A Novel Laminin-Binding Protein Mediates Microbial-Endothelial Cell Interactions and Facilitates Dissemination of Lyme Disease Pathogens

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Borrelia burgdorferi conserved gene products BB0406 and BB0405, members of a common B. burgdorferi paralogous gene family, share 59% similarity. Although both gene products can function as potential porins, only BB0405 is essential for infection. Here we show that, despite sequence homology and coexpression from the same operon, both proteins differ in their membrane localization attributes, antibody accessibility, and immunogenicity in mice. BB0406 is required for spirochete survival in mammalian hosts, particularly for the disseminated infection in distant organs. We identified that BB0406 interacts with laminin, one of the major constituents of the vascular basement membrane, and facilitates spirochete transmigration across host endothelial cell barriers. A better understanding of how B. burgdorferi transmigrates through dermal and tissue vascular barriers and establishes disseminated infections will contribute to the development of novel therapeutics to combat early infections.

Keywords. Borrelia burgdorferi; BB0406; pathogen dissemination; laminin.

Lyme disease is a common vector-borne disease in North America and Europe that is inflicted by a group of atypical bacterial pathogens, Borrelia burgdorferi sensu lato [1–3]. The microbe is maintained in a complex enzootic infection cycle that involves Ixodes ticks and a vertebrate reservoir host. During blood meal engorgement, a tick infected with B. burgdorferi can transmit the pathogen to the host dermis. The spirochetes multiply at the tick-bite site and subsequently migrate to distant organs. After the initial infection of the dermal inoculation site, it remains unknown precisely how a fraction of spirochetes can invade the vasculature and then extravasate from the vessel to colonize distant organs.

The colonization of the host organs in humans, like the joints, heart, and central nervous system, can result in an array of serious clinical manifestations, such as Lyme arthritis, carditis, and neuroborreliosis [4]. Antibiotic treatment is usually effective, although a subset of antibiotic-treated hosts can later experience a variable set of clinical symptoms, known as posttreatment Lyme disease syndrome [5, 6]. When infested via B. burgdorferi–infected ticks or inoculated via syringe, many laboratory animals [7], including C3H mice [8, 9] are susceptible to B. burgdorferi infection, which may mimic many aspects of clinical Lyme disease [2]. Currently, a vaccine to prevent Lyme disease in humans is unavailable. Therefore, a better understanding of the infection process of B. burgdorferi, especially how the bacteria evade immune responses at the dermal inoculation site and disseminate through host vasculature, is fundamental to the development of new intervention strategies against the infection.

Hematogenous dissemination of a blood-borne pathogen is a critical initial process that remains poorly understood. The dissemination of spirochetes in the mammalian host requires temporal regulation of several virulence determinants, particularly surface proteins and adhesins, which can interact with host ligands like extracellular matrix (ECM) molecules, aiding in B. burgdorferi–vascular system interactions, transmigration, and tissue colonization [10]. For example, B. burgdorferi BBK32 interacts with host fibronectin and glycosaminoglycan molecules, facilitating borreliadhesion to vasculature via stabilization of the bacterial-endothelial interaction [11–14]. Similarly, an outer membrane (OM) surface protein, P66, has
both porin and adhesin functions [15, 16], particularly as an integrin-binding protein, and has demonstrated its ability to promote spirochete extravasation [17]. Further studies are required to identify *B. burgdorferi* OM proteins that promote vascular interactions and transmigration, which will help in our understanding of spirochete dissemination events, pathogenesis, and the development of preventive strategies.

The *B. burgdorferi* bb0406 gene product is cotranscribed with 2 immediately upstream genes that encode bb0404 and bb0405 [18, 19]. The latter gene has recently been characterized as a transmembrane protein that facilitates spirochete infection in mammals [18]. BB0406 and BB0405 share 59% similarity, are grouped into the same paralogous gene family, and are highly conserved in Lyme disease pathogens. Both BB0405 and BB0406 are capable of forming pores in large unilamellar vesicles, suggesting their potential functions as porins [19]. In the current article, we report that BB0406 is a laminin-binding protein, which facilitates spirochete transmigration through the host endothelial barrier, thereby aiding the spirochete dissemination and survival in various distant organs in mammals.

