OspA Antibodies Inhibit the Acquisition of *Borrelia burgdorferi* by *Ixodes* Ticks

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**Is*odes* ticks are infected by *Borrelia burgdorferi* when larvae feed on spirochete-infected mice. We studied the acquisition of *B. burgdorferi* by larval ticks, characterized the production of outer surface protein A (OspA) by spirochetes entering larvae, and examined the effects of OspA antibodies on the establishment of *B. burgdorferi* infections in ticks. Most larvae were infected by spirochetes 24 to 48 h after placement on mice. OspA antibodies stained the first spirochetes observed in larvae, suggesting that OspA is synthesized early during the colonization of the vector. When OspA antibodies were administered to *B. burgdorferi*-infected mice and larvae were then placed on the animals, the severity of larval infection and the number of infected ticks (7 of 16) were decreased compared with that of controls (15 of 16). The inhibitory effects of OspA antibodies were observed with passive antibody transfer as well as active host-generated immunity. The lower larval infection rate observed in the presence of OspA antibodies was exacerbated after the larval molt since only 1 of 12 nymphs was infected, and none of the mice that were fed upon by these nymphs became infected with *B. burgdorferi*. Therefore, an OspA antibody response in mice altered the reservoir competence of the vertebrate host by inhibiting the movement of *B. burgdorferi* from the host to the vector.

*Borrelia burgdorferi*, the bacterium responsible for Lyme disease, is a vector-borne spirochete that is maintained in the northeastern United States by a natural cycle involving wild rodents and *Ixodes* ticks. Larval ticks acquire the infection when they feed on *B. burgdorferi*-infected mice. The spirochetes are transtadially inherited through the molt, and feeding nymphal and adult ticks transmit *B. burgdorferi* to vertebrates. The goal of the current study was to characterize the transfer of spirochetes from infected rodents to larval ticks. Specifically, experiments were performed to determine the length of time needed for spirochetes to enter feeding larval ticks and to characterize the synthesis of *B. burgdorferi* outer surface protein A (OspA) by spirochetes entering larval ticks. Experiments were also performed to determine the effects of OspA antibodies on the transfer of spirochetes from the host to the vector.

Most of the studies on the interactions of *B. burgdorferi* with its arthropod vector have concentrated on the events that occur when infected nymphal and adult ticks transmit the spirochetes to vertebrate hosts (2, 4, 22). In nymphs, *B. burgdorferi* is present in the lumen of the gut (4). When ticks feed, the spirochetes multiply in the gut, cross the gut epithelium into the hemocoel, migrate to the salivary glands, and invade the host (7, 16, 18, 22).

Studies have also revealed that *B. burgdorferi* expresses different genes during nymphal feeding. OspA, -B, and -C are some of the major antigens synthesized by *B. burgdorferi*. In unfed nymphal ticks, OspA and OspB are produced while OspC is rarely detected (8, 11, 21). When infected ticks feed on a rodent host, the majority of spirochetes in the tick clear OspA and OspB and synthesize OspC (8, 21). Thus, as spirochetes move from the vector into the mammalian host, they change their coat structure by replacing, at least in part, OspA and OspB with OspC. Mice immunized with OspA are protected from infection because OspA antibodies kill *B. burgdorferi* in the tick gut and prevent transmission of spirochetes out of the vector (8). OspA antibodies do not eliminate spirochetes from mice with already established spirochete infections (9), presumably because spirochetes in mice rarely synthesize this protein (8, 15).

To complete the life cycle, *B. burgdorferi* has to leave the rodent host and colonize feeding larval ticks. Since spirochetes in mice do not appear to produce OspA while those in nymphal ticks do, the OspA protein has to be synthesized again at some point during the establishment of infection in the tick. The goal of the current study was to characterize the time course of *B. burgdorferi* acquisition by feeding larvae and to determine when spirochetes within larvae produced OspA. Experiments were also performed to examine the effects of OspA antibodies in the host on the movement of spirochetes from the host to the vector.

**MATERIALS AND METHODS**

**Mice and *B. burgdorferi*.** Pathogen-free C57BL/6J (B6) mice were purchased from the National Institutes of Health, Bethesda, Md. The spirochete used was a clonal population of *B. burgdorferi* N40 (passage 3). Spirochetes were cultured in Barbour-Stoenner-Kelly II (BSKII) medium (1).

**Maintenance and infection of ticks.** All ticks used in this study were derived from a second-generation colony of ticks maintained at the Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, Conn. The laboratory-reared larvae are free of *B. burgdorferi* infection. The colony was established from locally collected *Ixodes scapularis* adults fed upon New Zealand White rabbits. To infect ticks with *B. burgdorferi*, approximately 100 uninfected larvae were placed on an infected C57BL/6J mouse and allowed
to feed to repletion. Larvae were kept in humid chambers at 21°C until they molted into nymphs.

