

# Vector-Host Interactions in Disease Transmission

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## Abstract

Tick-borne spirochetes include borreliae that cause Lyme disease and relapsing fever in humans. They survive in a triangle of parasitic interactions between the spirochete and its vertebrate host, the spirochete and its tick vector, and the host and the tick. Until recently, the significance of vector-host interactions in the transmission of arthropod-borne disease agents has been overlooked. However, there is now compelling evidence that the pharmacological activity of tick saliva can have a profound effect on pathogen transmission both from infected tick to uninfected host, and from infected host to uninfected tick. The salivary glands of ticks provide a pharmacopoeia of anti-inflammatory, anti-haemostatic and anti-immune molecules. These include bioactive proteins that control histamine, bind immunoglobulins, and inhibit the alternative complement cascade. The effect of these molecules is to provide a privileged site at the tick-host interface in which borreliae and other tick-borne pathogens are sheltered from the normal innate and acquired host immune mechanisms that combat infections. Understanding the key events at the tick vector-host interface, that promote spirochete infection and transmission, will provide a better understanding of the epidemiology and ecology of these important human pathogens.

## The Tick-Borne Parasite Triangle

The survival of tick-borne spirochetes is dependent on a triangle of interactions (Figure 1). Most studies have focussed on the interactions between the spirochete and its vertebrate host, for example, the pathogenesis of infections in humans (van Dam *et al.*, 1993), or the replication of the spirochete in the tick vector (Gern *et al.*, 1990). Studies on the interactions between tick vector and vertebrate host have examined the host range of different vector species and determined the relative roles of different vertebrate species in supporting the tick vector population (Matuschka *et al.*, 1992; Oliver, 1996). A comparatively new area of research is the study of the vector-host interface, ie. the site of tick attachment and feeding. This is the site at which the tick-borne spirochete passes from an invertebrate to a vertebrate host system (or vice versa), and is a key step in the transmission dynamics and indeed the survival of the spirochete (Wikel, 1999).

Most published animal studies on arthropod-transmitted pathogens have relied on infecting the vertebrate species by needle and syringe inoculation of the pathogen. However, it has now well-documented that the results of such artificial inoculation can differ dramatically from those observed following natural vector-borne transmission (Belkaid *et al.*, 1998). These differences may be due to the effect of vector saliva on the feeding site and/or the variations in pathogen phenotype between vertebrate and invertebrate states. For example, early development work on a vaccine for Lyme disease involved protection studies in which immunised mice were inoculated with the etiological agent, *Borrelia burgdorferi*. The realisation that the host response to *B. burgdorferi* infection differed significantly in animals inoculated with *in vitro* cultured spirochetes compared with animals infected via *B. burgdorferi*-infected ticks has led to a change in approach (Schwan, 1996). Vaccine development studies for Lyme disease now rely on challenging immunised animals with infected ticks (de Silva and Fikrig, 1997). In fact, many studies on vertebrate host responses to *B. burgdorferi* infections that involve artificial inoculation of the spirochete are of questionable value.

## Feeding Strategy of the Tick Vector

The tick vectors of Lyme disease spirochetes are ixodid species of the genus *Ixodes* whereas relapsing fever spirochetes are transmitted by argasid species of the *Ornithodoros* genus (Table 1). Ixodid species generally take a single bloodmeal at each parasitic developmental stage: larva, nymph, adult (female). The bloodmeal is huge, resulting in a weight gain of  $\geq 100$ -fold, and consequently takes several days (or even weeks) to complete. By contrast, argasid species have several blood-feeding and non-feeding nymphal stages. Their bloodmeals are comparatively small, with weight gains  $< 10$ -fold, and are

