

## **Molecular Adaptation of *Borrelia burgdorferi* in the Murine Host**

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### **Abstract**

An analysis of expression of 137 lipoprotein genes on the course of murine infection revealed a two-step molecular adaptation by *Borrelia burgdorferi*, the Lyme disease spirochete. For the first step, regardless whether the initial inocula of *B. burgdorferi* expressed either all (cultured spirochetes) or less than 40 (host-adapted spirochetes) of the 137 lipoprotein genes, the spirochetes were modulated to transcribe 116 of the genes within 10 d after being introduced to the murine host. This step of adaptation was induced by the microenvironment of the host tissue. During the second step, which was forced by host immune selection pressure and occurred between 17 and 30 d after infection, *B. burgdorferi* down-regulated most of the lipoprotein genes and expressed less than 40 of the 137 genes. This novel adaptation mechanism could be a critical step for *B. burgdorferi* to proceed to chronic infection, as the pathogen would be cleared at the early stage of infection if the spirochetes failed to undergo this process.

Key words: Lyme disease • immune evasion • lipoprotein • gene expression • DNA microarray

### **Introduction**

*Borrelia burgdorferi*, the Lyme disease spirochete, is maintained within a complex enzootic life cycle involving the tick vector and the mammal. The spirochete adapts to these diverse environments, in part, by selective gene expression. Environmental cues such as temperature (1, 2), pH (2, 3), and nutrients or chemicals (4, 5) 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 (6). The most dramatic modifications that occur as *B. burgdorferi* migrates from ticks to the mammalian host involve lipoprotein gene expression, such as the down-regulation of outer surface protein A (OspA) and the up-regulation of OspC (7, 8). The genome of *B. burgdorferi* contains more than 150 open-reading frames that encode putative lipoproteins, more than any other known bacterium (9), and many are surface exposed or have direct contact with the environment. These lipoproteins play major roles in pathogenesis and immunity and are likely to be involved in *B. burgdorferi* adaptation through the spirochete life cycle.

### **Materials and Methods**

**Spirochete Isolate.** *B. burgdorferi* B31 clone 5A11 (a gift from Steven Norris, University of Texas, Houston, TX) was cultivated in Barbour-Stoenner-Kelly H complete medium at 33°C (Sigma-Aldrich). This isolate carries 21 linear and circular plasmids (10).

**Mouse Inoculation with Cultured Spirochetes.** Both C3H/HeN (C3H) and C3H/HeN-SCID (SCID) mice (4 to 6-wk-old; National Institutes of Health, Bethesda, MD) were given one single intradermal injection of 10<sup>5</sup> spirochetes that were grown to stationary phase. Mice were killed at intervals of 1 to 8 wk, starting at 10 d after needle inoculation. Ears and skins were frozen immediately in liquid nitrogen for RNA preparation.

**Mouse Inoculation with Host-adapted Spirochetes.** Donor C3H mice were infected by needle inoculation for 4 mo. Ear tissues were cut into small pieces and implanted into the skin at the back of naive C3H mice. Infected mice were killed at 11 to 40 d after infection. Ears and skins were frozen in liquid nitrogen for RNA preparation.

**RNA Preparation from Cultured Spirochetes and Mouse Tissues.** Total RNA was purified from spirochetes grown to mid-logarithmic phase or mouse ears and skins using the RNeasy Mini Kit (QIAGEN). To remove DNA contamination, RNA samples were digested in solution with RNase-free DNase I (Life Technologies) at 37°C for 3 h, and then loaded to the RNeasy Mini columns and further treated with RNase-free DNase (QIAGEN) for an additional 30 min at room temperature. Digested samples were repurified and analyzed for potential DNA contamination by PCR.

