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Borrelia burgdorferi Changes Its Surface Antigenic Expression in Response to Host Immune Responses

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The Lyme disease spirochete, *Borrelia burgdorferi*, causes persistent mammalian infection despite the development of vigorous immune responses against the pathogen. To examine spirochetal phenotypes that dominate in the hostile immune environment, the mRNA transcripts of four prototypic surface lipoproteins, decorin-binding protein A (DbpA), outer surface protein C (OspC), BBF01, and VlsE, were analyzed by quantitative reverse transcription-PCR under various immune conditions. We demonstrate that *B. burgdorferi* changes its surface antigenic expression in response to immune attack. *dbpA* expression was unchanged while the spirochetes decreased *ospC* expression by 446 times and increased BBF01 and *vlsE* expression up to 20 and 32 times, respectively, under the influence of immune pressure generated in immunocompetent mice during infection. This change in antigenic expression could be induced by passively immunizing infected severe combined immunodeficiency mice with specific *Borrelia* antisera or OspC antibody and appears to allow *B. burgdorferi* to resist immune attack.

Lyme disease, caused by *Borrelia burgdorferi*, is a complex multisystem disorder that can result in arthritis, neurological abnormalities, carditis, and cutaneous lesions such as erythema migrans and acrodermatitis chronica atrophicans (48). *B. burgdorferi* infection in humans and mice may persist despite the development of vigorous immune responses against the pathogen (47). *B. burgdorferi* has approximately 150 lipoprotein genes (8, 16), and some may be anchored to the spirochetal outer membrane to form an antigenic layer that protects the bacterium from, and directly interacts with, the environment (19). Surface-exposed lipoproteins may render the spirochetes susceptible to specific lipoprotein antibodies in vitro, in the arthropod vector, and in the mammalian host (11, 22, 32, 37, 43, 45). While lipoproteins may stimulate innate responses via Toll-like receptors 1 and 2, enhancing both humoral and cellular immune responses to *B. burgdorferi* (2, 52), little is known about how this extracellular bacterium survives in the hostile immune environment during mammalian infection.

B. burgdorferi adapts to diverse environments in the tick and mammal during its life cycle, in part by selective gene expression. Environmental cues such as temperature (42, 49), pH (7), nutrients or chemicals (3, 53), and others (5, 39, 44) influence *B. burgdorferi* gene expression in vitro, and the cultivation of *B. burgdorferi* in dialysis membrane chambers implanted into rat peritoneal cavities up-regulates several spirochetal genes (1). The most dramatic modifications that occur as *B. burgdorferi* migrates from ticks to a mammal involve lipoprotein gene expression, such as the down-regulation of outer surface protein A (OspA) and the up-regulation of OspC that occurs during tick feeding (38, 46). These changes occur among populations of spirochetes; however, individual spirochetes that express OspA, OspC, both, or neither may be detected (38). Investigating how changes in surface antigenic expression of *B. burgdorferi* contribute to its resistance to immune attack during persistent mammalian infection will provide insights into the pathogenesis of Lyme disease.

B. burgdorferi exhibits tissue-specific gene expression during mammalian infection (36). It persistently expresses *ospC* during infection of immunodeficient mammals (27, 29). The development of OspC antibody, however, preferentially selects for spirochetes that do not abundantly express OspC in immunocompetent mice. It is likely that antibodies to many lipoprotein antigens can place an immune selection pressure on spirochetes that express these antigens (29). We now examine spirochetal phenotypes that are selected for under the influence of host responses by quantitatively analyzing the mRNA transcripts of four prototypic surface-exposed lipoproteins, decorin-binding protein A (DbpA) (18, 20), OspC (33, 51), BBF01 (13, 15), and VlsE (Vmp-like sequence, expressed) (25, 54), during murine infection.

MATERIALS AND METHODS

Spirochete and mouse strains. *B. burgdorferi* B31 clone 5A11 (a gift from Steven Norris, University of Texas, Houston) was cultivated in Barbour-Stoenner-Kelly H complete medium at 33°C (Sigma Chemical Co., St. Louis, Mo.). BALB/c wild-type and BALB/c background severe combined immunodeficiency (SCID) mice were purchased from the Jackson Laboratory (Bar Harbor, Maine). B-cell-deficient mice on a BALB/c background were generated as previously described (9). All mice were 4 to 8 weeks old when they were infected.

Mouse inoculation and passive immunization. Ten wild-type, 10 B-cell-deficient, and 70 SCID mice were given one single intradermal injection of 10^5 cultured spirochetes. All of the infected wild-type and B-cell-deficient mice and 30 of the infected SCID mice were sacrificed at 12 days to 4 months postinfection. The rest of the SCID mice were used for passive immunization experiments. Heart, joint, and skin tissues (not from the inoculation site) were harvested and

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TABLE 1. qPCR primers and probes

Gene	Primer	Probe
flaB	Forward 5′-GAGTTTCTGGTAAGATTAATGCTC-3′ Reverse 5′-CATTTAAATTCCCTTCTGTTGTCTGA-3′	5′-AGAGGTTTGTCACAAGCTTCTAGAAATACTTCAAAGGC-3′
dbpA	Forward 5′-CTTAAACTAACTATACTTGTTAAC-3′ Reverse 5′-AATGTCTTTAGCGCTTCGTTC-3′	5′-TTATATCATGTGGACTAACAGGAGCAAC-3′
ospC	Forward 5′-TACGGATTCTAATGCGGTTTTAC-3′ Reverse 5′-GTGATTATTTTCGGTATCCAAACCA-3′	5′-TGAAGCGTTGCTGTCATCTATAGATGAAATTGCTGCT-3′
BBF01	Forward 5′-TAGCACAACATGCTCCAAACTC-3′ Reverse 5′-ACAATTTCCTCTTTTACTTCTGGGA-3′	5′-ATCCGATGGAAAACCTGTTCCTGGGGAC-3′
vlsE	Forward 5′-CTTATACTTTTCATTATAAGGAGACGATG-3′ Reverse 5′-GCCTCTGCTACTAACCCAC-3′	5′-GCCAAGTTGCTGATAAGGACGACCCAAC-3′
actin	Forward 5′-CATCATGAAGTGTGACGTTGAC-3′ Reverse 5′-GCATCCTGTCAGCAATGCC-3′	5′-GTATGCCAATACAGTGCTGTCTGGTGGTACCAC-3′

immediately frozen in liquid nitrogen. Frozen samples were stored at −70°C until DNA and RNA were isolated.