**MATERIALS AND METHODS**

**Bacteria, Mice, and Ticks**

The *B. burgdorferi* isolates B31-A3, 297, and N40, and the *Borrelia garinii* isolate PBi, were grown in Barbour-Stoenner-Kelly-H medium [18]. C3H/HeN mice were purchased from the National Institutes of Health and Charles River Laboratories. Animal experiments were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee and Institutional Biosafety Committee. The ticks used were originated from a colony that is maintained in the laboratory.

**Polymerase Chain Reaction**

The primers sequences are listed in Supplementary Table 1. For gene expression analysis, the C3H mice (3 animals per group) were infected with an intradermal inoculum of *B. burgdorferi* (10⁵ cells per mouse), and 14 days after infection, tissues were collected. Infected mice were parasitized by larval ticks (25 ticks per mouse) that were collected after repletion. After molting, nymphs were allowed to engorge on mice (25 ticks per animal) and were collected at 24, 48, and 96 hours after attachment. Total RNA was isolated from tissues using TRIzol reagent (Invitrogen), reverse-transcribed to complementary DNA (cDNA), and treated with DNase (NEB). The relative abundances of the bb0406 transcripts, normalized against flaB copies, were analyzed by means of quantitative polymerase chain reaction (qPCR) using cDNA samples, as detailed elsewhere [18]. The spirochete burdens in the tissues were assessed by measuring copies of the flaB transcripts through qPCR, using cDNA samples, and by normalizing against the corresponding β-actin levels [18].

**Generation of Recombinant BB0406, Antiserum, and Immunoblotting**

The bb0406 gene was cloned into pET28a using specific primers (Supplementary Table 1), and the recombinant protein was produced in *Escherichia coli*, as described elsewhere [18]. BB0406 antisera were generated in mice, and the titer and specificity were assessed using enzyme-linked immunosorbent assay (ELISA) and Western blotting analysis, respectively, as described elsewhere [18].

**Generation of bb0406 Deletion Mutants**

The bb0406 mutants were generated via homologous recombination by replacing bb0406 open reading frame with a kanamycin resistance cassette, as detailed elsewhere [20]. The upstream and downstream DNA fragments of bb0406 were cloned into plasmid pXL10601 and electroporated into *B. burgdorferi*. Transformants were selected with kanamycin (350 μg/mL) and analyzed for the desired integration of the kanamycin resistance cassette, loss of bb0406 transcripts, and absence of polar effects on the transcription of the surrounding genes. Two bb0406 deletion clones (designated as Mut1 and Mut2), retaining identical plasmids as wild-type isolates (except for nonessential plasmid lp 5), were used for further experiments. For in vitro growth analysis, spirochetes were diluted to a density of 10⁴ cells/mL, grown to the stationary phase (approximately 10⁵ cells/mL), and counted by means of dark-field microscopy using a Petroff-Haussler cell counter.

**Phenotypic Analysis of Genetically Modified bb0406 Isolates**

To examine the phenotypes of bb0406 mutants, groups of 3 mice were infected with either wild-type isolates or either of the mutant clones (10⁵ spirochetes per animal) by intradermal inoculation. Tissue samples were collected at 7, 14, and 21 days after infection. Pathogen burdens in murine tissues were evaluated using qPCR. Portions of skin and spleen were cultured in Barbour-Stoenner-Kelly medium to recover viable spirochetes. For studies addressing the acquisition of pathogens in ticks, mice were infected with borrelial isolates and, after 12 days, infected with naïve nymphs (25 ticks per group). Ticks were collected after repletion, and pathogen burdens were assessed using qPCR. For transmission studies, naturally infected nymphs or nymphs microinjected with *B. burgdorferi* were fed on naïve mice (5 ticks per mouse, 3 mice per group). Engorged ticks were subjected to qPCR analyses of their spirochete levels. Mice were euthanized 14 days after tick feeding, and tissues were assessed for spirochete burdens by qPCR and culture analysis.

For the intravenous injection study, groups of mice (3 animals per group) were intravenously infected with borrelial isolates (10⁵ spirochetes per mouse). The pathogen burdens in the bloodstream and tissues were determined on day 14 of infection, using qPCR. For the hyperinfection study, separate groups of mice (3 animals per group) were infected intradermally with wild-type *B.burgdorferi* (10⁵ spirochetes per mouse) or Mut1 or Mut2 clones (10⁵ spirochetes per mouse). The spirochete burdens were assessed by means of qPCR at the injection site and in murine tissues on day 14 of infection.
Motility and Bactericidal Assays
Spirochete motility was evaluated by swimming plate assays, as described elsewhere [21], using a nonmotile flaB mutant [22] as controls. BB0406 antibodies were tested for bactericidal activities against B. burgdorferi using a regrowth assay, as described elsewhere [23].