**cDNA Preparation.** RNA preparations were first annealed with reverse oligonucleotide primers of *flaB* and all the 137 investigated lipoprotein genes in the presence of reverse transcription (RT) buffer (Stratagene). All primer sequences are available

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by request. dNTPs and reverse transcriptase were added (Stratagene) and transcription was conducted at 42°C for 1 h. RNA-cDNA hybrids were denatured at 95°C for 5 min and RNA chains were hydrolyzed by digestion with 1.0 N NaOH at 65°C for 15 min. After neutralization with 1.0 N HCl, cDNA was purified using the Quik PCR Product Purification Kit (QIAGEN).

**PCR.** For each PCR reaction volume of 50 µl, cDNA that was converted from 25 ng of total spirochetal RNA, 250 ng RNA prepared from SCID mice with acute or chronic infection or from C3H mice with acute infection, or 2,500 ng RNA from C3H mice with chronic infection was applied. The amount of cDNA that was used for each PCR reaction was determined by twofold serial dilution PCR of the *flaB* gene.

**Southern Blotting Confirmation of PCR Products by Microarray Hybridization.** All PCR products were assessed by agarose gel electrophoresis. Those that showed expected sizes were combined and purified using the Quik PCR Product Purification Kit (QIAGEN). Purified DNAs were incorporated with aminoallyl dUTP by nick translation (Life Technologies), fluorescently labeled with the Alexa Fluor 647 kit (Molecular Probes, Inc.), and allowed to hybridize to the lipoprotein DNA microarray (11). The array was scanned with an Axon GenePix 4000A Array Scanner (Axon Instruments, Inc.).

**Sequencing of PCR Products.** PCR products of selective genes were gel-purified using the QIAquick Gel Extraction Kit (QIAGEN). Purified DNAs were sequenced.

**Passive Immunization of SCID Mice with Anti-*B. burgdorferi* Antisera.** SCID mice were infected with cultured *B. burgdorferi* B31 and received three subcutaneous injections of 100-µl pooled mouse antiserum or prebled (control) at 11, 13, and 15 d. Antisera were collected from C3H mice that had been infected with cultured spirochetes for 1 to 6 mo, while prebled was drawn from naive C3H mice. SCID mice were killed at 2 d after the last passive immunization and ear tissues were frozen in liquid nitrogen for RNA preparation.

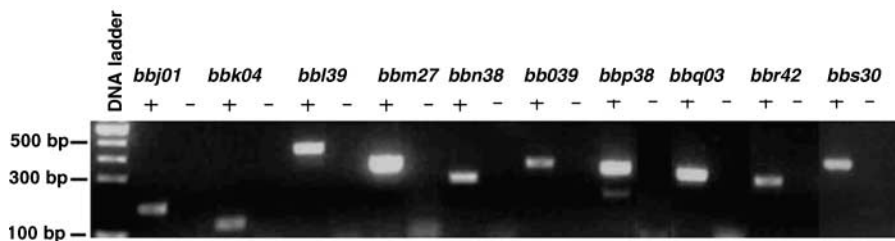
## Results

**Lipoprotein Gene Expression during In Vitro Cultivation.** To investigate the molecular adaptation of *B. burgdorferi* in different environments, we first examined the expression of 137 putative lipoprotein genes during in vitro cultivation. Total RNA was prepared from cultured spirochetes. To determine if any DNA contamination was present, following two DNase digestion treatments, the RNA preparation was amplified by both PCR and RT-PCR using each primer pairs of the first putative lipoprotein genes carried by all of the 20 lipoprotein-carrying genetic elements: one chromosome and 19 plasmids (9). Both RT-PCR and PCR products were assessed by agarose gel electrophoresis. The results obtained from plasmids J (lp38), K (lp36), L (cp32-8), M