OspC monoclonal antibody preparation and passive immunization. The hybridoma cell line B5 was generated as described previously (34). Secreted OspC monoclonal antibody isotyped as immunoglobulin G2a (IgG2a) is able to protect mice against a tick-transmitted infection with *B. burgdorferi* B31 and to reduce *ospC* expression in infected SCID mice. The antibody was purified from mouse ascites fluid with use of a protein G column (Pierce Chemical Company, Rockford, Ill.). Purity and concentration were assessed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and the Bio-Rad protein assay kit (Bio-Rad Laboratories, Richmond, Calif.). Ten SCID mice were infected for 4 months as described above, and each subcutaneously received 24 μg of OspC monoclonal antibody every 2 days for three doses. Another 10 mice were each given three 24-μg doses of purified healthy mouse IgG2a (Sigma) as a control. All animals were sacrificed 3 days later after the last passive immunization. Heart, joint, and skin tissues were collected and stored as described above.

Anti-*Borrelia* serum preparation and passive immunization. To prepare anti-*Borrelia* sera, BALB/c mice were infected with cultured B31 5A11 spirochetes as described above. Blood was drawn between 2 and 4 months postinfection, and sera were isolated, pooled, and stored at −20°C. To prepare prebled sera, blood was collected from uninfected BALB/c mice and sera were isolated, pooled, and stored. Ten SCID mice were infected for 4 months as described above, and each subcutaneously received 100 μl of anti-*Borrelia* sera every 2 days for six doses. Another 10 infected mice were each given six 100-μl doses of prebled murine sera as a control. All animals were sacrificed 3 days later after the last passive immunization. Heart, joint, and skin tissues were collected and stored as described above.

RNA and DNA preparation. Frozen heart, joint, and skin samples were transferred in liquid nitrogen and ground thoroughly with a mortar and pestle. An appropriate amount of tissue powder was transferred into a 500-μl polypropylene tube for DNA preparation with use of the DNeasy Mini kit according to the manufacturer's instructions (Qiagen Inc., Valencia, Calif.). RNA was isolated from the remaining powder with use of Trizol reagent (Invitrogen Life Technologies, Carlsbad, Calif.). To ensure that there was no DNA contamination, RNA preparations were first digested in solution with RNase-free DNase I (Life Technologies, Inc., Gaithersburg, Md.) at 37°C for 2 h and then loaded onto the RNeasy Mini columns and further treated with RNase-free DNase I (Qiagen) for an additional 20 min at room temperature. Doubly digested samples were repurified and analyzed for potential DNA contamination by PCR amplification of the *flaB* gene.

cDNA preparation. The DNA-free RNA preparation was first annealed with the reverse oligonucleotide primer mixture of *flaB* (5′-ATTCCAAGCTCTTCA GCTG-3′), *dbpA* (5′-CATTGCTGAAAATTCACCAC-3′), *ospC* (5′-CAGCAT CAGTAACACCTTC-3′), BBF01 (5′-CCCTTGAGTAAGGAAACTAC-3), and *vlsE* (5′-CGTCGTACTACTTATATCGC-3′) genes at 65, 60, 55, 50, and 45°C each for 1 min, in the presence of reverse transcription (RT) buffer (Invitrogen). Deoxynucleoside triphosphates and SuperScript II RNase H$^-$ reverse transcriptase (Invitrogen) were added, RT was conducted at 42°C for 1 h, and then the reaction mixture was inactivated at 95°C for 5 min according to the manufacturer's instructions.

Quantitative PCR (qPCR) DNA concentration standards. *flaB*, *dbpA*, *ospC*, and BBF01 DNA concentration standards were prepared from cultured *B. burgdorferi*. B31 5A11 spirochetes were grown to stationary phase in Barbour-Stoenner-Kelly H complete medium, counted in a Petroff-Hausser counter (Hausser Scientific Partnership, Horsham, Pa.), and harvested by centrifugation at 15,000 × *g* for 10 min. Resultant pellets were digested with proteinase K (Qiagen) at 55°C for 2 h, inactivated at 95°C for 10 min, and diluted at 10^0 to 10^5 spirochetal DNA copies/μl.

To generate an actin DNA concentration standard, primers (forward, 5′-TG AGCGGTTCCGGTGTCC-3′; reverse, 5′-CAGTGAGGCCAGAATGGA-3′) were designed to amplify a 292-bp internal fragment of the actin gene by PCR with use of murine DNA as a template. To prepare a *vlsE* DNA concentration standard, a spirochetal DNA preparation was PCR amplified to generate a 330-bp internal fragment within the 5′-conserved region of the *vlsE* gene with use of primers (forward, 5′-TTTCATTATAAGGAGACGATGA-3′; reverse, 5′-CG TCGTACTACTTATATCGC-3′). *Taq* polymerase was purchased from Roche Diagnostics Co. (Indianapolis, Ind.). A PCR program with the following parameters was used: 95°C for 5 min; 95°C for 40 s, 50°C for 1 min, and 72°C for 40 s, 50 cycles; and 72°C for 10 min. PCR products were purified using the Quick PCR product purification kit (Qiagen). Purity was examined by agarose gel electrophoresis. DNA concentrations were determined by measuring the optical density at a 260-nm wavelength, converted to copy number, and diluted at 10^2 to 10^7 DNA copies/μl for actin DNA quantification and at 10^0 to 10^5 DNA copies/μl for *vlsE* cDNA quantification. The *vlsE* DNA concentration standard was prepared in this way since the qPCR efficiency of spirochetal DNA as a template was very low, probably because of this gene being too close to the right telomere of lp28-1 (54).