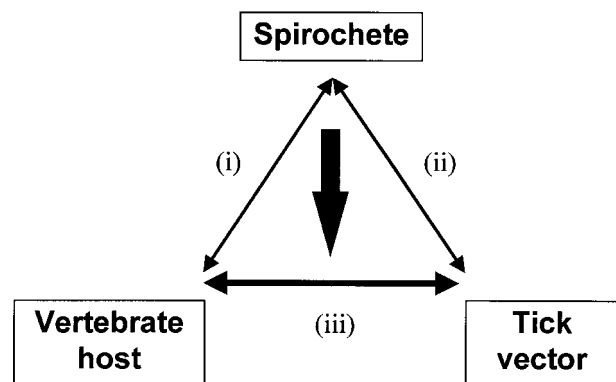


Figure 1. The triangle of interactions between tick-borne spirochetes, their vectors and vertebrate hosts. (i) spirochete-host interactions; (ii) spirochete-tick interactions; and (iii) host-tick interactions (the skin site of tick attachment and blood-feeding). The vertical arrow represents interactions of the spirochete with the host-tick interface.

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Table 1. Tick-Borne Spirochetes

Spirochete	Tick vector	Vertebrate host
Lyme disease spirochetes:		
<i>Borrelia burgdorferi</i> sensu lato	<i>Ixodes dentatus</i> , <i>I. hexagonus</i> , <i>I. neotomae</i> , <i>I. pacificus</i> , <i>I. ricinus</i> , <i>I. persulcatus</i> , <i>I. scapularis</i> (= <i>I. dammini</i> ), <i>I. spinipalpis</i> , <i>I. uriae</i>	rodents, birds, medium-sized mammals
Relapsing fever spirochetes <sup>1</sup> :		
<i>Borrelia caucasica</i>	<i>Ornithodoros verrucosus</i> <sup>3</sup>	rodents
<i>B. crocidurae</i>	<i>O. erraticus</i>	rodents
<i>B. duttonii</i>	<i>O. moubata</i>	humans
<i>B. hermsii</i>	<i>O. hermsii</i> <sup>2</sup>	rodents <sup>2</sup>
<i>B. hispanica</i>	<i>O. erraticus</i>	rodents
<i>B. latyschewii</i>	<i>O. tartakovskyi</i>	rodents
<i>B. mazzottii</i>	<i>O. talaje</i> <sup>3</sup>	rodents
<i>B. persica</i>	<i>O. tholozani</i>	rodents
<i>B. parkeri</i>	<i>O. parkeri</i>	rodents
<i>B. turicatae</i>	<i>O. turicata</i>	rodents
<i>B. venezuelensis</i>	<i>O. rudis</i> <sup>3</sup>	rodents
Other tick-borne spirochetes:		
<i>B. anserina</i>	<i>Argas persicus</i>	birds
<i>B. theileri</i> <sup>4</sup>	<i>Rhipicephalus evertsi</i> , <i>Boophilus</i> spp.	ungulates

<sup>1</sup> adapted from (Dennis 1998)

<sup>2</sup> chipmunks and tree squirrels

<sup>3</sup> not recognized species names (Pittaway 1991)

<sup>4</sup> Bishop 1995

completed within less than one hour (typically 20 min). Larvae of many *Ornithodoros* species differ in that they take a single bloodmeal, feeding for several days, whereas for some species (e.g. *O. moubata*) the larvae do not feed. For a general reference on tick biology see Sonenshine 1991.

Following host location and selection of a preferred feeding site (rodent-feeding *Ixodes* immatures often feed on the ears), the elaborate feeding process begins. Using its chelicerae, the tick saws through the keratinous layer of the skin and inserts its mouthparts (hypostome) into the epidermis, in some cases penetrating into the dermis. Many ixodid species secrete a milky cement which polymerises around the hypostome, securing the tick to the skin and providing a gasket that prevents leakage of tick saliva or host fluids. Ticks are pool feeders, forming a feeding lesion within the epidermis from which they take up their bloodmeal. For ixodid species, most of the time spent attached to the host is spent in the slow-feeding phase. During this period, the feeding pool is created and the tick prepares to expand its cuticle to accommodate the enormous bloodmeal. Only during the last day or so of attachment do they enter the fast-feeding phase when most of their bloodmeal is taken up. Feeding alternates between salivation and sucking. The process of detachment is poorly understood. Somehow ixodid species detach their mouthparts, leaving the cement core embedded in the skin. Tick cement comprises a number of glycosylated proteins, which have similarities to collagen (Mulenga *et al.*, 1999) and keratin (A.R. Trimnell, G.C. Paesen, and P.A. Nuttall, unpublished data), major constituents of vertebrate skin.

