**REVIEW**

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# Tick Saliva in Anti-Tick Immunity and Pathogen Transmission

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**ABSTRACT.** When feeding on vertebrate host ticks (ectoparasitic arthropods and potential vectors of bacterial, rickettsial, protozoal, and viral diseases) induce both innate and specific acquired host-immune reactions as part of anti-tick defenses. In a resistant host immune defense can lead to reduced tick viability, sometimes resulting in tick death. Tick responds to the host immune attack by secreting saliva containing pharmacologically active molecules and modulating host immune response. Tick saliva-effected immunomodulation at the attachment site facilitates both tick feeding and enhances the success of transmission of pathogens from tick into the host. On the other hand, host immunization with antigens from tick saliva can induce anti-tick resistance and is seen to be able to induce immunity against pathogens transmitted by ticks. Many pharmacological properties of saliva described in ticks are shared widely among other blood-feeding arthropods.

*Abbreviations*

| | | | |
|---|---|---|---|
| BP | -binding protein | OspA | outer surface protein A |
| DbpA | decorin-binding protein A | $PGE_2$ | prostaglandin $E_2$ |
| DTH | delayed-type hypersensitivity | $PGI_2$ | prostaglandin $I_2$ |
| FXa | coagulation factor Xa | RGD | Arg–Gly–Asp amino-acid ligand sequence |
| HBP | histamine-binding protein | salp | salivary proteins |
| IFN-γ | interferon-γ | SAT | saliva-activated transmission |
| Ig | immunoglobulin | TAI | tick-adhesion inhibitor |
| IL | interleukin | TAP | tick anticoagulant peptide |
| MIF | macrophage migration inhibitory factor | TGF-β | transforming growth factor β |
| NK | natural killer cells | $T_H1$ | helper T cell subtype 1 |

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## 1 INTRODUCTION

Ticks are obligate, blood-feeding ectoparasites of vertebrates. They reside on the host only when feeding. *Ixodidae* and *Argasidae* are the two major tick families. *Ixodidae* are called "hard ticks" because of their sclerotized dorsal plate, and are the most important family in terms of numbers and medical importance. *Argasidae* are called "soft ticks" because of their flexible cuticle. Ixodids require days to complete feeding; during this time they remain firmly attached to the host, whereas *Argasidae* are rapid feeders, requiring a few hours or less for feeding to repletion, without firm attachment. Each feeding stage of ixodid life cycle may utilize a separate host, often of different species. An individual argasid, as a "nest parasite", generally feeds on the same host or host species through its life span of one or more years (Bergman 1996; Parola and Raoult 2001).

## 2 MAIN FUNCTION OF TICK SALIVARY GLANDS IS TO RETURN EXCESS WATER AND SECRETE PHARMACOLOGICALLY ACTIVE MOLECULES INTO THE VERTEBRATE HOST

Salivary glands can be defined as organs that synthesize and secrete products which assist in the acquisition of food. When on the host, tick concentrates the blood nutrients by returning excess water and ions, two major constituents of tick saliva, from the blood meal *via* saliva back into the host. About 33–50 % of all fluid ingested is excreted back to the host *via* salivary glands (Bowman *et al.* 1997). When salivating into the feeding wound, pathogens presented in tick's saliva are transmitted into the host; this is the most common route of tick-derived pathogen transmission (Stich *et al.* 1993). Salivary gland of ixodid tick synthesizes and secretes cement that firmly secures the tick to the host *via* mouthparts (Bishop *et al.* 2002). When off-host, salivary gland secretes hygroscopic solution that absorbs water from air and prevents the tick from dehydrating (Sauer *et al.* 1996; Meyer-Konig *et al.* 2001).

Ticks have to pierce their host's skin to obtain their meal. This triggers several repair reactions in the host, including blood clotting, platelet aggregation, blood vessel contraction, increased vascular permeability, and leukocyte chemotaxis to the injury site. Pharmacological components in tick saliva (for a brief summary *see* Table I) facilitate feeding by antagonizing or modifying their host's inflammatory response and hemostatic processes. When feeding, tick salivary glands enlarge their volume and become active. Enlargement and activation of salivary glands is supported by production of "feeding proteins" (Wang *et al.* 1999*a*). During attachment, the spectrum of proteins salivated into the wound changes (Sanders *et al.* 1996). Differences in protein production not only between different tick species, but also among individuals of the same species or developmental stage can be observed (Wang *et al.* 1999*b*; Lawrie and Nuttall 2001). Also the activity of tick-derived immunoactive factors can vary depending on the particular host species parasitized (Lawrie *et al.* 1999).

