INFECTION AND IMMUNITY, June 2002, p. 3300–3303 Vol. 70, No. 6
0019-9567/02/$04.00+0 DOI: 10.1128/IAI.70.6.3300–3303.2002

DNA Microarray Assessment of Putative *Borrelia burgdorferi* Lipoprotein Genes

Fang Ting Liang,[1] F. Kenneth Nelson,[2] and Erol Fikrig[1]*

Section of Rheumatology, Department of Internal Medicine, School of Medicine,[1] and Department of Molecular, Cellular, and Developmental Biology,[2] Yale University, New Haven, Connecticut 06520-8031

Received 5 December 2001/Returned for modification 19 February 2002/Accepted 14 March 2002

A DNA microarray containing fragments of 137 *Borrelia burgdorferi* B31 putative lipoprotein genes was used to examine Lyme disease spirochetes. DNA from *B. burgdorferi* sensu stricto B31, 297, and N40; *Borrelia garinii* IP90; and *Borrelia afzelii* P/Gau was fluorescently labeled and hybridized to the microarray, demonstrating the degree to which the individual putative lipoprotein genes were conserved among the genospecies. These data show that a DNA microarray can globally examine the genes encoding *B. burgdorferi* lipoproteins.

Lyme disease is caused by genetically diverse spirochetes collectively termed *Borrelia burgdorferi* sensu lato. This complex includes several genospecies, of which at least three cause significant disease in humans: *B. burgdorferi* sensu stricto, *Borrelia garinii*, and *Borrelia afzelii* (17, 23). All North American pathogenic strains that have been identified are *B. burgdorferi* sensu stricto. In Europe, all three genospecies are found, with *B. garinii* and *B. afzelii* being the most prevalent. Manifestations of Lyme disease vary geographically, depending in part on the predominant genospecies. Lymphocytoma, acrodermatitis chronica atrophicans, and encephalomyelitis are mainly found in Europe, whereas disseminated early infection and arthritis are more common in the United States (1, 22, 25). *B. burgdorferi* sensu stricto strain B31 has a genome consisting of a linear chromosome and 21 linear and circular plasmids, which carries approximately 1,700 open reading frames (ORFs) (4, 7). Approximately 150 ORFs encode putative lipoproteins, more than any other known bacterium (4, 7). Nineteen of these putative lipoproteins have homologues that have been already identified in other organisms (7). Some of the remaining lipoproteins have demonstrated roles in *B. burgdorferi* pathogenesis, including enabling the spirochetes to adhere to decorin (8, 21), fibronectin (5, 6, 18, 19), or the tick midgut (14, 15) or to evade the immune system (26) or complement (12).

Putative lipoprotein gene DNA microarray. To efficiently study the putative lipoprotein genes among *B. burgdorferi* isolates, a lipoprotein DNA microarray was developed. *B. burgdorferi* B31 clone 5A11, which contains all 21 known plasmids (20), was grown to the stationary phase in Barbour-Stoenner-Kelly H medium at 33°C. The spirochetes were isolated, and DNA was purified by using the DNeasy Mini kit (Qiagen Inc., Valencia, Calif.). The genomic sequence of *B. burgdorferi* B31 was downloaded from The Institute for Genomic Research website (http://www.tigr.org), and 137 pairs of primers were designed to amplify a 150- to 500-bp internal fragment of each lipoprotein gene. As a control, *flaB* primers were also designed. Since some gene families share sequence identity, it is not possible to individually differentiate these genes by hybridization. For example, the *rev* family consists of three members (*bbc10*, *bbm27*, and *bbp27*) and two of them are almost identical (*bbm27* and *bbp27*) (4, 7). Therefore, two pairs of primers were designed for this family. One pair was unique for *bbc10* while the second pair was specific for both *bbm27* and *bbp27*. Similarly, only two pairs of primers were used for the eight members of the *mlp* family. Each pair only amplified one of the eight ORFs. All PCR primer sequences are available by request. PCR products were assessed by agarose gel electrophoresis, and all showed the expected sizes. Amplified DNA was purified, dissolved to a concentration of 0.05 μg/μl in 50% dimethyl sulfoxide, and spotted in triplicate on CMT-GAPS aminosilane-coated slides (Corning Inc., Corning, N.Y.) by using a Virtek SDDC-2 Arrayer (Toronto, Canada). To cross-link DNA to the microarray, printed slides were treated with 200 mJ of UV. The array was then blocked in 25% formamide plus 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) plus 0.1% sodium dodecyl sulfate (SDS) plus 1% bovine serum albumin at 42°C for 45 min, washed with distilled water at room temperature, and spun dry at 500 × *g* for 3 min.

