Novel Microbial Virulence Factor Triggers Murine Lyme Arthritis

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(See the editorial commentary by Radolf on pages 877–9.)

*Borrelia burgdorferi* bba57 is a conserved gene encoding a potential lipoprotein of unknown function. Here we show that bba57 is up-regulated in vivo and is required for early murine infection and potential spirochete transmission process. Although BBA57 is dispensable for late murine infection, the mutants were unable to induce disease. We show that BBA57, an outer membrane and surface-exposed antigen, is a major trigger of murine Lyme arthritis; even in cases of larger challenge inocula, which allow their persistence in joints at a level similar to wild-type spirochetes, bba57 mutants are unable to induce joint inflammation. We further showed that BBA57 deficiency reduces the expression of selected “neutrophil-recruiting” chemokines and associated receptors, causing significant impairment of neutrophil chemotaxis. New approaches to combat Lyme disease may include strategies to interfere with BBA57, a novel virulence factor and a trigger of murine Lyme arthritis.

Keywords. *Borrelia burgdorferi*; Lyme disease; virulence; inflammation.

*Borrelia burgdorferi*, the agent of Lyme disease, is a common cause of tick-borne diseases in the United States and many other regions across the globe [1]. The pathogen is transmitted to mammalian dermis via the bite of an infected *Ixodes* tick. *B. burgdorferi* then disseminates to distant cutaneous sites and a variety of internal organs including the joints, heart, and bladder [2, 3]. The clinical manifestations of Lyme borreliosis are well characterized and may involve a pathognomonic skin rash, arthritis, carditis, and neurological symptoms [3]. The most frequent complications of Lyme borreliosis involve the joints, as in the absence of antibiotic therapy, approximately 60% of infected patients develop Lyme arthritis [4]. In most cases, antibiotic therapy results in cure; however, some patients may develop a form of antibiotic-resistant chronic arthritis that is unrelated to persistent infection.

The pathogenesis of Lyme arthritis has been widely studied in animal models, most notably in mice, which mimic certain pathological episodes attributed to human disease [5]. *B. burgdorferi* infection in murine joints triggers marked inflammatory responses, involving both innate and adaptive immune components [4, 6]. In infected mice, the development of joint inflammation is orchestrated by a number of inflammatory cytokines and chemokines that influence the infiltration of neutrophils into the joint space [7–9]. Although neutrophil recruitment is necessary to control *B. burgdorferi* infection [10], interference with infiltration of these granulocytes via genetic depletion of certain chemokine receptors has been shown to attenuate the severity of arthritis [11].

Similar to the pathogenic mechanism in arthritis, host inflammatory responses in the cardiac tissue results in carditis, which occurs in 4%–10% of patients [12–16], and has also been studied in murine models.
Unlike what is observed in joints, the inflammatory infiltrate observed in myocardial lesions consists of >70% mononuclear cells [17], and the severity of the disease is consistent with augmented expression of selected CCL chemokines and their receptors [3, 18, 19]. Together, these studies demonstrated that the severity of arthritis or carditis is correlated with spirochete persistence in infected tissues, induction of specific immunomodulators, and subsequent recruitment of immune cells. However, the potential existence or the identity of specific microbial trigger(s), such as surface antigen(s) that induce the cells are recruited into B. burgdorferi safety Committee. An national Animal Care and Use Committee and Institutional Bio in accordance with the guidelines of the Institu- the National Institutes of Health. Animal experiments were performed, as described elsewhere [20]. For assessment of joint RNA (1 μg) was used for quantitative RT-PCR (qRT-PCR) analysis with iQ5 thermal cycler (Bio-Rad), as de- tailed elsewhere [20].

**Generation of Recombinant BBA57 and Production of Antiserum**

The bba57 ORF was cloned into pGEX-6P-1 (GE Healthcare) using primers (Supplementary Table 1), and the recombinant protein without predicted N-terminal leader sequence was produced in *Escherichia coli*. Purification and enzymatic cleavage of the glutathione S-transferase fusion protein was performed as described elsewhere [21]. Murine antiserum against recombinant BBA57 was produced as detailed elsewhere [22]. The titer and specificity were evaluated by using enzyme-linked immunosorbent assay and immunoblotting, as described elsewhere [23].

**Preparation of Outer Membrane Vesicles and Immunoblotting Assay**

The outer membrane (OM) vesicles of *B. burgdorferi* were isolated as described elsewhere [24]. Immunoblotting was performed as described elsewhere [25], using proteins (0.3 μg/ lane) in a 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis gel, which was transferred onto a membrane and probed with BBA57, OspA or FlaB antibodies at a dilution of 1:4000, 1:4000, or 1:2000, respectively.

