INFECTION AND IMMUNITY, June 2005, p. 3313–3321 Vol. 73, No. 6
0019-9567/05/$08.00+0 doi:10.1128/IAI.73.6.3313–3321.2005

Immunoglobulin-Regulated Expression of *Borrelia burgdorferi* Outer Surface Protein A In Vivo

Emir Hodzic, Stefan Tunev, Sunlian Feng, Kim J. Freet, and Stephen W. Barthold*

Center for Comparative Medicine, Schools of Medicine and Veterinary Medicine, University of California Davis, Davis, California 95616

Received 2 November 2004/Returned for modification 22 December 2004/Accepted 14 January 2005

Borrelia burgdorferi*, the agent of Lyme disease, down-regulates outer surface protein A (OspA), which is abundantly expressed in ticks, during infection of the mammalian host. In this study we examined the signals that may be responsible for maintaining the OspA-negative state of spirochetes during infection. Transcription of *ospA* mRNA was found in tissues of C3H-severe combined immunodeficient (C3H-*scid*) mice, but not immunocompetent C3H mice, inoculated with cultured *B. burgdorferi*, tick-borne spirochetes, and host-adapted spirochetes. Transcription was more frequent at 4 weeks than at 1 week. Transcription was present at the host-tick interface as early as 24 h after tick attachment but declined at 48 and 72 h. Thus, *ospA* mRNA transcription in distant tissues and at later times in C3H-*scid* mice is probably due to up-regulation during infection. Adoptive lymphocyte transfer from naïve C3H mice to infected C3H-*scid* mice resulted in OspA seroconversion, confirming OspA expression in the host. Passive transfer of normal mouse serum, immunoglobulin M (IgM) from normal mouse serum, or IgG from normal mouse serum into infected C3H-*scid* mice resulted in down-regulation of *ospA*, but transfer of normal mouse serum depleted of immunoglobulin did not influence *ospA* mRNA transcription. Collectively, our results indicate that *ospA* mRNA transcription in the host is regulated by nonspecific immunoglobulin, which may be a natural antibody.

Lyme borreliosis in humans, as well as in domestic and wild animals, is caused by spirochetes belonging to the *Borrelia burgdorferi* sensu lato complex, including *B. burgdorferi* sensu stricto in North America. *B. burgdorferi* is transmitted to and acquired from its hosts by hard tick species belonging to the *Ixodes persulcatus* complex, which includes *Ixodes scapularis* and *Ixodes pacificus* in North America, *Ixodes ricinus* in Europe, and *Ixodes persulcatus* in Eastern Europe and Asia (2, 38, 49). In the United States, a wide variety of birds and mammals serve as reservoir hosts, but the principal reservoir host is the white-footed mouse, *Peromyscus leucopus*, and the white-tailed deer, *Odocoileus virginianus*, plays a critical role as a host for adult ticks (2). The life cycle of *I. scapularis* requires at least 2 years, involving larval, nymphal, and adult stages that must feed on hosts while also surviving seasonal weather variations. *B. burgdorferi* must also persevere through and adapt to these various conditions.

The ability of *B. burgdorferi* to survive and adapt to these markedly changing conditions is believed to be facilitated by differential expression of various gene products, particularly outer surface proteins (Osps). A notable example is OspA, a major 31-kDa lipoprotein that is abundantly expressed by *B. burgdorferi* in the midgut of unfed ticks and by spirochetes grown in artificial media but is generally not expressed during infection of mammalian hosts. OspA has been the subject of intensive investigation since its initial discovery (3, 10, 22, 26, 37, 52). Among the factors that have been shown to modulate OspA expression are temperature (34, 48), pH (51, 52), cocultivation with tick cells (34), exposure to tick hemolymph (20), the presence of anti-OspA antibody within feeding ticks (17), the presence of natural antibody (9), and serum starvation (1).

More germane to the role of OspA in the infectious cycle and the reason for investigating its expression under various conditions is the fact that OspA is highly dynamic in the tick and the host. In unfed infected ticks (nymphs and adults) spirochetes are restricted to their midgut and express abundant OspA (16), whereas feeding by the tick stimulates spirochetes to rapidly multiply and migrate to the salivary glands but significantly down-regulates OspA (13, 14, 47, 48). Immunization of hosts against OspA protects against tick-borne infection by killing OspA-expressing spirochetes in the tick midgut during the initial stages of feeding (22). When spirochetes are transmitted to naïve hosts, they do not express OspA (24, 30, 40) and are therefore no longer vulnerable to OspA immunity (15). These dynamics are reflected in the sera of most patients and animals following tick-borne infection with *B. burgdorferi*. Such sera do not contain OspA antibody, whereas sera from experimental animals inoculated by syringe with high doses of cultured *B. burgdorferi*, which expresses OspA, contain OspA antibodies (5, 6, 8, 19, 23, 36, 41, 42).

In contrast to these findings, OspA antibody has been found in the sera of some human patients late in the course of infection, suggesting OspA expression (19, 27, 28). Furthermore, serial serum samples from Lyme disease patients have revealed seroconversion to OspA coinciding with the severity and onset of arthritis, as well as the duration of arthritis (27, 28). The mechanism for this paradoxical event has not been determined, but one possible explanation is that OspA expression can be increased by spirochetes maintained in an inflammatory environment in vivo (12). In addition to OspA antibody in some patients with chronic infections and disease, low levels

* Corresponding author. Mailing address: Center for Comparative Medicine, University of California at Davis, One Shields Avenue, Davis, CA 95616. Phone: (530) 752-1245. Fax: (530) 752-7914. E-mail: swbarthold@ucdavis.edu.

of OspA antibody, primarily immunoglobulin M (IgM), have been detected in many patients during early infection, but these antibodies tend to be complexed with antigen, and the response appears to be transient (43–45).

In the immunocompetent mouse model of Lyme disease, seroconversion to OspA and *ospA* transcription have not been detected during infection following tick-borne infection or infection with host-adapted spirochetes (7, 11, 15, 23–25, 42). We recently published seemingly contrary studies that found low levels of *ospA* mRNA in the skin, hearts, and tibiotarsal joints of C3H-severe combined immunodeficient (C3H-*scid*) mice inoculated by syringe with cultured *B. burgdorferi*. Furthermore, *ospA* mRNA transcription was most commonly found after 2 or more weeks of infection and at sites distant from the inoculation site, suggesting that *ospA* mRNA transcription took place by disseminating spirochetes within the host (25).

To further explore this phenomenon, we assessed *B. burgdorferi ospA* mRNA transcription in C3H-*scid* mice following syringe inoculation, tick-borne inoculation, or inoculation with host-adapted *B. burgdorferi* and examined the antibody responses in infected C3H-*scid* mice that were adoptively reconstituted with lymphocytes from immunocompetent mice. The results led to investigation of the effect of non-OspA (nonspecific) antibody on *ospA* mRNA transcription. Our studies suggest that host innate immunity, mediated through immunoglobulins, is involved in modulation of OspA expression in vivo.

MATERIALS AND METHODS

Mice. C3H/Smn.CIcrHsd-*Prkdc*^{scid} (C3H-*scid*) and C3H/HeN (C3H) specific-pathogen-free mice (3 to 5 weeks old) were purchased from Harlan Sprague-Dawley, Inc. (Indianapolis, Ind.) and Frederick Cancer Research Center (Frederick, MD), respectively. Mice were housed in isolator cages, given food and water ad libitum, and killed by carbon dioxide narcosis, followed by cardiocentesis.

