Toll-Like Receptors 1 and 2 Heterodimers Alter Borrelia burgdorferi Gene Expression in Mice and Ticks

Erol Fikrig, Sukanya Narasimhan, Girish Neelakanta, Utpal Pal, Manchuan Chen, and Richard Flavell

Borrelia burgdorferi, the agent of Lyme disease, is recognized by Toll-like receptor (TLR) 1 and 2 heterodimers. Microarray analysis of in vivo B. burgdorferi gene expression in murine skin showed that several genes were altered in TLR1/2-deficient animals compared with wild-type mice. For example, expression of bbe21 (a gene involved in B. burgdorferi lp25 plasmid maintenance) and bb0665 (a gene encoding a glycosyl transferase) were higher in TLR1/2-deficient mice than in control animals. In contrast, messenger RNA levels for bb0731 (a spoJ-like gene) and bba74 (a gene encoding a periplasmic protein) were lower in TLR1/2-deficient mice than in wild-type animals. The expression profiles of some of these genes were altered similarly in B. burgdorferi–infected ticks fed on control or TLR1/2-deficient mice. Quantitative reverse-transcription polymerase chain reaction analysis supported the microarray analysis and suggested that spirochete gene expression is altered by the milieu created by specific host TLRs, both in the murine host and in the arthropod vector.
13–16]. Furthermore, myeloid differentiation primary response protein 88 (MyD88)–deficient mice have greater difficulty controlling spirochetes than do TLR1- or TLR2-deficient animals [17–19]. MyD88 serves as the adapter molecule for several TLRs, suggesting that additional receptors are also involved in the identification of B. burgdorferi.

The spirochete uses several strategies to circumvent these immune responses. When it enters the host via a tick bite, B. burgdorferi begins its standoff with the host immune responses by exploiting tick salivary proteins spit into the bite site [20, 21]. Soon after entry into the host, the spirochete transcriptome and proteome undergo further changes as they adapt in the host, using their own proteins (such as the complement regulator–acquiring surface proteins) to defuse [22] and extracellular matrix binding-proteins to escape [23] host immune reactions. Liang et al [24] have suggested that the expression profile of several Borrelia genes—including ospc, bbf01, and vlsE [25]—might be altered by host humoral responses. Crowley and Hubner [26] have shown that an in vivo inflammatory environment induces the expression of outer surface protein A by unknown molecular mechanisms. Work by Anguita et al [27] has indicated that the recombination of the vlsE locus, an important aspect of immune evasion, might be influenced by interferon γ-mediated inflammation in the host. Because the engagement of spirochete lipoproteins with TLRs is a critical initiator of host immune responses to Borrelia [28], TLR-mediated signals might influence spirochete gene expression. In the present study, we examined how gene expression in the Lyme disease agent is altered by TLR1/2-mediated signals, using TLR1/2 heterodimer recognition of B. burgdorferi as a model.

METHODS

B. burgdorferi, mice, and Ixodes scapularis. Virulent, low-passage, clonal B. burgdorferi N40 was used. TLR1/2 heterodimers cannot form in TLR1/2-, TLR2-, or TLR1-deficient animals. The functional defect in these animals is similar because TLR1/2 heterodimers are required for the recognition of B. burgdorferi lipoproteins. TLR1/2- and TLR1-deficient mice on a B6 background were used in all experiments [11], and we collectively refer to the TLR1/2 heterodimer–deficient animals as TLR1/2-deficient mice. Control B6 mice were purchased from the National Institutes of Health. Mice were infected by means of a single subcutaneous inoculum of \( 1 \times 10^4 \) spirochetes; 2 weeks later, mice were killed and tissue specimens collected. For inoculation of mice with host-adapted spirochetes to assess the virulence of B. burgdorferi, donor B6 wild-type and TLR1/2-deficient mice (5 animals in each group) were infected by means of needle inoculation, as described above. Two weeks after infection, ear punch samples from TLR1/2-deficient and wild-type mice were implanted into the skin of naive wild-type B6 mice (5 mice in each group), as described elsewhere [24]. Mice were killed 14 days after infection, and bladders and skin were collected for DNA preparation and for quantitative polymerase chain reaction (PCR).

B. burgdorferi–infected I. scapularis nymphs were maintained at a tick colony at Yale. Eight to 10 B. burgdorferi N40–infected I. scapularis nymphs were placed on each control or TLR1/2-deficient mouse (5 mice in each group), and ticks were fed to repletion. Guts and salivary glands dissected from fed ticks were pooled into groups of 5 ticks for RNA extraction and complementary DNA (cDNA) synthesis, as described elsewhere [29]. Guts from unfed N40-infected nymphs were also processed for cDNA synthesis, as described above.

