Tick–host interactions and their immunological implications in tick-borne diseases

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The family of ticks are important vectors of pathogens which can cause a variety of diseases in different species, including humans. In recent years the incidence of diseases transmitted by ticks has risen. This seems to be partly due to the expansion of the tick vector into urban areas. Pathogens transmitted by ticks include viruses, bacteria and protozoans. Ticks serve not only as transmitters of pathogens, they also allow development of the infectious agent inside. In some diseases, like Lyme disease, this can be important for the transmission of the pathogen. Together with the pathogen, the tick injects saliva components into the host which can modulate or impair the local immune response of the host, and which can facilitate pathogen evasion into the host tissue. As a reaction of the host immune system to the tick bite and to the transmission of potential pathogens, the tick itself is exposed to the innate and adaptive immune system of the host. This action of the host immune system against the transmitted pathogen can be utilized in immunization strategies aiming to sterilize the pathogen-infested tick.

The complex interactions of the pathogen with the tick vector and the host, and the tick with the host provide a basis for the development of disease-preventive strategies aiming to control the emerging diseases transmitted by ticks.

Some of the major viral infections spread by Ixodes ticks are European tick-borne encephalitis and the clinically more severe Russian spring–summer encephalitis. Other viral infections, such as Crimean–Congo haemorrhagic fever transmitted by Hyalomma sp., occur sporadically throughout Africa, Asia and Europe. The genus Flavivirus in the family Flaviviridae consists of small enveloped viruses. Several members of this genus, such as the tick-borne encephalitis (TBE) virus, the dengue virus (DEN1–4), Japanese encephalitis (JE) virus and yellow fever (YF) virus are arthropod-borne human pathogens.

Another flavivirus, which is transmitted by Haemaphysalis ticks, is causing Kyasanur forest disease in India, claiming many victims annually. The flavivirus genome is approximately 11 kb and encodes the three structural proteins capsid (C), membrane (M) and envelope (E), and several nonstructural proteins. The flavivirus is internalized via receptor-mediated endocytosis followed by fusion of the viral membrane with the endosomal membrane. The E protein of flavivirus is a major determinant of virulence since it plays a critical role in mediation of receptor-binding and membrane-fusion. Holbrook et al. used Longt (LGT) virus as a model system for tick-borne flaviviruses to investigate the interaction between the viral attachment protein and the host-cell receptor. LGT virus is assigned to the TBE virus serocomplex. The principal mechanism utilized by enveloped viruses to facilitate viral entry employs membrane fusion processes. Upon binding, the virus receptor complex is internalized and packaged into endosomes. The internal pH of the endosome is eventually decreased, causing an irreversible conformational shift in the flavivirus E protein which is thought to expose a fusion peptide within the E protein that penetrates the endosomal membrane, resulting in the release of virus particle into the cytosol. Among the flavivirus nonstructural glycoproteins, only NS1 glycoprotein can be found on the surfaces of the infected cell and in the culture medium. Extracellular and surface NS1 protein induce the production of antibodies that cannot neutralize the virus, because nonstructural glycoprotein remains absent in the virions. NS1 is one of the most conserved flaviviral proteins. Association of the NS1 flavivirus nonstructural glycoprotein with intracellular membranes and co-localization with the viral dsRNA replicative form suggest a role in viral RNA replication.

Lyme disease is one of several bacterial diseases transmitted by Ixodes ticks. It is caused by Borrelia
B. burgdorferi sensu lato and occurs in the United States, Europe and Asia. The tick-borne Rocky Mountain spotted fever is a life-threatening rickettsial disease widespread throughout the United States, caused by Rickettsia rickettsii and transmitted by Dermacentor ticks. Another rickettsial infection, Fievre boutonneuse, occurs mainly in Europe and is caused by Rickettsia conorii. In South Africa, Amblyomma ticks transmit Rickettsia afgaece. The diseases monocytic and granulocytic human ehrlichiosis, which have gathered significant attention recently, are caused by Ehrlichia chaffeensis and Ehrlichia phagocytophila species, respectively. E. chaffeensis is transmitted by Amblyomma americanum, whereas E. phagocytophila is transmitted by Ixodes scapularis in USA and by Ixodes rincus in Europe (Table 1).

A third family of pathogens, protozoans, play a minor role in disease transmission by ticks compared to bacteria and viruses. Babesia divergens and Babesia microti, causative agents of babesiosis are transmitted by Ixodes ticks both in USA and Europe [15, 16].

In the pathogenesis of diseases mentioned above, the tick is positioned between different species transmitting the pathogen. However, the tick is clearly not just a crawling hypodermic needle and syringe with regard to the transmission of tick-borne pathogens. In part, tick-transmitted infectious agents have to undergo various stages of developmental cycles within the vector before they can be transmitted. The pathogenic, infectious microorganisms can express some of their structural or functional compounds especially during development in the vector. These molecules are not necessarily present during the infection of the mammalian host. In the case of B. burgdorferi, the outer surface lipoprotein OspA is expressed by B. burgdorferi within the unfed tick. It is downregulated upon the initiation of blood-feeding of the tick vector [17]. As OspA expression is being reduced, expression of lipoprotein OspC is being upregulated by Borrelia burgdorferi. Likewise, the 6.6 kDa outer membrane-associated lipoprotein of B. burgdorferi is expressed predominantly during the tick phase of spirochete life cycle. Thus, there is evidence that B. burgdorferi has to adapt its metabolism and gene expression repertoire in order to transverse from the ‘cold-blooded’ ticks to the ‘warm-blooded’ mammals.