Probe DNA was purified from cultured *B. burgdorferi* sensu stricto B31, 297, and N40; *B. garinii* IP90; and *B. afzelii* P/Gau or from murine spleens (control). Aminoallyl dUTP (Molecular Probes, Inc., Eugene, Oreg.) was incorporated by nick translation, fluorescently labeled with the Alexa Fluor 546 kit (B31 DNA) or the Alexa Fluor 647 kit (mouse, 297, N40, IP90, and P/Gau DNA) from Molecular Probes, purified, and lyophilized. A pair of fluorescently labeled DNA probes (one was B31 and the other was mouse, 297, N40, IP90, or P/Gau DNA) plus 10 μg of blocking mouse DNA (*Mbo*I digested) were dissolved in 15 μl of hybridization buffer (25% formamide, 5× SSC, and 0.1% SDS) and denatured at 95°C for 5 min. The heated samples were centrifuged at 8,000 × *g* for 2 min and applied to a preblocked microarray. The slide was covered with a coverslip and incubated in a humid chamber at 42°C for 16 to 20 h. The hybridized slide was stringently washed in hybridization buffer for 3 min and then in 2× SSC plus 0.1% SDS for an additional 3 min at 42°C. The slide was transferred into 0.2× SSC plus 0.1% SDS for 3 min, into 0.2× SCC for 1 min, and into 100% ethanol for 30 s at room temperature and spun dry.

* Corresponding author. Mailing address: Section of Rheumatology, Department of Internal Medicine, Yale University School of Medicine, 604 LCI, 333 Cedar St., New Haven, CT 06520-8031. Phone: (203) 785-2453. Fax: (203) 785-7053. E-mail: erol.fikrig@yale.edu.

TABLE 1. Conservation of putative lipoprotein genes that have homologues in other organisms

Gene	Potential function	Conservation in strain[a]:			
		B. burgdorferi		*B. garinii* IP90	*B. afzelii* P/Gau
		297	N40		
bb0088	GTP-binding protein	HC	HC	HC	HC
bb0098	DNA mismatch repair protein	HC	HC	HC	LC
bb0100	Glutamate racemase	HC	HC	HC	HC
bb0122	Translation elongation factor TS	HC	HC	HC	HC
bb0141	Membrane fusion protein	HC	HC	HC	HC
bb0300	Cell division protein	HC	HC	HC	HC
bb0341	Glu-tRNA amidotransferase, subunit B	HC	HC	HC	C
bb0372	Glutamyl-tRNA synthetase	HC	HC	C	C
bb0387	Ribosomal protein S12	HC	HC	HC	C
bb0489	Ribosomal protein L24	HC	HC	HC	HC
bb0536	Zinc protease	HC	HC	HC	HC
bb0620	β-Glucosidase	HC	HC	HC	C
bb0621	4-Methyl-5(β-hydroxyethyl)-thiazole monophosphate biosynthesis protein	HC	HC	LC	C
bb0652	Protein export membrane protein	HC	HC	HC	HC
bb0687	Phosphomevalonate kinase	HC	HC	C	C
bb0785	Stage V sporulation protein G	HC	HC	HC	HC
bb0828	DNA topoisomerase	HC	HC	HC	HC
bba34	Oligopeptide ABC transporter	HC	HC	HC	M
bbb16	Oligopeptide ABC transporter	HC	HC	HC	LC

[a] HC, highly conserved; C, conserved; LC, less conserved; M, may exist.

The hybridized microarray was scanned with an Axon GenePix 4000A array scanner, and raw data were captured with GenePix 3.0 software (Axon Instruments, Foster City, Calif.).