Microarray analysis. Amplification of spirochete transcripts in infected mice was performed using DECAL (differential expression analysis using a custom-amplified library), as described elsewhere [30]. Groups of 5 TLR1/2-deficient and control mice were infected with \( 1 \times 10^4 \) B. burgdorferi per mouse, and infection was confirmed by PCR or culture. Animals were killed on day 14 after spirochete challenge. Positive selection, amplification of spirochete transcripts, normalization, and random-prime labeling to generate biotin-labeled probes were done as described elsewhere [30]. The normalized probes were used to hybridize duplicate B. burgdorferi whole-genome nylon membrane arrays [31]. After the arrays were probed with DECAL-enriched cDNA, the hybridization was scored visually by 3 independent observers, and spots corresponding to gene elements were given a score from 0 to 3 (in increments of 0.5), on the basis of the intensity of hybridization [30]. A gene was considered differentially expressed when at least a 2-fold increase or decrease in hybridization score was noted (eg, from 1.5 to 3.0 or from 1 to 2) between experimental and control arrays. The expression of flaB was unchanged, as judged by a score of 0.5 in both the arrays. Spot pairs corresponding to differentially expressed genes were quantitatively analyzed using ImageJ software (version 1.32), a public-domain image-processing program (available at: http://rsb.info.nih.gov/ij). The arrays were scanned using an HP LaserJet4010 scanner and were saved as an uncompressed TIFF image. Pixel values for the selected spot pairs were then measured in ImageJ. Ratios of the mean pixel intensities of experimental spot pairs and respective control spot pairs were normalized to the pixel intensity ratios of flaB in each array, to derive normalized fold-change values for gene expression. Digital analysis was congruent with the visual analysis.

PCR. B. burgdorferi–infected murine tissues and spirochete-infected ticks were dissected, and total RNA was processed for quantitative reverse-transcription PCR using the iQ SYBR Green Supermix (Bio-Rad), as described elsewhere [29]. The following primers were used: for tick actin, 5′-GGCGAGCTAGCAG-3′ (forward) and 5′-GGTATCGTGTCGACTC-3′ (reverse); for flaB, 5′-TTCAATCAGGTAACGCAAC-3′ (for-
Figure 1. Microarray analysis of *Borrelia burgdorferi* gene expression in Toll-like receptor (TLR) 1 and 2–deficient mouse skin. Shown is a representative autoradiogram image of the nylon arrays probed with DECAL-enriched RNA from the skin of TLR1/2-deficient (A) and wild-type (B) mice infected with *B. burgdorferi*. Spots corresponding to bbe21 (1), bb0665 (2), bba74 (3), and bb0731 (4) are indicated.

Plasmid profile analysis. Both wild-type and TLR1/2-deficient mice were injected by needle with in vitro–cultivated spirochetes, as described above. Fourteen days after infection, bladders were harvested and processed for DNA extraction using a DNeasy kit (Qiagen). Plasmid profiles were analyzed using the primers described by Parveen et al [33], and levels were normalized to those of *flaB*.

In silico analysis. DNA and protein sequences of *B. burgdorferi* genes were obtained from the Comprehensive Microbial Resource Web site (http://cmr.jcvi.org/tigr-scripts/CMR/CmrHomePage.cgi). Homology searches and protein subcellular location predictions were conducted using the BLAST (http://blast.ncbi.nlm.nih.gov) and PSORTb (http://www.psort.org/psortb) analysis tools, respectively.

RESULTS

Alteration of spirochete gene expression in *B. burgdorferi*–infected TLR1/2-deficient mice. TLR1 and TLR2 recognize *B. burgdorferi* lipoproteins, thereby initiating a signaling cascade that results in host cell activation and cytokine release. We determined here whether TLR-mediated responses, as a counterbalance, influence pathogen gene expression. *B. burgdorferi* microarrays were used to examine spirochete mRNAs in the skin during infection of TLR1/2-deficient and control mice.