Acquisition of spirochetes by larval ticks. (i) Studies with tick-infected mice. Eight mice were infected with *B. burgdorferi* by placing 10 infected nymphal ticks on each mouse. Thirty days after infection, four mice were passively immunized with OspA antibody and the four remaining mice were passively immunized with a control serum. The OspA antiserum used in passive transfer experiments was a rabbit serum raised against a recombinant OspA-glutathione transferase fusion protein. Although the OspA serum protects mice from tick-borne infection (8), it does not clear infection in mice with established infections (10). The control serum was raised against glutathione transferase. The sera were administered to mice by injecting 100 μl intraperitoneally and 100 μl subcutaneously.

Twenty-four hours after passive immunization, the mice were anesthetized with ketamine and xylazine and 100 larvae were placed on each mouse. Some of the larvae in the process of feeding (days 1, 2, and 3) as well as some of the replete larvae (days 4, 6, and 10) were tested for spirochetes. Individual larvae were tested by either immunofluorescence (see below) or an enzyme-linked immunosorbent assay (ELISA; see below). The larvae from the OspA antibody-treated group were tested by immunofluorescence only. The remaining replete larvae (those not tested for spirochetes) were kept in humid chambers and allowed to molt to nymphs.

Two weeks after molting, 12 nymphs from each group were tested by immunofluorescence to determine the infection rate. Nymphs from each group were also tested for infectious spirochetes by allowing the nymphs to feed on naive mice. Six mice were used for each group, and two nymphs were placed on each mouse. Two weeks after the nymphs detached, the mice were killed and checked for infection by culturing selected tissues in BSKII medium as described previously (9).

(ii) Studies with syringe-infected mice. Two groups of four mice each were infected by syringe with a large (10⁷ spirochetes/mouse) or small (10³ spirochetes/mouse) inoculum. Four weeks later, 100 larvae were placed on each mouse. The larvae were allowed to feed to repletion and detach from the mice. After all the larvae were collected, the mice were killed and the serum was stored. The presence of OspA antibodies in the serum was determined by immunoblotting with nitrocellulose strips containing antigens from cultured spirochetes as described previously (9). Briefly, the strips were incubated with the infected mouse serum (1:100 dilution), OspA MAbs CII.78 (1:50 dilution), or a murine anti-OspC serum (1:100 dilution) for 1 h at ambient temperature. The primary antibody incubation was followed by several washes and an incubation with alkaline phosphatase-conjugated goat anti-mouse antibodies. The blots were washed several times and then developed for 5 min in nitroblue tetrazolium-5-bromo-4-chloro-3-indolyl phosphate (NBT-BCIP; Stratagene, La Jolla, Calif.).

**RESULTS**

Acquisition of *B. burgdorferi* by larval ticks. To determine the time needed for spirochetes to enter the vector, larvae were placed on an infected mouse and examined for spirochetes 1, 2, 3, and 10 days later. The larvae were assessed for the presence of *B. burgdorferi* and the intensity of infection by use of a FITC-conjugated polyclonal rabbit serum raised against *B. burgdorferi* (Table 1, control antibody-treated group). Twenty-four hours after tick placement, 2 of 12 larvae had low-level infections. At 48 and 72 h, 10 of 12 and all 12 larvae, respectively, were infected, and the majority of the ticks had moderate to heavy-grade infections. Thus, most of the larvae acquired spirochetes between 24 and 48 h after placement on the host.

One potential problem with the use of the FITC-conjugated rabbit anti-*B. burgdorferi* serum to determine the intensity of...
spirochete infection within larvae is that antibodies in the rodent host may bind to spirochetes and interfere with the diagnostic reagent. However, interference from mouse antibody appeared to be minimal because spirochetes in larval homogenates were not stained with FITC-conjugated goat anti-mouse secondary antibody alone, indicating that the spirochetes in the larval gut are not coated heavily with mouse antibody (data not shown).

**Ospa synthesis by *B. burgdorferi* entering larval ticks.** Since spirochetes in mice do not appear to produce Ospa while those in nympha ticks do, the Ospa protein has to be synthesized again at some point during the establishment of infection in the tick (3, 8, 15). Larvae that had fed for different times were probed with Ospa MAb CH1.78. *B. burgdorferi* spirochetes were stained by the Ospa MAb at the earliest time at which spirochetes were observed in feeding larvae (day 1) as well as at all subsequent intervals (Table 1, control antibody-treated group) (by IFA), indicating that Ospa is produced early during the colonization of the larval gut by spirochetes.

