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Borrelia burgdorferi Population Kinetics and Selected Gene Expression at the Host-Vector Interface

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By using real-time quantitative PCR, the population dynamics and gene transcription of *Borrelia burgdorferi* were examined in ticks and skin of mice during acquisition of the infection from mice by ticks and during transmission of the infection from ticks to mice. Population dynamics were determined by using a *flaB* DNA target. A quantitative analysis of *flaB*, *ospA*, *ospC*, *dbpA*, and *arp* transcription was also performed. The results revealed that both uninfected larval and nymphal *Ixodes scapularis* ticks acquired *B. burgdorferi* as early as 1 day after attachment and that the sizes of spirochete populations within ticks increased during feeding. In addition, all gene targets revealed that there was RNA transcription during feeding. Similar events occurred within infected nymphal ticks feeding on uninfected hosts. Transmission from infected nymphal ticks to mice could be detected within 1 day after attachment. Analysis of skin during the first 3 days after attachment of infected ticks revealed rising numbers of spirochetes but minimal gene transcription. In contrast, the skin of mice with established infections revealed static populations of spirochetes and active but stable transcription of *flaB*, *ospC*, *dbpA*, and *arp*. There were consistent reductions in the number of spirochetes in the skin at the tick attachment sites compared to the number of spirochetes in the skin at nontick sites, but there were no differences in gene expression between tick and nontick skin sites. Evidence of *ospA* transcription in skin could be found 1 day after tick attachment but not thereafter.

Borrelia burgdorferi, the causative agent of Lyme disease, is transmitted to and acquired from its hosts by *Ixodes* spp. hard ticks. The principal *Ixodes* vectors include *Ixodes ricinus* in Europe, *Ixodes persulcatus* in Eastern Europe and Asia, and *Ixodes scapularis* and *Ixodes pacificus* in North America (35, 43). Acquisition and transmission are processes that involve the tick, the host, and the pathogen in reciprocal interactions with one other. After attachment, ticks induce host local inflammatory responses and immune responses against components within their saliva. To counteract these responses, tick saliva also contains substances that suppress or divert host immune responses (31, 46).

In addition to these vector and host processes, *B. burgdorferi* undergoes dynamic changes within the vector during both acquisition and transmission and within vertebrate hosts after transmission. These changes include striking variations in antigen expression. For example, *B. burgdorferi* is limited to the midgut of resting (unfed) nymphal and adult ticks (5) and usually express outer surface protein A (OspA) and very rarely OspC (18). After ticks attach and begin feeding, the spirochetes rapidly multiply (12), down-regulate or shed OspA, and up-regulate OspC during their migration to the salivary glands (41). Upon transmission, the spirochetes stay in the skin at the attachment site for several days and then disseminate throughout the vertebrate host (42). Early in infection, spirochetes express OspC but not OspA (11, 30, 37). In addition to these two lipoproteins that seem to be involved in adaptation to different environments, there is mounting evidence that genes encoding different antigens are differentially expressed or repressed in response to a variety of environmental cues during pathogen-tick-host interactions (7, 8, 13, 22, 26, 27, 32, 38, 39, 41, 44, 45).

The purpose of the present investigation was to examine *B. burgdorferi* population dynamics and differential gene expression at the host-vector interface by quantitative DNA and RNA analysis of selected prototype genes. The genes that were investigated for expression included those that encode OspA and OspC, two well-defined lipoproteins with known differential expression; decorin binding protein A (DbpA), a 22-kDA highly immunogenic antigen that is expressed during infection (15); arthritis-related protein (Arp), a 37-kDa lipoprotein that elicits antibodies that selectively induce arthritis resolution (14); and FlaB, a structural component of flagellin, which is presumed to be constitutively expressed by spirochetes in both ticks and hosts during infection (2).

MATERIALS AND METHODS

Mice. C3H/HeN specific-pathogen-free mice that were 3 to 5 weeks old were purchased from Frederick Cancer Research Center (Frederick, Md.). The mice were housed in an isolation room, provided food and water ad libitum, and killed by carbon dioxide asphyxiation. At the time of necropsy, urinary bladder and ear samples were cultured from each mouse, as described previously (4). At necropsy, mice inoculated with *B. burgdorferi* were all confirmed to be culture positive, while none of the control mice was culture positive.

***B. burgdorferi*.** A clonal strain of *B. burgdorferi* sensu stricto (cN40) was used (4). *B. burgdorferi* was cultured in BSK II medium and enumerated as described previously (24). In this study, mice were inoculated intradermally with 10^4 mid-log-phase spirochetes at the base of the tail.