The *B. burgdorferi* gene expression profile 2 weeks after infection in TLR1/2-deficient and control mice was mostly similar, at least within the limitations of the capabilities of the microarray and DECAL procedures. However, a small subset
of genes were differentially expressed (Figure 1). Approximately 20 spirochete genes demonstrated enhanced expression (on the basis of an increase of 1.5-fold or more in hybridization score, as judged by visual and digital analysis of pixel intensities) in TLR1/2-deficient animals compared with wild-type mice (Table 1). Genes that showed increased expression in wild-type mice (increase of 1.5-fold or more in hybridization score) compared with TLR1/2-deficient animals were also apparent (Table 2). A second separate experiment yielded the same result. Two up-regulated and 2 down-regulated genes in TLR1/2-deficient mice were selected to establish the paradigm that TLR1/2-mediated responses alter spirochete gene expression. To examine the effect that the host immune status has on both tick and vertebrate host milieu, we prioritized genes on the basis of our ability to unambiguously detect their expression in N40-infected murine and tick host. Genes that belonged to large paralogous families were not prioritized, because sequence identity often confounds PCR and array analysis. On the basis of these criteria, we focused on bbe21 and bb0665, which showed markedly increased expression in TLR1/2-deficient animals compared with wild-type mice during murine Lyme borreliosis, and on bb0731 and bba74, which showed markedly decreased expression.

**Table 1. *Borrelia burgdorferi* Genes Expressed at Higher Levels in Toll-Like Receptor 1 and 2–Deficient Mice than in Wild-Type Mice**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function, homology, or category</th>
<th>Cellular location</th>
<th>Fold increase in expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>bbe21</td>
<td>Maintenance of lp25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>No prediction</td>
<td>3.30</td>
</tr>
<tr>
<td>bb0665</td>
<td>Conserved Hypothetical&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Cytoplasmic membrane</td>
<td>2.80</td>
</tr>
<tr>
<td>bbs02</td>
<td>Hypothetical&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Cytoplasm</td>
<td>3.20</td>
</tr>
<tr>
<td>bbo15</td>
<td>Hypothetical&lt;sup&gt;b&lt;/sup&gt; (putative B12-binding protein)</td>
<td>Cytoplasm</td>
<td>2.32</td>
</tr>
<tr>
<td>bbo02</td>
<td>Hypothetical&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Cytoplasm</td>
<td>3.30</td>
</tr>
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<td>Hypothetical&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Cytoplasm</td>
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<td>Cytoplasm</td>
<td>3.20</td>
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<td>DNA metabolism&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>bb0154</td>
<td>Protein and peptide trafficking&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Cell envelope/biosynthesis and degradation</td>
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<td>1.64</td>
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<tr>
<td>bb0715</td>
<td>Cell envelope/biosynthesis and degradation</td>
<td>Cytoplasm</td>
<td>1.50</td>
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**NOTE.** Cellular locations were predicted using PSORTb software. Fold increases in expression were computed on the basis of the ratios of mean pixel intensity scores (normalized to flaB) assigned for each element by ImageJ software comparison of the nylon arrays and represent fold increases in hybridization. Boldface type indicates genes analyzed in the present study.

<sup>a</sup> Gene function derived from the literature.

<sup>b</sup> Gene functions as annotated by the J. Craig Venter Institute (Maryland).

**Increased expression of *B. burgdorferi* bbe21 and bb0665 and decreased expression of bb0731 and bba74 in TLR1/2-deficient mice.** In each experiment, 5 TLR1/2-deficient mice and 5 control mice were challenged with *B. burgdorferi*, and each mouse was individually examined for spirochete gene expression. Consistent with earlier observations [13], the *B. burgdorferi* burden was higher in TLR1/2-deficient mice than in control mice (Figure 2A), and joint inflammation was evident in all the animals. At 2 weeks, when spirochete numbers are high in the dermis, *B. burgdorferi* bbe21 and bb0665 mRNA levels were significantly higher in the skin of TLR1/2-deficient mice than in wild-type mice (*P* < .05) (Figure 2B). In contrast, bb0731 and bba74 levels were significantly decreased in TLR1/2-deficient animals compared with control mice (*P* < .05) (Figure 2B). This expression profile was also observed in the bladder (Figure 2C), and differences in the expression of all 4 genes were statistically significant. These results confirmed the initial microarray results (Tables 1 and 2).

