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PHARMACOLOGICALLY ACTIVE COMPOUNDS IN TICK SALIVARY GLANDS

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Abstract — Ticks are obligate blood-feeding arthropods. To feed successfully, ticks inject saliva into the feeding lesion in the host skin. Tick saliva contains a wide array of pharmacologically active compounds that counteract host defense systems (hemostasis, inflammation, immune responses) and ensure successful feeding and survival of the ectoparasites. The structural-functional relationships of some of the salivary compounds have been described, but current research on tick sialomes indicates that the diversity of tick salivary molecules is very wide and needs furher extensive exploration. Tick saliva offers a rich source of biologically active compounds with potential use as pharmaceuticals.

Key words: Acari, ticks, salivary glands, hemostasis, immunomodulation

INTRODUCTION

Ticks are obligate blood feeding ectoparasites. Apart from taking a blood meal from their hosts, ticks transmit a wide variety of pathogens, including viruses, bacteria, rickettsiae, and protozoa that cause diseases in humans and animals. To acquire a blood meal, ticks insert their highly specialized mouthparts through the host skin and anchor them in the skin by attachment cement (Sonenshine, 1991). Fast-feeding soft ticks (Argasidae) feed rapidly with deep penetration of the host skin and cause considerable damage (Binnington and Kemp, 1980), while slow-feeding hard ticks (Ixodidae) can penetrate the epidermis either superficially, e.g., *Rhipicephalus* (*Boophilus)* spp., or more deeply, e.g., *Amblyomma* spp. (Sonenshine, 1991; Bowman et al., 1997). After attachment of the tick to the host and probing for blood, capillaries and small blood vessels are lacerated, host cells are ruptured, and an extensive hemorrhage forms at the feeding lesion in the host dermis. The volume of the ingested blood as well as the duration of feeding are development stage- and species-specific. Hard ticks may require several days to weeks to complete their blood meal, whereby tick females may ingest more blood than 100-times their initial body weight (Sauer et al., 1995).

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Ticks succeed in completing blood meal thanks to the presence of a wide range of physiologically active molecules in their salivary glands. These molecules developed during the host-parasite co-evolution and are crucial to overcome hemostatic and immune responses of the hosts and, in addition, they support transmission of tick-borne pathogens (Wikel, 1996; Bowman et al., 1997; Brossard and Wikel, 2004; Nuttall and Labuda, 2004; Ramamoorthi et al., 2005).

The salivary glands of ticks are multifunctional complex organs. In free-living ticks, salivary glands assist in the absorption of water vapor from unsaturated air. Salivary glands enable the feeding ticks to concentrate blood nutrients by returning excess water and ions via saliva to the host. Moreover, salivary glands of ticks produce a 'cocktail' of molecules to regulate secretion of salivary proteins and modulate host defense mechanisms (Ribeiro and Francischetti, 2003; Valenzuela, 2004). The anatomy, morphogenesis, and physiology of tick salivary glands have been described in a number of reviews (e.g., Sonenshine, 1991; Sauer et al., 1995, 2000; Bowman and Sauer, 2004). After a tick attaches to a host, expression of a series of new genes and synthesis of proteins is initiated in their salivary glands that reflect the stages of the feeding process. Argasid ticks feed rapidly and penetrate deeply in the host skin and do not secrete factors enabling strong attachment to the host. In contrast, almost all ixodid ticks produce cement proteins that ensure firm attachment of the tick to the host and seal the area around the mouth parts to the wound site. As feeding progresses, the amount of secreted saliva increases and salivary glands undergo a remarkable and rapid structural reorganization. At the peak of the feeding process, the glands can increase 25-fold in size and protein content. Once the tick is engorged and detaches, the glands degenerate through a process of cell apoptosis.

The insertion of the tick hypostome into the host skin causes damage to the epidermis and rupture of blood vessels. Mechanical damage to the host skin would normally lead to formation of a hemostatic plug by activation of the coagulation cascade and vessel contraction, as well as to inflammatory responses. Such responses of the host would disrupt tick feeding and cause rejection of the tick with detrimental consequences to tick viability and reproduction. However, tick saliva contains compounds that counteract host hemostatic, inflammatory, and immune responses and enable ticks to feed for days to weeks at one site (e.g., Ribeiro and Francischetti, 2003; Andrade et al., 2005; Steen et al., 2005; etc.).