***B. burgdorferi*.** A clonal strain of *B. burgdorferi* sensu stricto (cN40) was grown in modified Barbour-Stoenner-Kelly (BSK II) medium (2) at 33°C. At the time of necropsy, tissues (blood, inoculation site, and urinary bladder) were cultured in modified BSK II medium, as described previously (6), to confirm the infection status of each mouse.

Mouse inoculation. For syringe inoculation, 10^4 *B. burgdorferi* cN40 spirochetes at the mid-log phase in 0.1 ml of BSK II medium were inoculated intradermally at the dorsal thoracic midline into each mouse. For tick-borne inoculation, five nymphal ticks that were infected with *B. burgdorferi* cN40 were placed on the dorsal thoracic midline and allowed to attach and feed to repletion. For infection with host-adapted spirochetes, 3-mm punches were obtained from ears of infected C3H mice using disposable dermal biopsy punches (Premiere Medical Supply) at 3 weeks after syringe inoculation. The punches were placed in BSK II medium and transplanted beneath the skin of the dorsal thoracic midline through a pocket watch incision, as described previously (4, 7). Recognizing that doses could not be exactly the same with these different types of inocula, we attempted to roughly equilibrate inocula for all of these methods to the 10^4-spirochete dose inoculated by syringe. For tick inoculation we utilized nymphal ticks with a mean of 5×10^4 spirochetes per tick (unknown numbers were transmitted), and the 3-mm-diameter ear transplants contained a mean of 1.8×10^4 spirochetes, based upon quantitative DNA analysis (see below).

Ticks. *I. scapularis* ticks were obtained as field-collected adults from southern Connecticut (cordially provided by Durland Fish, Yale University, New Haven, CT), and they produced uninfected larvae for experimental use. All larvae were derived from a single cohort for the experiments described in this study, and a sample of the cohort was confirmed to be uninfected by *B. burgdorferi flaB* PCR. To generate infected nymphs, larvae were allowed to engorge on C3H mice that had been infected with *B. burgdorferi* for 2 weeks following syringe inoculation, as described previously (25). A sample of the infected nymphal tick cohort revealed that 97% of the ticks were PCR positive for *B. burgdorferi flaB.*

Quantitative analysis of *B. burgdorferi* DNA and mRNA. Tissue samples were weighed, snap-frozen in liquid nitrogen immediately after collection, pulverized, and homogenized, and then they were split equally for use for DNA and RNA extraction. DNA was extracted using DNeasy tissue kits according to the manufacturer's instruction for tissues (QIAGEN, Valencia, CA), and total RNA was purified using RNeasy mini kits according to the manufacturer's instruction for tissues (QIAGEN, Valencia, CA). The copy number of each *B. burgdorferi* target gene was expressed per mg of tissue. Samples used for RNA analysis were homogenized with a QIAshredder and then treated with RNase-free DNase I prior to elution. 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 use. For synthesis of cDNA from extracted total RNA, reverse transcription was performed utilizing TaqMan reverse transcription reagents (PE Biosystems, Foster City, CA). cDNA was 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. Real-time PCR was optimized and utilized to quantify *B. burgdorferi* population dynamics targeting *flaB* DNA, as well as to evaluate *ospA* transcriptional activity, targeting *ospA* cDNA, as described previously (25).

Histology. Hearts and joints (knees, tibiotarsi) were fixed in 10% neutral buffered formalin. Joints were demineralized in decalcifying solution. Formalin-fixed tissues were embedded in paraffin, sectioned at 5 μm, and then stained with hematoxylin using standard histologic techniques.

Serology. An antibody capture enzyme-linked immunosorbent assay (ELISA) was used to determine serum IgM and IgG titers to mouse IgG (heavy and light chains) (Jackson Immuno-Research Laboratories, Inc., West Grove, PA), *B. burgdorferi* cN40 lysate, and recombinant OspA. Briefly, ELISA plates (Nunc ImmunoMax Maxi-Sorp, Wiesbaden, Germany) were coated with 1 μg/ml of rat anti-mouse IgG (Jackson Immuno-Research Laboratories, Inc.), *B. burgdorferi* lysate, or recombinant cN40 OspA (21) in carbonate buffer and incubated overnight at 4°C. After the plates were washed and after nonspecific binding was blocked with 1% bovine serum albumin, twofold serial dilutions of sera were titrated in the plates, which were incubated overnight at 4°C. The plates were washed again and then incubated with alkaline phosphatase-conjugated rat-anti mouse IgG(H+L) (Jackson Immuno-Research Laboratories, Inc.). After a final wash, wells were incubated with 1 mg of alkaline phosphatase substrate (Sigma, St. Louis, Mo.) per ml for color development. Absorbance was read with an ELISA reader (Molecular Devices, Sunnyvale, CA) at a test wavelength of 405 nm. Each assay included positive and negative controls for serum. The cutoff point for positive reactions was established at 3 standard deviations above normal mouse serum or control antigen background.

Purification of serum immunoglobulins. Normal mouse serum was obtained from specific-pathogen-free female C3H mice (3 to 5 weeks old). To equilibrate serum to buffer, serum was dialyzed overnight at 4°C against binding buffer (0.1 M sodium phosphate, 0.15 M sodium chloride, pH 7.2). For separation of IgM, the serum was applied to an AminoLink Plus (Pierce Biotechnology, Inc., Rockford, IL) column with immobilized rat anti-mouse IgM antibody. Bound fractions of IgM were eluted with 0.1 M glycine, pH 2.7. For separation of IgG, the remaining serum was applied consecutively to Affinity Pak immobilized protein L (Pierce) and protein G HiTrap affinity (Amersham Biosciences Corporation, Piscataway, NJ) columns. Bound fractions of IgG were eluted with 0.1 M glycine, pH 2.7. The immunoglobulin content of all fractions (normal mouse serum, serum devoid of immunoglobulins, IgG and IgM fractions) was assessed by quantitative ELISA. The concentrations of immunoglobulin in the IgG and IgM fractions were adjusted to the levels detected in the normal mouse serum.

Statistics. Statistical comparisons of mean numbers of spirochetes per mg of tissue for infected C3H-*scid* and C3H mice were made using StatView (PowerPC version; SAS Institute Inc., Cary, NC). Multiple-comparison analyses were performed with a one-way analysis of variance, followed by a least-difference post hoc test. *P* values lower than 0.05 were considered significant.

RESULTS

Comparison of *ospA* mRNA transcription in C3H-*scid* mice infected by syringe or with tick-borne spirochetes. An experiment was performed not only to confirm previous findings (24) for *ospA* mRNA transcription in syringe-inoculated C3H-*scid* mice but also to determine if *ospA* mRNA transcription was due to the fact that mice were inoculated with cultured spirochetes or if tick-borne spirochetes elicited the same effect. Ten C3H-*scid* mice were infected with *B. burgdorferi* by allowing