Additional factors of great importance in tick feeding and pathogen transmission are pharmacologically active molecules in the saliva of the tick. These bioactive substances include anticoagulants, inhibitors of platelet aggregation, vasodilators and suppressors of the immune defence of the host [18]. Ticks as well as other blood-feeding arthropods in general are indeed ‘smart pharmacologists’ [19]. The vector environment and pharmacological properties of the tick saliva demonstrate that the arthropod is not a passive partner in the vector–host–pathogen relationship.

### Table 1. Major tick species, pathogen, disease and the geographical distribution

<table>
<thead>
<tr>
<th>Tick species</th>
<th>Pathogen</th>
<th>Transmitted disease</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ixodes ricinus</td>
<td>B. afzelii</td>
<td>Lyme borreliosis</td>
<td>Europe, North Africa</td>
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<td></td>
<td>B. garinii</td>
<td>Lyme borreliosis</td>
<td>Europe, North Africa</td>
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<td>B. valaisiana</td>
<td>Lyme borreliosis</td>
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<td>Europe, North Africa</td>
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<td></td>
<td>Ehrlichia phagocytophila</td>
<td>Monocytic human ehrlichiosis</td>
<td>Europe, North Africa</td>
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<td></td>
<td>TBE virus</td>
<td>Tick-borne encephalitis</td>
<td>Europe, North Africa</td>
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<tr>
<td></td>
<td>Babesia divergens</td>
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<td>B. burgdorferi</td>
<td>Lyme borreliosis</td>
<td>USA, Southeastern Canada</td>
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<tr>
<td></td>
<td>Babesia microti</td>
<td>Babesiosis</td>
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<td></td>
<td>E. phagocytophila</td>
<td>Monocytic human ehrlichiosis</td>
<td>USA, Southeastern Canada</td>
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<td>B. burgdorferi</td>
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<td>B. burgdorferi</td>
<td>Lyme borreliosis</td>
<td>USA, Southeastern Canada</td>
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<td>Hyalomma marginatum</td>
<td>Rickettsia rickettsii</td>
<td>Rocky Mountain spotted fever</td>
<td>USA, Southern Europe, Russia</td>
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<tr>
<td>Dermacentor anderson</td>
<td>Colorado tick fever virus</td>
<td>Rocky Mountain spotted fever</td>
<td>USA, Southern Europe, Russia</td>
</tr>
<tr>
<td>Haemaphysalis concinna</td>
<td>Tick-borne encephalitis virus</td>
<td>Rocky Mountain spotted fever</td>
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<td>H. spinigera</td>
<td>Kyasanur forest disease virus</td>
<td>Tick-borne encephalitis</td>
<td>India, Sri Lanka</td>
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<td>Amblyomma americanum</td>
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<td>Fievre boutonneuse</td>
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<td>A. variegatum</td>
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<td>Crimean–Congo haemorrhagic fever</td>
<td>Uganda, Senegal, Nigeria, Central African Republic</td>
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<td>Soldado virus</td>
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<td>O. moubata</td>
<td>Borrelia duttoni</td>
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<td>O. hermsi</td>
<td>B. hermsi</td>
<td>Relapsing fever</td>
<td>Western USA</td>
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obstacles in their quest for a blood meal. For example, vertebrates have developed several sophisticated mechanisms for preventing blood loss. Yet blood-feeding arthropods, which require blood for nutrition, egg production and survival, have evolved equally powerful methods for bypassing these haemostatic mechanisms. In addition to the circumvention of haemostatic mechanisms, blood-feeding ticks must also combat host inflammatory/immune counterattacks. Blood-feeding ectoparasitic ticks inject their saliva, when they probe for a bloodmeal. Tick saliva contains a wide array of bioactive proteins and lipid molecules exhibiting a range of pharmacological properties to thwart the defence mechanisms elicited by the host in response to the tick bite. Bioactive compounds in tick saliva were recently reviewed in detail by Bowman et al. Blood-feeding ticks have evolved salivary immunomodulatory factors that prevent the vertebrate host from rejecting the ticks and/or becoming sensitized to the vasomodulatory factors of saliva that facilitate blood feeding. These mechanisms inadvertently enhance pathogen transmission by ticks. As the tick feeds, blood vessels are ruptured that proceed to drain into the feeding lesion and provide the tick with a pool of blood for their nourishment. Normally, one would expect the blood flow from such severed blood-vessels to be quickly arrested by host haemostatic processes (platelet aggregation, coagulation and vasoconstriction). The initial stage of this haemostasis would involve circulating platelets adhering to the damaged blood vessel wall, becoming activated and then aggregating to form a plug in the gap and provide a surface for coagulation processes and fibrin clot. Platelets exposed to the collagen in damaged subendothelium become activated and release ADP, thus amplifying further platelet attraction and causing platelet aggregation. Tick saliva contains a variety of compounds that can prevent the functional activity of collagen, thrombin and fibrinogen proteins.

The host coagulation process is activated through extrinsic (collagen-activated) and intrinsic (tissue factor-activated) pathways of the coagulation cascade. Both converge at the activation of factor X and conversion of prothrombin to thrombin. Activated thrombin cleaves the plasma protein fibrinogen, generating fragments that aggregate to form the fibrin clot. Most tick anticoagulants target blood-clotting factor Xa, thrombin or both. An FXa inhibitor from the tick Ornithodoros savignyi has been identified and characterized. In ticks, a specific FXa inhibitor was identified from both the salivary glands and whole body extracts. FXa inhibitors were also purified from the salivary glands of tick species, Rhicopechus appendiculatus and Hyalomma truncatum. The saliva of the lone star tick A. americanum inhibited both FXa and thrombin, while the salivary gland contained a specific thrombin inhibitor (12 kDa), designated americanin. In addition, FXa inhibitor was purified from the whole body extract of the soft tick Ornithodoros moubata. Another inhibitor of FXa was also isolated from the nympha of the camel tick Hyalomma dromedarii. This inhibitor is a potent anticoagulant because it prolonged both the activated partial thromboplastin time and the prothrombin time of the camel plasma. In this study conducted by Ibrahim et al., it was found that FXa inhibitor from the nympha of H. dromedarii inhibited both thrombin and FXa but not other serine proteases like trypsin and chymotrypsin, a cysteine protease (papain), an acid protease (pepsin) or the bacterial protease subtilisin. Savignin, a thrombin inhibitor isolated from the O. savignyi has recently been characterized. Most haematophagus arthropods like Ixodes dammini, O. moubata and O. savignyi inhibit the platelet aggregation by secreting the enzyme salivary apyrase that rapidly breaks down ADP. Apyrase, however, is not found in A. americanum.

