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REVIEW ARTICLE

Blood meal acquisition by ticks ; molecular advances and implications for vaccine development

Albert Mulenga[1], Akiko Tsuda[1], Chihiro Sugimoto[2] and Misao Onuma[1]*

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Abstract

In their quest for a blood meal, hematophagous arthropods must first defeat the host's hemostatic defense. Following injury as it occurs when hematophagous arthropods insert their proboscis into host skin to feed, the host will attempt to stop excessive blood loss through its hemostatic defense mechanism involving platelet aggregation, blood clotting and vasoconstriction. To acquire a full blood meal hematophagous arthropods inject an arsenal of bioactive enzymes which ultimately overpower the host's hemostatic defense. We have looked at a selected number of studies on the molecular biology of arthropod anti-hemostatic proteins and developed commentaries on the suitability of these molecules as target tick vaccine antigens.

Key words : hemostasis, platelet aggregation, vasodilation, tick vaccine development

Irrespective of differences in host preferences and feeding periods, hematophagous arthropods are confronted with similar obstacles in their quest for food. When an arthropod inserts its proboscis into host skin, it causes injury to blood vessels resulting in blood loss. To limit excessive blood loss, the host responds to this injury by activating the hemostatic defense mechanism including vasoconstriction, platelet aggregation and blood coagulation[31,46,49]. To facilitate acquisition of a full blood meal with subsequent disease transmission, hematophagous arthropods secrete and inject into the host's skin a cocktail of bioactive substance, which ultimately overcomes the hemostatic mechanism

[1] Laboratory of Infectious Diseases, Graduate School of Veterinary Medicine, Hokkaido University, Kitaku, Kita 18, Nishi 9, Sapporo 060-0818, Japan
[2] The National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, 080-8555, Japan

*Corresponding author
Phone ; +81-11-706-5215
Fax ; +81-11-706-5217
E-mail ; monuma@vetmed.hokudai.ac.jp

