Tick–host interactions: saliva-activated transmission

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SUMMARY

The skin site at which ticks attach to their hosts to feed is the critical interface between the tick and its host, and tick-borne pathogens. This site is highly modified by the pharmacologically active molecules secreted in tick saliva. For pathogens, it is an ecologically privileged niche that many exploit. Such exploitation is referred to as saliva-activated transmission (SAT) – the indirect promotion of tick-borne pathogen transmission via the actions of bioactive tick saliva molecules on the vertebrate host. Here we review evidence for SAT and consider what are the most likely candidates for SAT factors among the tick pharmacopoeia of anti-haemostatic, anti-inflammatory and immunomodulatory molecules identified to date. SAT factors appear to differ for different pathogens and tick vector species, and possibly even depend on the vertebrate host species. Most likely we are searching for a suite of molecules that act together to overcome the redundancy in host response mechanisms. Whatever they turn out to be, the quest to identify the tick molecules that mediate SAT is an exciting one, and offers new insights to controlling ticks and tick-borne diseases.

Key words: Saliva-activated transmission, tick saliva, pathogen transmission.

TICK–HOST–PATHOGEN INTERACTIONS

The relationships between tick-borne pathogens, their tick vectors and diverse vertebrate hosts, can be represented by a triangle of parasitic interactions (Fig. 1). The interactions are between (1) pathogen–tick, (2) pathogen–host, and (3) tick–host. In (1) the pathogen interacts with its vector, infecting and replicating within tick cells or extracellular spaces (including those of the gut, haemocoel and salivary glands). The ability of a particular tick species to act as a vector depends on whether the pathogen can survive and overcome several 'barriers' within the tick, e.g. the environment within the midgut where the pathogen is initially taken up in the blood meal, the gut infection barrier, and the salivary gland infection barrier. The role of the tick immune system in controlling infections is largely unknown, as is the nature of any conspecific or heterospecific interactions between strains or genotypes of these pathogens during multiple infections of an individual tick.

Interaction (2) also involves infection: the pathogen interacts with its vertebrate host, infecting and replicating extracellularly or within cells. The outcome of infection depends on the tropism of the pathogen for specific host cell or tissue types, or locations (e.g. nervous tissue, joints), and its pathogenic phenotype, and on the age and immune status of the host, and genetic background.

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The third component of the triangle (Fig. 1, interaction 3) is the interaction between the tick and its host. Although this is the non-infective face of the triangle, the skin site at which ticks attach to feed is the cross-roads for pathogens. It is the initial site at which pathogens gain access to either their host or vector: the tick–host–pathogen interface. This cross-roads is particularly busy for tick-borne pathogens because blood feeding of ticks is such a complex and comparatively long and highly ordered process, especially in ixodid species. Attachment and feeding takes several days to complete (minutes to hours for argasids), and involves sawing through the epidermis by means of toothed chelicerae, inserting the mouthparts (barbed hypostome and chelicerae) into the resulting wound site which are then (for ixodids) cemented in place, followed by the formation of a feeding pool resulting from tick and host activities. For ixodid species, the majority of the blood meal is not taken up until the last day of attachment (Kemp, Stone & Binnington, 1982). Such a profound physical and chemical assault on the host should provoke strong haemostatic, inflammatory and immune responses. However, despite the host's armoury of rejection mechanisms, the tick manages to remain attached and achieve engorgement. Successful feeding of ticks relies on a pharmacy of chemicals located in their complex salivary glands and secreted, in tick saliva, into the feeding pool (see chapters by Brossard & Wikel and Valenzuela in this Supplement).

Increasing evidence indicates that the survival of tick-borne pathogens depends on their ability to exploit the pharmacological activities of tick saliva molecules. This is depicted in Fig. 1 by the broad vertical arrow representing the interaction of the

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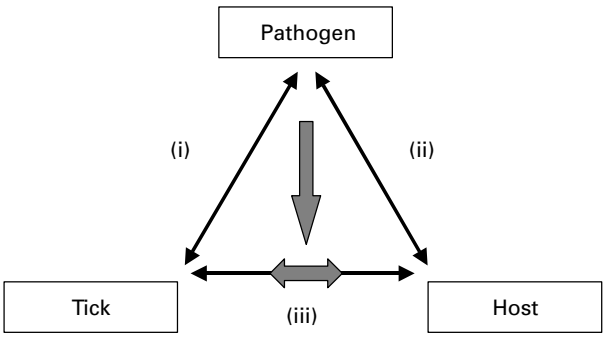


Fig. 1. The triangle of interactions between tick-borne pathogens, their tick vectors and vertebrate hosts. (1) pathogen–tick interactions; (2) pathogen–host interactions; and (3) tick–host interactions. The vertical arrow represents interactions of a pathogen with tick–host interactions at the skin site of tick attachment and blood-feeding (the tick–host–pathogen interface) and the double-headed arrow represents the cross-roads for pathogens at which they pass from infected tick vector to vertebrate host, or from infected host to tick vector.

pathogen with the modified tick–host interface. Why do we use such a forceful representation? Because it demonstrates our view of pathogen adaptation to the very specific environment created by the intimate and dramatic interplay between host and vector, and concentrated in the tick feeding site. By exploiting this unique ecological niche – the feeding site – tick-borne pathogens are making an easier living for themselves, increasing their chances of survival in nature.

