Chapter 1
Exploring the Sialomes of Ticks

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Abstract Ticks (Acarina) are obligate blood-feeding arthopods that vector human and animal pathogens, causing typhus, Lyme disease, Rocky Mountain spotted fever, tick-borne relapsing fever, babesiosis, Q fever, arboviruses, anaplasmosis, and ehrlichiosis. Among the specializations required for this peculiar diet, tick saliva, a fluid once believed to be relevant only for lubrication of mouthparts and water balance, is now well known to be a cocktail of potent antihemostatic, anti-inflammatory, and immunomodulatory molecules that helps these arthropods obtain a blood meal from their vertebrate hosts. The repertoire of pharmacologically active components in this cocktail is impressive as well as the number of targets they specifically affect. These salivary components change the physiology of the host at the bite site, and, consequently, some pathogens transmitted by ticks take advantage of this change and become more infective. Tick salivary proteins have therefore become an attractive target to control tick-borne diseases. Recent advances in molecular biology, protein chemistry, and computational biology are accelerating the isolation, sequencing, and analysis of a large number of transcripts and proteins from the saliva of different ticks. Many of these newly isolated genes code for proteins with homology to known proteins allowing identification or prediction of their function. These and other molecules from genome and proteome sequences offer an exciting possibility to identify new vaccine antigens, potential biopharmaceuticals, antimicrobial peptides, and other novel human therapeutics.

Keywords Sialomes • Ticks • Acarina • Sialotranscriptome • Sialoproteome • Pharmacologically active components

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Abbreviations

ADP	Adenosine diphosphate
APTT	Activated partial thromboplastin time
ATP	Adenosine triphosphate
BIP	B-cell inhibitory proteins
BmAP	*Boophilus microplus* anticoagulant protein
BmTI-A	*Rhipicephalus microplus* trypsin inhibitor-A
BPTI–Kunitz	Basic protease inhibitor–Kunitz type
cAMP	Cyclic adenosine monophosphate
Dc	Dendritic cell
ECM	Extracellular matrix
ETC	Extrinsic tenase complex
FIXa	Factor IXa
FVIII	Factor VIII
FX	Factor X
FXa	Factor Xa
GP IIb–IIIa	Glycoprotein IIb–IIIa
IC_{50}	The concentration of an inhibitor where the response (or binding) is reduced by half
IFN	Interferon
IL	Interleukin
Ir-CPI	*Ixodes ricinus* contact phase inhibitor
IRS-2	*I. ricinus* serine proteinase inhibitor (serpin)
Isac	*I. scapularis* anticomplement
ISL929	*Ixodes scapularis* salivary proteins
MIF	Macrophage migration inhibitory factor
NCBI	National Center for Biotechnology Information
NK	Natural killer
OmCI	*Ornithodoros moubata* complement inhibitor
PGE2	Prostaglandin E2
PGF2a	Prostaglandin F2
PT	Prothrombin time
RaHBP	*Rhipicephalus appendiculatus* histamine-binding salivary protein
Salp	Salivary protein
SAT	Saliva-assisted transmission
SG	Salivary gland
SGE	Salivary gland extract
SHBP	serotonin- and histamine- binding protein
TAI	Tick adhesion inhibitor
TAP	Tick anticoagulant peptide
TdPI	Tick-derived protease inhibitor
TF	Tissue factor
tHRF	Tick histamine release factor
TT	Thrombin time

1.1 Introduction

Ticks are hematophagous ectoparasites of terrestrial vertebrates and are of great medical and veterinary importance, mainly because they are vectors of diseases affecting humans, livestock, and companion animals. Moreover, tick feeding can cause direct damage to their hosts such as significant blood loss as well as paralysis, toxicosis, irritation, and allergy [1]. Tick saliva contains a cocktail of potent pharmacologically active components able to disarm the host hemostatic system [2, 3] and alter the inflammatory and host immune responses [4, 5]. The molecules present in tick saliva range from lipids to large proteins and represent a plethora of biological activities (antihemostatic, anti-inflammatory, and immunomodulatory molecules). These molecules modify the physiology of their hosts at the tick-bite site, allowing these ectoparasites to obtain a blood meal from the host [3, 6–8]. In addition to its role in feeding and other functions related to ion and water handling, tick saliva may potentiate the transmission and establishment of tick-borne pathogens, and therefore, immune responses to tick saliva can confer protection against pathogen transmission [8–11]. Adaptation of ticks to their natural hosts has resulted in their ability to modulate the host immune and hemostatic response with their saliva. However, with non-natural hosts, tick feeding often results in immune and allergic responses, presumably to the injected salivary proteins, resulting in tick rejection [12]. Accordingly, the identification and characterization of tick salivary proteins may lead to the discovery of novel pharmacological agents, and it may help in the identification of potential vaccine candidates to control tick-borne diseases [8, 13].

Recently, salivary gland transcriptomic and proteomic analyses of several hard and soft ticks have been performed, providing data sets that are invaluable for a better understanding of tick sialomes and the immunobiology at the tick–host–pathogen interface [7, 14, 15]. Three main surprises arose with this approach:

1. The first was that the repertoire of tick salivary gland transcripts and proteins is much more broad and complex than anticipated, containing hundreds to thousands of different proteins, many of which are novel, since they produce no similarities to other proteins in the databases, such as the nonredundant database of the National Center for Biotechnology Information (NCBI). The authors of those studies classified the salivary transcripts and proteins they found as putative secreted or possible housekeeping groups and then into different groups according to their known or predicted biological function. Most such putative secreted proteins have unknown functions but, if secreted into their hosts, probably have antihemostatic, anti-inflammatory, immunomodulatory, or even anti-angiogenic or antimicrobial activity [15]. Regarding the probable housekeeping proteins identified, the authors suggested that their sequences may help to identify novel secreted protein families if identified in proteome experiments.
2. Another surprise was that the most abundant tick salivary proteins are members of multigene families. For some of these protein families, it is known that they are differentially expressed as feeding progresses; thus, at the last day of feeding, the tick is producing a different family member in saliva than that produced at the first day and may thus be evading the host immune response.

3. The third surprise, and challenge, was that we cannot anticipate at all the function of the majority of the tick salivary proteins. Indeed, for any tick species with a known transcriptome, less than 5 % of the proteins have been expressed and their function verified. Whole protein families await functional identification.

1.2 How Ticks Evade the Host

1.2.1 Tick Compounds Affecting Host Hemostasis

Hemostasis is an efficient mechanism that controls blood loss following vascular injury. Ticks attempting to obtain a blood meal face the vertebrate host's hemostatic system whose role is to prevent blood loss after tissue injury. The three branches of the hemostatic system, vasoconstriction (reduction of the blood flow), platelet aggregation (formation of the platelet plug), and the blood coagulation cascade (formation of the blood clot), pose a real threat to ticks when obtaining blood from the host. These three branches are well interconnected, making hemostasis a redundant system. The redundancy of the system is exemplified during platelet activation; these cells are essential in forming the platelet plug, but additionally, when activated, they release two potent vasoconstrictors, serotonin, and thromboxane A2, resulting in a decrease of blood flow. Additionally, activated platelets expose a negative charge on their membrane comprising exposed phosphatidyl serine phospholipids. These phospholipids are used for the formation of protein complexes such as the "tenase complex" composed of factor VIII (FVIII), factor IXa (FIXa), and factor X (FX), which is required for the formation of factor Xa (FXa) and the activation of the blood coagulation cascade. Another example of redundancy is in the blood coagulation branch. In addition to being a crucial enzyme in this cascade, thrombin cleaves the thrombin receptor on platelets, causing them to activate and aggregate. Therefore, the hemostatic system poses interesting problems for blood feeders, including specificity and redundancy [16].

How did ticks solve these problems? Ticks have been in the business of blood feeding for a long time. At least 120 Ma of evolution and adaptation to their host's hemostatic system have created a repertoire of potent bioactive salivary molecules with vasodilatory, antiplatelet, and anticoagulant activities [17, 18]. In many cases, molecules display more than one antihemostatic activity to combat the specificity and redundancy of the hemostatic system. Differences in the antihemostatic repertoires suggest that antihemostatic mechanisms in hard and soft ticks evolved independently [19]. 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 antihemostatic capacities have been exhaustively explored and described [18]. Examples of the different molecules which have been characterized from the saliva of different ticks and examples of their potent biological activities will be described in this chapter (Table 1.1).

Table 1.1 Molecules characterized from the saliva of different tick species and their most potent biological activities

Tick species	Molecule	Molecular weight	Target and/or function	Nature of biochemical	References
Vasodilatation					
Ixodes scapularis	Prostacyclin ($PG1_2$)		Vasodilatation	Eicosanoïde	[20]
	tHRF	20 kDa	Vasodilatation	Ligand of histamine	[21]
Ixodes ricinus	IRS-2	38 kDa	Cathepsine G, chymase	Serpin	[22]
Amblyomma americanum	Prostaglandin E_2 and $F_2\alpha$	352.4 kDa	Vasodilatation	Prostaglandins	[23, 24]
Boophilus microplus	Prostaglandin E_2		Vasodilatation	Prostaglandin	[25]
Platelet aggregation inhibitors					
Argasidae	Apyrase		ATP, ADP	Apyrase	[26, 27]
Ornithodoros moubata	Moubatin	17 kDa	Collagen receptor	Lipocalin	[28]
	Disaggregin	60 kDa	Antagonist of integrin	Peptide	[29]
Ornithodoros savignyi	Savignygrin	14 kDa	Antagonist of integrin	Disintegrin	[30]
	Apyrase				[31]
	Savignin		Antithrombin		[32]
Ornithodoros moubata	Apyrase				[33]
	Disagregin				[29]
	Moubatin				[34]
	Tick adhesion inhibitor (TAI)				[35]
Ixodes scapularis	Apyrase	62 kDa	ATP, ADP	Enzyme	[36]
Ixodes scapularis, Ixodes pacificus	Ixodegrins	14 kDa	Antagonist of integrin	Peptide	[37]
Ixodes ricinus	IRS-2	42 kDa	Thrombin	Lipocalin	[22]
Haemaphysalis longicornis	Longicornin	16 kDa	Collagen receptor	Lipocalin	[38]
Dermacentor variabilis	Variabilin (GPIIa–IIIb antagonist)	5 kDa	Antagonist of integrin	Lipocalin	[39]
	Madanin 1 and 2				[40]

(continued)

Table 1.1 (continued)

Tick species	Molecule	Molecular weight	Target and/or function	Nature of biochemical	References
Amblyomma americanum	Americanin		Thrombin		[41]
Boophilus microplus	Antithrombin				[42]
Anticoagulation and fibrinolysis					
Ornithodoros moubata	TAP	7 kDa	FXA	Peptide anticoagulant	[43]
	Ornithodorin		Thrombin		[44]
Ornithodoros savignyi	Savignin	14 kDa	Thrombin	Peptide	[32]
	Tap-like protein	7 kDa	FXA	Peptide anticoagulant	[45]
	BSAP1–BSAP2		TF pathway inhibitor		[46]
Ixodes scapularis	Ixolaris	14 kDa	TF pathway inhibitor	Protein	[47]
	Salp14	15 kDa	TF pathway inhibitor	Protein	[48]
	TIX-5	7 kDa	Inhibitor FXa-mediated FV activation	Factor X inhibitor	[49]
Ixodes ricinus	Ir-CPI	3–4 kDa	Intrinsic pathway, fibrinolysis	Peptide	[50]
Amblyomma variegatum	Variegin		Thrombin	Lipocalin	[51]
Amblyomma americanum	FXa inhibitor		FXa inhibitor		[52]
	Americanin		Thrombin		[41]
Amblyomma cajennense	Amblyommine-X	7 kDa	FXa	RGD peptide	[53]
Haemaphysalis longicornis	Madanin 1 and 2	7 kDa	Thrombin	Peptide	[40]
	Haemaphysalin		FxII/XIIa		[54]
	Longistatin	23 kDa	Fibrinolysis	Peptide	[55]
Rhipicephalus appendiculatus	65 kDa protein	65 kDa	Factor Xa inhibitor	Prothrombinase complex	[56]
Rhipicephalus (Boophilus) microplus	BmAP	14 kDa	Thrombin	Anticoagulant protein	[42]
	Boophilin	1.7 kDa	Thrombin, trypsin, plasmin	Protein	[57]

	Microphilin	1.8 kDa	Thrombin	Protein	[58]
Boophilus calcaratus	Calcaratin	14.5 kDa	Thrombin	Protein	[59]
Dermacentor andersoni	Inhibitor of FV and FVII		Inhibitor of FV and FVII		[60]
Hyalomma truncatum	FXa inhibitor		FXa inhibitor		[61]
Complement inhibitors					
Ornithodoros moubata	OmCI	16.8 kDa	C5, prevention of interaction of C5 with C5 convertase	Complement inhibitor	[62]
I. scapularis	Salp20	48 kDa	Interacts with C3 convertase		[6]
	Isac	18.5 kDa	Alternative complement pathway, interacts with C3 convertase		[6]
I. ricinus	IRAC I, II, Isac paralogues		Alternative complement pathway, interacts with C3 convertase		[63]
Immunosuppression/immunomodulation					
Ixodes scapularis	Salp15	15 kDa	Impairs IL-2 production and T-cell proliferation, binds *B. burgdorferi* OspC, protects the spirochete from antibody-mediated killing	Protein	[64, 65]
	IL-2-binding protein		Inhibits proliferation of human T cells and CTLL-2 cells		[66]
	ISL 929 et ISL 1373	10 kDa	Impair adherence of polymorphonuclear leukocytes	Protein	[67]