The feeding process of argasid species is completed much more rapidly, and they do not secrete cement. Unlike

ixodids, argasid ticks excrete coxal fluid (from glands associated with the first pair of legs) that can act as an additional route of spirochaete transmission. However, for spirochetes transmitted by ixodid or argasid ticks, the principal route of transmission is via tick saliva (Ribeiro *et al.*, 1987; Schwan, 1996).

### Immunomodulation at the Tick-Host Interface

The physical and chemical assault by the feeding tick, on its host, provokes the three arms of the host's defences: hemostasis (blood coagulation, platelet aggregation, and vasoconstriction), inflammation, and immunity (innate and acquired). Ticks counterattack by secreting a remarkable variety of bioactive proteins, peptides, and non-peptidic molecules in their saliva. These molecules have anti-haemostatic, anti-inflammatory, or anti-immune properties (Table 2). In most cases the tick molecules responsible for particular pharmacological activities have not been identified, studies having been based on *in vitro* assays using crude salivary gland extracts (SGE) or, more rarely, tick saliva.

### Saliva Activated Transmission (SAT)

The immunomodulatory properties of tick saliva are thought to be responsible for the phenomenon known as saliva-activated transmission (SAT). In ticks, this phenomenon has been most extensively studied with viruses (Table 3). For example, when guinea pigs infested with uninfected *I. ricinus* nymphs were infected with tick-borne encephalitis virus (TBEV) by needle and syringe inoculation of the virus, only 10% of the ticks became infected. By contrast, 52%

Table 2. Pharmacological Activities of Salivary Gland Extracts or Saliva of *Ixodes* and *Ornithodoros* Tick Vectors of Spirochetes

Activity	Target	Reference
<i>Ixodes</i> species:		
Anti-hemostatic	ADP	(Ribeiro <i>et al.</i> , 1985)
	prostaglandin receptor	(Ribeiro <i>et al.</i> , 1985)
Anti-inflammatory	prostacyclin receptor	(Ribeiro <i>et al.</i> , 1988)
	thrombin	(Hoffmann <i>et al.</i> , 1991)
	anaphylatoxins	(Ribeiro and Spielman, 1986)
	bradykinin	(Ribeiro <i>et al.</i> , 1985)
	histamine	G.C. Paesen, unpublished data
Anti-immune	alternative complement system	(Ribeiro, 1987; Lawrie <i>et al.</i> , 1999; Valenzuela <i>et al.</i> , 2000)
	neutrophils	(Ribeiro <i>et al.</i> , 1990)
	splenic T lymphocytes	(Urioste <i>et al.</i> , 1994)
	interleukin 2	(Urioste <i>et al.</i> , 1994)
	macrophages, nitric oxide	(Urioste <i>et al.</i> , 1994)
	interferon $\alpha$	(Hajnicka <i>et al.</i> , 2000)
	immunoglobulin G	(Wang and Nuttall, 1999)
<i>Ornithodoros</i> species:		
Anti-hemostatic	thrombin	(Nienaber <i>et al.</i> , 1999; Schaffer <i>et al.</i> , 1991; Waxman <i>et al.</i> , 1990)
	platelet aggregation	(Ribeiro <i>et al.</i> , 1985; Ribeiro <i>et al.</i> , 1991; Keller <i>et al.</i> , 1993; Waxman and Connolly, 1993; Karczewski <i>et al.</i> , 1994; Karczewski <i>et al.</i> , 1995)
Anti-immune	factor Xa	(Joubert <i>et al.</i> , 1998)
	ADP	(Mans <i>et al.</i> , 1998)
	complement	(Astigarraga <i>et al.</i> , 1997)