SALIVA-ACTIVATED TRANSMISSION (SAT)

Promotion of tick-borne pathogen transmission, via the actions of tick saliva components on the host, has been termed saliva-activated transmission (SAT). It represents the pathogen's quest for saliva-mediated host modulation, represented by the large vertical arrow in Fig. 1. The term SAT was first coined to describe the promotion of Thogoto virus transmission by SGE of *Rhipicephalus appendiculatus* (Nuttall & Jones, 1991). Since then, the phenomenon has been demonstrated for a number of tick-borne pathogens (Tables 1 and 3). Direct and indirect evidence of SAT has also been reported for several insect-borne pathogens including *Leishmania* spp. and their sandfly (*Lutzomyia*, *Phlebotomus*) vectors (Titus & Ribeiro, 1988; Belkaid *et al*. 1998), La Crosse virus and *Aedes triseriatus* mosquitoes (Osorio *et al*. 1996), Cache Valley virus and *Aedes aegypti*, *Aedes triseriatus* and *Culex pipiens* mosquitoes (Edwards, Higgs & Beaty, 1998), vesicular stomatitis virus and *Simulium vittatum* blackflies (Mead *et al*. 2000), and *Orientia tsutsugamushi* (the aetiological agent of scrub typhus) and *Leptotrombidium deliense* and *Blankaartia acuscutellaris* mite larvae (Frances *et al*. 2000).

Experimentally, SAT is demonstrated by enhanced transmission of infectivity when the pathogen plus salivary gland extract (SGE) is syringe-inoculated into a host, compared with the level of infectivity when the pathogen alone is injected. For example, comparison of pooled experimental data for Thogoto virus infection reveals the attenuating effect of SGE (Table 2). Experimental inoculation of guinea pigs with a mixture of the virus plus SGE of partially fed *Rhipicephalus appendiculatus* or *Amblyomma variegatum* female ticks increased the number of nymphs that became infected approximately 10-fold compared with inoculation with virus alone. Similar direct evidence of SAT has been reported for tick-borne encephalitis virus (TBEV), the Lyme disease spirochaetes *Borrelia afzelii*, *B. burgdorferi* sensu stricto, and *B. lusitaniae*, and *Franciscella tularensis* (Table 1).

Indirect evidence of SAT is provided by observations of efficient non-systemic transmission between infected and uninfected ticks co-feeding on the same host (Table 3). The first unequivocal example was demonstrated by the non-viraemic transmission of Thogoto virus (Jones *et al*. 1987). Remarkably, virus transmission from infected to uninfected ticks co-feeding on non-viraemic guinea pigs was more efficient than transmission on hamsters that exhibited high levels of viraemia. Non-viraemic transmission has been shown for several other tick-borne viruses, including TBEV, Crimean-Congo haemorrhagic fever virus, Louping ill virus, Bhanja virus, Palma virus and West Nile virus (Table 3). A possible example exists for Kyansanur forest disease virus transmitted by *Argas persicus* feeding on a domestic chick (Singh, Goverdhan & Bhat, 1971). Comparable non-systemic transmission has been reported for *B. burgdorferi* ss, *B. garinii* and *B. afzelii* (Table 3). Transmission of *Ehrlichia ruminantium*, the causative agent of heartwater in cattle, between infected and uninfected ticks co-feeding on tortoises, is another possible example of non-systemic transmission (Bezuidenhout, 1987). By contrast, *Anaplasma marginale*, the tick-borne rickettsial pathogen of cattle, and the agent of human granulocytic ehrlichiosis (*Anaplasma phagocytophilum*), do not appear to utilize co-feeding non-systemic transmission (Hodzic *et al*. 2001; Kocan & de la Fuente, 2003).

Results of co-feeding experiments using pathogen-immune natural rodent hosts also support the concept of SAT (Labuda *et al*. 1997*b*). These animals supported TBEV transmission in the presence of virus-specific neutralizing antibodies. In sharp contrast, immunization with specific tick-derived antigens significantly diminished TBEV transmission and surprisingly, also increased the survival of mice following an otherwise lethal infective *I. ricinus* tick bite (Labuda, unpublished observation). The protective effect of anti-tick immunity against TBEV infection (which has been observed with other

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Table 1. Tick-borne pathogens showing direct evidence of saliva-activated transmission

Pathogen	Tick species source of SGE	Reference
Viruses:		
Tick-borne encephalitis virus	*Ixodes ricinus*, *Dermacentor reticulatus*, *D. marginatus*, *Rhipicephalus appendiculatus*	(Alekseev *et al.* 1991; Labuda *et al.* 1993*b*)
Thogoto virus	*Rhipicephalus appendiculatus*, *R. evertsi*, *Amblyomma cajennense*, *A. hebraeum*, *A. variegatum*, *Boophilus microplus*, *Hyalomma dromedarii*, *H. marginatum rufipes*	(Jones *et al.* 1989, 1992*a*, *b*, *c*)
Bacteria:		
Borrelia afzelii	*Ixodes ricinus*	(Pechova *et al.* 2002)
Borrelia burgdorferi sensu stricto	*Ixodes scapularis*	(Zeidner *et al.* 2002)
Borrelia lusitaniae	*Ixodes ricinus*	(Zeidner *et al.* 2002)
Franciscella tularensis	*Ixodes ricinus*	(Krocoka *et al.* 2003)

pathogen-vector systems) underpins the concept of transmission-blocking vaccines (see chapter by Willadsen in this Supplement).