qPCR. qPCR analyses were performed using the iCycler (Bio-Rad Laboratories, Hercules, Calif.). The Platinum *Taq* DNA polymerase High Fidelity kit was purchased from Invitrogen. The sequences of primers and internal probes of *flaB*, *dbpA*, *ospC*, BBF01, *vlsE*, and actin genes are listed in Table 1. Taqman TAMRA probes were ordered from Applied Biosystems (Foster City, Calif.). Amplification was performed in a 50-μl final volume in individual wells of a 96-well iCycler iQ PCR plate (Bio-Rad). Twelve wells of each plate were assigned for DNA standards at six different concentrations in duplicate. Each cDNA or DNA sample was amplified in duplicate for the *flaB*, *dbpA*, *ospC*, BBF01, *vlsE*, or actin gene. A PCR program with the following parameters was used: 95°C for 5 min and 50 cycles of 95°C for 30 s and 60°C for 1 min. The mean cDNA copy numbers of *flaB*, *dbpA*, *ospC*, BBF01, and *vlsE* transcripts of each cDNA pool and mean DNA copy numbers of *flaB* and actin genes of each DNA sample were automatically calculated from duplicate wells with use of the iCycler software. Tissue spirochete burdens were calculated as *flaB* DNA copy numbers per 10^6 actin DNA copies. Lipoprotein gene expression levels were presented as *dbpA*, *ospC*, BBF01, or *vlsE* mRNA copy numbers per 10^3 *flaB* mRNA transcripts.

Statistical analysis. Data were analyzed using Microsoft Excel (Redmond, Wash.) software. A two-tailed Student *t* test was used to analyze qPCR and quantitative RT-PCR data. A *P* value of <0.05 was considered to be a significant difference.

RESULTS

The tissue microenvironment alters the antigenic profile of *B. burgdorferi*. To investigate the influence of adaptive immune pressure on *B. burgdorferi*, an analysis of lipoprotein gene expression in selective tissues from spirochete-infected SCID mice was first performed. Mice were sacrificed at 12 and 120 days postinfection, time points representative of acute and persistent infection, respectively, and expression of four representative lipoproteins and the tissue spirochete burdens were investigated in the heart, joints, and skin. These lipoproteins, DbpA, OspC, BBF01, and VlsE, were investigated since they are considered to be expressed in vivo and surface exposed and may be involved in pathogenesis and immunity to infection (13, 15, 17, 18, 20, 25, 54). These selected tissues were investigated for different reasons: skin represents the tissue of initial entry and exit of *B. burgdorferi*, and both the heart and joints are major sites of inflammation in the murine model of Lyme disease (4). At 12 days postinfection, populations of spirochetes showed vastly different expression levels of *dbpA*, *ospC*, BBF01, and *vlsE* in tissues (Fig. 1). *B. burgdorferi* more actively transcribed *dbpA* in the joints than in the heart ($P = 1.0 \times 10^{-4}$) and skin ($P < 0.008$). *ospC* was more abundantly expressed in the skin than in the heart ($P < 0.04$) and joints ($P < 0.05$). BBF01 was expressed at much higher levels in the joints than in the heart ($P = 4.2 \times 10^{-5}$), and *vlsE* was transcribed more actively in the joints than in the heart ($P = 4.9 \times 10^{-10}$) and skin ($P = 2.3 \times 10^{-9}$). Within 12 days after infection, *B. burgdorferi* might not have fully adapted to the tissue microenvironments. Spirochetal gene expression was also examined at a later time point. At 4 months, the expression levels of three of these four genes dramatically changed. *B. burgdorferi* increased *ospC* transcription in the heart ($P = 1.3 \times 10^{-4}$) but reduced its expression in joints ($P = 8.4 \times 10^{-8}$). Spirochetes increased BBF01 expression in the joints ($P = 5.1 \times 10^{-7}$) and skin ($P = 0.004$) and *vlsE* expression in the skin ($P = 0.007$). The differences in transcription of *dbpA*, *ospC*, and *vlsE* have been reflected by previous differences in the levels of these proteins in these tissues (10). These data indicate that the tissue microenvironment influences spirochetal phenotypes in the absence of adaptive immune pressure. The *B. burgdorferi* load also differed in the tissues examined at 12 days postinfection (Fig. 2). The joints provided the best environment for *B. burgdorferi* replication since the spirochete numbers were similar in this tissue at 12 days and 4 months ($P > 0.10$). At the same time, the spirochetal burdens in the heart were only 1.9% ($P = 2.4 \times 10^{-9}$) and in the skin were 29.4% ($P = 8.5 \times 10^{-5}$) of those detected at 4 months (Fig. 2). Taken together, these data indicate that the tissue microenvironments affect the spirochete growth as well as influence the *B. burgdorferi* lipoprotein gene expression.

Immune responses change spirochetal surface antigenic expression. The immune system consists of numerous key components that directly interact with foreign invaders and protect the host from microbial infections. To persist in the immune environment, *B. burgdorferi* must respond to lethal attack by the immune system. We used SCID, B-cell-deficient, and immunocompetent mice to investigate how *B. burgdorferi* adapts to various immune environments. B-cell-deficient and wild-type mice were infected for 4 months to allow immune responses to fully develop and *B. burgdorferi* to adapt. As a control, SCID mice were infected for 4 months. Gene expression was investigated and is presented in Fig. 3. Of the four

