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The *dbpBA* Locus of *Borrelia burgdorferi* Is Not Essential for Infection of Mice[▽]

Yanlin Shi, Qilong Xu, Sunita V. Seemanapalli, Kristy McShan, and Fang Ting Liang*

Department of Pathobiological Sciences, Louisiana State University, Baton Rouge, Louisiana 70803

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The Lyme disease spirochete *Borrelia burgdorferi* expresses a broad array of adhesive molecules, including the decorin-binding proteins A and B (DbpA and DbpB), which are believed to play important roles in mammalian infection. The *dbpBA* locus was deleted; resulting mutants were able to infect both immunodeficient and immunocompetent mice, indicating that neither DbpA nor DbpB is essential for the infection of mammals, although the DbpAB deficiency may significantly attenuate infectivity potential.

The Lyme disease spirochete *Borrelia burgdorferi* expresses a broad array of adhesive molecules (4), including the decorin-binding proteins A and B (DbpA and DbpB) (2, 11–13), the fibronectin-binding protein BBK32 (23, 24), Bgp (*Borrelia* glycosaminoglycan-binding protein) (20, 21), and P66 (3, 6). The lipoproteins DbpAB and BBK32 bind to components of the mammalian extracellular matrix (ECM) decorin and fibronectin, respectively (2, 11, 23, 24); the outer membrane proteins P66 and Bgp bind to either integrin $\alpha_{IIb}\beta_3$ (3, 6) or other ECM components, glycosaminoglycans (20, 21). A recent study showed that BBK32 also interacts with glycosaminoglycans (9). The interactions of these proteins with host ligands have been proposed to play crucial roles in dissemination, tissue colonization, and/or persistence during *B. burgdorferi* infection of mammalian hosts.

DbpA and DbpB were among the first identified adhesive molecules of *B. burgdorferi* (11); DbpA is probably the best-characterized borrelial adhesin (2, 22). The two lipoproteins are encoded within a two-gene operon (12) which is located on the plasmid lp54 (10). DbpA and DbpB are not expressed by *B. burgdorferi* in the tick vector (13) but are up-regulated during mammalian infection (5, 17, 18), suggesting a potential role of the lipoproteins in the infection of mammals. However, it is unknown if these two lipoproteins are required for the infection of mammalian hosts, which is the focus of the current study.

Generation of *dbpAB* mutants. To delete the *dbpBA* locus, a disruption plasmid was first constructed. A 2,047-bp fragment covering a partial sequence of the open reading frame (ORF) BBA20, the entire ORFs BBA21, BBA22, and BBA23, and a partial sequence of *dbpA* (BBA24) was amplified by use of primers P1F and P1R (Fig. 1A; Table 1). A second, 2,145-bp fragment, including a partial sequence of *dbpB* (BBA25), the entire ORFs BBA26, BBA27, BBA28, and BBA29, and a partial sequence of the ORF BBA31, was amplified using primers P2F and P2R. The two PCR products were pooled, purified using a QIAquick PCR purification kit (QIAGEN Inc., Valencia, CA), digested with NheI, repurified, and ligated. The resultant product was used as a template and amplified by nested PCR using primers P3F and P3R. The PCR product was purified, digested with Acc65I, and cloned into the TA vector pNCO1T as described previously (7) to generate pNCO1T::*dbpA′B′*, which cannot replicate in the borrelial system. A gentamicin cassette (*aacC1*) was amplified by use of primers P5F and P5R (Fig. 1B; Table 1) from the shuttle vector pBSV2G (a gift from P. Rosa and P. Stewart), which confers gentamicin resistance both in *Escherichia coli* and in *B. burgdorferi* (8). The amplicon was purified, digested with XbaI, and cloned into pNCO1T::*dbpA′B′* to complete the construction of the disruption plasmid pNCO1T::*dbpA′B′*::*aacC1*. The insert within the plasmid was sequenced to ensure it was as designed.