## 3 TICK SALIVARY FACTORS FACILITATE FEEDING BY PREVENTING THE HOST'S HEMOSTATIC PROCESS

As the tick feeds, blood vessels are ruptured, proceed to drain into the feeding lesion and provide the tick with a pool of blood from which to imbibe. Such blood flow is normally rapidly arrested by the host's hemostatic process. This process, which could hinder tick feeding, involves hemostatic plug forming, activation of the plasma coagulation cascade and vessel contraction that can seal the injury site.

Hemostasis is initiated when platelets respond to subendothelial collagen exposed at the injury site and become activated. ADP is released from damaged cells and by activated platelets, thus amplifying further platelet attraction and causing platelet aggregation. Tick saliva contains apyrase inhibiting platelet aggregation by hydrolyzing ATP and ADP to AMP and monophosphate (Ribeiro *et al.* 1985; Titus and Ribeiro 1990; Mans *et al.* 2000). Tick salivary $PGI_2$ increases the concentration of cAMP in platelets, inhibits the secretion of ADP, and such inhibits aggregation and cause disaggregation of those platelets that have aggregated (Bowman *et al.* 1996; Pedibhotla *et al.* 1997).

Platelet activation and ADP release is followed by thrombin production and activation of the coagulation pathway. Blood coagulation (plug formation) occurs through intrinsic or extrinsic pathways and involves a series of proteolytic reactions that ultimately lead to the thrombin-catalyzed conversion of soluble fibrinogen into an insoluble fibrin mesh that holds the platelet plug in place. Tick saliva-derived anticoagulants TAP (Jordan *et al.* 1990; Waxman *et al.* 1990) and ixolaris (Francischetti *et al.* 2002) inhibit coagulation factor Xa (FXa), a serine proteinase catalyzing formation of thrombin in both coagulation pathways, while ornithodorin (van de Locht *et al.* 1996), americanin (Zhu *et al.* 1997), ixin (Hoffmann *et al.* 1991) and an unnamed 60-kDa-protein fraction (Horn *et al.* 2000) are specific thrombin inhibitors. The function of some tick anticoagulants is yet unclear (Mulenga *et al.* 2001).

Activated platelets express surface adhesion receptor proteins, collectively known as integrins that enable cell–cell and cell–matrix interactions. Tick-derived disintegrin-like peptides savignygrin in argasid (Mans *et al.* 2002) and variabilin in ixodid ticks (Wang *et al.* 1996) contain the integrin recognition motif RGD used for binding to integrins and can inhibit platelet aggregation by preventing the binding of other ligands to a platelet receptor. There is no amino-acid sequence similarity between variabilin and savignygrin, and the position of the RGD motif is completely different. This suggests that platelet aggregation inhibitors with RGD-like motifs have evolved after the divergence of hard and soft ticks. This implies that the main tick families have adapted to their blood feeding environments independently (Mans *et al*. 2002). Disagregin (Karczewski *et al.* 1994; Karczewski and Connolly 1997) and TAI (Karczewski *et al.* 1995) both

lack the RGD motif, and inhibit platelet aggregation by preventing binding to ligands by a distinct mechanism from disintegrin-like peptides. The inhibition mechanism of moubatin, an inhibitor of platelet activation by collagen, is yet unclear (Keller *et al.* 1993; Waxman and Connoly 1993).