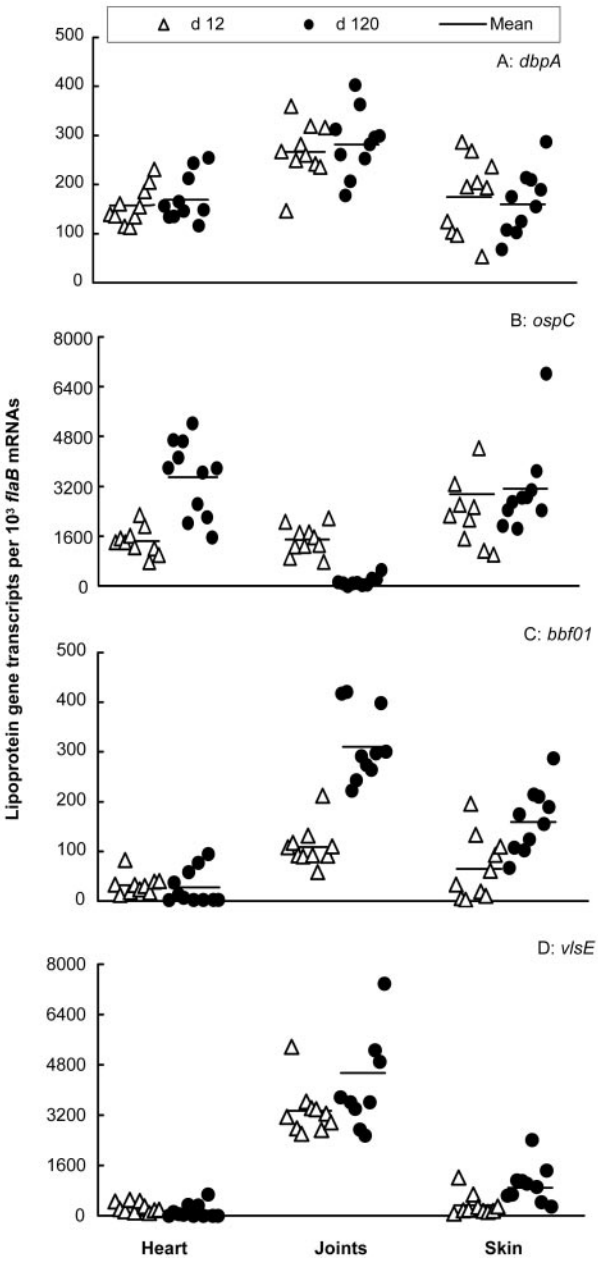

FIG. 1. Tissue milieus modulate spirochetal antigenic profiles. Twenty SCID mice were infected with *B. burgdorferi* and euthanized at either 12 days ($n = 10$) or 4 months ($n = 10$). RNA samples were prepared from heart, joint, and skin tissues and converted to cDNA. *flaB*, *dbpA*, *ospC*, BBF01, and *vlsE* expression levels were analyzed by qPCR and presented as *dbpA*, *ospC*, BBF01, and *vlsE* mRNA copy numbers per 10^3 *flaB* mRNA copies.

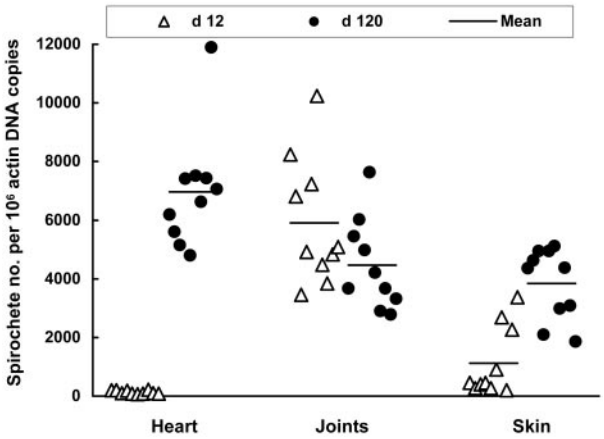

FIG. 2. Tissue spirochetal burdens in the absence of immune pressure. DNA samples were prepared from the heart, joints, and skin of the 20 SCID mice that were used to generate data for Fig. 1. Spirochetal *flaB* and mouse actin DNAs were quantified by qPCR. The data are expressed as *flaB* DNA copy numbers per 10^6 actin DNA copies.

lipoprotein genes examined, only expression of *dbpA* was not affected by adaptive immune pressure. The presence of cellular immunity significantly reduced *ospC* expression in both the joints ($P < 0.01$) and skin ($P = 1.2 \times 10^{-4}$) and BBF01 expression in the joints ($P = 2.7 \times 10^{-3}$) and increased BBF01 and *vlsE* expression in both the heart ($P = 4.1 \times 10^{-4}$ and 2.7×10^{-4}, respectively) and skin ($P < 0.02 \times 10^{-5}$ and 7.6×10^{-5}, respectively). The most dramatic adaptation occurred under the influence of both humoral and cellular immune responses. *B. burgdorferi* decreased *ospC* expression 388, 9.3, and 446 times in the heart ($P = 7.7 \times 10^{-8}$), joint ($P = 2.9 \times 10^{-3}$), and skin ($P = 1.9 \times 10^{-6}$) tissues of wild-type mice compared to SCID mice, respectively. In contrast, adaptive immune pressure increased BBF01 expression 20 and 1.8 times in the heart ($P = 5.8 \times 10^{-8}$) and skin ($P = 6.4 \times 10^{-3}$), respectively, and *vlsE* expression 32 and 4.1 times in these two tissues ($P = 5.3 \times 10^{-8}$ and 0.02), respectively. *B. burgdorferi* significantly reduced both BBF01 ($P = 2.6 \times 10^{-3}$) and *vlsE* ($P = 0.023$) expression in the joints of wild-type mice. Overall, adaptive immune pressure substantially changed the surface antigenic expression of *B. burgdorferi*, given the fact that these four lipoproteins are surface-exposed antigens.

Although the presence of T cells significantly affected *B. burgdorferi* adaptation, cellular immunity was not able to control the tissue spirochetal burdens in the heart ($P > 0.68$) and joints ($P > 0.70$) (Fig. 4). T cells did reduce the bacterial loads of the skin tissue by 41% ($P < 0.003$). In contrast, humoral immunity played the dominant role in controlling tissue spirochetal burdens, leading to 258-, 24-, and 34-fold reductions in the heart ($P = 1.9 \times 10^{-9}$), joint ($P = 7.4 \times 10^{-8}$), and skin ($P = 1.7 \times 10^{-8}$) tissues, respectively, of wild-type mice compared to SCID mice.