During the prolonged feeding period, the tick requires that blood vessels draining into feeding lesion remain patent. From the tick’s perspective, it would be advantageous that this blood flow could be increased, as and when necessary. Prostaglandins, namely prostaglandin E2 (PGE2) and prostaglandin D2 (PGD2) and, to a lesser extent, PGD2 are extremely potent vasodilators, causing the vascular smooth muscle to relax and blood flow to increase. Though prostaglandins are potent vasodilators, in general they do not cause increased microvascular permeability. PG12 is the most potent inhibitor of platelet aggregation and ADP secretion. Indeed PG12, can also cause the disaggregation of those platelets that have already aggregated. Importantly, potent vasoconstricting peptides, the endothelins, are released by vascular endothelium in response to mechanical injury, shear stress, stretch, turbulent flow or inflammatory mediators and can cause extreme vasoconstriction locally. Vasoconstriction is an important factor for haemostasis. It is expected that endothelins are released into the feeding lesion. Prostaglandins in tick saliva (PGD2, PGE2 and PG12) counter the endothelin-induced vasoconstriction. Further factors in tick saliva ameliorate the response of rejection, elicited by the inflammatory and immune system of the host. Pain and itch responses of the host, which may lead to increased host grooming are suppressed by a bradykinin-deactivating dipeptidyl carboxypeptidase and a histamine-binding protein. The protective functions of vector saliva on pathogens may also have the effect of potentiating the transmission of pathogens to hosts through the alteration of the immunological microenvironments at the feeding site or lymph nodes draining the site of attachment. Complement and antibody production, as well as cytokine expression of macrophages and T-lymphocytes are all targeted by tick saliva. These immunomodulatory factors are important in pathogen transmission. The etiological agent of Lyme disease B. burgdorferi develops in the midgut of the tick at the
Host immune responses to tick infestation

Host immune responses to salivary gland-derived molecules in the case of ixodid ticks can be triggered because ticks acquire a blood meal over a period of days. The salivary glands of the vector families Ixodidae and Amblyomminae are significantly different in their structure and function. The cell number, ultrastructure and chemical compound of secretory inclusions are also significantly different. Differences in chemical compounds of saliva and intensity of production in the periods of feeding influence protective reactions of host organisms. Ectoparasitic insects stimulate the array of host innate and specific acquired immune responses. Many host ectoparasitic arthropod associations are characterized by the development of an acquired resistance, which could potentially reduce infestation. Host-acquired resistance to tick infestation is characterized by a reduced engagement weight of the tick, a reduced number of ova production, fewer viable ova, prevention of moulting and tick death. The immunological basis of host immunity to ticks is well established. Development of cellular infiltrates, particularly basophils and eosinophils, at tick attachment sites provides strong evidence for an inflammatory reaction to ticks by selected host species. Many investigators have reported that host-acquired resistance to tick infestation involves numerous regulatory and effector components of the immune system: Antigen-presenting cells (APCs, particularly Langerhans cells in the epidermis), B- and T-lymphocytes, cytokines, circulatory and haemocytotropic antibodies, and granulocytes with the bioactive mediators derived from these cells. Langerhans cells are capable of trapping tick antigens in the epidermis and then migrating to draining lymph nodes to function as APCs. Basophils and eosinophils are prominent infiltrating granulocytes at tick-bite sites on repeatedly infested guinea pigs and cattle. Tick-infested animal models develop anti-tick antibodies, which are capable of binding to Fc receptors on basophils. The arming of basophils with these tick-specific antibodies can result in the release of a myriad of biologically active molecules from those cells, after making complexes of surface-bound antibodies with tick immunogens or the ticks themselves. Histamine, a basophil-derived molecule, inhibits the tick salivation and engorgement. Furthermore, complement activation plays a role in the expression of acquired resistance to ticks. The numerous biological activities generated by activation of either the alternative or the antibody-dependent classical complement pathways can contribute to the expression of anti-tick immunity of the host.

T-lymphocytes are critically important as regulators and effectors of the host immune responses to ticks. Cutaneous basophil hypersensitivity, the basis of influx of basophils at tick-attachment sites, is a form of delayed type of hypersensitivity mediated by Th1 lymphocytes. A number of tick-host interactions are characterized by the ability of tick salivary gland-derived molecules to stimulate the antigen-specific proliferation of lymphocytes from infested animals. Immunophenotypic analysis of lymphocytes infiltrating tick-attachment sites revealed that CD4+ cells predominated over CD8+ lymphocytes.

