Displaced tick–parasite interactions at the host interface

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SUMMARY

Reciprocal interactions of parasites transmitted by blood-sucking arthropod vectors have been studied primarily at the parasite–host and parasite–vector interface. The third component of this parasite triangle, the vector–host interface, has been largely ignored. Now there is growing realization that reciprocal interactions between arthropod vectors and their vertebrate hosts play a pivotal role in the survival of arthropod-borne viruses, bacteria, and protozoa. The vector–host interface is the site where the haematophagous arthropod feeds. To obtain a blood meal, the vector must overcome the host's inflammatory, haemostatic, and immune responses. This problem is greatest for ixodid ticks which may imbibe as much as 15 ml blood whilst continuously attached to their host for 10 days or more. To feed successfully, the interface between tick and host becomes a battle between the host's mechanisms for combating the tick and the tick's armoury of bioactive proteins and other chemicals which it secretes, via saliva, into the feeding lesion formed in the host's skin. Parasites entering this battlefield encounter a privileged site in their vertebrate host that has been profoundly modified by the pharmacological activities of their vector's saliva. For example, ticks suppress natural killer cells and interferons, both of which have potent antiviral activities. Not surprisingly, vector-borne parasites exploit the immunomodulated feeding site to promote their transmission and infection. Certain tick-borne viruses are so successful at this that they are transmitted from one infected tick, through the vertebrate host to a co-feeding uninfected tick, without a detectable viraemia (virus circulating in the host's blood), and with no untoward effect on the host. When such viruses do have an adverse effect on the host, they may impede their vectors' feeding. Thus important interactions between ticks and tick-borne parasites are displaced to the interface with their vertebrate host – the skin site of blood-feeding and infection.

Key words: arthropod vectors, blood feeding, ticks, immunomodulation.

THE VECTOR-BORNE PARASITE TRIANGLE

Many parasites that infect vertebrates rely on blood-sucking arthropods (e.g. mosquitoes and ticks) for their transmission. Often such parasites replicate in the arthropod vector as they pass from the bloodmeal in the gut, through the arthropod's body, to the salivary glands. The parasite is then transmitted to the vertebrate host via the arthropod's saliva which is secreted into the feeding lesion as the vector takes a bloodmeal.

The interactions between parasite, arthropod vector, and vertebrate host may be usefully considered as a parasite triangle (Fig. 1). In this, three parasitic interactions exist: (i) infection of the vertebrate host by the parasite (which may be a pathogen); (ii) infection of the arthropod by the parasite; and (iii) blood-feeding of the arthropod vector (an ectoparasite) on the vertebrate host. Reciprocal interactions of parasites transmitted by haematophagous arthropod vectors have been studied primarily at the parasite–host (i) and parasite–vector interface (ii). The third component of this parasite triangle, the vector–host interface (iii), has been largely ignored. Now there is a growing realization that reciprocal interactions between arthropod vectors and their vertebrate hosts play a pivotal role in the survival of arthropod-borne viruses (arboviruses), bacteria, and protozoa.

RECIPROCAL VECTOR–HOST INTERACTIONS

In order to feed, haematophagous arthropods must overcome their hosts' inflammatory, haemostatic and immune responses. The problem is greatest for ixodid ticks which may process as much as 4000 mg of host blood whilst continuously attached to their host for 10 days or more (Kaufman, 1989). Many features of tick feeding behaviour account for their effectiveness as parasite vectors. Indeed, ticks are second only to mosquitoes in their medical importance as disease vectors, and ticks transmit the greatest variety of pathogens of any blood-sucking arthropod.

Ticks are 'pool feeders', sawing and tearing their way into the dermis, and sucking the fluids that are exuded into the resulting wound through the food canal of their complex mouthparts (Sonenshine, 1991). Generally, they require a bloodmeal to develop from one stage to the next (from larva, to nymph, and then to adult), and to produce eggs. Ixodid ticks may feed for a few days or up to 2 weeks, cementing their hypostome into the skin to act as a holdfast; argasid ticks feed more quickly (except for larvae), and take smaller but a greater number of bloodmeals.