Transformed Human Brain Microvascular Endothelial Cell and Electric Cell-Substrate Impedance Sensing Assays
To test the effects of B. burgdorferi on the integrity of vascular barriers, we used an in vitro model of the human blood-brain barrier [24–26] based on transformed human brain microvascular endothelial cell (THBMEC) monolayers and electric cell-substrate impedance sensing (ECIS; Applied Biophysics). About 10⁶ THBMECs were grown in M199 medium, as detailed elsewhere [25], seeded in gold microelectrode arrays (8W10E+; Applied Biophysics), and exposed to 1–2 × 10⁸ wild-type B. burgdorferi or mutants at a multiplicity of infection of 1:5–10. The resistance changes of THBMEC monolayers were monitored for 24 hours and normalized to control M199 medium–treated cells.

Transwell Assay
The assay used an in vitro model of the human blood-brain barrier represented by THBMECs grown on Transwell plates (Costar) with permeable membrane inserts, as described elsewhere [24]. After 24–36 hours of THBMEC growth, when the cellular resistance reached maximal steady-state values, 5–10 × 10⁵ spirochete cells were added to the inserts at a multiplicity of infection of 5–10 and incubated for 24–48 hours. Spirochetes that crossed the THBMEC monolayers were collected from the bottom chambers, centrifuged, and used for qPCR to quantify transmigrated B. burgdorferi as a function of the total input.

Assays for B. burgdorferi–Extracellular Matrix Interaction
The binding of B. burgdorferi proteins to various ECM molecules was analyzed using assays detailed elsewhere, such as ELISAs [27] and surface plasmon resonance [28]. Binding of B. burgdorferi cells to laminin was determined using methods reported elsewhere [27, 29].

Statistical Analysis
Data are presented as means and standard errors of the means of ≥2 independent experiments. Significant differences between samples were determined using Student 2-tailed t tests or 1-way analysis of variance, using GraphPad Prism 5.01 software, following logarithmic transformation of the data. Differences were considered significant at P < .05.

RESULTS

bb0406 Encodes a Weakly Immunogenic Antigen Predominantly Expressed During Early Murine Infection
The bb0406 mRNA is detectable in several borrelial isolates and strains in culture (Figure 1A, upper panel), although the protein remains nearly undetectable (Figure 1A, lower panel). Expression of bb0406 is induced at 37°C in cultured cells (Figure 1B, left panel) and in ticks during spirochete transmission, but the transcripts are also detectable in various murine tissues (Figure 1B, right panel), with the most dramatic expression during early infection (Figure 1C). A low but noticeable antibody response against BB0406 is detectable in infected mice (Figure 1D), suggesting a poor abundance, or a weak immunogenicity and subsurface nature, of the antigen [30].

BB0406 Supports B. burgdorferi Dissemination and Survival in Mammalian Hosts
To study the role of BB0406 in B. burgdorferi virulence, we generated bb0406-deficient B. burgdorferi, defined by a loss in expression of the target gene, but with no polar effects on the surrounding genes, including bb0405 and bb0407 (Figures 2A–C and Supplementary Figure 1). Despite repeated attempts, we were unable to complement the gene; therefore, for all subsequent studies, we used 2 independent clones of isogenic bb0406 mutants (Supplementary Figure 2). The bb0406 mutants did not reflect apparent growth defects (Supplementary Figure 1D).

Next, mice were infected with equal numbers (10⁶ cells per animal) of wild-type or bb0406 mutant isolates (2 independent clones, Mut1 or Mut2), via needle inoculation. Similar to wild-type cells, the bb0406 mutants survived at the skin inoculation site until day 7, but their levels decreased at day 14, both at the skin inoculation site (Figure 2A, upper panel) and at distant skin or other organs (Figure 2A, lower panel). We noted that the wild-type and bb0406 mutants induced comparable antibody responses, further supporting mutant survival during early infection (Supplementary Figure 3A). In agreement with a previous study [19], we found that anti-BB0406 antibodies have bactericidal activities (Supplementary Figure 3B). The detection of low levels of bb0406 mutants in distant tissues could be due to their impaired ability to disseminate through blood (Figure 2B). Consequently, the survival defect of the bb0406 mutants in mice also affected their entry or acquisition in ticks (Figure 2C).