Further indirect evidence of SAT is based on *in vitro* studies in which SGE is shown to promote infections by tick-borne pathogens. For the Lyme disease spirochaete, SGE of unfed female *I. ricinus* stimulated growth of *B. garinii* cultures *in vitro* while SGE of unfed female *D. reticulatus*, a non-competent vector of the Lyme disease spirochaete, did not (Rudolf & Hubalek, 2003). *In vitro* studies with murine macrophages showed that killing of *B. afzelii* spirochaetes was inhibited by SGE of *I. ricinus* females fed for 5 days (Kuthejlova *et al.* 2001). As macrophages represent one of the the first lines of host defence against this spirochaete, suppression of their antimicrobial activity may well contribute to SAT. A stimulatory chemotactic effect of SGE from *I. scapularis* on *B. burgdorferi* ss was observed using a modified U-tube chemotaxis assay (Shih, Chao & Yu, 2002). This apparent chemotactic potential of SGE may contribute to the acquisition of Lyme disease spirochaetes by feeding ticks, which also can be considered a form of SAT. Thus the effect of saliva at the feeding site of uninfected ticks may act as a magnet, drawing spirochaetes to the site. The mechanism for this, if it occurs, is unknown.

In vitro treatment of bovine lymphocytes with SGE of *R. appendiculatus* enhanced their susceptibility to infection by *Theileria parva* sporozoites (Shaw, Tilney & McKeever, 1993). Curiously, the effect was observed with SGE from unfed ticks as well as ticks that had fed for 4 days, and it occurred within only 90 min of treatment. Studies are needed to determine whether the apparent stimulating effect on *T. parva* invasion of host cells reflects an ability of the tick vector to promote transmission of this important pathogen. Tick SGE has even been shown to accelerate replication of vesicular stomatitis virus, an arthropod-borne virus (arbovirus) not transmitted by ticks (see *Cytokine inhibitors*, below).

Table 2. Thogoto virus infection of ticks via different transmission routes

Transmission route	% ticks infected* (no. of experiments)
Syringe inoculation: virus	6% (6)
Syringe inoculation: virus + SGE	58% (7)
Co-feeding with infected ticks	85% (11)

* Infection of *Rhipicephalus appendiculatus* nymphs feeding on guinea pigs that were either inoculated with virus ± SGE or co-infested with Thogoto virus infected ticks. Modified from Nuttall, 1998.

An interesting approach to investigating whether tick-induced immunomodulation affects tick-borne pathogen infection was reported for *Anaplasma phagocytophilum*, the aetiological agent of human granulocytic ehrlichiosis (Borjesson *et al.* 2002). Mice were infected via syringe inoculation and then infested with uninfected *Ixodes scapularis* nymphs. At the same time as the infestation, a suture was placed through the skin, distant from the ticks. The suture served as a non-specific inflammatory source and acted as a positive control for the specific inflammation induced by the feeding ticks. A marked increase in bacteraemia and rate of *A. phagocytophilum* infection of neutrophils were observed following tick feeding. The increased bacteraemia was not explained in terms of a specific tick-induced modulatory mechanism, nevertheless, this approach warrants further investigation.

All the direct and indirect evidence for SAT involves ixodid tick species, with one clear exception (Table 3). Whether this indicates a greater capacity of ixodid species for SAT remains to be determined, as comparatively few studies have been reported with argasid species. The one exception is the reported non-viraemic transmission of West Nile virus (WNV) between co-feeding infected and uninfected *Ornithodoros moubata* (Lawrie *et al.* 2004). This report is unusual because WNV is regarded as a

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Table 3. Tick-borne pathogens showing indirect evidence of saliva-activated transmission (non-systemic transmission)

Pathogen	Tick species involved	Reference
Viruses:		
Bhanja virus	*Dermacentor marginatus*, *Rhipicephalus appendiculatus*	(Labuda *et al*. 1997*a*)
Crimean-Congo haemorrhagic fever virus	*Hyalomma marginatum*	(Gordon, Linthicum & Moulton, 1993; Zeller *et al*. 1994)
Louping ill virus	*Ixodes ricinus*	(Jones *et al*. 1997)
Tick-borne encephalitis virus	*Ixodes persulcatus*, *I. ricinus*, *Dermacentor marginatum*, *D. reticulatus*, *Rhipicephalus appendiculatus*	(Alekseev & Chunikhin, 1990; Labuda *et al*. 1993*a*, *c*)
Thogoto virus	*Rhipicephalus appendiculatus*, *Amblyomma variegatum*	(Jones *et al*. 1987, 1990*b*)
Palma virus	*R. sanguineus*, *Dermacentor reticulatus*, *D. marginatus*	(Labuda *et al*. 1997*a*)
West Nile virus	*Ornithodoros moubata*	(Lawrie *et al*. 2004)
Bacteria:		
Borrelia afzelii	*Ixodes ricinus*	(Richter *et al*. 2002)
Borrelia burgdorferi sensu stricto	*Ixodes scapularis*	(Gern & Rais, 1996; Patrican, 1997)
Borrelia garinii	*Ixodes persulcatus*	(Sato & Nakao, 1997)

mosquito-borne virus although epidemiological evidence indicates it can be maintained in nature by argasid species (see chapter by Labuda & Nuttall in this Supplement).