Ticks. Adult *I. scapularis* ticks were field collected in southern Connecticut and were cordially provided by Durland Fish of Yale University, New Haven, Conn. These adults produced uninfected larvae for experimental use. All larvae were derived from a single cohort for the experiments described in this paper. To generate infected and uninfected nymphs, larvae were allowed to attach to and

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TABLE 1. Oligonucleotide PCR primers and TaqMan internal probes for *B. burgdorferi* genes targeted in this study

Gene	Primer		Length (bp)	Probe	
	Designation	Sequence (5′→3′)		Designation	Sequence (5′→3′)[a]
flaB	FL-571F	GCAGCTAATGTTGCAAATCTTTTC	107	FL-611P	AAACTGCTCAGGCTGCACCGGTTC
	FL-677R	GCAGGTGCTGGCTGTTGA			
ospA	OSPA-288F	TGAAGGCGTAAAAGCTGACAAA	142	OSPA-266P	CAATTTTGAACGATCTAGGTCAAACCACACTTGA
	OSPA-369R	TTCTGTTGATGACTTGTCTTTGGAA			
ospC	OSPC-108F	TGTTAAAGGGCCTAATCTTACAGAAATAA	128	OSPC-147P	TACACAATCTAACGCAGTTGTTCTGGCCGT
	OSPC-235R	TACCAATAGCTTTGGTAGCAAGTTCAT			
dbpA	P22-172F	GATAACAATGTAAATTTTGCTGCCTTT	95	P22-203P	ATAGTGAAACAGGTAGCAAGGTATCAGAAAATTCATTCA
	P22-266R	GTAGCTCGCACTTTTGCTTCAAG			
arp	P37-663F	TACACACCCCATATTTGATCACATTACT	105	P37-705P	TCCCGGACAAGATTCTATATCCAATACATGGG
	P37-767R	TTGCTATCACCACCAATTTCAAGT			

[a] The probes were labeled at the 5′ end with the fluorescent reporter dye 6-carboxy-fluorescein and at the 3′ end with the fluorescent quencher dye 6-carboxy-tetramethyl-rhodamine.

engorge on C3H mice that had been infected with *B. burgdorferi* for 2 weeks or were allowed to feed on uninfected mice (controls). Engorged larvae were collected from infected and uninfected mice separately and then allowed to molt and harden into nymphs. Ten percent of the pools of molted infected and uninfected nymphs were tested by PCR (*flaB* DNA) (see below) to verify the infection status and the prevalence of infection. Of the nymphs derived from larvae that fed on infected mice (65 individual samples), 96.92% were PCR positive; none of the nymphs derived from larvae that fed on uninfected mice (47 individual samples) was PCR positive.

***B. burgdorferi* quantitative detection.** Uniform samples of shaved skin were obtained with 3-mm-diameter disposable biopsy punches (Premiere Medical Products). Skin samples were weighed immediately after collection. Skin samples were snap frozen in liquid nitrogen, pulverized, and homogenized, and then they were split equally for DNA and RNA extraction. Paired ticks from each mouse were processed for DNA and RNA extraction. DNA was extracted with DNeasy tissue kits used according to the manufacturer's instruction for tissues and insects (Qiagen, Valencia, Calif.). The copy number of each *B. burgdorferi* target gene was expressed per milligram of skin or per tick. For quantitative analysis of DNA extracted from skin samples and from ticks, real-time PCR was used, as described previously (25). Three oligonucleotides, two primers, and a probe for each *B. burgdorferi* target gene were selected by using Primer Express software (PE Biosystems, Foster City, Calif.), as shown in Table 1.