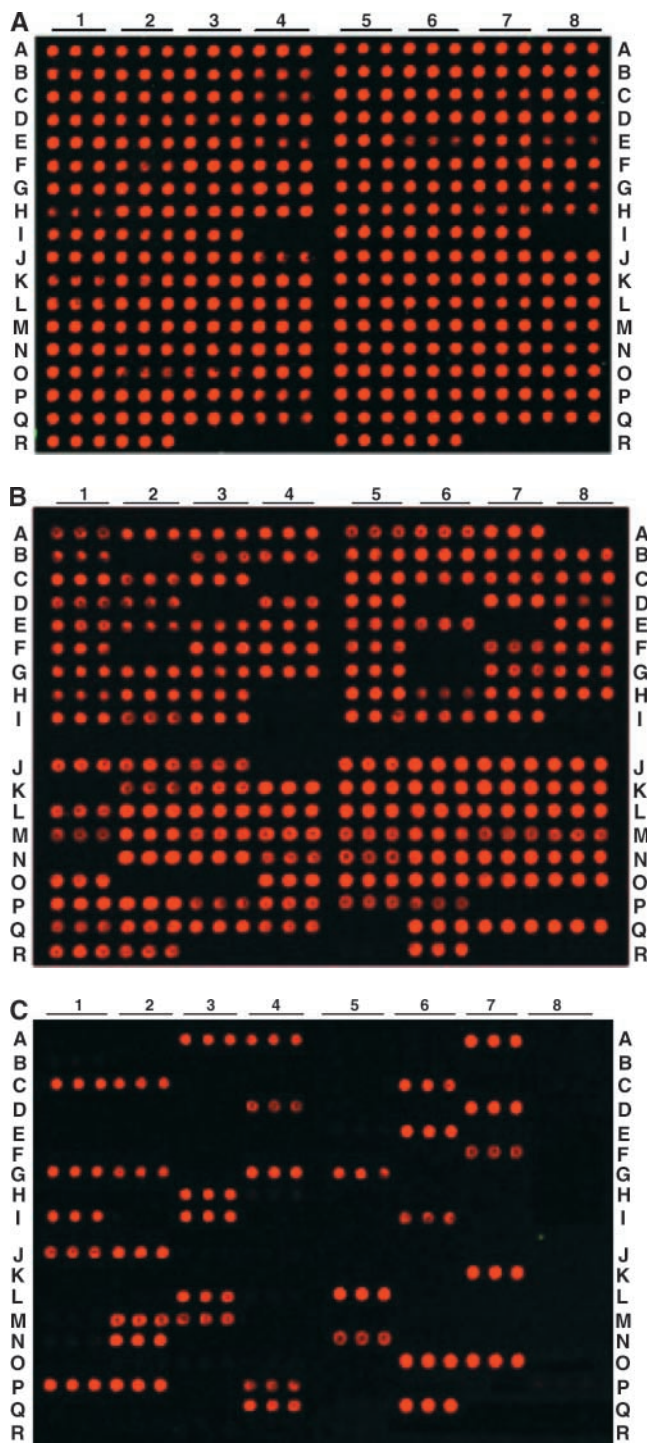
(cp32-6), N (cp32-9), O (cp32-7), P (cp32-1), Q (lp56), R (cp32-4), and S (cp32-3) were selectively presented in Fig. 1. The RT-PCR products only showed amplicons with expected sizes, indicating that DNA contamination was not detectable. Then, the 117 remaining lipoprotein genes were individually amplified by RT-PCR, and the *flaB* gene was used as a control and also amplified. Resultant products were assessed by agarose gel electrophoresis and all showed the expected sizes. To more definitively examine the gene expression, *flaB* and all 137 lipoprotein PCR products were combined, purified, fluorescently labeled, and allowed to hybridize to a lipoprotein DNA microarray. In vitro cultivated spirochetes expressed virtually all of these 137 (putative) lipoprotein genes (Fig. 2 A). These data were confirmed in a separate experiment.