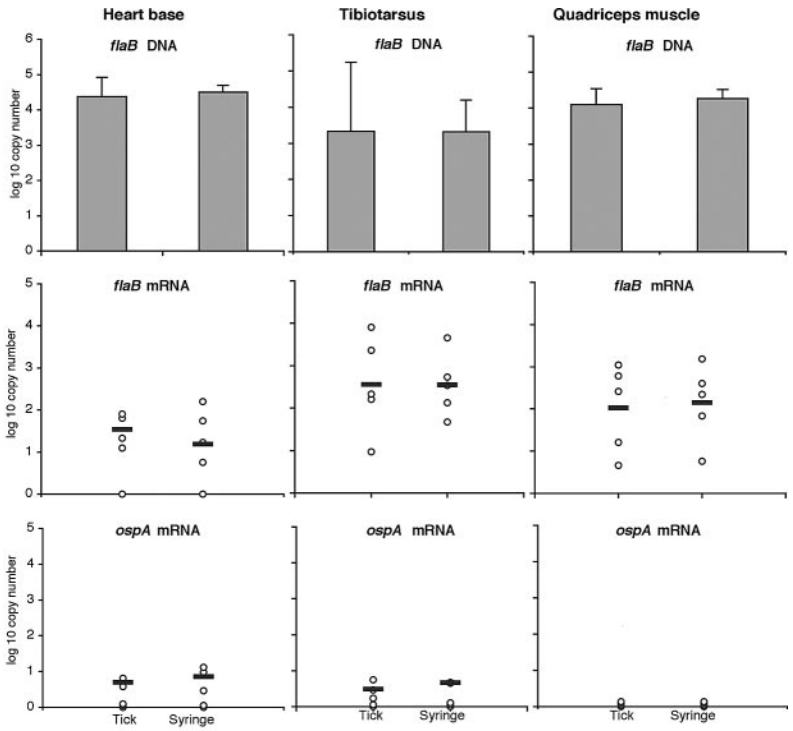

FIG. 1. Quantitative analysis of *flaB* DNA (means ± standard deviations) (top panels), *flaB* mRNA (middle panels), and *ospA* mRNA (bottom panels) in heart base, tibiotarsus, and quadriceps muscle of C3H-*scid* mice at 4 weeks after tick-borne or syringe infection with *B. burgdorferi* cN40. The values are expressed per milligram of tissue.

five infected nymphal ticks to feed to repletion. Two days after the ticks were placed on these mice, an additional 10 C3H-*scid* and five C3H mice were syringe inoculated. The staggered timing of inoculation approximately equilibrated the timing of infection, to reflect the delay of spirochete entry after tick attachment (25, 39). At 1 week after inoculation (9 days after tick attachment), five tick-infected and five syringe-infected C3H-*scid* mice were necropsied. At 4 weeks after inoculation, five syringe-infected C3H mice, five syringe-infected C3H-*scid* mice, and five tick-infected C3H-*scid* mice were necropsied. At necropsy, tissues were cultured to verify infection, and heart base, tibiotarsus, and quadriceps femoris muscle samples were used for PCR analysis (*B. burgdorferi flaB* DNA, *flaB* mRNA, and *ospA* mRNA). Quadriceps muscle was included in order to compare an infected tissue without significant inflammation to the tibiotarsus, which manifested arthritis.

At 1 week, hearts from four of five syringe-infected and four of five tick-infected mice, tibiotarsal tissue from four of five syringe-infected and one of five tick-infected mice, and quadriceps muscle from three of five syringe-inoculated mice had detectable *flaB* DNA (data not shown). At 4 weeks, all mice, regardless of the inoculum, had detectable *flaB* DNA in all three tissues. In all *flaB* DNA-positive samples, transcriptional activity of *flaB* mRNA was detectable. In contrast, *ospA* mRNA transcription was detected in only one syringe-infected heart sample at 1 week. At 4 weeks, low levels of *ospA* mRNA transcription were detected in hearts from three of five syringe-infected and two of five tick-infected mice and in tibiotarsi from two of five syringe-infected and three of five tick-infected mice but not in quadriceps muscle (Fig. 1). Collectively, three of five syringe-infected and three of five tick-inoculated mice had detectable *ospA* mRNA transcription in one or more tissues at 4 weeks. These results confirm previous studies which demonstrated that *ospA* mRNA transcription occurred during infection of C3H-*scid* mice (24). The data also suggest that *ospA* mRNA transcription, albeit low and not detectable in all samples, is more frequent after 4 weeks of infection than after 1 week and therefore likely to reflect up-regulation during infection of the host. Furthermore, the presence of *ospA* mRNA transcription in inflamed hearts and joints, but not in uninflamed muscle, supports the observation of other workers (12) that inflammation stimulates *ospA* mRNA transcription.

Transcription of *ospA* mRNA at the vector-host interface in C3H-*scid* mice. The experiment described above suggested that *ospA* mRNA transcription occurred following tick-borne infection. We next examined the skin of C3H-*scid* mice at 24, 48, and 72 h after tick attachment to determine if *ospA* mRNA transcription was present during early transmission (Fig. 2). Fifteen C3H-*scid* mice were each infested with six nymphal ticks, and two tick attachment sites were collected from each mouse for groups of five mice at 24, 48, and 72 h after tick attachment. Of the samples that were positive for *flaB* DNA, 4 of 10 samples (from five *flaB* DNA-positive mice) were positive for *ospA* mRNA transcription at 24 h; one of nine samples (from four *flaB* DNA-positive mice) were positive at 48 h; and none of six samples (from three *flaB* DNA-positive mice) were positive at 72 h. These results confirm previous findings for tick-borne infection in C3H mice (24). They extend the findings to C3H-*scid* mice and indicate that spirochetes initially transcribe *ospA* mRNA at 24 h in the tick attachment sites

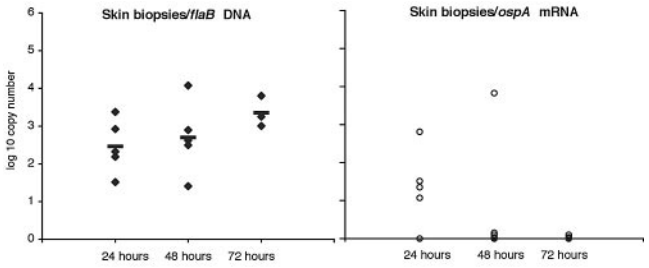

FIG. 2. Quantitative analysis of *B. burgdorferi* cN40 *flaB* DNA (means ± standard deviations) (left panel) and *ospA* mRNA (right panel) in skin of C3H-*scid* mice at the site of tick attachment at 24, 48, and 72 h. The values are expressed per milligram of tissue.

following tick-borne transmission, but spirochetes appear to down-regulate *ospA* mRNA transcription by 48 to 72 h, despite rising numbers of spirochetes in the skin in an immunodeficient host.

Transcription of *ospA* mRNA in C3H-*scid* mice inoculated with host-adapted spirochetes. In the next experiment we sought to determine if *ospA* mRNA transcription is up-regulated in C3H-*scid* mice following infection by transplant of ear tissue containing host-adapted spirochetes from immunocompetent C3H mice (in which *ospA* mRNA transcription does not take place). Ten C3H mice were inoculated by syringe with *B. burgdorferi*. Three weeks later, these donor mice were necropsied, and the infection status was confirmed by culture. All donor mice were culture positive. Ear tissues from the donor mice were transplanted into C3H-*scid* mice. At 1 and 4 weeks after inoculation, groups of five and four C3H-*scid* mice, respectively, were necropsied, tissues were cultured to verify infection, hearts and joints were processed for histology, and the heart base, tibiotarsal joint, and quadriceps muscle were collected for PCR analysis. All mice which received transplants were culture positive.

Carditis and arthritis were present in the four mice examined at 4 weeks but were not evident in the five mice at 1 week. Although *flaB* mRNA transcription was present in hearts and joints of all five mice at 1 week, no *ospA* mRNA transcription was detected at this time. In contrast, *ospA* mRNA transcription was found in three heart samples and one joint sample at 4 weeks (Fig. 3). These results suggest that host-adapted spirochetes (derived from immunocompetent C3H mice), which do not express OspA, appear to up-regulate *ospA* mRNA transcription during infection of C3H-*scid* mice.

