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Accelerated Infectivity of Tick-Transmitted Lyme Disease Spirochetes to Vector Ticks

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We determined whether the span of infectivity of Lyme disease spirochetes (*Borrelia burgdorferi*) to vector ticks varies with the mode of infection in laboratory mice. Noninfected larval deer ticks were permitted to feed on two strains of spirochete-infected mice that had been naturally (via tick bite) and parenterally (via needle injection) infected with *B. burgdorferi* 2, 4, or 8 weeks earlier, and engorged ticks were dissected and examined for spirochetes by direct immunofluorescence microscopy. After initial infection, spirochetal infectivity to ticks was less efficient in needle-infected mice than in mice infected via tick bites. Tick-transmitted spirochetes develop more rapidly from the skin of infected mice and do not induce a strong antispirochete antibody response during the early stage of infection.

In nature, the etiologic agent of Lyme disease (*Borrelia burgdorferi*) is transmitted solely via the bites of vector ticks (ticks related to the *Ixodes ricinus* complex). Spirochetes are delivered directly to the skin of the infested host and remain localized for several days in the skin of a rodent host (19). These spirochetes seem better adapted to movement in the ground substance of the skin than in the blood or lymph vascular system (10). Indeed, skin has been proved to be the most consistent site for spirochete isolation during the course of Lyme disease infection (1, 7, 18, 22). Although dissemination of spirochetes from their site of deposition differs according to the strain of laboratory mouse (19, 21), the efficiency of transmission from mice to ticks correlates directly with duration of attachment of the infecting ticks (12, 18).

Infectivity of Lyme disease spirochetes (*B. burgdorferi*) is determined by the initial route of inoculation, and spirochetal infection in mice can be established by intradermal inoculation of only a few spirochetes (5). Skin may, therefore, provide a facilitating site in the initial phase of infection. Moreover, the profound anti-inflammatory effects (14) associated with the components of tick saliva may enhance the infectivity and transmission of tick-delivered Lyme disease spirochetes. Although the infectivity of Lyme disease spirochetes to vector ticks is influenced by various host factors (18, 20), the effect of mode of infection on spirochetal infectivity to vector ticks remains to be defined.

It may be that the spirochetal infectivity to vector ticks differs according to the mode of infection in mice. To explore this possibility, we permitted laboratory-reared noninfected larval *Ixodes scapularis* (formerly *Ixodes dammini*) ticks to feed on spirochete-infected mice that had previously been infected either by natural tick bites or by intradermal inoculation of live spirochetes, and we compared the spans of spirochetal infectivity to vector ticks of these mice. In particular, we determined whether tick-transmitted Lyme disease spirochetes disseminate more accessibly in the skin after deposition by an infecting tick.

The spirochetal isolate (JD1) of *B. burgdorferi* and noninfected larval deer ticks used in this study were originally isolated from naturally infected ticks and derived from adult ticks collected from the site of endemicity of Crane Wildlife Reservation (Ipswich, Mass.) as described previously (18). Both the tick colony and spirochetes had been introduced to and maintained in our laboratory since November 1994. Spirochete-infected nymphs were derived from the engorged larvae that had fed on spirochete-infected mice, and spirochetes used to infect mice were reisolated from infected nymphs and maintained in BSK-H medium (Sigma Co., St. Louis, Mo.; catalog no. B3528) for fewer than five passages.

Groups of 4-week-old outbred (CD-1) and inbred (C3H/HeNCrj) spirochete-free mice were purchased from the animal supply centers of the National Defense Medical Center (Taipei, Taiwan) and National Cheng Kung University Medical Center (Tainan, Taiwan), respectively. All mice were ear tagged and caged by group according to the mode of infection.

For parenteral infection, six mice of each group were gently anesthetized with 0.2 ml of sodium pentobarbital (1%) and were inoculated intradermally with 2×10^4 (measured by Petroff-Hausser counting chamber, catalog no. 3900) live *B. burgdorferi* (JD1) organisms in a 0.1-ml volume with a 26-gauge needle. In naturally infected groups, mice were exposed to five to seven spirochete-infected nymphal deer ticks and each mouse had at least five ticks feed to repletion. Tick-infested mice were caged individually over water, as described previously (18), and detached ticks were collected and examined for the presence of spirochetes within their gut.