Anti-*Borrelia* antibodies change spirochetal surface antigenic expression. To examine whether specific antibodies in the absence of B and T cells are able to modify the surface antigenic expression and to reduce the tissue spirochetal burdens, antiserum transfer was conducted. SCID mice were infected with *B. burgdorferi* for 4 months, an interval in which the

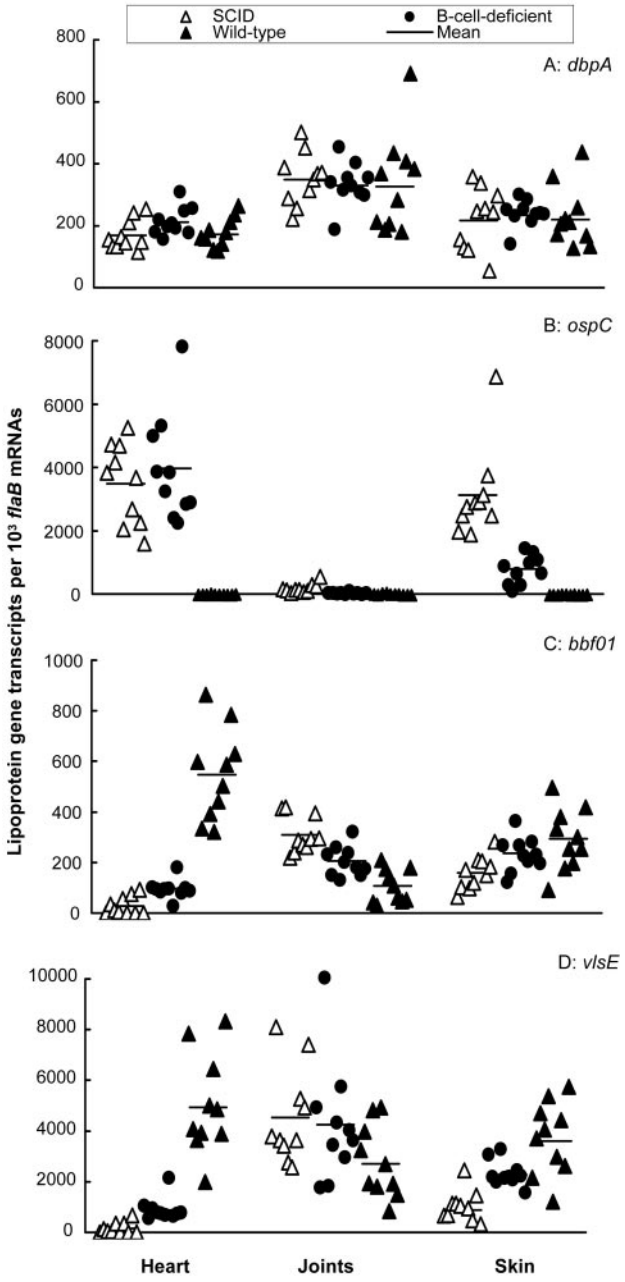

FIG. 3. Immune responses change surface antigenic expression. A group of 10 SCID, B-cell-deficient, or wild-type mice were infected for 4 months. RNA samples were prepared from heart, joint, and skin tissues and converted to cDNA. *flaB*, *dbpA*, *ospC*, BBF01, and *vlsE* expression levels were analyzed by qPCR and presented as *dbpA*, *ospC*, BBF01, and *vlsE* mRNA copy numbers per 10^3 *flaB* mRNA copies.

spirochetes had adapted to the host, and then passively immunized with either prebled mouse sera as a control or anti-*Borrelia* sera. Like the immune pressure generated in the immunocompetent mice, passive transfer did not influence *dbpA* expression (Fig. 5). The administration of specific antibodies altered the spirochetal surface antigenic expression similarly to what had been noted in immunocompetent mice. These included decreased *ospC* expression in the heart (76-fold, $P =$

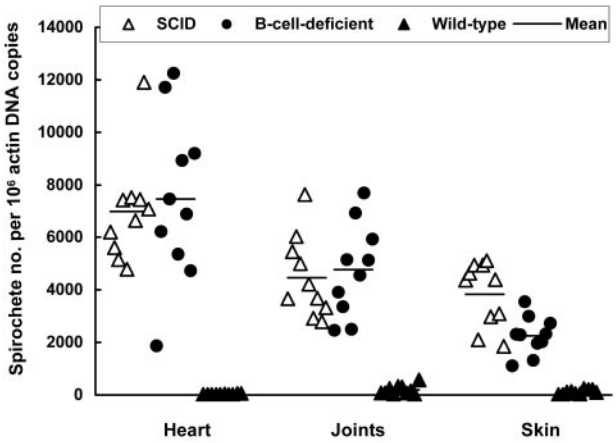

FIG. 4. Influence of immune pressure on tissue spirochetal burdens. DNA samples were prepared from the heart, joints, and skin of the SCID ($n = 10$), B-cell-deficient ($n = 10$), and wild-type ($n = 10$) mice that were used to generate data for Fig. 3. Spirochetal *flaB* and mouse actin DNAs were quantified by qPCR. The data are expressed as *flaB* DNA copy numbers per 10^6 actin DNA copies.

4.5×10^{-7}), joint (4-fold, $P = 0.04$), and skin (58-fold, $P = 0.001$) tissues and increased BBF01 and *vlsE* expression in both the heart (17- and 44-fold; $P = 1.7 \times 10^{-6}$ and 0.02, respectively) and skin (3.5- and 6-fold, respectively; $P = 0.01$ and 1.6×10^{-8}, respectively) tissues. However, the transferred specific antiserum was not able to reduce the expression of either BBF01 ($P = 0.20$) or *vlsE* ($P = 0.45$) in the joint tissue.

The specific antisera also significantly decreased the tissue spirochetal burdens (Fig. 6). These included the tissue spirochetal reductions of 81, 12, and 17 times in the heart ($P = 4.2 \times 10^{-9}$), joints ($P = 5.0 \times 10^{-6}$), and skin ($P = 9.1 \times 10^{-5}$), respectively. The passive transfer (Fig. 6) was not as effective as the immune pressure developing in the immunocompetent mice in controlling the tissue spirochetal burden, probably because the procedure could not provide an antibody titer as high as the immune response induced by infection.

OspC monoclonal antibody changes spirochetal surface antigenic expression. Our previous study has shown that OspC antibody selects against OspC-expressing spirochetes in the skin tissue (27). In the present study, we examined whether the specific antibody changed expression of other lipoprotein genes and tissue spirochetal burdens. SCID mice were infected with *B. burgdorferi* for 4 months and passively immunized with either mouse IgG2a as a control or OspC monoclonal antibody. The antibody decreased *ospC* expression 2.5, 26, and 158 times in the heart ($P = 0.001$), joints ($P = 0.003$), and skin ($P = 9.5 \times 10^{-8}$), respectively (Fig. 7). Although *dbpA* expression was not affected in the transfer study, the passive immunization significantly increased BBF01 transcription by 18, 1.5, and 5.5 times in the heart ($P = 0.002$), joints ($P = 0.003$), and skin ($P = 3.0 \times 10^{-6}$), respectively. To respond to the attack by OspC antibody, *B. burgdorferi* also increased *vlsE* expression 20 times both in the heart ($P = 0.02$) and in the skin ($P = 1.6 \times 10^{-8}$) but not in the joints ($P > 0.42$).