The tick-inflicted injuries trigger several repair reactions in the host, including blood clotting, platelet aggregation and blood vessel contraction

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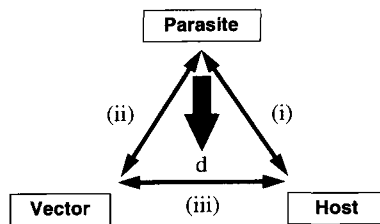

Fig. 1. Representation of the vector-borne parasite triangle. Interactions indicated by double-headed arrows at the interface of (i) pathogen (endoparasite)–host, (ii) pathogen–vector, and (iii) vector (ectoparasite)–host. Single arrow to d, displaced interactions of the pathogen with its vector.

(the three components of haemostasis). Such host reactions are exacerbated if immunity develops to tick salivary antigens (Rechav, 1992; Wikel, 1996). Ticks respond by secreting numerous pharmacologically-active substances in their saliva to facilitate feeding by antagonizing or modifying the host's haemostatic response (Table 1). They include anticoagulants, platelet aggregation inhibitors and vasodilators (prostaglandins) that are secreted in tick saliva. Host immune mechanisms may reduce feeding success of ticks by enhancing inflammatory reactions. To counter the host response, saliva of some ticks also has anti-inflammatory, immunosuppressive and anti-complement properties (Table 1).

One novel family of proteins isolated from ticks, that appear to modulate the host response, are the immunoglobulin-binding proteins (IGBPs). For some time it has been known that a small fraction of the immunoglobulins taken up by ticks in their bloodmeal passes through the tick midgut into the haemocoel, retaining biological activity (Ben-Yakir *et al.* 1986). This observation has been exploited in the development of tick vaccines (Sauer, McSwain & Essenberg, 1994). Recently, it has been shown that immunoglobulins which pass into the haemocoel are subsequently excreted in the tick's saliva during feeding (Wang & Nuttall, 1994). Equally surprising was the demonstration of IGBPs in tick haemolymph and salivary glands. To date, IGBPs have been found in all ixodid tick species examined (Wang & Nuttall, 1995). The presence of IGBPs in both tick haemolymph and salivary glands may provide the mechanism by which ticks excrete, via their saliva, those host IgGs that evade digestion and enter the haemolymph. Such a mechanism for clearing potentially harmful antibodies helps to explain how Thogoto virus-infected ticks survived after feeding on a bloodmeal containing specific anti-Thogoto virus antibodies (Jones & Nuttall, 1989*a*). If ticks can handle host immunoglobulins in such a way that they 'neutralize' the ability of antibodies to bind to their specific epitopes – whether they belong to a parasite infecting the tick, or to the tick *per se* – then such a strategy would have obvious benefits to both vector and vector-borne parasite. However, IGBPs may have an even more intriguing role in tick feeding and pathogen transmission. When guinea pigs were immunized against IGBP-MC, a male specific salivary gland protein, or when feeding males were inoculated *in situ* with antibodies to IGBP-MC, the feeding of the male's mated female was impaired (Wang *et al.* 1997). Hence male *Rhipicephalus appendiculatus* appear to 'mate guard', protecting the mated female against the host, possibly through local immunosuppression mediated by IGBPs.

Two immunomodulatory activities of tick saliva have obvious potential benefits for tick-borne viruses. Both natural killer (NK) cells and interferons provide the innate mammalian defences against viral infection. Extracts of the salivary glands of feeding ticks (*Dermacentor reticulatus*, *R. appendiculatus* and *Ixodes ricinus*) suppressed the NK cell activity of effector cells from human blood, and the anti-viral action of mouse interferon α/β, measured *in vitro* (Kubes *et al.* 1994; Hajnicka *et al.* 1997 and unpublished data). The components of tick saliva responsible for these immunomodulatory activities, and their role in facilitating tick blood-feeding, are undetermined. However, NK cells are a major source of type 2 interferon (interferon γ), providing an important step in the cytokine cascade that directs a type 1 T helper (TH1) cell response. By suppressing NK cell activity, ticks may modulate the TH1 response to enhance their feeding performance and, incidentally, they may enhance tick-borne parasite transmission (Wikel & Bergman, 1997).

The numerous biological activities associated with tick saliva, which change as feeding progresses, demonstrate that the feeding process of ticks is a dynamic reciprocal interaction between the host's defences and the tick's counterattack. As a result, any parasite transmitted by the feeding tick enters a skin site that is profoundly influenced by the outcome of these vector–host interactions.