The composition of tick saliva is complex and redundant in some cases and reflects complex and redundant host defense responses. Tick saliva contains inhibitors of blood coagulation and platelet aggregation, as well as vasodilatory and immuno-modulatory substances (Ribeiro et al., 1993; Wikel and Alarcon-Chaidez, 2001; Brossard and Wikel, 2004; Andrade et al., 2005; Steen et al., 2005). The blood-feeding strategy of ticks on one hand, and the pool and mode of action of the pharmacologically active compounds contained in their saliva and salivary glands on the other hand, are mostly species-specific. The activity, mechanisms of action, and characteristics of these compounds have been studied more intensively during the last two decades. The aim of such studies is to prepare the active compounds

in recombinant form, with their perspective usage as pharmaceuticals. In addition, elucidation of the mechanisms of interaction between ectoparasites and their hosts can lead to the discovery of new vaccine targets against ticks and the pathogens ticks transmit (Titus et al., 2006; Maritz-Olivier et al., 2007; Hovius et al., 2008).

In this minireview, the main host defense responses affected by ticks and some of the tick strategies to overcome host responses are listed. However, the range of biologically active compounds in tick salivary glands is much wider, and intensive research on tick sialomes and transciptosomes is in progress.

TICK COMPOUNDS AFFECTING HOST HEMOSTASIS

Hemostasis is an efficient mechanism that controls blood loss following vascular injury. Research on the mechanisms through which ticks inhibit host hemostasis led to the discovery and identification of a variety of compounds with diverse biological activities (Arocha-Pinango et al., 1999; Kazimírová, 2007), but differences in the anti-hemostatic repertoires suggest that anti-hemostatic mechanisms in hard and soft ticks evolved independently (Mans et al., 2008). Saliva of the same tick species can contain simultaneously more antihemostatic molecules, inhibiting different arms of the hemostatic system. However, it is important to note that the antihemostatic repertoire in ticks differs between species as well as across genera, and there is no tick species whose full anti-hemostatic capacities have been exhaustively explored and described (Kazimírová, 2007).

Vasodilators

Following laceration of blood vessels by tick mouthparts, arachidonic acid is released by activated platelets and is converted by other platelet enzymes into thromboxane A_2, a platelet-aggregating, platelet-degranulating, and vasoconstricting substance. Activated platelets also release serotonin which, together with thromboxane A_2, is responsible for early vasoconstriction in local inflammation caused by tissue injury. To antagonize vasoconstrictors produced by the host on the site of tissue injury, vasodilators are secreted by ticks to the feeding pool. To date, only non-proteinaceous vasodilatory compounds have been identified in tick saliva. They include lipid derivatives such as prostacyclin and prostaglandins (Ribeiro et al., 1988; Bowman et al., 1988).

Inhibitors of platelet aggregation

Platelet aggregation represents the initial and most immediate stage of hemostasis. Following vascular injury, platelets adhere to the subendothelial tissue, then become activated by agonists such as collagen, thrombin, ADP, and thromboxane A_2. Agonists bind to specific receptors on the surface of platelets and initiate a long and highly complex chain of intracellular chemical reactions that lead to platelet aggregation and formation of a hemostatic plug. The platelet aggregation cascade is targeted by ticks at several stages (see Table 1, Kazimírová, 2007). Ticks target either ADP via salivary apyrase that hydrolyzes the phosphodiester bonds of ATP and

ADP and inhibits ADP-induced platelet aggregation (Mans et al., 2002a), or prevent activation of platelets by collagen (Waxman and Connolly, 1993; Cheng et al., 1999). Interaction between fibrinogen and the GPIIb-IIIa complex is the important final step to platelet aggregation. Accordingly, tick saliva contains disintegrin-like peptides that block the binding of adhesive proteins to GPIIb–IIIa (Mans et al., 2002b; Wang et al., 1996) or inhibit platelet aggregation by preventing binding to ligands by mechanisms distinct from disintegrin-like peptides (Karczewski et al., 1994).

Inhibitors of the blood-coagulation cascade

Blood coagulation involves a series of enzymatic reactions where an inactive proenzyme (coagulation factor) is converted to an active form, which then activates the next proenzyme in the series. Thrombin is involved in the final common pathway of the coagulation cascade and converts fibrinogen into fibrin clot, but also regulates the activity of blood coagulation factors and stimulates platelet reactions. A number of inhibitors of serine proteases involved in the coagulation cascade have been identified and characterized from ticks, of which thrombin and FXa are the most common targets (Table 1). The majority of inhibitors identified so far have been proteins that display a variety of molecular masses, targets, and inhibitory mechanisms.

