologie, Veterinärmedizinische Fakultät, Universität Leipzig, Leipzig, Germany*

Invariable region $(IR)_6$, an immunodominant conserved region of VlsE, the antigenic variation protein of *Borrelia burgdorferi,* is currently used for the serologic diagnosis of Lyme disease in humans and canines. A longitudinal assessment of anti-IR_6 antibody levels in *B. burgdorferi*–infected rhesus monkeys revealed that this level diminished sharply after antibiotic treatment (within 25 weeks). In contrast, antibody levels to P39 and to whole-cell antigen extracts of *B. burgdorferi* either remained unchanged or diminished less. A longitudinal analysis in dogs yielded similar results. In humans, the anti-IR_6 antibody titer diminished by a factor of $\geqslant 4$ in successfully treated patients and by a factor of <4 in treatment-resistant patients. This result suggests that the quantification of anti-IR_6 antibody titer as a function of time should be investigated further as a test to assess response to Lyme disease therapy or to determine whether a *B. burgdorferi* infection has been eliminated.

The number of annually reported cases of Lyme disease in the United States has increased ~25-fold since national surveillance began in 1982. A mean of 12,451 Lyme disease cases was reported annually in 1993–1997 [1]. At the same time, tangible progress has been made in the prevention and diagnosis of this emerging infectious disease. A commercial vaccine is available [1], and efforts to improve diagnostic serologic analysis have yielded algorithms to aid in the standardization of serologic methods [2]. In addition, promising diagnostic procedures based on recombinant proteins of *Borrelia burgdorferi* [3], the spirochete that causes Lyme disease, and on synthetic peptides [4] have been developed that may replace the 2-tiered serology currently recommended by the Centers for Disease Control and Prevention (CDC) [2].

As for chemotherapy, although most patients respond to treatment [5], the question of when a patient with Lyme disease may be considered to be cured continues to be a matter of controversy. Research is in progress at the National Institutes of Health (NIH) to try to discern whether the so-called posttreatment Lyme disease syndrome (PTLDS; i.e., the occurrence of persistent signs and symptoms of disease, despite the administration of what is currently considered to be adequate antibiotic therapy) is due to ongoing active borrelial infection, to a postinfectious syndrome, to irreversible sequelae of earlier tissue injury, or to a condition altogether unrelated to Lyme disease [6].

Most recently, an NIH-funded randomized, double-blinded, placebo-controlled, multicenter phase 3 antibiotic treatment trial of patients with PTLDS yielded no significant difference in the percentage of patients who thought their symptoms had improved, gotten worse, or stayed the same between the antibiotic treatment and placebo groups. Considering that patients in this trial were subjected to a 30-day-long course of intravenous (iv) ceftriaxone (2.0 g once a day) followed by 60 days of oral doxycycline (200 mg/day [100 mg twice a day]) [7], the result either calls into question the infectious etiology of PTLDS or suggests that, in a manner as yet unexplained, spirochetes have become inaccessible to or immune to the effects of antibiotics. These ambiguities underscore the need for a reference standard to assess cure of a *B. burgdorferi* infection. If such were available, it would be possible not only to ascertain whether treatment of early Lyme disease was successful, thereby preventing the transition to the late, more intractable form of the disease, but also to distinguish among the possible PTLDS etiologies.

During the 1980s and early 1990s, several attempts were made to evaluate whether the antibody response to *B. burg-*

Received 12 April 2001; revised 12 June 2001; electronically published 30 August 2001.

Presented in part: 14th International Conference on Lyme Disease and Other Tick-Borne Disorders, Hartford, Connecticut, April 2001.

Study patients gave informed consent for punch biopsies.

Financial support: Centers for Disease Control and Prevention (U50/CCU606604); National Institutes of Health (RR-00164).

Reprints or correspondence: Dr. Mario T. Philipp, Dept. of Parasitology, Tulane Regional Primate Research Center, Tulane University Health Sciences Center, 18703 Three Rivers Rd., Covington, LA 70433 (Philipp@tpc.tulane.edu).

The Journal of Infectious Diseases 2001; 184:870–8

0022-1899/2001/18407-0008$02.00

dorferi could be used as an indicator of infection status. Longitudinal analyses of serum titers, measured both by fluorescence and by ELISA, were performed. The consensus from these studies is either that IgG (or IgM) antibody titers do not change significantly after treatment or, when they do, the changes do not correlate with presence or absence of cure. In these ELISAs, whole-cell extracts of *B. burgdorferi* were used as antigen [8, 9].

Recently, a sensitive and specific serologic test for Lyme disease based on the detection of antibody to a conserved immunodominant region of VlsE was developed [4, 10–12]. VlsE, the antigenic variation lipoprotein of *B. burgdorferi,* has 2 invariable domains at the amino and carboxyl termini, respectively, and a central variable domain [13]. The variable domain contains 6 variable regions, VR_I–VR_{VI}, and 6 invariable regions, IR_1–IR_6 [13]. The latter remain unchanged during antigenic variation [13] and are conserved among strains and genospecies of *B. burgdorferi* sensu lato [10]. IR_6 is the most conserved of the IRs and is immunodominant both in monkeys and in humans infected with *B. burgdorferi* [10]. The serologic test developed is based on an ELISA that uses a peptide (C_6) as antigen. This peptide reproduces the sequence of IR_6 of the IP90 strain of the European genospecies *B. garinii* [4].

During a longitudinal assessment of the antibody response to C_6 in rhesus monkeys infected with *B. burgdorferi,* we noticed that the anti-C_6 antibody level diminished sharply after the animals were treated with antibiotics. In untreated animals, in contrast, this level remained essentially unchanged several years after infection [4]. This prompted us to compare the rate of decrease of anti-C_6 antibody levels with that of antibodies to P39 (BmpA) [14] and to whole-cell antigen extracts of *B. burgdorferi.* We further compared these rates in *B. burgdorferi*–infected antibiotic-treated dogs and humans. Here we describe the results of these studies.