To quantify the copy number of each DNA gene target, plasmid standards were prepared in order to create absolute standard curves. The primers for the *flaB* gene (nucleotides 87 to 119 and 659 to 677) amplified a 591-bp fragment. The primers for the *ospA* gene (nucleotides 164 to 184 and 655 to 672) amplified a 509-bp fragment. The primers for the *ospC* gene (nucleotides complementary to DNA flanking regions approximately 30 bp upstream and downstream of the gene) amplified a 730-bp fragment. The primers for the *dbpA* gene (nucleotides 1 to 21 and 563 to 582) amplified a 582-bp fragment. The primers for the *arp* gene (nucleotides 1 to 18 and 952 to 978) amplified a 978-bp fragment. Each fragment was cloned into the pCR2.1 vector (Invitrogen, Carlsbad, Calif.). To create standard curves for each gene, plasmid DNA was used as the template and serially diluted 10^9-fold in triplicate. The threshold cycles for quantification were defined from baseline cycles for each gene. Each plasmid dilution, representing the plot of threshold cycle value versus the log of copy number, was included in each PCR, which was then used to calculate an unknown amount of DNA in examined samples. The analytical sensitivity for each target gene was in the range from 1 to 10^9 copies. To check the sensitivity of real-time PCR, tissue samples from naïve mice were spiked with a dilution series of *B. burgdorferi* (10^6 to 10^1 spirochetes). From each spiked tissue sample, the target genes were amplified, and the copy numbers were 81 to 88.5% of the input. The expected yield of purified DNA from tissue samples is around 80%, so the loss of 11.5 to 19% of the spirochetes was within the range desired.

Analysis of mRNA. Total RNA from each sample was purified by using RNeasy mini kits according to the manufacturer's instruction for tissues or insects (Qiagen). Samples were homogenized with QIAshredder, and prior to elution they were treated with RNase-free DNase I. The concentration and purity of extracted RNA were determined by measuring the A_{260} and A_{280}. Extracted total RNA was stored at −80°C until it was used. The extracted total RNA was used for two separate reactions, one to synthesize cDNA and the other to test for DNA contamination. The reverse transcription step was performed by utilizing TaqMan reverse transcription reagents (PE Biosystems) with (for the cDNA synthesis reaction) and without (for the control reaction) reverse transcriptase. cDNA synthesis and control reaction mixtures were amplified in a standard heat block instrument after incubation for 10 min at 25°C following transcription at 48°C for 30 min and inactivation at 95°C for 5 min. Following cDNA synthesis, a real-time PCR was performed in new tubes to which cDNA and control preparations were added, as described above. DNA standard curves created for each target gene were not used for absolute quantification of RNA because there was no control for the efficiency of the reverse transcription. Therefore, standard curves were created with in vitro-transcribed RNA by using the RiboProbe in vitro transcription system (Promega, Madison, Wis.). The concentration of purified transcribed RNA was measured by absorbance and then converted to the number of copies by using the molecular weight of the RNA. Serial dilutions of in vitro-transcribed RNA were prepared in triplicate. The analytical sensitivity for each target gene was in the range from 10^1 to 10^9 copies.

Experimental plan. In the first experiment we examined *B. burgdorferi* acquisition kinetics and patterns of selected gene expression in uninfected nymphal ticks feeding on infected hosts. Sixteen C3H mice were infected with *B. burgdorferi* by intradermal inoculation, and eight mice were sham inoculated with BSK II medium. Two weeks after inoculation, each mouse was infested with four uninfected nymphal ticks. At zero time and 1, 2, and 3 days after tick attachment, four infected and two uninfected mice were killed. Paired skin samples were taken from the tick sites and from contralateral nontick attachment sites. At the same time, feeding ticks were removed from each mouse, and two ticks from each mouse were processed for DNA and RNA extraction. The remaining nymphal ticks were collected after engorgement, left in a humidify chamber to molt, and then 10 days later collected and used for DNA and RNA extraction.

To confirm the findings obtained in the first experiment and to explore *B. burgdorferi* gene expression in larval ticks, 16 mice were infected for 2 weeks and then infested with four noninfected nymphal ticks each; also, eight infected mice were infested with approximately 50 larval ticks each. Skin samples and feeding nymphal ticks were collected from mice infested with nymphal ticks as described above. Six feeding larval ticks were removed from each mouse at zero time and on days 1, 2, and 3 after tick attachment (eight pools of six larvae each per interval). The remaining larval ticks were collected after engorgement, left in a humidify chamber to molt, and then collected and used for DNA and RNA extraction 10 days later.

In an effort to examine population kinetics and gene expression profiles in infected ticks feeding on uninfected mice (in contrast to the experiments described above, in which uninfected ticks feeding on infected hosts were examined), 16 uninfected mice were each infested with four *B. burgdorferi* infected nymphal ticks. At zero time and 1, 2, and 3 days after tick attachment, paired skin samples from the tick sites and from contralateral nontick sites of four mice at each time point were collected as described above. The ticks and tissues were processed as described above. The experiment was repeated by using the same number of mice and the same procedure.