**Proteinase K Accessibility Assay and Triton X-114 Phase Partitioning**

The proteinase K digestion assay was performed as detailed elsewhere [21]. Triton X-114 phase partitioning assays were performed as described elsewhere [26].

**Generation of bba57 Mutant and Complemented Isolates of B. burgdorferi**

BBA57-deficient *B. burgdorferi* was generated via homologous recombination by replacing bba57 ORF with a kanamycin-resistance (*kanAn*) cassette [20]. The DNA fragments flanking the bba57 upstream and downstream were PCR amplified using primers P1–P4 and inserted into 2 multiple-cloning sites flanking the *kanAn* cassette in plasmid pXLF10601 (pXLF-bba57). The construct was transformed into *B. burg- dorferi*, selected with kanamycin (350 μg/mL), and finally screened using primers to confirm the desired integration of *kanAn* cassette. A bba57 deletion clone retaining the same set of plasmids as the wild-type isolate was used for further experiments.

Genetic complementation of bba57 mutant was achieved by reinsertion of bba57 gene into the mutant. A fragment, including bba57 and its promoter region, was cloned into pKFSS1 [27], which contains a streptomycin-resistance cassette (*aadA*). An

**METHODS**

**B. burgdorferi, Mice, and Ticks**

An infectious isolate of *B. burgdorferi*, B31-A3, was used. Four- to six-week-old C3H/HeN mice were purchased from the National Institutes of Health. Animal experiments were performed in accordance with the guidelines of the Institu- tional Animal Care and Use Committee and Institutional Bio- safety Committee. An *Ixodes scapularis* colony was derived from adult ticks collected from several sites in Connecticut. The ticks were not tested for additional pathogens other than *B. burgdorferi*.

**Polymerase Chain Reaction Analysis**

The primers used in polymerase chain reaction (PCR) are shown in Supplementary Table 1. For studying bba57 expression in the vector, infected nymphs (25 ticks/mouse) were parasitized on naive mice and were collected between 24–96 hours after attachment and pooled together. To assess bba57 expression in hosts, mice (5 animals/group) were infected with spirochetes (10⁵ cells/mouse). Tissues were collected at 1–4 weeks and pooled by tissue type. For measurement of chemokine transcript levels, joint tissues from infected mice were collected. DNA-free RNA samples were isolated from tissues, and quantitative reverse-transcription (RT) PCR analyses were performed, as described elsewhere [20]. For assessment of *B. burgdorferi* levels in tissues, *flaB* transcripts were measured and then normalized to the level of mouse β-actin transcripts [21].

**PCR Array**

The PCR array for mouse cytokines was performed using the manufacturer’s protocol (Inflammatory Cytokines & Receptors RT² Profiler PCR Arrays, Qiagen-SuperArray Bioscience), which comprised 84 murine chemokines, cytokines and receptors related to inflammation. Briefly, mice were infected with *B. burgdorferi*, and joints were harvested 3 weeks after infection. Joint RNA (1 μg) was used for quantitative RT-PCR (qRT-PCR) analysis with iQ5 thermal cycler (Bio-Rad), as de- tailed elsewhere [20].
insert containing bba57-aadA gene fusion was subcloned into plasmid pXLF-bba57, as detailed earlier [30]. The construct was electroporated into the bba57 mutant and selected using antibiotics. A bba57-complemented clone that contained the same plasmid profiles as the wild type was chosen for further study. For in vitro growth analysis, spirochetes were diluted to a density of $10^6$ cells/mL, grown until stationary phase (approximately $10^8$ cells/mL) and counted by dark-field microscopy every 24 hours with a Petroff-Hausser cell counter [25].

**Phenotypic Analysis of bba57 Mutant and Complemented Isolates**

For the analysis of *B. burgdorferi* infectivity in hosts, mice (5 animals/group) were injected with *B. burgdorferi* isolates through needle inoculation intradermally in the lower right quadrant of the abdomen upstream from the joint. Tissues were isolated at various time points, and pathogen levels were measured using qRT-PCR [20]. The swelling in joints was measured using a digital caliper [20]. In addition, nymphs were infected with wild-type spirochetes or genetically manipulated *B. burgdorferi* via microinjection, and the ability of pathogens to transmit to naive hosts was assessed as detailed elsewhere [21].