Inhibitors of thrombin – Several specific direct thrombin inhibitors with various modes of action have been characterized in salivary glands of both soft and hard ticks (e.g., Hoffmann et al., 1991; Zhu et al., 1997; Nienaber et al., 1999; Iwanaga et al., 2003; Horn et al., 2000; Motoyashiki et al., 2003; see Table 1). Recently, a new direct thrombin inhibitor, variegin, was characterized from the tropical bont tick *Amblyomma variegatum* with structural similarity to, but much more potent than, hirulog, a 20-amino-acid synthetic thrombin inhibitor based on the natural leech peptide hirudin (Koh et al., 2007).

Inhibitors of factor X activation – The tick anticoagulant peptide (TAP) from saliva of the soft tick *Ornithodoros moubata* is the most intensively studied tick anticoagulant. TAP has some homology with Kunitz type inhibitors, but is a highly specific, reversible competitive inhibitor of factor Xa (Waxman et al., 1990). The soft tick *Ornithodoros savignyi* also contains a FXa inhibitor with 46% identity to TAP (Joubert et al., 1998), and in addition BSAP1 and BSAP2, which are inhibitors of the extrinsic pathway of the coagulation cascade (Ehebauer et al., 2002). An anticoagulant from *Rhipicephalus appendiculatus* saliva probably targets components of the prothrombinase complex different from FXa (Limo et al., 1991). Inhibitors of FV and FVII have been described for *Dermacentor andersoni* (Gordon and Allen, 1991). Ixolaris, a tissue factor (TF) pathway inhibitor belonging to a novel group of tick anticoagulants, was isolated from *Ixodes scapularis* (Francischetti et al., 2002). A 9.8 kDa anticoagulant protein that inhibits the intrinsic pathway and shows homology to Salp14 and Salp9Pac is also present in saliva of *I. scapularis*. Recombinant Salp14 prolongs APTT and specifically inhibits factor Xa (Narasimhan et al., 2002). These proteins probably belong to a novel family of anticoagulants with related functions.

Table 1. Examples of pharmacologically active molecules in tick salivary glands and saliva. Explanations to Table 1. Refs.: [1] Ribeiro et al. (1998); [2] Bowman et al. (1996); [3] Mans et al. (2002a); [4] Waxman and Connolly (1993); [5] Karczewski et al. (1994); [6] Mans et al. (2002b); [7] Cheng et al. (1999); [8] Wang et al. (1996); [9] Waxman et al. (1990); [10] Nienaber et al. (1999); [11] Ehebauer et al. (2002); [12] Francischetti et al. (2002); [13] Narasimhan et al. (2002); [14] Hoffmann et al. (1991); [15] Zhu et al. (1997); [16] Koh et al. (2007); [17] Iwanaga et al. (2003); [18] Kato et al. (2005); [19] Limo et al. (1991); [20] Sant Anna Azzolini et al. (2003); [21] Horn et al. (2000); [22] Motoyashiki et al. (2003); [23] Gordon and Allen (1991); [24] Paesen et al. (1999); [25] Sangamnetdej et al. (2002); [26] Paesen et al. (2007); [27] Jaworski et al. (2001); [28] Nunn et al. (2005); [29] Valenzuela et al. (2000); [30] Tyson et al. (2007); [31] Daix et al. (2007); [32] Bergman et al. (2000); [33] Anguita et al. (2002); [34] Ramamoorthi et al. (2005); [35] Gillespie et al. (2001); [36] Leboulle et al. (2002); [37] Kotsyfakis et al. (2006); [38] Hannier et al. (2004); [39] Yu et al. (2006); [40] Frauenschuh et al. (2007)