Statistics. Statistical comparisons between infected and uninfected mice or ticks were made by using a Student's *t* test (SPSS, version 6.1 for Macintosh; SPSS Inc., Chicago, Ill.). A multiple-comparison analysis was performed by using a one-way analysis of variance, followed by a least-squares difference post hoc test. Calculated *P* values of <0.05 were considered significant.

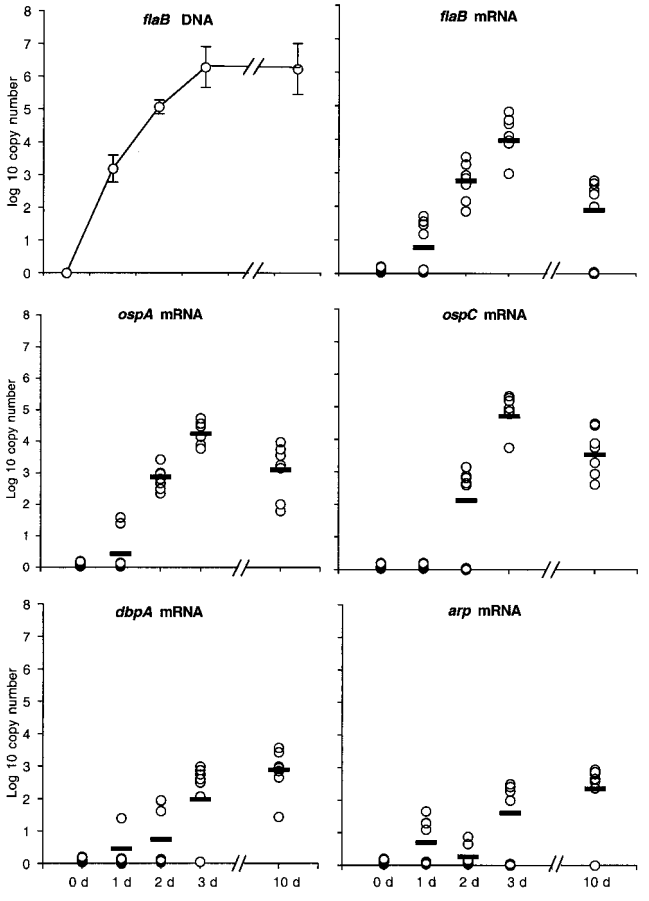

FIG. 1. Mean copy number ± standard deviation for *flaB* DNA in uninfected nymphal ticks fed on infected mice and mRNA levels of *flaB*, *ospA*, *ospC*, *dbpA*, and *arp* genes in the same ticks at different times after attachment. Ticks were tested at zero time and 1, 2, and 3 days after attachment, as well as after 10 days (postengorgement). The horizontal bars indicate mean copy numbers.

RESULTS

Spirochete population kinetics and gene expression in uninfected ticks feeding on infected mice. Uninfected nymphal ticks that fed on infected mice began acquiring spirochetes during the earliest sampling interval (day 1) after attachment (Fig. 1). Based upon *flaB* DNA copy numbers, the number of spirochetes within nymphal ticks increased significantly during feeding and then plateaued through molting. Fewer spirochetes were detected in larval ticks than in nymphal ticks (commensurate with the smaller size of the larval ticks), but the population kinetics were similar (data not shown).

In both nymphal and larval ticks, *flaB* and *ospA* RNA were first detected within 1 day of tick attachment; the levels rose between day 1 and 2 after attachment and then declined at day 10. *ospC* RNA was first detected on day 2 after tick attachment, and the levels rose between day 2 and day 3 after attachment and then declined by day 10 (Fig. 1; data for larval ticks not shown). A somewhat different pattern was observed for *dbpA* and *arp* RNA, whose levels seemed to increase gradually during feeding and molting of nymphal ticks. Since all gene targets were analyzed by using the same cDNA samples, the relative differences in expression patterns suggested different kinetics. When they were compared to spirochete numbers (based upon *flaB* DNA), the data suggested that *flaB*, *ospA*, and *ospC* expression rose with the proliferation or acquisition of spirochetes in feeding ticks on days 1 through 3 and then declined relative to the numbers of spirochetes on day 10, when the spirochete numbers had plateaued. In contrast, the levels of *dbpA* and *arp* both increased gradually through day 10.