The immune system has high costs of maintenance and production during an attack of an infectious agent. The expression of the immune function against the pathogen also depends on the condition of the individual. Blanco et al. reported the condition-dependent immune defence in the magpie (Pica pica). They conducted a study to find out whether the size of organs of the immune system varies in relation to ectoparasitism. They found that the spleen size directly correlates to the amount of ectoparasitism. The bursa fabricius and spleen are the major organs of the immune system of birds. In young birds, the bursa produces the B-cells, which then migrate to lymphoid tissues. The bursa fabricius atrophies in birds at the onset of sexual maturity due to the effect of adrenal and sex hormones. Ectoparasites may stimulate an immune response in the host even when they do not penetrate deeply into the body. This response may play a role in regulating parasite load of the host. The organs of the immune system show changes in the size depending on the abundance and impact of the infecting parasites. Intensively parasitized or diseased individuals may eventually develop abnormally enlarged organs (such as hypertrophied spleen, a condition termed splenomegaly) because of the increased antiparasite defence functions of the hosts. Individuals in good health condition may invest more energy in the capacity of their immune system to fight against infection than those in poor health condition.
Ticks and host cytokines

Cytokines play a central role in regulation of immune responses. Many pathogens have developed the ability to modulate cytokine networks to facilitate their survival in the presence of host immune defence. The fact that ticks can influence host cytokine production, should not be surprising, considering the days required for a blood-feeding tick to engorge. The skin and draining lymph nodes of BALB/c mice infected with I. ricinus nymphs were examined by in situ hybridization for the expression of mRNA for interferon-gamma (IFN-γ), interleukin-2 (IL-2) and interleukin-4 (IL-4) during three successive infestations. At 72 h after tick attachment to a previously uninfested host, lymph-node sections showed expression of IL-2 and IFN-γ while IL-4 was not detected. Skin biopsies obtained during a primary infestation contained fewer than 5% cells positive for either IL-4 or IFN-γ mRNAs and no detectable IL-2 mRNA. Cytokine mRNA profiles changed in the skin during the second and third infestation. IL-2 mRNA was detectable in 20–50% and IFN-γ mRNA in more than 50% of skin cells during the second and third infestation. IL-4 mRNA was detected in 20–50% of skin cells during the second and third infestation and 5–20% during the third infestation. A relative dominance of IL-2 and IFN-γ expression in skin biopsies seems to demonstrate a predominantly Th1-lymphocyte-mediated delayed type of hypersensitivity as an initial host reaction to tick feeding.

Culture supernatants of lymphocytes taken from axillary and branchial lymph nodes of BALB/c mice infected one to three times with I. ricinus nymphs were evaluated by enzyme-linked immunosorbant assay for their ability to produce IL-4 and IFN-γ after in vitro stimulation with T-cell mitogen concanavalin (ConA). Nine days after the first infestation, lymphocytes collected from lymph nodes draining the infestation site produced high levels of IL-4 and low levels of IFN-γ, while similar cells from lymph nodes, which are not draining an infestation site, did not produce either cytokine. The amount of IL-4 produced by lymphocytes from the lymph nodes draining the attachment site remained elevated, when assayed at nine days after the beginning of the third infestation, while IFN-γ levels increased during the third exposure. Control cells did not produce either IL-4 or IFN-γ. It seems that during the initial attachment of the tick to the host, a Th1-mediated immune reaction dominates at the site of tick bite, whereas after several days of attachment of the tick to the host, the immune response turns towards Th2-mediated immunity. This polarization of Th1-mediated immunity towards Th2-mediated immunity seems to be due to tick and host interactions. The relative importance of different cytokines likely fluctuates during the development and expression of primary and memory cell-driven immune responses to immunogens introduced by a feeding tick.

Suppression of the host immune system by ticks or tick-transmitted pathogens

The ability of ectoparasitic arthropods to suppress the innate and acquired host immune defence adds another layer of complexity to the relationship of vector-borne disease-causing pathogens with their mammalian host. The host immune-effector elements that are suppressed by the ectoparasitic ticks are those that also play an important role in host defence to vector-borne infectious agents. The complexity of interactions of tick vectors, mammalian hosts and vector-borne pathogens indicates that the arthropod vector should be included, or at least considered, in any model system for the study of vector-borne diseases. For example, studying infection by needle inoculation of arthropod-transmitted pathogens is clearly neglecting the potential contribution of important vector-derived factors in pathogen transmission, establishment and subsequent disease pathogenesis.

The simplest explanation of a tick-induced immunosuppression of the host may be antigenic competition. New molecules are expressed in salivary glands of ticks during the process of feeding. Barriga et al. showed that hosts could respond to at least 44 tick antigens during infestation. They found that the responses of infested calves to different antigens of Boophilus microplus ticks were independent from each other.

There are components in the tick saliva that cause immunosuppression. The saliva or salivary gland homogenate of several tick species has been shown to severely impair T-cell functions. The impairment seems to be due to, at least in part, the reduced local production of IL-2 (refs 31, 85, 87) and IFN-γ (ref. 88). These cytokines can be vital for the development of an effective immune response at the ‘feeding’ lesion, including recruitment, activation and proliferation of immune cells causing an inflammatory response (Figure 1).

It has been observed that salivary gland extracts of several tick species, including Ixodes scapularis profoundly inhibit the proliferative response of mitogen-stimulated T-lymphocytes. Gillespie et al. reported that tick saliva contains a protein named soluble IL-2-binding factor. The arthropod salivary IL-2-binding capacity provides a simple mechanism for the suppression of T-cell proliferation as well as for the activity of immune effector cells that are responsive to IL-2 stimulation. The identification of a secreted IL-2-binding factor is quite novel for an ectoparasitic system. IL-2-binding factor in I. scapularis saliva represents the first evidence for direct IL-2-targeting by any parasite or pathogen of mammals. Various mechanisms of subverting the host immune system through their cytokines and chemokines have evolved in several classes of pathogens. Ectoparasites such as I. scapularis can succeed the strategy of direct subversion of a central signalling cytokine because they do not multiply within the host.
It is known that vaccination, in part, depends on specific antibody-mediated immunoreaction that damages the parasite. Immunoglobulin molecules of the host can pass through the gut barriers into the haemolymph of the ectoparasitic tick while retaining antibody activity. Research on the Ixodid tick *R. appendiculatus* revealed that host immunoglobulin-G in tick was excreted via salivation of the tick during the feeding process. Immunoglobulin-binding proteins in tick haemolymph and salivary glands are thought to be responsible for such excretion. This discovery of an immunoglobulin excretion system in ticks indicates that they have developed sophisticated mechanisms to protect themselves from the antibody attack of the host.