of the same batch of ticks became infected when they fed on guinea pigs inoculated with a mixture of the virus plus an extract prepared from the salivary glands of uninfected adult female *I. ricinus* that had fed for 5 days on an uninfected host (Labuda *et al.*, 1993). The ability of SGE or saliva to promote the transmission of arthropod-borne pathogens (ie. SAT) has been demonstrated unequivocally for certain tick-borne viruses and for sandfly-transmitted leishmania. Additionally, there is circumstantial evidence that other arthropod-borne pathogens, including *B. burgdorferi*, exploit SAT to promote their transmission. For viruses, indirect evidence of SAT is provided by their ability to be transmitted from infected to uninfected vectors, feeding together (co-feeding) on a host that develops no patent signs of viremia. This latter phenomenon has been termed non-viraemic transmission (Jones *et al.*, 1987) and is thought to result from SAT.

The basic mechanism underlying SAT is the ability of the vector-borne pathogen to exploit the pharmacological properties of its vector's saliva. To date, the SAT factor has not been identified for any of the pathogens shown in Table 3. Indeed, it is not known whether the SAT factor comprises one or more than one saliva molecule. Studies with Thogoto virus showed that the SAT factor is not present in the salivary glands of uninfected ticks (*Rhipicephalus*

*appendiculatus*), but that it accumulates in the salivary glands and is secreted in saliva as feeding progresses. Maximal SAT activity was shown by SGE and saliva of uninfected adult *R. appendiculatus* that had been feeding for 5-8 days, after which SAT activity decreased (Jones *et al.*, 1992b). The dynamics of SAT activity for Thogoto virus and its tick vector, *R. appendiculatus*, suggest that the active saliva ingredient is not an anti-haemostatic, anti-inflammatory or anti-complement factor, as these activities are expressed early during the feeding period, in parallel with activation of the matching host responses.

The SAT factor probably differs for different pathogens and vector species. Thus, SAT of TBEV is shown by *I. ricinus* and *R. appendiculatus* SGE, whereas SAT of Thogoto virus occurs with SGE of *R. appendiculatus* but not of *I. ricinus* (Jones *et al.*, 1992a). Interestingly, SAT activity may correlate with vector competence. Both *I. ricinus* and *R. appendiculatus* are competent vectors of TBEV although *R. appendiculatus* is not a natural vector, whereas Thogoto virus is transmitted by *R. appendiculatus* but not by *I. ricinus* (Jones *et al.*, 1992a).

Studies similar to those involving viruses and SAT have not been undertaken with tick-borne spirochetes. The most comparable studies are those reported by Zeidner and colleagues (1996). In these, tick-borne *B. burgdorferi* infection of mice was reduced by treatment with three cytokines: either tumour necrosis factor alpha (TNF $\alpha$ ), interleukin-2 (IL-2) or interferon gamma (IFN $\gamma$ ). This observation indicates that anti-cytokine activity of tick saliva may promote *B. burgdorferi* transmission. To date, anti-cytokine activity detected in SGE from *Ixodes* ticks has been recorded for interferon alpha (IFN $\alpha$ ) which is not known to affect *B. burgdorferi* (Hajnicka *et al.*, 2000).

The identity of SAT factors awaits a better understanding of the components of tick saliva. Ideally, we need to undertake manipulative experiments with single saliva molecules. For proteins and large peptides, this requires cloning and sequencing tick saliva molecules, and producing them in recombinant form. Studies so far suggest

Table 3. Vector-Borne Pathogens Demonstrating or Implicated in Saliva-Activated Transmission

Pathogen	Tick-borne	Insect-borne
viruses	Crimean-Congo haemorrhagic fever	Cache Valley
	Palma	La Crosse
	Tick-borne encephalitis	
	Thogoto	Vesicular stomatitis
bacteria	<i>Borrelia burgdorferi</i>	
protozoa	<i>Theileria parva</i>	<i>Leishmania</i> spp.

this will be an enormous task, as there appear to be tens if not hundreds of different molecules secreted by each tick species.