DETERMINANTS OF SAT

For tick-borne viruses, the SAT factor promotes transmission through its activity on the host rather than by a direct effect on the virus. The most compelling evidence for this is from studies with Thogoto virus. When Thogoto virus was mixed with SAT-active SGE and then assayed in cell culture and mice, viral infectivity was unchanged (Jones, Hodgson & Nuttall, 1989, 1990*a*). This suggests that the enhancing factor is not a proteolytic enzyme that cleaves a viral surface protein to expose a more infectious virus particle, as occurs with some insect-borne viruses (Borucki *et al*. 2002; Takamatsu *et al*. 2003). However, more compelling is the observation that SAT was observed when SGE and Thogoto virus were inoculated at different times into guinea pigs (Jones, Kaufman & Nuttall, 1992*b*). When SGE was inoculated into the skin of tick-infested guinea pigs, and then Thogoto virus was inoculated in the same site at the same time or 1, 2, 3 or 4 days later, the number of nymphs infected was increased compared with the controls inoculated with virus alone. In fact, yields of infected ticks were highest when the virus was inoculated 2 or 3 days after inoculation of SGE. Similar results were obtained in the converse experiment, in which SGE was inoculated after the virus, although the window for enhancement appeared to be shorter. The key to successful enhancement in these experiments was the inoculation of virus and SGE into the same skin site; when inoculated at different sites, the numbers of infected nymphs fell to levels observed when guinea pigs were inoculated with virus alone (Jones *et al*. 1989). Immunomodulatory effects of tick saliva that may mediate SAT and explain these observations are considered in the next section (Candidate SAT Factors).

Tick-borne transmission studies undertaken with laboratory animals suggest a correlation between pathogen, vector competence and SAT. Thus, SAT was demonstrated with TBEV and SGE from *Ixodes ricinus* and *Rhipicephalus appendiculatus*; both tick species can transmit the virus (although *R. appendiculatus* is not a natural vector). However, Thogoto virus showed SAT with SGE or saliva of *R. appendiculatus* (its natural vector species) but not with SGE from *I. ricinus*, a non-competent vector species that is unable to transmit Thogoto virus following *per os* infection (Jones *et al*. 1989, 1992*a*; Labuda *et al*. 1993*b*). A similar observation has been made for the Lyme disease spirochaete. SGE of *I. ricinus* enhanced the spirochaete load of *B. lusitaniae* but not *B. burgdorferi* ss, whereas *I. scapularis* SGE specifically increased levels of *B. burgdorferi* ss, but did not affect the dissemination of the related *B. lusitaniae* (Zeidner *et al*. 2002).

The apparent correlation between SAT and vector competence is somewhat contradicted by the fact that tick SGE accelerates the replication of vesicular stomatitis virus, *in vitro* (Hajnicka *et al*. 1998). This insect-borne virus has no known tick vector. By contrast, attempts to show SAT with Dugbe virus and Kadam virus, using SGE from their natural tick vector species and a variety of mammalian species as hosts, have been unsuccessful (Steele & Nuttall, 1989; L. D. Jones personal communication).

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Table 4. Candidate SAT factors

Saliva molecules	Activities that may benefit tick-borne pathogens
Histamine-binding proteins (histacalins)	Suppression of inflammatory responses including recruitment of neutrophils and eosinophils
Complement inhibitors	Suppression of inflammatory and immune responses
Immunoglobulin-binding proteins	Protection against antibodies
Cytokine inhibitors:	
Type 1 interferon inhibitors	Inhibition of anti-viral activities
IL-2 binding proteins	T cell suppression
IL-8 binding proteins	Anti-neutrophil activity
Leukocyte modulators:	
B cell modulators	Reduced antibody and cytokine production
T cell modulators	Polarisation to a Th2 response
Natural killer cell suppressors	Inhibition of anti-viral activities
Phagocyte modulators	Inhibition of microbicidal activity

Together, these observations on the determinants of SAT demonstrate that the interface between vector and host, with which the pathogen interacts (indicated by the vertical arrow, Fig. 1), is highly complex, remarkably specific and considerably variable. Both the vector and the vertebrate host species of the pathogen, and possibly even the strain of the pathogen, have roles to play in determining whether SAT occurs.

The implications of SAT and non-systemic transmission for the survival of tick-borne pathogens in nature are not considered in this review. However, this important aspect of tick–host–pathogen interactions has been discussed in other publications (see Randolph *et al*. 1996, 1999; Nuttall & Labuda, 2003).

CANDIDATE SAT FACTORS

To date, the SAT factor has not been identified for any of the pathogens shown in Tables 1 and 3. Indeed, it is not known whether the SAT factor of a particular tick-borne pathogen comprises one or more than one saliva molecule. Studies with Thogoto virus demonstrated that the SAT factor is not present in the salivary glands of unfed ticks 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 female *A. variegatum* that had been feeding for 5 to 8 days, after which SAT activity declined (Jones *et al*. 1992*b*). For adult female *R. appendiculatus*, maximum SAT activity was shown by SGE of ticks that had been feeding for 6 days, whereas SGE from adult female *Boophilus microplus*, collected at a feeding weight range of 3 to 250 mg, all showed SAT and with no obvious peak (Jones *et al*. 1989; Jones, Matthewson & Nuttall, 1992*c*). Whatever the one-host tick species, *B. microplus*, produces that promotes Thogoto virus transmission appears to be present for most, if not all, of the adult female feeding period. The dynamics of SAT activity for Thogoto virus and its tick vector, *R. appendiculatus*, suggest that the active saliva ingredient is probably 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. Physico-chemical analysis indicates that the SAT factor for Thogoto virus is one or more proteins or peptides (Jones *et al*. 1990*a*).