CO-FEEDING PARASITE TRANSMISSION

The first hint that tick-borne transmission of a parasite involved more than simply a needle and syringe inoculation came from studies with Thogoto virus. Transmission experiments using *R. appendiculatus*, the African brown ear tick, and hamsters demonstrated that Thogoto virus fulfils the criteria for an arbovirus (Davies, Jones & Nuttall, 1986). Hamsters are highly susceptible to Thogoto virus, developing high levels of viraemia reaching 10^8 plaque-forming units (PFU) per ml blood after syringe-inoculation with comparatively low viral doses; death occurs 5–6 days post-infection. By contrast, guinea pigs inoculated with the virus show no clinical signs, and typically no viraemia. Nevertheless, when Thogoto virus-infected and uninfected ticks were allowed to feed together on guinea pigs,

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Table 1. Bioactive peptides/proteins secreted in tick saliva

Constituent	Properties	Tick species	Reference
Anti-complement factor	49 kDa; inhibitor of the alternative complement pathway	*Ixodes dammini**	(Ribeiro, 1987)
Tick anticoagulant peptide (TAP)	7 kDa; inhibitor of blood coagulation factor Xa	*Ornithodoros moubata*	(Waxman *et al.* 1990)
Anticoagulant	Thrombin inhibitor	*Ixodes holocyclus*	(Anastopoulos, Thurn & Broady, 1991)
Anticoagulant	65 kDa; anticoagulant	*Rhipicephalus appendiculatus*	(Limo *et al.* 1991)
Moubatin	17 kDa; inhibits platelet aggregation response to collagen	*Ornithodoros moubata*	(Waxman & Connolly, 1993)
Disagregin	7 kDa; platelet aggregation inhibitor	*Ornithodoros moubata*	(Karczewski, Endris & Connolly, 1994)
Calreticulin	58 kDa; function unknown	*Amblyomma americanum*	(Jaworski *et al.* 1995)
Tick adhesion inhibitor (TAI)	15 kDa; blocks adhesion to collagen	*Ornithodoros moubata*	(Karczewski *et al.* 1995)
Immunoglobulin binding protein (IGBP-MC)	21 kDa; binds IgG	*Rhipicephalus appendiculatus*	(Wang *et al.* 1997)
Histamine-binding proteins	21–24 kDa; bind histamine	*Rhipicephalus appendiculatus*	(Paesen, unpublished)

* Species designation cited in manuscript.

Table 2. Co-feeding transmission of TBE virus on natural hosts

Host		Ticks			
Species*	No. of animals	No. added	Yield	Yield infected	% infected
Field mouse	8	320	217	147	46
Bank vole	8	320	130	36	11
Pine vole	6	240	17	12	5
Hedgehog	2	80	48	2	3
Pheasant	5	300	97	0	0

* Field mouse, *Apodemus flavicollis* (6 animals) and *A. agrarius* (2 animals); bank vole, *Clethrionomys glareolus*; pine vole, *Pitymys subterraneus*; hedgehog, *Erinaceus europaeus*; pheasant, *Phasianus colichicus*.

most of the uninfected ticks became infected even though they were physically separated from the infected ticks, and the guinea pigs showed no detectable viraemia. Indeed, more uninfected nymphal ticks became infected by co-feeding with infected adult ticks on non-viraemic guinea pigs than by co-feeding on highly viraemic hamsters (Jones *et al.* 1987). The minimum overlap in the co-feeding period was at least 3 days; when fed together for a shorter duration, the numbers of nymphs that became infected were reduced (Jones & Nuttall, 1989*b*). Transmission between co-feeding ticks was inhibited when the guinea pig hosts were previously immunized against Thogoto virus (Jones & Nuttall, 1989*a*), and was reduced when the animals were immune to tick infestation (Jones & Nuttall, 1990). Although these results with Thogoto virus challenged the emphasis placed on viraemia in arbovirus transmission, they were obtained with an atypical arbovirus and an artificial laboratory model. However, the generality of non-viraemic transmission has now been tested with other tick-borne viruses and with natural hosts.