Materials and Methods

Antibiotic treatment, serum collection, and serologic analysis in monkeys and dogs. Seven 2–4-year-old rhesus macaques *(Macaca mulatta)* of both sexes were inoculated with the JD1 strain of *B. burgdorferi* sensu stricto by tick bite, as described elsewhere [15]. At postinoculation (PI) week 12, the animals were treated orally with doxycycline for 60 days (2 mg/kg twice a day). Peak and trough levels of serum doxycycline were determined, to ensure that the MIC had been reached. The doxycycline concentration at the peak was 7 times the MIC (0.3 mg/L); at the trough it was at or below the MIC. All of the animals were culture positive before treatment, as determined by cultivation of skin biopsy samples within the first 4 weeks after infection. Tissue cultivation was done as described elsewhere [16]. Serum samples were collected weekly for 40 weeks.

We used 3 serologic assays with these serum specimens: C_6 ELISA, standard Lyme disease diagnostic ELISA, and P39 ELISA. The C_6 ELISA was done as described elsewhere [4]. The standard ELISA, using whole-cell extract of *B. burgdorferi* as antigen, is available commercially (MarDx) and was performed according to the manufacturer's instructions. Antigen for the P39 ELISA was a purified recombinant fusion protein that consisted of a fragment of BmpA, lacking the first 12 aa of the mature form of this protein, fused to the maltose-binding protein (MBP) of *Escherichia coli.* The *BmpA* gene was isolated from *B. burgdorferi* strain JD1 [17].

The optimum antigen and antibody conjugate concentrations were determined by checkerboard analysis. Immunoassay flat-bottom plates (Costar) were coated with 1 μg/mL of BmpA MBP or with MBP alone in a 0.1 *M* carbonate buffer, pH 9.6, and were incubated for 3 h at 37°C in a humidified incubator. Unbound antigen and unbound reagents from each of the ensuing incubations were removed by 3 washes with PBS containing 0.05% Tween 20 (PBS-T). Subsequent incubations were for 1 h at 37°C with (1) 0.2 mL/well of a 3% (vol/vol) blocking solution of liquid gelatin (Norland Laboratories) in PBS-T; (2) 0.05 mL/well of monkey serum at a dilution of 1:200; or (3) 0.1 mL/well of a 1:14,000 dilution of horseradish peroxidase–labeled anti–human IgG antibody (γ-chain specific; catalog no. 074-1002; Kirkegaard & Perry). Plates were developed for 10 min by adding 0.1 mL/well of a solution that contained tetramethylbenzidine chromogen and hydrogen peroxide, as prepared by the manufacturer (catalog no. 50-76-00; Kirkegaard & Perry). The reaction was stopped with 0.1 mL/well of 0.1 *N* phosphoric acid, and the optical density (OD) was read at 450 nm in a Tecan Spectra II ELISA reader (SLT Lab Instruments). Net OD values were obtained by subtracting OD values obtained with MBP as antigen from OD values obtained with BmpA-MBP.

Serum samples from 4 additional rhesus macaques were serially assessed with the C_6 ELISA. These animals had been inoculated with *B. burgdorferi* B31 spirochetes for another study [15] and were never treated with antibiotics.

We tested serial serum samples from 16 dogs (specific pathogen–free 6-week-old beagles of both sexes) that were infected with *B. burgdorferi* by exposure to feral ticks. Infection was confirmed in all of the dogs by culture and by polymerase chain reaction (PCR), as described elsewhere [18]. Starting on day 120 of the experiment, 3 groups of 4 dogs each were treated with doxycycline, ceftriaxone, or azithromycin, respectively, for 30 consecutive days. The remaining 4 animals were not treated and served as controls. Blood samples were collected every 2 weeks and were tested for anti–*B. burgdorferi* antibodies by a kinetic ELISA with whole-cell *B. burgdorferi* extract as antigen. Details of these procedures have been described elsewhere [18]. For the present study, serial serum samples from ceftriaxone-treated dogs and from control group animals were assessed for anti-C_6 antibody with the C_6 ELISA, as described elsewhere [12].

Serum samples from patients with early Lyme disease. We analyzed serum samples from Lyme disease patients participating in two clinical studies: the Lyme disease culture study of New York Medical College [19] and the "azithromycin trial" [20]. Patients in the first group were eligible for participation if they had $\geqslant$1 erythema migrans (EM) rashes (as defined in CDC surveillance criteria [21]) and had undergone a 2-mm skin biopsy. Patients with a positive culture of skin and/or blood samples were evaluated at baseline, at 7–10 and 21–28 days, at 3, 6, and 12 months, and annually thereafter. At these times, patients were interviewed and examined and a blood sample was taken. All of the patients were treated with antibiotics as per recommendations of the Infectious Diseases

Society of America [5]. They were considered to be cured if they were free of the signs and symptoms shown at presentation and had no additional clinical evidence of infection.

The serum samples from the azithromycin trial had been serially collected from 7 culture-confirmed patients with early Lyme disease during the first year after their treatment at the Marshfield clinic, Marshfield, Wisconsin [20]. The azithromycin trial was a double-blinded, randomized, controlled trial in which the efficacies of the antibiotics azithromycin and amoxicillin in the treatment of EM were compared. Serum specimens were collected serially at varying times for 26 weeks to >1 year after presentation [20]. In addition, a group of 4 serum samples from patients with early Lyme disease who presented with EM were obtained from the A. I. duPont Hospital for Children, Wilmington, Delaware. Serum specimens were obtained at presentation and ~6 months later. Patients were treated with cefuroxime axetil for 20 days, and all were free of signs and/or symptoms by week 12 after presentation.