**Histopathological Evaluation**

Murine joints and hearts were collected 21 days after *B. burgdorferi* infection, fixed in 10% buffered formalin, and processed for hematoxylin-eosin staining [20]. The histopathological specimens were evaluated in double-blinded fashion, as detailed elsewhere [20, 25]. The diameters of individual joints were recorded before *B. burgdorferi* inoculation, and the increase in ankle diameters was recorded weekly. Inflammation in the heart was assessed in the mice of same age group. Immunohistochemical detection of peroxidase was performed as detailed elsewhere [28].

**Chemotaxis Assay**

An assay to examine neutrophil migration was performed using a 48-well microchamber (Neuro Probe) [29]. Cell-free supernatants, prepared from joints collected 3 weeks after infection, were placed in the lower chamber in the presence or absence of anti-CXCL5 (Cedarlane Labs) or control antibodies, which was separated from the upper chamber by a 5-µm pore polycarbonate membrane. Neutrophils were isolated from murine bone marrow [29], placed on the upper chamber (5 x $10^4$ cells/well), and allowed to migrate into the membrane, which was stained using Diff-Quik system (Dade Behring). Each well-associated membrane area was scored by using light microscopy to count the intact cells present in 10 random fields.

**Flow Cytometry**

Homogenized joints were digested with type I collagenase (1 mg/mL), centrifuged, resuspended in PBS, and stained with either anti–Gr-1 (eBioscience) or isotype immunoglobulin G (IgG) followed by a phycoerythrin-labeled secondary antibody (BD Biosciences). After washing, joint cells (approximately $10^6$ cells/mouse) were resuspended in 4% paraformaldehyde and individually analyzed with a flow cytometer (FACSArria II; BD Biosciences). Unstained cells were used as negative controls.

**Statistical Analysis**

Results are expressed as the mean ± standard error of the mean. The significance of the difference between the mean values of groups was evaluated by 2-tailed Student’s t test.

**RESULTS**

**bba57 Encoding of Surface-Exposed OM Antigen and Expression in Infected Mice and Ticks**

Our recent studies identified a handful of *B. burgdorferi* genes, potentially encoding OM antigens that are differentially expressed during bacterial growth in culture, murine hosts, or the arthropod vector [20, 30]. Additional expression analysis of bba57, a gene located on the linear plasmid lp54 and encoding a predicted membrane lipoprotein, showed that bba57 is induced in vivo during spirochete infection of both murine hosts and tick vectors (data not shown). Further analysis of bba57 expression in representative tick- and mammal-specific phases of infection indicated that the gene is up-regulated in fed larvae, unfed and fed nymphs, and multiple tissues of infected mice (Figure 1A). We show that BBA57 is an OM protein; it is detectable in isolated OM vesicles (Figure 1B) and fractionated into the detergent-enriched phase when subjected to Triton X-114 phase partitioning analysis (Figure 1C). In addition, proteinase K digestion analysis demonstrated that BBA57 was exposed on the spirochete surface (Figure 1D).

**BBA57 in Early Stages of Murine Infection and Potential Transmission Between Vectors and Hosts**

We next assessed the contribution of BBA57 in supporting spirochete persistence in vivo by creating bba57 deletion mutants as well as genetically complemented isolates (Figure 2A–E). Genetic manipulation of bba57 had no recordable defects for spirochete survival in culture as displayed by a growth pattern in bba57 mutants and bba57-complemented isolates similar to that of wild-type spirochetes (Figure 2G). However, analysis of mutants in mice via needle inoculation (10^5 spirochetes/mouse, 5 animals/group) indicated that bba57 deletion impaired the ability of the spirochetes to persist in joints as well as in additional tissues during the early stages (first 1–2 weeks) of infection (Figure 3A; P < .01). However, by the third week, the levels of bba57 mutants were enhanced in
most tissue locations except for joints (Figure 3A), where the levels of mutants remained significantly decreased throughout the time course of the study. Although the role of BBA57 in supporting spirochete persistence in the vector is currently unknown, a study using *bba57* mutant–infected ticks generated via microinjection of cultured spirochetes showed that, unlike wild-type *B. burgdorferi* or *bba57*-complemented isolates, *bba57* mutants were unable to transmit to naive mice (Figure 3B), strongly suggesting that BBA57 supports pathogen transmission from ticks to naive hosts.