Tick-borne encephalitis (TBE) virus is the most medically important arbovirus in Europe (Gresikova & Calisher, 1988). Initial studies reported non-viraemic transmission between infected and uninfected ticks feeding together on non-viraemic guinea pigs (Alekseev & Chunikhin, 1990; Alekseev *et al.* 1991; Labuda *et al.* 1993*a*). In nature, the principal European vector of TBE virus, *Ixodes ricinus* (the wood or sheep tick), feeds on a wide range of species: immature stages commonly infest small mammals, birds and medium-sized mammals such as squirrels and hares, whilst adults prefer larger mammals, e.g. deer, sheep, goats. To determine whether non-

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viraemic transmission occurs with natural host species, field mice (*Apodemus flavicollis* and *A. agrarius*), bank voles (*Clethrionomys glareolus*), pine voles (*Pitymys subterraneous*), hedgehogs (*Erinaceus europaeus*) and pheasants (*Phasianus colchicus*) were captured in the wild. Individual animals that had no evidence of prior TBE virus infection (i.e. no detectable neutralizing antibodies to the virus) were infected with *I. ricinus* ticks retained in two neoprene chambers attached to the back of the animals. In chamber 1 were placed two infected adult female ticks, 2 uninfected males, and 20 uninfected nymphs; chamber 2 contained 20 uninfected nymphs only. After a feeding period of 4 days, the animals were killed and ticks and selected host tissues assayed for virus. Hedgehogs and pheasants were comparatively resistant to infection and TBE virus transmission. In contrast, pine voles were highly susceptible to infection: three of the 6 pine voles died before the ticks completed engorgement and the remaining individuals showed comparatively high levels of virus in their spleens, lymph nodes, brain and blood. Although most of the engorged nymphs from pine voles were infected, only a few of them fed successfully and hence the net yield of infected ticks was low. In striking contrast, field mice showed comparatively low levels of virus infection but produced the greatest yield of infected ticks (Table 2). Remarkably, 3/6 *A. flavicollis* field mice had no detectable viraemia and yet 58% (47/81) of ticks that fed on these individuals became infected.

The results of co-feeding TBE virus-infected and uninfected ticks on wild vertebrate hosts point strongly towards non-viraemic transmission occurring in nature. Transmission of Crimean–Congo haemorrhagic fever virus and louping ill virus between infected and uninfected ticks co-feeding on natural non-viraemic hosts adds weight to this claim (Zeller, Cornet & Camicas, 1994; Jones *et al.* 1997). Moreover, comparative estimates of the basic reproductive rate (R_0) indicate that non-viraemic transmission between co-feeding ticks, rather than classical viraemic transmission, is the main mechanism by which TBE virus survives in its natural ecosystem (Randolph, Gern & Nuttall, 1996). This conclusion was reinforced by evidence that TBE virus was transmitted between infected and uninfected ticks co-feeding on natural hosts immune to the virus, although transmission efficiency was reduced (Labuda *et al.* 1997). Thus the natural host population that is immune to TBE virus is capable of repeatedly supporting virus transmission and thereby contributing significantly to the population of infected ticks.

The phenomenon of non-viraemic transmission has parallels in the transmission of other tick-borne parasites. *Rickettsia* (formerly *Cowdria*) *ruminantium*, the cause of heartwater in cattle, was transmitted from infected to uninfected ticks co-feeding on tortoises (Bezuidenhout, 1987). Similarly, *Borrelia burgdorferi*, the bacterial agent of Lyme disease, was transmitted to uninfected ticks co-feeding spatially with infected ticks in the absence of a systemic infection. Such non-systemic transmission was demonstrated initially using laboratory mice (Gern & Rais, 1996) and subsequently with sheep which are natural hosts of *B. burgdorferi* in parts of the UK (Ogden, Nuttall & Randolph, 1997).

SALIVA-ACTIVATED TRANSMISSION (SAT)

Results described above clearly demonstrate the different picture generated by experiments in which attempts are made to mimic natural conditions of tick-borne virus transmission (i.e. infected and uninfected ticks feeding together on the same individual host), compared with the more conventional method of infecting animals by needle and syringe inoculation. Such differences are particularly evident in the numbers of ticks that become infected. For example, when *R. appendiculatus* nymphs fed on non-viraemic guinea pigs infested with Thogoto-virus infected adult ticks, 14-times more nymphs became infected than when the nymphs fed on guinea pigs syringe-inoculated with Thogoto virus.