Specificity and normalization of microarray hybridization. To determine hybridization specificity, labeled murine (control) and B31 spirochetal DNAs were allowed to bind to the microarray. The ratios of 635-nm (murine DNA) and 532-nm (B31 DNA) emission values for each spot on the microarray were <0.2; therefore, readings below this value were considered to be insignificant. Normalization was achieved by adjusting both channels of laser intensity to reach a ratio of approximately 1 when the 635- and 532-nm emission values of *flaB*, a highly conserved *B. burgdorferi* gene, were used as calibration standards for each individual microarray. The degree of conservation was determined with DNA probes from B31 and one test strain (297, N40, IP90, or P/Gau) hybridized to each spot on the microarray and was calculated as a ratio of the 635- and 532-nm emission values. The values ranged from 0 to approximately 1.0. When the ratio obtained with two hybridized DNA probes was >0.8, 0.6 to 0.79, 0.4 to 0.59, 0.2 to 0.39, or <0.2, the gene was defined as highly conserved, conserved, less conserved, may exist, or may not exist, respectively. Three separate experiments demonstrated that the microarray data were highly reproducible.

Assessment of putative lipoprotein genes among *B. burgdorferi* genospecies. There are 19 lipoproteins that have homologues whose functions have been identified in other organisms (7). All of these genes were highly conserved in the American strains (Table 1). Sixteen of the genes were highly conserved in the European strain IP90. Although only 10 of the 19 genes were highly conserved in *B. afzelii* P/Gau, this strain shared all of these 19 genes. These genes were generally more conserved in the respective strains than were their counterparts that are unique to *B. burgdorferi*, regardless of whether they were located on the chromosome or on a plasmid of the spirochetes.

Of the 137 putative lipoprotein genes investigated in this study, 62 are located on the chromosome. Like B31, American strains 297 and N40 and European isolate IP90 had all 62 of these genes. These genes were highly conserved in the American strains except for two genes, *bb0424* and *bb0844*, in strain N40 (Tables 1 and 2). Most of the 62 genes were also highly conserved in European strain IP90. The second European strain, P/Gau, might lack 3 of the 62 genes (*bb0224*, *bb0321*, and *bb0424*). Interestingly, these were 3 of the 5 least conserved genes (*bb0224*, *bb0321*, *bb0424*, *bb0806*, and *bb0844*) in

TABLE 2. Conservation of putative chromosomal lipoprotein genes

Gene	Conservation in strain[a]:			
	B. burgdorferi		*B. garinii* IP90	*B. afzelii* P/Gau
	297	N40		
bb0003	HC	HC	HC	C
bb0028	HC	HC	HC	C
bb0038	HC	HC	HC	LC
bb0070	HC	HC	HC	HC
bb0071	HC	HC	HC	C
bb0142	HC	HC	HC	HC
bb0155	HC	HC	HC	C
bb0185	HC	HC	HC	HC
bb0213	HC	HC	C	LC
bb0224	HC	HC	LC	N
bb0227	HC	HC	HC	C
bb0298	HC	HC	HC	HC
bb0309	HC	HC	HC	HC
bb0321	HC	HC	LC	N
bb0324	HC	HC	C	C
bb0352	HC	HC	HC	LC
bb0365	HC	HC	C	C
bb0382	HC	HC	HC	C
bb0383	HC	HC	C	HC
bb0384	HC	HC	HC	C
bb0385	HC	HC	HC	HC
bb0398	HC	HC	C	M
bb0424	HC	C	M	N
bb0458	HC	HC	HC	C
bb0464	HC	HC	HC	HC
bb0475	HC	HC	C	C
bb0524	HC	HC	HC	C
bb0542	HC	HC	C	LC
bb0553	HC	HC	HC	HC
bb0606	HC	HC	C	HC
bb0628	HC	HC	HC	HC
bb0644	HC	HC	HC	HC
bb0664	HC	HC	HC	LC
bb0689	HC	HC	HC	C
bb0696	HC	HC	HC	HC
bb0740	HC	HC	HC	LC
bb0758	HC	HC	C	C
bb0760	HC	HC	HC	HC
bb0806	HC	HC	LC	LC
bb0813	HC	HC	C	C
bb0815	HC	HC	HC	LC
bb0823	HC	HC	HC	HC
bb0832	HC	HC	C	LC
bb0840	HC	HC	HC	LC
bb0844	HC	M	LC	M

[a] HC, highly conserved; C, conserved; LC, less conserved; M, may exist; N, may not exist.