Similar to needle-borne infections (Figure 2A), bb0406 mutants were also unable to establish tick-transmitted infections, although ticks that were artificially infected with equal numbers of either the wild type or bb0406 mutant displayed similar spirochete levels (Supplementary Figure 4A). However, despite a robust antibody response comparable to that in mice infected with wild-type spirochetes (Supplementary Figure 4B), a significantly lower level of bb0406 mutants was detected in mice after 14 days of tick engorgement (Figure 2D). Histological analyses of the tick-bite sites show no obvious differences in the migration of immune cells, suggesting that the function of BB0406 is unlikely to be related to evasion of the host’s innate immunity (Supplementary Figure 5). Consistent with low pathogen levels, none of the organs retrieved from mice infected with bb0406 mutants were positive for B. burgdorferi, as measured by cultures using skin and spleen tissues (data not shown). Collectively, these results indicate that BB0406 is important for
the establishment of spirochete infections in mice, including their hematogenous dissemination.

**BB0406 Assists Spirochete Dissemination Through Murine Vasculature**

Because previous studies indicated that a greater mutant inoculum could rescue the phenotypic defects in tissue survival [31], we performed an infection experiment with a higher level of inoculum with bb0406 mutants. Both mutant clones were severely impaired in their abilities to establish infection in disseminated tissues when mice were intradermally injected a 1000-times-higher dose of the mutant clones (10^8 cells per animal), compared with the wild type (10^5 cells per animal) (Figure 3A and B). We next assessed whether the tissue dissemination defect of bb0406 mutants could be restored when they are directly introduced in the blood. Equal levels of mutants or wild-type isolates were introduced into murine blood vessels by intravenous injection. Although similar spirochete levels were detected in the blood (Figure 3C), the burden of bb0406 mutants in distant murine organs was significantly lower, compared with the wild type, indicating that BB0406 might assist in pathogen dissemination from blood to tissues (Figure 3D). These results strongly suggest that BB0406 is not needed for spirochete survival in the blood but that it may facilitate pathogen transmigration from the blood vessels. The deficiency in the migration of bb0406 mutants is not due to spirochete motility or chemotaxis defects, as suggested by results of a swim plate assay (Supplementary Figure 6).
BB0406 supports *Borrelia burgdorferi* dissemination and persistence in mammalian hosts. A, Spirochete burden at various murine tissues. Mice (3 animals per group) were inoculated intradermally with equal numbers (10^5 spirochetes per mouse) of *B. burgdorferi* B31 wild-type (WT) cells (white bar) or either of 2 isogenic independent clones of bb0406 mutants (Mut1 or Mut2) (black bar). Upper panel, spirochete burdens at the dermal injection sites were assessed on day 7 (left), which indicated a similar level in all groups (*P* > .05), while on day 14, the pathogen burden in mutants was significantly lower compared with the WT (right). Lower panel, Decreased levels of mutant isolates in distant skin and other organs 14 days after infection. *P* < .05. B, Spirochete burden in blood. The levels of WT or mutant isolates were analyzed in the blood on day 8 after infection using quantitative polymerase chain reaction (qPCR). *P* < .05. C, Spirochete acquisition in ticks. Mice (3 animals per group) were injected with WT or mutant isolates (10^7 spirochetes per mouse) for 12 days and then allowed to be parasitized by naive ticks (25 ticks per group). The *B. burgdorferi* burdens in fed ticks were analyzed by measuring copies of flaB transcripts and normalizing to tick β-actin using qPCR. The levels of bb0406 mutants were significantly decreased in ticks compared with the WT. *P* < .05. D, bb0406 is required for transmission of spirochetes from ticks. Nymphal ticks were microinjected with either WT or bb0406 mutants (Mut1 and Mut2) and then allowed to engorge on naive mice (5 ticks per mouse and 3 animals per group). At 14 days after tick feeding, the spirochete burdens in murine tissues were assessed via qPCR by measuring copies of flaB transcripts and normalizing against mouse β-actin. The spirochete burdens in mice infected with Mut1 and Mut2 (black bar) were significantly lower than in mice infected with WT (white bar). *P* < .05.