Serum samples from patients with late Lyme disease and PTLDS. Patients with late Lyme disease included arthritis patients ($n = 11$) from the duPont Hospital for Children who had been treated either with amoxicillin or with doxycycline for 4 weeks and were free of signs and symptoms at the time of the last blood sample. The time between blood samplings varied between 4 and 76 weeks. The first sample was taken at presentation. In addition, serum samples from 1 patient with chronic treatment-resistant Lyme arthritis and from 2 PTLDS patients were serially obtained at the research hospital of the National Institute of Allergy and Infectious Diseases (NIAID; Bethesda, MD).

Serologic analysis of human serum specimens. C_6 ELISA with human serum samples was performed as described elsewhere [4]. Anti-C_6 antibody titers were determined by 2-fold serial dilutions. The end point was defined as the first dilution at which the OD value was below the cutoff line. The latter was set at the mean OD value of 10 serum specimens from patients living in an area where Lyme disease is not endemic plus 3 times the SD of that mean. Standard ELISA values and titers were determined by using *B. burgdorferi* whole-cell antigen extracts, as described elsewhere [22].

Results

Course of C_6 antibody response in monkeys. The longitudinal analysis of serum antibody levels in monkeys yielded a striking result. Although antibody levels determined with the 3 assays (C_6 ELISA, whole-cell antigen [standard] ELISA, and P39 ELISA [figure 1*A,* 1*B,* and 1*C,* respectively]) increased gradually in all animals during the initial course of infection, they declined at markedly different rates after antibiotic treatment. Between weeks 12 and 21, when the animals were treated with antibiotics, antibody levels to both C_6 and whole-cell antigens declined, except in animal AA68, in which the standard ELISA OD remained essentially unchanged (figure 1*B, arrow*). As mentioned, the decline in the OD values was more pronounced with the C_6 ELISA than with the standard ELISA. Anti-P39 antibody levels were not determined in this period, except for animal V134 (figure 1*C, arrow*). Surprisingly, by PI week 34 (13 weeks after treatment termination), the anti-C_6 antibody levels had reached the background level for this assay (figure 1*A*). In contrast, the standard ELISA OD had not yet reached its corresponding cutoff line by PI week 40. In addition, for 5 of the 7 animals in the study, the antibody levels, as measured by the standard ELISA, had reached a plateau that was 1.5–4 times the cutoff value (figure 1*B*). The response to P39 followed a similar pattern. With the exception of the 2 animals that did not respond to this antigen, the OD value of the P39 ELISA had reached a plateau of 5–10 times the cutoff value for this assay by PI week 31 (figure 1*C*). In fact, the anti-P39 antibody levels appeared to be essentially unaffected by the doxycycline treatment.

Levels of anti-C_6 antibody are fairly stable in the absence of antibiotic treatment. We previously determined that the anti-C_6 antibody response in chronically infected monkeys remains high and at a level essentially unchanged for >3 years, the longest period studied [4]; however, in monkeys, during a short period at the beginning of the infection process, the anti-C_6 antibody level transiently diminished. This dip in the C_6 ELISA OD was observed in virtually every infected animal between PI weeks 5 and 12 and was unrelated to antibiotic treatment. Figure 2 shows the serial C_6 ELISA OD values in 4 rhesus monkeys infected (via tick bite) with *B. burgdorferi* B31. The OD values dip by PI weeks 6–12. The dip also is visible in the experiment shown in figure 1*A* in animals V822, V760, V134, and AA68. In the remaining 3 animals (AL93, AK53, and AA67), the dip may have overlapped the doxycycline treatment.

Course of anti-C_6 antibody response in dogs. In a previous investigation, Straubinger et al. [18] surveyed the serial antibody response to whole-cell antigen extracts of *B. burgdorferi* in the same dogs that were used in the present study. Their survey and ours included time points before and after ceftriaxone treatment. Such antibody levels, which were determined by kinetic ELISA, had reached a plateau at ~60% of the highest level reached in the study period by PI week 47 (i.e., 6 months after the termination of treatment) [18]. In contrast, the anti-C_6 antibody level had already reached a plateau at this time, essentially at background level (figure 3). Hence, as with monkeys, the anti-C_6 antibody response waned faster and more radically after antibiotic treatment than the antibody response to whole-cell antigens.

Before antibiotic treatment, spirochetes could be cultured from each skin sample of all the dogs during the first 120 days of infection. In contrast, after treatment, only control dogs yielded skin biopsy samples that were culture positive, and only rarely were skin samples PCR positive in treated dogs [18]. At postmortem examination, no treated dog yielded tissue samples that were culture positive, whereas untreated dogs yielded multiple tissue samples that were culture positive for *B. burgdorferi* [18]. Thus, the antibiotic either greatly reduced or totally eliminated the infectious burden of treated dogs, in comparison with untreated dogs. Concomitantly, the anti-C_6 antibody decreased almost to background levels in treated dogs but remained at