As mentioned in the preceding section, the limited evidence to date indicates that the SAT factor differs for different pathogens and vector species. The SAT factor may even differ with different vertebrate host species, a possibility that has not been explored. Nevertheless, all the evidence for SAT factors points to the bioactive molecules present in the salivary glands and secreted in saliva. These saliva molecules are responsible for countering host haemostatic, inflammatory and immune responses. Although their precise role and mode of action in tick-borne pathogen transmission is unknown, a number of these bioactive molecules are likely to affect the host in a way that is beneficial to pathogen transmission (Table 4). Their isolation and activities are described in this Supplement (see chapters by Brossard & Wikel and Valenzuela). Here we consider certain tick saliva molecules and immunomodulatory activities as SAT factor candidates for promoting tick-borne pathogen transmission.

Anti-haemostatic molecules

Tick anticoagulant and antiplatelet proteins and peptides seem unlikely candidates for SAT factors. However, a study of the acquisition of *Anaplasma phagocytophilum* by *Ixodes scapularis* nymphs feeding

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on infected mice identified a role for the formation of small haemorrhages within the feeding pool (Borjesson *et al*. 2002). Furthermore, the interface between coagulation and inflammation (involving serine protease receptors) suggests opportunities for a potential role (Cirino *et al*. 2000). Vasodilators are more obvious candidates but most vasodilatory activity of ticks has been attributed to prostaglandins, for which a role in pathogen transmission has not been identified. The nature of vasoactive compounds in the salivary glands of *Rhipicephalus appendiculatus* and *Dermacentor reticulatus* has not been identified (Rajská and Labuda, unpublished observations).

Anti-inflammatory molecules

Histamine-binding proteins. Histamine is a key mediator of inflammation and also affects immune functions. It is secreted by mast cells, basophils and (in some species) blood platelets. In mast cells, histamine is stored within granules and released following activation of the cells by crosslinking of IgE receptors. Apart from its role in the brain, this biogenic amine is believed to function as a primitive means of controlling parasites, hence its abundance in the gastrointestinal tract and in skin (Stebbings, 1974). Following extracellular release, histamine binds to its receptors, triggering a cascade of inflammatory and immune responses that result in swelling (oedema), redness (erythema) and irritation. The importance of histamine as a mediator in successful anti-tick responses, particularly in previously exposed hosts that have developed an immune (anamnestic) response, has been demonstrated in several host species, including cattle, rabbits and guinea pigs (Willadsen, Wood & Riding, 1979; Brossard, 1982; Wikel, 1982).

Histamine plays a role in allergies and other inflammatory diseases. Most drugs that control the action of histamine are receptor antagonists, acting on one of the histamine receptors. Ticks have adopted a different strategy to control the effects of histamine: they produce saliva molecules that bind histamine directly (Paesen *et al*. 1999, 2000). 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. Histacalins have been isolated from several ixodid tick species including *Rhipicephalus appendiculatus* and *Ixodes hexagonus*. However, they have not been detected in the important vector species, *I. ricinus* (although anti-histamine activity has been detected) and *O. moubata* (G. C. Paesen, personal communication). Homologues of the *R. appendiculatus* histacalins have been identified in the salivary glands of *I. scapularis* (see the chapters by Brossard & Wikel and Valenzuela in this Supplement) but it remains to be determined whether they bind histamine. In addition, a histamine release factor homologue is secreted by at least one ixodid tick species, suggesting ticks have a multifaceted control mechanism for histamine (Mulenga *et al*. 2003).

The apparent absence of histacalins from *I. ricinus* and *O. moubata*, if confirmed, suggests that they are not SAT factor candidates for tick-borne pathogens transmitted by these two important vector species. However, this does not preclude a role for histacalins in SAT or a role for other anti-histamine molecules. A site of inflammation is a hostile environment for invading pathogens. Anti-histamines are likely to benefit tick-borne pathogens even if they are not the key factor in facilitating their transmission. Histamine upregulates certain cytokines such as tumour necrosis factor alpha (TNFα), a suppressor of *B. burgdorferi* infections, and activates natural killer (NK) cells, which have antiviral activity. Through the H_4 receptor, histamine is involved in leukocyte trafficking, including chemotaxis of eosinophils and mast cells, and recruitment of neutrophils (Takeshita *et al*. 2003). In a mouse model of allergic asthma, a histacalin of *R. appendiculatus* largely abrogated this inflammatory disease (Couillin *et al*. 2004). If ticks can effectively manipulate the many functions of histamine, all tick-borne pathogens would probably benefit to some degree.

Complement inhibitors. The complement system is the principal effector arm of the humoral immune system in vertebrates, involved in inflammation and innate immunity. The two activation pathways (classical and alternative) comprise parallel cascades that converge on the C3 protein resulting in complement activation via the formation of similar catalytic C3 and C5 convertases (Law & Reid, 1995). Complement activation results in the release of the anaphylatoxins, C3a and C5a, by the C3 and C5 convertases, respectively. These acute inflammatory peptides induce damage by recruiting and stimulating granulocytes to release proinflammatory mediators, tissue degradative enzymes, and oxygen free radicals (Ember, Jagels & Hugli, 1998). The anaphylatoxins also increase adhesion molecule and inflammatory cytokine expression (Kohl, 2001). The terminal membrane attack complex of the complement system can disrupt pathogens or infected host cells and has proinflammatory activity. Additional effects of complement activation include opsonisation and phagocytosis of pathogens, clearance of immune cell complexes by recruitment of macrophages, and increased efficiency of antigen presentation to B cell receptors. Clearly, complement is as much a threat to tick-borne pathogens as it is to ticks.