TABLE 3. Conservation of putative plasmid lipoprotein genes

Gene	Conservation in strain[a]:				Gene	Conservation in strain[a]:			
	B. burgdorferi		*B. garinii* IP90	*B. afzelii* P/Gau		*B. burgdorferi*		*B. garinii* IP90	*B. afzelii* P/Gau
	297	N40				297	N40		
bba04	HC	HC	LC	M	*bbh32*	HC	N	N	N
bb05	HC	HC	C	M	*bbi16*	HC	HC	M	N
bb07	HC	HC	HC	M	*bbi28*	HC	HC	M	N
bb14	HC	HC	HC	LC	*bbi29*	HC	HC	LC	N
bb15	HC	HC	C	M	*bbi32*	HC	HC	LC	N
bb16	HC	HC	LC	N	*bbi34*	HC	HC	M	N
bb24	HC	HC	M	N	*bbi42*	HC	C	M	M
bb25	HC	HC	LC	N	*bbj01*	HC	HC	LC	M
bb33	HC	HC	LC	M	*bbj09*	M	C	HC	N
bb36	HC	HC	C	M	*bbj34*	LC	C	LC	N
bb57	HC	HC	C	M	*bbj36*	M	C	HC	N
bb59	HC	HC	LC	N	*bbj51*	M	C	M	N
bb60	HC	HC	LC	M	*bbk04*	HC	LC	C	C
bb62	HC	HC	LC	N	*bbk07*	HC	HC	M	N
bb64	HC	HC	LC	N	*bbk12*	HC	HC	M	N
bb65	HC	HC	LC	M	*bbk19*	HC	C	LC	N
bb66	HC	HC	LC	N	*bbk32*	HC	HC	HC	N
bb73	HC	HC	M	N	*bbk47*	M	HC	M	N
bbb08	HC	HC	C	M	*bbk49*	M	C	M	N
bbb09	HC	HC	HC	LC	*bbk53*	HC	LC	LC	M
bbb14	HC	HC	C	M	*bbl39*	C	C	C	N
bbb19	LC	LC	C	M	*bbl40*	HC	LC	M	N
bbb27	HC	HC	C	M	*bbm27*	LC	HC	M	N
bbc10	LC	N	N	N	*bbm38*	LC	LC	C	N
bbd10	HC	HC	HC	LC	*bbn38*	LC	C	LC	N
bbd24	HC	C	C	M	*bbn39*	HC	C	M	N
bbe06	HC	C	LC	N	*bbo39*	HC	HC	LC	M
bbe08	LC	HC	LC	N	*bbo40*	C	C	M	N
bbe09	HC	HC	LC	N	*bbp38*	HC	HC	HC	N
bbe28	M	HC	M	N	*bbq03*	HC	LC	C	C
bbe31	HC	HC	M	N	*bbq05*	C	M	M	N
bbf20	HC	N	HC	M	*bbq35*	HC	LC	M	N
bbf32	M	M	C	M	*bbq47*	M	LC	M	N
bbg-02	HC	HC	M	N	*bbr42*	HC	HC	LC	M
bbg25	LC	HC	HC	M	*bbs30*	HC	HC	LC	N
bbh01	HC	M	M	N	*bbs41*	LC	LC	C	M
bbh18	HC	N	M	N					

[a] HC, highly conserved; C, conserved; LC, less conserved; M, may exist; N, may not exist.

strain IP90. The fourth least conserved gene was *bb0844*, which also was the least conserved in strain N40 (Table 2).