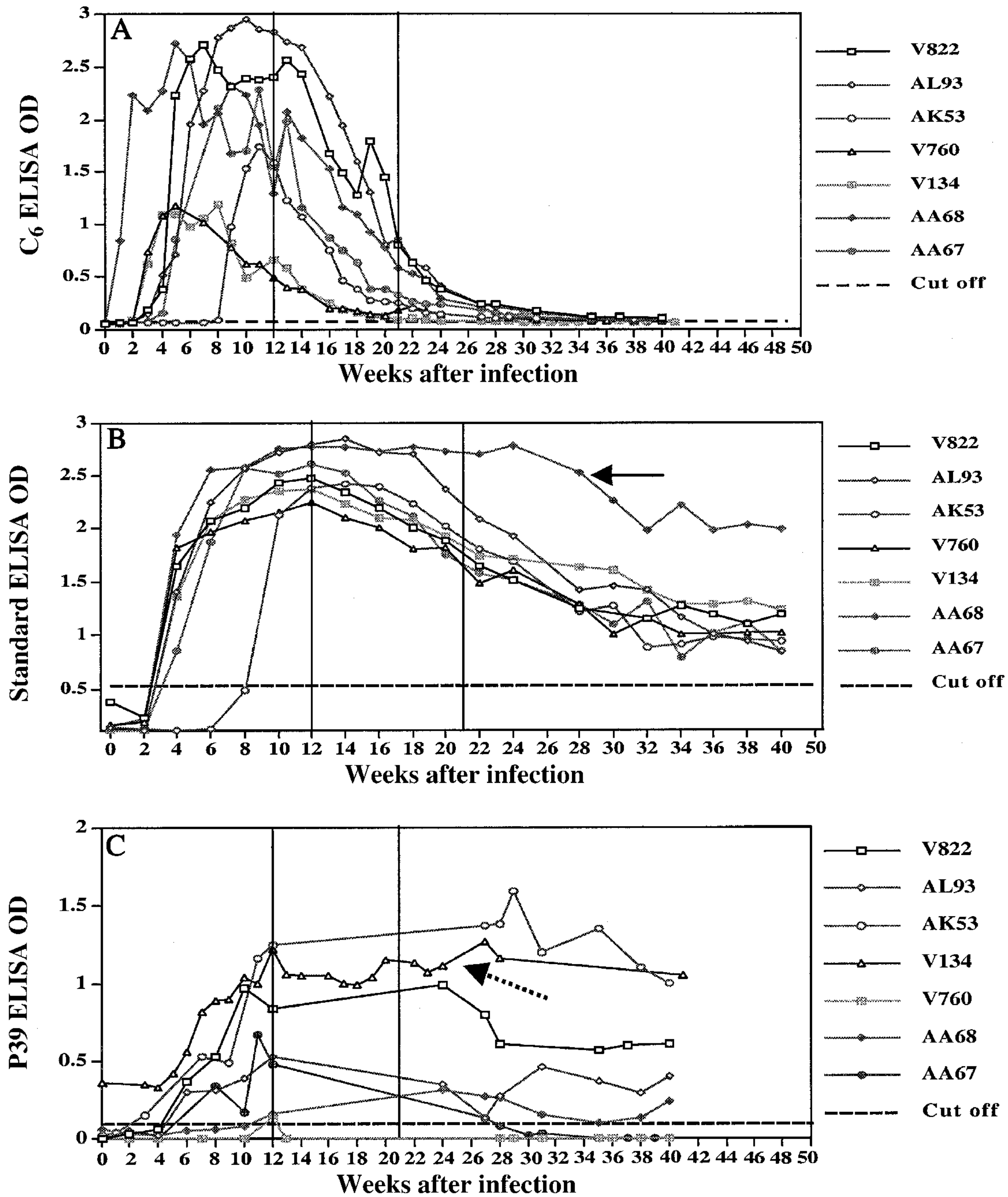

Figure 1. Longitudinal assessment of anti-C_6 *(A),* anti–whole-cell antigen extract *(B),* and anti-P39 *(C)* antibody response in rhesus monkeys infected with *Borrelia burgdorferi* JD1 and treated with doxycycline. *Vertical lines,* times of initiation and termination of treatment. *B* and *C,* Arrows indicate standard ELISA curve for animal AA68 and P39 antibody curve for animal V134, respectively (see text). Cutoff line was set at mean optical density (OD) value of preinfection serum samples plus 3 × SD of the mean.

the highest levels in control animals throughout the study period (figure 3).

Course of anti-C_6 antibody response in humans. We next compared the decline in anti-C_6 antibody titer with the decline in antibody titer to whole-cell extracts in 4 patients with early Lyme disease and in 11 patients with late Lyme disease seen at the duPont Hospital for Children, who responded to treatment. In all but 1 case, the anti-C_6 antibody titer fell faster than the anti–whole-cell antigen titer. Interestingly, the anti-C_6 antibody titer decreased by a factor of ⩾4 in all of the patients

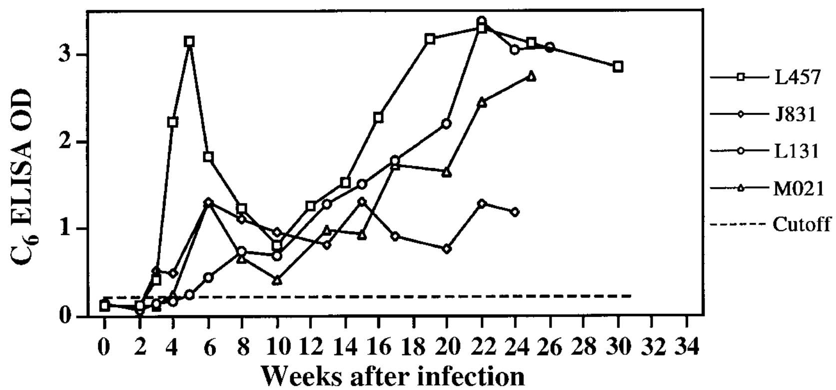

Figure 2. Serial anti-C_6 antibody response in 4 rhesus macaques (L457, J831, L131, and M021) infected with *Borrelia burgdorferi* B31. OD, optical density.

when assessed at >20 weeks after presentation. The geometric mean decline in anti-C_6 antibody titer was 9.1 (range, 4–32) when the second serum sample was obtained ⩾21 weeks after presentation and 4.0 (range, 1–8) when obtained at ⩽20 weeks. Similar results were obtained with serum samples obtained at presentation and at years 1, 2, 4, or 5 after treatment from 9 patients with early Lyme disease seen at the New York Medical College. These patients were free of signs and symptoms at the later visit. Regardless of the time elapsed, at the time of the last visit, the anti-C_6 antibody titer had fallen by a factor of ⩾4 (median, 9.3; range, 4–64). When serum specimens from the duPont Hospital patients who were tested for anti-C_6 antibody were tested by standard (whole-cell antigen) ELISA, the geometric mean decline in titer for samples collected ⩾21 weeks after presentation was 1.6 (range, 1–4) and for specimens collected before that time likewise was 1.6 (range, 1–8). These results, and those with experimental animals, suggest that the rate of decline in anti-C_6 antibody titer, unlike that of antibody to whole-cell antigen, could serve as an indirect indicator of spirochetal burden (i.e., a test to assess response to Lyme disease therapy or to assess whether a *B. burgdorferi* infection had been eliminated).