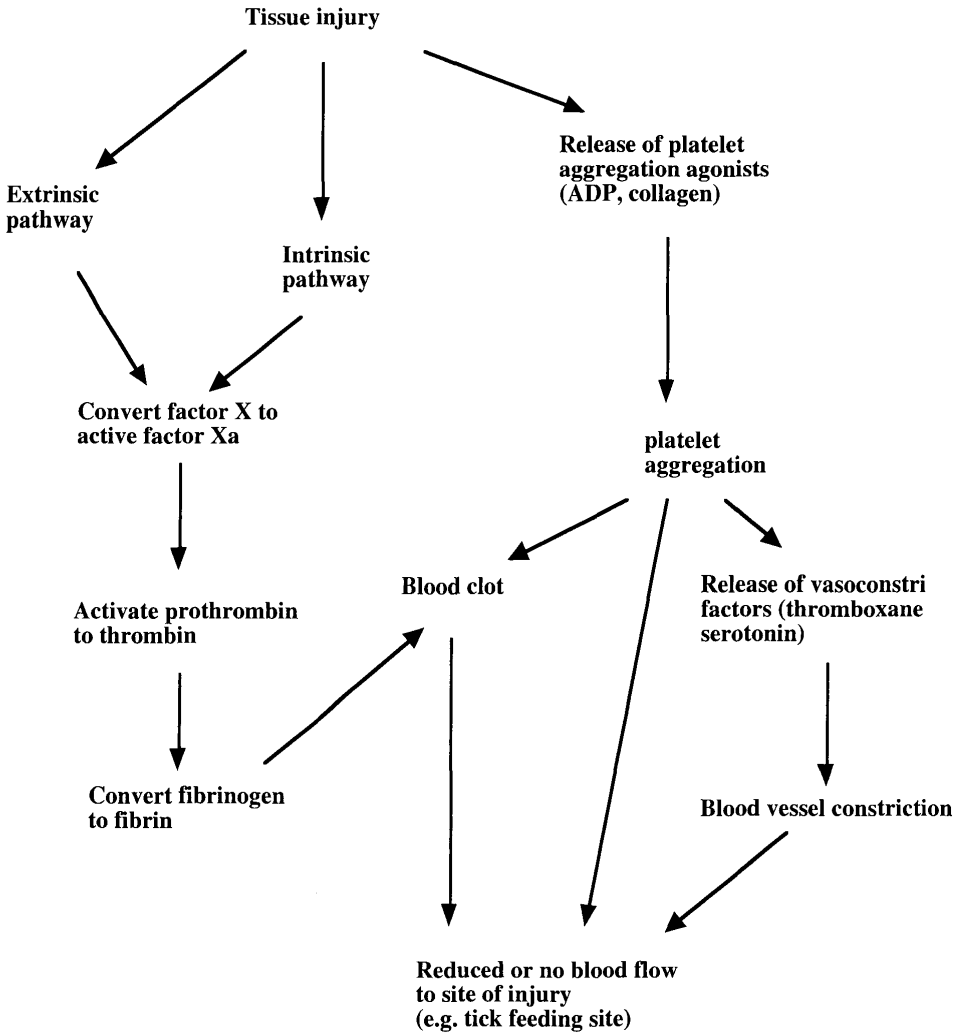

Fig. 1 Simplified diagram of hemostasis (adapted from Law et al.[31]). The figure shows how tissue injury as it occurs during tick feeding can activate blood coagulation, platelet aggregation, and vessel constriction.

of the host. For people working on development of tick vaccines, stopping the tick's ability to acquire a full blood meal and transmit disease pathogens is usually a primary objective and thus tick proteins involved in modulation of host hemostatic defense are regarded as potential target for tick vaccine antigens. The tick salivary gland is believed to be the major source of the majority of these proteins[51]. Our group[40,42,55] and others[41] have cloned tick salivary gland proteins, although most of them were not functionally characterized and hence their biological significance has not been established. While the entire picture of how hematophagous arthropods obtain their blood meal is yet to be fully known, an appreciable amount of data exists on the molecular aspects of bioactive agents used by hematophagous arthropods to modulate host hemostasis for a blood meal acqusition. Since arthropod anticoagulation has been a subject of previous reviews[9,31,46], we have restricted ourselves here to studies on blood meal acquisition by both hard and soft ticks. We have arranged this review around the three general areas of hemostasis as depicted in Fig. 1, namely, anticoagulants and anti-platelet factors as well as vasodilators. The attractive-

ness of hematophagus arthropod-derived anti-hemostatics is either their potential application as pharmaceuticals or target antigens for tick vaccine development being advanced here. Our comments in this review are biased towards the possibility of targeting arthropod modulators of host hemostasis as candidates for tick vaccine development.

Hemostasis

Fig. 1 shows a simplified diagram of hemostasis. Blood coagulation occurs through two pathways, the intrinsic and extrinsic, both which through independent mechanisms, lead to the conversion of Factor X to Factor Xa[31]. The intrinsic pathway is triggered by exposure of blood to subendothelial components such as collagen. For the extrinsic pathway, blood coagulation is triggered by the release of tissue thromboplastin from injured cells. In both pathways, a series of reactions culminates in activation of Factor X to Factor Xa which in turn converts prothrombin to thrombin, the enzyme that produces fibrin from fibrinogen. Fibrin is essential in formation and stabilization of the hemostatic plug at the site of injury. Platelets play an important role in hemostasis as an integral component of the initial hemostatic plug that forms at the site of injury[46]. For platelets to participate in hemostatic plug formation they must first be activated by an agonist. Physiologically platelets are activated by factors released from injured cells including adenosine diphosphate (ADP) and collagen as well as thrombin[32]. Following injury, mammalians also constrict venules to limit blood loss. Vasoconstriction can be promoted by serotonin and thromboxane A 2 released by aggregating platelets and other inflammatory cells, such as neutrophils and mast cells[46,49].

Anticoagulants

The general view of arthropod-derived anticoagulants is that these molecules have activity against one of the 12 factors of the blood coagulation cascade and that their activity will result in aborted progression of the coagulation cascade as well as formation of the fibrin clot. Presence of anticoagulant activity in hematophagous arthropods directed against several points of the coagulation cascade have been demonstrated biochemically[9].

Sequence data of most reported arthropod-encoded anticoagulants is however still limited and thus whether or not hematophagous arthropods use related proteins to modulate host hemostasis is still a matter of speculation. Table 1 summarizes some of the characterized anticoagulants from ticks.