**Lipoprotein Gene Expression on the Course of Infection in Immunodeficient Mice.** We next examined lipoprotein gene expression in infected SCID mice. 10 animals were infected with cultured *B. burgdorferi* and killed at 10, 17, 24, 31, 45, 60, 75, 100, 130, and 180 d. Total RNA was prepared and amplified by RT-PCR. PCR products were analyzed by agarose gel electrophoresis and microarray hybridization. *B. burgdorferi* quickly down-regulated 21 of the lipoprotein genes within 10 d after introduction into the immunodeficient host (Fig. 2 B). These down-regulated genes included *bb0098*, *bb0424*, *bb0464*, *bb0475*, *bb0664*, *bb0740*, *bb0840*, *bba15*, *bba62*, *bbb08*, *bbe06*, *bbe08*, *bbe28*, *bbg25*, *bbh32*, *bbi28*, *bbi32*, *bbk04*, *bbo40*, *bbq05*, and *bbr42*. The 116 remaining genes were persistently transcribed throughout the entire 6-mo study period, as their mRNA transcripts were detectable by RT-PCR in all of the nine mice that were killed at 17 to 180 d after infection (data not shown). Neither *bbo40* (*erpM*) or *bbr42* (*erpY*) amplicons were revealed by agarose gel electrophoresis but these genes' DNA spots were reactive on the microarray (Fig. 2 B), probably due to the cross-hybridization of other *erp* gene PCR products with these genes' DNA fragments on the microarray. The sequences of the *erp* family share high percentages of identity (9). Although the *ospA* (*bba15*) mRNA transcript was not detected, surprisingly, *ospB* (*bba16*) was continuously expressed throughout infection. The *ospB* PCR product was sequenced to confirm that this gene was transcribed (data not shown).

**Lipoprotein Gene Expression on the Course of Infection in Immunocompetent Mice.** As immune selection pressure could influence the gene expression of surface-exposed, Ab-targeted lipoproteins (12), we investigated *B. burgdorferi* lipoprotein expression under immune selection pressure. RNA



**Figure 1.** Amplification of selected *B. burgdorferi* lipoprotein genes that are expressed on the plasmids. Total RNA was prepared from cultured *B. burgdorferi* and analyzed by both PCR (-) and RT-PCR (+). PCR products were separated by ethidium bromide-incorporated agarose gel, and the lack of a product in the (-) column demonstrated that DNA was not present in the RNA preparation.



**Figure 2.** Lipoprotein gene expression of *B. burgdorferi* in in vitro cultivation (A), infected SCID mice (B), and C3H mice (C). *flaB* and 137 lipoprotein gene fragments were amplified from *B. burgdorferi* B31 DNA and printed in triplicate on the microarray. The location of each DNA fragment was listed on Fig. 3, and locations I4, I8, R3, R4, R7, and R8 were blank. RNA was prepared from cultured spirochetes (A), the ear tissues of SCID mice that had been infected with cultured *B. burgdorferi* for 10 d (B), or the ear tissues of C3H mice that had been infected with cultured *B. burgdorferi* for 24 d (C), and amplified by RT-PCR. Purified PCR products were fluorescently labeled and hybridized to the microarray.

was prepared from 10 immunocompetent C3H mice that were infected with cultured spirochetes for 10 to 180 d, and analyzed by RT-PCR and microarray hybridization. *B. burgdorferi* expressed a similar set of lipoprotein genes as detected in infected SCID mice (Fig. 2 B) when the mouse was examined at 10 d after infection. At 17 d, however, most of the 116 lipoprotein genes that were expressed at 10 d were down-regulated by immune selection pressure elicited by infection (data not shown). Less than 40 of the lipoprotein genes were continuously expressed when infected C3H mice were examined at 24, 31, 45, 60, 73, 100, 130, and 180 d after infection (Fig. 2 C). These results demonstrated that early and chronic infections constitute two remarkably different environments for *B. burgdorferi* adaptation and the development of immune selection pressure in the host significantly impacts lipoprotein gene expression.