**Table I.** Sample of pharmacologically active molecules in tick saliva

| Kind | Name | Tick family | Function | Characterization |
|---|---|---|---|---|
| Anticoagulants | TAP | *Argasidae* | factor Xa inhibitor | serine proteinase inhibitor |
| | ornithodorin | *ditto* | thrombin inhibitor | similar to TAP |
| | ixolaris | *Ixodidae* | inhibitor of factor X (FX) activation | similar to tissue factor pathway inhibitor (TFPI) |
| | americanin | *ditto* | thrombin inhibitor | protein |
| | ixin | *ditto* | thrombin inhibitor, antitrypsin activity | probably miniprotein |
| | – | *ditto* | thrombin inhibitor | 60-kDa protein |
| | – | *ditto* | probably anticoagulant | serine proteinase |
| Platelet aggregation inhibitors | apyrase | both | hydrolysis of ATP and ADP | 5′-nucleotidase |
| | savignygrin | *Argasidae* | binding to integrins prevents cell adhesion | disintegrin-like peptide |
| | variabilin | *Ixodidae* | binding to integrins prevents cell adhesion | disintegrin-like peptide |
| | disagregin | *Argasidae* | inhibitor of platelet adhesion to fibrinogen | lacks RGD motif |
| | moubatin | *ditto* | inhibits platelet activation by collagen | 17-kDa protein |
| | TAI | *ditto* | blocks the adhesion of platelets to collagen | 15-kDa protein |
| Prostaglandins (PG) | $PGA_2$/$PGB_2$, $PGD_2$, $PGE_2$, $PGF_2$, $PGI_2$ | *Ixodidae* | vasodilatation, inhibitor of platelet aggregation, proliferation suppressor, stimulates secretion of tick bioactive proteins, *etc*. | prostaglandin |
| Complement inhibitors | isac | *ditto* | similar to complement factor H, complement alternative pathway inhibition | distinct from complemental factor H |
| Immunomodulators | iris (*Ixodes ricinus* immunosuppressor) | *ditto* | immunosuppression, immunomodulation | similar to pig leukocyte elastase inhibitor |
| | salp 15 | *ditto* | inhibition of IL-2 production | weak similarity with inhibin A (TGF-β family) |
| Binding proteins | IL-2BP | *ditto* | inhibits T-cells by IL-2 binding, possibly causes T-cell anergy | protein |
| | – | *ditto* | inhibits IL-8 induced chemotaxis by binding IL-8 | protein |
| | HBPs | *ditto* | suppress inflammation by histamine or serotonin binding, possibly other unknown function | lipocalins with 1 or 2 binding sites |
| | IGBPs | *ditto* | bind IgG, play a role in IgG excretion through tick salivary glands | possibly glycoproteins |
| Cytokine homologue | MIF homologue | *ditto* | inhibits the migration of human macrophages similarly to human MIF | protein sequence 40 % identical to human MIF |
| Others | salp 25D | *ditto* | antioxidant | glutathione peroxidase homologue |

When activated platelets degranulate, release of ADP (which recruits more platelets to the developing plug), serotonin and thromboxane $A_2$ causes the vessel to constrict around the platelet plug and restrict blood flow. Tick $PGE_2$ and $PGI_2$ are potent vasodilators causing the vascular smooth muscle to relax and the blood flow to increase without increased plasma leakage and associated pain (Bowman *et al.* 1996). Another

function for $PGE_2$ in tick salivary glands is to mobilize $Ca^{2+}$, which is connected with stimulation of secretion of bioactive proteins into the tick's saliva during feeding (Qian *et al.* 1998).

Tick saliva contains ample antihemostatic factors that can block hemostasis at all three points – at hemostatic plug formation, activation of plasma coagulation cascade and vessel contraction that can seal the injury site. All three hemostatic events can stop blood flow into the feeding wound and hinder successful tick feeding.

## 4 INFLAMMATION OR ANTIBODY PRODUCTION IS A KEY TO TICK REJECTION. TICK ACTIVELY MODULATES VERTEBRATE ANTI-TICK IMMUNITY