Spirochete kinetics and gene expression in host skin of infected mice in response to uninfected tick feeding. Skin samples obtained from infected mice at the nymphal tick attachment sites and from the nontick sites at each time point were all PCR positive (Fig. 2). None of the samples from uninfected mice were positive for *flaB* DNA. Based upon *flaB* DNA copy numbers, there were consistently fewer spirochetes per milligram of skin tissue at the tick attachment sites than at the nontick sites on days 2 and 3 ($P = 0.044$ and $P = 0.026$, respectively, as determined by paired Student's *t* test). There

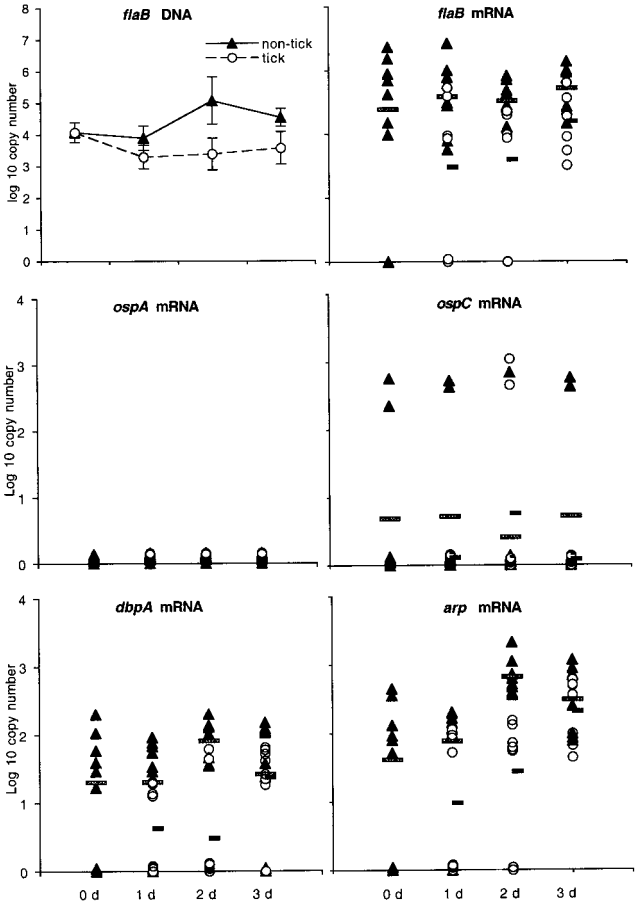

FIG. 2. Mean copy number ± standard deviation for *flaB* DNA per milligram of skin tissue at the attachment sites of uninfected ticks and at nontick sites of infected mice and mRNA levels of *flaB*, *ospA*, *ospC*, *dbpA*, and *arp* genes in the same skin samples at different times after tick attachment. Tick sites and nontick sites were tested at zero time and 1, 2, and 3 days after tick attachment. Mice were infected for 2 weeks prior to tick attachment. The long horizontal bars indicate mean copy numbers at the nontick sites, and the short horizontal bars indicate mean copy numbers at the tick sites.

was also a significant decrease in the number of spirochetes at the tick attachment site on day 1 compared to the number at zero time ($P = 0.045$). These results suggested that there was active uptake of spirochetes by ticks but no notable proliferation of spirochetes within host skin in response to tick attachment and feeding.

There were no apparent differences in the levels of *flaB*, *ospA*, *ospC*, *dbpA*, and *arp* RNA in skin at tick attachment sites and at nontick sites of infected mice at zero time and 1, 2, and 3 days after tick attachment (Fig. 2). As expected, *ospA* RNA was not detected in skin at any time. *ospC* RNA was inconsistently detected in skin from some sites but not all sites (mostly at the nontick sites). *flaB, dbpA,* and *arp* RNA were detected in nearly all skin samples. These findings suggested that there were no differences in gene expression by spirochetes in the skin at tick attachment sites and nontick sites in mice with established infections.

Spirochete population kinetics and gene expression in infected ticks feeding on uninfected mice. Based upon quantification of spirochetal *flaB* DNA, attachment and feeding of nymphal ticks on uninfected mice stimulated spirochetal proliferation within the feeding ticks after attachment and the results were significant on day 1 ($P = 0.0036$), day 2 ($P = 0.026$), or day 3 ($P = 0.0005$) compared to zero time (Fig. 3). Evaluation of *B. burgdorferi* gene expression in infected ticks prior to and during feeding revealed RNA transcription of *flaB*, *ospA*, *ospC*, *dbpA*, and *arp* at all times after tick attachment (Fig. 3). The peak mRNA levels for all targets were observed on day 2 after tick attachment, and there were decreases on day 3 (Fig. 3).