Transcription of *ospA* mRNA and seroconversion to OspA in infected C3H-*scid* mice adoptively reconstituted with lymphocytes from immunocompetent C3H mice. Transcription of *ospA* mRNA occurred in C3H-*scid* mice but not in C3H mice, suggesting that *ospA* mRNA transcription is influenced by innate or acquired host immune factors. Furthermore, although these results indicate that *ospA* mRNA transcription occurred, they do not necessarily prove that OspA translation and expression occurred. In an effort to prove that OspA protein expression occurred, C3H-*scid* mice were infected and then immunologically reconstituted and tested for seroconversion to OspA. Twenty C3H-*scid* mice were syringe inoculated with *B. burgdorferi*. At 2 weeks after inoculation, 10 C3H-*scid* mice were reconstituted by intraperitoneal inoculation with 2×10^7

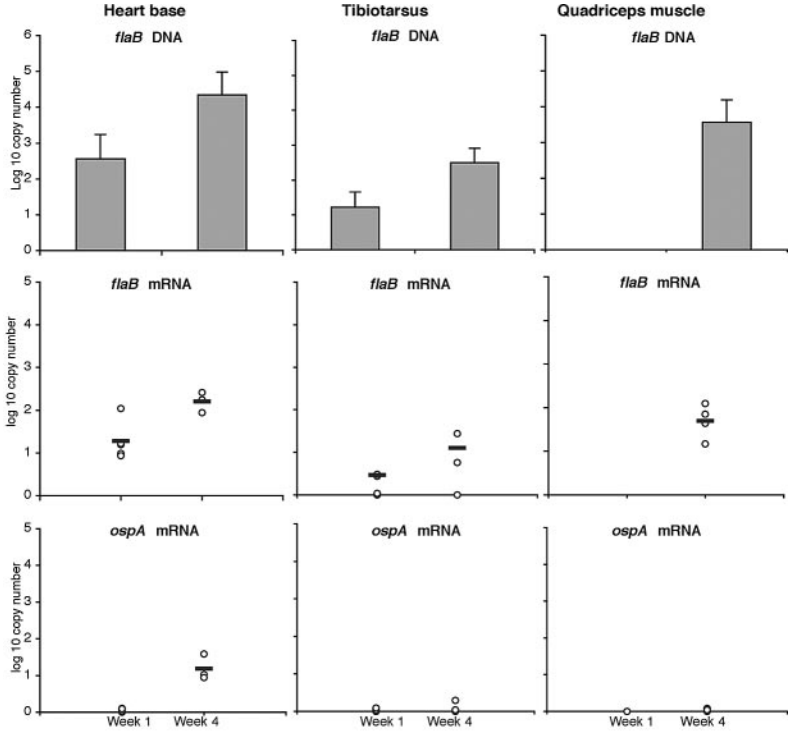

FIG. 3. Quantitative analysis of *flaB* DNA (means ± standard deviations) (top panels), *flaB* mRNA (middle panels), and *ospA* mRNA (bottom panels) in heart base, tibiotarsus, and quadriceps muscle of C3H-*scid* mice at 1 and 4 weeks after infection by transplantation of ear tissue containing host-adapted *B. burgdorferi* cN40 spirochetes from immunocompetent C3H mice. The values are expressed per milligram of tissue.

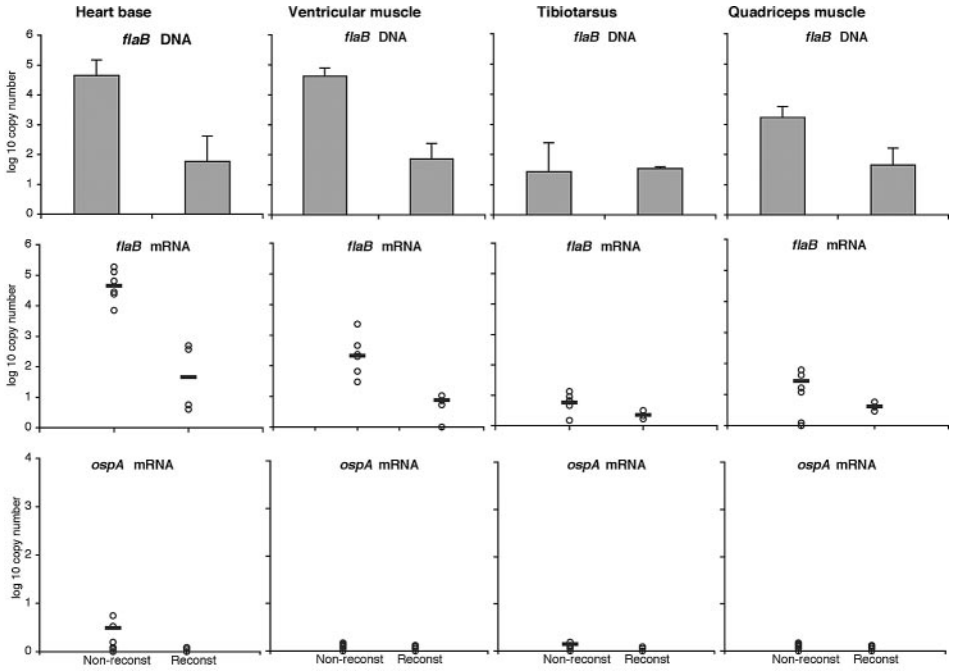

FIG. 4. Quantitative analysis of *flaB* DNA (means ± standard deviations) (top panels), *flaB* mRNA (middle panels), and *ospA* mRNA (bottom panels) in heart base, ventricular muscle, tibiotarsus, and quadriceps muscle of C3H-*scid* mice at 6 weeks after reconstitution with normal C3H lymphocytes (Reconst) or after no reconstitution (Non-reconst). The values are expressed per milligram of tissue.

splenocytes and 1×10^7 lymph node cells (total, 3×10^7 cells) from naïve C3H mice. At 4 weeks after inoculation, five nonreconstituted C3H-*scid* mice were necropsied. At 6 weeks after inoculation, five nonreconstituted and five reconstituted (4 weeks after adoptive transfer) mice were necropsied, and at 8 weeks after inoculation, five reconstituted mice were necropsied (6 weeks after reconstitution). The infection status was confirmed by culture. Hearts, joints, and muscles were analyzed by PCR, and serum was tested for antibody. In this experiment, heart base was compared to ventricular muscle (inflamed versus adjacent noninflamed tissue), and the tibiotarsus was compared to quadriceps muscle (inflamed versus adjacent noninflamed tissue).

At necropsy, all mice from both groups (reconstituted and nonreconstituted) were culture and PCR positive. Carditis and arthritis were present in nonreconstituted C3H-*scid* mice at 4 and 6 weeks, and the arthritis was severe. Inflammatory lesions were present in the heart base but not in the ventricular muscle and were present in the tibiotarsus but not in the quadriceps muscle. Carditis and arthritis were less severe in reconstituted mice at 6 weeks and were regressing at 8 weeks (data not shown). Nonreconstituted C3H-*scid* mice were examined at 4 weeks to verify transcription of *ospA* mRNA at a time at which the reconstituted mice would be developing acquired immunity 2 weeks following adoptive transfer. Transcription of *ospA* mRNA was verified in one or more tissues from four of five mice (two of five heart bases; three of five tibiotarsi) (Fig. 4). Thus, it could be assumed that similarly inoculated but reconstituted C3H-*scid* mice were likely to immunologically recognize OspA, if it was translated into protein.