Although OspC antibody effectively reduced *ospC* expression in the heart, joints, and skin, it significantly reduced the tissue spirochetal burdens only in the heart (125-fold, $P = 8.4$

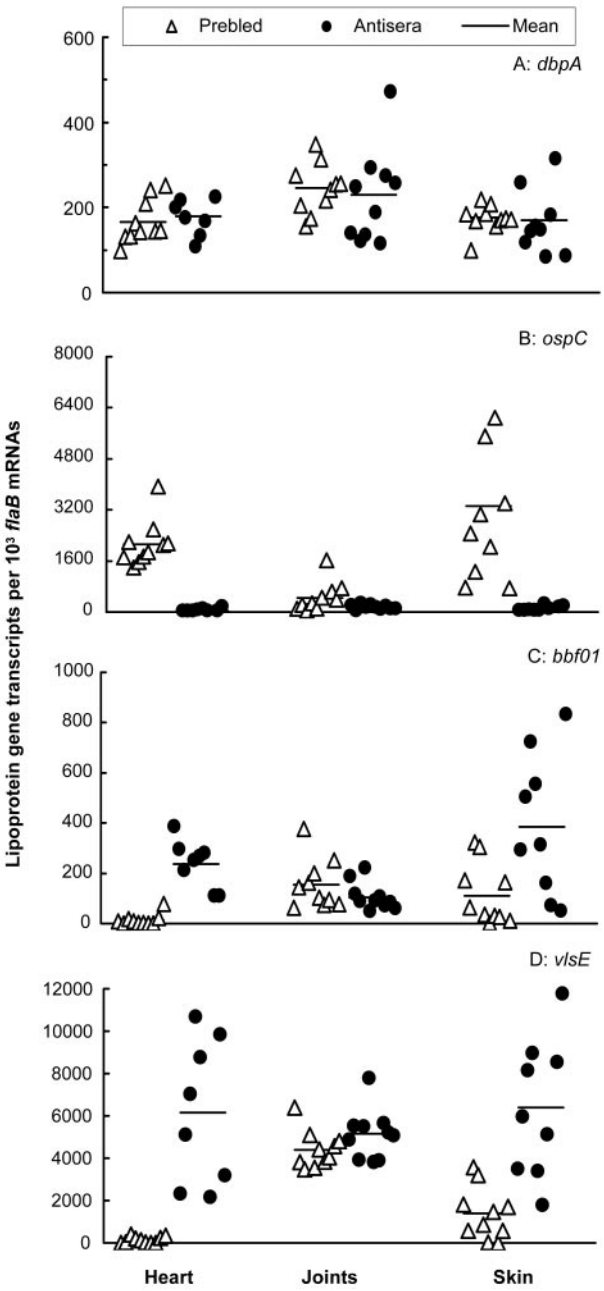

FIG. 5. Anti-*Borrelia* antibodies change surface antigenic expression. Twenty SCID mice were infected with *B. burgdorferi* for 4 months and then passively immunized with prebled mouse sera ($n = 10$) or murine anti-*Borrelia* antisera ($n = 10$). RNA samples were prepared from heart, joint, and skin tissues and converted to cDNA. *flaB*, *dbpA*, *ospC*, BBF01, and *vlsE* expression levels were analyzed by qPCR and presented as *dbpA*, *ospC*, BBF01, and *vlsE* mRNA copy numbers per 10^3 *flaB* mRNA copies. Spirochetal mRNA was not detectable in two heart samples and one skin sample of the passively immunized group, consistent with the results of no spirochetal DNA being detected in these tissues.

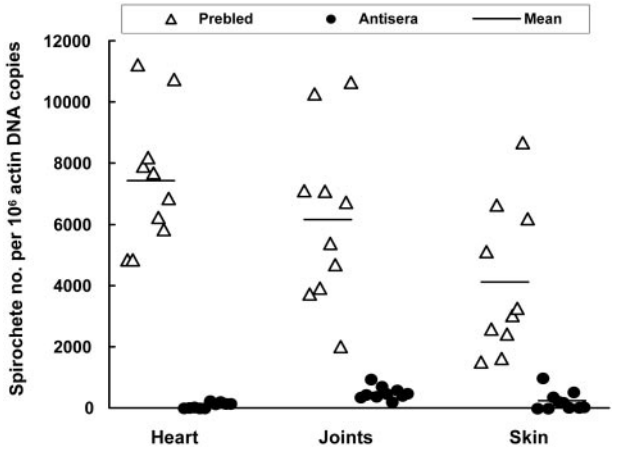

FIG. 6. Influence of anti-*Borrelia* sera on tissue spirochetal burdens. DNA samples were prepared from the heart, joints, and skin of the 20 SCID mice that were used to generate data for Fig. 5. Spirochetal *flaB* and mouse actin DNAs were quantified by qPCR. The data are expressed as *flaB* DNA copy numbers per 10^6 actin DNA copies.

$\times 10^{-12}$). There were no significant differences in the spirochete number in the joints ($P = 0.70$) and skin ($P = 0.19$) between the two treatment groups (Fig. 8).

DISCUSSION

The present study examined the influence of various in vivo conditions on the expression of four prototypic surface lipoprotein antigens and showed that the tissue milieus shape the surface antigenic expression of *B. burgdorferi*, especially in the immune environment. In the absence of immune pressure, *B. burgdorferi* presented itself in multiple phenotypes depending on the tissues where it resided and the time courses over which infection persisted. For instance, *B. burgdorferi* increased *ospC* expression 2.4 times in the heart from 12 days to 4 months postinfection while it reduced *ospC* expression 8.0-fold in the joints. In contrast, spirochetes increased BBF01 expression 2.8 times in the joints during the same period. The immune response is an overwhelming force that may select for spirochetes with a modified surface antigenic expression in all the tissues, and these alterations may help *B. burgdorferi* evade immune attack. The study also examined the role of both humoral and cellular immune responses in controlling the tissue spirochetal burdens. Although cellular immunity does not directly kill extracellular bacteria, it triggers inflammatory responses that are harmful to foreign invaders and recruits and activates phagocytes to clear infection. Our study indicates that cellular immunity affects the tissue spirochetal burdens only in the skin. Humoral immunity, in contrast, is able to effectively reduce the tissue spirochetal burdens in all of the tissues examined.