The mechanism underlying non-viraemic transmission was unknown until the report that *Leishmania* infectivity was enhanced by salivary gland extracts from sandflies (Titus & Ribeiro, 1988). Based on this observation, syringe inoculation experiments were undertaken in which Thogoto virus was mixed with an extract prepared from the salivary glands of uninfected feeding ticks. Surprisingly, 10-times more nymphs became infected compared to the numbers of nymphs infected by feeding on guinea pigs inoculated with virus alone (Table 3). As with non-viraemic virus transmission between co-feeding infected and uninfected ticks, none of the inoculated guinea pigs showed a detectable viraemia (Jones, Hodgson & Nuttall, 1989). The enhancement of virus transmission was only observed when the virus inoculum was mixed with extracts from salivary glands of feeding ticks, and was not observed with salivary glands from unfed ticks, or with extracts of any other tick organ. This was the first evidence that non-viraemic transmission of a tick-borne virus involves a component(s) of tick salivary glands.

Physico-chemical analysis indicated that the factor in salivary gland extract that enhanced Thogoto virus transmission was one or more proteins or peptides (Jones *et al.* 1989; Jones, Hodgson & Nuttall, 1990). When the virus was mixed with the salivary gland extract and then assayed in cell culture and mice, viral infectivity was unchanged, suggesting that the enhancing factor was neither a proteolytic enzyme nor some other component that acted directly on the virus.

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Table 3. Comparison of Thogoto virus transmission

Mode of transmission*	% recipient ticks infected (no. animals)
Syringe inoculation: virus	6 (6)
Syringe inoculation: virus + SGE	58 (7)
Co-feeding with infected ticks	85 (11)

* Uninfected guinea pigs infested with uninfected (recipient) *Rhipicephalus appendiculatus* nymphs. The animals were either inoculated with virus ± uninfected *R. appendiculatus* salivary gland extract (SGE) or co-infested with infected ticks.

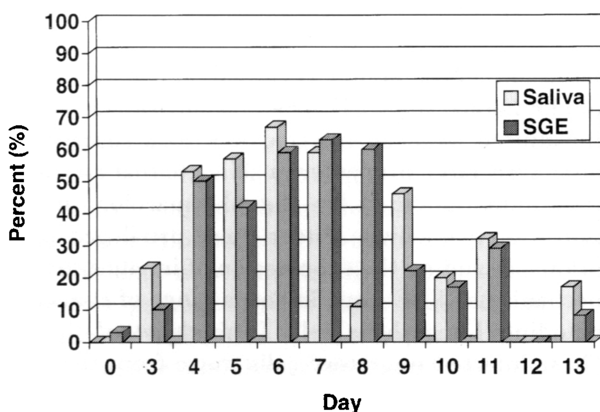

Fig. 2. Comparison of saliva activated transmission (SAT) induced by saliva or salivary gland extract [adapted from (Jones *et al.* 1992*b*)]. Mean percentages of nymphs that became infected while feeding on guinea pigs inoculated with saliva or extracts of the respective salivary glands of uninfected ticks that had been feeding for the indicated number of days.

The enhancing effect was only observed when salivary gland extract and Thogoto virus were inoculated into the same skin site on the guinea pig; when inoculated at different sites, the numbers of nymphs that became infected fell to levels observed when guinea pigs were inoculated with virus alone (Jones *et al.* 1989). In addition to being highly localized, the enhancing effect was comparatively long lasting. Thus, when Thogoto virus was inoculated into the skin of tick-infested guinea pigs, and then salivary gland extract was inoculated in the same site either at the same time, or 1, 2 or 3 days later, the number of nymphs infected was increased compared with the infected tick numbers obtained from tick-infested animals inoculated with virus alone (Jones, Kaufman & Nuttall, 1992*b*). Similar results were obtained in the converse experiment, in which Thogoto virus was inoculated after the salivary gland extract, although the duration of enhancement appeared to be shorter.