The *B. burgdorferi* B31 BBE02 disruptant 5A18NP1 (a gift from H. Kawabata and S. Norris) was grown in 50 ml of Barbour-Stoenner-Kelly H (BSK-H) complete medium (Sigma Chemical Co., St. Louis, MO) at densities of 5×10^7 to 1×10^8 cells/ml (mid- to late exponential phase), harvested, washed, and transformed with 8.0 μg of the disruption plasmid DNA under standard electroporation conditions (26, 28). 5A18NP1 was utilized for its high transformability resulting from the disruption of the gene BBE02, a putative restriction-modification gene (14). The cells were allowed to recover in 20 ml of BSK-H complete medium at 33°C for 18 h. After a gentamicin concentration of 50 μg/ml was added, the suspension was transferred into 96 PCR tubes (200 μl/tube). Aliquots were incubated at 33°C for 10 days; live spirochetes were examined under a dark-field microscope and found in 10 of the 96 tubes. Approximately 30 μl of gentamicin resistance culture was transferred to 1.4 ml of BSK-H medium in a 1.5-ml microcentrifuge tube and grown to near-stationary phase at 33°C. Spirochetes were harvested from 500 μl of culture by centrifugation at 13,000 × *g* for 10 min at room temperature, washed twice with excess volumes of phosphate-buffered saline (pH = 7.3) to remove the residual disruption plasmid DNA, and resuspended in 500 μl of deionized H_2O. One microliter of suspension was used as a DNA source for the examination of the *aacC1* cassette by PCR using primers P5F and P5R (Fig. 1B; Table 1). The cassette was found in 7 of the 10 clones. Spontaneous mutations most likely contributed to the three clones lacking the *aacC1* cassette but developing gentamicin

* Corresponding author. Mailing address: Department of Pathobiological Sciences, Louisiana State University, Skip Bertman Drive at River Road, Baton Rouge, LA 70803. Phone: (225) 578-9699. Fax: (225) 578-9701. E-mail: fliang@vetmed.lsu.edu.

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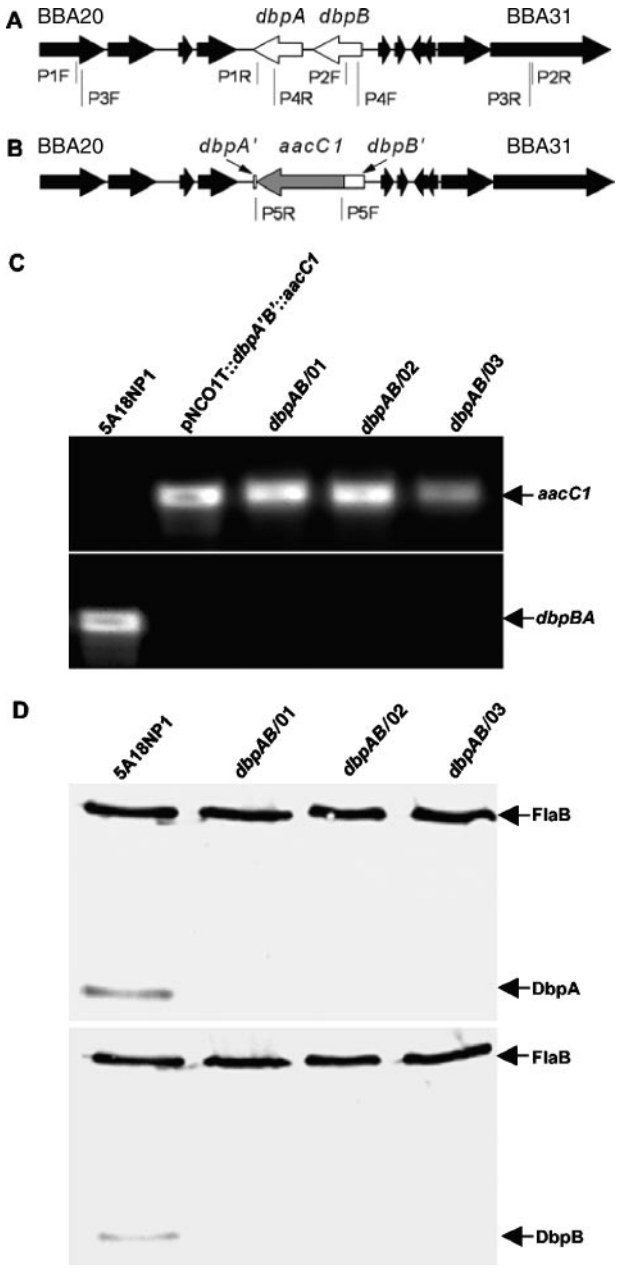