Activation of host complement occurs when ticks feed, contributing to resistance to tick infestation (Wikel & Allen, 1977; Allen, Khalil & Graham,

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1979). Not surprisingly, ticks have evolved complement inhibitors. Saliva or SGE of several *Ixodes* spp. (*I. scapularis*, *I. ricinus*, *I. hexagonus* and *I. uriae*) inhibit the alternative complement pathway (Ribeiro & Spielman, 1986; Ribeiro, 1987; Astigarraga *et al.* 1997; Lawrie, Randolph & Nuttall, 1999) while *Ornithodoros* spp. target both the classical and alternative pathways (Astigarraga *et al.* 1997; Nunn *et al.* 2004). The complement inhibitor of *I. scapularis* is a 18·5 kD protein that appears to destabilise the active convertase, C3bBb (Valenzuela *et al.* 2000). Attempts to isolate an homologous protein from *I. ricinus* have been unsuccessful but activity was characterized as strongly inhibiting generation of the C3a anaphylatoxin (Lawrie, Sim & Nuttall, 2004). The complement inhibitor of *O. moubata* is a 17 kD lipocalin-like protein, which prevents production of the C5a but not the C3a anaphylatoxin (Nunn *et al.* 2004). Characterization of complement inhibitors from other tick species is needed to determine whether the different strategies (targeting the alternative pathway alone or inhibiting both alternative and classical pathways) is a feature of ixodid compared with argasid species, and its impact on pathogen transmission.

For *I. ricinus*, anti-complement activity is demonstrated by SGE from unfed as well as feeding ticks (Lawrie *et al.* 1999). This reflects the fact that activation of the alternative complement cascade is one of the first events following tick attachment, and a constant threat throughout tick feeding. However, the existence of significant anti-complement activity in unfed *I. ricinus* SGE indicates the complement inhibitor is not the SAT factor of TBEV, as SGE from unfed adult female *I. ricinus* does not demonstrate SAT activity (Labuda *et al.* 1993*b*). Nevertheless, complement inhibitors should be explored in relation to transmission of Lyme disease spirochaetes.

Complement sensitivity is a major influence in Lyme disease ecology and pathogenicity (Kurtenbach *et al.* 1998; van Dam, 2002; see also chapter by Piesman & Gern in this Supplement). When different members of the *Borrelia 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 survives in avian serum but is rapidly lysed by rodent serum. By contrast, *B. afzelii* survives rodent serum but is killed by avian serum (Kurtenbach *et al.* 1998). Similar observations have been made for *B. bissettii* (Ullmann *et al.* 2003). The borreliacidal effect is due to the activity of the alternative complement pathway, and specific mechanisms appear to mediate resistance (Kurtenbach *et al.* 1998; Hellwage *et al.* 2001; Pausa *et al.* 2003). Several *B. garinii* strains are sensitive to human serum (van Dam *et al.* 1997). Despite this sensitivity, *B. garinii* is able to infect humans and cause neuroborreliosis. This may well be a property of particular *B. garinii* strains. But 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?

If tick complement inhibitors promote transmission and aid survival of Lyme disease spirochaetes (and other tick-borne pathogens), another influential factor could be the host specificity of anti-complement activity (Lawrie *et al.* 1999). For example, the cosmopolitan *I. ricinus* demonstrates anti-complement activity against a range of mammalian (including human) and avian hosts. By contrast, the hedgehog tick, *I. hexagonus*, has high levels of activity against hedgehog serum, but no anti-complement activity against serum from avian species. However, complement inhibitors cannot be the only SAT factor for *B. burgdorferi* sl as *I. ricinus* (adult females) SGE can inhibit red deer (*Cervus scoticus*) serum complement and yet deer (including red deer) are not hosts for the Lyme disease spirochaete although they are important hosts for the principal *Ixodes* vector species (reviewed by Piesman & Gern in this Supplement).

Immunomodulators

Immunoglobulin-binding proteins. When ticks feed, a small proportion of host plasma proteins escape digestion and pass through the gut wall, into the haemocoel. These host proteins include immunoglobulin G (IgG) molecules, some of which may be pathogen-specific antibodies (Fujisaki, Kamio & Kitaoka, 1984; Jasinskas, Jaworski & Barbour, 2000). The fate of host immunoglobulins that enter the tick haemocoel was unknown until the discovery that adult female *R. appendiculatus* excrete intact IgG in their saliva (Wang & Nuttall, 1994). Further investigations revealed a family of immunoglobulin G-binding proteins (IGBPs) in the haemolymph and salivary glands of adult *R. appendiculatus*, and proteins with similar activity in other ixodid species, including *I. ricinus* and *Amblyomma variegatum*, and a sequence homologue in *I. scapularis* (Wang & Nuttall, 1999; Packila & Guilfoile, 2002). Although IGBPs have not been reported for argasid tick species, circumstantial evidence suggests they may occur (Minoura, Chinzei & Kitamura, 1985).

The prevalence and abundance of IGBPs indicate that they play an important role in blood feeding. Possibly they provide a Tick Immunoglobulin Excretion System (TIES) that enables ticks to ferry potentially damaging antibodies safely through the haemocoel to their salivary glands from where they are excreted (Wang & Nuttall, 1999). If such a TIES exists, it may benefit tick-borne pathogens by

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protecting them (and possibly the infected tick) from pathogen-specific antibodies taken up in the blood-meal. An indication of such protection was obtained in studies of Thogoto virus infection of *R. appendiculatus* ticks in the presence of antibody to Thogoto virus (Jones & Nuttall, 1989).