Prostaglandins are also well established immunosuppressants. They can suppress IFN-γ (refs 92, 93) and IL-2 production and also can inhibit bioactivity of IL-2 on IL-2-dependent cells by reducing the expression of IL-2 receptors in these cells. By these actions, prostaglandins can inhibit T-cell function. *I. dammini* saliva was shown to inhibit IL-2 production by T-cells and investigators tentatively concluded that this inhibition could be accounted for by the PGE₂ content of the saliva samples. A latter report indicated that PGE₂ content of *B. microplus* saliva was the major component responsible for observed inhibition of T-cell proliferation. However, a contradictory report showed that in case PGE₂ was removed from *I. dammini* saliva by microfiltration, the resultant PGE₂-free saliva showed similar suppression of T-cell proliferation as did the non-altered saliva, leading to the conclusion that salivary PGE₂ plays a minor role in the immunosuppressive activity of tick saliva. These contradictory findings have to be viewed in the context of a multitude of pathogenic factors that are present in tick saliva and are potentially immunosuppressive. The production of a variety of immunosuppressive compounds other than prostaglandins in the tick salivary glands has been demonstrated by the inhibition of IL-1 and tumour necrosis factor-α (TNF-α) production by macrophages, and which is not an action of PGE₂ (ref. 92). However, it is clear that the amount of PGE₂ reported in tick saliva seems to be sufficient to cause an

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**Figure 1.** Tick–pathogen–host interactions. After a tick bite saliva components of the tick influence the host’s systems of complement, coagulation and immunity. In addition, the pathogen can exhibit tissue infiltration and local immunosuppression while persisting at the site of the bite. The host in turn mounts a sustained reaction of the innate and adaptive immune system against the pathogen.
impairment of some aspects of the immune response. Prostaglandins are intricately involved in the induction of pain and inflammation. Thus, it seems obvious that salivary prostaglandins injected into the host should cause pain and subsequently, the host would recognize the feeding tick. This scenario should increase the likelihood that the tick is dislodged by host-grooming. However, studies of pain and inflammatory events during tick-bite illustrate that ticks render the proinflammatory properties of prostaglandins ineffective. Tick salivary prostaglandins are likely to exhibit ‘anti-inflammatory actions’ at the feeding site. Following the detection of tissue injury, the inflammatory response involves three interrelated processes, namely vasodilation, oedema formation and leukocyte accumulation. Inflammatory mediators (histamine, serotonin, kinins and components of the complement system) are rapidly released at the site of tissue injury by the vascular endothelium and by attracted inflammatory cells. Local vasodilation causes an increased blood flow and engorged post-capillary venules become more permeable. These combined effects lead to a rise in the amount of fluid leaving the blood vessel into the extravascular space. The extravasated fluid overloads the lymphatic clearance process resulting in swelling or oedema and pressure-related pain. Leukocytes, namely neutrophils, are attracted to the inflammatory sites by complement components, migrate through the capillary wall and then, for example, release enzymes by undergoing respiratory burst. Biocidal superoxide, hydrogen peroxide and radicals are generated. Such neutrophil activity could be detrimental to an attached tick.

Several investigators have reported the presence of immunomodulatory substances in tick saliva. In addition to PGE₂, other molecules with immunosuppressive properties⁸⁵, like a 49 kDa molecule that inhibits the activation of the alternative complement pathway, and an unknown anaphylatoxin inactivating substance that prevents the complement-mediated inflammation have been demonstrated. By acting directly on the immune response or indirectly on its effector mechanisms, the inoculation of these materials may suppress the immune response of the host, preventing rejection of the ticks. Saliva of I. scapularis inhibits the deposition of complement component C3b and C5b, thus inhibiting initiation and sequential activation of the components of the alternative pathway of complement activation. In addition, phagocytosis of substances coated with complement can be inhibited⁹⁹. Saliva-activated transmission of Borrelia afzelii was demonstrated using salivary gland extract (SGE) from I. ricinus ticks and C3H mice. Injection of Borrelia spirochetes together with SGE increased the level of bacteraemia. SGE from I. ricinus ticks inhibited the killing of B. afzelii spirochetes by murine macrophages. SGE also reduced the production of two major defence molecules of phagocytes, superoxide and nitric oxide. Suppression of macrophage microbicidal mechanisms contributes to the inhibitory effect of tick saliva on the killing of B. afzelii spirochetes, thus facilitating the transmission of the pathogen⁹⁹. A 36-kDa immunosuppressant protein (Da-p36) was isolated from the salivary glands of feeding female ixodid ticks Dermacentor andersoni⁹⁶. A recent study demonstrated that salivary gland extracts of a partially engorged female tick Dermacentor reticulatus can reduce the killing of tumour cells by human natural killer (NK) cells in vitro. The NK cell population of the cytokine IFN-γ is important in deviation of immune responses towards Th₁ pattern, which is an important part of acquired resistance to pathogens, particularly viruses. In addition, ticks exploit other aspects of T-lymphocyte function to reduce host immune responses to feeding. Cytokines are messenger molecules that orchestrate the complex cross-talk and actions of the immune system. Ticks have evolved mechanisms to suppress T-lymphocyte cytokine production. Salivary gland extracts of D. andersoni suppressed the production of Th₁ lymphocyte cytokines IL-1β and IFN-γ by lymphocytes from uninfested mice. Likewise, the saliva of I. scapularis inhibited splenocyte production of IL-2 (ref. 85). Subsequent studies have revealed a general pattern of I. scapularis infection causing a downregulation of Th₁ cytokines and a polarization towards a Th₂ profile in latter stages of tick-bite. In comparison, salivary gland extract from I. ricinus tick shows an inhibitory effect on Th₁ and stimulatory effect on Th₂ cytokine elaboration, and in this way SGE polarizes the cytokine profile towards the Th₂ side. IL-1 produced by macrophages, promotes the secretion of IL-2 and the formation of IL-2 receptors by T-lymphocytes. Interaction of IL-2 with its receptor is essential for proliferation and differentiation of T-helper lymphocytes, and thus initiates specific immune responses. TNF-α produced predominantly by macrophages has multiple functions like the activation and congregation of macrophages, neutrophils and other immunocompetent cells. IL-2 and IFN-γ produced by T-lymphocytes are major promoters of cell mediated immunity (Th₁ lymphocyte-dependent reaction). In contrast, IL-4 and IL-5 are promoters of humoral immunity (Th₂ lymphocyte-dependent reaction). IL-10 is a strong inhibitor of cell-mediated immunity. Salivary factors that interfere with the orchestra of host cytokines have ample possibilities to alter host immunity.