An Ospa antigen-capture ELISA (5, 6) was also used to detect and quantify the total amount of Ospa within each feeding larvae (Table 1, control antibody-treated group). None of the ticks was positive in the ELISA before day 2. The ELISA is probably not sensitive enough to detect the low-grade infections detected by IFA on days 1 and 2. On days 3 and 4, four of six and all nine of the larvae tested, respectively, were positive. By days 6 and 10, the numbers of positive larvae dropped to three of seven and two of five ticks, respectively. Between days 3 and 10, the mean amount of Ospa within larvae ranged from 168 to 758 pg. We have previously reported that cultured spirochetes contain about 1.1 fg of Ospa/spirochete (6). However, this correlation observed for spirochetes grown in culture may not apply to spirochetes within feeding larvae because spirochetes change the amount of Ospa on their surfaces at different stages of their life cycle (8).

**Acquisition of *B. burgdorferi* by larval ticks in the presence of passively administered Ospa antibody in the blood meal.** Since Ospa was synthesized early during the movement of spirochetes from the host to larval ticks (Table 1, Ospa antibody-treated group) (determined by IFA), experiments were carried out to determine whether Ospa antibodies inhibited the acquisition of spirochetes by the vector. The majority of larvae feeding on Ospa antiserum-treated animals acquired spirochetes between days 1 and 2 of feeding, and by day 3, 10 of the 12 larvae exposed to Ospa antibodies had spirochetes (Table 1, Ospa antibody-treated group). Thus, in both the control and Ospa antibody-treated groups (Table 1), the majority of larvae acquired spirochetes between days 1 and 2. However, in contrast to the control group, larvae from the anti-Ospa-treated group had low-grade infections (Table 1, Ospa antibody-treated group). On day 3, all of the infected larvae from the anti-Ospa-treated group had low-grade infections while the majority of larvae from the control group had heavy-grade infections (Table 1, Ospa antibody-treated group) ($P < 0.002$; chi-square test, 2 degrees of freedom). On day 10, compared to the larvae of the control group, the larvae from the anti-Ospa-treated group had fewer infected larvae (7 of 16 versus 15 of 16) ($P < 0.005$; chi-square test, 1 degree of freedom) as well as fewer spirochetes in the infected ticks. Thus, Ospa antibodies reduced the number of permanently infected larvae and the severity of infection in the ticks.

**Effects of Ospa antibody on the ability of *B. burgdorferi* to survive the larval molt.** Experiments were carried out to examine whether spirochetes that persisted within larvae in the presence of Ospa antibodies were able to survive the larval molt and infect mice during nymphal feeding. Larvae that fed on mice treated with anti-Ospa serum and control antibodies were allowed to molt to the nymphal stage. The nymphs were examined for the presence of spirochetes. All 12 of the nymphs from the control group were infected. In contrast, 1 of 12 nymphs from the Ospa antibody-treated group had *B. burgdorferi* spirochetes ($P < 0.001$; chi-square test, 1 degree of freedom). Transtadial transmission of *B. burgdorferi* was markedly reduced in larvae infected in the presence of Ospa antibodies.

The observation that most of the nymphs from the anti-Ospa-treated group were uninfected was confirmed by allowing the nymphs to feed on naive C57 mice. Four of the six mice fed upon by the control nymphs were infected, whereas none of the six mice fed upon by the nymphs from the anti-Ospa-treated group were infected ($P < 0.02$; chi-square test, 1 degree of freedom). The presence of Ospa antibody in the larval blood meal abrogated, or severely decreased, the infectivity of nymphal ticks.

**Acquisition of *B. burgdorferi* by larval ticks following an active host-generated immune response to Ospa.** Instead of relying on experiments with passive antibody transfer alone, we also performed experiments to examine the effect of a host-generated immune response against Ospa on the acquisition of spirochetes by larvae. Mice infected by tick bite or at low doses (<10<sup>4</sup> bacteria) rarely develop Ospa antibodies. Mice infected with high doses of *B. burgdorferi* (>10<sup>5</sup> bacteria) do develop Ospa antibodies, presumably because of the large amount of the Ospa antigen in the initial inoculum (12). Two groups of four mice each were challenged with 10<sup>7</sup> or 10<sup>5</sup> organisms. Both groups of mice had antibodies against a variety of common antigens, including OspC and flagellin. In addition to these common bands, mice infected with a high dose of spirochetes had antibodies against Ospa, OspB, and another unidentified antigen (which, in a blot, appeared as a band below that of Ospa) (Fig. 1).

Larvae were allowed to engorge to repletion on the low- and high-dose-infected groups of animals. Ten days after detachment from the mice, the ticks were examined to determine the number of infected larvae as well as the severity of infection (Fig. 2). Fifteen of 16 and all 16 of the larvae from the low- and high-dose groups of mice, respectively, were infected. However, a striking difference was noted in the severity of infection between the two groups (Fig. 2). Larvae from the low-dose group of mice that had not seroconverted to Ospa had mostly heavy-grade infections (11 of 15), while mice from the high-dose group that had seroconverted to Ospa (12 of 16) primarily had moderate-grade infections (Fig. 2) ($P < 0.005$; chi-square test, 2 degree of freedom). Thus, as in the case with the passive transfer experiments, the severity of infection in larvae was reduced in the presence of an active Ospa antibody response, although the effect was more pronounced in the passive transfer studies.