Most tick-borne pathogens, including viruses, are transmitted to a vertebrate host in the saliva secreted by the feeding tick vector (Kaufman & Nuttall, 1996). Because the enhancing factor in the salivary glands of feeding ticks appears to act within the skin of the vertebrate host, rather than directly on the virus, this factor is probably secreted in tick saliva. To test this hypothesis, saliva was collected from uninfected adult female *R. appendiculatus* removed from uninfected guinea pigs at different days of feeding. After collecting saliva, each tick was dissected and the uninfected salivary glands removed and prepared as a salivary gland extract. Each saliva and equivalent salivary gland extract was mixed separately with Thogoto virus and inoculated into different tick-infested guinea pigs. The enhancing activity of saliva and salivary gland extract showed similar dynamics (Fig. 2). There was a gradual increase in the number of recipient ticks that became infected, reaching a maximum with saliva or salivary gland extracts collected from uninfected ticks that had fed for 6 days, and then followed by a decline (Jones *et al.* 1992*b*). The one discrepancy was at day 8 of feeding when saliva showed a reproducible drop in enhancing activity, the reason for which is unknown. Nevertheless, the obvious similarity in activity profiles strongly suggests that the virus transmission enhancing factor is synthesized in the salivary glands during tick feeding and secreted into the skin feeding lesion in tick saliva. The phenomenon was named saliva-activated transmission (SAT) (Nuttall & Jones, 1991).

The SAT factor is produced by uninfected feeding ticks; furthermore, enhancement of virus transmission has no obvious direct benefits for the tick. These two observations indicate that the SAT factor is independent of virus infection. Most likely, the function of the SAT factor is to modulate the skin site of tick attachment and thereby facilitate feeding. Tick-borne viruses that demonstrate SAT appear to have co-evolved with their vectors and vertebrate hosts to exploit the unique environment of the vector–host interface.

In addition to Thogoto virus, SAT has been demonstrated experimentally with TBE virus (Alekseev *et al.* 1991; Labuda *et al.* 1993*b*) and Dhori virus (L. D. Jones, unpublished data). Interestingly, SAT has only been demonstrated with arthropod species that are competent vectors of a particular tick-borne virus. For example, salivary gland extracts of *I. ricinus* ticks do not show SAT with Thogoto virus (for which *I. ricinus* is not a competent vector) but SAT occurs with TBE virus for which *I. ricinus* is the principle European vector species (Jones *et al.* 1992a; Labuda *et al.* 1993*b*). This implies that the mechanism underlying SAT differs for different vector–virus associations. Enhancement of *Leishmania* spp. transmission by salivary gland extracts of phlebotomine sandfly vectors suggests that SAT is not confined to ticks and arboviruses (Titus & Ribeiro, 1988; Samuelson *et al.* 1991).

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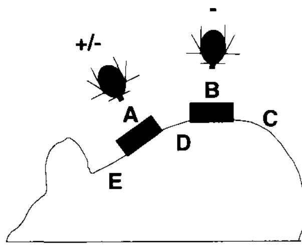

Fig. 3. Experimental design to investigate the importance of skin in co-feeding tick transmission of TBE virus (Labuda *et al.* 1996, 1997). Skin site A: infected adult and uninfected nymphal ticks co-feeding within retaining chamber A. Skin site B: uninfected nymphal ticks feeding in chamber B. Skin sites C, D and E: uninfested and untreated sites differing in their proximity to the infected ticks.

ROLE OF SKIN IN VIRUS TRANSMISSION

Early events during arbovirus infections of mammalian hosts are poorly understood. Most studies have involved needle and syringe inoculation with relatively high doses of the virus, often by unnatural routes (e.g. intracerebral inoculation of day-old mice), and with highly susceptible laboratory animals. For arboviruses, the general picture to emerge from such studies involves viral replication at the inoculation site and in draining lymph nodes, producing a transient viraemia. Newly synthesized virus then spreads to other tissues where replication occurs producing a secondary viraemia (Monath & Heinz, 1996).

Marked differences in the course of infection following syringe inoculation compared with tick-borne virus transmission were highlighted in studies with TBE virus (Labuda *et al.* 1996). Wood mice (*A. sylvaticus*), bank voles and laboratory strains of mice inoculated intradermally with high doses of TBE virus had a detectable viraemia and infection at the skin site of inoculation, 24 h post-inoculation (p.i.), which were cleared by 72 h p.i. A different picture emerged when wild and laboratory rodents were exposed to co-feeding *I. ricinus* ticks. Viraemia took longer to develop and the localized skin infection persisted. In these experiments, infected adult and uninfected nymphal ticks were placed in one retaining chamber (at skin site A), and uninfected nymphs were placed in a second chamber (skin site B) on each animal (Fig. 3). Virus transmission from infected to uninfected ticks co-feeding on natural hosts was correlated with infection of the localized skin site of tick feeding and not with development of a detectable viraemia. Moreover, viraemia did not result in a generalized skin infection. Rather, virus was recruited preferentially to the skin site of tick feeding (site B) and generally was not detected in uninfested skin sites (sites C and D, Fig. 3).