Seventy-five putative lipoprotein genes, located on *B. burgdorferi* B31 plasmids, were also included in this study. Strain 297 had all of the genes, while N40 and IP90 lacked 4 (*bbc10*, *bbf20, bbh18*, and *bbh32*) and 2 (*bbc10* and *bbh32*) of these 75 genes, respectively (Tables 1 and 3). Most of these genes were highly conserved in strains 297 and N40 but less conserved in IP90. *B. afzelii* P/Gau shared approximately 30 of these 75 genes, and most of them were not conserved.

B. burgdorferi B31 contains 21 linear and circular plasmids, 19 of which carry at least one putative lipoprotein gene, including plasmids A (lp54), B (cp26), C (cp9), D (lp17), E (lp25), F (lp28-1), G (lp28-2), H (lp28-3), I (lp28-4), J (lp38), K (lp36), L (cp32-8), M (cp32-6), N (cp32-9), O (cp32-7), P (cp32-1), Q (lp56), R (cp32-4), and S (cp32-3) (4, 7). Linear plasmids T (lp5) and U (lp21) do not carry any suspected lipoprotein genes. The DNA microarrays contained gene fragments amplified from these 19 lipoprotein gene-carrying plasmids. Examination of *bbc10*, the only suspected lipoprotein gene on cp9, would suggest that N40 and IP90 may lack this plasmid (Table 3). Lack of conservation of this gene in strains N40 and IP90 or loss of the cp9 plasmid during in vitro cultivation might, however, also explain the low ratios of bound DNA probes of these two strains (3). In contrast, lp54 contains 19 lipoprotein genes. Therefore, it is more likely that the detection or absence of these genes in the DNA microarray would reflect the presence or lack of this plasmid. Palmer and colleagues studied the distribution of linear plasmids with a set of different markers and suggested that strains N40, IP90, and P/Gau lack lp56 (16). In *B. burgdorferi* B31, this plasmid contains 4 lipoprotein genes, *bbq03*, *bbq05*, *bbq35*, and *bbq47* (4, 7), all of which can be detected in both N40 and IP90 with our microarray (Table 3). Plasmid gene arrangements among spirochetes could potentially account for these differences. For example, *vlsE* is carried by lp28-1 in strain B31 (26) but may be located on a different size plasmid in other isolates (13, 24). When *vlsE* was used as the marker for lp28-1, our studies indicated that both IP90 and P/Gau contained this plasmid, in contrast to Palmer and colleagues' result that both isolates lack lp28-1 (16). Therefore, the complete sequencing of individual

plasmids is the only valid method of determining which lipoprotein genes are present on specific plasmids.

The *B. burgdorferi* B31 genome has 17 chromosomal lipoproteins with homologues whose functions have been defined in other organisms (7), and our data suggest that these genes were very conserved in the spirochetes tested. In addition, *bba15* (*ospA*, which facilitates tick attachment) (14, 15), *bbb19* (*ospC*) (2), and *bbf32* (*vslE*, involved in immune evasion) (26) may play important roles in the *B. burgdorferi* enzootic life cycle and are generally detectable in all of the genospecies. Plasmid loss might partially explain why the extrachromosomal genes were less conserved and few plasmid genes were detected in isolate P/Gau. However, these data on the chromosomal genes should not be affected by this phenomenon. In fact, our studies strongly indicated that most of the chromosomal lipoprotein genes were significantly less conserved in *B. afzelii* P/Gau than other strains, including the European isolate IP90. This may provide a reasonable explanation for the observations reported by other researchers that antigenic proteins are highly divergent among European isolates (9–11).

We have developed a DNA microarray to examine 137 lipoprotein genes of five *B. burgdorferi* isolates. Our results suggest that the DNA microarray can be used globally to examine the putative lipoprotein genes among *B. burgdorferi* isolates, including the three major *B. burgdorferi* sensu lato genospecies, *B. burgdorferi* sensu stricto, *B. garinii*, and *B. afzelii*. The *B. burgdorferi* lipoprotein gene microarray should prove useful in the genetic studies of geographic collections of *B. burgdorferi*, the examination of *B. burgdorferi* lipoprotein gene expression under different in vitro conditions, and the expression of *B. burgdorferi* lipoproteins throughout the *B. burgdorferi* life cycle in the arthropod vector and the mammalian host.