(continued)

Table 1.1 (continued)

Tick species	Molecule	Molecular weight	Target and/or function	Nature of biochemical	References
	Sialostatin L, L 2	12.5 kDa	Inhibits cathepsin L activity	Protein	[68]
Ixodes ricinus	Iris	43 kDa	Iris modulates T lymphocyte and macrophage responsiveness, induces Th2-type responses	Protein	[69, 70]
	Bip		Inhibitor of B-cell proliferation	Protein	[71]
	Ir-LBP		Neutrophil	Protein	[72]
Dermacentor andersoni	P36	36 kDa	T-cell inhibitor	Protein	[73]
Hyalomma asiaticum	BIF	13 kDa	Inhibits LPS-induced proliferation of B cells	Protein	[74]
	Hyalomin A, B		B suppresses host inflammatory responses (modulation of cytokine secretion, detoxification of free radicals)	Peptide	[75]
Rhipicephalus appendiculatus	Japanin	17.7 kDa	Reprogrammes DC responses	Protein	[76]
Dermacentor reticulatus	SHBP	22 kDa	Histamine- and serotonin-binding protein	Protein	[77]
Rhipicephalus appendiculatus	RaHBP(M), RaHBP(F)	21 kDa	Histamine-binding proteins	Protein	[78]
Rhipicephalus appendiculatus	TdPI	13.5 kDa	Tryptase inhibitor	Peptidase	[79]
Amblyomma americanum	MIF	17 kDa	Inhibitor of macrophage migration	Protein	[80]
Rhipicephalus sanguineus	Ado, PGE2	352.4 kDa	Modulate host inflammatory responses	Saturated fatty acid	[81]

Chemokine binding					
Rhipicephalus sanguineus	Evasin-1	10.46 kDa	Chemokines CCL3, CCL4, CCL18, CXCL1	Protein	[82, 83]
	Evasin-3,	7 kDa			
	Evasin-4	12.03 kDa	CCL5 et CCL11		
Wound healing, angiogenesis					
Ixodes ricinus	Metalloprotease		Inhibits angiogenesis	Enzyme	[84]
Haemaphysalis longicornis	Haemangin		Inhibits angiogenesis	Protein	[85]
	HLTnI; troponin I-like molecule		Inhibits angiogenesis	Protein	[86]

1.2.1.1 Tick Vasodilators

Ticks are able to disarm the vasoconstriction branch of the hemostatic system by the presence of salivary vasodilators. The latest discovered are molecules which increase blood flow by antagonizing vasoconstrictors produced by the hemostatic system following tissue injury.

All known tick salivary vasodilators reported to date are nonproteinaceous vasodilatory compounds. They include lipid derivatives such as prostacyclin and prostaglandins [20, 87]. Examples of salivary vasodilators (Table 1.1) from the hard tick, *Ixodes scapularis*, are a salivary arachidonic acid lipid derivative prostacyclin [20] and prostaglandin E2 (PGE2) [36]. The latest molecule is a short-acting vasodilator and also an inhibitor of platelet aggregation that exerts its effect by increasing cyclic adenosine monophosphate (cAMP) in smooth muscle cells resulting is vasorelaxation. The saliva of the lone star tick, *Amblyomma americanum*, also contains the vasodilator PGE2 and, additionally, PGF2a [23, 24]. The presence of PGE2 has also been reported in *Rhipicephalus* (formerly *Boophilus*) *microplus* [25], *Haemaphysalis longicornis*, and *I. holocyclus* [88]. However, a tick histamine release factor (tHRF), secreted in *I. scapularis* saliva [21], and a novel *I. ricinus* serine proteinase inhibitor (serpin), IRS-2, which inhibits cathepsin G and chymase [22], probably also act as modulators of vascular permeability [8].

1.2.1.2 Tick Inhibitors of Platelet Aggregation

Platelet aggregation represents the initial and most immediate stage of defense to avoid blood loss during tissue injury (hemostasis). Following vascular injury, platelets adhere to the subendothelial tissue and then become activated by agonists such as collagen, thrombin, adenosine diphosphate (ADP), and thromboxane A2. 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 to form the platelet plug, promote clotting, and release vasoconstrictor substances. The platelet aggregation cascade is targeted by ticks at several stages [Table 1.1; 18]. The ability of ticks to counteract the platelet aggregation cascade occurs in several stages [89]. Thus, ticks target ADP via salivary apyrase, which hydrolyzes the phosphodiester bonds of ATP and ADP or inhibits ADP-induced platelet aggregation [31], or prevent activation of platelets by collagen [28, 38]. Interaction between fibrinogen and the GPIIb–IIIa complex is the important final step to platelet aggregation.

Integrin αIIbβ3 (glycoprotein IIb–IIIa, GPIIb–IIIa) is an inactive receptor on resting platelets which, when activated, regulates aggregation and adhesion of platelets [90]. This glycoprotein receptor binds fibrinogen resulting in a platelet–fibrinogen–platelet interaction or platelet aggregation by fibrinogen cross-linking. ADP secreted by activated platelets provokes integrin and Ca2+−dependent platelet aggregation. Thrombin, ADP, and adrenalin increase the receptor affinity to their ligands (plasma protein, fibrinogen, and von Willebrand factor), which are responsible for binding

to platelets during aggregation. Accordingly, tick saliva contains disintegrin-like peptides that block the binding of adhesive proteins to GPIIb–IIIa receptor [30, 39], therefore inhibiting the platelet–fibrinogen–platelet interaction, even if platelets are activated [29, 30, 39]. This antiplatelet strategy is used by the soft ticks, *Ornithodoros moubata* and *O. savignyi*. These ticks contain proteins named disagregin (7 kDa) and savignygrin, respectively, which bind to GPIIb–IIIa in platelets [29, 31]. Disagregin uses a motif that is different from known GPIIb–IIIa antagonists to bind to the receptor, whereas savignygrin uses the classical Arg–Gly–Asp (RGD) motif to bind to GPIIb–IIIa. The saliva of the hard tick, *Dermacentor variabilis*, contains a protein named Variabilin (4.9 kDa) which has an RGD motif and blocks this receptor. However, this peptide has little sequence homology to other GPIIb–IIIa antagonists [39]. Additionally, Ixodegrins from *I. pacificus* and *I. scapularis* display sequence similarity to Variabilin, with two additional cysteines in the RGD position [37], but their disintegrin activity has yet to be confirmed [89].

It is interesting to note that when different tick species use the same strategy to counteract a biological activity, they may still use different proteins. However, in addition to the previously described integrin *O. moubata* produces Moubatin (17 kDa), a salivary antiplatelet factor which belongs to the lipocalin family of beta-barrel structures that, in general, bind small hydrophobic molecules [34, 91]. Salivary proteins with lipocalin structure have also been described in the tick, *Rhipicephalus appendiculatus* [78]. In addition, activation of platelets by collagen is prevented, for example, by Moubatin, a specific inhibitor of collagen stimulated platelet activation from *O. moubata*, whereas tick adhesion inhibitor (TAI) identified in the same tick species inhibits the adhesion of platelets to matrix collagen [28, 35]. Another inhibitor of collagen-mediated platelet aggregation, Longicornin, was isolated from the hard tick *Haemaphysalis longicornis* [38]. However, Longicornin does not bind directly to collagen fibers and does not affect platelet adhesion to collagen, indicating that the inhibitor, similarly to Moubatin, shares a common receptor with collagen.

The strategy used by most blood feeders to block platelet aggregation is to destroy or hydrolyze the platelet agonist ADP. This is achieved by the presence of the salivary enzyme, apyrase (EC 3.6.1.5), which hydrolyses the phosphodiester bonds of nucleoside triphosphates and diphosphates but not monophosphates. Apyrase activity has been reported in the saliva of many ticks including *I. scapularis*, *O. moubata* [33, 36], and *O. savignyi* [92]. Apyrase from *R. microplus* belongs to the 5'-nucleotidase family [93]. On the other hand, apyrase activity has not been detected in the saliva of, for example, *A. americanum* [23], but increased prostaglandin levels in the saliva of this tick inhibit platelet aggregation by preventing ADP secretion during platelet activation [23, 94].

Thrombin, the protease activated at the end of the blood coagulation cascade, is a potent agonist of platelet activation. Salivary antithrombins from soft ticks, including *O. moubata* and *O. savignyi*, have been characterized as anticoagulants as well as inhibitors of platelet aggregation induced by thrombin [32, 95]. The serpin IRS-2 from *I. ricinus* inhibits both cathepsin G- and thrombin-induced platelet aggregations [22].

1.2.1.3 Tick Inhibitors of the Blood Coagulation Cascade

Blood coagulation involves a series of enzymatic reactions whereby 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, which in turn cleaves fibrinogen into fibrin. Polymerization of fibrin results in blood clot formation. A number of inhibitors of serine proteases involved in the coagulation cascade are the most characterized entities from the saliva of ticks. Anticoagulants from ticks can be classified based on four mechanisms of action: thrombin inhibitors, inhibitors of activated factor X (FXa), inhibitors of the extrinsic tenase complex (ETC), and contact system protein inhibitors [96], with thrombin and FXa being the most common targets.

Inhibitors of Thrombin

A strategy employed by ticks to inhibit the blood coagulation cascade is to block thrombin activity. Thrombin is the last enzyme in the blood coagulation cascade and is a strong agonist for platelet aggregation. Several specific direct thrombin inhibitors with various modes of action have been characterized in the salivary glands of both soft and hard ticks [32, 40, 42, 52, 59, 95, 164; Table 1.1]. Variegin, characterized from *A. variegatum*, has structural similarity to, but is much more potent than, hirulog, a 20-amino-acid synthetic thrombin inhibitor based on the natural leech peptide hirudin [51]. In addition, Boophiline [57] and Rhipilin-1 [97] have been described in the saliva of the ticks *R. microplus* and *R. haemaphysaloides*, respectively. Americanin, the salivary antithrombin from *A. americanum*, is a specific, reversible, and a slow tight-binding inhibitor of thrombin [52]. The salivary antithrombin from *O. savignyi* is a 12.4 kDa protein named Savignin [32] which is a slow, tight-binding inhibitor of thrombin and interacts with the active site as well as with the binding exosite of this protease [30]. Savignin is 63 % identical to Ornithodorin, the salivary antithrombin from *O. moubata* [44]. Soft tick antithrombins insert their N-terminal residues into the thrombin active site inhibiting the activity of this protease, whereas traditional Kunitz-type inhibitors use a central, reactive loop. In addition, various other peptides with protease-inhibiting activity, such as Microphilin [58] and anticoagulant protein (BmAP) [42] from *R. microplus* or Calcaratin [59] from *Boophilus calcaratus*, are not ranked in any of the previous groups.

Inhibitors of Factor Xa

The tick anticoagulant peptide (TAP) from the saliva of the soft tick, *O. moubata*, is the most intensively studied soft tick anticoagulant [43]. TAP has some homology with Kunitz-type inhibitors, with a molecular mass of 6.977 kDa, but is a highly

specific, reversible competitive inhibitor of factor X activation [FXa; 62]. FXa is involved in the activation of thrombin, hence, the importance of blocking the activity of this protease for hematophagous arthropods. TAP binds FXa with a dissociation constant of 180 pM. The soft tick, *O. savignyi*, also contains an FXa inhibitor with 46 % identity to TAP [45]. Moreover, FXa inhibitors are reported from the saliva of the lone star tick, *A. americanum* [41], and from the saliva of *Hyalomma truncatum* [61]. Amblyomin-X recombinant protein derived from an *A. cajennense* transcript encoding a protein containing an N-terminal Kunitz-type domain and a C-terminus with no homology to any known sequences was also found to inhibit FXa [53]. Salp14, a protein belonging to the salivary protein (Salp) family, was identified in saliva of *I. scapularis* and specifically inhibits the FXa active site [48, 98].

Two anticoagulants have been identified from the salivary glands of the tick, *O. savignyi*. These two anticoagulants, termed BSAP1 and BSAP2, have molecular masses of 9.3 and 9.2 kDa, respectively, and are inhibitors of the extrinsic pathway of the blood coagulation cascade [46]; no sequence information is available for them yet. An anticoagulant from *R. appendiculatus* saliva probably targets components of the prothrombinase complex different from FXa [56]. Inhibitors of FV and FVII have been described for *D. andersoni* [60].

Inhibitors of the Extrinsic Tenase Complex (ETC)

Ixolaris, a tissue factor (TF) pathway inhibitor belonging to a novel group of tick anticoagulants, was isolated from *I. scapularis* [47, 99]. Ixolaris is a small protein (9.8 kDa) of 140 amino acids containing ten cysteines and two Kunitz-type domains [47]. It inhibits the intrinsic pathway and shows homology to Salp14 and Salp9Pac, also present in saliva of *I. scapularis*. Recombinant Salp14 prolongs activated partial thromboplastin time (APTT) and specifically inhibits factor Xa [48]. These proteins probably belong to a novel family of anticoagulants with related functions.