Species	Molecule	Target and/or function	Ref.
Ixodes scapularis	Prostacyclin	Vasodilation	[1]
Amblyomma americanum	Prostaglandins	Vasodilation	[2]
		Platelet aggregation inhibitors	
Soft ticks (Argasidae)	Apyrase	ATP, ADP	[3]
Ornithodoros moubata	Moubatin	Collagen receptor	[4]
Ornithodoros moubata	Disagregin	Integrin antagonist	[5]
Ornithodoros savignyi	Savignygrin	Integrin antagonist	[6]
Haemaphysalis longicornis	Longicomin	Collagen receptor	[7]
Dermacentor variabilis	Variabilin	Integrin antagonist	[8]
		Anticoagulants	
Ornithodoros moubata	TAP, Kunitz-type serine protease inhibitor	FXa	[9]
Ornithodoros savignyi	Savignin	Thrombin	[10]
Ornithodoros savignyi	BSAP1; BSAP2	Extrinsic pathway	[11]
Ixodes scapularis	Ixolaris, Kunitz-type serine protease inhibitor	FVIIa-TF, FXa	[12]
Ixodes scapularis	Novel anticoagulant with homology to Salp	Intrinsic pathway	[13]
Ixodes ricinus	Ixin	Thrombin	[14]
Amblyomma americanum	Americanin	Thrombin	[15]
Amblyomma variegatum	Variegin	Thrombin	[16]
Haemaphysalis longicornis	Madanin-1; Madanin-2	Thrombin	[17]
Haemaphysalis longicornis	Haemaphysalin	FXII/XIIa	[18]
Rhipicephalus appendiculatus	65 kDa protein	Prothrombinase complex	[19]
Rhipicephalus sanguineus	RsTI, Kunitz-type serine protease inhibitor	Plasmin, neutrophil elastase	[20]
Boophilus microplus	BmAP	Thrombin	[21]
Boophilus calcaratus	Calcaratin	Thrombin	[22]
Dermacentor andersoni		FV, FVII	[23]
Rhipicephalus appendiculatus	RaHBP(M), RaHBP(F)-1,2	Histamine-binding proteins	[24]
Dermacentor reticulatus	SHBP	Histamine- and serotonin-binding protein	[25]
Rhipicephalus appendiculatus	TdPI	Tryptase inhibitor	[26]
Amblyomma americanum	MIF	Inhibitor of macrophage migration	[27]
		Complement inhibitors	
Ornithodoros moubata	OMCI	C5, prevention of interaction of C5 with C5 convertase	[28]
Ixodes scapularis	Isac	Alternate complement pathway, interacts with C3 convertase	[29]

Table 1. Continued.

Species	Molecule	Target and/or function	Ref.
Ixodes scapularis	Salp 20	C3 convertase	[30]
Ixodes ricinus	IRAC I, II, Isac paralogs	Alternate complement pathway, interacts with C3 convertase	[31]
		Immunosuppressors	
Dermacentor andersoni	P36	T cell inhibitor	[32]
Ixodes scapularis	Salp15	Impairs IL-2 production and T cell proliferation; binds *B. burgdorferi* OspC, protects the spirochete from antibody-mediated killing	[33] [34]
Ixodes scapularis	IL-2 binding protein	Inhibits proliferation of human T cells and CTLL-2 cells	[35]
Ixodes ricinus	Iris	Modulates T lymphocyte and macrophage responsiveness, induces Th2 type responses; inhibitor of hemostasis	[36]
Ixodes scapularis	Sialostatin L	Inhibits cathepsin L activity	[37]
Ixodes ricinus	BIP	Inhibitor of B cell proliferation	[38]
Hyalomma asiaticum	BIF	Inhibits LPS-induced proliferation of B cells	[39]
		Chemokine binding	
Rhipicephalus sanguineus	Evasin-1	Chemokines CCL3, CCL4, and CCL18	[40]

BmTI-A, a kallikrein and elastase inhibitor of the BPTI-Kunitz type, is present in *Boophilus microplus* larvae (Tanaka et al., 1999). The inhibitor increases APTT, but not PT or TT. Recently, a plasma kallikrein-kinin system inhibitor named haemaphysalin was identified in *Haemaphysalis longicornis* (Kato et al., 2005). The inibitor interferes with reciprocal activation between factor XII and prekallikrein.

Additional anti-hemostatic activities – Apart from antiplatelet factors and anticoagulants, a number of biological activities which may be related to host hemostasis have been described in the saliva of ticks. Fibrinolytic activity due to the presence of a metalloprotease was detected in saliva of *I. scapularis*. The role of salivary metalloproteinases in tick feeding appears to be related to their antifibrinogen- and antifibrin-specific activities (Francischetti et al., 2003). Kunitz-type serine proteinase inhibitors (RsTI, M.W. between 8 and 18 kDa) were isolated from larvae of *Rhipicephalus sanguineus* (Sant Anna Azzolini et al., 2003). They target plasmin and neutrophil elastase and their role in hemostasis is predicted to be similar to the serine proteinase inhibitors such as found, for example, in *R. (Boophilus) microplus* (see Tanaka et al., 1999).