**BBA57 as Microbial Trigger of Murine Lyme Arthritis**

Although a significant level of *bba57* mutants persisted in all tested murine organs, including joints (Figure 3A), the mutants failed to induce measurable inflammation in the joints or in the heart (Figure 4). Because severity of murine Lyme arthritis is correlated with the number of colonizing pathogens in joint tissues, we next examined whether a larger challenge inoculum of *bba57* mutants could enhance joint colonization, and in that case, induce more severe arthritis. Groups of 5 mice were individually challenged with either $10^5$ wild-type cells or $10^6$–$10^8$ mutants. Three weeks after challenge, pathogen levels in joints were determined with qRT-PCR. No significant differences in the numbers of *bba57* mutants and wild-type spirochetes in joint tissues were evidenced in mice challenged with either $10^7$ or $10^8$ *bba57* mutants (Supplementary Figure 1A; *P* > .05). Surprisingly, unlike wild-type spirochetes, even a 1000-fold higher initial inoculum of *bba57* mutants was unable to induce joint swelling (data not shown) and accordingly, caused less-obvious histopathological signs of arthritis (Supplementary Figure 1B).

We then assessed whether *bba57* mutants produce a delayed inflammation or fail to induce significant arthritis. To explore this question, mice were infected with wild-type spirochetes ($10^5$ cells/mouse) and a 100-fold higher level of *bba57* mutants ($10^7$ cells/mouse). The pathogen burden and the
degree of joint swelling were monitored weekly through 6 weeks of infection. Except for week 1, a similar level of bba57 mutants and wild-type B. burgdorferi was detected at all tested time points (P > .05; Figure 5A). However, compared with wild-type spirochetes, bba57 mutants induced minimal swelling in the ankle joints until 6 weeks after infection.
Taken together, these data suggest that the presence of BBA57 is directly correlated with the genesis of Lyme arthritis.

Effect of BBA57 Deficiency on Expression of Selected Chemokine Genes Associated With Neutrophil Chemotaxis In Vivo

Host cytokine responses are implicated in the genesis of Lyme arthritis [7, 9, 11]. We therefore assessed whether deficiency of BBA57 alters the expression profiles of host inflammation–related cytokine genes. Groups of mice (3 animals/group) were infected with 10⁵ wild-type spirochetes or 100-fold higher bba57 mutants (10⁷ cells/mouse), and the expression of a panel of 84 host inflammation–related cytokine genes in joints was analyzed 3 weeks after infection by means of PCR arrays. Genes encoding neutrophil chemoattractants, CXCL5 and CXCL1, and their receptors on neutrophils, IL8rb (CXCR2), were among the most dramatically down-regulated cytokine genes in murine joint tissues infected with bba57 mutants (Supplementary Figure 2).

Next, independent infection experiments were performed to examine expression profiles of selected chemokines in murine joints with qRT-PCR during the course of B. burgdorferi infection. The results confirmed that the expression levels for the chemokine genes CXCL1 and CXCL5 and their receptor, CXCR2, were dramatically down-regulated in the joint during the first 3 weeks in bba57 mutant–infected mice compared with wild-type spirochetes or bba57-complemented isolates.

(P < .05; Figure 3B). Taken together, these data suggest that the presence of BBA57 is directly correlated with the genesis of Lyme arthritis.
In contrast, another CXCR2 ligand gene, MIP-2, displayed significant up-regulation in the bba57 mutant–infected joints (*P < .05; Figure 6D).

Role of BBA57 in Recruiting Neutrophils in Infected Murine Joints

Deficiency of BBA57 significantly reduces the expression of chemokines that are involved in neutrophil chemotaxis in infected joints (Figure 6). We next assessed whether neutrophil recruitment is impaired in joints colonized by bba57 mutants. Because peroxidase is one of the markers of granulocytes like neutrophils, immunohistochemical analysis was performed using joint tissues after 3 weeks of infection. As reflected by the diminished peroxidase staining, less infiltration of neutrophils was apparent in joints infected with bba57 mutants than in those infected with wild-type spirochetes or bba57-complemented isolates (Figure 7A). Furthermore, cells were isolated from infected joint tissues, stained with neutrophil surface marker Gr-1 and examined by flow cytometry (Figure 7B). Compared with wild-type spirochetes (61.07% ± 2.4%) or bba57–complemented isolates, the percentage of Gr-1+ cells in bba57 mutants was significantly reduced (**P < .01; Figure 7B).