We thank Steven Norris (University of Texas, Houston) for providing clonal isolate B31 5A11.

This study was supported by grants from the National Institutes of Health and the American Heart Association. E.F. is the recipient of a Burroughs Wellcome Clinical Scientist Award in Translational Research.

REFERENCES

1. **Ackermann, R., P. Hörstrup, and R. Schmidt.** 1984. Tick-borne meningopolyneuritis (Garin-Bujadoux, Bannwarth). Yale J. Biol. Med. **57:**485–490.
2. **Baranton, G., G. Seinost, G. Theodore, D. Postic, and D. Dykhuizen.** 2001. Distinct levels of genetic diversity of *Borrelia burgdorferi* are associated with different aspects of pathogenicity. Res. Microbiol. **152:**149–156.
3. **Barbour, A. G.** 1988. Plasmid analysis of *Borrelia burgdorferi*, the Lyme disease spirochete. J. Clin. Microbiol. **26:**475–478.
4. **Casjens, S., N. Palmer, R. V. Vugt, W. M. Huang, B. Stevenson, P. Rosa, R. Lathigra, G. G. Sutton, J. Peterson, R. J. Dodson, D. Haft, E. Hickey, M. Gwinn, O. White, and C. M. Fraser.** 2000. A bacterial genome in flux: the twelve linear and nine circular extrachromosomal DNAs in an infectious isolate of the Lyme disease spirochete *Borrelia burgdorferi*. Mol. Microbiol. **35:**490–516.
5. **Fikrig, E., S. W. Barthold, W. Sun, W. Feng, S. R. Telford III, and R. A. Flavell.** 1997. *Borrelia burgdorferi* P35 and P37 proteins, expressed in vivo, elicit protective immunity. Immunity **6:**531–539.
6. **Fikrig, E., W. Feng, S. W. Barthold, S. R. Telford III, and R. A. Flavell.** 2000. Arthropod- and host-specific *Borrelia burgdorferi bbk32* expression and the inhibition of spirochete transmission. J. Immunol. **164:**5344–5351.
7. **Fraser, C. M., S. Casjens, W. M. Huang, G. G. Sutton, R. Clayton, R. Lathigra, O. White, K. A. Ketchum, R. Dodson, E. K. Hickery, M. Gwinn, B. Dougherty, J. F. Tomb, R. D. Fleischmann, D. Richardson, J. Peterson, A. R. Kerlavage, J. Quackenbush, S. Saizberg, M. Hanson, R. V. Vugt, N. Palmer, M. D. Adams, J. Gocayne, J. Weidman, T. Utterback, L. Watthey, L. McDonald, P. Artiach, C. Bowman, S. Garland, C. Fujii, M. D. Cotton, K. Horst, K. Roberts, B. Hatch, H. O. Smith, and J. C. Venter.** 1997. Genomic sequence of a Lyme disease spirochete, *Borrelia burgdorferi*. Nature **390:**580–586.
8. **Gao, B. P., S. J. Norris, L. C. Rosenberg, and M. Höök.** 1995. Adherence of *Borrelia burgdorferi* to the proteoglycan decorin. Infect. Immun. **63:**3467–3472.
9. **Hauser, U., G. Lehnert, R. Lobentanzer, and B. Wilske.** 1997. Interpretation criteria for standardized Western blots for three European species of *Borrelia burgdorferi* sensu lato. J. Clin. Microbiol. **35:**1433–1444.
10. **Hauser, U., G. Lehnert, and B. Wilske.** 1999. Validity of interpretation criteria for standardized Western blots (immunoblots) for serodiagnosis of Lyme borreliosis based on sera collected throughout Europe. J. Clin. Microbiol. **37:**2241–2247.
11. **Hauser, U., H. Krahl, H. Peters, V. Fingerle, and B. Wilske.** 1998. Impact of strain heterogeneity on Lyme disease serology in Europe: comparison of enzyme-linked immunosorbent assays using different species of *Borrelia burgdorferi* sensu lato. J. Clin. Microbiol. **36:**427–436.