Spirochete population kinetics and gene expression in host skin of uninfected mice in response to infected-tick feeding. Twenty-four hours after tick attachment, *flaB* DNA was detected in the skin at the tick attachment sites in four of eight mice. All tick attachment sites were *flaB* DNA positive at later times, and the spirochete numbers rose between days 1 and 2 (Fig. 4). Thus, attachment and feeding stimulated spirochete proliferation within the ticks, and transmission of spirochetes from the ticks to the hosts could be detected as early as 1 day after tick attachment. Analysis of nontick sites revealed dissemination of spirochetes, based upon the presence of *flaB* DNA in skin samples from two of eight mice at 3 days after tick attachment but not at earlier times.

Despite the detectable presence of approximately the same amount of spirochetal DNA at the tick attachment sites after 1 day and thereafter, the gene expression was remarkably low (Fig. 4) compared to that of spirochetes in the skin of mice with established infections (Fig. 2). The most striking finding was the lack of *flaB* RNA expression at tick attachment sites 1, 2, and 3 days after tick attachment. *ospA* transcription was detected transiently during early transmission (on day 1), in contrast to *ospC* RNA, which was detected after 3 days but not earlier (on days 1 and 2). *dbpA* and *arp* RNA were either absent or detected at low levels. These results suggest that during the early stages of entry into the host, spirochetes are not particularly active until the dissemination stage. The 3-day interval, in which *ospC* was up-regulated to levels found during disseminated infection (Fig. 2), may represent the beginning of this process. Comparison of *dbpA* and *arp* gene expression early in infection (Fig. 4) and *dbpA* and *arp* gene expression during disseminated infection (Fig. 2) suggested that these genes are up-regulated between 3 days and 2 weeks.

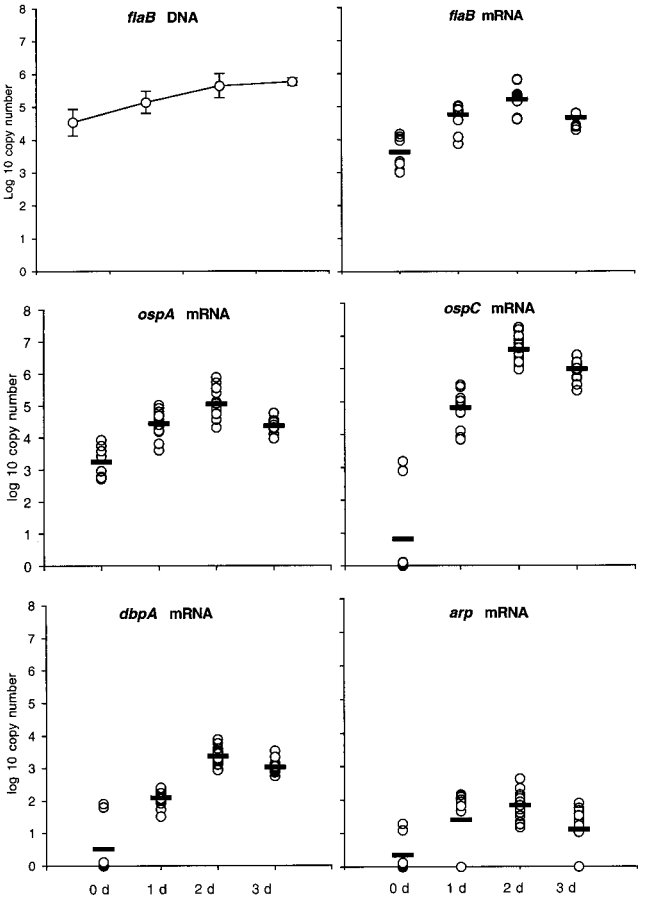

FIG. 3. Mean copy number ± standard deviation for *flaB* DNA in infected nymphal ticks fed on uninfected mice and mRNA levels of *flaB*, *ospA*, *ospC*, *dbpA*, and *arp* genes in the same ticks at different times after attachment. Ticks were tested at zero time and 1, 2, and 3 days after attachment. The horizontal bars indicate mean copy numbers.