Inhibitors of Protein Contact System

Rhipicephalus microplus trypsin inhibitor-A (BmTI-A) is a kallikrein and elastase inhibitor of the BPTI–Kunitz type [100]. The inhibitor increases APTT but does not prolong prothrombin time (PT) or thrombin time (TT). In addition, a plasma kallikrein–kinin system inhibitor named haemaphysalin was identified in *H. longicornis* [54]. This inhibitor interferes with reciprocal activation between factor XII and prekallikrein. A contact phase inhibitor (Ir-CPI) present in *I. ricinus* salivary glands inhibits the intrinsic coagulation pathway and, to a much lesser extent, fibrinolysis in vitro [50].

1.2.2 Additional Tick Salivary Anti-hemostatic Activities

Many biological activities which may be related to host hemostasis have been described in tick saliva.

A fibrinolytic activity has been detected in *I. scapularis* saliva which is due to the presence of a metalloprotease. The role of salivary metalloproteases in tick feeding appears to be related to their antifibrinogen- and antifibrin-specific activities [101]. These proteolytic activities are metal dependent and target gelatin, fibrin, fibrinogen, and fibronectin but not collagen or laminin. These activities may confer additional anticoagulant activity by preventing the formation of the fibrin clot or dissolving the already formed blood clot. Kunitz-type serine proteinase inhibitors (RsTI, 8–18 kDa) were isolated from the larvae of *R. sanguineus* [102]. Their role in hemostasis is predicted to be similar to serine proteinase inhibitors such as those found, for example, in *R. microplus* [100], and they target plasmin and neutrophil elastase.

Serine protease inhibitors with similarity to the insect serpin family have also been discovered in ticks [103, 104]. Tick serpins might also interact with host defense responses, including hemostasis.

Calcium-binding proteins belonging to the calreticulin family are also present in tick saliva. They may play a modulating role in host hemostasis through binding calcium ions required as coagulation enzyme cofactors [105].

Phospholipase A2, most probably responsible for the hemolytic activity of saliva, has been detected in *A. americanum* [106]. This salivary activity hydrolyzes arachidonyl phosphatidylcholine and is activated by submicromolar calcium. It has been suggested that this phospholipase (55 kDa) may be involved in producing PGE2 from host substrates and that it may also be responsible for the hemolytic activity reported in *A. americanum* saliva [107].

Ixodes scapularis saliva has been reported to inhibit key pro-inflammatory activities of neutrophils such as aggregation following activation by anaphylatoxins, the release of enzymes, production of oxygen radicals, or the phagocytosis of bacteria [108].

Moreover, anti-IL-8 activity was reported from the saliva of *D. reticulatus*, *A. variegatum*, *R. appendiculatus*, *H. inermis*, and *I. ricinus* [109].

Histamine is a highly potent inflammatory mediator and a vasoactive factor which binds to H1 and H2 receptors, causing edema and erythema by dilating and increasing the permeability of small blood vessels. Additionally, histamine is a regulator of the T-cell response [110]. *Rhipicephalus appendiculatus* has a set of novel salivary histamine-binding proteins with a lipocalin structure [78]. Interestingly, *R. appendiculatus* histamine-binding proteins are beta-barrel structures with two binding sites instead of one binding site for hydrophobic molecules. Similar proteins were identified in salivary glands of *I. scapularis* [111] and *A. americanum* [112].

Another mediator of the inflammatory response, serotonin, is secreted by tissue mast cells (in rodents) and has similar activities to histamine. A serotonin-binding protein (22 kDa) was isolated from *D. reticulatus* salivary glands [77]. This protein

is similar in structure to the *R. appendiculatus* histamine-binding protein with two binding sites, one of which binds histamine, while the other is slightly larger and is able to accommodate and bind serotonin [165].

Ixodes scapularis has a salivary protein that specifically inhibits the alternative pathway of the complement cascade [113]. Isac (*I. scapularis* anticomplement) is an 18 kDa protein that inhibits the formation of C3 convertase, which acts as a regulator of the complement cascade [6]. However, the sequence of Isac is not homologous to any known complement cascade regulator. Longistatin, a plasminogen activator identified recently in *H. longicornis*, was found to hydrolyze fibrinogen and delay fibrin clot formation [55].

1.2.3 Tick Compounds and Angiogenesis

Angiogenesis is characterized by the invasion, migration, and proliferation of smooth muscle and endothelial cells, a process that involves sprouting of new capillaries from existing blood vessels. It is a highly regulated process, essentially in many physiologic conditions, including development, reproduction, and wound repair. Vascular cell adhesion molecules appear to contribute to its regulation, and several pathologic conditions have been related to unregulated angiogenesis, as in tumor development [114]. Disintegrins have been characterized as platelet aggregation inhibitors that can prevent adhesion of tumor cell lines to extracellular matrix (ECM) components. Saliva from *I. scapularis* has been reported as a potent inhibitor of angiogenesis [115]. Relatively few disintegrins have been molecularly cloned and expressed (Table 1.2); therefore, salivary disintegrin inhibitors of angiogenesis remain a relatively unexplored field of investigation with great promise for a range of medical applications.

Ticks are the most important source of disintegrins among arthropods. In fact, ticks must inhibit the interaction of cells with ECM components during the long feeding period as part of the mechanism by which they keep blood flowing through its proboscis. Below we detail the salivary disintegrins which have been characterized molecularly or functionally in ticks. These disintegrins are described in Table 1.2.

1.2.3.1 Disintegrins and Functions

Variabilin

Variabilin is present in the SGs of the hard tick *Dermacentor variabilis* and inhibits platelet aggregation induced by ADP ($IC_{50} \sim 150$ nM), collagen, and thrombin receptor peptide SFLLRNP. It also blocks platelet adhesion to fibrinogen. It is a potent antagonist of the fibrinogen receptor integrin αIIbβ3 and the vitronectin receptor αvβ3 [39].

Table 1.2 Tick salivary disintegrins [116]

Name [reference]	Tick species	Mol wt	IC50	R/S/P[a]	Tripeptide	Cell target	Integrin
Variabilin [39]	*D. variabilis*	5	157 nM	N/N/Y	RGD	+2.5	αIIbβ3
ISL929/1373 [29]	*Ixodes* sp.	10	[d]	Y/N/Y	[d]	Neutrophils	αMβ2[d]
Monogrin [117]	*A. monolakensis*	10	150 nM	Y/N/Y	RGD	Platelets	αIIβ3
Tick antiplatelet inhibitor (TAI) [35][b]	*O. moubata*	15	8 nM	N/N/Y	[d]	Platelets EC	α2β1, α1β1
Disagregin [29, 118]	*O. moubata*	6	104 nM	N/N/Y	RED	Platelets	αIIbβ3
Ixodegrin [37][c]	*Ixodes* sp.	7	[d]	N/N/N	RGD	Platelets[d]	αIIββ3
Savignygrin [30]	*O. savignyi*	7	130 nM	N/N/Y	RGD	Platelets	αIIbβ3

[a]*R* obtained in recombinant form, *S* structure available, *P* inhibition of cell function tested with recombinant or purified proteins, *Mol wt* molecular weight (approximate), *EC* endothelial cell, *NIF* neutrophil inhibitory factor, *HPI* hookworm platelet inhibitor, *TAI* tick adhesion inhibitor
[b]TAI has not been molecularly identified
[c]Ixodegrin has not been expressed or purified
[d]IC50, or integrin specificity unknown, or not confirmed

Disagregin

Disagregin is a 6 kDa protein from the SGs of *O. moubata* that potently blocks ADP-induced platelet aggregation (IC_{50} ~ 150 nM) [29]. In addition, disagregin inhibits platelet aggregation by different agonists, blocks platelet adhesion to fibrinogen, binds to resting and ADP-activated platelets, and also binds integrin αIIbβ3 in activated platelets with K_D ~ 40 nM. Cross-linking experiments also demonstrated binding of disagregin to integrin αIIbβ3. In contrast, disagregin does not affect endothelial cell adhesion to vitronectin, which is mediated by integrin αvβ3 [29].

Savignygrin

Savignygrin is a platelet aggregation inhibitor purified from the soft tick *O. savignyi* and is similar to disagregin. It inhibits platelet aggregation induced by ADP (IC_{50} ~ 130 nM), collagen, thrombin receptor-activating peptide, and epinephrine. It also blocks binding of α-CD41 to platelets, binding of αIIbβ3 to fibrinogen, and adhesion of platelets to fibrinogen, suggesting it targets the fibrinogen receptor. Savignygrin forms a complex with both αIIbβ3 subunits, and this complex formation is unaffected by the activation state. This disintegrin belongs to the BPTI family of serine protease inhibitors and presents the integrin RGD-recognition motif on the substrate-binding loop of the Kunitz fold [31]. Additionally, savignygrin can

promote disaggregation—which is an inhibition of platelet aggregation at a post-aggregation level—through occupation of the αIIbβ3 receptor. Savignygrin-like molecules have also been cloned from the soft tick *O. coriaceus* [115].

Monogrin

Monogrin was purified from the SGs of the soft tick *Argas monolakensis*. Both recombinant and purified monogrins block ADP-induced platelet aggregation (IC_{50} ~ 150 nM) but not initiation of shape change. Monogrins were found to interact with integrin αIIbβ3 by surface plasmon resonance [19].

Ixodegrin

This family was named after identification of *I. pacificus* [37] and *I. scapularis* putative cysteine-rich proteins with an RGD or KGD domain indicative of proteins that interfere with fibrinogen binding to platelets, acting as platelet aggregation inhibitors. Ixodegrins display sequence similarity to variabilin. Recently, a protein described from the SGs of the tick *Amblyomma variegatum* showed similarities to *I. scapularis* ixodegrins [15]. Ixodegrin remains to be produced in a heterologous system to confirm its functional activity.

Tick Antiplatelet Inhibitor (TAI)

TAI (~15 kDa) has been purified from *O. moubata* SGs but has not been molecularly cloned. It inhibits platelet adhesion to soluble collagen under static conditions (IC_{50} ~ 8 nM) without affecting the onset or maximum aggregation triggered by collagen or other platelet agonists. TAI also affects endothelial cell adhesion to collagen and has partial inhibitory activity for fibronectin-mediated platelet adhesion. Further, it outcompetes anti-α2β1 monoclonal antibody Gi9 binding to platelets, suggesting it is an integrin α2β1 antagonist [35].

ISL929/1373

ISL929 and ISL1373 are two *I. scapularis* salivary proteins that have been described as neutrophil inhibitors. Expression of both molecules is induced upon tick feeding and mostly expressed in the salivary gland. Recombinant ISL929 and ISL1373 appear to reduce expression of β2 integrins and to decrease production of superoxide by neutrophils in vitro. Furthermore, mice immunized with both proteins had increased number of neutrophils at the site of attachment, suggesting that they interfere with inflammation in vivo [15].

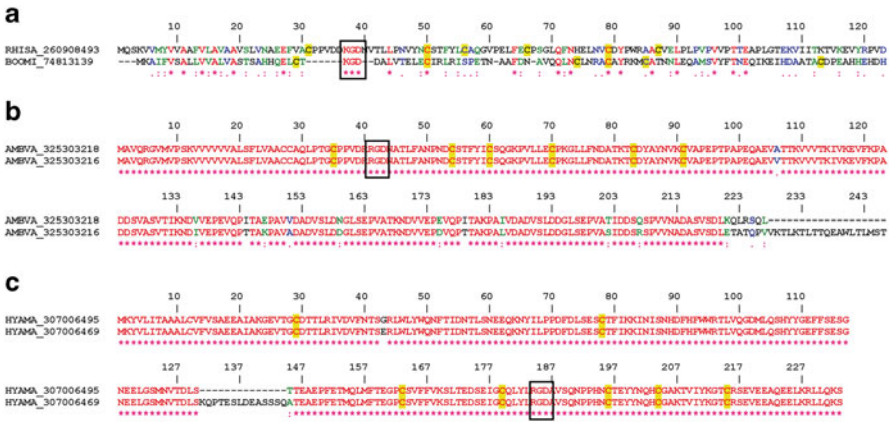

Fig. 1.1 ClustalW alignment for putative KGD (**a**) and RGD (**b** and **c**) disintegrins from metastriate ticks [116]

As described in Sect. 1.2.3.1, tick disintegrins exhibit substantial complexity and variability. In Sect. 1.2.3.2, we will focus on the structure of the different families of disintegrins, including both known and putative salivary disintegrins.