Several serine protease inhibitors with similarity to the serpin family were discovered in ticks (Mulenga et al., 2001, 2003). Tick serpins might also interact with host defense responses, including hemostasis. However, the targets and mode of action of tick serpins have yet to be elucidated.

Calcium-binding proteins with sequence homology to the calreticulin family

are also present in tick saliva. Tick calreticulins may play a modulating role in host hemostasis through binding calcium ions required as coagulation enzyme cofactors (Jaworski et al., 1995). Phospholipase A2, most probably responsible for the hemolytic activity of saliva, was detected in *Amlyomma americanum* (Bowman et al., 1997).

TICKS AND THE HOST IMMUNE RESPONSE

Host cellular innate immune responses and the complement system are the first lines of defense against invading pathogens. The complement system comprises a group of serum proteins that can be activated by different pathways. Activation of the complement system leads to generation of molecules with various biological activities in inflammation and opsonization and lysis of invading pathogens. The adaptive immune response is triggered when activated antigen-presenting cells migrate to lymphoid tissues where they present antigens to T cells, which play a central role in cellular immune responses at the site of infection or assist in the activation of B cells and the generation of an antigen-specific humoral response (Janeway et al., 1999). Tick salivary compounds can modulate both innate and acquired immunity of the hosts to protect themselves from inflammation and host immune responses (Gillespie et al., 2000; Leboulle et al., 2002; Brossard and Wikel, 2004; Valenzuela, 2004). Complex tick–host molecular interactions are considered as a competition between host defenses against the ectoparasite and tick evasion strategies. Some hosts develop resistance to tick feeding, while others develop no protective immunity to tick infestations. Thus, host resistance or susceptibility depends on the tick–host association and can most likely be explained by tick-induced modulation of the host cytokine network (Andrade et al., 2005; Hajnická et al., 2005).

The *in vitro* effect of tick saliva and salivary gland extracts on host immune effector cells like granulocytes, macrophages, natural killer (NK) cells, and T and B cells has been extensively documented (e.g., Ramachandra and Wikel, 1992; Kubeš et al., 1994; Ferreira and Silva, 1998; Schoeler et al., 2000; Gwakisa et al., 2001; Hannier et al., 2003; Mejri et al., 2002). Repeated tick infestations and salivary gland extracts are known to suppress production of macrophage proinflammatory cytokines and the secretion of Th1 cytokines, whereas they upregulate Th2 cytokines, indicating a Th2 polarization of the host immune response (e.g., Ferreira and Silva, 1999; Mejri et al., 2001). Tick-mediated suppression of Th1 lymphocyte reactivity may inhibit expansion of antigen-specific T cell clones, differentiation of B cells, activation of macrophages, and natural killer cell activity. The tick-induced Th2 cytokine profile seems to be advantageous for survival of the tick because of the anti-inflammatory effect of Th2 cytokines. In addition, anti-inflammatory mechanisms may also enhance the transmission of tick-borne pathogens (Schoeler and Wikel, 2001; Wikel and Alarcon-Chaidez, 2001). It is suggested that various tick salivary compounds may have competing activities during infestation, and the amount of saliva injected may also influence tick feeding and pathogen transmission (Mejri et al. 2001, 2002).

Despite relatively extensive knowledge on tick-induced host immunomodula-

tion, only a few active molecules have been identified and characterized in tick salivary glands (Bergman et al., 2000; Valenzuela et al., 2000; Gillespie et al., 2001; Jaworski et al., 2001; Anguita et al., 2002; Leboulle et al., 2002; Hannier et al., 2004; Frauenschuh et al., 2007). Understanding of the molecular basis of the strategies used by ticks to evade host resistance and immune mechanisms that lead to host protection will probably open new possibilities to design vaccines for tick control (Wikel and Alarcon-Chaidez, 2001).