Figure 4. Deficiency of Borrelia burgdorferi BBA57 decreases the severity of arthritis and carditis in mice. A, Assessment of joint swelling in B. burgdorferi–infected mice. Arthritis was evaluated in mice infected with B. burgdorferi isolates by assessment of joint swelling measured with a digital caliper. Differences in joint swelling between bba57 mutants (white bars) and wild-type (black bars) or bba57-complemented (gray bars) isolates were significant at all time points (*P < .05). Bars represent means ± standard errors of the mean from 2 independent infection experiments. Abbreviations: bba57−, bba57 mutants; bba57 Com, bba57-complemented isolates; WT, wild-type B. burgdorferi. B, C, Representative of histological findings in joints (B) and hearts (C) isolated from mice infected with wild-type B. burgdorferi, bba57 mutants, or bba57-complemented isolates, as observed after 3 weeks of infection. Left panels are lower-resolution images (joint, ×10 [bar, 160 µm]; heart, ×4 [bar, 400 µm]); right panels are higher-resolution images (joint, ×20 [bar, 80 µm]; heart, ×10 [bar, 160 µm]) of selected areas (insets in left panels). Arrows indicate infiltration of immune cells. Note that in the cardiac tissue (C), the inflammatory process for mutants, compared with wild-type spirochetes, is minimal. D, Quantitative evaluation of carditis. At least 10 random sections from each spirochete group were scored for severity of carditis, on a scale of 0–3. Differences between bba57 mutants and wild-type or bba57–complemented isolates are significant (*P < .01).
complemented isolates (48.2% ± 3.6%), significantly fewer Gr-1–positive cells were accumulated in joints infected with bba57 mutants (22.7% ± 1.5%; P < .01).

Finally, we adopted an established chemotaxis assay to demonstrate that BBA57 is involved in B. burgdorferi–induced neutrophil migration in vitro. Cell-free supernatants were prepared from joint tissues infected with wild-type spirochetes or mutants and used to assess neutrophil chemotaxis. Data indicated that neutrophil chemotaxis was significantly impaired by bba57 mutants, compared with wild-type spirochetes or bba57-complemented isolates (P < .01; Figure 7C). The inflation of neutrophils was significantly inhibited by pretreatment with CXCL5 antibody, but not by isotype IgG (Figure 7C), suggesting that CXCL5 is an important chemokine in mediating the migration of neutrophils. Taken together, these results suggest that BBA57 is involved in the induction of selected chemokines within the joint tissues. These chemokines probably recruit neutrophils, ultimately contributing to the genesis of joint inflammation associated with Lyme arthritis.

DISCUSSION

Outer membrane (OM) antigens that are induced in vivo may assist in B. burgdorferi persistence in the host [20]. Our study suggests that a novel OM protein, BBA57, facilitates pathogen persistence in vivo either by assisting the pathogen to evade host innate immune defenses or by contributing to an unknown physiological requirement that is pronounced during early host infection or during transmission between the vector and hosts. In mammals, B. burgdorferi infection may elicit robust inflammatory responses, resulting in a multi-organ illness. Several factors are likely to account for such responses including pathogen levels in infected organs, strain virulence, and host-specific immune responses [4, 9].

In animal models including C3H mice, the spirochetes are known to induce marked edema and inflammation in the synovium, which is mainly reflected in the ankle joints and visible within the first 2 weeks of infection [2, 31]. This time point also corresponds to the dissemination of spirochetes from the skin into the joint [5]. However, paradoxical to the
current dogma suggesting that the genesis and severity of Lyme arthritis correlate with pathogen burden in joints, our results indicate that the gravity of joint inflammation is influenced by a specific microbial molecule. We show that in experimental cases where levels of BBA57-deficient pathogens are similar to the level of isogenic wild-type spirochetes in joints, the mutants are still unable to induce arthritis. This suggests that the presence of BBA57 (as a surface microbial antigen) influences the inflammatory outcome in the host. Although joint inflammation constitutes the focus of our study, bba57 mutants were also unable to induce severe inflammatory responses in the heart; thus, the possible role of BBA57 in triggering Lyme carditis is a subject for future investigation.