**Molecular Adaptation of *B. burgdorferi* during Murine Infection.** To further investigate the influence of immune selection pressure on *B. burgdorferi* adaptation, spirochetes that had been adapted under immune selection pressure were used for inoculation. Donor mice were first infected with cultured *B. burgdorferi*. 4 mo later, after the spirochetes had adapted and expressed less than 40 lipoprotein genes (Fig. 3), ear tissues were taken from the donors and transplanted into nine naive immunocompetent mice. *B. burgdorferi* re-expressed all of the 83 down-regulated lipoprotein genes when five recipient mice were examined at 11 to 22 d after tissue transplantation (Fig. 3). This reexpression persisted for ~2 wk. *B. burgdorferi* redown-regulated all of these re-expressed genes within 5 wk after the initial transplantation (Fig. 3), as their mRNA transcripts became undetectable when four recipient mice were examined at 33 d or later. These results further confirmed the influence of immune pressure on *B. burgdorferi* adaptation.

It took 5 wk for host-adapted *B. burgdorferi* to finish the adaptation to the immune selection pressure but only 3.5 wk for the cultured spirochetes to complete the same process (Figs. 2 C and 3). The temporal difference may be due to the fact that cultured *B. burgdorferi* expressed all of the lipoproteins while the host-adapted spirochetes had down-regulated all of the adaptable genes and thus must re-expresses to trigger the immune response. In addition, the spirochete inoculation dose may also be a factor as the tissue implantation introduced fewer organisms than the needle inoculation.

**Dominant Role of the Humoral Response in *B. burgdorferi* Adaptation.** *B. burgdorferi* is primarily an extracellular pathogen and the humoral response has been shown to play a critical role in immunity and disease regression. To carefully examine the role of Abs in *B. burgdorferi* molecular adaptation, four infected SCID mice were passively immunized with antisera from infected immunocompetent mice and two received normal mouse sera as controls. Almost all the lipoprotein genes that were down-regulated by immune selection pressure in immunocompetent mice were no longer expressed in the four passively immunized SCID mice but expressed in the two controls (data not shown). Several of the genes, however, were not down-regulated in the passive