1.2.3.2 Disintegrins and Comparatives Structures

Disintegrins from Metastriate Ticks (*Dermacentor*, *Rhipicephalus*, and *Amblyomma*)

KGD and RGD Disintegrin Family

Two distinct KGD disintegrins from the salivary glands of *Rhipicephalus* ticks have been identified as mucin-like proteins (Fig. 1.1a). RGD disintegrins from *Amblyomma* sp. which have been identified as chitin-binding peritrophins (midgut protein) are shown in Fig. 1.1b. Figure 1.1c displays two sequences from *Haemaphysalis* sp. salivary gland which belong to the lipocalin family, one of them having an insertion between amino acids 130 and 145 [116]. Perhaps the RGD in these proteins is adapted for integrin recognition. *Dermacentor* sp. salivary glands are characterized by the presence of two highly related members, including 14 cysteines, and a KGD found between cysteines 10 and 11 [116]. Interestingly, a shorter sequence from *R. appendiculatus* was found to display a high degree of similarity to the other two members from *Dermacentor* sp.; the KGD is also located between two cysteines. Perhaps these molecules have evolved to interact with β3 integrins.

KTS/RTS Disintegrin Family

A family of KTS disintegrins has been found in *A. americanum* salivary gland (Fig. 1.2). These proteins belong to the Kunitz family of protein inhibitors. There are abundant transcripts coding for putative disintegrins with which the KTS tripeptide appears to

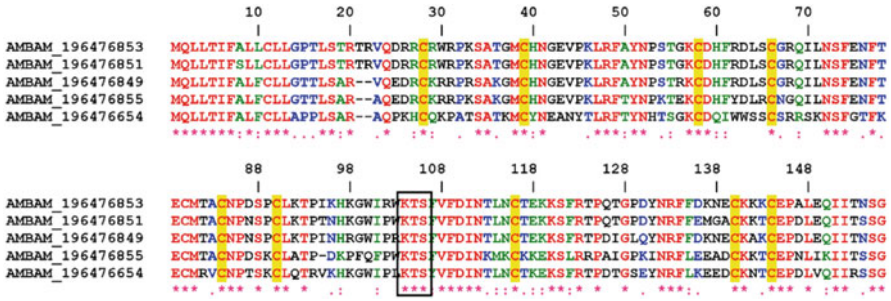

Fig. 1.2 ClustalW alignment for putative KTS disintegrins from *Amblyomma* sp. ticks [116]

be properly placed to interact with α1β1 integrins, assuming the specificity is the same as reported for the viperidae[1] KTS [119]. These molecules contribute to blocking endothelial cell adhesion to collagen and to assisting in the inhibition of angiogenesis and host response to injury [115]. Three other molecules with KTS or RTS motifs were, respectively, found in *Amblyomma* or *Rhipicephalus* sp. [116].

Duodegrins

Bioinformatic analysis identified several duodegrin sequences with more than one tripeptide motif. In some proteins, VGD and RTS motifs exist, while in others, RED and VGD motifs were identified [116]. These sequences code for cysteine-rich proteins of high molecular weight in the midgut of ticks and include the protein, BM86, which is used as a vaccine against tick infestation [120, 121]. While its function is unknown, it might be related to protection of the tick gut against host neutrophil attack.

Disintegrins from Prostriate Ticks (*Ixodes* sp.)

RGD, KGD, and VGD Disintegrin Family

Short proteins from *Ixodes* sp. which display a typical RGD flanked by cysteines 5 and 6 are shown in Fig. 1.3a. They have been classified as putative secreted salivary proteins, since they have no match to other proteins. Likewise, a second family of putative RGD secreted sialogenins is presented in Fig. 1.3b. In Fig. 1.3c, two related putative disintegrins are aligned, one of which (IXOSC_67083633) is named ixodegrin-2A [37]. In addition, KGD and VGD motifs, shown in Fig. 1.4a, were reported in ixodid ticks [116, 122].

[1] The Viperidae (vipers) are a family of venomous snakes found all over the world, except in Antarctica, Australia, New Zealand, Ireland, Madagascar, Hawaii, various other isolated islands, and north of the Arctic Circle.

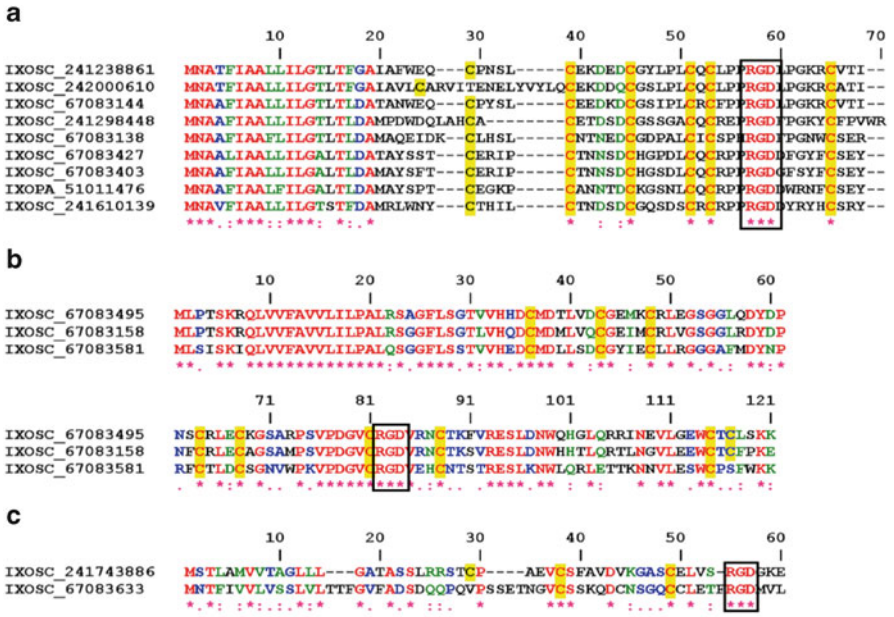

Fig. 1.3 ClustalW alignment for putative RGD (**a**) and RGD (**b** and **c**) disintegrins from Ixodidae ticks [116]

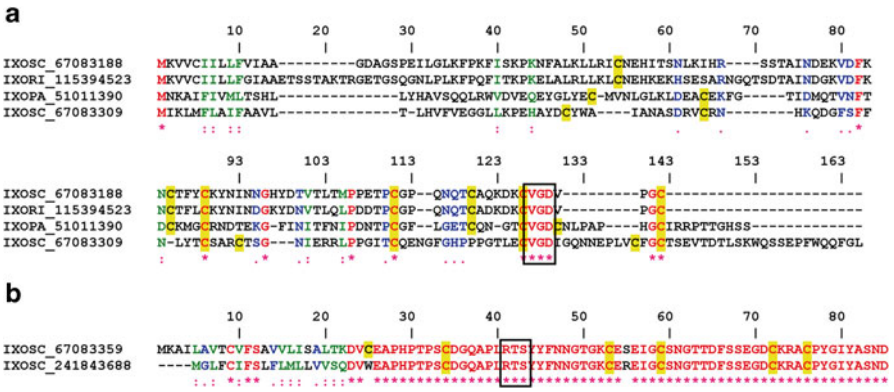

Fig. 1.4 ClustalW alignment for putative VGD (**a**) and RTS (**b**) disintegrins from Ixodidae ticks [116]

RTS Disintegrin Family

One sequence was found to display RTS motif properly flanked by cysteines, suggesting that this molecule might work as a disintegrin targeting α1β1 (Fig. 1.4b). This is a putative secreted protein without database hits. As reported for other RTS disintegrins, this sequence may also contribute to blockade of angiogenesis by tick

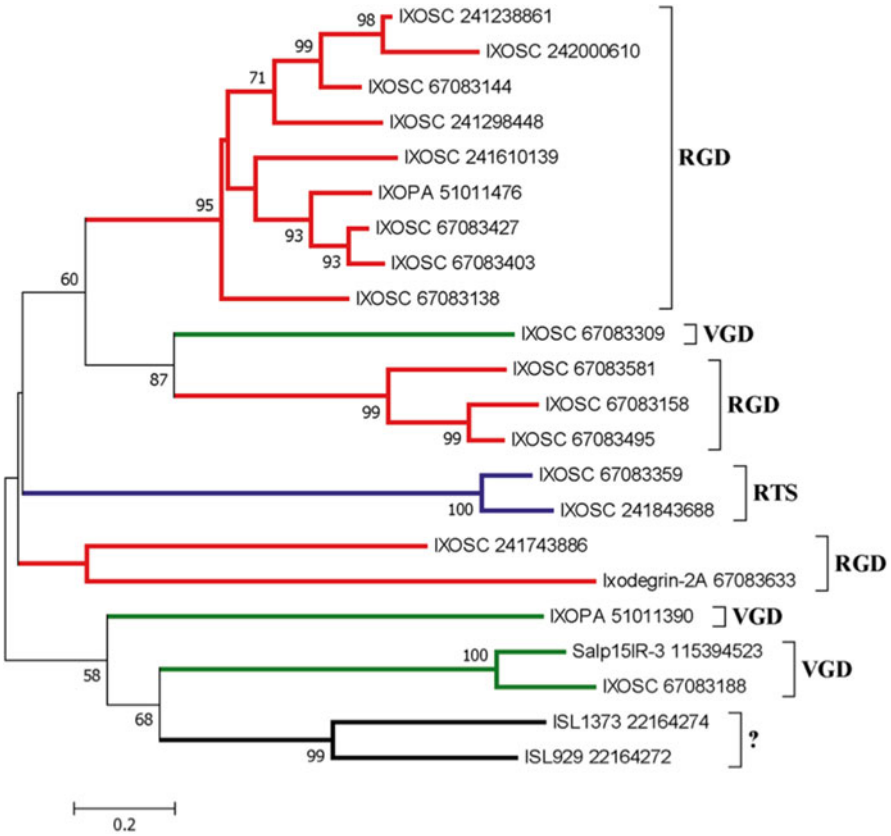

Fig. 1.5 Neighbor-joining phylogram for the Ixodidae sequences presented in Figs. 1.5 and 1.6. The numbers in the phylogram nodes indicate percent bootstrap support for the phylogeny. The bar at the bottom indicates 20 % amino acid divergence in the sequences [116]

saliva [115]. Figure 1.5 displays a phylogenetic tree containing several salivary disintegrins from Ixodidae. It is clear that they are positioned in a separate clade as different families.

Disintegrins from *Ornithodoros* sp.

The repertoire of anti-hemostatics in hard ticks which feed for several days differs significantly from those found in soft ticks [7, 15, 19, 37, 117, 123, 124]. Two short RGD disintegrins from *O. parkeri* (Fig. 1.6a) have been identified as savignygrin-like-1 and -2 [124]. They are likely platelet aggregation inhibitors. Figure 1.6b contains two other sequences containing RGD motifs from *O. coriaceus* [123] belong to the lipocalin family of proteins and have eight cysteines. It remains unclear whether they target platelets, neutrophils, or endothelial cells until they are obtained in recombinant form for further experimentation.

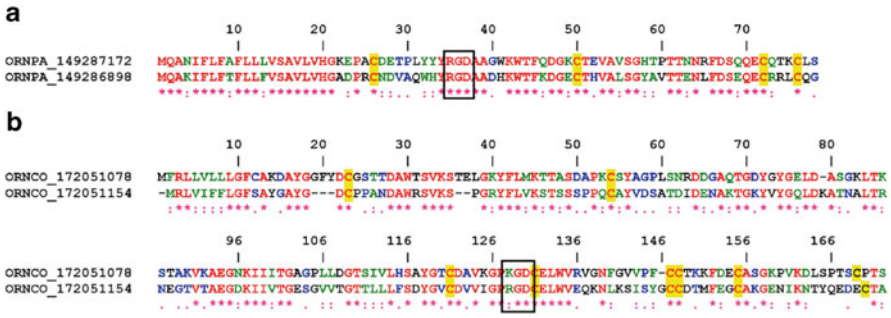

Fig. 1.6 ClustalW alignment for putative RGD (**a**) and K/RGD disintegrins, (**b**) from *Ornithodoros* ticks [116]

1.2.4 Tick Compounds and Host Immunity

The first lines of defense against invading pathogens are host cellular innate immune responses and the complement system. Tick salivary compounds can modulate both innate and acquired immunity of the hosts to protect themselves from inflammation and host immune responses [5, 16, 69, 125, 166]. 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 [126, 127]. Repeated tick infestations and salivary gland extracts are known to suppress production of macrophage pro-inflammatory cytokines and the secretion of Th1 cytokines, whereas they upregulate Th2 cytokines, indicating a Th2 polarization of the host immune response [128, 129].

Despite relatively extensive knowledge of tick-induced host immunomodulation, only a few active molecules have been identified and characterized in tick salivary glands [5, 6, 64, 69, 71, 73, 80, 82].

1.2.4.1 Innate Immune Responses

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, like neutrophils [108], NK cells [130], macrophages [131], T cells [73, 132], and dendritic cells [133]. Moreover, tick saliva contains a variety of inhibitory activities directed against many pro-inflammatory cytokines such as IL-2 and chemokines (CCL2/MCP-1, CCL3/MIP-1α, CCL5/RANTES, and CCL11/eotaxin) [127]. Evasins, a family of chemokine-binding proteins, have been detected in *R. sanguineus* ticks [71]. This family show selectivity to different chemokines: Evasin-1 binds to CCL3, CCL4, and CCL18; Evasin-3 binds to CXCL8 and CXCL1; and Evasin-4 binds to CCL5 and CCL11 [71, 81]. In addition, Evasin-3-like activities were described for

other metastriate tick species, which provide other evidence that ticks control host neutrophil functions during feeding.