Deletion of bb0406 Impairs *B. burgdorferi* Dissemination Through Human Endothelial Cell Layers

We next used an in vitro model to directly test the hypothesis of whether BB0406 facilitates *B. burgdorferi* transmigration through endothelial barriers. We examined the spirochete transmigration through an endothelial barrier consisting of immortalized THBMECs, which share characteristics of primary microvascular endothelial cells. The results indicate that both bb0406 mutant clones induced markedly different THBMEC resistance, compared with wild-type *B. burgdorferi* (Figure 4A), and are also impaired in their abilities to migrate through the endothelial barrier (Figure 4B), further suggesting that BB0406 facilitates pathogen dissemination through host vasculature.

BB0406 Is a Laminin-Binding Protein

We next examined the possibility that BB0406 may interact with ECM proteins, particularly those that are associated with the host vasculature. To explore this, we tested recombinant BB0406 and another control membrane protein for their abilities to bind fibronectin, type I collagen, type IV collagen, elastin, laminin, heparan sulfate, chondroitin 4-sulfate, dermatan sulfate, and chondroitin 6-sulfate (and bovine serum albumin, as a control), using qualitative ELISA. The results indicate that BB0406 predominantly interacts with laminin and heparan sulfate (Figure 5A), although an unsaturated binding with the latter ligand was recorded (Figure 5B). The binding affinity between BB0406 and laminin was evaluated using quantitative ELISA (Figure 5B) and further confirmed with an independent
surface plasmon resonance assay (Figure 5C). We next examined whether the BB0406 deficiency results in the impairment of the spirochete binding to laminin. To explore this, the wild-type or bb0406 mutant clones were separately incubated with laminin-coated microtiter wells (or bovine serum albumin, as a control), and bound cells were measured. Significantly decreased bindings of both clones (Mut1 and Mut2) to laminin were recorded (Figure 5D).

DISCUSSION

The B. burgdorferi OM protein BB0406 is cotranscribed with another OM protein, BB0405, with 59% similarity [18, 19]. Although both proteins are capable of forming a membrane pore [19], only BB0405 was shown to support spirochete infection in mammals [18, 19]. In contrast to the prevailing notion that BB0406 has a redundant role in infection [19], we show that the protein facilitates spirochete survival in host tissues, possibly assisting in hematogenous dissemination of the pathogen via interaction with laminin, which is the major functional component of the basement membrane surrounding blood vasculature [32]. Because previous studies [19] and our current data show that the anti-BB0406 antibodies also have bactericidal properties, interference with the function of BB0406 or the disruption of its interaction with laminin may lead to novel strategies to combat B. burgdorferi infection.

Our data suggesting that BB0406 is expressed at low levels in vitro is supported by findings of previous mass spectrometry studies [33], which show that the protein remained undetectable in the OM of B. burgdorferi. Therefore, the bactericidal activity of anti-BB0406 antibodies against cultured spirochetes [19] is puzzling and may occur via the steric hindrance of antibodies, which may bind to off-target yet homologous proteins, such as BB0405. Notably, despite being a subsurface protein [30], BB0406 induces a detectable antibody response during murine infection (Figure 1D), whereas the highly antigenic and surface-exposed protein BB0405 failed to generate such responses. This

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**Figure 3.** Hyperinfection or intravenous injection does not rescue infectivity of bb0406 mutants. A, Spirochete burdens at the dermal inoculation sites after a high infection dose. Mice (3 animals per group) were infected either with wild-type (WT) (10⁶ spirochetes per mouse) or a 1000-fold higher inoculum dose of the mutant clones (10⁸ spirochetes per mouse). After 14 days of infection, the levels of mutant isolates were measured by quantitative polymerase chain reaction (qPCR), which indicated a significantly lower level compared with the WT. *P < .05. B, bb0406 mutants fail to persist in mice even after a higher level of inoculum. Mice were injected with the Mut1 or Mut2 isolate (10⁸ spirochetes per animal), as detailed in A, and pathogen levels in distant murine tissues were determined by qPCR after 14 days of infection. Levels of the bb0406 mutants were significantly lower in all mouse tissues, as compared with the WT. *P < .05. C, Pathogen burden in blood. Mice (3 animals per group) were intravenously injected with either WT or bb0406 mutants (Mut1 and Mut2) (10⁶ spirochetes per mouse). On day 14, spirochete burdens in the blood were determined by qPCR. Spirochetes persisted at high levels in blood, day 14. D, Intravenous injection did not rescue BB0406 mutant infectivity in mice. After 14 days of infection via intravenous injection, as detailed in C, the levels of Borrelia burgdorferi in murine tissues were analyzed using qPCR. Levels of the bb0406 mutants were significantly lower in all mouse tissues, compared with the WT. *P < .05. Error bars represent means and standard errors of the mean for 3 independent experiments.
differential development of host antibodies may be the result of differences in the posttranscriptional or posttranslational regulation of BB0405 and/or BB0406. In any case, either BB0405 [18, 19] or BB0406 is independently required for murine infectivity, especially at the disseminated phase of infection.