We used a xenodiagnostic procedure to determine the infectivity of Lyme disease spirochetes to vector ticks in both strains of spirochete-infected mice that had been naturally (via tick bites) and parenterally (via needle injection) infected. Thus, infected mice of each group were restrained individually in a small wire cage, and laboratory-reared noninfected larval deer ticks (60 to 80 for each mouse) were randomly placed on these mice until repletion at 2, 4, or 8 weeks after infection. After xenodiagnosis, engorged larval deer ticks from each mouse were collected and dissected at 1 to 2 weeks thereafter, and all ticks were examined for spirochetal infection by a direct immunofluorescent antibody assay, as described previously (18). The infection rates of engorged ticks were analyzed by chi-square test.

As indicated in Table 1, spirochetes can be detected in

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TABLE 1. Spirochetal infectivity to vector ticks of mice parenterally infected with *B. burgdorferi* compared with that of mice infected via the bites of spirochete-infected ticks

Animal strain and mode of infection	Time after infection (wk)	Spirochete infection in ticks[a]	
		No. of ticks tested	% of ticks infected
Inbred C3H			
Intradermal	2	240	43
	4	180	76
	8	180	83
Tick bite	2	240	75
	4	180	86
	8	180	90
Outbred CD-1			
Intradermal	2	240	40
	4	180	72
	8	180	82
Tick bite	2	240	72
	4	180	82
	8	180	87

[a] Engorged larval ticks were examined for the presence of spirochetes 1 to 2 weeks thereafter by direct immunofluorescent antibody assay, and six mice for each time period were tested.

engorged larvae that had fed on mice only 2 weeks after initial infection, and the infection rate of ticks increased markedly to a higher percentage (>70%) at 4 weeks thereafter, regardless of the mouse strain. In addition, spirochetal infectivity to ticks in tick-infected mice was more efficient than in mice infected via needle inoculation ($P < 0.01$, chi-square test, 2 df; $\chi^2 = 9.579$ and 10.948 for C3H and CD-1 mice, respectively). Thus, the span of spirochetal infectivity to ticks varies with the mode of infection in mice.

To determine the dissemination of spirochetes and spirochetemia in mice, we assessed whether the ability to isolate Lyme disease spirochetes from tissues varies with the mode of infection as well as the strain of mouse. Thus, the full depth of the pinna of ear skin was excised by means of 4-mm biopsy punches at 2, 4, or 8 weeks after mice had been either naturally or parenterally infected and was transferred to a well of a microdilution plate (Corning model 25860) containing BSK-H medium, as described previously (13). The plate was incubated at 34°C in a humidified CO_2 incubator (Nuaire, Inc., Plymouth, Minn.) and was examined for spirochetes weekly for 8 weeks by dark-field microscopy. Blood samples were also taken from the retro-orbital sinus of each mouse at 2, 4, or 8 weeks after infection for cultivation of the spirochetes by using sterile Pasteur pipettes (Kimble Glass, model 72050) and were subsequently transferred to blood collection tubes (Vacutainer 6495; Becton Dickinson, Hong Kong) containing 5 ml of culture medium with the antibiotics rifampin (50 μg/ml), amphotericin B (2.5 μg/ml), and phosphomycin (20 μg/ml), respectively. The identities of the spirochetes isolated from these tissues were verified by a fluorescein isothiocyanate-conjugated hyperimmune polyclonal anti-*B. burgdorferi* (JD1) rabbit serum (19) and by monoclonal antibodies against OspA and flagellin antigens (3, 4).

Results indicated in Table 2 reveal that spirochetes can be cultured from the skin of both strains of mice as early as 2 weeks following infection, and that is more efficient when the mice were infected longer than 4 weeks. In contrast, only one spirochete culture was developed out of the blood samples, regardless of the mode of infection and strain of mouse. In addition, spirochetes developed more rapidly from the tissues of tick-infected mice compared with those of mice infected via needle injection. These results suggest that spirochetes may disseminate more accessibly in the skin of the host after deposition by an infected tick, and spirochetemia is rarely observed in infected hosts.

We also determined whether the humoral immune response against Lyme disease spirochetes differs with the mode of infection as well as the strain of mouse. Sera sampled from mice at 2, 4, or 8 weeks after infection were serially diluted from 1:25 to 1:3,200 and were applied in duplicate to the wells of *B. burgdorferi*-coated immunofluorescent antibody assay slides. After incubation, a fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin G (catalog no. F8264; Sigma Co.), at a dilution of 1:50, was used for determining the titers of antibodies, and results were presented as the greatest dilution of a test serum that reacted with the conjugate.