**DISCUSSION**

One of the objectives of this study was to determine the time taken for *B. burgdorferi* to infect feeding larvae. The majority of larvae feeding on an infected rodent acquired *B. burgdorferi* following a 24- to 48-h lag. The events at the vector-host interface responsible for the lag in the acquisition of spirochetes by larvae remain to be clarified. Once infected, the number of organisms within a larva continued to increase during and after the blood meal (Table 1). Piesman and colleagues (17) have reported on a maximum of around 2,500 to 3,000 spirochetes per larva about 2 weeks postrepletion. The multiplication of *B. burgdorferi* within the larvae and possibly...
OspA antibodies administered to the host interfered with the acquisition of spirochetes by larval ticks. The fact that soon after placement (days 1 and 2) the spirochete numbers in larvae were similar in OspA antibody-treated and control groups suggests that OspA antibodies do not significantly hinder the initial movement of spirochetes from mice to larvae. The antibodies appear to affect spirochete survival and growth in the larval gut after the initial establishment of infection. Spirochetes in the larval gut may be vulnerable to the anti-

FIG. 1. Mice infected by syringe with a large inoculum of *B. burgdorferi* seroconvert to OspA. Mice were infected by syringe with a large (10⁷ spirochetes) or small (10³ spirochetes) inoculum. The presence of OspA antibodies in the serum was determined by immunoblotting. The strips were probed with the following antisera: normal mouse serum (lane 1), OspA MAb CIIL.78 (lane 2), serum from mouse immunized with OspC (lane 3), serum from mice infected with 10⁷ *B. burgdorferi* spirochetes (lanes 4 to 7), and serum from mice infected with 10³ *B. burgdorferi* spirochetes (lanes 8 to 11).

FIG. 2. Mice infected by syringe with a large inoculum of *B. burgdorferi* transmit spirochetes with a reduced efficiency to ticks. Mice were infected by syringe with a large (10⁷) or small (10³) inoculum of spirochetes (open and shaded bars, respectively), and 4 weeks later, larvae were placed on the mice. Replete larvae were examined for spirochetes by DFA. To distinguish larvae with low-grade, moderate and heavy-grade infections, the numbers of spirochetes in 10 (45× objective) microscope fields were counted. Larvae with <10, 11 to 50, and >50 spirochetes were labeled as having low-grade, moderate, and heavy-grade infections, respectively.
immune anti-OspA serum, may account for the differences in the magnitude of the effect. Kurtenbach and his colleagues have also investigated the effects of elicited immune responses on the acquisition of _B. burgdorferi_ by _Ixodes ricinus_, the European vector of Lyme disease, and observed lower numbers of spirochetes within larvae feeding on rodents with OspA and OspB antibodies in comparison to larvae feeding on mice without OspA and OspB antibodies (14).

An observation that has remained enigmatic is that although OspA is an abundant surface antigen produced by _B. burgdorferi_ within the vector and spirochetes grown in culture, rodents infected by tick bite rarely seroconvert to OspA (3). _B. burgdorferi_ appears to prevent the host from mounting an immune response against OspA by clearing this protein from the surface during host infection (8, 15). The results of this study provide an explanation as to why it would be advantageous for the spirochete to prevent the reservoir host from mounting an immune response against OspA. OspA antibodies in infected rodent hosts decreased the acquisition and maintenance of spirochetes by larval ticks. The development of antibodies against OspA during host infection may also account for why certain hosts are better reservoirs of _B. burgdorferi_ than others. The white-footed mouse (_Peromyscus leucopus_), the main reservoir of _B. burgdorferi_ in the northeastern United States, rarely develops antibodies against OspA (3). In contrast, humans, who are not a reservoir host for _B. burgdorferi_, can sometimes develop OspA antibodies early in infection or in the later stages of disease (13, 20). More studies with both competent and incompetent reservoirs of _B. burgdorferi_ are required to understand the effects of host immunity on the horizontal spread of this pathogen.

Our results demonstrating that OspA antibodies prevent larvae from acquiring productive infections are relevant when the different uses of OspA-based vaccines are considered. OspA vaccines used in the wild reservoir host will be particularly effective in reducing the infection burden in nature because the vaccine prevents both vector-to-host and host-to-vector transmission. OspA-based vaccines have been approved for veterinary use, and they are currently being evaluated for use in humans. The results presented here should stimulate work aimed at developing OspA-based vaccines for use among wild animal reservoirs of _B. burgdorferi_.

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**REFERENCES**