**No alteration in plasmid profile and infectivity of spirochetes in TLR1/2-deficient mice.** Altered *B. burgdorferi* gene expression could reflect a population of avirulent spirochetes that had been enriched in the TLR1/2-deficient host because
of dampened immune responses. To address this, we first examined the plasmid profile of *B. burgdorferi* cultured from the bladders of wild-type and TLR1/2-deficient animals. Spirochetes from both control and experimental animals were positive by PCR for all the detectable plasmids assessed (data not shown). Levels of lp25, a plasmid critical for spirochete virulence in both mammals and ticks [7], were similar in control and experimental groups and were comparable with the virulent low-passage N40 strain used in this study (Figure 3A). Furthermore, we grafted skin from *B. burgdorferi*–infected TLR1/2-deficient and wild-type mice onto naive wild-type mice. We reasoned that if the TLR1/2-deficient mice were enriched for avirulent spirochetes, then these *B. burgdorferi* would be eliminated in the wild-type host. Mice that received skin grafts from TLR1/2-deficient mice demonstrated spirochete burdens comparable to those in mice that received skin grafts from wild-type mice (Figure 3B).

**Lower *B. burgdorferi bba74 and bb0731 expression in ticks fed on TLR1/2-deficient mice.** We then determined whether TLR1 and TLR2 influenced *bbe21*, *bb0665*, *bb0731*, and *bba74* mRNA levels in the vector by allowing ticks to engorge on TLR1/2-deficient or wild-type mice. The *B. burgdorferi* burden in the midguts of ticks fed on TLR1/2-deficient animals was significantly higher than the spirochete burden in ticks fed on control animals (Figure 4A). Spirochetes made substantially less *bbe21*, *bb0731*, and *bba74* mRNA, but not *bb0665* mRNA, in unfed ticks than in engorged vectors (Figure 4B–4E). *B. burgdorferi* *bbe21* and *bb0665* expression in the midguts of ticks was not altered by TLR1/2 deficiency of the murine host (Figure 4B and 4C). Expression of *bba74* and *bb0731* was significantly lower in the midguts of ticks fed on TLR1/2-deficient mice than in ticks fed on control animals (Figure 4D and 4E). Expression of *bbe21* and *bb0665* was increased and expression of *bba74* and *bb0731* was decreased in the salivary glands of ticks fed on TLR1/2-deficient mice compared with that in ticks fed on control animals. The expression levels of all 4 genes examined in this study were ~500–1000-fold higher in nymphal ticks compared with those in the murine host.
DISCUSSION

Spirochete-mediated stimulation of TLR1 and TLR2 causes a cascade of events that results in the generation of cellular and humoral immune responses that diminish *B. burgdorferi* burden in vivo [13]. The roles played by TLR2, TLR5, TLR9, and the major TLR adaptor MyD88 in the clearance of *Borrelia* has been defined [34]. While earlier efforts suggested that *Borrelia* gene expression may be modulated by humoral immunity [35], studies have not determined whether pathogen gene expression in vivo is altered by key components of host innate immunity, such as TLRs. We observed that the expression of a subset of

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**Figure 2.** *Borrelia burgdorferi* levels and selected gene expression in mice. **A**, Quantitative reverse-transcription polymerase chain reaction (qRT-PCR) assessment of flaB levels relative to mouse β-actin levels as a measure of viable *Borrelia* burden. **B** and **C**, qRT-PCR assessment of bbe21, bb0665, bba74, and bb0731 transcripts in the skin (**B**) and bladders (**C**) of control and Toll-like receptor (TLR) 1 and 2–deficient mice 2 weeks after infection. Asterisks (*) indicate a significant (*P* < .05, Student *t* test) increase in *Borrelia* burden, a significant increase in bbe21 and bb0665 expression, and a significant decrease in bb0731 and bba74 expression in TLR1/2–deficient mice compared with control mice. Data are means ± standard errors for values from 5–8 animals.
spirochete genes were enhanced or decreased in TLR1/2-deficient animals compared with wild-type mice (Tables 1 and 2). TLR1/2 deficiency did not predominantly influence the expression of *Borrelia* genes encoding lipoproteins, the predominant ligands for TLR1/2 activation [28]. Differences in the humoral responses apparently modified the expression of several lipoprotein-encoding genes, including *ospC*, *bfb01*, and *vlsE* [24, 25]. The present study used a TLR1/2-deficient host, but *Borrelia* components also engage with other TLRs [36] and would continue to trigger the other TLRs, as in the wild-type host. We chose to examine *bbe21*, *bb0665*, *bb0731*, and *bb74* expression profiles in greater detail, because mRNA levels of these genes were consistently altered in replicate experiments in TLR1/2-deficient mice compared with controls and because their expression is readily detectable in the tick vector.