When tick feeds on naïve host, the cellular infiltrate is first dominated by neutrophils followed by mononuclear cells, later a small amount of basophils and eosinophils can be observed (Gill 1986). When infestation is repeated, basophils and eosinophils dominate in the dermal infiltrate, and degranulation of basophils and mast cells can be observed (den Hollander and Allen 1985; Gill 1986). Most cells infiltrating the attachment site are inhibited by tick saliva. NK cells (Kubeš *et al.* 1994, 2002; Kopecký and Kuthejlová 1998), neutrophils (Ribeiro *et al.* 1990), macrophages (Urioste *et al.* 1994; Kopecký and Kuthejlová 1998) and mostly T cells (Ramachandra and Wikel 1992; Bergman *et al.* 2000; Kovář *et al.* 2001, 2002) reduce many of their activities when they come in contact with tick saliva. $PGE_2$ produced by tick salivary glands has been shown to inhibit the host's mononuclear cells (Inokuma *et al.* 1994). Products of some of the above infiltrating cells are inhibited by tick-derived antioxidant salp 25D (Das *et al.* 2001). Moreover, the release of chemotactic factors in the sensitized animal causes also infiltration of $CD4^+$ $T_H$-lymphocytes (Mbow *et al.* 1994) and Langerhans cells (Nithiuthai and Allen 1984; Mbow *et al.* 1994) to the attachment site during tick re-infestation. Tick-derived inhibition of anaphylatoxin production (Ribeiro and Spielman 1986), secretion of proteins binding IL-8 (controls the movement and activity of neutrophils) (Hajnická *et al.* 2001) and action of tick-produced homologue of MIF (inhibits macrophage migration) (Jaworski *et al.* 2001), can block the generation of chemoattractants and limit cellular infiltrate at the feeding site (Ribeiro and Spielman 1986).

During infection or tick infestation, cellular (inflammation, $T_H1$) or humoral (antibody production, $T_H2$) immune response can be activated. Activation of $T_H1$ cells can lead to macrophage activation and can be associated with DTH which, together with basophil activation, often leads to tick rejection (Ferreira and Silva 1999; Falcone *et al.* 2001). Tick saliva-induced IL-4 up-regulation (Schoeler *et al.* 1999, 2000) together with IL-12 down-regulation (Ferreira and Silva 1999) brings about stimulation of the $T_H2$ pattern together with dow-nregulation of $T_H1$ cells (Kovář *et al.* 2001, 2002). Down-regulation of IL-2 (Ferreira and Silva 1999), a typical $T_H1$ cytokine and T-cell autocrine stimulator, corresponds with reduced T cell proliferation (Kovář *et al.* 2001, 2002). Iris protein in tick saliva could be one of the major factors responsible for modulated cytokine production and lymphocyte inhibition (Leboulle *et al.* 2002). Together with salp15 causing decreased IL-2 production (Anguita *et al.* 2002) tick produces IL-2BP, which decreases the amount of IL-2 protein in the feeding lesion. Such a tick-caused lack of IL-2 could lead to T-cell inhibition or anergy in activated T cells (Gillespie *et al.* 2001; Anguita *et al.* 2002). IFN-γ is another $T_H1$ derived cytokine that is clearly down-regulated by tick saliva (Kopecký *et al.* 1999; Kovář *et al.* 2001, 2002). $T_H2$ response leads to a susceptible state to both tick and tick-transmitted pathogens (Ferreira and Silva 1999; Ogden *et al.* 2002), probably thanks to the tick immunoglogulin excretion system (Wang and Nuttall 1995*a*) and direct inhibition of antibody production (Matsumoto *et al.* 2001). IgBP, a part of the tick self-defense, bind imbibed vertebrate IgG passed through tick's gut barrier into the hemolymph, and are thought to help the tick with its excretion *via* salivation (Wang and Nuttall 1994, 1995*a*,*b*, 1999; Rechav and Nuttall 2000).

The only antibody isotype that seems to be effective in natural anti-tick immunity are specific IgE antibodies, which can bind to Fc receptors on basophils and mast cells. These "armed" mast cells and basophils release bioactive molecules, such as histamine, that can inhibit tick salivation and engorgement (den Hollander and Allen 1985; Wikel 1996). Extensive local basophil degranulation is often associated with tick rejection (Brown *et al.* 1982). Soluble receptors of histamine, tick HBP are thought to suppress inflammation by preventing histamine to reach the target cell (Paesen *et al.* 1999). The role of HBP in anti-tick immunity is unclear since experiments showed that histamine binding is probably not its primary function (Paesen *et al.* 2000; Sangamnatdej *et al.* 2002).