Table 1. Anti-hemostatic agents characterized from hard ticks

Tick species	Protein size (Mr, kDa) / coagulation cascade target	Reference
R. appendiculatus	65 kDa / factor Xa	[33]
B. microplus	60 kDa anti-thrombin	[21]
	67 kDa nucleotide-hydrolysing enzyme	[34]
D. variabilis	5 kDa anti-platelet aggregation	[58]
H. analoticum	16 kDa anti-factor Xa	[24]
A. americanum	16 kDa anti-factor Xa	[64]

Apart from a few studies that reported tick anti-coagulatant activity directed against factors V and VIII in *Dermacentor andersoni*[18] and thrombin in *Amblyomma americanum*[64], *Ixodes ricinus*[20] or *Boophilus microplus*[21], the majority of reported anticoagulants from tick saliva are directed against factor Xa (see Table 1). It is not known whether this indicates the fact that arthropods predominantly encode factor Xa-directed anticoagulants or that there is a bias by researchers when choosing assays used during the purification or isolation process. Although available sequence data is too limited to make any conclusive comments on presence or absence of genetic diversity on arthropod anticoagulants, existing data (see Table 1) is indicative of possible diversity among tick-encoded anticoagulants. For instance, anticoagulant activity directed against fXa was linked to a 65 kDa protein in *Rhipicephalus appendiculatus*[33], a 16 kDa protein in *Hyalomma analoticum*[24] and in *Amblyomma americanum*[63]. Similarly, anticoagulant activity directed against thrombin was associated to 60 and 16 kDa proteins in *B. microplus*[21] and *A. americanum*[63], respectively. In a related study, Joubert et al.[25] described a 7 kDa factor Xa-directed anticoagulant in *Ornithodoros savinyi* that showed 78% similarity at the amino acid level to a 6 kDa anti-factor Xa from *O. moubata*[59]. The observed diversity, if consistent, could be a drawback with regard to suitability of tick anticoagulants as target antigens for tick vaccine development, in that identifying a good vaccine candidate against one tick species will have no bearing on other tick species. It is also worth mentioning that this will only be a problem in geographic regions, like Africa for instance, where livestock is likely to be infested by more than one tick species at a time. Under such circumstances limited diversity of the target vaccine antigen will be highly desirable.

Platelet aggregation inhibitors

Studies on hemostasis by Hovig et al.[22] and Mustard and Packham[43] were indicative of the fact that platelet aggregation is pivotal in stopping bleeding from small lacerated blood vessels as occurs during arthropod feeding. Haemostasis of large blood vessels is impaired when coagulation is impaired. However, in small blood vessels coagulation seems to have a negligible role, as bleeding time of small blood vessels is not affected when coagulation is inhibited by heparin injection[43]. Abolished platelet function, however, results in severe bleeding disorders, even from small skin lesions[43]. Given the fact that the arthropod proboscis is less likely to cause injury to a large blood vessel but more likely to smaller blood vessels, it is logical to assume that inhibition of platelet aggregation by hematophagous arthropods is pivotal in their quest for a blood meal. Platelet aggregation is induced by a wide range of stimuli including adenosine diphosphate (ADP) released by injured cells, collagen fibrils of exposed subendothelial tissues, thrombin produced after activation of the coagulation cascade and platelet aggregation factor (PAF) released by leucocytes[49]. Inhibitors of platelet aggregation have been described from a number of ticks. In discussing advances on platelet aggregation antagonists, we have subdivided the topic based on the mode of function of the platelet antagonist, including (a) degradation of the ADP agonist (adenosine triphosphate (ATP)-diphosphorhydrolase (apyrase) enzymes), (b) inhibition of platelet adhesion to collagen and (c) inhibition of fibrinogen receptor function.