The difficulty with the TIES hypothesis is that it does not explain why ticks have such an elaborate system for transporting biologically active immunoglobulins through their body; a simpler solution would be to ensure that all immunoglobulins in the blood-meal 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). Whether such an effect aids in the transmission or acquisition of tick-borne pathogens by feeding ticks remains to be explored.

Cytokine inhibitors. Cytokines are the chemical mediators of inflammation and immunity. The type 1 interferons are a cytokine superfamily comprising four subfamilies of which IFNα and IFNβ are induced by viral infections. Most cell types produce them. A common inducer is dsRNA, a product of viral replication. Infected cells synthesise and release IFN, which then binds to IFN receptors of neighbouring cells, triggering more that 50 genes via the JAK/STAT signalling pathway (Stark *et al.* 1998). Several factors are activated (including dsRNA-dependent protein kinase R, the 2′–5′ oligoadenylate synthestase, and the Mx proteins) that create an antiviral state in which inhibition occurs at different stages of viral replication and assembly (Goodbourn, Didcock & Randall, 2000).

Arboviruses are not generally recognised as strong inducers of IFNα/β and there have been few (if any) studies of IFN induction following vector-borne virus transmission. A notable exception as a strong IFN inducer is vesicular stomatitis virus, an insect-borne rhabdovirus. This virus was used to study the effect of tick SGE on viral replication *in vitro*, although it is not tick-borne. Cultures of mouse L cells were treated with SGE from partly fed adult *R. appendiculatus* or *D. reticulatus* and then infected with low doses of vesicular stomatitis virus (Hajnicka *et al.* 1998). Viral yield was increased by 100- to 1000-fold 16–23 h post-infection compared with untreated cultures, and corresponded with the earlier appearance of vesicular stomatitis virus nucleocapsid protein. This was the first published evidence that tick SGE can promote virus replication *in vitro*. A follow-up study showed that the effect was likely to result from inhibition of the antiviral effect of IFN by salivary gland products (Hajnicka *et al.* 2000). The suppressive effect was most apparent when cells were treated with SGE prior to virus infection, suggesting that the effect may be directed against the IFNα/β receptor rather than a direct interaction with IFN.

IFNα/β-induced viral resistance is mediated by antiviral factors such as Mx gene products that are active against viruses of several different families including the Orthomyxoviridae, Bunyaviridae and Togaviridae. The interferon-induced mouse Mx1 protein has intrinsic antiviral activity against influenza A and B viruses, and the tick-borne orthomyxovirus, Thogoto virus (Haller *et al.* 1995). Mice carrying the *Mx1* gene are resistant to Thogoto virus infection by needle injection. However, they are susceptible to tick-borne virus challenge (non-viraemic transmission from infected to uninfected co-feeding ticks) and, to a lesser degree, injection of virus mixed with tick SGE (Dessens & Nuttall, 1998). These data are consistent with the ability of tick SGE to interfere with the antiviral action of IFNα/β. Cells exposed to tick saliva in the skin of $Mx1^+$ mice may not respond to released interferon (through the putative effect of the saliva on the IFNα/β receptor) thus preventing induction of the Mx1 protein (a large nuclear GTPase) and the resulting antiviral state. The biological significance of these observations is unknown, particularly as Thogoto virus produces a potent IFNα/β antagonist of its own (Hagmaier *et al.* 2003). However, it is curious that *Mx1*-based resistance is found in approximately 75% of wild mice and yet no murine influenza viruses have been recorded, begging the question why mice should have a potent defence mechanism against influenza A and B viruses (Haller, Acklin & Staehli, 1987; Haller *et al.* 1995). Inhibition of IFNα/β appears to be a good candidate for the SAT factor of Thogoto virus. However, studies to date have been confined to *D. reticulatus* and need to be extended to the vector of Thogoto virus, *R. appendiculatus*.

In addition to the action on IFN (described above), several studies have reported effects of tick feeding or tick salivary gland products on cytokine expression or activities (see chapter by Brossard & Wikel in this Supplement). Many of the effects on cytokine activities appear to be due to a wealth of cytokine binding molecules produced by ixodid tick species and secreted in their saliva (Gillespie *et al.* 2001; Hajnická *et al.* 2001 and unpublished data). Cytokine binders react with IL-2, IL-4, IL-8, MCP-1, MIP-1α, RANTES and eotaxin, and probably more. The IL-2 binding protein of *I. scapularis* provides a mechanism for suppressing T cell proliferation and other IL-2-stimulated immune responses (Gillespie *et al.* 2001). The IL-8 binder of *D. reticulatus* out-competes IL-8 receptors on neutrophils, inhibiting IL-8-induced chemotaxis of neutrophils (Hajnická *et al.* 2001). Biological

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activities of other putative cytokine binders have not yet been investigated. Nevertheless, the apparent strategy of manipulating the cytokine network most likely overcomes redundancy in this innate immune system, and should greatly facilitate blood feeding.

By binding different cytokines, ixodid ticks provide a potential gateway for tick-borne pathogens. To what extent this gateway is exploited remains to be determined. The only indication to date is that inoculation of C3H/HeJ mice with a mixture of TNF-α, IFN-γ and IL-2 at the time of tick feeding suppressed *B. burgdorferi* transmission by *I. scapularis*, suggesting that cytokine manipulation by ticks might aid borrelia transmission (Zeidner *et al*. 1996). Clearly, the extent to which ticks manipulate the cytokine network, and the consequences for tick-borne pathogen transmission, need to be explored.