12. **Hellwage, J., T. Meri, T. Heikkilä, A. Alitalo, J. Panelius, P. Lahdenne, I. J. T. Seppälä, and S. Meri.** 2001. The complement regulator factor H binds to the surface protein OspE of *Borrelia burgdorferi*. J. Biol. Chem. **276:**8427–8435.
13. **Kawabata, H., F. Myouga, Y. Inagaki, N. Murai, and H. Watanabe.** 1998. Genetic and immunological analyses of Vls (VMP-like sequences) of *Borrelia burgdorferi*. Microb. Pathog. **24:**155–166.
14. **Pal, U., A. M. de Silva, R. R. Montgomery, D. Fish, J. Anguita, J. F. Anderson, Y. Lobet, and E. Fikrig.** 2000. Attachment of *Borrelia burgdorferi* within *Ixodes scapularis* mediated by outer surface protein A. J. Clin. Investig. **106:**561–569.
15. **Pal, U., R. R. Montgomery, D. Lusitani, P. Voet, V. Wevnanys, S. E. Malawista, Y. Lobet, and E. Fikrig.** 2001. Inhibition of *Borrelia burgdorferi*-tick interaction in vivo by outer surface protein A antibody. J. Immunol. **166:**7398–7403.
16. **Palmer, N., C. Fraser, and S. Casjens.** 2000. Distribution of twelve linear extrachromosomal DNAs in natural isolates of Lyme disease spirochetes. J. Bacteriol. **182:**2476–2480.
17. **Postic, D., M. V. Assous, P. A. Grimont, and G. Baranton.** 1994. Diversity of *Borrelia burgdorferi* sensu lato evidenced by restriction fragment length polymorphism of rrf (5S)-rrl (23S) intergenic spacer amplicons. Int. J. Syst. Bacteriol. **44:**743–752.
18. **Probert, W. S., and B. J. B. Johnson.** 1998. Identification of a 47 kDa fibronectin-binding protein expressed by *Borrelia burgdorferi* B31. Mol. Microbiol. **30:**1003–1015.
19. **Probert, W. S., J. H. Kim, M. Höök, and B. J. B. Johnson.** 2001. Mapping the ligand region of *Borrelia burgdorferi* fibronectin-binding protein BBK32. Infect. Immun. **69:**4129–4133.
20. **Purser, J. E., and S. J. Norris.** 2000. Correlation between plasmid content and infectivity in *Borrelia burgdorferi*. Proc. Natl. Acad. Sci. USA **97:**13865–13870.
21. **Roberts, W. C., B. A. Mullikin, R. Lathigra, and M. S. Hanson.** 1998. Molecular analysis of sequence heterogeneity among genes encoding decorin binding protein A and B of *Borrelia burgdorferi* sensu lato. Infect. Immun. **66:**5275–5285.
22. **Steere, A. C., N. H. Bartenhagen, J. G. Craft, G. J. Newman, D. W. Rahn, L. H. Sigal, P. N. Spieler, K. S. Stenn, and S. E. Malawista.** 1983. The early clinical manifestations of Lyme disease. Ann. Intern. Med. **99:**76–82.
23. **Wang, G., A. van Dam, I. Schwartz, and J. Dankert.** 1999. Molecular typing of *Borrelia burgdorferi* sensu lato: taxonomic, epidemiological, and clinical implications. Clin. Microbiol. Rev. **12:**633–653.
24. **Wang, G., A. van Dam, and J. Dankert.** 2001. Analysis of a VMP-like sequence (*vls*) locus in *Borrelia garinii* and Vls homologues among four *Borrelia burgdorferi* sensu lato species. FEMS Microbiol. Lett. **15:**39–45.
25. **Weber, K., G. Schierz, B. Wilske, and V. Preac-Mursic.** 1984. European erythema migrans disease and related disorders. Yale J. Biol. Med. **57:**13–21.
26. **Zhang, J. R., J. M. Hardham, A. G. Barbour, and S. J. Norris.** 1997. Antigenic variation in Lyme disease Borreliae by promiscuous recombination of VMP-like sequence cassettes. Cell **89:**275–285.

Editor: D. L. Burns