FIG. 1. Generation of *dbpAB* mutants. (A) Diagram of the *dbpBA* locus and adjacent ORFs. The two-gene operon is located within lp54, which carries 76 ORFs, BBA01 to BBA76. The two open arrows represent *dbpA* and *dbpB*; the filled arrows denote BBA20 to BBA23 and BBA26 to BBA31. The binding sites of eight primers, i.e., P1F to P4F and P1R to P4R, are also indicated. (B) Diagram of the disrupted *dbpBA* locus, showing major portions of the *dbpA* and *dbpB* genes replaced with a gentamicin cassette (*aacC1*). The gray arrow represents the *aacC1* cassette flanked by small residual sequences (*dbpA′* and *dbpB′*) of the genes *dbpA* and *dbpB* (open bars). The binding sites of primers P5F and P5R are also indicated. (C) PCR analysis of *dbpAB* mutants. 5A18NP1 spirochetes, the disruption plasmid pNCO1T::*dbpA′B′*::*aacC1*, and three *dbpAB* mutants were used as DNA sources and subjected to PCR amplification using primers P5F and P5R (top) or primers P4F and P4R (bottom). (D) Immunoblot analysis of *dbpAB* mutants. 5A18NP1 and three *dbpAB* mutants were analyzed by immunoblotting probed with a mixture of FlaB MAb and mouse antisera raised against recombinant DbpA (top) or DbpB (bottom).

TABLE 1. Primers used in the study

Primer name	Primer sequence (5′ to 3′)[a]
P1F	AAATGCCCTTGTATGTAGCAATTACA
P1R	CAGCTAGCAAAAATAACTAATAAACAACAT
P2F	TTGCTAGCTTCTCTTAAGGCTAGTCCA
P2R	AAGCTTAAGACCTGCTATCTTACAA
P3F	AAGGTACCAATAACAGCAGAGAAAT
P3R	TAGGTACCTGCTATCTTACAAAAAGACT
P4F	CGCATGTAGTATTGGATTAG
P4R	CATTGCTGAAAATTCACCAC
P5F	TATCTAGAGCTTCAAGGAAGATTTCC
P5R	AAATCTAGACGCTCAGTGGAACGAA

[a] P1R and P2F, P3F and P3R, and P5F and P5R contain restriction enzyme sites NheI, Acc65I, and XbaI (underlined), respectively.

resistance; these clones were discarded. The presence of lp28-1, the plasmid essential for infection in immunocompetent hosts (15, 25), was examined by PCR as described previously (29); three of the clones maintaining lp28-1 were chosen for the study and were designated *dbpAB*/01, *dbpAB*/02, and *dbpAB*/03.

The plasmid contents of the three selected clones were surveyed by PCR and further confirmed by microarray hybridization as described previously (29) and are presented in Table 2. The insertion of the *aacC1* cassette was reconfirmed by PCR using primers P5F and P5R (Fig. 1C, top); the deletion of the *dbpA* and *dbpB* genes was shown by PCR using primers P4F and P4R (Fig. 1C, bottom). The lack of DbpA and DbpB expression was confirmed by immunoblotting probed with a mixture of FlaB monoclonal antibody (MAb) and antisera raised against either recombinant DbpA or DbpB (Fig. 1D). The FlaB MAb was developed by Barbour et al. (1); the DbpA and DbpB antisera were produced by immunizing mice with recombinant DbpA or DbpB emulsified with Freund's complete (first injection) or incomplete (remaining injections) adjuvant.

The *dbpBA* locus is not essential for infection of either SCID or wild-type mice. Mice with severe combined immunodeficiency (SCID) were first used to examine whether the *dbpBA* locus is required for infection. For each of the three *dbpAB* mutants, two SCID mice on a BALB/c background (provided by the Division of Laboratory Animal Medicine at Louisiana State University, Baton Rouge, LA) were inoculated via one single intradermal/subcutaneous injection of 10^5 organisms. An additional five mice were challenged with the parental

TABLE 2. The *dbpBA* locus is not required for infection of immunodeficient mice[a]

Inoculum	Plasmids missing	No. of cultures positive/total no. of specimens examined			
		Heart	Joint	Skin	All sites
5A18NP1	lp28-4, lp56, lp5	5/5	5/5	5/5	15/15
dbpAB/01	cp9, lp28-4, lp56, lp5	2/2	2/2	2/2	6/6
dbpAB/02	cp9, lp28-4, lp56, lp5	2/2	2/2	2/2	6/6
dbpAB/03	cp9, lp28-4, lp56, lp21, lp5	2/2	2/2	2/2	6/6

[a] Each of the three *dbpAB* clones was inoculated into two SCID mice, and the parental clone 5A18NP1 was inoculated into five mice as a control. All animals were sacrificed 1 month after inoculation. Heart, tibiotarsal joint, and skin specimens were harvested and cultured for spirochetes in BSK-H complete medium.