Initial assessment of C_6 ELISA as a test of response to therapy or of cure. To examine the C_6 ELISA as a test of response to therapy or cure, we used serum samples from patients who had unmistakable signs and symptoms of treatment-resistant Lyme disease. We first assessed patients with early culture-confirmed Lyme disease who had participated in the azithromycin trial [20] and whose serum specimens were serially collected during the first posttreatment year. All but 1 of the 7 patients

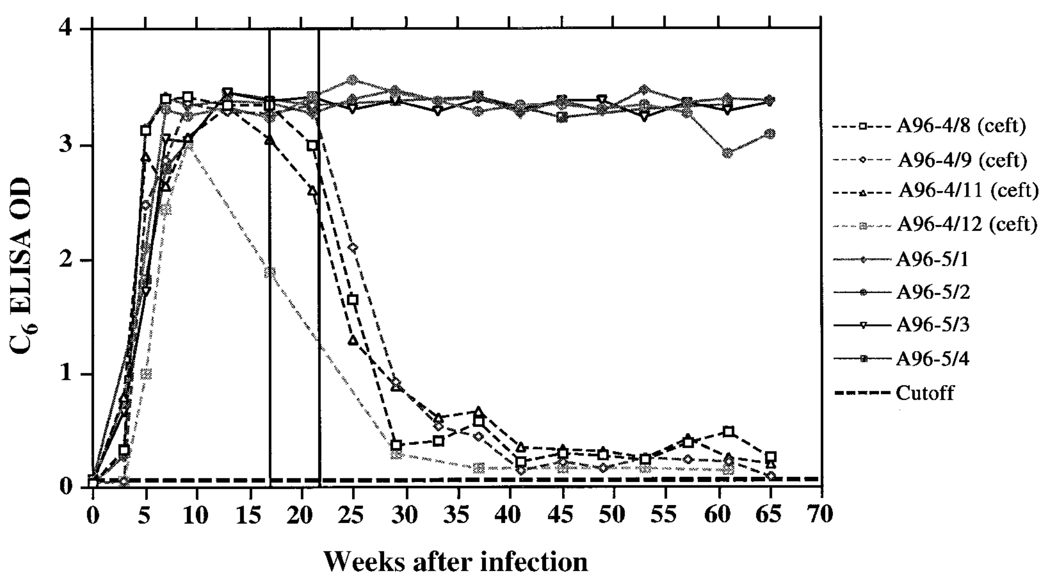

Figure 3. Anti-C_6 antibody response in dogs infected with *Borrelia burgdorferi* and treated with ceftriaxone (ceft; *dotted lines*) or not treated *(solid lines)*. *Vertical lines,* times of initiation and termination of treatment. Cutoff was set at mean optical density (OD) value of preinfection serum samples plus 3 × SD of the mean.

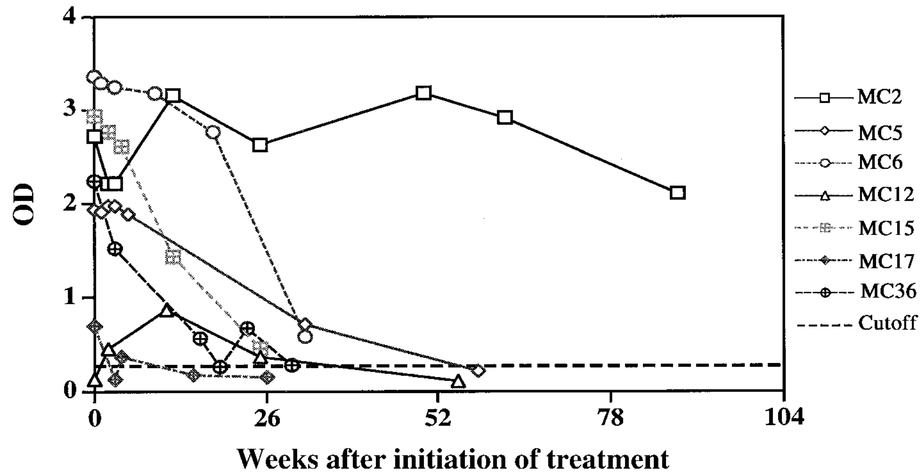

Figure 4. Anti-C_6 antibody level as function of time after treatment in antibiotic-treated patients from the Marshfield clinic (Marshfield, WI). Cutoff was defined as mean optical density (OD) value of serum samples from 10 patients from a hospital in an area where Lyme disease is not endemic plus 3 × SD of the mean.

were successfully treated. Patient MC2, in whom initial treatment failed, received iv ceftriaxone 1 year after treatment with azithromycin. This patient had intense migratory joint pain that started at 7 months and a small right knee effusion at 11 months that was positive for *B. burgdorferi* by PCR [23]. Except for patient MC2, whose anti-C_6 antibody level remained essentially unchanged for 1 year, the antibody levels of all patients either reached the cutoff line or likely would have done so shortly after the last time point we assessed (figure 4). As before, during a 6-month posttreatment period, titers diminished by a factor of ⩾4 for all of the patients who were free of symptoms, except for patient MC17, whose initial serum sample had a low OD value (figure 4). The geometric mean decline in titer for cured patients was 8.6 (range, 3–128). In contrast, the serum titer of patient MC2, who was treatment resistant, increased 4-fold instead of diminishing.