Complement also plays a role in the expression of acquired resistance to ticks (Ribeiro 1987). Activation of the alternative pathway of complement is often associated with anti-tick resistance (Wikel 1979; Papatheodorou and Brossard 1987). Tick salivary anticomplement protein isac inhibits complement cascade

activation by interacting with the C3 convertase and inhibiting interaction of factor B with C3b, similarly as does natural complement regulator, factor H. In contrast to it, isac does not have any effect on the classical pathway of complement. No sequence similarity was found between isac and factor H (Lawrie *et al.* 1999; Valenzuela *et al.* 2000). Since complement factor C5a possesses a strong chemotactic activity and causes degranulation of basophils and mast cells, nonspecific activation of complement protein C5 by tick saliva is another way how anti-tick imflammatory reaction can be elicited (Gordon and Allen 1991).

Many other unnamed, not well identified, factors have been recently described in tick saliva. Sometimes, they are characterized by function and molar mass (Mejri *et al.* 2001), sometimes nucleotide sequence is described in dosens of proteins and function will be characterized in the future (Das *et al.* 2001; Valenzuela *et al.* 2002).

## 5 SALIVA-ACTIVATED TRANSMISSION AND VACCINE DEVELOPMENT

In previously exposed (sensitized) hosts, tick feeding evokes a rapid and intense immune response against tick-acquired resistance. In a host expressing acquired resistance, cutaneous basophil hypersensitivity, the basis for the basophil influx, a form of DTH mediated by $T_H1$ lymphocytes occurs at the attachment site (Falcone *et al.* 2001), as discussed above. Host acquired resistance to tick infestation is characterized by reduced engorgement weight, increased duration of feeding, reduced numbers of ova produced, fewer viable ova, and prevention of moulting, resulting in tick death.

Another potent anti-tick immunological reaction could be induced with tick-derived antigens serving as vaccine components. This represents one of the most promising alternatives to chemical control and has the advantages of target-species specificity, environmental safety, and lack of human health risk (Kemp *et al.* 1989; Rodriguez *et al.* 1994, 1995*b*). Anti-tick vaccine could be asserted in domestic animals, because the bite of some tick species may be associated with a severe reaction and can lead to lower commercial production or death in the case of massive infestation (Sing *et al.* 1983; Lacombe *et al.* 1999). Trager (1939) carried out the first experimental vaccination against the tick 60 years ago and nowadays, a glycoprotein Bm86 isolated from tick gut is the sole antigen in anti-tick commercial vaccine used for cattle farming (Rodriguez *et al.* 1995*a*,*b*; Willadsen *et al.* 1995). Unfortunately, tick resistance to Bm86-based vaccination has been recently reported (De La Fuente *et al.* 2000). Recently, particular attention has been focused on tick saliva immunogens as vaccine components (Wikel 1996; Jittapalapong *et al.* 2000).

As discussed above, ticks secrete a variety of substances to allow their feeding to proceed. These factors provide a better environment for the pathogen to gain an advantage in establishing an infection in the new host. Natural route of infection is more efficient in transmission of the pathogen to the vertebrate host than syringe injection (Gern *et al.* 1993). The phenomenon, called saliva-activated transmission (SAT), consists in the promotion of transmission of many tick-borne pathogens: Thogoto virus (Jones *et al.* 1992), tick-borne encephalitis virus (Hajnická *et al.* 2000), *Theileria parva* (Shaw *et al.* 1993), *Francisella tularensis* (Kročová *et al.* 2003) and *Borrelia burgdorferi* (Pechová *et al.* 2002). On the other hand, a host previously exposed to ticks can be resistant to subsequent tick-transmitted pathogen infection (Wikel *et al.* 1997) which means that the host's immune response to tick antigens can block pathogen transmission. Tick saliva also enhances the possibility of pathogen transmission between ticks sharing one host (Labuda *et al.* 1997). That gives promising perspectives in the development of transmission-blocking vaccine (Wikel *et al.* 1997; Mulenga *et al.* 1999; Sharma *et al.* 2001). Such a saliva-derived vaccine protein component giving good protection against transmitted pathogen was shown in the sand fly-*Leishmania* model, which is intensively studied in contrast to the tick-*Borrelia* one (Enserink 2001). Better characterization of tick saliva proteins and other components can help in finding a similar protein in tick saliva.