A dipeptidyl carboxypeptidase activity was found to account for the salivary kininase activity of *I. scapularis* [134]. In fact, bradykinin and histamine are important mediators of itch and pain and can found to stimulate host grooming and removal of the feeding ticks. However, tick salivary kininases hydrolyze circulating kinins (e.g., bradykinin). Hard ticks also produce amine-binding proteins of the lipocalin family. 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 *R. appendiculatus* [78, 165], and the gene for a protein that binds both serotonin and histamine (SHBP) was identified in *D. reticulatus* [77]. In addition, 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 remodeling [79]. Ticks also produce proteins that mimic host proteins to evade the host immune response [11]. A tick macrophage migration inhibitory factor (MIF) has been described in *A. americanum* [80]. It inhibits the migration of macrophages and most probably protects the tick from macrophage attack [11].

1.2.4.2 The Complement System

The complement system links the innate and adaptive responses of the host immune system and is activated via three main pathways (alternative, classical, and lectin pathway). The alternative pathway is the major line of defense against pathogens and ticks [11]. Several molecules with anticomplement activities were identified in tick salivary glands. Isac, Salp20, and Isac-1 from *I. scapularis* [6, 62] and the Isac paralogues IRAC I and II from *I. ricinus* [79, 135] inhibit specifically the formation of the C3 convertase of the alternative pathway by blocking binding of complement factor B to complement C3b. In addition, OmCI (*O. moubata* complement inhibitor) belonging to proteins of the lipocalin family has been the first natural complement inhibitor isolated from a soft tick that specifically targets the C5 activation step in the complement cascade [136; Table 1.1].

1.2.4.3 Acquired Immune Responses

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 [11]. Moreover, ticks have evolved ways to alter production of T-lymphocyte cytokines. Generally, it has been reported that tick saliva polarizes the host immune response toward 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) [5, 137, 138]. The inhibition of T-cell

responsiveness to mitogens could result from the direct effect of salivary gland proteins on lymphocytes or from their production of IL-10, while upregulation of IL-4 and IL-10 probably leads to the development of a Th2 response [4, 132, 137, 138].

A 36 kDa protein (p36) present in the saliva of feeding *D. andersoni* has been characterized as a T-cell inhibitor [73; Table 1.1]. An immunosuppressor, Iris, was detected in *I. ricinus* females [69]. Iris suppresses T-lymphocyte proliferation, induces a Th2-type immune response, and inhibits the production of pro-inflammatory cytokines (IL-6 and TNF-alpha). A 15 kDa salivary gland protein from *I. scapularis* (Salp15) 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 [64, 122]. 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 the saliva of *I. scapularis* [66]. Sialostatin L, a protein with inhibitory action against cathepsin L that displays anti-inflammatory properties and inhibits proliferation of cytotoxic T lymphocytes, was also found in the saliva of *I. scapularis* [68].

B-cell inhibitory proteins (BIP and BIF) were identified in *I. ricinus* and *Hyalomma asiaticum asiaticum*, respectively [71, 74]. Apart from substances modulating the host immune responses, ticks also produce immunoglobulin-binding proteins that protect them primarily from ingested host immunoglobulins [139].

1.2.5 Tick Saliva and Pathogen Transmission

Pathogens (bacteria, viruses, piroplasms, etc.) are transmitted from the salivary glands of the tick to the host via salivary fluid. Some pathogens co-injected with saliva may be more infective because the blood feeders' saliva changes the physiology of the host at the feeding site by injecting an array of bioactive molecules. In addition, anti-inflammatory mechanisms may also enhance the transmission of tick-borne pathogens [136, 137]. 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 [129, 139; Table 1.3]. The saliva of the tick, *D. reticulatus*, promoted vesicular stomatitis virus growth in vitro [162], while the saliva of *D. reticulatus*, *I. ricinus*, and *R. appendiculatus* enhanced tick-borne encephalitis virus transmission [141]. In another example, the saliva of the tick, *R. appendiculatus*, enhanced *Thogotovirus* transmission [140] and, in combination with interleukin-2, increased *Theileria parva* infection in lymphocytes [163]. *I. ricinus* saliva increased bacteremia (*Borrelia afzelii*) in C3H mice [142] and exacerbated the proliferation of the bacterium *Francisella tularensis* in mice [146]. Finally, there is evidence that the pathogen *B. 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 [65].

Table 1.3 Examples of saliva-assisted transmission (SAT) of tick-borne pathogens [11]

Pathogen	Tick species	SAT factor	Effect	References
THOV	*Rhipicephalus appendiculatus*	SGE[a]	Enhanced transmission and infectivity	[140]
TBEV	*Ixodes ricinus*	SGE	Enhanced transmission and infectivity	[141]
Borrelia afzelii	*I. ricinus*	SGE	Accelerating effect on spirochete proliferation in the host, suppression of pro-inflammatory cytokines	[142]
Borrelia burgdorferi s.s.	*I. ricinus*	SGE	Accelerating effect on spirochete proliferation in the host	[143]
B. burgdorferi s.s.	*I. ricinus*	Saliva	Increased spirochete load in host skin, increased transmission to ticks	[144]
Borrelia lusitaniae	*I. ricinus*	SG[b] lysate	Increase of spirochete loads in target organs	[145]
B. burgdorferi s.s.	*I. scapularis*	SG lysate	Increase of spirochete loads in target organs	[145]
Francisella tularensis	*I. ricinus*	SGE	Accelerates proliferation of the bacteria in the host	[146]
THOV	*R. appendiculatus*		Non-viremic transmission	[147]
TBEV	*I. ricinus*		Non-viremic transmission	[148]
Borrelia afzelii	*I. ricinus*		Co-feeding transmission	[149]
B. burgdorferi s.s.	*I. ricinus*		Co-feeding transmission	[150]
B. burgdorferi s.s.	*I. scapularis*		Co-feeding transmission	[151]
TBEV	*I. ricinus*	Saliva	In vitro modulation of infection rate of DCs[c] and production of cytokines	[152]
B. afzelii	*I. ricinus*	SGE	Anti-inflammatory activities	[153]
B. afzelii	*I. ricinus*	SGE	Impairment of signal pathways in DCs	[154, 155]
		SGE	Impairment of DC functions	[156]
B. burgdorferi	*I. ricinus*	Tick feeding	Modulation of skin innate immunity	[157]
	I. ricinus		BIP inhibition of B lymphocyte proliferation induced by *B. burgdorferi* lipoproteins OspA and OspC	[158]
B. burgdorferi	*I. ricinus*		Salp15 Iric-1, a Salp15 homologue, binds to OspC of *B. burgdorferi* s.s., *B. garinii*, and *B. afzelii*	[159]

(continued)

Table 1.3 (continued)

Pathogen	Tick species	SAT factor	Effect	References
B. burgdorferi	*I. scapularis*		Salp15, immunosuppressive functions, binds to OspC of *B. burgdorferi*, protects the spirochete from antibody-mediated killing, facilitates transmission and replication of the spirochete	[65]
			Salp25D, antioxidant, facilitates the acquisition of spirochetes by the vector from an infected mammalian host	[160]
			Salp20, inhibits complement, facilitates pathogen survival	[6]
			P8, lectin complement pathway inhibitor, facilitates pathogen transmission	[161]
Anaplasma phagocytophilum	*I. scapularis*		Salp16, facilitates migration of the pathogen to salivary glands	[171]

[a]*SGE* salivary gland extract
[b]*SG* salivary gland
[c]*Dcs* dendritic cells

1.3 Conclusion

The area of tick saliva research has taken a great leap forward in recent years. Molecular biology and high-throughput approaches are increasing our knowledge of the proteins present in the salivary glands of ticks. This new information together with the vast knowledge acquired over the last three decades on the pharmacology of tick saliva and immune responses to tick salivary proteins has the potential to open new venues to the understanding of tick saliva on blood feeding and pathogen transmission. Understanding of the molecular basis of the strategies used by ticks to evade host resistance and immune mechanisms that lead to host protection offers great promise to engender new strategies for the use of tick salivary antigens as vaccines to control vector-borne diseases.

References

1. Jongejan, F., & Uilenberg, G. (2004). The global importance of ticks. *Parasitology, 129*, S3–S14.
2. Ribeiro, J. M. C. (1987). Role of saliva in blood-feeding by arthropods. *Annual Review of Entomology, 32*, 463–478.
3. Ribeiro, J. M. C. (1995). Blood-feeding arthropods: Live syringes or invertebrate pharmacologists? *Infectious Agents and Disease, 4*, 143–152.
4. Wikel, S. K. (1999). Tick modulation of host immunity: An important factor in pathogen transmission. *International Journal of Parasitology, 28*, 851–859.
5. Gillespie, R. D., Mbow, M. L., & Titus, R. G. (2000). The immunomodulatory factors of blood feeding arthropods saliva. *Parasite Immunology, 22*, 319–331.
6. Valenzuela, J. G., Charlab, R., Mather, T. N., & Ribeiro, J. M. C. (2000). Purification, cloning and expression of a novel salivary anti-complement protein from the tick, *Ixodes scapularis. Journal of Biological Chemistry, 275*, 18717–18723.
7. Francischetti, I. M., Sa-Nunes, A., Mans, B. J., Santos, I. M., & Ribeiro, J. M. (2009). The role of saliva in tick feeding. *Frontiers in Bioscience, 14*, 2051–2088.
8. Chmelar, J., Calvo, E., Pedra, J. H. F., Francischetti, I. M., & Kotsyfakis, M. (2012). Tick salivary secretion as a source of anti-hemostatics. *Journal of Proteomics, 75*, 3842–3854.
9. Randolph, S. E. (2009). Tick-borne disease systems emerge from the shadows: The beauty lies in molecular detail, the message in epidemiology. *Parasitology, 136*, 1403–1413.
10. Nuttall, P. A., & Labuda, M. (2004). Tick-host interactions: Saliva-activated transmission. *Parasitology, 129*, S177–S189.
11. Kazimírová, M., & Štibrániová, I. (2013). Tick salivary compounds: Their role in modulation of host defenses and pathogen transmission. *Cellular and Infection Microbiology, 43*, 1–19.
12. Ribeiro, J. M. C. (1989). Role of saliva in tick/host interactions. *Experimental and Applied Acarology, 7*, 15–20.
13. Maritz-Olivier, C., Christian Stutzer, C., Jongejan, F., Neitz, A. W. H., & Gaspar, A. R. M. (2007). Tick anti-hemostatics: Targets for future vaccines and therapeutics. *Trends in Parasitology, 23*, 397–407.
14. Karim, S., Singh, P., & Ribeiro, J. M. (2011). A deep insight into the sialotranscriptome of the gulf coast tick, *Amblyomma maculatum. PLoS ONE, 6*(12), e28525.
15. Ribeiro, J. M., Anderson, J. M., Manoukis, N. C., Meng, Z., & Francischetti, I. M. (2011). A further insight into the sialome of the tropical bont tick, *Amblyomma variegatum. BMC Genomics, 12*, 136–147.
16. Valenzuela, J. G. (2004). Exploring tick saliva: From biochemistry to "sialomes" and functional genomics. *Parasitology, 129*, S83–S94.
17. Arocha-Piñango, C. L., Marchi, R., Carvajal, Z., & Guerrero, B. (1999). Invertebrate compounds acting on the hemostatic mechanism. *Blood Coagulation and Fibrinolysis, 10*, 43–68.
18. Kazimírová, M. (2007). Chapter VIII. Bioactive compounds in ticks acting on host thrombohemostasis. In V. Wiwanitkit (Ed.), *Thrombohemostatic disease research* (pp. 95–113). New York: Nova Science Publishers, Inc.
19. Mans, B. J., Andersen, J. F., Francischetti, I. M. B., Valenzuela, J. G., Schwan, T. G., Pham, V. M., Garfield, M. K., Hammer, C. H., & Ribeiro, J. M. C. (2008). Comparative sialomics between hard and soft ticks: Implications for the evolution of blood-feeding behavior. *Insect Biochemisry and Molecular Biology, 38*, 42–58.
20. Ribeiro, J. M. C., Makoul, G. T., & Robinson, D. R. (1988). *Ixodes dammini*: Evidence for salivary prostacyclin secretion. *Journal of Parasitology, 74*, 1068–1069.