Three additional serum samples from patients with either chronic treatment-resistant Lyme arthritis or PTLDS who were treated at the NIAID research hospital were assessed. Patient 1 (with PTLDS) was a 36-year-old man who had an EM rash in April 1996 and fever, fatigue, myalgias, and arthralgias. In July 1996, he developed right knee arthritis, followed by right ankle and left elbow arthritis. Both standard ELISA and Western blot were positive. He was treated with iv ceftriaxone for 1 month after the July visit. One month after finishing the therapy, the symptoms returned (myalgias, arthralgias, fatigue, difficulty concentrating, short-term memory loss, anxiety, and irritability). He was treated with amoxicillin but did not improve. The relative invariance of the anti-C_6 antibody titer in patient 1, despite the repeated treatment, correlates with the persistence of symptoms. Blood samples were collected at roughly 6-month intervals (table 1).

Patient 4 (with treatment-resistant Lyme arthritis), a 76-year-old woman, was seen early in 1998. She had right knee pain followed by recurrent bilateral knee arthritis. In December 1998, she was serologically tested for Lyme disease, with positive ELISA and Western blot results. She was treated iv with ceftriaxone for 1 month with no improvement and was later switched to oral doxycycline, also with no improvement. At a visit to the NIH in April 1999, PCR of the synovial fluid was positive for *B. burgdorferi.* Her anti-C_6 antibody titer remained essentially unchanged from immediately after extensive treatment with ceftriaxone to 8 months later, when symptoms persisted (table 1).

Patient 7 (with PTLDS), a 35-year-old man, presented in July 1997 with severe depression. Brain magnetic resonance imaging showed multiple white matter lesions. Lyme ELISA and IgG Western blot analyses were positive. Symptoms of depression had started ∼1990. He had a history of right facial palsy in 1985, followed by multiple episodes of recurrent bilateral knee arthritis from 1987 to 1989. He was treated iv for 3 weeks with ceftriaxone (completed 13 August 1994). One year after treatment, his symptoms had not resolved, and his anti-C_6 antibody titer was unchanged (table 1). Thus, in the 3 patients with treatment-resistant Lyme disease or PTLDS, there was a strict correlation between presence of symptoms and change of anti-C_6 antibody titers by a factor of <4.

Discussion

We have shown that the antibody response to IR_6, as measured by the C_6 ELISA, wanes rapidly after antibiotic treatment of a *B. burgdorferi* infection in experimental animals and humans. Both anti-C_6 antibody levels, as measured by the ELISA OD, and, of more importance, antibody titers decreased more quickly than antibody to *B. burgdorferi* whole-cell extracts. This happened regardless of host species and was not the trivial consequence of comparing antibody level elicited against mul-

Table 1. Serum samples from patients with late treatment-resistant Lyme arthritis or posttreatment Lyme disease syndrome.

Patient, sampling date	C_6 titer	Comment(s)
Patient 1		
1/28/97	12,800[a]	Previously treated with ceftriaxone and amoxicillin; no antibiotics at evaluation
5/29/97	6400	Completing treatment with ceftriaxone; partial improvement of symptoms
11/17/97	6400	Worsening symptoms
6/10/99	6400[b]	Retreated with ceftriaxone and doxycycline without improvement; when receiving clarythromycin and hydroxychloroquine, symptoms partially improved
Patient 4		
4/28/99	12,800[a]	Persistent bilateral knee arthritis after ceftriaxone for 1 month and doxycycline for 3 months; PCR of synovial fluid *Borrelia burgdorferi* positive
6/28/99	12,800	Completed 6 weeks of iv ceftriaxone
7/11/99	12,800	Bilateral knee arthritis remained; PCR of synovial fluid weakly positive
3/16/00	6400[b]	Persistent arthritis; negative synovial PCR
Patient 7		
8/20/97	25,600[a]	3 Weeks of ceftriaxone treatment completed 1 week before evaluation
9/11/97	12,800–25,600	Antibiotics discontinued; persistent complaints
7/7/98	25,600[b]	New enhancing lesion present on brain MRI; when retreated with iv ceftriaxone for 4 weeks (2–3 weeks completed before evaluation), no improvement of symptoms but lesion resolved on MRI

NOTE. C_6 titer ratio (initial:final) was 2 for patients 1 and 4 and 1 for patient 7. iv, intravenous; MRI, magnetic resonance imaging; PCR, polymerase chain reaction.
[a] Initial.
[b] Final.

tiple antigens with that elicited against a single one, as evidenced by the persistent response to P39 observed in antibiotic-treated monkeys. In fact, the anti-P39 response was remarkably stable, when compared with the anti-C_6 response.

In dogs and monkeys with untreated *B. burgdorferi* infections, the response to C_6 remains stable. In serum specimens from treatment-resistant Lyme disease patients, the C_6 antibody titer also remained fairly constant. We submit, therefore, that the anti-C_6 antibody response should be further investigated as a possible test to assess response to therapy or cure from a *B. burgdorferi* infection. Such a test could be used not only to ascertain whether treatment of early Lyme disease is successful, thereby preventing the transition to the late, more intractable form of the disease, but also to distinguish among the possible etiologies of PTLDS. A test for cure of spirochetal infection has long been available for syphilis. Antibiotic treatment efficacy in patients with syphilis is assessed by using the quantitative forms of the VDRL test or the rapid plasma reagin test. Both tests are based on the quantification of serum antibody that reacts with a mixture of cardiolipin, cholesterol, and lecithin [24]. Quantitative reactions are reported in terms of the highest (last) dilution at which the specimen is fully reactive. Treatment is considered successful if titer declines ⩾4-fold [24].

The development of such a test for Lyme disease has been elusive, perhaps because the focus has been antibody to whole-cell antigen extracts [8, 9, 25]. Many of these antigens, including P39, are probably efficient elicitors of B cell memory. Periodic restimulation of B memory cells with antigens is thought to be required for the long-term maintenance of both an antibody response and the memory B cell pool [26, 27]. Thus, availability of antigens in the form of immune complexes is a prerequisite for the maintenance of long-term serum antibody responses. These antigens are stored in folliculodendritic cells (FDCs), which are highly adapted to serve as deposit for antigen-antibody complexes that drive the B cell differentiation process [28]. FDCs are thought to sequester antigens in an immunogenic form for up to several years, making antigens available for the restimulation of memory cells and the maintenance of serum antibody levels [26, 28].