Although a redundant role of BB0406 in murine infection was proposed [19], a different mutagenesis strategy was used, which likely contributed to disparate phenotypic outcomes. Whereas our study created a cleaner mutant via the deletion of a single targeted gene (bb0406), we were unable to complement the isolate; therefore, we used 2 independent mutant clones to analyze the phenotype. In contrast, the published study created a dual mutant by ablating the expression of 2 genes (both bb0405 and bb0406) and then genetically complementing either gene to generate 2 independent isolates that reexpressed either BB0405 or BB0406. Of note, native BB0406 displays a spectacular differential expression in vivo that varies both spatially (Figure 1B) and temporally (Figure 1C). Shrestha et al [19] used a constitutively active borrelial promoter (flaB) that overexpressed bb0406, and it is possible that an abnormally regulated gene product may affect the normal physiology of the organism, therefore affecting its virulence in mice. In either case, the outcomes of both studies highlighted that both BB0405 and BB0406 are expressed and maintained in B. burgdorferi and thus are likely to have distinct or overlapping functions. Both genes, which are substantially similar, have variable but detectable expression in ticks and mammalian hosts. Therefore, it is tempting to speculate that these gene products may perform similar functions at different stages of the spirochete life cycle using conserved regions, and/or that they perform distinctly different functions in spirochete infectivity.

The vascular system is the main route of dissemination for tick-borne pathogens like B. burgdorferi, which colonizes several internal organs in mammals. Such hematogenous dissemination is a multistep process including adhesion, tethering, dragging, stationary adhesion, and extravasation [34]; BB0406 is likely to be
involved in one or more of these processes. The BB0406-laminin interaction could be critical for the first step of bacterial adhesion to the basement membrane of the blood vessel. Because one of the major structural and functional constituents of the vascular basement membrane, the binding of laminin to BB0406 could compromise the integrity of the vessel wall, thereby facilitating pathogen passage through the endothelial barrier. Considering that laminin is involved in interactions with many other ECM proteins, including integrins and collagens [35, 36], its binding to spirochetes (via BB0406) could disrupt a plethora of its other functions that are relevant to cell attachment, differentiation, and movement. On the other hand, *B. burgdorferi* is known to produce additional laminin-binding proteins [37, 38], which may interact with specific target laminin and contribute to pathogen survival and pathogenesis. This remains a possibility, because there are ≥15 isoforms of laminin in the basement membranes of the vessel walls of arterioles, capillaries, postcapillary venules, and venules, which display enormous variability in structure and expression [39].

In addition to the BB0406-laminin interaction, there are other OM proteins known to support spirochete dissemination through host vasculature. For example, BBK32, a spirochete adhesin that interacts with multiple host ECM molecules like fibronectin and glycosaminoglycans [11, 13, 14, 40], is involved in the early steps of *B. burgdorferi* endothelial adhesion and interactions with the host microvasculature [41]. In addition, the OM protein P66, an integrin-binding borreliid porin, was recently reported to assist in spirochete and vascular interactions, vascular transmigration, and extravasation, via P66-integrin interactions [17]. Many other bacterial porin and transporter proteins are known to bind specific ECM proteins that promote microbial adherence [42]. In fact, the structures, surface locations, and abundances of bacterial porin and transporter proteins have likely contributed to their evolution as multifunctional proteins that ultimately support bacterial virulence. Future investigations into these intriguing mechanisms—in particular, how specific OM proteins, like BB0406, support
the intravasation and extravasation of pathogens, in immunocompetent or in immunodeficient hosts (like SCID mice)—will greatly enrich our knowledge of the biology of tick-borne infections and host immune responses and will inform the development of effective measures to control these diseases.

**Supplementary Data**

Supplementary materials are available at The Journal of Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

**Notes**

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