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Dynamic changes in Lyme disease spirochetes during transmission by nymphal ticks

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Abstract. Ticks are not crawling needles, merely delivering infectious agents to vertebrate hosts. A sophisticated interplay takes place between ticks, pathogens, and vertebrate hosts. The relationship between *Ixodes* ticks and the Lyme disease spirochetes they transmit involves subtle changes in spirochete populations that maximize their chances of being transmitted. An understanding of this complex interplay will, hopefully, allow the development of new tools to block transmission of tick-borne agents.

Key words: ticks, Lyme disease, salivary glands, *Borrelia burgdorferi*

Introduction

Dr Willy Burgdorfer and colleagues first described Lyme disease spirochetes in ticks in 1982. In this seminal publication (Burgdorfer *et al.*, 1982), unfed female *Ixodes scapularis* were found to contain spirochetes distributed mainly in their midgut, with occasional spirochetes seen in the hindgut and rectal ampule. Spirochetes were not observed in the salivary glands or other tissues. Subsequently, Burgdorfer *et al.* (1989) noted that although the majority of unfed adult *I. scapularis* and *I. ricinus* had spirochetes restricted mainly to their midguts, a minority (<5%) had a more generalized or systemic infection, with small numbers of spirochetes observed in central ganglion, Malphigian tubules, salivary glands, and ovary. Furthermore, Burgdorfer *et al.* (1989) suggested that ticks with infections restricted to the midgut were efficient vectors of Lyme disease spirochetes because they regurgitate gut fluids during the feeding process.

The fact that some ticks had *Borrelia burgdorferi* infections restricted to the midgut whereas others had more generalized infections, including the hemolymph and salivary glands, was confirmed by electron microscopy (Benach *et al.*, 1987; Zung *et al.*, 1989). Ribeiro *et al.* (1987) noted that

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spirochetes in unfed female *I. scapularis* were restricted to the midgut, but once feeding started spirochetes could soon be detected in the hemolymph and could be detected in actual saliva by day 3 of feeding. Attention quickly shifted to tick salivary glands as the source of infectious *B. burgdorferi*.

An experimental model evolved for elucidating the dynamics of tick-transmitted infection, utilizing nymphal *I. scapularis* and North American strains of *B. burgdorferi sensu stricto*. Spirochete transmission was not efficient until nymphs had fed for >48 h (Piesman *et al.*, 1987; des Vignes *et al.*, 2001). Spirochetes were found to multiply rapidly in the midgut during the first 2 days of feeding, whereupon they traveled from the midgut, through the hemolymph, to salivary glands, and eventually to the host. Salivary gland homogenates derived from feeding nymphal *I. scapularis* became infectious after the ticks had fed for 60 h (Piesman, 1995). Schwan *et al.* (1995), as well as Schwan and Piesman (2000), noted that spirochetes in the tick midgut changed their outer surface coat from OSPA to OSPC during the first 2 days of tick feeding. Ohnishi *et al.* (2001) subsequently reported that midgut inhabiting spirochetes predominately expressed OSPA, while spirochetes in the salivary glands expressed OSPC or expressed neither OSPA nor C. Spirochetes in the host dermis attached to the hypostome of ticks removed from hosts had a spirochete population similar to that seen in the salivary glands. The development of quantitative PCR tools (q-PCR) has provided new methods for studying spirochete populations in specific organs of the tick.

Materials and Methods

Nymphal *I. scapularis* infected with the B31 strain of *B. burgdorferi sensu stricto* were produced as previously described (Piesman, 1993). These ticks were allowed to feed on ICR mice for defined intervals. Ticks tissues were dissected and prepared for q-PCR as previously described (Piesman *et al.*, 2001). All animals used in these experiments were handled according to approved protocols on file with the CDC/DVBID Animal Use Committee.

Results

Q-PCR was used to assay the number of spirochetes before nymphal *I. scapularis* attached to hosts, as well as during days 1, 2, 3, and 4 of attachment. Spirochete numbers in tick midguts increased (six-fold) from 998 per tick before attachment to 5884 at 48 h of attachment (Piesman *et al.*, 2001). Spirochetes in tick salivary glands increased >17-fold during feeding, from 1.2 per salivary gland pair before feeding to 20.8 at 72 h postattachment. The period

of the most rapid increase in the number of spirochetes in the salivary glands (48–60 h of attachment) coincides with the time period when ticks transmit *B. burgdorferi sensu stricto* to vertebrate hosts. The movement of spirochetes from tick midguts to tick salivary glands during feeding may represent a specific adaptation on the part of Lyme disease spirochetes to maximize their chances of being transmitted while minimizing the time period spent in the relatively hostile environment of tick salivary glands compared to milder conditions in tick midguts.

Discussion

A paradigm for spirochete transmission can be proposed wherein spirochetes remain confined primarily to the midgut in unfed nymphs. As the nymphs begin to feed, spirochetes multiply in the midgut until they downregulate OSPA. A tick midgut protein that binds OSPA may have the effect of holding this spirochete population captive until they stop expressing OSPA (Pal *et al.*, 2000). Once the spirochetes have eliminated OSPA from their surface they are free to travel through the hemolymph, to the salivary glands, and to the host. Quantitative molecular based studies, using real-time PCR techniques have shown that extremely small numbers of spirochetes are in tick salivary glands before they initiate feeding (Piesman *et al.*, 2001). These spirochetes may not be infectious (Ohnishi *et al.*, 2001) or there may be too few of them to initiate infection at the time the tick is removed. Interestingly, Crippa *et al.* (2002) suggested that removing the tick during feeding, and removing the localized dermal tissue attached to the hypostome, could preempt infection because the spirochetes have not had time to disperse away from the feeding site.

Initial molecular studies to determine the messenger RNA (mRNA) levels of spirochetes in ticks and hosts have been conducted (Gilmore *et al.*, 2001; Hodzic *et al.*, 2002). We conducted studies (unpublished) of the mRNA profile of spirochete populations in tick midguts and salivary glands to shed light on how spirochetes adapt to maximize their chances for transmission by ticks. As expected, mRNA of OSPA was associated primarily with spirochetes in the midgut, whereas salivarian spirochetes intensely produced mRNA of OSPC. Interestingly, vlsE was another mRNA associated primarily with spirochetes in salivary glands. This fits the picture wherein both OSPC and vlsE are thought to play an important role in host adaptation.

Similar studies should be conducted with European genospecies. Studies by Kahl *et al.* (1998), Fingerle *et al.* (2002) and Crippa *et al.* (2002) have shown that one cannot generalize from the study of one genospecies of *B. burgdorferi* to another without experimental observations, since the

transmission dynamics of *B. burgdorferi sensu stricto* appears to differ from that of *B. afzellii*.

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