DISCUSSION

During their life cycle, ticks feed as larvae on small rodent reservoir hosts, thereby acquiring *B. burgdorferi* spirochetes. Spirochetes remain in the ticks as they molt into nymphs and adults. Examination of flat (unfed) nymphal ticks used in this study revealed that the mean number of spirochetes was 5×10^4 cells per tick, which correlated with the number that we have found in nymphal ticks used for several other experiments and is in accordance with similar results reported by other workers (6). As shown by other workers (12) and in the present study, feeding stimulates spirochetes to rapidly multiply within ticks, as observed in infected ticks during feeding. Our results indicate that feeding ticks acquire spirochetes within 1 day after attachment to infected hosts and that hosts acquire spirochetes within 1 day after attachment of infected ticks. Other workers have shown that there is transmission within 1 day, but at a very low rate (36). The differences are likely to be due to

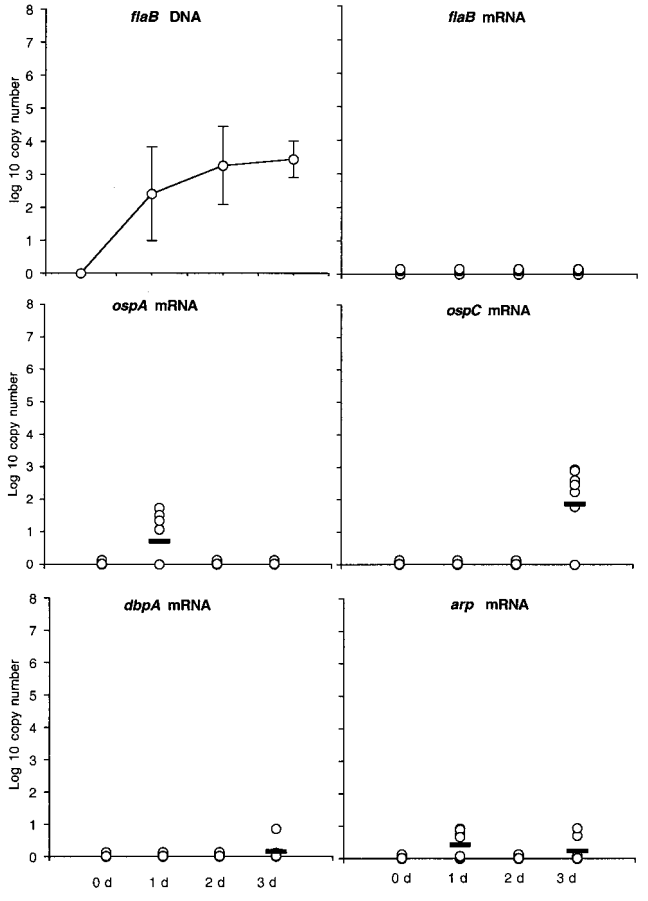

FIG. 4. Mean copy number ± standard deviation for *flaB* DNA per milligram of skin tissue at the attachment sites of infected ticks from uninfected mice and mRNA levels of *flaB*, *ospA*, *ospC*, *dbpA*, and *arp* genes in the same skin samples at different times after tick attachment. The horizontal bars indicate mean copy numbers.

spirochete isolation and experimental variables, but the data demonstrate that transmission can be quite rapid.

Using quantitative PCR, we have previously found significantly lower numbers of spirochetes at tick attachment sites than at nontick sites for mice with established infections (24). The present study confirmed this observation, suggesting that spirochetes actively leave the site of tick attachment (and move into the tick). The diminished number of spirochetes in the skin at the tick attachment site correlates with the concomitant acquisition of spirochetes by a feeding tick. Investigating these findings further, we assessed expression of selected genes in the skin at the tick attachment sites and at nontick skin sites. No apparent differences in levels of transcription of *flaB*, *ospA*, *ospC*, *dbpA*, or *arp* were observed. These results suggested that tick attachment and feeding do not influence RNA transcriptional activity of spirochetes and do not recruit spirochetes to a tick site. Thus, acquisition of spirochetes by feeding ticks appears to be a passive process for spirochetes.

On the other hand, interaction of spirochetes with the tick and/or the host results in dynamic spirochetal changes, including genetic variation and shifts in protein expression through both antigenic variation and antigenic modulation (reviewed in reference 3). Certainly, some of these changes are means of avoiding immune clearance, but in the absence of immune pressure, it is likely that changes in protein expression allow spirochete adaptation to the markedly varied environments of the tick and the host. A variety of environmental stimuli can invoke shifts in gene and protein expression in vitro (7, 8, 13, 27, 32, 38, 39, 41, 44, 45). Differential protein expression is also likely to facilitate spirochete transmission from the tick midgut to tick salivary glands and to the host's dermis during feeding (34).