21. Dai, J., Narasimhan, S. Z., Liu, L., Wang, P., & Fikrig, E. (2010). Tick histamine release factor is critical for *Ixodes scapularis* engorgement and transmission of the Lyme disease agent. *PLoS Pathogen, 6*, e1001205. doi:10.1371/journal.ppat.1001205.
22. Chmelar, J., Oliveira, C. J., Rezacova, P., Francischetti, I. M., Kovarova, Z., Pejler, G., et al. (2011). A tick salivary protein targets cathepsin G and chymase and inhibits host inflammation and platelet aggregation. *Blood, 117*, 736–744.
23. Ribeiro, J. M. C., Evans, P. M., MacSwain, J. L., & Sauer, J. (1992). *Amblyomma americanum*: Characterization of salivary prostaglandins E2 and F2 alpha by RP-PLC/bioassay and gas chromatography-mass spectrometry. *Experimental Parasitology, 74*, 112–116.
24. Aljamali, M., Bowman, A. S., Dillwith, J. W., Tucker, J. S., Yates, G. W., Essenberg, R. C., & Sauer, J. R. (2002). Identity and synthesis of prostaglandins in the lone star tick, *Amblyomma americanum* (L.), as assessed by radio-immunoassay and gas chromatography/mass spectrometry. *Insect Biochemisry and Molecular Biology, 32*, 331–341.
25. Dickinson, R. G., O'hagan, J. E., Shotz, M., Binnington, K. C., & Hegarty, M. P. (1976). Prostaglandin in the saliva of the cattle tick *Boophilus microplus. Australian Journal of Experimental Biology & Medical Science, 54*, 475–486.
26. Mans, B. J., Louw, A. I., Gaspar, A. R. M. D., & Neitz, A. W. H. (1998). Purification and characterization of apyrase from the tick, *Ornithodoros savignyi. Comparative Biochemistry and Physiology. Part B, Biochemistry and Molecular Biology, 120*, 617–624.
27. Schuijt, T. J., Bakhtiari, K., Daffre, S., Deponte, K., Wielders, S. J., Marquart, J. A., et al. (2013). Factor Xa activation of factor V is of paramount importance in initiating the coagulation system: Lessons from a tick salivary protein. *Circulation, 128*, 254–266.
28. Waxman, L., & Connolly, T. M. (1993). Isolation of an inhibitor selective for collagen-stimulated platelet aggregation from the soft tick *Ornithodoros moubata. Journal of Biological Chemistry, 268*, 5445–5449.
29. Karczewski, J., Endris, R., & Connolly, T. M. (1994). Disagregin is a fibrinogen receptor antagonist lacking the Arg-Gly-Asp sequence from the tick, *Ornithodoros moubata. Journal of Biological Chemistry, 269*, 6702–6708.
30. Man, B. J., Louw, A. I., & Neitz, A. W. H. (2002). Savignygrin, a platelet aggregation inhibits or from the soft tick *Ornithodoros savignyi*, present the RGD integrin recognition motif on the Kunitz-BPTI fold. *Journal of Biological Chemistry, 277*, 21371–21378.
31. Mans, B. J., Louw, A. I., & Neitz, A. W. H. (2002). Evolution of hematophagy in ticks: Common origins for blood coagulation and platelet aggregation inhibitors from soft ticks of the genus *Ornithodoros. Molecular Biology and Evolution, 19*, 1695–1705.
32. Nienaber, J., Gaspar, A. R. M., & Neitz, A. W. H. (1999). Savignin, a potent thrombin inhibitor isolated from the salivary glands of the tick *Ornithodoros savignyi* (*Acari: Argasidae*). *Experimental Parasitology, 93*, 82–91.
33. Ribeiro, J. M. C., Endris, T. M., & Endris, R. (1991). Saliva of the soft tick, *Ornithodoros moubata*, contains anti-platelet and apyrase activities. *Comparative Biochemistry and Physiology Part A, 100*, 109–112.
34. Keller, P. M., Waxman, L., Arnold, B. A., Schultz, L. D., Condra, C., & Connolly, T. M. (1993). Cloning of the cDNA and expression of moubatin, an inhibitor of platelet aggregation. *Journal of Biological Chemistry, 268*, 5450–5456.
35. Karczewski, J., Waxman, L., Endris, R. G., & Connolly, T. M. (1995). An inhibitor from the argasid tick *Ornithodoros moubata* of cell adhesion to collagen. *Biochemical and Biophysical Research Communications, 208*, 532–541.
36. Ribeiro, J. M. C., Makoul, G. T., Robinson, D. R., & Spielman, A. (1985). Antihemostatic, anti-inflammatory, and immunosuppressive properties of the saliva of a tick, *Ixodes dammini. Journal of Experimental Medicine, 161*, 332–344.
37. Francischetti, I. M. B., Pham, V. M., Mans, B. J., Andersen, J. F., Mather, T. N., Lane, R. S., et al. (2005). The transcript-tome of the salivary glands of the female western black-legged tick *Ixodes pacificus* (*Acari: Ixodidae*). *Insect Biochemistry and Molecular Biology, 35*, 1142–1161.

38. Cheng, Y., Wu, H., & Li, D. (1999). An inhibitor selective for collagen-stimulated platelet aggregation from the salivary glands of hard tick *Haemaphysalis longicornis* and its mechanism of action. *Science in China. Series C, Life Sciences, 42*, 457–464.
39. Wang, X., Coons, L. B., Taylor, D. B., Stevens, S. E., & Gartner, T. K. (1996). Variabilin, a novel RGD-containing antagonist of glycoprotein IIb-IIIa and platelet aggregation inhibit or from the hard tick *Dermacentor variabilis. Journal of Biological Chemistry, 271*, 17785–17790.
40. Iwanaga, S., Okada, M., Isawa, H., Morita, A., Yuda, M., & Chinzei, Y. (2003). Identification and characterization of novel salivary thrombin inhibitors from the ixodidae tick, *Haemaphysalis longicornis. European Journal of Biochemistry, 270*, 1926–1934.
41. Zhu, K., Sauer, J. R., Bowman, A. S., & Dillwith, J. W. (1997). Identification and characterization of anticoagulant activities in the saliva of the lone star tick, *Amblyomma americanum* (L). *Journal of Parasitology, 83*, 38–43.
42. Horn, F., Coutinhodos Carlos, P., & Termignoni, C. (2000). *Boophilus microplus* anticoagulant protein: An anti-thrombin inhibitor isolated from the cattle tick saliva. *Archives of Biochemistry and Biophysics, 384*, 68–73.
43. Waxman, L., Smith, D. E., Arcuri, K. E., & Vlasuk, G. P. (1990). Tick Anti-coagulant Peptide (TAP) is a novel inhibitor of blood coagulation factor Xa. *Science, 248*, 593–596.
44. Van de Locht, A., Stubbs, M. T., Bode, W., Friedrich, T., Bollschweiler, C., Hoffken, W., et al. (1996). The ornithodorin-thrombin crystal structure a key to the TAP enigma. *EMBO Journal, 15*, 6011–6017.
45. Joubert, A. M., Louw, A. I., Joubert, F., & Neitz, A. W. H. (1998). Cloning nucleotide sequence and expression of the gene encoding factor Xa inhibitor from the salivary glands of the tick, Ornithodoros savigny*i. Experimental and Applied Acarology, 22*, 603–619.
46. Ehebauer, M. T., Mans, B. J., Gaspar, A. R. M., & Neitz, A. W. H. (2002). Identification of extrinsic blood coagulation pathway inhibitors from the tick *Ornithodoros savignyi* (*Acari: Argasidae*). *Experimental Parasitology, 101*, 138–148.
47. Francischetti, I. M. B., Valenzuela, J. G., Andersen, J. F., Mather, T. N., & Ribeiro, J. M. C. (2002). Ixolaris, a novel recombinant tissue factor pathway inhibitor (TFPI) from the salivary gland of the tick, *Ixodes scapularis*: identification of factor X and factor Xa as scaffolds for the inhibition of factor VIIa/Tissue factor complex. *Hemost. Thromb. Vascular Biology, 99*, 3602–3612.
48. Narasimhan, S., Koski, R. A., Beaulieu, B., Anderson, J. F., Ramamoorthi, N., Kantor, F., et al. (2002). A novel family of anticoagulants from the saliva of *Ixodes scapularis. Insect Molecular Biology, 11*, 641–650.
49. Nunn, M. A., Sharma, A., Paesen, G. C., Adamson, S., Lissina, O., Willis, A. C., et al. (2005). Complement inhibitor of C5 activation from the soft tick *Ornithodoros moubata. Journal of Immunology, 174*, 2084–2091.
50. Decrem, Y., Rath, G., Blasioli, V., Cauchie, P., Robert, S., Beaufays, J., et al. (2009). Ir-CPI, a coagulation contact phase inhibitor from the tick *Ixodes ricinus*, inhibits thrombus formation without impairing hemostasis. *Journal of Experimental Medicine, 206*, 2381–2395.
51. Koh, C. Y., Kazimirova, M., Trimnell, A., Takac, P., Labuda, M., Nuttall, P. A., et al. (2007). Variegin a novel fast and tight binding thrombin inhibitor from the tropical bont tick. *Journal of Biological Chemistry, 282*, 29101–29113.
52. Zhu, K., Bowman, A. S., Brigham, D. L., Essenberg, R. C., Dillwith, J. W., & Sauer, J. R. (1997). Isolation and characterization of americanin, a specific inhibitor of thrombin, from the salivary glands of the lone star tick *Amblyomma americanum* (L.). *Experimental Parasitology, 87*, 30–38.
53. Batista, I. F. C., Ramos, O. H. P., Ventura, J. S., Junqueira-de- Azevedo, I. L. M., Ho, P. L., & Chudzinski-Tavassi, A. M. (2010). A new Factor Xa inhibitor from *Amblyomma cajennense* with a unique domain composition. *Archives of Biochemistry and Biophysics, 493*, 151–156.

54. Kato, N., Iwananga, S., Okayama, T., Isawa, H., Yuda, M., & Chinzei, Y. (2005). Identification and characterization of the plasma kallikrein-kinin system inhibitor, haemaphysalin, from hard tick, *Haemaphysalis longicornis. Thrombosis and Haemostasis, 93*, 359–367.
55. Avraamides, C. J., Garmy-Susini, B., & Varner, J. A. (2008). Integrins in angiogenesis and lymphangiogenesis. *Nature Reviews Cancer, 8*, 604–617.
56. Limo, M. K., Voigt, W. P., Tumbo-Oeri, A., Njogu, R. M., & Ole-Moi Yoi, O. N. (1991). Purification and characterization of an anticoagulant from the salivary glands of the ixodid tick *Rhipicephalus appendiculatus. Experimental Parasitology, 72*, 418–429.
57. Macedo-Ribeiro, S., Almeida, C., Calisto, B. M., Friedrich, T., Mentele, R., Stürzebecher, J., et al. (2008). Isolation, cloning and structural characterization of boophilin, a multifunctional Kunitz-type proteinase inhibitor from the cattle tick. *PLoS ONE, 3*, e1624.
58. Ciprandi, A., de Oliveira, S. K., Masuda, A., Horn, F., & Termignoni, C. (2006). *Boophilus microplus*: Its saliva contains microphilin, a small thrombin inhibitor. *Experimental Parasitology, 114*, 40–46.
59. Motoyashiki, T., Tu, A. T., Ayimov, D. A., & Ibragim, K. (2003). Isolation of anti-coagulant from the venom of tick, *Boophilus calcaratus*, from Uzbekistan. *Thrombosis Research, 110*, 235–241.
60. Gordon, J. R., & Allen, J. R. (1991). Factors V and VII anticoagulant activities in the salivary glands of feeding *Dermacentor andersoni* ticks. *Journal of Parasitology, 77*, 167–170.
61. Joubert, A. M., Crause, J. C., Gaspar, A. R., Clarke, F. C., Spickett, M., & Neitz, A. W. (1995). Isolation and characterization of an anticoagulant present in the salivary glands of the bont-legged tick, *Hyalomma truncatum. Experimental and Applied Acarology, 19*, 79–92.
62. Tyson, K., Elkins, C., Patterson, H., Fikrig, E., & deSilva, A. (2007). Biochemical and functional characterization of Salp20, an *Ixodes scapularis* tick salivary protein that inhibits the complement pathway. *Insect Molecular Biology, 16*, 469–479.
63. Guo, X., Booth, C. J., Paley, M. A., Wang, X., DePonte, K., Fikrig, E., Narasimhan, S., & Montgomery, R. R. (2009). Inhibition of neutrophil function by two tick salivary proteins. *Infection and Immunity, 77*, 2320–2329.
64. Hannier, S., Liversidge, J., Sternberg, J. M., & Bowman, A. S. (2004). Characterization of the B-cell inhibitory protein factor in *Ixodes ricinus* tick saliva: A potential role in enhanced *Borrelia burgdorferi* transmission. *Immunology, 113*, 401–408.
65. Mans, B. J., Louw, A. I., Gaspar, A. R. M. D., & Neitz, A. W. H. (1998). Apyrase activity and platelet aggregation inhibitors in the tick *Ornithodoros savignyi. Experimental and Applied Acarology, 22*, 353–366.
66. Kotsyfakis, M., Sa-Nunes, A., Francischetti, I. M., Mather, T. N., Andersen, J. F., & Ribeiro, J. M. C. (2006). Anti-inflammatory and immune suppressive activity of sialostatin L, a salivary cystatin from the tick *Ixodes scapularis. Journal of Biological Chemistry, 281*, 26298–26307.
67. Prevot, P. P., Adam, B., Boudjeltia, K. Z., Brossard, M., Lins, L., Cauchie, P., et al. (2006). Anti-hemostatic effects of a serpin from the saliva of the tick *Ixodes ricinus. Journal of Biological Chemistry, 281*, 26361–26369.
68. Yu, D., Liang, J., Yu, H., Wu, H., Xu, C., Liu, J., et al. (2006). A tick B-cell inhibitory protein from salivary glands of the hard tick, *Hyalomma asiaticum asiaticum. Biochemical and Biophysical Research Communications, 343*, 585–590.
69. Brossard, M., & Wikel, S. K. (2008). Tick immune biology. In A. S. Bowman & P. A. Nuttall (Eds.), *Ticks: Biology, disease and control* (pp. 186–204). Cambridge/New York: Cambridge University Press.
70. Beaufays, J., Adam, B., Menten-Dedoyart, C., Fievez, L., Grosjean, A., Decrem, Y., et al. (2008). Ir-LBP, an *Ixodes ricinus* tick salivary LTB4-bindinglipocalin, interferes with host neutrophil function. *PLoS ONE, 3*, e3987.
71. Frauenschuh, A., Power, C. A., Déruaz, M., Ferreira, B. R., DaSilva, J., Teixeira, M. M., et al. (2007). Molecular cloning and characterization of a highly selective chemokine-binding pro-