(a) *Apyrase enzymes*

Apyrase is a ubiquitous enzyme that has

been found in humans, plants, and hematophagous arthropods[8,9] and functions to hydrolyze ATP and ADP or other nucleotides[31]. Apyrase enzymes hydrolyze pyrophosphate bonds from ATP and ADP to release AMP and orthophosphates[48]. Intracellular concentrations of both ATP and ADP are on the millimolar range whereas extracellular concentrations are below the micromolar range[48]. During the process of blood feeding, the arthropod proboscis causes injury to host cells leading to release of intracellular ADP and ATP. Extracellular ADP is a potent physiological agonist for platelet aggregation[15,47,50]. For extracellular ATP, its functions are diverse including stimulation of cell proliferation[17] and differentiation[12] as well as induction of proinflammatory activities of neutrophils[15]. ATP has also been shown to modulate the responses of immune cells including T and B lymphocytes[44] as well as mast cells[10]. Additionally ATP can also influence the release of proinflammatory cytokines such as interleukin-1 (IL-1) from macrophages[15,16]. The release of nucleotides by injured cells as occurs during tick feeding is most likely an inbuilt protection mechanism by mammalian hosts to prevent excessive blood loss and to initiate protective inflammatory responses at the arthropod's feeding site. Since apyrase can degrade both ADP and ATP and therefore block platelet aggregation and inflammatory responses, the elevated levels of apyrase enzymes that have been observed in arthropod saliva are postulated to be a counter measure by blood feeding arthropods like ticks to modulate host defense[31,46]. Studies on tick-encoded apyrase are limited. Apyrase activity has been described from *Ixodes damini* (now widely known as *Ixodes scapularis*)[49], *O. moubata*[47] and *O. savinyi*[36,37]. In other studies, Willadsen et al.[61,62] detected, purified and determined the partial amino acid sequence of a 67 kDa nucleotide-hydrolyzing enzyme (5'-nucleotidase) from *Boophilus microplus*. This cDNA encoding this enzyme has been cloned and the recombinant functionally characterized[34,35]. Valenzuela et al.[56] indicated that despite the common ability by apyrases to hydrolyze ATP and ADP with approximately same activity levels, apyrase enzymes from different organisms show differences in primary structures and optimum conditions for their activities. For people working on tick vaccine development, the interest in apyrase enzymes is the possibility to target these enzymes as candidate antigens for tick vaccine development. Studies addressing this possibility are either not reported or have not been done. In a lone study by Mathews et al.[38] apyrase enzyme activity was inhibited by antibodies from BALB/C mice that were immunized to *Anopheles stephensi* mosquito saliva by repeated mosquito bites. However one disappointing observation from the study by Mathews et al.[38] was the fact that mosquitoes feeding on immunized mice had no deficiency in probing these mice for a blood meal, even in the face of high titers of anti-apyrase antibodies. The implications of this study could be indicative of the fact that hematophagous arthropods might be using an elaborate multimolecule system to suppress host hemostasis and may not be inhibited by targeting a single protein. This clearly indicates that hematophagous arthropods do have an asernal of bioactive enzymes that may require a lot of work to defeat.

(b) *Inhibitors of collagen-stimulated platelet aggregation*

In addition to ADP released from damaged cells, platelet aggregation is also initiated by platelets coming into contact with sub-endothelial collagen in damaged blood vessels[46]. This mechanism ensures that the he-

mostatic plug is formed at the site of injury and therefore preventing excessive blood loss. The activation of platelets by collagen is an important component of the natural mechanism to arrest bleeding[11,43]. The significance of collagen-stimulated platelet aggregation in stopping bleeding following injury is exemplified by prolonged bleeding times and in some cases bleeding disorders in patients who have demonstrated defects in their platelet responsiveness to collagen *ex vivo*[11]. A search of literature yielded only a limited number of studies on inhibitors of collagen-stimulated platelet aggregation. Karczewski et al.,[28] and Waxman and Connoly[59] respectively purified 16 kDa (tick adhesion inhibitor, TAI) and 17 kDa (moubatin) inhibitors of collagen-stimulated platelet aggregation from *Ornithodoros moubata*. The cDNA encoding moubatin has been cloned and functionally characterized[29]. Inhibitors of collagen-stimulated platelet aggregation from ticks are probably the least studied as indicated by the limited number of articles on this topic. It will be very interesting to explore the potential of collagen -stimulated platelet aggregation as target tick vaccine antigens.