Among the four lipoprotein genes examined, *dbpA* appeared to be the only one that was constantly expressed in all the tissues regardless of immune pressure, suggesting that DbpA may be important for mammalian infection and/or not amenable to host antibodies in vivo. Alternatively, *B. burgdorferi* may lack the ability to regulate this gene during murine infection, although evidence indicates that it is coregulated with *ospC*

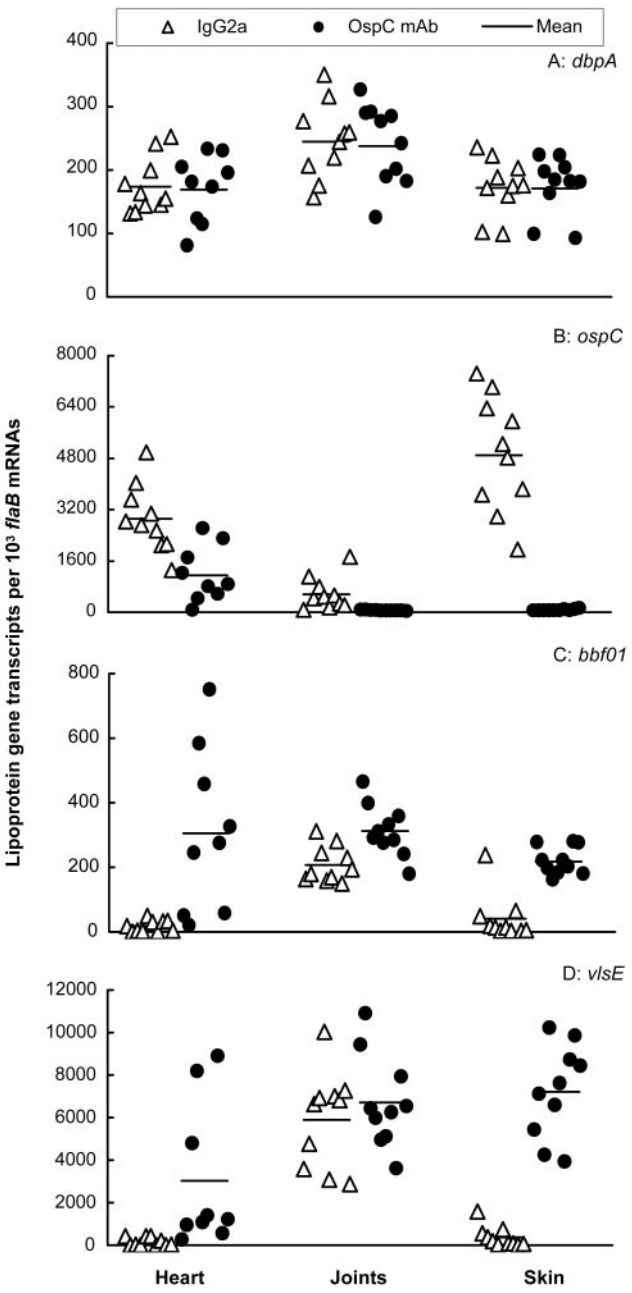

FIG. 7. OspC monoclonal antibody changes surface antigenic expression. Twenty SCID mice were infected with *B. burgdorferi* for 4 months and then passively immunized with either mouse IgG2a ($n = 10$) or OspC monoclonal antibody ($n = 10$). RNA samples were prepared from heart, joint, and skin tissues and converted to cDNA. *flaB*, *dbpA*, *ospC*, BBF01, and *vlsE* expression levels were analyzed by qPCR and presented as *dbpA*, *ospC*, BBF01, and *vlsE* mRNA copy numbers per 10^3 *flaB* mRNA copies. Spirochetal mRNA was not detectable in one heart specimen of the passively immunized group, consistent with the result of no spirochetal DNA being detected in this tissue.

during in vitro cultivation (21). *ospC* was abundantly expressed in both heart and skin in the absence of adaptive immune pressure. Unlike *ospC*, both BBF01 and *vlsE* were more actively expressed in the joints than in the heart and skin. In fact, *B. burgdorferi* transcribed both BBF01 and *vlsE* below a detect-

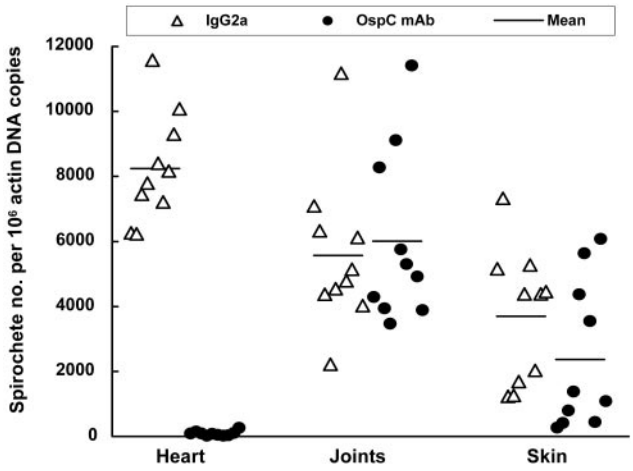

FIG. 8. OspC monoclonal antibody selectively reduces tissue spirochetal burdens in the heart. DNA samples were prepared from the heart, joints, and skin of the 20 SCID mice that were used to generate data for Fig. 7. Spirochetal *flaB* and mouse actin DNAs were quantified by qPCR. The data are expressed as *flaB* DNA copy numbers per 10^6 actin DNA copies.