Regardless of the mouse strain, immunoglobulin G titers became elevated gradually at 2 weeks after parenteral infection and increased markedly to a higher level (>1:1,000) at 4 weeks following infection. In contrast, antispirochete antibodies in sera collected from mice that were bitten by infected nymphs do not rise to a significant level (>1:500) until 4 weeks after infection (data not shown). These results suggest that tick-delivered Lyme disease spirochetes do not induce a strong antispirochete antibody response during the early stage of infection.

The efficiency of transmission of Lyme disease spirochetes from infected hosts to vector ticks may depend on the initial way of entry of these spirochetes. In our observations, spirochetal infectivity to larval *I. scapularis* (formerly *I. dammini*) ticks is almost twofold more efficient in mice infected via tick bite than in mice infected via needle injection during the early stage of infection (2 weeks after inoculation). Similar observations were also reported with *I. ricinus* larvae (8). However, the spirochetal infectivity to vector ticks in the parenterally infected mice was shown to be three- to fivefold more efficient in engorged *I. scapularis* larvae than in engorged *I. ricinus* larvae. It is more likely that the different route of inoculation by syringe injection may account for the discrepancy in spiro-

TABLE 2. Efficiency of cultivation of Lyme disease spirochetes from tissues of naturally and parenterally infected mice[a]

Mode of infection and animal strain	Time after infection (wk)	% of mice from which spirochetes were cultured (no. of culture-positive mice/no. of mice tested)	
		Blood	Ear skin
Infected bites			
C3H	2	0	50 (3/6)
	4	0	67 (4/6)
	8	0	83 (5/6)
CD-1	2	0	33 (2/6)
	4	0	67 (4/6)
	8	0	83 (5/6)
Intradermal			
C3H	2	17 (1/6)	17 (1/6)
	4	0	33 (2/6)
	8	0	67 (4/6)
CD-1	2	0	0
	4	0	33 (2/6)
	8	0	67 (4/6)

[a] The mice had been exposed to infected nymphal ticks or injected intradermally with low-passage cultured spirochetes.

chetal infectivity to larval vector ticks. Indeed, the intradermal route of inoculation of spirochetes is far more efficient than the subcutaneous route in response to the optimal dose for infection as well as the induction of disease (5). Accordingly, the spirochetal infectivity to vector ticks in parenterally infected mice differs markedly from that in mice infected via tick bites.

The mechanisms responsible for modulating spirochetal infectivity to ticks remain elusive. Although a host-derived anti-tick immune response had been proposed (24), the inhibition of feeding by attached larval deer ticks on tick-infected mice was not obvious (18–20). Another possible mechanism involves the development of an immune response specifically against spirochete antigens (16), as well as the quantity of spirochete-specific antibodies (11). Current evidence, however, indicates that mammalian hosts infected via tick bite generally raise no such prominent antibody responses (8, 15, 19, 23). It is possible that delayed antibody production against spirochetes may account for the relatively high infectivity to ticks of tick-infected mice. In addition, recent studies also indicate that anti-OspA immunity in immunized mice can be circumvented by host-adapted spirochetes (6), and spirochete antigens tend to be changed during the course of infection and tick feeding (9, 17). Thus, differential spirochetal infectivity to ticks in tick-infected mice may be attributed to the span of induction of spirochete-specific antibodies.

It is assumed that an infecting tick delivers a lesser number of spirochetes to the skin of an infested host and that the intensity of spirochetes in the skin of infected mice may affect the efficiency of transmission of Lyme disease spirochetes to ticks. Our results indicate that spirochetes can be more promptly cultured from the skin of tick-infected mice than from that of parenterally infected mice, and spirochetes are rarely cultured from the blood of both strains of mice. This discrepancy in cultivation of spirochetes from tissues, therefore, may be related to the persistence of spirochetes in the skin after initial inoculation. Indeed, spirochetes are more accessible in the skin than in the blood after deposition by an infecting tick (19–21), and the span of time during which spirochetes accumulate at the site of tick feeding may also vary. In addition, the feeding apparatus of *Ixodes* ticks is adapted for imbibing accumulated tissue fluids from a cavity created in the host's skin (2). Nevertheless, on the basis of our observations, we suggest that spirochetes may disseminate more accessibly in the skin after deposition by an infecting tick and that spirochetemia is not often found.

In summary, our observations indicate that spirochetal infectivity to vector ticks varies with the mode of infection in mice and that mice infected via tick bites are more infectious to the subsequent feeding ticks, regardless of prominent antispirochete host immunity. The factors that affect spirochetal infectivity to vector ticks, however, remain to be further identified.

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