tein from the tick *Rhipicephalus sanguineus. Journal of Biological Chemistry, 282*, 27250–27258.
72. Wu, J., Wang, Y., Liu, H., Yang, H., Ma, D., Li, J., et al. (2010). Two immune regulatory peptides with antioxidant activity from tick salivary glands. *Journal of Biological Chemistry, 285*, 16606–16613.
73. Jaworski, D. C., Jasinskas, A., Metz, C. N., Bucala, R., & Barbour, A. G. (2001). Identification and characterization of a homologue of the pro-inflammatory cytokine, macrophage migration inhibitory factor in the tick, *Amblyomma americanum. Insect Molecular Biology, 10*, 323–331.
74. Wang, H., & Nuttall, P. A. (1999). Immunoglobulin-binding proteins in ticks: New target for vaccine development against a blood- feeding parasite. *Cellular and Molecular Life Sciences, 56*, 286–295.
75. Preston, S. G., Majtán, J., Kouremenou, C., Rysnik, O., Burger, L. F., Cabezas Cruz, A., et al. (2013). Novel immune modulators from hard ticks selectively reprogramme human dendritic cell responses. *PLoS Pathogen, 9*, e1003450.
76. Oliveira, C. J., Sa-Nunes, A., Francischetti, I. M., Carregaro, V., Anatriello, E., Silva, J. S., et al. (2011). Deconstructing tick saliva: Non-protein molecules with potent immune modulatory properties. *Journal of Biological Chemistry, 286*, 10960–10969.
77. Ribeiro, J. M. C. (1987). *Ixodes dammini*: Salivary anti-complement activity. *Experimental Parasitology, 64*, 347–353.
78. Paesen, G. C., Adams, P. L., Harlos, K., Nuttall, P. A., & Stuart, D. I. (1999). Tick histamine-binding proteins: Isolation, cloning and three dimensional structure. *Molecular Cell, 3*, 661–671.
79. Couvreur, B., Beaufays, J., Charon, C., Lahaye, K., Gensale, F., Denis, V., et al. (2008). Variability and action mechanism of a family of anti-complement proteins in *Ixodes ricinus. PLoS ONE, 3*, e1400.
80. Anguita, J., Ramamoorthi, N., Hovius, J. W., Das, S., Thomas, V., Persinski, R., et al. (2002). Salp15, an *Ixodes scapularis* salivary protein, inhibits CD4(+) T cell activation. *Immunity, 16*, 849–859.
81. Déruaz, M., Frauenschuh, A., Alessandri, A. L., Dias, J. M., Coelho, F. M., Russo, R. C., et al. (2008). Ticks produce highly selective chemokine binding proteins with anti-inflammatory activity. *Journal of Experimental Medicine, 205*, 2019–2031.
82. Kubeš, M., Fuchsberger, N., Labuda, M., Žuffová, E., & Nuttall, P. A. (1994). Salivary gland extracts of partially fed *Dermacentor reticulatus* ticks decrease natural killer cell activity *in vitro. Immunology, 82*, 113–116.
83. Decrem, Y., Beaufays, J., Blasioli, V., Lahaye, K., Brossard, M., Vanhamme, L., et al. (2008). A family of putative metalloproteases in the salivary glands of the tick *Ixodes ricinus. FEBS Journal, 275*, 1485–1499.
84. Islam, M. K., Tsuji, N., Miyoshi, T., Alim, M. A., Huang, X., Hatta, T., et al. (2009). The Kunitz-like modulatory protein haemangin is vital for hard tick blood-feeding success. *PLoS Pathogen, 5*, e1000497.
85. Fukumoto, S., Sakaguchi, T., You, M., Xuan, X., & Fujisaki, K. (2006). Tick troponin I-like molecule is a potent inhibitor for angiogenesis. *Microvascular Research, 71*, 218–221.
86. Karczewski, J., & Connolly, T. M. (1997). The interaction of disagregin with the platelet fibrinogen receptor, glycoprotein IIb-IIIa. *Biochemical and Biophysical Research Communications, 241*, 744–748.
87. Bowman, A. S., Dillwith, J. W., & Sauer, J. R. (1996). Tick salivary prostaglandins: Presence, origin, and significance. *Parasitology Today, 12*, 388–396.
88. Inokuma, H., Kemp, D. H., & Willadsen, P. (1994). Prostaglandin E2 production by the cattle tick (*Boophilus microplus*) into feeding sites and its effect on the response of bovine mononuclear cells to mitogen. *Veterinary Parasitology, 53*, 293–299.
89. Francischetti, I. M. B. (2010). Platelet aggregation inhibitors from hematophagous animals. *Toxicon, 56*, 1130–1144.
90. Ferguson, J. J., & Zaqqa, M. (1999). Platelet glycoprotein IIb/IIIa receptor antagonists: Current concepts and future directions. *Drugs, 58*, 965–982.

91. Mans, B. J., Louw, A. I., & Neitz, A. W. (2003). The major tick salivary gland proteins and toxins from the soft tick, *Ornithodoros savignyi* are part of the tick lipocalin family: Implications for the origins of tick toxicoses. *Molecular Biology and Evolution, 20*, 1158–1167.
92. Mans, B. J., Coetzee, J., Louw, A. I., Gaspar, A. R. M., & Neitz, A. W. H. (2000). Disaggregation of aggregated platelets by apyrase from the tick *Ornithodoros savignyi* (*Acari: Argasidae*). *Experimental and Applied Acarology, 24*, 271–282.
93. Liyou, N., Hamilton, S., Elvin, C., & Willadsen, P. (1999). Cloning, expression of ecto 5′-nucleotidase from cattle tick *Boophilus microplus. Insect Molecular Biology, 8*, 257–266.
94. Bowman, A. S., Sauer, J. R., Zhu, K., & Dillwith, J. W. (1995). Biosynthesis of salivary prostaglandins in the lone star tick, *Amblyomma americanum. Insect Biochemisry and Molecular Biology, 25*, 735–741.
95. Hoffmann, A., Walsmann, P., Riesener, G., Paintz, M., & Markwardt, F. (1991). Isolation and characterization of a thrombin inhibitor from the tick *Ixodes ricinus. Pharmazie, 46*, 209–212.
96. Koh, C. Y., & Kini, R. M. (2009). Molecular diversity of anticoagulants from haematophagous animals. *Thrombosis and Haemostasis, 102*, 437–453.
97. Gao, X., Shi, L., Zhou, Y., Cao, J., Zhang, H., & Zhou, J. (2011). Characterization of the anticoagulant protein Rhipilin-1 from the *Rhipicephalus haemaphysaloides* tick. *Journal of Insect Physiology, 57*, 339–343.
98. Narasimhan, S., Montgomery, R. R., DePonte, K., Tschudi, C., Marcantonio, N., Anderson, J. F., et al. (2004). Disruption of *Ixodes scapularis* anticoagulation by using RNA interference. *Proceedings of the National Academy of Sciences of the United States of America, 101*, 1141–1146.
99. Francischetti, I. M. B., Mather, T. N., & Ribeiro, J. M. C. (2004). Penthalaris a novel recombinant five-Kunitz tissue factor pathway inhibitor (TFPI) from the salivary gland of the tick vector of *Lyme* disease, *Ixodes scapularis. Thrombosis and Haemostasis, 91*, 886–898.
100. Tanaka, A. S., Andreotti, R., Gomes, A., Torquato, R. J. S., Sampaio, M. U., & Sampaio, C. A. M. (1999). A Double-headed serine protease inhibitor—Human plasma kallikrein and elastase inhibitor— From *Boophilus microplus larvae. Immunopharmacology, 45*, 171–177.
101. Francischetti, I. M. B., Mather, T. N., & Ribeiro, J. M. C. (2003). Cloning of a salivary gland metalloprotease and characterization of gelatinase and fibrin(ogen)lytic activities in the saliva of the Lyme disease tick vector *Ixodes scapularis. Biochemical and Biophysical Research Communications, 305*, 869–875.
102. Sant Anna Azzolini, S., Sasaki, S. D., Torquato, R. J., Andreotti, R., Andreotti, E., & Tanaka, A. S. (2003). *Rhipicephalus sanguineus* trypsin inhibitors present in the tick larvae: Isolation, characterization, and partial primary structure determination. *Archives of Biochemistry and Biophysics, 417*, 176–182.
103. Mulenga, A., Sugino, M., Nakajima, M., Sugimoto, C., & Onuma, M. (2001). Tick-encoded serine proteinase inhibitors (serpins); potential target antigens for tick vaccine development. *Journal of Veterinary Medical Science, 63*, 1063–1069.
104. Mulenga, A., Tsuda, A., Onuma, M., & Sugimoto, C. (2003). Four serine proteinase inhibitors (serpin) from the brown ear tick, *Rhipicephalus appendiculatus*; cDNA cloning and preliminary characterization. *Insect Biochemisry and Molecular Biology, 33*, 267–276.
105. Jaworski, D. C., Simmen, F. A., Lamoreaux, W., Coons, L. B., Muller, M. T., & Needham, G. R. (1995). A secreted calreticulin protein in ixodid tick *(Amblyomma americanum*) saliva. *Journal of Insect Physiology, 41*, 369–375.
106. Bowman, A. S., Gengler, C. L., Surdick, M. R., Zhu, K., Essenberg, R. C., Sauer, J. R., et al. (1997). A novel phospholipase A2 activity in saliva of the lone star tick, *Amblyomma americanum* (L.). *Experimental Parasitology, 87*, 121–132.
107. Zhu, K., Dillwith, J. W., Bowman, A. S., & Sauer, J. R. (1997). Identification of hemolytic activity in saliva of the lone star tick (Acari: Ixodidae). *Journal of Medical Entomology, 34*, 160–166.