Gene	Location	Donor mouse	Recipient mouse			Gene	Location	Donor mouse	Recipient mouse		
			d 11	d 22	d 33				d 11	d 22	d 33
bb0003	A5	-	+	+	-	bba25	O7	+	+	+	+
bb0028	J5	-	+	+	-	bba33	F8	-	+	+	-
bb0038	A6	-	+	+	-	bba34	O8	-	+	+	-
bb0070	J6	-	+	+	-	bba36	D1	-	+	+	-
bb0071	A7	+	+	+	+	bba57	M1	-	+	+	-
bb0088	J7	-	+	+	-	bba59	D2	-	+	+	-
bb0098	A8	-	+	+	-	bba60	M2	+	+	+	+
bb0100	J8	-	+	+	-	bba62	D3	-	-	-	-
bb0122	B5	-	+	+	-	bba64	M3	+	+	+	+
bb0141	K5	-	+	+	-	bba65	D4	+	+	+	+
bb0142	B6	-	+	+	-	bba66	M4	-	+	+	-
bb0155	K6	-	+	+	-	bba73	E1	-	+	+	-
bb0185	B7	-	+	+	-	bbb08	N1	-	-	-	-
bb0213	K7	+	+	+	+	bbb09	E2	-	+	+	-
bb0224	B8	-	+	+	-	bbb14	N2	+	+	+	+
bb0227	K8	-	+	+	-	bbb16	E3	-	+	+	-
bb0298	C5	-	+	+	-	bbb19	N3	-	+	+	-
bb0300	L5	+	+	+	+	bbb27	E4	-	+	+	-
bb0309	C6	+	+	+	+	bbc10	N4	-	+	+	-
bb0321	L6	-	+	+	-	bbd10	F1	-	+	+	-
bb0324	C7	-	+	+	-	bbd24	O1	-	+	+	-
bb0341	L7	-	+	+	-	bbe06	F2	-	-	-	-
bb0352	C8	-	+	+	-	bbe08	O2	-	-	-	-
bb0365	L8	-	+	+	-	bbe09	F3	-	+	+	-
bb0372	A1	-	+	+	-	bbe28	O3	-	-	-	-
bb0382	J1	+	+	+	+	bbe31	F4	-	+	+	-
bb0383	A2	-	+	+	-	bbf20	O4	-	+	+	-
bb0384	J2	+	+	+	+	bbf32	G5	+	+	+	+
bb0385	A3	+	+	+	+	bbq02	P5	-	+	+	-
bb0387	J3	-	+	+	-	bba25	G6	-	-	-	-
bb0398	A4	+	+	+	+	bbh01	P6	-	+	+	-
bb0424	J4	-	+	+	-	bbh18	G7	-	+	+	-
bb0458	B1	-	+	+	-	bbh32	P7	-	-	-	-
bb0464	K1	-	-	-	-	bbi16	G8	-	+	+	-
bb0475	B2	-	-	-	-	bbi28	P8	-	-	-	-
bb0489	K2	-	+	+	-	bbi29	H5	-	+	+	-
bb0524	B3	-	+	+	-	bbi32	Q5	-	-	-	-
bb0536	K3	-	+	+	-	bbi34	H6	-	+	+	-
bb0542	B4	-	+	+	-	bbi42	Q6	+	+	+	+
bb0553	K4	-	+	+	-	bbi01	H7	-	+	+	-
bb0606	C1	+	+	+	+	bbi09	Q7	-	+	+	-
bb0620	L1	-	+	+	-	bbi34	H8	-	+	+	-
bb0621	C2	+	+	+	+	bbi36	Q8	-	+	+	-
bb0628	L2	-	+	+	-	bbj51	I5	-	+	+	-
bb0644	C3	-	+	+	-	bbk04	R5	-	-	-	-
bb0652	L3	+	+	+	+	bbk07	I6	+	+	+	+
bb0664	C4	-	-	-	-	bbk12	R6	-	+	+	-
bb0687	L4	-	+	+	-	bbk19	I7	-	+	+	-
bb0689	D5	-	+	+	-	bbk32	G1	+	+	+	+
bb0696	M5	-	+	+	-	bbk47	P1	+	+	+	+
bb0740	D6	-	-	-	-	bbk49	G2	+	+	+	+
bb0758	M6	-	+	+	-	bbk53	P2	+	+	+	+
bb0760	D7	+	+	+	+	bbi39	G3	-	+	+	-
bb0785	M7	-	+	+	-	bbi40	P3	-	+	+	-
bb0806	D8	-	+	+	-	bbm27	G4	+	+	+	+
bb0813	M8	-	+	+	-	bbm38	P4	+	+	+	+
bb0815	E5	-	+	+	-	bbn38	H1	-	+	+	-
bb0823	N5	+	+	+	+	bbn39	Q1	-	+	+	-
bb0828	E6	+	+	+	+	bbn39	H2	-	+	+	-
bb0832	N6	-	+	+	-	bbo40	Q2	-	-	-	-
bb0840	E7	-	-	-	-	bbn38	H3	+	+	+	+
bb0844	N7	-	+	+	-	bba03	Q3	-	+	+	-
bba04	E8	-	+	+	-	bba05	H4	-	-	-	-
bba05	N8	-	+	+	-	bba35	Q4	+	+	+	+
bba07	F5	-	+	+	-	bbq47	I1	+	+	+	+
bba14	O5	-	+	+	-	bbq42	R1	-	+	+	-
bba15	F6	-	-	-	-	bbq30	I2	-	+	+	-
bba16	O6	+	+	+	+	bbq41	R2	-	+	+	-
bba24	F7	+	+	+	+	flaB	I3	+	+	+	+