In the absence of classic endotoxins, such as lipopolysaccharide, spirochete lipoproteins are considered a trigger of host inflammatory responses [32, 33] that are mediated by specific toll-like receptors (TLR), TLR1 and TLR2, in conjunction with CD14 [34–36], although TLR-independent receptor responses are also important for B. burgdorferi–induced inflammation [37–40]. Spirochete lipids are inherently proinflammatory [32, 33, 41] and thus it remains possible that the potential lipid moiety of BBA57 helps induce host inflammatory responses. However, the borrelial OM probably harbors numerous additional lipoproteins [42], including abundant antigens, such as BmpA, that are selectively induced during joint infection [23]. Thus, attenuation of inflammatory responses in joints infected with bba57 mutants may not be solely attributable to the deficiency of membrane lipids (resulting from deletion of lipoprotein BBA57). This speculation is supported, in part, by the identical protein profiles in the wild-type spirochetes and bba57 mutants (Figure 2F), as well as by our observation that an enhancement of the mutant burden, and thus potential increase in the availability of overall borrelial membrane lipids in joint tissues, failed to augment significant inflammatory responses. Because phagocytosis of B. burgdorferi is critical for induction of host inflammatory responses [43–45], it is tempting to speculate that BBA57, as a microbial surface protein, could play a role in the binding or uptake by resident phagocytic cells, thereby triggering the chemokine output that initiates the local inflammatory process.

A hallmark of murine Lyme arthritis is severe polysynovitis characterized by synovial proliferation and predominant infiltration of neutrophils [5]. Similarly in humans, joint fluid...
obtained from infected children reflected a large numbers of leukocytes, with a predominance of neutrophils [46]. In vitro studies demonstrate that spirochetes can induce certain adhesion molecules and promote transendothelial migration of neutrophils [47]. The select set of cytokines, such as chemokines CXCL5 and CXCL1 and their cognate receptor CXCR2, are largely responsible for neutrophil chemotaxis [48]. In fact, the severity of murine Lyme arthritis correlates with the production of CXCL1, along with other chemokines in the joints, and requires neutrophil recruitment via CXCR2 [8]. In our study, a decreased expression of chemokines CXCL1 and CXCL5 in bba57 mutant–infected mice was also accompanied by reduced infiltration of neutrophils in joints. We thus speculate that BBA57 could serve as one of the predominant microbial molecular triggers inducing the expression of specific chemokines and/or their receptor(s) in yet-to-be-identified cell types that may recruit neutrophils in joint tissues, thereby influencing the pathogenesis of Lyme arthritis. Furthermore, other cytokines that could be associated with the development of arthritis [49, 50], such as CCL17 and CCL20, are also regulated in joint tissues infected with bba57 mutants (Supplementary Figure 2). Because Lyme arthritis is a complex outcome of multifactorial host responses [4, 9], additional host factor(s) may also be responsible for the genesis of host inflammatory immune responses against the pathogen. Strategies to interfere with microbial triggers that promote host inflammation and

Figure 7. BBA57 deficiency affects neutrophil recruitment in infected joint tissues. A, Immunohistochemical identification of neutrophil granulocytes in infected joint tissue sections. Joint issues infected with wild type, bba57-complemented (bba57 Com) or bba57 mutant (bba57−) Borrelia burgdorferi were harvested 3 weeks after spirochete inoculation; samples were serially sectioned and either stained with hematoxylin-eosin (left panels) or probed with a rabbit anti-peroxidase antibody followed by biotin-labeled secondary antibody and dianinobenzidine tetrahydrochloride (right panels). Arrows indicate massive infiltration of granulocytes that remained positive peroxidase labeling. Abbreviations: bba57− bba57 mutants; bba57 Com, bba57-complemented isolates; WT, wild-type B. burgdorferi. B, Flow cytometry–based detection of neutrophils in infected joint tissues. Cells were isolated from murine joints infected with indicated groups of spirochetes, stained with antibodies against Gr-1 or isotype immunoglobulin G followed by a phycoerythrin-labeled secondary antibody, and then assessed with flow cytometry. Cells without staining were used as negative controls. Data represent percentage of Gr-1–positive cells in relation to total number of cells isolated from the corresponding joints; a representative histogram of 3 reproducible experiments is shown. Significantly fewer neutrophils were evident in joints infected with bba57 mutants compared with wild-type or bba57-complemented isolates (P<.01). Abbreviation: IgG, immunoglobulin G. C, Assessment of neutrophil chemotaxis in vitro. Tissue supernatants were extracted from spirochete-infected joints and used in the chemotaxis assay in the presence or absence of anti-CXCL5 antibody or isotype IgG. Accumulation of neutrophils was assessed using the Diff-Quik labeling system. Bars represent means ± standard errors of the mean from 3 independent experiments. Significantly higher levels of neutrophil migration were detected for wild-type and bba57-complemented spirochetes than for bba57 mutants (*P<.05), which can be partially inhibited by CXCL5 antibody (**P<.05) but not isotype IgG.
block the function of surface antigens required for efficient pathogen transmission, such as BBA57, may contribute to the development of new therapeutic and preventive approaches to combat Lyme disease.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyrighted. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

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