(c) *Inhibitors of fibrinogen receptor function*

For activated platelets to eventually participate in formation of the initial hemostatic plug, they must first become competent to bind fibrinogen and the von Willebrand factor (vWF)[23]. The concerted action of physiological agonists of platelet aggregation activates specific signaling pathways, generating a number of second messengers leading to the functional expression of integrin $a_{11}b_3$ (glycoprotein[GP]IIb-IIIa receptor complex), the fibrinogen receptor on platelets[57]. Cross-linking of platelets by the multivalent binding of either vWF or fibrinogen to stimulated receptors on platelets mediates platelet aggregation[57] and this is the final common response of activated platelets to most agonists[53]. The role of fibrinogen receptors on platelet function should be very critical in that deficiencies or dysfunctions may result in aborted hemostatic plug formation and prolonged bleeding. For instance people with inherited defects or mutations in the fibrinogen GP IIa-IIIb receptor complex have prolonged cutaneous bleeding time[1,4,14,54]. In other studies monoclonal antibodies against the fibrinogen receptor complex prevented platelet aggregation by preventing binding of the vWF or fibrinogen to the GP Iib-IIIa receptor complex[2]. Given the fact that hematophagous arthropods need to have a sustained flow of blood to their feeding site for them to acquire a full blood meal, it is not surprising therefore for arthropod saliva to contain inhibitors of fibrinogen receptors or modulators. A limited number of studies have identified inhibitors of fibrinogen binding receptors from ticks. Disagregin, a 6 kDa protein lacking the consensus Arg-Gly-Asp cell recognition sequence that is apparently present in all fibrinogen antagonists, was purified and cloned from *Ornithodoros moubata*[27]. Disagregin inhibited platelet aggregation induced by a wide range of agonists by competitively inhibiting binding of to the GP IIb-IIIa receptor complex[26,27]. In a related study, a 5 kDa novel inhibitor of the GP IIb-IIIa fibrinogen receptor complex named as variabilin was isolated from *Dermacentor variabilis*[58]. Because of presence in its primary structure of the cell recognition sequence (Arg-Gly-Asp), variabilin was thought to be structurally similar to other fibrinogen antagonists, such as decorsin and ornatin from leeches[39,53] as well as disintegrins from snakes[3,19,52]. Despite the limited number of studies reviewed here, there are indications for functional diversity of tick-encoded fibrinogen antagonists with one group of an-

tagonists mediating its functions through the Arg-Gly-Asp cell recognition motif (e.g. variabilin[58]) and the other group through a mechanism yet to be described (e.g. disagregin[27]).

Vasodilators

In addition to development of a hemostatic plug, mammals can also stop excessive loss of blood through constriction of blood vessels supplying the site of injury. Through vasoconstriction, the host can stop the arthropod from feeding to repletion by limiting the blood volume flowing to the feeding site. To facilitate feed to repletion, hematophagous arthropods inject vasodilators into skins of their hosts. The bioactive substances, induce dilation of constricted venules that leads to increased volume of blood flowing into the feeding site and therefore ensuring that the arthropod feeds to repletion. A greater diversity of vasodilators has been found in the salivary glands of several blood-feeding arthropods[7,13,56]. In ticks however, induction of vasodilation has so far only been linked to prostaglandins[6]. Tick saliva of many tick species, including *Amblyomma americanum, Ixodes scapularis* and *Boophilus microplus, Hyalomma analoticum excavatum* were shown to contain extremely high levels of prostaglandin, far higher than in mammalian inflammatory exudates[6]. Prostaglandins are known potent inducers of vasodilation and were proposed mechanism to help the tick feed to repletion by increasing the amount of blood flow to the feeding site[30]. Additionally prostaglandins were also shown to be involved in immunosupression observed in tick infested animals[5,6] as well as secretion of bioactive enzymes[45,63]. It is not known at present whether ticks also encode homologues of peptide vasodilators that have been identified from short-term blood feeding arthropods[13,56]. The contribution of vasodilation towards acquisition of a full blood meal in tick is not clearly known. Understanding this aspect will be useful in ascertaining the importance of vasodilators as target antigens for tick vaccine development.

Conclusion

Acquisition of the blood meal is central towards the role of ticks as successful vectors of animal disease pathogens and it is the goal of most tick vaccine development studies to attempt to stop ticks from feeding to repletion. Unfortunately the molecular-biological aspects of blood meal acquisition by ticks remains largely uncharacterized. It will be of great interest to undertake molecular biology studies of the tick vector for us to understand and establish the molecular basis of tick-host interactions. Advances in this area will lead to development of the much needed immunological tick control methods.

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