To examine events within the skin at the cellular level, mice were exposed to co-feeding TBE virus-infected and uninfected *I. ricinus* ticks (Labuda *et al.* 1996). Skin explants were removed from sites of tick feeding (sites A and B) and from uninfested skin sites (sites E and C; Fig. 3) and incubated floating on culture medium. Numerous leucocytes were observed to migrate from the skin explants of tick feeding sites. TBE viral antigen was detected in both migratory Langerhans cells and neutrophils; in addition, the migratory monocytes/macrophages produced infectious virus. These results illustrate the important role that the skin site of tick feeding plays in both virus transmission from infected (donor) ticks, and virus acquisition by uninfected (recipient) co-feeding ticks.

MODEL FOR NON-VIRAEMIC VIRUS TRANSMISSION

Based on observations reviewed above, a transmission model for TBE virus was developed. When transmitted from an infected tick to a susceptible host, TBE virus first enters a skin site that has been profoundly modified by the pharmacological activities of tick saliva. Under these privileged conditions, including the suppression of innate antiviral mechanisms, TBE virus infects a range of skin cells including Langerhans cells (Labuda *et al.* 1996). These infected migratory cells move from the skin site of infected tick feeding to the draining lymph nodes. There they interdigitate with lymphocytes which in turn are programmed to become skin lymphocytes (Austyn, 1992). The primed lymphocytes migrate to the skin, attracted to sites disturbed by tick feeding. If the trafficking lymphocytes have acquired the infection, possibly from the interdigitating Langerhans cells, they will carry the virus to the skin sites where uninfected ticks are feeding. This will allow the virus to be vectored through the vertebrate host independently of a viraemia. Such a model explains why tick-borne pathogens pass so efficiently from infected to uninfected ticks feeding together on a non-systemically infected host. The validity of this model needs to be tested.

RECIPROCAL TICK–PARASITE INTERACTIONS AT THE HOST INTERFACE

The exploitation by tick-borne pathogens of tick-induced modulation of the skin feeding represents a displaced interaction between the pathogen and its vector (Fig. 1*d*). But is this interaction reciprocated? As stated above, the SAT factors that promote pathogen transmission are produced by uninfected ticks, and there are no obvious benefits to ticks in promoting SAT. However, studies on the co-feeding transmission of TBE virus with natural wildlife hosts suggest otherwise (Labuda *et al.* 1993*c*). In these studies, pine voles were found to be highly susceptible to TBE virus infection; indeed, half of the group of pine voles died following exposure to

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TBE virus-infected ticks. Of a total of 240 nymphal ticks placed on the pine voles, only 7% fed successfully; by contrast, 68% of nymphs placed on field mice completed engorgement (Table 2). In pine voles, TBE virus established a systemic infection, with high titres of virus in the brain and blood whereas, in field mice, the infection was limited and viraemia was low or undetectable. These contrasting results suggest that low virulence of TBE virus in field mice enables tick vectors to complete their relatively long feeding period. Thus SAT may minimise any detrimental effect of tick-borne pathogens on their host, thereby benefiting the tick vector. In this case, the displaced tick-pathogen interaction at the host interface is reciprocal.

FUTURE CHALLENGES

As illustrated above, the host response to tick blood-feeding is modulated by a rich cocktail of bioactive ingredients in tick saliva. There is ample evidence that one or more of the modulatory saliva-induced effects is exploited by tick-transmitted parasites. The challenge now is to identify the key activity that promotes pathogen transmission, and isolate and characterize the active saliva component. Probably more than one modulatory effect, and more than one bioactive saliva protein or other biochemical component, facilitates pathogen transmission. It is also likely that different salivary substances will play key roles in the transmission of different tick-borne pathogens. For example, factors that paralyse the action of interferon may be most relevant for tick-borne viruses, whereas anti-complement factors may play a key role in the transmission of tick-borne borrelia. Identification and characterization of modulatory saliva components will not only provide important insights into vertebrate mechanisms for controlling parasites (including tick ectoparasites and tick-borne pathogens), but offer opportunities for new and sustainable strategies of disease control.

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