B. burgdorferi antigens that can stimulate a sustained serum antibody response long after spirochetes have been killed must be molecules that are both abundant and stable in the host milieu and possibly are released (or shed) by live (or dead) spirochetes so as to easily form immune complexes. Above all, they should not be subjected to rapid turnover by the spirochete. This last attribute is the one we believe is key, for antigens that are rapidly turned over by living spirochetes shortly after their synthesis are probably nonabundant and unstable and/or disappear rapidly after spirochetal death. Production of antibodies to such antigens would not be maintained after spirochetal death, as the antigens would never reach the FDC reservoir.

VlsE might be one such rapidly turned over antigen. VlsE antigenic variation is achieved by a mechanism of recombination whereby fragments from a central portion of the *vlsE* gene are recombined with fragments from a string of 15 DNA cassettes located upstream from the *vlsE* locus [13]. The recombination mechanism is so promiscuous that, on average, and within 4 days of an infection in immune-competent C3H/HeN mice, as many as 11.0 predicted amino acid changes occur per variant [29]. To take advantage of this speedy recombination mechanism, the *vlsE* gene is probably transcribed at a high rate, and, as a consequence, there must also be in place a mechanism of fast turnover of already synthesized and membrane-associated VlsE molecules. This would avoid membrane crowding and, of most importance, the continued expression of "old" variant molecules against which antibody may have been elicited. We therefore hypothesize that VlsE is an antigen that is rare or not present in dead spirochetes in vivo and thus is never well represented among the antigens in FDCs. Antibody to VlsE should persist in the circulation in close correlation with the presence of viable spirochetes. Thus, the rapid decline in anti-C_6 antibody after antibiotic treatment would be a direct consequence of the constraints we postulate as part of the VlsE antigenic variation mechanism—the swift turnover of VlsE molecules.

An alternative explanation for the posttreatment decline in anti-C_6 antibody levels relates to the possibility that C_6 (VlsE?)

might be a T cell–independent (TI) antigen. By using mice lacking both $\alpha\beta^+$ and $\gamma\delta^+$ T cells, McKisic and Barthold [30] recently identified several *B. burgdorferi* antigens that can elicit an antibody response during infection in such animals [30]. Proteins of molecular masses of 21, 32, 34, 39, 58, and 66 kDa were among the TI antigens identified [30]. The mitogenic effect of bacterial lipoproteins on B cells is well known [31–34]. It is thus conceivable that an organism such as *B. burgdorferi,* in which 11% of the genome encodes potential lipoproteins [35], can generate an array of TI (and T cell–dependent) antibody responses. In preliminary experiments, we verified that normal mice infected with *B. burgdorferi* and subsequently treated with ceftriaxone experience a decline in anti-C_6 antibody levels similar to those observed in monkeys and dogs. In contrast, antibody levels to P39 remained essentially unchanged (M.T.P., M.B.J., and J.E.P., unpublished data). If normal immunocompetent mice can mount primarily TI antibody responses to the IR_6, as detected by the C_6 peptide ELISA, it is possible that such responses would be short-lived after antibiotic treatment, since some TI antigens are poor elicitors of B cell memory responses [36, 37]. We are now testing this alternative hypothesis.

In conclusion, the swift waning of the anti-C_6 antibody response in the wake of antibiotic treatment, as seen both in experimental animals and in humans, suggests that the quantification of C_6 antibody titer as a function of time may serve as a test to assess whether a *B. burgdorferi* infection has been eliminated. We are in the process of further evaluating the validity of this contention in an expanded retrospective study by using serum samples from patients with both early and late Lyme disease. If this study yields a satisfactory result, it will be followed by a prospective evaluation.

Acknowledgment

We gratefully acknowledge Gary Wormser, New York Medical College, for providing serum samples.