Comparing the spirochete loads for different treatment groups of mice at week 6 (Fig. 4) revealed significantly higher spirochete numbers (*flaB* DNA) in the heart base ($P = 0.0007$), ventricular muscle ($P = 0.00027$), and quadriceps muscle ($P = 0.00001$) of nonreconstituted mice than in the tissues of reconstituted mice. In contrast, reconstitution did not significantly reduce spirochete numbers in tibiotarsal joints ($P = 0.37$), despite resolving arthritis. Quantitative analysis of *flaB* mRNA revealed a pattern that followed the quantitative *flaB* DNA dynamics for both groups of mice and for all tissues. Transcription of *ospA* mRNA was detected in the heart base in two of five mice and in the tibiotarsus of three of five mice (four of five mice tested had *ospA* transcription in one or both sites) but not in the ventricular muscle or quadriceps muscle. None of the reconstituted mice had detectable *ospA* mRNA transcription in any of the tissues. These results confirmed that *ospA* mRNA transcription occurs in the absence of immunity. Furthermore, they support the observation that *ospA* mRNA transcription tends to occur in tissues with inflammation (heart base and tibiotarsus), in contrast to adjacent, noninflamed tissues (ventricular muscle and quadriceps muscle).

IgM and IgG were detected in all reconstituted mice at high titers (>1:25,000), confirming that there was successful reconstitution. Antibodies against *B. burgdorferi* lysates were detected in all reconstituted C3H-*scid* mice, with the highest titers in mice necropsied at 8 weeks (data not shown). When plates were coated with OspA and probed with sera from reconstituted C3H-*scid* mice, OspA antibodies were detected at a titer of 1:100 in two of five mice necropsied at 8 weeks (data not shown). Thus, detection of OspA antibodies in reconstituted C3H-*scid* mice confirmed that the low levels of *ospA* mRNA transcription in C3H-*scid* mice are accompanied by OspA translation, based upon seroconversion.

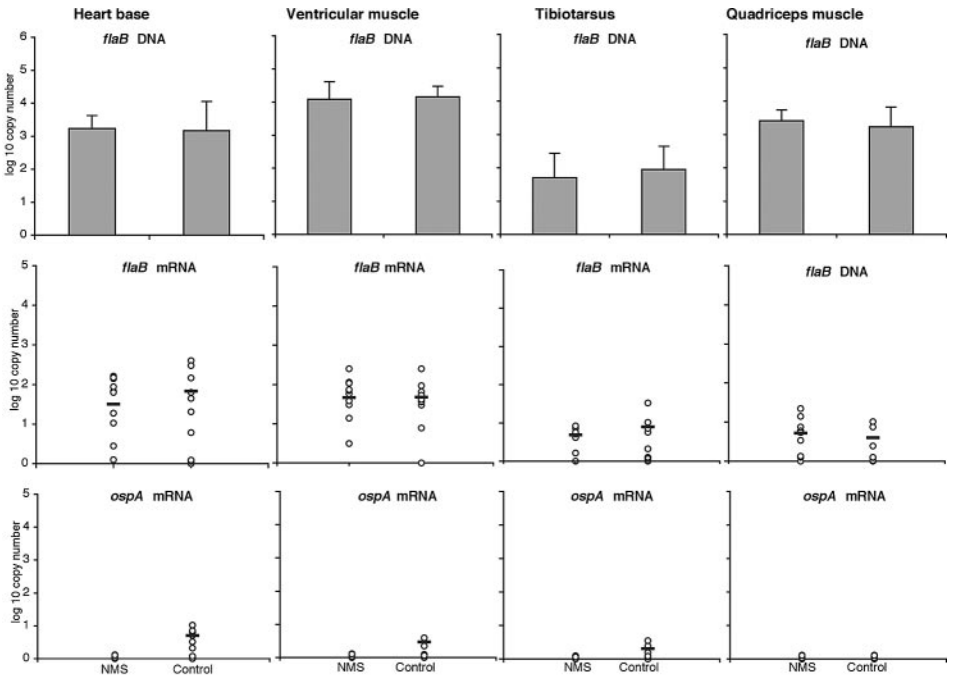

FIG. 5. Quantitative analysis of *flaB* DNA (means ± standard deviations) (top panels), *flaB* mRNA (middle panels), and *ospA* mRNA (bottom panels) in heart base, ventricular muscle, tibiotarsus, and quadriceps muscle of C3H-*scid* mice treated with normal C3H mouse serum (NMS) at the time of and at 2 weeks after inoculation with *B. burgdorferi* cN40 and of C3H-*scid* mice receiving no serum (control). The values are expressed per milligram of tissue.

Effect of normal serum on *ospA* mRNA transcription in infected C3H-*scid* mice. Other workers (9) have shown that natural antibodies in naïve mice interact with spirochetes in feeding ticks and influence OspA expression within the tick. Therefore, the effect of normal mouse serum on *ospA* mRNA transcription in infected C3H-*scid* mice was investigated. Twenty C3H-*scid* mice were syringe inoculated with *B. burgdorferi*. Ten of the mice were each injected intraperitoneally with 1 ml of normal (uninfected) C3H mouse serum at the time of inoculation and at 2 weeks, and the other 10 mice received no serum. At 4 weeks, mice were necropsied, tissues were cultured to verify infection, and tissues (heart base, ventricular muscle, tibiotarsus, and quadriceps muscle) were processed for *B. burgdorferi* DNA and RNA analysis.

All mice were culture positive. The numbers of spirochetes, based upon *flaB* DNA copy numbers, and the levels of *flaB* mRNA transcription in each tissue were equivalent in the two groups (serum treated and untreated) of C3H-*scid* mice (Fig. 5). Transcription of *ospA* mRNA was found in the heart base of 6 of 10 untreated mice, in the ventricular muscle of 2 of 10 untreated mice, and in the tibiotarsus of 4 of 10 untreated mice. Collectively, 7 of 10 untreated mice had *ospA* mRNA transcription in one or more tissues. No *ospA* mRNA transcription was detected in the quadriceps muscle from any of the untreated mice. In contrast, no *ospA* mRNA transcription was detected in the heart base, ventricular muscle, tibiotarsus, or quadriceps muscle of mice treated with normal mouse serum (10 mice had no *ospA* mRNA transcription in any tissue). Thus, these results suggest that components of serum from naïve, immunocompetent mice influence *ospA* mRNA transcription in the host.

Effect of normal mouse serum and immunoglobulin fractions on *ospA* mRNA transcription in infected C3H-*scid* mice. Next, we attempted to localize the effector for down-regulation of *ospA* mRNA transcription to the immunoglobulin fraction of serum. Twenty C3H-*scid* mice were syringe inoculated with *B. burgdorferi* and divided into four equal groups of five mice. Group 1 received 1 ml of normal mouse serum at the time of inoculation and at 2 weeks. Group 2 received 1 ml of normal mouse serum that was depleted of immunoglobulin. Group 3 received 1 ml of the IgG fraction from normal mouse serum. Group 4 received 1 ml of the IgM fraction from normal mouse serum. At 4 weeks, mice were necropsied, tissues were cultured to verify infection, and the heart base, ventricular muscle, tibiotarsus, and quadriceps muscle were processed for *B. burgdorferi* DNA and RNA analysis.

The various treatments had no effect upon *B. burgdorferi flaB* DNA levels or *flaB* mRNA transcription. For the 20 mice and 20 tissue samples from each mouse, *ospA* mRNA transcription was found only in tissues from three mice (two of three heart base samples, one of three tibiotarsus samples) treated with serum that was depleted of immunoglobulin (Fig. 6). Furthermore, these results confirmed the effect of normal mouse serum and nonspecific immunoglobulins on *ospA* mRNA transcription.