### The Tick Pharmacopoeia

Attempts to isolate and characterise the components of tick saliva have so far only revealed the tip of the iceberg. Nevertheless, substantial progress has been made in understanding the molecular strategies adopted by ticks to counter the three arms of the host response, *viz* haemostasis, inflammation, and cellular and humoral immunity. The following considers a few examples that may have significance for tick-borne spirochete transmission.

#### Anti-Hemostatic Molecules

Anti-hemostatic molecules were thought to play a role in arthropod-borne pathogen transmission following the discovery that maxadilan, a peptide secreted by sandflies (*Lutzomyia* spp.) appeared to enhance *Leishmania* transmission (Theodos *et al.*, 1991; Titus and Ribeiro, 1988). However, this may not be the case (Warburg *et al.*, 1994).

One of the first anti-hemostatic molecules identified in tick saliva was prostaglandin (Ribeiro *et al.*, 1985). Prostaglandins play a role in vasodilation and anti-platelet aggregation, as well as immunomodulation. They are produced from arachidonic acid taken up in the bloodmeal by feeding ticks, and are not stored in the salivary glands. Hence any transmission-enhancing properties of SGE are not likely to be due to the actions of prostaglandins.

#### Anti-Inflammatory Molecules

The important inflammatory mediator, histamine (a biogenic amine), is produced and stored in mast cells, basophils and (in some species) blood platelets. On extracellular release, histamine binds to its receptors, triggering a cascade of inflammatory and immune responses that result in swelling (oedema), redness (erythema), and irritation. Histamine is an important mediator in successful anti-tick responses, particularly in previously exposed hosts that have developed an immune (anamnestic) response (Brossard, 1982; Wikel, 1982).

The importance of histamine in tick blood-feeding is reflected in the wealth of histamine-binding proteins that ticks have evolved to counter its activities (Paesen *et al.*, 1999). Tick histamine-binding proteins are structurally related to a large family of barrel-shaped ligand-binding proteins known as lipocalins. They have been named histacalins. Their high affinity for histamine enables them to out-compete histamine receptors and thereby prevent a histamine-mediated inflammatory response (Paesen *et al.*, 1999 and unpublished data). Histacalins have been isolated from tick species belonging to a number of ixodid genera, including *Ixodes hexagonus*, however they have not been detected in *I. ricinus* (the most important European vector of *B. burgdorferi*) although anti-histamine activity has been detected (G.C. Paesen, unpublished data).

Histacalins have not so far been detected in argasid tick species. However, a lipocalin is produced by the salivary glands of *O. moubata* but this molecule, moubatin, inhibits blood platelet aggregation (Paesen *et al.*, 1999). The use of a common molecular structure to perform different functions in tick saliva illustrates the enormity of

the task of characterising the tick pharmacopoeia.

The apparent absence of histacalins from *I. ricinus* and *O. moubata*, if confirmed, suggests that they do not play a role in the tick-borne transmission of spirochetes. Nevertheless, a site of inflammation is a hostile environment for invading pathogens. Anti-inflammatory mediators are likely to benefit spirochetes, even if they are not the key factor in facilitating their transmission. In particular, histamine upregulates certain cytokines such as TNF (see preceding section), and activates natural killer cells, components of the innate immune system. Suppression of these effects by tick anti-histamines is likely to aid spirochete transmission.

#### Immunomodulators

The molecules responsible for at least two immunomodulatory activities relevant to tick-borne spirochete transmission have been isolated and partially characterised: immunoglobulin-binding proteins (IGBPs) and a complement inhibitor.