Innate immune responses

Innate immune responses represent the first line of immune defense of the hosts to local injury and involve complement, acute phase proteins, neutrophils, macrophages, mast cells, basophils, eosinophils, dendritic cells, and NK cells. Complement components, prostaglandins, leukotrienes, chemokines, and cytokines contribute to the recruitment of inflammatory cells to the site of injury (e.g., Andrade et al., 2005). Normally, the consequences of prolonged feeding of an ectoparasite would be local inflammation and rejection. However, ticks produce compounds that inhibit the pro-inflammatory functions of most cells infiltrating the attachment site, e.g., neutrophils (Ribeiro et al., 1990), NK cells (Kubeš et al., 1994), macrophages (Kopecký and Kuthejlová, 1998), T cells (e.g., Ramachandra and Wikel, 1992; Bergman et al., 2000), and dendritic cells (Cavassani et al., 2005). Recruitment of specific leukocyte populations during the inflammatory response is triggered by chemokines, which are key mediators of the inflammatory response against parasites. The chemokine CXCL8 (IL-8) is a chemo-attractant for neutrophils. Anti-IL-8 activity was reported from saliva of various ticks (Hajnická et al., 2001). Morover, tick saliva contains a variety of inhibitory activities directed against other pro-inflammatory cytokines such as IL-2 and chemokines (CCL2/MCP-1, CCL3/MIP-1α, CCL5/RANTES, and CCL11/eotaxin) (Hajnická et al., 2005). Evasins, a family of chemokine-binding proteins, have been detected in *Rhipicephalus sanguineus* ticks, and Evasin-1 - a CC chemokine-binding protein highly specific for CCL3, CCL4, and CCL18 (PARC) - was characterized (Frauenschuh et al., 2007).

Bradykinin and histamine are important mediators of itch and pain and could stimulate host grooming and removal of the feeding ticks. Tick salivary kininases hydrolyze circulating kinins (e.g., bradykinin). A dipeptidyl carboxypeptidase activity was found to account for the salivary kininase activity of *Ixodes scapularis* (Ribeiro and Mather, 1998). Hard ticks also produce amine-binding proteins of the lipocalin family that minimize host responses to local inflammation. A male-specific histamine-binding salivary protein [RaHBP(M)] and two female-specific histamine-binding salivary proteins [RaHBP(F)-1, 2] were isolated from the saliva of *Rhipicephalus appendiculatus* (Paesen et al., 1999), and the gene for a protein that binds both serotonin and histamine (SHBP) was identified in *Dermacentor reticulatus* (Sangamnetdej et al., 2002). Recently, a tick-derived protease inhibitor (TdPI) has been described and characterized from *R. appendiculatus* that suppresses the activity of human β-tryptases, mast cell-specific serine proteases with roles in inflammation and tissue remodelling (Paesen et al., 2007). Ticks also produce pro-

teins that mimic host proteins to evade the host immune response. Tick macrophage migration inhibitory factor (MIF) is a peptide produced by the salivary glands of the hard tick *Amblyomma americanum* (Jaworski et al., 2001) that inhibits the migration of macrophages and most probably protects the tick from macrophage attack.

The complement system – links the innate and adaptive responses of the host immune system and is activated via three main pathways, of which the alternative pathway is the major line of defense against invading pathogens and is also involved in the resistance to ticks. Tick saliva contains a number of molecules that specifically inhibit factor 3 (C3) convertase (Isac, IRAC I, II, Salp 20, ISAC-1) or C5 activation (OMCI) (see Hovius et al., 2008; Couvreur et al., 2008; and citations therein; and Table 1).

Acquired immune responses

During the first exposure to ticks, immunoglobulin and T cell-mediated immune responses are induced in the hosts. Salivary immunogens are processed by Langerhans cells located in the epidermis and presented to immunocompetent lymphocytes (Schoeler and Wikel 2001; Andrade et al., 2005). Antigen-presenting cells can also transport immunogens to draining lymph nodes and promote antibody and cell-mediated responses. The DTH (delayed type hypersensitivity) response which characteristic influx of lymphocytes and macrophages, basophils, and eosinophils is often observed at the tick feeding site and homocytotropic antibodies are produced. Memory B and T lymphocytes are generated. In resistant hosts, the presence of reactive antibodies and effector T lymphocytes assures a rapid response to infestation and can impair tick feeding, while in natural tick-host associations ticks have evolved to overcome host immune responses (Ribeiro, 1995).