![Figure 3. Borrelia burgdorferi Ip25 levels and virulence in Toll-like receptor (TLR) 1 and 2–deficient mice. Shown are the results of quantitative polymerase chain reaction assessment of Ip25 levels in spirochetes from the bladders of wild-type and TLR1/2-deficient mice (A) and of flaB levels as a measure of *Borrelia* burden in the skin and bladders of wild-type mice that received skin grafts from *Borrelia* as a measure of *Borrelia* survival in both the vector and host.](image)

The gene *bbe21*, a member of the paralogous family 57, may participate in plasmid replication [6, 37]. Stewart et al [38] have also suggested a role for BBE21 in the replication of Ip25. Ip25 is essential for *Borrelia* survival in both the vector and host milieu [7], providing nicotinamidase (encoded by the gene *pncA* [BBE22]), a key enzyme for the synthesis of the cofactor nicotinamide adenine dinucleotide [39]. We observed *bbe21* up-regulation coincident with active spirochete replication in the arthropod and the TLR1/2-deficient mammalian host. Whether the preferential increase in *bbe21* expression in TLR1/2-deficient mice relates to increased spirochete burden remains to be determined.

The gene *bb0665*, a chromosomal gene, encodes for a 32-kDa protein predicted to contain a putative glycosyl transferase domain that might be involved in the transfer of sugar from UDP-glucose, UDP-N-acetyl-galactosamine, GDP-mannose, or CDP-abequose to a range of substrates (http://cmr.jcvi.org/tigr-scripts/CMR/CmrHomePage.cgi). *Borrelia* also contains several low-molecular-weight glycosylated lipids that are potentially immunogenic [28, 40, 41]. The decrease in *bb0665* expression in control mice compared with TLR1/2-deficient mice (Figure 2) might help the spirochetes to remain less “visible” to the host immune surveillance. More recently, Yang and Li [42] have shown that *bb0665*, along with *bb0666* and *bb0667*, is part of an operon termed *pami*. It has been suggested that *bb0666* encodes a putative N-acetylmuramyl-l-alanine amidase involved in peptidoglycan synthesis critical for cell division [42]. The product of *bb0665*, as part of the *pami* operon, could be involved in transferring sugars to the peptidoglycan layer during cell wall biosynthesis and cell division. One would expect to observe increased *bb0665* expression in TLR1/2-deficient mice, in which the spirochete burden is increased compared with control mice.

The gene *bb74*, which is contained on the linear plasmid 54, encodes for a 257-aa protein recently shown to be a periplasmic protein associated with the outer membrane [43]. Although *bb74* expression was increased in vitro when *Borrelia* was temperature shifted from 23°C to 37°C [31, 44], it was repressed under mammal-like conditions simulated using the dialysis membrane chamber implant model [45] and in vitro by increasing the temperature in the presence of blood in the culture medium [46]. Mulay et al [47] have demonstrated that *bb74* expression, like that of *ospA*, is turned off in the murine host, repression that is apparently mediated by the *Borrelia* alternative sigma factor RpoS. In the present study, we observed *bb74* transcripts in the murine host, although the expression levels of *bb74* decreased ~1000-fold in the murine host compared with that in fed nymphs (Figure 2). Although the present study used B6 mice, Mulay et al [47] used C3H/HeJ mice. Unlike B6 mice, C3H/HeJ mice encode a defective TLR4 protein, which leads to an impaired TLR4 response [48] that could account for the observed difference between the 2 studies. Interestingly, expression of *bb74* was reduced ~50-fold in TLR1/2-deficient B6 mice compared with that in wild-type B6 mice.
Figure 4. *Borrelia burgdorferi* bbe21, bb0665, bb0731, and bba74 expression in *Ixodes scapularis*. Shown are the results of quantitative reverse-transcription polymerase chain reaction assessment of flaB levels as a measure of viable *Borrelia* burden (A) and of bbe21 (B), bb0665 (C), bba74 (D), and bb0731 (E) transcripts in the unfed tick (unfed), in the midguts (MG) of ticks engorged on control (fed MG), in the salivary glands (SG) of ticks engorged on control (fed SG), in the midguts of ticks engorged on Toll-like receptor (TLR) 1 and 2–deficient mice (fed MG TLR1/2<sup>−/−</sup>), and in the salivary glands of ticks engorged on TLR1/2-deficient mice (fed SG TLR1/2<sup>−/−</sup>). Asterisks (*) indicate a significant (\( P<.05 \), Student t test) increase in *Borrelia* burden and a significant decrease in bba74 and bb0731 expression in ticks fed on TLR1/2-deficient mice compared with those fed on control mice. Data are means ± standard errors from a single experiment that was representative of duplicate experiments.