Pathogens, which are transferred to the host by the tick will have to cope with the attack of the immune system of the host. At first contact, the innate immune system and, in particular, the complement system play a key role in the elimination of microorganisms after entrance into the human host. Kraiczy et al. reported that Borrelia develops strategies to inactivate host defence mechanisms. By investigating serum susceptibility of Borrelia, it was found that B. afzelii isolates are serum-resistant, whereas the majority of B. burgdorferi sensu stricto isolates display an intermediate serum-sensitive phenotype. Borrelia garinii isolates, however, are killed effectively by complement and therefore regarded as serum-sensitive. Two
outer surface proteins of Borrelia (27.5 and 20.7 kDa) have been identified that interact directly with FHL-1/ reconnitin and factor H, the two major regulators of the alternative complement pathway. These borrelial proteins are termed as CRASPs (complement regulator-acquiring surface proteins). CRASPs were detectable only in serum-resistant borrelia and, accordingly, binding of FHL-1/ reconnitin and factor H only occurred with serum-resistant borrelia isolates. Thus, CRASP represents an important mechanism of immune evasion, primarily of B. afzelii. In addition, serum-factors that are capable of killing serum-resistant Borrelia have been identified. Borrelial activity is mostly observed in the sera of patients within the third stage of Lyme disease, and consists of antibody and complement action. This observation suggested that not all borreliolar antigens are able to induce a borrelial antibody response of the host.

Parasite dispersal and spread of tick-borne diseases

Parasite dispersal is likely to be one of the most important processes influencing the dynamics and coevolution of host–parasite interactions. The antagonistic interaction between parasites and their hosts seems to be an important force for shaping the evolutionary and ecological dynamics of a species. Recently, the contribution of birds in the ecology of B. burgdorferi sensu lato has become more obvious. Evidence of the reservoir competence of particular bird species has been obtained by using tick xenodiagnosis. B. burgdorferi sensu lato circulates not only in terrestrial environments involving I. ricinus and birds as vectors, but also in marine environment involving Ixodes uriae and seabirds. Migratory birds contribute to the spread of B. burgdorferi sensu lato and of infected tick vectors along migration routes. It is emerging that the dynamics in the ecology of Lyme borreliosis is largely host-driven, and selection and migration are major forces shaping the population structure of B. burgdorferi. Phylogenetic analysis of B. burgdorferi sensu lato indicated a high rate of migration for bird-associated genotypes. A study of migrating birds to an offshore New England island revealed that bird migration promotes long distance dispersal of I. dammini from areas where they are endemic to those where they are not. The contribution of migratory and resident birds to the dispersion of Lyme disease varies depending on tick infestation and population density. In England, approximately 20 million farm-reared pheasants (Phasianus colchicus) are released into the wild for recreational shooting. In addition, wood-mice, squirrels and deer provide feeding to considerable numbers of I. ricinus, the European vector of B. burgdorferi sensu lato. For ticks themselves, migration abilities are limited. Dispersal among host populations relies on host movement. Gyllén et al. reported that migratory birds not only carry infected ticks, but are infected by spirochetes for several months. This infection can be passed onto other ticks. Recently, it was investigated whether the bird, redwing thrush (Turdus iliacus) could carry Borrelia garrini and reactivate it under stressful conditions by simulating its migration. Stress, especially strenuous exercise, can suppress the immune system. Hormonal regulation of the immune system, particularly by increased circulating glucocorticoids, may also adversely affect immunocompetence and activate latent infections. Stress hormones are increased in birds during migration. It has been reported that stress migration could impair a bird’s defence against B. garrini and so increase the degree of spirochetemia. Migratory restlessness in redwing thrushes was shown to reactivate a latent borrelia infection. As thrushes and other birds often travel great distances during migration, this reveals a new mechanism for facilitating the long-distance spread of Lyme disease. Ticks anywhere along a migration route, can feed on migrants with reactivated infections and become infected themselves, in turn passing the disease to other organisms. Natural mixed infection of ticks by different pathogens is a normal and commonly occurring phenomenon. There is no report on the existence of antagonistic relationships between spirochetes and rickettsiae in ticks. The absence of such an antagonism between different pathogens in tick vectors could facilitate infections of the human host. The large-scale movement of seabirds and the subsequent dispersal of I. uriae ticks is an important evolutionary factor for the parasite and its different vertebrate and invertebrate hosts.