A variety of tick species have been found to suppress *in vitro* proliferation of lymphocytes induced with T and/or B cell mitogens. Tick-induced immunosuppression of the host is also characterized by decreased primary antibody responses to T cell-dependent antigens. Moreover, ticks have evolved ways to deviate T lymphocyte cytokines. Generally, it has been reported that tick saliva polarizes the host immune response towards a Th2 type profile characterized by downregulation of Th1 cytokines (IL-2, IFN-γ) and enhanced production of Th2 cytokines (IL-4, IL-5, IL-6, IL-10, IL-13) (see reviews by Gillespie et al., 2000; Schoeler and Wikel, 2001; Wikel and Alarcon-Chaidez, 2001; and references therein). Inhibition of T cell responsiveness to ConA could result from the direct effect of salivary gland proteins on lymphocytes or from their production of IL-10, while up-regulation of IL-4 and IL-10 probably leads to the development of a Th2 response (Ramachandra and Wikel, 1992; Wikel, 1999; Schoeler and Wikel, 2001; Wikel and Alarcon-Chaidez, 2001).

Several T-cell inhibitors have been identified in ticks (Table 1). A 36 kDa protein (p36) present in the saliva of feeding *Dermacentor andersoni* has been characterized and cloned (Bergman et al., 2000). Iris (an immunosuppressor) was detected in the salivary glands of feeding *Ixodes ricinus* females (Leboulle et al., 2002). Expression of Iris was induced in the salivary glands of *I. ricinus* during the feeding process,

and Iris was secreted into the tick saliva. It suppresses T lymphocyte proliferation, induces a Th2 type immune response, and inhibits the production of pro-inflammatory cytokines (IL-6 and TNF-alpha). Salp15, a 15 kDa salivary gland protein from *Ixodes scapularis*, is another feeding-induced protein that inhibits the activation of T cells. Salp15 specifically binds to the CD4 molecules on CD4+ T (helper) cells, which results in inhibition of T cell receptor-mediated signaling, leading to reduced IL-2 production and impaired T cell proliferation (Anguita et al., 2002; Garg et al., 2006). There is evidence that the pathogen *Borrelia burgdorferi* in *I. scapularis* might use Salp15 during transmission to a vertebrate host, as it specifically interacts with *B. burgdorferi* outer surface protein C and the binding of Salp15 protects *B. burgdorferi* from antibody-mediated killing *in vitro* (Ramamoorthi et al., 2005).

A secreted IL-2-binding protein that suppresses T cell proliferation and the activity of other immune effector cells responsive to IL-2 stimulation was detected in saliva of *I. scapularis* (Gillespie et al., 2001). Sialostatin L, a protein with inhibitory action against cathepsin L that displays antiinflammatory properties and inhibits proliferation of cytotoxic T lymphocytes, was also found in saliva of *I. scapularis* (Kotsyfakis et al., 2006).

Ticks can benefit from suppression of B cell responses by inhibiting specific anti-tick antibody responses that could lead to rejection by the host. B cell inhibitory proteins (BIP and BIF) were identified in *I. ricinus* and *Hyalomma asiaticum asiaticum,* respectively (Hannier et al., 2004; Yu et al., 2006). In addition to modulation of the host responses by B cell inhibitors, pathogens like *Borrelia burgdorferi* might also benefit from BIP-mediated B cell suppression.

Apart from substances modulating the host immune responses, ticks produce immunoglobulin-binding proteins that protect the tick primarily from ingested host immunoglobulins (Wang and Nuttall, 1999).

CONCLUDING REMARKS

Ticks have adapted to blood feeding by counteracting host hemostasis, inflammation, and immune responses. Tick salivary glands represent a rich source of various pharmacologically active compounds that facilitate blood feeding and interfere with host defense systems. In addition to antihemostatics and immunomodulators, ticks produce a number of other molecules with a wide range of biological activities, such as components of the cement cone, cardiotoxic factors and neutotoxins, various enzymes and enzyme inhibitors, and protein homologs (see reviews, e.g., by Valenzuela, 2004; Nuttall and Labuda, 2004; Mans and Neitz, 2004; Andrade et al., 2005; Steen et al., 2006; Tu et al., 2005). Novel tick-derived molecules may be useful in the development of pharmaceuticals for treatment of a number of hemostatic disorders, cardiovascular diseases, and disorders of the immune system. However, the isolation and identification of salivary molecules is often slow and difficult. High-throughput approaches involving molecular biology techniques, proteomics, and functional genomics have been introduced to study salivary components of

ticks and will make it possible to test predicted and novel functions of tick molecules (Valenzuela, 2002, 2004).

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