Figure (2), suggesting that bba74 expression may be additionally influenced by the immune status of the host.

The gene bb0731, a chromosomal gene, encodes for a predicted 13-kDa protein that contains domains related to the plasmid partitioning the protein ParB and that is a parologue of the *Borrelia* gene bb0434, which encodes the stage 0 sporulation protein J (Spo0J). The Spo0J family of proteins are thought to play a vital role in coupling developmental transcription to cell cycle progression, acting as a biological switch regulating vegetative growth and sporulation [49]. *Borrelia* does not sporulate; however, its transcriptional status changes in different milieus, remaining quiescent (as in unfed tick guts) or actively replicating (as in feeding ticks) [50]. The increased levels of bb0731 transcripts in immunocompetent control mice compared with TLR1/2-deficient mice might be inversely coupled to the spirochete’s ability to replicate actively in response to altered environment.

Grafting of skin from TLR1/2-deficient mice onto wild-type mice produced spirochete burdens comparable to those observed in mice that received skin grafts from wild-type mice (Figure 3B). This finding demonstrated that spirochetes in TLR1/2-deficient mice were virulent and ruled out the possibility that differential expression of spirochete genes in TLR1/2-deficient animals was not an artifact of selection of spirochetes expressing a set of preferred genes.

Spirochetes in the vector are passively exposed to the TLR receptors and to host immune molecules (including cytokines) when ticks take a blood meal. We therefore examined whether the TLR1/2-deficient blood meal would influence the expression of bbe21, bb0665, bba74, and bb0731 within the tick. Expression of bbe21 and bb0665 in the tick gut did not change significantly when ticks were fed on TLR1/2-deficient or control animals (Figure 4B and 4C). This departure from observations made in the murine host suggests that the role played by ar-
throppod-specific signals in the expression of these genes in the tick gut is predominant. However, the decrease in bba74 and bb0731 transcripts in nymphs fed on TLR1/2-deficient mice compared with nymphs fed on wild-type mice indicates the potential influence that the host immune system has on the expression of bba74 in the tick gut (Figure 4D and 4E). Expression levels of bbe21 and bb0665 were increased and expression levels of bba74 and bb0731 were decreased in the salivary glands of ticks fed on TLR1/2-deficient mice (Figure 4B–4E), a trend that mirrors the profiles observed in the murine host (Figure 2). These observations reveal a facet of host-pathogen interactions that occurs within the vector, perhaps to better prepare the outgoing pathogen for optimal survival in the competent host.

The interplay between pathogens and their vertebrate and invertebrate hosts is complex. The present study shows that B. burgdorferi gene expression is influenced by specific TLR-mediated innate immune responses, perhaps to enhance spirochete survival. Although a decade has passed since the sequencing of the genome of B. burgdorferi [6], the functions of the major portion of the genome are not understood. This limits our ability to infer the physiological significance of differential expression of genes and frustrates transcriptome analysis of the genome of B. burgdorferi. Despite this limitation, a theme appears to emerge from the in silico analysis of the 4 genes differentially expressed between the transcriptome and the functional genome of B. burgdorferi [6], the functions of the major portion of the genome are not understood. This limits our ability to infer the physiological significance of differential expression of genes and frustrates transcriptome analysis of B. burgdorferi. Despite this limitation, a theme appears to emerge from the in silico analysis of the 4 genes differentially expressed in TLR1/2-deficient mice. The products of the genes bb0665, bb0731, and bbe21, but not bba74, appear to be involved in the modulation of spirochete replication, consistent with the obvious differences in spirochete numbers between the wild-type and TLR1/2-deficient mice. Importantly, the present study identifies a cluster of genes regulated by TLR1/2-mediated signals. Future studies might reveal additional gene clusters altered by other TLRs and unfold “functional” gene clusters in the context of host innate immunity, helping to bridge the gap between the transcriptome and the functional genome of B. burgdorferi. The molecular mechanisms that direct this change of expression are likely multifactorial. Understanding the mechanisms by which these microbial changes are generated may lead to new strategies to combat infection with B. burgdorferi and other agents of disease.

**Acknowledgments**

We are grateful to Ira Schwartz for providing the B. burgdorferi genome arrays, and we deeply appreciate his critical scientific input during the preparation of the manuscript. We thank Kathleen DePonte and Deborah Beck for technical assistance.

**References**