TABLE 3. The *dbpBA* locus is not required for infection of immunocompetent mice[a]

Expt no.	Inoculum	No. of cultures positive/total no. of specimens examined				No. of mice infected/total no. of mice inoculated
		Heart	Joint	Skin	All sites	
I	5A18NP1	5/5	5/5	5/5	15/15	5/5
	dbpAB/01	1/2	1/2	2/2	4/6	2/2
	dbpAB/02	1/3	3/3	3/3	7/9	3/3
	dbpAB/03	0/1	1/1	1/1	2/3	1/1
II	5A18NP1	5/5	5/5	5/5	15/15	5/5
	dbpAB/01	0/3	2/3	3/3	5/9	3/3
	dbpAB/02	1/3	2/3	2/3	5/9	2/3
	dbpAB/03	0/4	2/4	3/4	5/12	3/4

[a] Each of the three *dbpAB* clones was inoculated into one to three (in experiment I) or three or four (in experiment II) BALB/c mice, and 5A18NP1 was used as a control to infect five mice in each experiment. All animals were sacrificed 1 month after inoculation. Heart, tibiotarsal joint, and skin specimens (not from the inoculation site) were harvested and cultured for spirochetes in BSK-H complete medium.

clone 5A18NP1 as a control. All mice were euthanized 1 month after inoculation; heart, tibiotarsal joint, and skin specimens (not from inoculation sites) were aseptically collected for spirochete culture as previously described (29). Spirochetes were recovered successfully from each of the heart, joint, and skin specimens from all of the 11 mice, regardless of whether they received the parental clone or mutants (Table 2), indicating that neither DbpA nor DbpB is required for the infection of immunodeficient mice.

Next, six BALB/c mice (provided by the Division of Laboratory Animal Medicine at Louisiana State University, Baton Rouge, LA) were challenged with the three mutants via one single intradermal/subcutaneous injection of 10^5 organisms; an additional five mice received 5A18NP1 as a control. In a separate experiment, 10 animals were inoculated with the three mutants; an additional five mice were given the parental clone. All mice were euthanized 1 month later; 5A18NP1 spirochetes were recovered from each of the heart, joint, and skin specimens of all 10 mice from the two experiments (Table 3). Fourteen of the 16 mice inoculated with *dbpAB* mutants had at least one positive specimen, indicating that neither DbpA nor DbpB is required for the infection of immunocompetent mice. The mutants were not recovered from two inoculated mice probably because of attenuated infectivity resulting from the disruption of the *dbpBA* locus. Alternatively, genetic manipulating processes might have introduced unnoted defects, which, in turn, could be responsible for the negative culture results in 13 heart, 5 joint, and 2 skin specimens; a complementation study, which we have been unable to accomplish, may help clarify the issue. Another unaddressed issue is whether the DbpAB deficiency affects infectivity reflected by the 50% infective dose (ID_{50}). In the current study, up to 10^5 *dbpAB* bacteria, a dose that is 1,000-fold higher than the ID_{50} value of the parental clone 5A18NP1 (14), were inoculated into a mouse. Nevertheless, our study clearly shows that the *dbpBA* locus is not essential for the infection of either immunodeficient or immunocompetent mice, although the DbpAB deficiency may severely attenuate infectivity potential.

B. burgdorferi expresses multiple adhesins that potentially mediate interactions of spirochetes with ECM components and other host ligands, including DbpA, DbpB, BBK32, Bgp, and P66. Seshu et al. showed that a BBK32 deficiency increases the ID_{50} value in immunocompetent mice (27), consistent with a subsequent study by Li et al. indicating that the BBK32 gene is essential for the life cycle of *B. burgdorferi* neither in the tick vector nor in the murine host (16). Parveen et al. reported that Bgp is not required for the infection of immunodeficient mice, although it remains to be examined whether the adhesin is essential for the infection of immunocompetent mice (19). The current study shows that neither DbpA nor DbpB is required for the infection of immunodeficient or immunocompetent mice. The data presented in conjunction with studies by others (16, 19, 27) firmly demonstrate that the lack of one or two individual adhesins does not completely diminish the ability of *B. burgdorferi* to infect mammals, although these adhesive molecules as a whole may play an essential role in dissemination, tissue colonization, and/or persistence during mammalian infection.

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