DISCUSSION

The results of the current study reveal that *ospA* mRNA transcription takes place during infection of tissues in immunodeficient C3H-*scid* mice. The level of activity was low, and activity was not detectable in all mice or all samples. Collective

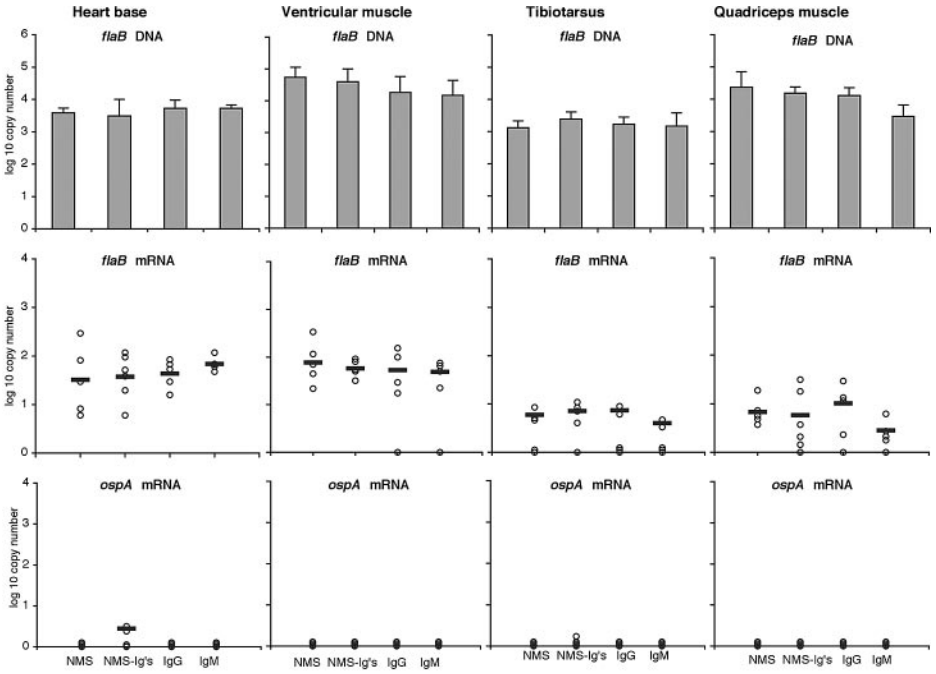

FIG. 6. Quantitative analysis of *flaB* DNA (means ± standard deviations) (top panels), *flaB* mRNA (middle panels), and *ospA* mRNA (bottom panels) in heart base, ventricular muscle, tibiotarsus, and quadriceps muscle of C3H-*scid* mice treated with normal C3H mouse serum (NMS), NMS depleted of immunoglobulin (NMS-Ig's), the IgG fraction of NMS (IgG), and the IgM fraction of NMS (IgM) at the time of and at 2 weeks after inoculation with *B. burgdorferi* cN40. The values are expressed per milligram of tissue.

analysis of the data, however, suggested that tissues with inflammation (heart base and tibiotarsus) favored *ospA* mRNA transcription. In contrast, noninflamed tissues (cardiac ventricular muscle and quadriceps muscle), which were consistently infected and contained spirochetes that were actively transcribing *flaB*, tended not to favor *ospA* mRNA transcription. These findings support observations of *ospA* mRNA transcription and OspA expression by spirochetes that were maintained in an inflammatory environment (12). In that study, spirochetes were maintained in dialysis tubing chambers implanted in the peritoneum of mice, which were then were subjected to intraperitoneal treatment with zymosan, a yeast cell extract that induces inflammation. Under such conditions, spirochetes increased transcription of *ospA* mRNA, as well as expressed increased amounts of OspA. In contrast to the current study, the spirochetes in the peritoneal chambers were culture derived and, even when host adapted, expressed OspA. Nevertheless, these two independent studies clearly demonstrate that OspA can be expressed in vivo and that an inflammatory environment favors *ospA* mRNA transcription and OspA expression by *B. burgdorferi*.

In other studies of *B. burgdorferi* infection of C3H-*scid* mice workers have not observed *ospA* mRNA transcription. Liang et al. (31) were unable to detect *ospA* mRNA transcription in C3H-*scid* mice using less sensitive microarray approaches. Crother et al. (11) were not able to detect OspA protein in detergent-phase tissue extracts from C3H-*scid* mice infected for 17 days with *B. burgdorferi* B31, but other proteins, including VlsE, OspC, and DbpA, were detectable. The inability to detect OspA was possibly due to the very small amounts of OspA in tissues, which the present data suggest, and possibly also due to the early time (17 days). Real-time PCR is a highly sensitive and quantitative means for detecting RNA transcripts in tissues of mice infected with *B. burgdorferi*, and it has recently been utilized to demonstrate dynamics of gene expression during infection in both immunocompetent and immunodeficient mice (24, 25, 32). Acquired immunity by the host has been shown to significantly reduce, but not eliminate, spirochetes from tissues, with commensurate reduction of transcription of several genes, including *flaB*, *ospC*, *dbpA*, *arp* (BBF01), *vlsE*, *fbp* (BBK32), *oppA-2*, and *p37-42* (BBK47). The only absolute reduction in gene transcription in all tissues under these circumstances was the reduction in *ospA* transcription (24). These results were confirmed in a recent study that examined *dbpA*, *ospC*, BBF01, and *vlsE* mRNA transcription, but that study involved fewer tissues and *ospA* mRNA transcription was not examined (32).

It has been generally accepted that *B. burgdorferi*, which expresses abundant amounts of OspA in unfed ticks, rapidly down-regulates *ospA* mRNA transcription during tick feeding upon a host (16, 33, 48). The signals for this profound change in surface protein expression are not known, but a recent study has shown that natural antibody in nonimmune mice is a significant factor (9). Ninety percent of spirochetes in the salivary glands of ticks feeding upon B-cell-deficient mice were shown to express OspA, in contrast to only 5% of spirochetes from ticks feeding upon naïve, immunocompetent mice. Furthermore, the spirochete numbers in the midguts of ticks were significantly reduced in ticks feeding upon immunocompetent mice compared to B-cell-deficient mice. Passive transfer of normal mouse serum into the B-cell-deficient mice was sufficient to induce these profound shifts in OspA expression. It has

been known for some time that natural IgM interacts with OspA on spirochetes grown in vitro (18, 50). Furthermore, sera from several different species of animals, which were not exposed to *B. burgdorferi*, have been shown to contain natural antibodies that can kill spirochetes in vitro in the presence of complement (29). Although we did not observe reductions in spirochete numbers in infected C3H-*scid* mice that were passively treated with normal mouse serum, our results complement these other studies by demonstrating the role of natural antibodies as a signal for *ospA* mRNA transcription modulation in vivo and maintenance of *ospA* down-regulation during persistent infection of the host.

The down-regulation of *ospA* mRNA transcription during tick feeding is not absolute, as some spirochetes in the salivary glands of feeding ticks have been shown to express OspA (35, 46–48). In a recent study examining *B. burgdorferi* population kinetics and gene expression at the host-vector interface, it was shown that spirochetes which initially enter the host at the site of tick feeding transiently transcribe *ospA* mRNA at 24 h but rapidly down-regulate *ospA* mRNA transcription by 48 h and beyond in immunocompetent C3H mice (25). The current study confirmed this observation, using C3H-*scid* mice. Thus, transient OspA expression explains the finding of early antibody responses to OspA, which tend to rapidly decline in both experimental animals and humans (43, 45). Studies of C3H-*scid* mice infected with cultured (24), host-adapted, or tick-borne spirochetes (current study) indicate that *ospA* mRNA transcription continues in the host in the absence of immune competence, and the critical signaling factor is natural immunoglobulin.