## 6 TICKS AND OTHER BLOOD-SUCKING ARTHROPODS

Hematophagy occurs in four orders of insects. There are varieties of feeding strategies among hematophagous arthropods. Duration of blood-meal acquisition can be measured in seconds to weeks, host blood can be obtained from the lumen of the blood vessel (solenophagy), or host blood can be imbibed from a hemorrhage created by physical rupturing of blood vessel (telmophagy), blood can form part of the diet in all life stages or only in the adult stage, or sexual dimorphism can play a role in the feeding habits. Blood-feeding and tissue-dwelling arthropods face significant threats to their survival from the host's hemostatic defenses, pain/itch sensations, and innate and specific acquired immune responses. Therefore, it is not surprising that both short-term and long-term blood-feeding arthropods have developed countermeasures to the

defense responses of their hosts and their saliva share many pharmacological and immunological activities. Tick saliva shares with the saliva of many other hematophagous arthropods many antihemostatic factors, such as anticoagulation inhibitors in mosquitoes (Waidhet-Kouadio *et al.* 1998), bugs (Lange *et al.* 1999) or in sand flies (Jacobs *et al.* 1990). Apyrase inhibiting platelet aggregation is part of the protein fraction of many blood-suckers including ticks, black flies (Cupp *et al.* 1995), sand flies (Valenzuela *et al.* 2001), mosquitoes (Champagne *et al.* 1995) and bugs (Sarkis *et al.* 1986). Similarly as in tick feeding, saliva of other blood-sucking arthropods modulates the immune response toward $T_H2$, and suppresses $T_H1$, as was observed in mosquitoes (Zeidner *et al.* 1999) or sand flies (Mbow *et al.* 1998). Tick saliva also shares a suppressive effect on lymphocyte proliferation with bugs (Kalvachová *et al.* 1999), sand flies (Titus 1998) or with black flies (Cross *et al.* 1993). As factors in arthropod saliva promote pathogen transmission, induction of host immune response against these factors can significantly impair pathogen transmission. Thus arthropod salivary factors, responsible for SAT, can serve as components of vaccine against tick-transmitted diseases. At least the SAT effect must be considered in the development of vaccine transmitted by blood-sucking arthopod vector, as described below. The above-described similarities among blood-feeders can facilitate in the interpretation of the obtained data.

## 7 VECTOR SALIVA IN PRACTICE

Since OspA-based vaccine against Lyme disease was shown not to provide sufficient protection, new vaccine candidates against *Borrelia* spirochetes are studied. One of them, borrelial DbpA, was shown to protect mice against subsequent borrelial infection (Cassatt *et al.* 1998; Feng *et al.* 1998; Hagman *et al.* 1998; Hanson *et al.* 1998). It has been shown that DbpA elicits a strong antibody response giving a sufficient protection against the challenge with a heterologous *B. burgdorferi* sensu stricto strain in mice (Hanson *et al.* 1998). As such, DbpA has emerged as a leading new candidate vaccinogen for Lyme disease. To extend their previous vaccination studies (Hagman *et al.* 1998), Hagman *et al.* (2000) checked the efficiency of DbpA vaccine against tick-transmitted *Borrelia* challenge. In this experiment, which mimics the natural mode of *Borrelia* transmission, DbpA totally failed to protect mice against borrelial infection. The question why DbpA failed in the tick transmission model, in contrast to needle inoculation, has not been solved. In nature, *Borrelia* spirochete is injected into the host body together with tick saliva. If we leave out the consideration of differences between *Borrelia* spirochete development in the natural vector and in a cultivation medium as discussed by Hagman *et al.* (2000), pharmacologically active molecules in tick saliva cause local vasodilatation and immunomodulation that significantly enhances the success of pathogen transmission (Pechová *et al.* 2002). This means that all vaccines against arthropod-borne diseases must be tested against a natural route of infection, or at least against syringe-injected pathogen mixed with vector saliva. Characterization of tick saliva proteins and other components can help in better understanding of vector-vertebrate host–pathogen interaction and, hopefully, in the development of a vaccine against such pathogens as borrelias.

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