able level in some of the heart and skin specimens, suggesting that these two genes are not essential for in vivo survival if adaptive immune pressure is absent. This is consistent with previous studies that show that plasmid lp28-1, which carries both BBF01 and *vlsE* (16), is required for persistent infection only of immunocompetent mice (24, 41, 50) and not of SCID mice (23, 40). These previous studies demonstrate that lp28-1 is unnecessary for in vivo survival of *B. burgdorferi* if adaptive immune pressure does not exist. Therefore, some or all of the genes on lp28-1 may play a critical role in immune evasion. Without them, *B. burgdorferi* would be cleared before a persistent infection could be established in immunocompetent mice. Although lp28-1 carries 32 open reading frames, BBF01 and *vlsE* may be the only two unique and functional genes, since the rest are either paralogues found on other borrelial genetic elements, frameshifted genes, or pseudogenes or encode less than 100 amino acids (16). Thus, these are the two most likely lipoprotein genes on lp28-1 that participate in the establishment of chronic infection in the immune environment. Interestingly, the present study showed that *B. burgdorferi* was able to increase expression of these two antigens by 32 times in both heart and skin tissues under adaptive immune selection pressure. Their expression levels were less affected in the presence of an immune response in the joints, probably because their transcriptional levels were already high in the absence of immune pressure.

B. burgdorferi VlsE undergoes vigorous antigenic variation during mammalian infection (55) and thus may not be easily targeted by protective antibodies. Not just the primary but the secondary and tertiary structures of VlsE are available (12). Its antigenic structure has also been extensively investigated (25, 26, 31). VlsE contains two invariable domains at the N and C termini that surround a central variable domain that consists of six variable regions and six invariable regions (25, 54). Both the invariable domains and regions are not exposed at the surface of the intact spirochete and thus cannot serve as targets of antibody when the bacterium is alive (12, 25, 28, 30). At least the C-terminal domain and two invariable regions are immunodominant and may serve as decoy epitopes to divert immune responses away from the variable regions (25, 28). Even so, a weak humoral response to the invariable regions is indeed detected during murine infection (35). This may be the reason why *vlsE* expression was somewhat reduced in the joints of infected immunocompetent mice compared with SCID mice. The transferred anti-*Borrelia* sera were not able to significantly reduce *vlsE* expression of the joint tissue, probably because the introduced specific antibodies had lower specificities for the variable regions of VlsE or contained an insufficient titer. OspC antibody did not affect *vlsE* expression at all in the joints since the preparation was free of other antibodies. However, it remains unaddressed whether antibodies to the variable regions of VlsE are bactericidal in the murine host.

The function of BBF01 is unknown. A previous study has shown that BBF01 antibody is able to reduce the severity of arthritis in infected SCID mice (13). However, the nature of BBF01 antibody activity remains to be addressed since the specific antibodies do not protect mice from infection nor affect the tissue spirochetal burdens (14). Although the present study cannot conclude if specific antibodies are able to target BBF01 in the heart and skin since the expression of this lipoprotein increased under immune pressure in these two tissues, our data indicate that the antigen may be targeted in the joints since the gene's transcription was significantly reduced by the immune response generated in immunocompetent mice. The transferred anti-*Borrelia* sera were not able to effectively reduce BBF01 expression, but OspC antibody significantly increased its expression in the joints, also suggesting that BBF01 may be targeted in this tissue. Anti-*Borrelia* sera did not significantly reduce BBF01 expression, probably because the passive transfer could not provide a sufficient anti-BBF01 titer, while OspC antibody increased BBF01 expression, probably because it could not confer immune selection pressure against BBF01-expressing spirochetes. BBF01 expression may be more protected against adaptive immune pressure in the skin and heart than in the joints, since this gene was more actively expressed in these two tissues than in the joints in the presence of adaptive immune pressure. There has been no evidence that BBF01 undergoes antigenic variation. The expression of this lipoprotein was elevated against adaptive immune pressure probably due to the existence of a host ligand that can interact with the antigen. Such interactions may interfere with specific antibody binding to the antigen. A differential expression of such ligands in different tissues may lead to varying resistance of BBF01 expression against immune-mediated selection. Extensive studies demonstrate that DbpA binds host ligands such as decorin (6, 18). This is consistent with the present study, which showed that *dbpA* expression was not significantly influenced by the immune response. In contrast, we have found that the interactions of DbpA with host decorin somewhat protect the lipoprotein's expression in the joint tissue of chronically infected wild-type mice compared to decorin-deficient mice (F. T. Liang et al., unpublished data).

In the absence of adaptive immune pressure, *B. burgdorferi* presents itself in different phenotypes depending on the tissues where it is nourished. For instance, OspC expressers dominate in the heart and skin while BBF01 and VlsE expressers flourish

in the joints. In response to immune pressure, *B. burgdorferi* phenotypes that express antibody-targeted antigens such as OspC may be selected against, and spirochetes that express non-antibody-targeted antigens, exemplified by VlsE and BBF01, may be more likely to persist. This antigenic adaptation appears to be essential for the spirochetes to survive as the host immune response develops and to proceed to persistent infection. The spirochetal phenotype with a high BBF01 and VlsE expression and a lower OspC expression dominates in all the tissues examined during chronic infection and thus may be the resistant form of *B. burgdorferi* in the hostile immune environment. It remains to be addressed whether this surface antigenic modification is solely due to spirochetal phenotypic changes.

ospC was more abundantly expressed in both the heart and skin than in the joints in the absence of immune pressure. OspC antibody virtually cleared OspC but was not able to significantly reduce the spirochetal burdens in the skin and joints, suggesting that this lipoprotein may not be required for the normal survival of *B. burgdorferi* in these tissues. In contrast, OspC antibody nearly cleared infection in the heart, suggesting that this antigen may be essential for spirochetal survival in this tissue. However, the present study cannot rule out the possibility that the unique niche of the heart tissue may persistently induce *ospC* expression and thus make *B. burgdorferi* more susceptible to the antibody, even though OspC is not required for the survival in this tissue.

The present study has clearly demonstrated that humoral immune pressure reduces *ospC* expression and increases BBF01 and *vlsE* expression to generate spirochetal phenotypes that are most likely to resist immune attack. To date, we can quantify only in vivo gene expression at the spirochetal population level in each selective tissue milieu and cannot examine gene expression by individual spirochetes within tissues. Immune selection of certain spirochetal phenotypes facilitates persistent infection, and the exact molecular mechanisms that govern the modification of the spirochetal surface antigenic expression in response to immune attack remain to be addressed.

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