Because of the relative paucity of spirochetes in tissues and the relatively low level of *ospA* mRNA transcription in infected mice, we attempted to prove OspA translation and expression indirectly with an adoptive transfer experiment. The results demonstrated that there was seroconversion of infected C3H-*scid* mice that were reconstituted with normal lymphocytes from naïve, immunocompetent C3H mice. However, seroconversion to OspA took place in only two of five mice that were successfully reconstituted. All five mice readily seroconverted to *B. burgdorferi*. The variable results are likely due to the fact that *ospA* mRNA transcription was down-regulated during the early phases of reconstitution because of sensitivity to natural immunoglobulin, thereby reducing or eliminating the antigenic stimulus at a time when full immunocompetence was affected. Nevertheless, we established the fact that some of the mice seroconverted specifically to OspA, thereby proving that OspA translation occurred in vivo.

The regulatory effector for *ospA* transcription in vivo, at least in part, is immunoglobulin. Our study compartmentalized the effector to either IgM or IgG. Nonspecific immunoglobulins, possibly natural antibody, have been shown to significantly influence OspA expression in the tick. In the previous study, as in the current study, the IgM and IgG fractions of normal mouse serum were tested, but only IgM was found to be effective at modifying OspA expression in ticks. It was speculated that the lack of effect of IgG on OspA expression in ticks may have been due to IgG binding proteins in tick saliva (9). It remains to be determined if the effects of immunoglobulins on *ospA* mRNA transcription and OspA expression are actually due to natural antibodies that are specifically reactive with OspA or due to some other mechanism. If specificity is required, this finding may provide insight into why OspA may be expressed in some Lyme disease patients who seroconvert to OspA during the late stages of infection.

ACKNOWLEDGMENT

This work was supported by PHS grant R01 AI26815 from the National Institute of Allergy and Infectious Diseases.

REFERENCES

1. **Alban, P. S., P. W. Johnson, and D. R. Nelson.** 2000. Serum-starvation-induced changes in protein synthesis and morphology of *Borrelia burgdorferi*. Microbiology **146:**119–127.
2. **Anderson, J. F., and L. A. Magnarelli.** 1993. Epizootiology of Lyme disease-causing borreliae. Clin. Dermatol. **11:**339–351
3. **Barbour, A. G., S. L. Tessier, and W. J. Todd.** 1983. Lyme disease spirochetes and ixodid tick spirochetes share a common surface antigenic determinant defined by a monoclonal antibody. Infect Immun. **41:**795–804.
4. **Barthold, S. W.** 1993. Antigenic stability of *Borrelia burgdorferi* during chronic infections of immunocompetent mice. Infect. Immun. **61:**4955–4961.
5. **Barthold, S. W., and L. K. Bockenstedt.** 1993. Passive immunizing activity of sera from mice infected with *Borrelia burgdorferi*. Infect. Immun. **61:**4696–4702.
6. **Barthold, S. W., M. S. de Souza, J. L. Janotka, A. L. Smith, and D. H. Persing.** 1993. Chronic Lyme borreliosis in the laboratory mouse. Am. J. Pathol. **143:**959–971.
7. **Barthold, S. W., E. Fikrig, L. K. Bockenstedt, and D. H. Persing.** 1995. Circumvention of outer surface protein A immunity by host-adapted *Borrelia burgdorferi*. Infect. Immun. **63:**2255–2261.
8. **Barthold, S. W., S. A. Levy, E. Fikrig, L. K. Bockenstedt, and A. L. Smith.** 1995. Serologic responses of dogs naturally exposed to or vaccinated against *Borrelia burgdorferi* infection. J. Am. Vet. Med. Assoc. **207:**1435–1440.
9. **Belperron, A. A., and L. K. Bockenstedt.** 2001. Natural antibody affects survival of the spirochete *Borrelia burgdorferi* within feeding ticks. Infect. Immun. **69:**6456–6462.
10. **Bergstrom, S., V. G. Bundoc, and A. G. Barbour.** 1989. Molecular analysis of linear plasmid-encoded major surface proteins, OspA and OspB, of the Lyme disease spirochaete *Borrelia burgdorferi*. Mol. Microbiol. **3:**479–486.
11. **Crother, T. R., C. I. Champion, X. Y. Wu, D. R. Blanco, J. N. Miller, and M. A. Lovett.** 2003. Antigenic composition of *Borrelia burgdorferi* during infection of SCID mice. Infect. Immun. **71:**3419–3428.
12. **Crowley, H., and B. T. Huber.** 2003. Host-adapted *Borrelia burgdorferi* in mice expresses OspA during inflammation. Infect. Immun. **71:**4003–4010.
13. **de Silva, A. M., and E. Fikrig.** 1997. Arthropod- and host-specific gene expression by *Borrelia burgdorferi*. J. Clin. Investig. **99:**377–379.
14. **De Silva, A. M., and E. Fikrig.** 1997. *Borrelia burgdorferi* genes selectively expressed in ticks and mammals. Parasitol. Today **13:**267–270.
15. **de Silva, A. M., E. Fikrig, E. Hodzic, F. S. Kantor, S. R. Telford III, and S. W. Barthold.** 1998. Immune evasion by tickborne and host-adapted *Borrelia burgdorferi*. J. Infect. Dis. **177:**395–400.
16. **de Silva, A. M., S. R. Telford III, L. R. Brunet, S. W. Barthold, and E. Fikrig.** 1996. *Borrelia burgdorferi* OspA is an arthropod-specific transmission-blocking Lyme disease vaccine. J. Exp. Med. **183:**271–275.
17. **de Silva, A. M., N. S. Zeidner, Y. Zhang, M. C. Dolan, J. Piesman, and E. Fikrig.** 1999. Influence of outer surface protein A antibody on *Borrelia burgdorferi* within feeding ticks. Infect. Immun. **67:**30–35.
18. **Dorward, D. W., E. D. Huguenel, G. Davis, and C. F. Garon.** 1992. Interactions between extracellular *Borrelia burgdorferi* proteins and non-*Borrelia*-directed immunoglobulin M antibodies. Infect. Immun. **60:**838–844.
19. **Dressler, F., J. A. Whalen, B. N. Reinhardt, and A. C. Steere.** 1993. Western blotting in the serodiagnosis of Lyme disease. J. Infect. Dis. **167:**392–400.
20. **Ewing, C., A. Scorpio, D. R. Nelson, and T. N. Mather.** 1994. Isolation of *Borrelia burgdorferi* from saliva of the tick vector, *Ixodes scapularis*. J. Clin. Microbiol. **32:**755–758.
21. **Feng, S., E. Hodzic, K. Freet, and S. W. Barthold.** 2003. Immunogenicity of *Borrelia burgdorferi* arthritis-related protein. Infect. Immun. **71:**7211–7214.
22. **Fikrig, E., S. R. Telford III, S. W. Barthold, F. S. Kantor, A. Spielman, and R. A. Flavell.** 1992. Elimination of *Borrelia burgdorferi* from vector ticks feeding on OspA-immunized mice. Proc. Natl. Acad. Sci. USA **89:**5418–5421.
23. **Gern, L., U. E. Schaible, and M. M. Simon.** 1993. Mode of inoculation of the Lyme disease agent *Borrelia burgdorferi* influences infection and immune responses in inbred strains of mice. J. Infect. Dis. **167:**971–975.
24. **Hodzic, E., S. Feng, K. J. Freet, and S. W. Barthold.** 2003. *Borrelia burgdorferi* population dynamics and prototype gene expression during infection of immunocompetent and immunodeficient mice. Infect. Immun. **71:**5042–5055.
25. **Hodzic, E., S. Feng, K. J. Freet, D. L. Borjesson, and S. W. Barthold.** 2002.