Effects of host immune responses on vector capacity

There has been little exploration how immunity of the host, whether induced by vaccination or naturally acquired, can influence the ability of ticks to transmit disease. Transmission of Pasteurella tularensis from infected ticks to rabbits was significantly reduced in rabbits resistant to infestation with D. andersoni. Similarly, repeated infestation of female BALB/c mice with I. scapularis significantly reduced the transmission of B. burgdorferi on subsequent infestation with borrelia-infected ticks. In parallel, it has been shown that antibody to OspA protein of B. burgdorferi generated by vaccination of the host with B. burgdorferi OspA inactivates borrelia within ticks and blocks transmission to mammals. It is of particular interest to consider transmission-blocking immunity in the prevention of tick-transmitted diseases. Transmission-blocking vaccines could be developed against a variety of parasites. ‘Sterilization’ of the tick might be achievable when the host-generated antibodies directed against the stages of the parasite are engorged by the
ticks. Reduction of babesia infection using a tick vaccine has been reported.27 In contrast, studies have shown that tick-transmitted viruses can be transmitted between ticks feeding on the same host, although 'immunity' to the virus was present in this host. Furthermore, there is evidence that tick saliva can promote virus growth.129

In contrast to viruses, in the initial stages of borrelia infection the pathogen is present locally in the skin (erythema migrans). Simultaneous feeding and subsequent cross-infection of the attached ticks do not seem to be relevant for borrelia infection.130 Even though downregulation of TNF-α, IFN-γ and IL-2 has been reported at the site of tick-bite, cytokines and chemokines are likely to influence pathogen–vector and pathogen–host interactions. For instance, systemic treatment of mice, which were infested with borrelia-infected I. scapularis, with one or all the three cytokines significantly reduced borrelia infection of these mice.131

Current status of vaccination against tick and tick-borne pathogens

Since tick resistance to chemicals used for tick control (acaricides) is a serious cause of concern, alternative measures for tick control are being developed. Acaricide resistance is highest in members of the genus Boophilus, however, it is also common in other ticks.132,133 An alternative method of vector control is artificial induction of immunologically-based resistance to infestation. Successful immunization against ticks can fulfill dual aims: decrease of the arthropod-burden and prevention of transmission of arthropod borne diseases.134 Initially, tick tissue extracts have been used for immunization strategies to induce resistance to Ixodid ticks.135 However, it is essential to define the antigenic molecules the host is responding to during infestation.136 Currently, it seems unlikely that native ectoparasite antigens could be produced in a cost effective way for immunization processes. Theoretically, there are two sources of protective antigens that could be envisaged.136 The first would be to use specific tick cells for vaccination. However, since the early vaccination trials using cells of A. americanum,137 there has been little reported research in this area. The second is through the use of recombinant antigens. However, problems in this process remain disulphide bond formation and glycosylation.138 Many proteins which are secreted into the aggressive environment of the digestive system of the parasite are heavily disulphide-bonded, and glycosylated. Expression systems, using baculovirus vectors, can carry out glycosylation to some extent. However, glycosylation in in vitro systems is unlikely to be immunologically identical with that of native parasite-derived proteins.131 It is impossible at present, to anticipate whether any or all of the protective immunological response to a native parasite antigen is directed to immunogenic oligosaccharides generated by glycosylation. There is evidence that glycosylation can be an important factor for the generation of host immunity.139 Work on the development of recombinant vaccines against the cattle tick B. microplus is one of the most significant advances to date in the use of concealed antigens to immunize cattle against ticks.140 Cattle infested with B. microplus produce antibodies to intrinsic membrane glycoproteins of the tick as well as to Bm86, a well-characterized concealed antigen from the tick gut. A vaccine based on Bm86 became the first ever anti-arthropod vaccine.141 Lodos et al. describe a vaccination model to simulate the effect of cattle vaccination with concealed antigens on Boophilus tick species.142 Simulation results showed that to achieve a higher level of tick control, an increase in the maximum antibody titre levels is important.142 Given the complexity of ticks, and the fact that they have developed defence and repair mechanisms, it is surprising that the immune response against a single antigen like Bm86 was shown to be effective. However, the immunological response to the single recombinant antigen is less effective than that to more complex mixtures of partially-purified antigens.143 For greater efficacy, it is likely that mixtures of recombinant antigens will be preferred in future vaccines. It is, therefore, important to show not only the effectiveness of particular antigens, but also their ability to act additively or synergistically with other antigens for greater efficacy.

In contrast to vaccination against ticks as target organisms, using the transmitted pathogen as a target has been evaluated with B. burgdorferi. After the discovery of the Lyme disease-causing pathogen, it became subsequently possible to culture B. burgdorferi in a complex liquid medium called Barbour–Stoenner–Kelly medium.144 In this culture medium, B. burgdorferi expresses abundant OspA, a species-specific lipoprotein, that together with flagellin accounts for approximately one-third of the total spirochetal proteins. The crystal structure of OspA has been reported to contain a single layer of beta-sheet connecting an N-terminal and a C-terminal globular domain. The central beta-sheet domain consists largely of polar amino acids.145 More than 100 proteins have been identified in the spirochete genome, including OspB, OspC, OspD, OspE and OspP.146 When, after two weeks of culture, spirochetes were injected into animals. Antibodies against flagellin, p39, and OspC could be detected initially. Thereafter, reactivity against OspA and OspB could be demonstrated.148 However, when host infection occurred naturally through the tick vector, humans, rhesus monkeys, dogs, mice and hamsters, either did not show sustained seroconversion to OspA or did not respond until several months after the onset of infection.149 Explanations for this weak antibody response to OspA after natural infection were found only after examination of the life cycle of spirochete. It was shown that B. burgdorferi changes the expression of OspA during attachment and
feeding of the ticks. During feeding, the spirochetes, which reside in the midgut of the tick, migrate to the tick’s salivary gland. The spirochete downregulates the expression of OspA and begins rapid synthesis of OspC. The downregulation of OspA before inoculation of the spirochete into the host is one explanation for the lack of anti-OspA antibody response in naturally-occurring infections. Downregulation of OspA also demonstrates that a vaccine directed against OspA must be specific for spirochetes inside arthropods. In 1990, an animal trial of an OspA vaccine was performed on a mouse model having cardiac and rheumatological manifestations of Lyme disease. Fikrig and colleagues reported that these mice, when immunized with recombinant OspA, were protected from natural infection. The mechanism of protection was a result of the formation of high titered antibodies to a conformational epitope in the C-terminal end of OspA. Direct anti-"Borrelia" activity of antibodies can be measured by assays detecting spirochetal immobilization, growth inhibition or killing. Antibody-induced inhibition or killing seems to be both complement-dependent and independent. Complement-mediated effects seem to be more efficient. Antibodies may also facilitate phagocytosis by macrophages/monocytes through binding onto Fc receptors. The use of fully lipidated OspA or OspA adsorbed to adjuvant increases antibody titres.