**Figure 3.** Molecular adaptation of *B. burgdorferi* in the murine host. C3H mice that had been infected with cultured *B. burgdorferi* for 4 mo were used as donors. Naive C3H mice received ear tissue implants and were killed 11, 22, and 33 d later. RNA was prepared from ears and analyzed for the expression of the 137 putative lipoprotein genes and *flaB* by RT-PCR and microarray hybridization. "Location," location of each gene's DNA fragment on the microarray; "+," expressed; "-", not expressed.

transfer studies including *bb0122*, *bb0155*, *bba34*, *bbe31*, *bb01*, *bbh18*, and *bbi29*. The fact that passive immunization could not provide as high a level of Ab as those elicited by infection might be the reason why some of the 83 down-regulated genes in infected immunocompetent mice were still expressed at a detectable level in the passively immunized SCID mice. Alternatively, the host cellular immune response may also play a role in *B. burgdorferi* adaptation.

## Discussion

*B. burgdorferi* expressed all of the 137 lipoprotein genes we investigated during in vitro cultivation (Fig. 2 A). Once the spirochete was introduced into the mammalian host, however, 21 of the lipoprotein genes were quickly down-regulated (Fig. 2 B). As expected, one of the down-regulated genes was *ospA*. *B. burgdorferi* does not prominently express

*ospA* during mammalian infection but overexpresses this lipoprotein within the tick vector (7, 8) to facilitate the adherence of *B. burgdorferi* to the tick midgut (13). When *OspA* is expressed in the reservoir host, it would elicit strong immune responses including the development of *OspA* Ab. As a consequence, acquisition of the spirochetes from mammals by larval ticks would be impaired or even blocked (14). Therefore, *B. burgdorferi* does not generally express *ospA* during infection of its natural mammalian host in order to maintain the enzootic cycle. *OspA* Ab may sometimes be found in patients with Lyme disease (15); however, humans are an aberrant host and not important for spirochete persistence. Most of the 20 remaining down-regulated lipoprotein genes are hypothetical. It remains to be addressed if they are, like *ospA*, also tick-specifically expressed.

A group of ~80 lipoprotein genes were persistently expressed during murine infection when no immune selec-

tion pressure was present. *ospC* (*bbb19*) is the best known member of this group. OspC has been identified as an Ab-targeted, surface-exposed Ag during murine infection (12). To avoid immune clearance by OspC Ab during mammalian infection, *B. burgdorferi* shuts down *ospC* (12). Although the other members of this group also appeared to be down-regulated by immune selection pressure elicited during infection, it remains to be addressed whether they are, like OspC, surface-exposed, Ab-targeted Ag. Some of them might be neither surface-exposed nor Ab-targeted but could be codown-regulated with other Ab-targeted Ag(s).

The third group of lipoproteins were expressed throughout mammalian infection. Some of them were significantly down-regulated by immune selection pressure but their expression was detectable. They may be critical for the survival of *B. burgdorferi* in the mammalian host. As expected, VlsE (BBF32) was continuously expressed since it is able to undergo antigenic variation during murine infection (16). Three of the four-member Bmp family (17), BmpB, BmpC, and BmpD (BB0382, BB0384 and BB0385), but not BmpA (BB0383), were also persistently found. Although the functions of these lipoproteins are unknown, they are homologous to TmpC, a putative outer or cytoplasmic membrane lipoprotein of *Treponema pallidum*, the spirochetal agent of syphilis (18), indicating the importance of these lipoproteins in the spirochetes' pathogenesis. The best known members of this group are decorin-binding proteins A and B (DbpA and DbpB; BBA24 and BBA25; reference 19) and fibronectin-binding protein (BBK32; reference 20). These three lipoproteins have been shown in vitro to be surface exposed and be able to bind extracellular matrices (19, 20). Recent evidence suggests that Dbps interact with their ligand and enhance the infectivity of *B. burgdorferi* during murine infection (21). It remains to be addressed if host decorin or fibronectin can coat *B. burgdorferi* via the Dbps and fibronectin-binding protein, and thus protect the pathogen from attack by specific Ab. ErpK (BBM38), ErpA (BBP38), and ErpX (BBQ47) were expressed in chronically infected immunocompetent mice, albeit some of them were transcribed at a very low level or even undetectable in some cases. Interestingly, like BBA24, BBA25, and BBK32, the Erp lipoproteins also are able to bind a host component, complement inhibitor factor H (22).