Leukocyte modulators. Numerous effects of tick saliva or SGE on T cell and macrophage function have been described *in vitro* (see chapter by Wikel & Brossard in this Supplement). Many of them are mediated through the effects on cytokines. The potential benefits to tick-borne pathogens are obvious but the real benefits of leukocyte modulators have not been elucidated. For example, neutrophils phagocytize *B. burgdorferi* hence the ability of their tick vector's saliva to inhibit neutrophil function could be highly significant (Ribeiro, Weis & Telford, 1990; Suhonen, Hartiala & Viljanen, 1998). Langerhans cells are thought to play a key role in non-viraemic transmission of TBEV (see 'The ''red herring'' hypothesis' in Nuttall & Labuda, 2003). Obviously the effect of SGE on Langerhans cells needs to be examined in the search for the SAT factor of TBEV. The function of the homologue of Macrophage Migration Inhibitory Factor (MIF, a mammalian pro-inflammatory cytokine) is unknown. It has been detected in both salivary glands and midgut tissues of unfed and feeding *A. americanum* adult females and, *in vitro*, inhibited macrophage migration (Jaworski *et al*. 2001). Potentially it may reduce macrophage microbicidal activity.

Tick infestation is characterized by polarization towards a Th2 response. This appears to benefit tick-borne pathogens such as *B. burgdorferi* and *Babesia bovis* (Zeidner *et al*. 1996, 1997; Christe, Rutti & Brossard, 2000; Goff *et al*. 2003). Indirect evidence of the potential benefit for TBEV was demonstrated by the reduction of co-feeding transmission in laboratory mice in which a Th1-like response was induced by immunization with a recombinant tick salivary gland protein (Labuda and Lickova, unpublished data). Mice repeatedly infested with *I. scapularis* nymphs, and showing a Th2-polarized response, became resistant to *B. burgdorferi* transmission indicating that the benefits of such polarization may be overcome by the host (Wikel *et al*. 1997).

Direct immunosuppression of B cells by *I. ricinus* SGE (Hannier *et al*. 2003) may explain the reduced ability of tick-infested hosts to produce antibodies (Wikel, 1985; Christe *et al*. 2000). Such immunosuppression is also likely to affect B cell production of immune regulatory cytokines such as IFNγ. B cells play a crucial role in antimicrobial immunity (Ochsenbein *et al*. 1999; Baumgarth, 2000). Tick-borne pathogens such as *B. burgdorferi* express surface B cell mitogens (Ma & Weis, 1993). Obviously, secretion of a B cell inhibitor in tick saliva, through inhibiting T cell-dependent and T cell-independent B cell activation, could provide a safe haven in the skin feeding site for tick-borne pathogens. As yet, the B cell inhibitor has not been characterized.

The role of natural killer (NK) cells includes control of viral infections through the killing of cells that express viral antigens on their surface. They may even kill extracellular bacteria. NK cells secrete cytokines, providing an important means by which innate immunity communicates with the acquired immune system (Lanier, 2000). Direct evidence of a role for NK cells during tick feeding is lacking, nevertheless tick salivary glands contain a protein that suppresses NK cell activity (Kubeš *et al*. 1994). Such activity was demonstrated with SGE from partially fed *Dermacentor reticulatus*, *Amblyomma variegatum* and *Haemaphysalis inermis*, but not from *Ixodes ricinus* or *Rhipicephalus appendiculatus* (Kubeš *et al*. 2002). The apparent absence of activity for the latter two important vectors suggests that control of NK cells does not play an important role in promoting tick-borne pathogen transmission, at least for these two tick species. However, a suppressive effect on NK cell activity was observed in a mouse model with SGE from partially fed female *I. ricinus* (Kopecky & Kuthejlova, 1998). Interactions between dendritic cells and NK cells, during the early stages of *B. burgdorferi* infection, influence development of a protective humoral response in mice (Mbow *et al*. 2001). Suppression of NK cell activity might thus affect *B. burgdorferi*, although possibly at a later stage than SAT.

CONCLUSIONS

The hunt for tick saliva molecules that promote pathogen transmission (the SAT factors) goes on. Most probably we are searching for a suite of molecules that act cooperatively. For ticks, cooperative salivary activity is the only way they can overcome redundancy in host protective systems to a degree that allows them to complete their luxurious meal. Cooperation balances the need, for example, to control blood flow and cell recruitment so that ticks have plenty to feed on but they do not have excessive host factors to fight against. Tick-borne pathogens apparently have evolved to exploit a combination of immunomodulatory activities in order to establish

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a crucial toehold in the site of tick feeding. Anti-tick vaccines may have to neutralize the suite of activities to be effective in blocking tick-borne pathogen transmission.

One specific difficulty in the quest for SAT factors is that, in most cases, SGE or saliva is taken from adult females. However, for many tick-borne pathogens, epidemiological data implicate nymphs as the key transmitters of these pathogens, and larvae as the acquirers of infections. The epidemiological role of adult females is to lay eggs and produce more acquirers (larvae) and transmitters (nymphs) of tick-borne pathogens. Thus more studies are needed on immature stages, to determine how their pharmacological prowess compares with that of their mothers. Isolation of candidate SAT factors, and testing their effects (as natural or synthetic chemicals, or recombinant proteins) on pathogen transmission (singly or as cocktails of different molecules), should help identify the key ingredients in the tick pharmacopoeia that promote pathogen transmission. The challenge then will be to determine how this information can be used to control ticks and tick-borne diseases.

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