##### IGBPs

For a long time, it was known that a small proportion of host immunoglobulins taken up in the bloodmeal of hematophagous arthropods, escapes digestion and passes through the gut wall into the haemocoel. The fate of these rogue immunoglobulins was unknown until the discovery that adult female *R. appendiculatus* excrete intact IgG in their saliva (Wang and Nuttall, 1994). Further investigations revealed a family of IGBPs in the hemolymph and salivary glands of adult *R. appendiculatus*, and proteins with similar activity in other ixodid species, including *I. ricinus* and *I. hexagonus*. Although IGBPs have not been reported for argasid tick species, circumstantial evidence suggests they may occur (Minoura *et al.*, 1985).

The prevalence and abundance of IGBPs indicate that they play an important role in bloodfeeding. Possibly they provide a tick immunoglobulin excretion system (TIES) that enables ticks to ferry potentially damaging antibodies safely through the hemocoel to their salivary glands whence they are excreted (Wang and Nuttall, 1999). If such a TIES exists, it may benefit spirochetes and other tick-borne pathogens by protecting them (and possibly the infected tick) from pathogen-specific antibodies taken up in the bloodmeal. Presumably this would benefit *B. burgdorferi* only after it has passed from the midgut where it is clearly susceptible to antibodies directed to the outer surface protein, OspA (Schwan, 1996). By contrast, *B. hermsii* quickly migrates from the midgut to the salivary glands when its tick vector feeds on a spirochetemic host (Schwan 1996), hence it may be exposed to residual anti-borrelia antibodies acquired in the preceding bloodmeal.

Despite the advantages of a TIES, it appears illogical that a tick should have such an elaborate system for transporting biologically active immunoglobulins through its body. A more obvious solution is to ensure that all immunoglobulins in the bloodmeal are degraded. The answer to this apparent conundrum is that the tick appears to use the excreted immunoglobulins against its host. Thus when guinea pigs were immunised with IGBPMC, a secreted IGBP from adult male *R. appendiculatus*, the feeding performance of its female mate was impaired. In this particular case, the male tick seems to use the host's immunoglobulins to induce local immunosuppression for

the benefit of the female, a novel form of 'mate guarding' (Wang *et al.*, 1998). If the tick vectors of spirochetes are similarly able to induce immunosuppression, possibly through local saturation of Fc receptors by excreted immunoglobulins, tick-transmitted spirochetes may benefit.

#### Complement Inhibitors

Inhibitory activity against complement has been demonstrated using SGE of both *Ixodes* spp. and *Ornithodoros* spp. (Table 2). For *Ixodes* spp., the inhibitory activity is directed against the alternative complement cascade. Interestingly, anti-complement activity shows host specificity for different tick species (Lawrie *et al.* 1999). Thus *I. hexagonus* has high levels of anti-complement activity against hedgehog serum (hedgehogs are its preferred host) and canine serum, but no activity against serum from pheasants or pigeons (on which it does not appear to feed). The cosmopolitan *I. ricinus* demonstrates anti-complement activity to a range of mammalian and avian hosts. Significant levels of anti-complement activity were demonstrated in SGE from both unfed and feeding *I. ricinus* (adult female). This may reflect the fact that activation of the alternative complement cascade, through the acute phase inflammatory response, is one of the first events following tick attachment, and presents a constant threat to the tick throughout engorgement.