Decorin-binding protein-A (DbpA) is another B. burgdorferi surface-exposed lipoprotein that has shown vaccine efficacy against experimental infection in a mouse model. B. burgdorferi continues to express DbpA but not OspA, after dermal inoculation and remains vulnerable to DbpA antibodies during the early stages of local and disseminating infection in mice. Of interest, DbpA is also an immunogenic antigen during human Lyme disease. Hanson et al. conducted a comparative study to observe the protective efficacies of DbpA and OspA, singly and in combination. They reported that DbpA and OspA in combination protected the host (mice) against higher B. burgdorferi challenge doses than a single antigen vaccine. The DbpA and OspA combinations proved to be more effective against different B. burgdorferi sensu lato isolates than single antigen vaccines. These findings open the way for a second generation of combination vaccines directly aiming against the Lyme disease spirochete.

The US Food and Drug Administration approved the first human vaccine against Lyme disease (LYMERix, SmithKline Beecham) in December 1998. Two vaccine preparations, LYMExrix and another recombinant OspA vaccine (Pasteur Merieux Connaught, Swiftwater, Pennsylvania), have been found safe in first and second phases of study. It is important to note that most of the trials were conducted in eastern United States, where B. burgdorferi sensu lato is the predominant species. There are many subspecies of Borrelia existing worldwide. Therefore, due to subspecies heterogeneity, the problem of cross-protection arises. It is still unclear whether and to what extent the vaccine will prove protective against other Borrelia subspecies. Perhaps, the most interesting aspect of OspA vaccines was that the vaccine-induced immune response took effect within the tick vector itself, before the causative spirochete could enter the host. This intravector mode of action is unique and opens the door to a new method of preventing tick-borne illness in humans. However, a drawback in the development of vaccines has been the marketing stop of LYMExrix due to minor demand.

TBE virus is a human pathogenic virus, and endemic in many parts of Europe and Asia, where it causes several cases of severe neurological illness every year. An interaction between a thermosensitive (ts) mutant and the wild-type (wt) of TBE virus in I. ricinus and R. appendiculatus ticks has been reported. The interaction was demonstrated by the lowered ability of wild-type virus to replicate in ticks previously infected by thermosensitive virus. The thermosensitive virus retained its thermosensitive phenotype throughout the persistent infection of both the ticks and tick cell line. Recently, it was shown that the envelope protein E of the TBE virus can be expressed from recombinant plasmids in different physical forms, depending upon the expression cassette used. Inoculation of mice with plasmids encoding protein E together with protein M precursor, prM has been shown to induce protective immune response in several immunization studies with different flaviviruses. Aberle et al. showed that a DNA construct that gives rise to a secreted particulate form of protein E of TBE virus was far superior to those generating predominantly intracellular or soluble secreted forms of the same antigen. The read-out of the study was the generation of high titres of neutralizing antibodies and a sterilizing immunity against virus challenge. They emphasized that not only the application method, but also the physical forms in which a protein antigen is expressed, has a strong influence on the type and extent of the immune response to DNA vaccines. Mandl et al. have conducted a study using in vitro synthesized infectious RNA as an attenuated live vaccine in another flavivirus model. Based on results obtained with a flavivirus (TBE), Wengler et al. proposed a novel, live, attenuated virus vaccination strategy consisting of the application of in vitro synthesized infectious RNA instead of live virus itself. When administered using the GeneGun, less than 1 ng of RNA was required to initiate replication of virus that was attenuated by a specifically engineered deletion, and this induced a protective immunity in laboratory mice. Because this approach uses RNA, it does not have the potential drawbacks of DNA vaccines, and thus combines the advantages of conventional live virus vaccines (e.g. mimicking natural infection and inducing long-lasting immunity) with those of nucleic acid vaccines (e.g. ease of production without a requirement for eukaryotic cell culture, stability and purity).
Conclusion

The aim of research in tick-host immunology is to develop immunological approaches to reduce the damage caused by tick-borne diseases. There is a need to characterize the antigens of ticks, which are responsible for pathogen transmission and which influence the emerging immunity of the host. Action of tick saliva components on the host include anticoagulatry activity, inhibition of complement activation, vasodilation, antiinflammatory action and immunosuppression. On the side of the pathogen, local immuno-suppression and pathogen persistence have been reported, in addition to systemic infection. The immunocompetent host is mounting a broad-range action against the tick and the transmitted pathogen, including local skin reactions and a response of the innate and adaptive immune system. Often these measures cannot prevent the manifestation of a systemic infection. Thus, anti-tick vaccines could provide an alternative to current methods for the control of ticks. Vaccination strategies for the control of ticks and the transmitted pathogens are likely to become increasingly important in the future.

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