References

1. Centers for Disease Control and Prevention. Recommendations for the use of the Lyme disease vaccine. MMWR Morb Mortal Wkly Rep **1999**;48:1–2.
2. Centers for Disease Control and Prevention. Recommendations for test performance and interpretation from the Second National Conference on Serologic Diagnosis of Lyme Disease. MMWR Morb Mortal Wkly Rep **1999**;44:590–1.
3. Lawrenz MB, Hardham JM, Owens RT, et al. Human antibody responses to VlsE antigenic variation protein of *Borrelia burgdorferi.* J Clin Microbiol **1999**;37:3997–4004.
4. Liang FT, Steere AC, Marques AR, Johnson BJ, Miller JN, Philipp MT. Sensitive and specific serodiagnosis of Lyme disease by enzyme-linked immunosorbent assay with a peptide based on an immunodominant conserved region of *Borrelia burgdorferi* VlsE. J Clin Microbiol **1999**;37:3990–6.
5. Wormser GP, Nadelman RB, Dattwyler RJ, et al. Practice guidelines for the treatment of Lyme disease. Clin Infect Dis **2000**;31(Suppl 1):S1–14.
6. Marques A. Protocol 96–I-0052. Protocol number 96-I-0052. Available at http://www.niaid.nih.gov/dir/labs/lci/lyme.htm.
7. Klempner MS, Hu LT, Evans J, et al. Two controlled trials of antibiotic treatment in patients with persistent symptoms and a history of Lyme disease. N Engl J Med **2001**;345:85–92.
8. Craft JE, Grodzicki RL, Steere AC. Antibody response in Lyme disease: evaluation of diagnostic tests. J Infect Dis **1984**;149:789–95.
9. Steere AC, Grodzicki RL, Kornblatt AN, et al. The spirochetal etiology of Lyme disease. N Engl J Med **1983**;308:733–40.
10. Liang FT, Alvarez AL, Gu Y, Nowling JM, Ramamoorthy R, Philipp MT. An immunodominant conserved region within the variable domain of VlsE, the variable surface antigen of *Borrelia burgdorferi.* J Immunol **1999**;163:5566–73.
11. Liang FT, Aberer E, Cinco M, et al. Antigenic conservation of an immunodominant invariable region of the VlsE lipoprotein among European pathogenic genospecies of *Borrelia burgdorferi SL.* J Infect Dis **2000**;182:1455–62.
12. Liang FT, Jacobson RH, Straubinger RK, Grooters A, Philipp MT. Characterization of a *Borrelia burgdorferi* VlsE invariable region useful in canine Lyme disease serodiagnosis by enzyme-linked immunosorbent assay. J Clin Microbiol **2000**;38:4160–6.
13. Zhang JR, Hardham JM, Barbour AG, Norris SJ. Antigenic variation in Lyme disease borreliae by promiscuous recombination of VMP-like sequence cassettes. Cell **1997**;89:275–85.
14. Simpson WJ, Cieplak W, Schrumpf ME, Barbour AG, Schwan TG. Nucleotide sequence and analysis of the gene in *Borrelia burgdorferi* encoding the immunogenic P39 antigen. FEMS Microbiol Lett **1994**;119:381–7.
15. Philipp MT, Lobet Y, Bohm RP Jr, et al. The outer surface protein A (OspA) vaccine against Lyme disease: efficacy in the rhesus monkey. Vaccine **1997**;15:1872–87.
16. Philipp MT, Aydintug MK, Bohm RP Jr, et al. Early and early disseminated phases of Lyme disease in the rhesus monkey: a model for infection in humans. Infect Immun **1993**;61:3047–59.
17. Ramamoorthy R, Povinelli L, Philipp MT. Molecular characterization, genomic arrangement, and expression of *bmpD,* a new member of the bmp class of genes encoding membrane proteins of *Borrelia burgdorferi.* Infect Immun **1996**;64:1259–64.
18. Straubinger RK, Straubinger AF, Summers BA, Jacobson RH. Status of *Borrelia burgdorferi* infection after antibiotic treatment and the effects of corticosteroids: an experimental study. J Infect Dis **2000**;181:1069–81.
19. Nadelman RB, Nowakowski J, Forseter G, et al. The clinical spectrum of early Lyme borreliosis in patients with culture-confirmed erythema migrans. Am J Med **1996**;100:502–8.
20. Luft BJ, Dattwyler RJ, Johnson RC, et al. Azithromycin compared with amoxicillin in the treatment of erythema migrans: a double-blind, randomized, controlled trial. Ann Intern Med **1996**;124:785–91.
21. Case definitions for public health surveillance: Lyme disease. MMWR Morb Mortal Wkly Rep **1990**;39:19–21.
22. Fawcett PT, Rose CD, Gibney KM. Comparative evaluation of adsorption with *Escherichia coli* on ELISA tests for Lyme borreliosis. J Rheumatol **1995**;22:684–8.
23. Melski JW, Reed KD, Mitchell PD, Barth GD. Primary and secondary erythema migrans in central Wisconsin. Arch Dermatol **1993**;129:709–16.
24. Larsen SA, Steiner BM, Rudolph AH. Laboratory diagnosis and interpretation of tests for syphilis [review]. Clin Microbiol **1995**;8:1–21.
25. Feder HM Jr, Gerber MA, Luger SW, Ryan RW. Persistence of serum antibodies to *Borrelia burgdorferi* in patients treated for Lyme disease. Clin Infect Dis **1992**;15:788–93.
26. Paul WE, ed. Fundamental immunology. 4th ed. Philadelphia: Lippincott-Raven, **1998**.
27. Gray D, Skarvall H. B-cell memory is short-lived in the absence of antigen. Nature **1988**;336:70–3.
28. Mandel THE, Phipps RP, Abbot A, Tew JG. The follicular dendritic cell: long term antigen retention during immunity. Immunol Rev **1980**;53:29–59.

29. Zhang JR, Norris SJ. Kinetics and in vivo induction of genetic variation of VlsE in *Borrelia burgdorferi.* Infect Immun **1998**; 66:3689–97.

30. McKisic MD, Barthold SW. T-cell–independent responses to *Borrelia burgdorferi* are critical for protective immunity and resolution of Lyme disease. Infect Immun **2000**; 68:5190–7.

31. Tai KF, Ma Y, Weis JJ. Normal human B lymphocytes and mononuclear cells respond to the mitogenic and cytokine-stimulatory activities of *Borrelia burgdorferi* and its lipoprotein OspA. Infect Immun **1994**; 62:520–8.

32. Ma Y, Weis JJ. *Borrelia burgdorferi* outer surface lipoproteins OspA and OspB possess B-cell mitogenic and cytokine-stimulatory properties. Infect Immun **1993**; 61:3843–53.

33. Snapper CM, Rosas FR, Jin L, Wortham C, Kehry MR, Mond JJ. Bacterial lipoproteins may substitute for cytokines in the humoral immune response to T cell–independent type II antigens. J Immunol **1995**; 155:5582–9.

34. Brenner C, Wroblewski H, Le Henaff M, Montagnier L, Blanchard A. Spiralin, a mycoplasmal membrane lipoprotein, induces T-cell–independent B-cell blastogenesis and secretion of proinflammatory cytokines. Infect Immun **1997**; 65:4322–9.

35. Fraser CM, Casjens S, Huang WM, et al. Genomic sequence of a Lyme disease spirochaete, *Borrelia burgdorferi.* Nature **1997**; 390:580–6.

36. Zhang J, Liu YJ, MacLennan IC, Gray D, Lane PJ. B cell memory to thymus-independent antigens type 1 and type 2: the role of lipopolysaccharide in B memory induction. Eur J Immunol **1988**; 18:1417–24.

37. Klein J. Immunology, the science of self-nonself discrimination. New York: John Wiley & Sons, **1982**.