Some of our findings strikingly differ from those that have been obtained with cultured *B. burgdorferi*. For instance, *ospA* and *ospB* are transcribed as a single mRNA unit in cultured *B. burgdorferi* (23). Our study indicated that *B. burgdorferi* expressed *ospB* but not *ospA* throughout murine infection. Hübner and colleagues have reported an RpoN-RpoS regulatory pathway that controls the expression of both *dbpA* and *ospC* (24). Our study, however, clearly showed that whereas *B. burgdorferi* expressed both genes in SCID mice, immune selection pressure shut down *ospC* but not *dbpA*. The differential expression of these two lipoproteins has already been reported in the tick vector (25). *B. burgdorferi* has coevolved with the mammalian (immunocompetent) host and tick vector for a long period of time. The in vitro artificial conditions probably constitute

an aberrant environment where *B. burgdorferi* changes protein expression profiles and can more easily lose plasmids, infectivity, and pathogenicity (26).

There are no existing mechanisms that can be used to explain how immune selection pressure down-regulates specific genes. A previous study indicates that *B. burgdorferi* is able to generate multiple phenotypes during murine infection (12). Different phenotypes may express unique sets of lipoproteins and these phenotypes can interconvert. *B. burgdorferi* infection elicits the immune responses including those to surface-exposed lipoproteins once they are expressed. The specific Abs selectively eliminate the phenotypes that express these Ab-targeted Ags. The Ab-targeted phenotypes are continuously generated and then eliminated during infection. Some of the lipoprotein genes might exhibit this "down-regulation phenomenon." Coexistence of multiple phenotypes of *B. burgdorferi* has already been observed in the tick vector (27) as well as in vitro cultivation (28). This selection hypothesis can explain some but not all of the observations that have been obtained to date. For instance, if Ab-targeted phenotypes were continuously generated, specific lipoprotein mRNAs could be positive by sensitive RT-PCR even though these newly "borne" spirochetes were immediately eliminated. Moreover, if Ab-targeted phenotypes were continuously generated, newly synthesized lipoproteins would constantly prime the immune system. As a consequence, the specific Ab response to Ab-targeted lipoproteins should be maintained. However, the analysis of the Ab response to selected lipoproteins, such as OspC, in infected white-footed mice and human patients with Lyme disease does not support this view. The OspC Ab titer substantially wanes while infection continues in white-footed mice (29). In human patients, the OspC Ab titer may fall below a detectable level as the infection persists, and then reappear months later (30). If OspC-expressing spirochetes were continuously generated, the anti-OspC response should persist throughout the infection. Therefore, at least, some of the lipoprotein genes could be turned off by specific Ab by mechanisms that have not been known yet.

Our study has clearly demonstrated a two-step process of molecular adaptation by *B. burgdorferi* during early murine infection. The first-step adaptation is induced by the microenvironment of the host tissues. Regardless whether cultured *B. burgdorferi*, which expressed all of the 137 lipoprotein genes, or host-adapted organisms, which expressed less than 40 of them, were used for inoculation, the spirochetes were modulated to transcribe the same set of 116 lipoprotein genes within 10 d after infection. During the second step of adaptation, immune selection pressure forced spirochetes to down-regulate more than 80 of these 116 genes. This adaptation is a fundamental for *B. burgdorferi* to establish chronic infection, as the pathogen would be cleared by the host immune system at the very early stage of infection if the spirochete failed to undergo the process.

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