108. Ribeiro, J. M. C., Weis, J. J., & Telford, S. R. (1990). Saliva of the tick *Ixodes dammini* inhibits neutrophil functions. *Experimental Parasitology, 70*, 382–388.
109. Hajnická, V., Kocáková, P., Slávikóvá, M., Slovák, M., Gašperík, J., Fuchsberger, N., et al. (2001). Anti-interleukin-8 activity of tick salivary gland extracts. *Parasite Immunology, 23*, 483–489.
110. Jutel, M., Watanabe, T., Klunker, S., Akdis, M., Thomet, O. A., Malolepszy, J., Zak-Nejmark, T., Koga, R., Kobayashi, T., Blaser, K., & Akdis, C. A. (2001). Histamine regulates T-cell and antibody responses by differential expression of H1 and H2 receptors. *Nature, 413*, 420–425.
111. Valenzuela, J. G., Francischetti, I. M. B., Pham, V. M., Garfield, M. K., Mather, T. N., & Ribeiro, J. M. C. (2002). Exploring the sialome of the tick *Ixodes scapularis. Journal of Experimental Biology, 205*, 2843–2864.
112. Aljamali, M. N., Bior, A. D., Sauer, J. R., & Essenberg, R. C. (2003). RNA interference in ticks: A study using histamine binding protein dsRNA in the female tick *Amblyomma americanum. Insect Molecular Biology, 12*, 299–305.
113. Anisuzzaman Islam, M. K., Alim, M. A., Miyoshi, T., Hatta, T., Yamajim, K., et al. (2011). Longistatin, a plasminogen activator, is key to the availability of blood-meals for ixodid ticks. *PLoS Pathogen, 7*, e1001312.
114. Francischetti, I. M., Mather, T. N., & Ribeiro, J. M. (2005). Tick saliva is a potent inhibitor of endothelial cell proliferation and angiogenesis. *Thrombosis and Haemostasis, 94*, 167–174.
115. Teresa, C. F., Assumpcao, J. M. C., Ribeiro, J. M., Ivo, M. B., & Francischetti, I. M. (2012). Disintegrins from hematophagous sources. *Toxins, 4*, 296–322.
116. Walsh, E. M., & Marcinkiewicz, C. (2011). Non-RGD-containing snake venom disintegrins, functional and structural relations. *Toxicon, 58*, 355–362.
117. Francischetti, I. M., Meng, Z., Mans, B. J., Gudderra, N., Hall, M., Veenstra, T. D., Pham, V. M., Kotsyfakis, M., & Ribeiro, J. M. (2008). An insight into the salivary transcriptome and proteome of the soft tick and vector of epizootic bovine abortion, *Ornithodoros coriaceus. Journal of Proteomics, 71*, 493–512.
118. Pechová, J., Štěpánová, G., Kovár, L., & Kopecký, J. (2002). Tick salivary gland extract-activated transmission of *Borrelia afzelii* spirochaetes. *Folia Parasitologica, 49*, 153–159.
119. Willadsen, P., Riding, G. A., McKenna, R. V., Kemp, D. H., Tellam, R. L., Nielsen, J. N., Lahnstein, J., Cobon, G. S., & Gough, J. M. (1989). Immunologic control of a parasitic arthropod. Identification of a protective antigen from *Boophilus Microplus. Journal of Immunology, 143*, 1346–1351.
120. Willadsen, P., & McKenna, R. V. (1991). Vaccination with ——concealed antigens: Myth or reality? *Parasite Immunology, 13*, 605–616.
121. Mans, B. J., Andersen, J. F., Schwan, T. G., & Ribeiro, J. M. (2008). Characterization of anti-hemostatic factors in the argasid, *Argas monolakensis*: Implications for the evolution of blood-feeding in the soft tick family. *Insect Biochemisry and Molecular Biology, 38*, 22–41.
122. Gillespie, R. D., Dolan, M. C., Piesman, J., & Titus, R. G. (2001). Identification of an IL-2 binding protein in the saliva of the Lyme disease vector tick, *Ixodes scapularis. Journal of Immunology, 166*, 4319–4326.
123. Francischetti, I. M., Mans, B. J., Meng, Z., Gudderra, N., Veenstra, T. D., Pham, V. M., & Ribeiro, J. M. (2008). An insight into the sialome of the soft tick, *Ornithodoros parkeri. Insect Biochemisry and Molecular Biology, 38*, 1–21.
124. Leboulle, G., Crippa, M., Decrem, Y., Mejri, N., Brossard, M., Bollen, A., et al. (2002). Characterization of a novel salivary immunosuppressive protein from *Ixodes ricinus* ticks. *Journal of Biological Chemistry, 277*, 10083–10089.
125. Andrade, B. B., Teixeira, C. R., Barral, A., & Barral-Netto, M. (2005). Haematophagous arthropod saliva and host defense system: At ale of tear and blood. *Anais da Academia Brasileira de Ciências, 77*, 665–693.
126. Hajnická, V., Vancová, I., Kocáková, P., Slovák, M., Gašperík, J., Slávikóvá, M., et al. (2005). Manipulation of host cytokine network by ticks: A potential gateway for pathogen transmission. *Parasitology, 130*, 333–342.

127. Ferreira, B. R., & Silva, J. S. (1999). Successive tick infestations selectively promote a T-helper2 cytokine profile in mice. *Immunology, 96*, 434–439.
128. Mejri, N., Franscini, N., Rutti, B., & Brossard, M. (2001). Th2 polarization of the immune response of Balb/c mice to *Ixodes ricinus* instars importance of several antigens in activation of specific Th2 sub populations. *Parasite Immunology, 23*, 61–69.
129. Bergman, D. K., Palmer, M. J., Caimano, M. J., Radolf, J. D., & Wikel, S. K. (2000). Isolation and molecular cloning of a secreted immuno-suppressant protein from *Dermacentor andersoni* salivary gland. *Journal of Parasitology, 86*, 516–525.
130. Kopecký, J., & Kuthejlová, M. (1998). Suppressive effect of *Ixodes ricinus* salivary gland extract on mechanisms of natural immunity *in vitro. Parasite Immunology, 20*, 169–174.
131. Ramachandra, R. N., & Wikel, S. K. (1992). Modulation of host-immune responses by ticks *Acari: Ixodidae*: Effects of salivary gland extracts on host macrophages and lymphocyte cytokine production. *Journal of Medical Entomology, 5*, 818–826.
132. Cavassani, K. A., Aliberti, J. C., Dias, A. R., Silva, J. S., & Ferreira, B. R. (2005). Tick saliva inhibits differentiation maturation and function of murine bone marrow-derived dendritic cells. *Immunology, 114*, 235–245.
133. Ribeiro, J. M. C., & Mather, T. N. (1998). *Ixodes scapularis*: Salivary kininase activity is a metallo dipeptidyl carboxypeptidase. *Experimental Parasitology, 89*, 213–221.
134. Paesen, G. C., Siebold, S., Harlos, K., Peacey, M. F., Nuttall, P. A., & Stuart, D. I. (2007). A tick protein with a modified Kunitz fold inhibits human tryptase. *Journal of Molecular Biology, 368*, 1172–1186.
135. Daix, V., Schroeder, H., Praet, N., Georgin, J.-P., Chiappino, I., Gillet, L., et al. (2007). *Ixodes* ticks belonging to the *Ixodes ricinus* complex encode a family of anti-complement proteins. *Insect Molecular Biology, 16*, 155–166.
136. Schoeler, G. B., & Wikel, S. K. (2001). Modulation of host immunity by haematophagous arthropods. *Annals of Tropical Medicine and Parasitology, 95*, 755–771.
137. Wikel, S. K., & Alarcon-Chaidez, F. J. (2001). Progress toward molecular characterization of ectoparasite modulation of host immunity. *Veterinary Parasitology, 101*, 275–287.
138. Garg, R., Juncadella, I. J., Ramamoorthi, N., Ashish Ananthanarayanan, S. K., Thomas, V., Rincón, M., et al. (2006). Cutting edge: CD4 is the receptor for the tick saliva immune-suppressor, Salp15. *Journal of Immunology, 177*, 6579–6583.
139. Hajnicka, V., Fuchsberger, N., Slovak, M., Kocakova, P., Labuda, M., & Nutall, P. A. (1998). Tick salivary gland extracts promote virus growth *in vitro. Parasitology, 116*, 533–538.
140. Shaw, M. K., Tilney, L. G., & Mckeever, D. J. (1993). Tick salivary gland extract and interleukin-2 stimulation enhances susceptibility of lymphocyte to infection by *Theileria parva* sporozoites. *Infection and Immunity, 61*, 1486–1495.
141. Jones, L. D., Hodgson, E., & Nuttall, P. A. (1989). Enhancement of virus transmission by tick salivary glands. *Journal of General Virology, 70*, 1895–1898.
142. Krocova, Z., Macela, A., Hernychova, L., Kroca, M., Pechova, J., & Kopecky, J. (2003). Tick salivary gland extract accelerates proliferation of *Francisella tularensis* in the host. *Journal of Parasitology, 89*, 14–20.
143. Horká, H., Cerná-Kýcková, K., Skallová, A., & Kopecký, J. (2009). Tick saliva affects both proliferation and distribution of *Borrelia burgdorferi* spirochetes in mouse organs and increases transmission of spirochetes to ticks. *International Journal of Medical Microbiology, 299*, 373–380.
144. Zeidner, N. S., Schneider, B. S., Nuncio, M. S., Gern, L., & Piesman, J. (2002). Coinoculation of *Borrelia* spp. with tick salivary gland lysate enhances spirochaete load in mice and is tick species-specific. *Journal of Parasitology, 88*, 1276–1278.
145. Krocová, Z., Macela, A., Hernychová, L., Kroca, M., Pechová, J., & Kopecký, J. (2003). Tick salivary gland extract accelerates proliferation of *Francisella tularensis* in the host. *Journal of Parasitology, 89*, 14–20.

146. Ramamoorthi, N., Narasimhan, S., Pal, U., Bao, F., Yang, X. F., Fish, D., et al. (2005). The Lyme disease agent exploits a tick protein to infect the mammalian host. *Nature, 436*, 573–577.
147. Labuda, M., Nuttall, P. A., Kozuch, O., Eleckova, E., Williams, T., Zuffova, E., et al. (1993). Non viraemic transmission of tick-borne encephalitis virus: a mechanism for arbovirus survival in nature. *Experientia, 49*, 802–805.
148. Richter, D., Allgöwer, R., & Matuschka, F. R. (2002). Co-feeding transmission and its contribution to the perpetuation of the Lyme disease spirochete *Borrelia afzelii. Emerging Infectious Diseases, 8*, 1421–1425.
149. Gern, L., & Rais, O. (1996). Efficient transmission of *Borrelia burgdorferi* between cofeeding *Ixodes ricinus* ticks (*Acari: Ixodidae*). *Journal of Medical Entomology, 33*, 189–192.
150. Piesman, J., & Happ, C. M. (2001). The efficacy of co-feeding as a means of maintaining *Borrelia burgdorferi*: A North American model system. *Journal of Vector Ecology, 26*, 216–220.
151. Fialová, A., Cimburek, Z., Iezzi, G., & Kopecký, J. (2010). *Ixodes ricinus* tick saliva modulates tick-borne encephalitis virus infection of dendritic cells. *Microbes and Infection, 12*, 580–585.
152. Severinová, J., Salát, J., Krocová, Z., Reznícková, J., Demová, H., Horká, H., et al. (2005). Co-inoculation of *Borrelia afzelii* with tick salivary gland extract influences distribution of immune competent cells in the skin and lymph nodes of mice. *Folia Microbiologica, 50*, 457–463.
153. Lieskovská, J., & Kopecký, J. (2012). Tick saliva suppresses IFN signalling in dendritic cells upon *Borrelia afzelii* infection. *Parasite Immunology, 34*, 32–39.
154. Lieskovská, J., & Kopecký, J. (2012). Effect of tick saliva on signalling pathways activated by TLR-2 ligand and *Borrelia afzelii* in dendritic cells. *Parasite Immunology, 34*, 421–429.
155. Slámová, M., Skallová, A., Páleníková, J., & Kopecký, J. (2011). Effect of tick saliva on immune interactions between *Borrelia afzelii* and murine dendritic cells. *Parasite Immunology, 33*, 654–660.
156. Kern, A., Collin, E., Barthel, C., Michel, C., Jaulhac, B., & Boulanger, N. (2011). Tick saliva represses innate immunity and cutaneous inflammation in a murine model of Lyme disease. *Vector Borne Zoonotic Diseases, 11*, 1343–1350.
157. Hannier, S., Liversidge, J., Sternberg, J. M., & Bowman, A. S. (2003). *Ixodes ricinus* tick salivary gland extract inhibits IL-10 secretion and CD69 expression by mitogen- stimulated murine splenocytes and induces hypo-responsiveness in B lymphocytes. *Parasite Immunology, 25*, 27–37.
158. Hovius, J. W., Schuijt, T. J., deGroot, K. A., Roelofs, J. J. T. H., Oei, A., Marquart, J. A., et al. (2008). Preferential protection of *Borrelia burgdorferi* sensu stricto by a Salp15 homologue in *Ixodes ricinus* saliva. *Journal of Infectious Diseases, 198*, 1189–1197.
159. Narasimhan, S., Sukumaran, B., Bozdogan, U., Thomas, V., Liang, X., DePonte, K., et al. (2007). A tick anti-oxidant facilitates the Lyme disease agent's successful migration from the mammalian host to the arthropod vector. *Cell Host & Microbe, 2*, 7–18.
160. Schuijt, T. J., Coumou, J., Narasimhan, S., Dai, J., Deponte, K., Wouters, D., et al. (2011). A tick mannose-binding lectin inhibitor interferes with the vertebrate complement cascade to enhance transmission of the Lyme disease agent. *Cell Host & Microbe, 10*, 136–146.
161. Sukumaran, B., Narasimhan, S., Anderson, J. F., DePonte, K., Marcantonio, N., Krishnan, M. N., et al. (2006). An *Ixodes scapularis* protein required for survival of *Anaplasma phagocytophilum* in tick salivary glands. *Journal of Experimental Medicine, 203*, 1507–1517.
162. Labuda, M., Jones, L. D., Williams, T., & Nuttall, P. A. (1993). Enhancement of tick-borne encephalitis virus transmission by tick salivary gland extract. *Medical and Veterinary Entomology, 7*, 193–196.

163. Pechova, J., Stepanova, G., Kovar, L., & Kopecky, J. (2002). Tick salivary gland extract-activated transmission of *Borrelia afzelii* spirochaetes. *Folia Parasitologica (Praha), 49*, 153–159.
164. Kazimírová, M., Jancinová, V., Petríková, M., Takáè, P., Labuda, M., & Nosál, R. (2002). An inhibitor of thrombin stimulated blood platelet aggregation from the salivary glands of the hard tick *Amblyomma variegatum* (*Acari: Ixodidae*). *Experimental and Applied Acarology, 28*, 97–105.
165. Sangamnatdej, S., Paesen, G. C., Slovak, M., & Nutall, P. A. (2002). A high affinity serotonin- and histamine binding lipocalin from tick saliva. *Insect Molecular Biology, 11*, 79–86.
166. Mejri, N., Rutti, B., & Brossard, M. (2002). Immunosuppressive effects of *Ixodes ricinus* tick saliva or salivary gland extracts on innate and acquired immune response of BALB/c mice. *Parasitology Research, 88*, 192–197.