Some workers have shown that spirochetes in flat (unfed) *I. scapularis* ticks express OspA but not OspC (33, 41), while other workers have reported spirochetal expression of OspC in unfed *I. ricinus* ticks (18, 30). In our study, we detected *ospA* RNA in all unfed nymphal ticks tested and *ospC* RNA in some of the unfed ticks tested. We found that during acquisition of spirochetes by both larval and nymphal ticks feeding on infected mice, *ospA* was transcriptionally up-regulated by spirochetes in feeding ticks but was not detected in the skin of the infected mice. These results confirm the premise that OspA is a tick-regulated antigen. OspA appears to facilitate spirochete attachment to the tick gut by binding to an *I. scapularis* protein (34). In a recent study that examined OspA expression in ticks by immunohistochemistry, it was found that spirochetes expressed OspA but not OspC in flat ticks and OspC expression increased during feeding, whereas OspA expression decreased (40). Our findings indicate that both *ospA* and *ospC* mRNA transcription occurred in uninfected ticks feeding on infected mice, as well as in infected ticks feeding on uninfected mice during the feeding process. The quantitative kinetics of these gene targets, as well as *flaB*, suggested that the increases parallel the population kinetics of spirochete proliferation within feeding ticks. Differences among studies can be readily explained by differences related to transcription, translation, and shedding of gene products. Our finding that there was decreased *ospA* and *ospC* transcription after molting (10 days) is in accordance with similar results showing that there was decreased OspA and OspC protein expression in ticks following feeding to repletion, as determined by other workers (33, 40). In vitro studies have shown that expression of OspA and expression of OspC increase in the presence of tick hemolymph and at a lower temperature (28, 32).

Less is known about the differential expression of genes or the effects of the gene products within the host, but it is logical that some gene products facilitate spirochete dissemination. For example, DbpA binds decorin on collagen (21), which is consistent with the strong dermatotropism of this organism. Immunization against DbpA has been shown to elicit protective immunity against syringe challenge but not tick challenge (15, 22, 23). An intriguing protein is Arp, which elicits an antibody response that is not protective but, when antiserum is passively transferred to infected mice, selectively resolves arthritis with no apparent effect upon carditis or the infection status (14). There are a number of other proteins that have possible biologic effects during host infection, including binding to fibronectin (20), proteoglycans (29), glycosphingolipids (1), plasminogen (10), and integrins (9).

With the optimization of RNA detection methods, differential *B. burgdorferi* gene expression is being examined in both the vector and the host. Transcription of *erpT* (which is not an *erp* gene or a gene encoding an E/F related protein but rather

is a truncated version of *arp*) and transcription of *bbk32* were both shown to be expressed in engorged ticks (16, 17). A more comprehensive study has recently been published, in which several genes, including *ospA*, *ospC*, *flaB*, *erpA*/*I*/*N*, *erpB*/*J*/*O*, *rev*, and *mlpA*, were shown to be transcriptionally active in both larval and nymphal ticks following feeding. Furthermore, *ospC*, *mlpA*, and *rev* transcription was not detected in the resting or flat-tick stage. Another gene that has been found to be expressed in the host, *bba64*, was not detected at any time in ticks (19). The gene targets that were chosen for analysis were targets shown to be regulated by environmental stimuli, including expression in the host during infection.

In the present study, we selected *flaB*, *ospA*, and *ospC* for similar reasons, but we focused on two immunodominant antigens (*dbpA* and *arp*) that invoke biologically relevant immune responses (protective immunity and arthritis-resolving immunity) during infection. In this study we utilized a more sensitive, real-time PCR method to examine both quantitative spirochete population kinetics and gene transcription. The sensitivity of this method allowed analysis of individual ticks rather than pools of ticks. This approach revealed a pattern of rising *flaB*, *ospA*, and *ospC* transcription during tick feeding, followed by a decline after molting. Similar patterns occurred in uninfected ticks that acquired the infection during feeding and in already infected ticks during the course of feeding. Feeding also stimulated *dbpA* and *arp* transcription within ticks. Transcription of *flaB*, *dbpA*, and *arp* was evident within the dermis of infected mice, whereas *ospC* transcription was found to be quite variable. Previous studies, in which the less sensitive reverse transcription-PCR method was used, revealed that *erpT* (truncated *arp*) was not transcribed during early infection (16). The sensitivity of real-time PCR allowed transient detection of low levels of *ospA* transcription in the host dermis after 1 day but not thereafter. Of all of the gene targets examined, transcription of *ospA* had the most striking on-off pattern in different environments. Results confirmed that *ospA* transcription does not occur in the host, and its transient presence early in infection is likely to be a residual event in spirochetes leaving a tick.

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