The alternative complement systems also presents a major threat to *B. burgdorferi* to the extent that complement sensitivity has been proposed as the key to Lyme disease ecology (Kurtenbach *et al.*, 1998). When different members of the *B. burgdorferi* sensu lato complex were incubated *in vitro* in serum from different vertebrate species, a striking correlation was observed with host susceptibility. Thus *B. garinii* strain ZQ 1 survived in avian serum but was rapidly lysed by rodent serum. By contrast, *B. afzelii* survived rodent serum but was killed by avian serum. The borreliacidal effect was shown to be due to activity of the alternative complement cascade (Kurtenbach *et al.*, 1998). Several *B. garinii* strains are sensitive to human sera (van Dam *et al.*, 1997). Despite this sensitivity to human complement, *B. garinii* is able to infect humans and cause neuroborreliosis. Could it be that anti-complement factors in tick saliva provide sufficient protection of *B. garinii* from the borreliacidal effects of human complement, enabling the tick-transmitted borreliae to seek out an immunoprotected site within the tick-bitten human host? The recent isolation of the alternative complement inhibitor from *I. scapularis* may allow us to answer this question (Valenzuela *et al.*, 2000). This 18 kD protein is believed to act in a similar fashion to factor H, interfering with the binding of factor B to C3b. Isolation of a recombinant form of this tick immunomodulator will greatly facilitate studies on its role in the transmission of Lyme disease spirochetes.

#### Co-Feeding Transmission of *Borrelia burgdorferi*

Although SAT has not been demonstrated for *B. burgdorferi*, circumstantial evidence suggests that it occurs, based on the demonstration of co-feeding transmission. Unlike co-feeding transmission of tick-borne viruses, in which transmission occurs from infected to uninfected ticks feeding together on a non-viraemic host at the same time, co-feeding transmission of *B. burgdorferi* may be spatial rather than temporal. One of the first studies of co-feeding

transmission of *B. burgdorferi* was undertaken with infected and uninfected *I. ricinus* feeding on laboratory mice (Gern and Rais, 1996). Uninfected larvae became infected when feeding at the same site (on the backs of mice) as infected nymphs while larvae feeding on the ears remained uninfected. Similar studies were reported for co-feeding transmission on sheep, the principal vertebrate host of *B. burgdorferi* in N.W. England (Ogden *et al.*, 1997). These observations indicate that uninfected ticks do not require systemically infected vertebrate hosts in order to become infected.

Studies on the non-viraemic transmission of TBEV indicate that co-feeding transmission involves specific translocation of the virus from the site of infected tick feeding to that of uninfected tick feeding (Labuda *et al.*, 1996). Selective skin tissue biopsies revealed infectious virus first at the site of infected tick feeding followed 24 h later at the site of uninfected tick feeding, but not at skin sites located between or adjacent to the two tick feeding sites. These observations suggest a 'push-pull' mechanism of transmission in which both the site of infected tick feeding and that of uninfected co-feeding ticks influence pathogen transmission. For virus translocated within infected cells, such a 'push-pull' concept appears plausible (Nuttall 1999). However, for self-locomotory borreliae, such a mechanism of co-feeding is more difficult to envisage. Either the borreliae perceive and respond to chemical cues within the host, or they hitch a ride on translocating host cells. The scarcity of *B. burgdorferi* in the skin of infected animals, compared with the efficiency by which feeding ticks become infected, led Schwan (1996) to comment that chemoattractants may exist in tick saliva that mobilize the spirochetes to sites of feeding ticks. Chemotaxis studies using *B. burgdorferi* and SGE of *I. ricinus* failed to demonstrate evidence of such attraction for cultured borreliae (P.A. Nuttall and L. Gern, unpublished data). In this context it is notable that neutrophils have been observed to phagocytose borreliae, however, it is not known what effect this has on borreliae viability.

#### Spirochetes and the Vector-host Interface

This brief and selective review presents evidence that the vector-host interface (the tick feeding site) is a unique ecosystem. The plethora of pharmacological activities mediated by the tick's pharmacy (its salivary glands) create an environment profoundly different from that of virgin skin. It is highly likely that tick-borne spirochetes have evolved to exploit at least some of these pharmacological effects. Indeed, they may even have 'learned' to manipulate the tick's pharmacy to benefit further their transmission and hence their survival. Clearly the tick-host interface is a highly complex but very exciting area for further research on the transmission triangle of tick-borne spirochetes.

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