Borrelia burgdorferi population kinetics and selected gene expression at the host-vector interface. Infect. Immun. **70:**3382–3388.
26. **Howe, T. R., L. W. Mayer, and A. G. Barbour.** 1985. A single recombinant plasmid expressing two major outer surface proteins of the Lyme disease spirochete. Science **227:**645–646.
27. **Kalish, R. A., J. M. Leong, and A. C. Steere.** 1993. Association of treatment-resistant chronic Lyme arthritis with HLA-DR4 and antibody reactivity to OspA and OspB of *Borrelia burgdorferi*. Infect. Immun. **61:**2774–2779.
28. **Kalish, R. A., J. M. Leong, and A. C. Steere.** 1995. Early and late antibody responses to full-length and truncated constructs of outer surface protein A of *Borrelia burgdorferi* in Lyme disease. Infect. Immun. **63:**2228–2235.
29. **Kurtenbach, K., H. S. Sewell, N. H. Ogden, S. E. Randolph, and P. A. Nuttall.** 1998. Serum complement sensitivity as a key factor in Lyme disease ecology. Infect. Immun. **66:**1248–1251.
30. **Leuba-Garcia, S., R. Martinez, and L. Gern.** 1998. Expression of outer surface proteins A and C of *Borrelia afzelii* in *Ixodes ricinus* ticks and in the skin of mice. Zentralbl. Bakteriol. **287:**475–484.
31. **Liang, F. T., F. K. Nelson, and E. Fikrig.** 2002. DNA microarray assessment of putative *Borrelia burgdorferi* lipoprotein genes. Infect. Immun. **70:**3300–3303.
32. **Liang, F. T., J. Yan, M. L. Mbow, S. L. Sviat, R. D. Gilmore, M. Mamula, and E. Fikrig.** 2004. *Borrelia burgdorferi* changes its surface antigenic expression in response to host immune responses. Infect. Immun. **72:**5759–5767.
33. **Montgomery, R. R., S. E. Malawista, K. J. Feen, and L. K. Bockenstedt.** 1996. Direct demonstration of antigenic substitution of *Borrelia burgdorferi* ex vivo: exploration of the paradox of the early immune response to outer surface proteins A and C in Lyme disease. J. Exp. Med. **183:**261–269.
34. **Obonyo, M., U. G. Munderloh, V. Fingerle, B. Wilske, and T. J. Kurtti.** 1999. *Borrelia burgdorferi* in tick cell culture modulates expression of outer surface proteins A and C in response to temperature. J. Clin. Microbiol. **37:**2137–2141.
35. **Ohnishi, J., J. Piesman, and A. M. de Silva.** 2001. Antigenic and genetic heterogeneity of *Borrelia burgdorferi* populations transmitted by ticks. Proc. Natl. Acad. Sci. USA **98:**670–675.
36. **Pachner, A. R., D. Dail, L. Li, L. Gurey, S. Feng, E. Hodzic, and S. Barthold.** 2002. Humoral immune response associated with Lyme borreliosis in non-human primates: analysis by immunoblotting and enzyme-linked immunosorbent assay with sonicates or recombinant proteins. Clin. Diagn. Lab. Immunol. **9:**1348–1355.
37. **Pal, U., A. M. de Silva, R. R. Montgomery, D. Fish, J. Anguita, J. F. Anderson, Y. Lobet, and E. Fikrig.** 2000. Attachment of *Borrelia burgdorferi* within *Ixodes scapularis* mediated by outer surface protein A. J. Clin. Investig. **106:**561–569.
38. **Peavey, C. A., and R. S. Lane.** 1995. Transmission of *Borrelia burgdorferi* by *Ixodes pacificus* nymphs and reservoir competence of deer mice (*Peromyscus maniculatus*) infected by tick-bite. J. Parasitol. **81:**175–178.
39. **Piesman, J.** 1993. Dynamics of *Borrelia burgdorferi* transmission by nymphal *Ixodes dammini* ticks. J. Infect. Dis. **167:**1082–1085.
40. **Porcella, S. F., and T. G. Schwan.** 2001. *Borrelia burgdorferi* and *Treponema pallidum*: a comparison of functional genomics, environmental adaptations, and pathogenic mechanisms. J. Clin. Investig. **107:**651–656.
41. **Roehrig, J. T., J. Piesman, A. R. Hunt, M. G. Keen, C. M. Happ, and B. J. Johnson.** 1992. The hamster immune response to tick-transmitted *Borrelia burgdorferi* differs from the response to needle-inoculated, cultured organisms. J. Immunol. **149:**3648–3653.
42. **Schaible, U. E., L. Gern, R. Wallich, M. D. Kramer, M. Prester, and M. M. Simon.** 1993. Distinct patterns of protective antibodies are generated against *Borrelia burgdorferi* in mice experimentally inoculated with high and low doses of antigen. Immunol. Lett. **36:**219–226.
43. **Schutzer, S. E., P. K. Coyle, J. J. Dunn, B. J. Luft, and M. Brunner.** 1994. Early and specific antibody response to OspA in Lyme disease. J. Clin. Investig. **94:**454–457.
44. **Schutzer, S. E., P. K. Coyle, L. B. Krupp, Z. Deng, A. L. Belman, R. Dattwyler, and B. J. Luft.** 1997. Simultaneous expression of *Borrelia* OspA and OspC and IgM response in cerebrospinal fluid in early neurologic Lyme disease. J. Clin. Investig. **100:**763–767.
45. **Schutzer, S. E., and J. Luan.** 2003. Early OspA immune complex formation in animal models of Lyme disease. J. Mol. Microbiol. Biotechnol. **5:**167–171.
46. **Schwan, T. G., R. H. Karstens, M. E. Schrumpf, and W. J. Simpson.** 1991. Changes in antigenic reactivity of *Borrelia burgdorferi*, the Lyme disease spirochete, during persistent infection in mice. Can. J. Microbiol. **37:**450–454.
47. **Schwan, T. G., and J. Piesman.** 2000. Temporal changes in outer surface proteins A and C of the Lyme disease-associated spirochete, *Borrelia burgdorferi*, during the chain of infection in ticks and mice. J. Clin. Microbiol. **38:**382–388.
48. **Schwan, T. G., J. Piesman, W. T. Golde, M. C. Dolan, and P. A. Rosa.** 1995. Induction of an outer surface protein on *Borrelia burgdorferi* during tick feeding. Proc. Natl. Acad. Sci. USA **92:**2909–2913.
49. **Spielman, A., J. F. Levine, and M. L. Wilson.** 1984. Vectorial capacity of North American *Ixodes* ticks. Yale J. Biol. Med. **57:**507–513.
50. **Whitmire, W. M., and C. F. Garon.** 1993. Specific and nonspecific responses of murine B cells to membrane blebs of *Borrelia burgdorferi*. Infect. Immun. **61:**1460–1467.
51. **Yang, X., T. G. Popova, K. E. Hagman, S. K. Wikel, G. B. Schoeler, M. J. Caimano, J. D. Radolf, and M. V. Norgard.** 1999. Identification, characterization, and expression of three new members of the *Borrelia burgdorferi* Mlp (2.9) lipoprotein gene family. Infect. Immun. **67:**6008–6018.
52. **Yang, X. F., U. Pal, S. M. Alani, E. Fikrig, and M. V. Norgard.** 2004. Essential role for OspA/B in the life cycle of the Lyme disease spirochete. J. Exp. Med. **199:**641–648.

Editor: J. T. Barbieri