Tick Spit Shines a Light on the Initiation of Coagulation

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Mammalian blood has numerous essential and well-known functions, including oxygen and nutrient delivery. This elixir is recognized by blood-feeding species of mosquitoes, ticks, fleas, lice, leeches, and bats that rely on blood meals for nutrition, life cycle progression, and survival. To obtain these blood meals that require minutes to a week or longer to complete, these blood-sucking creatures must thwart endogenous defense systems contained within blood—immune and procoagulant cells and plasma proteins that rapidly clot (within 3–4 minutes) to provide first-line defense against breaches in vascular integrity. In a fascinating display of evolutionary agility, hemovores have adapted elegant mechanisms to evade detection and to prevent blood coagulation by synthesizing an extensive armament of molecules with anesthetic, immunosuppressive, vasodilatory, anticoagulant, and profibrinolytic properties in mammals.1–3 Research characterizing the molecules generated by hemovores to bypass mammalian defense systems is available at http://circ.ahajournals.org.

These recombinant proteins, nymph feeding was reduced, and this reduction had fascinating consequences. Compared with tick nymphs fed on control rabbits, nymphs fed on rabbits immunized against tick salivary proteins were significantly smaller. Furthermore, this reduction in weight had a profound effect on the sexual maturation of ticks into adults. Schuijt et al6 observed that nymphs that reached ≥3.4 mg molted into female adults, whereas nymphs ≤3.3 mg molted into male adults.6 Notably, the smaller “male” nymphs were composed of 2 distinct populations, prompting speculation that the larger of these populations were “failed females” unable to reach sexual maturation. These data suggest that altering feeding in a way that even subtly decreases mean nymph size could profoundly alter adult sex ratios and decrease tick numbers in subsequent generations. These findings suggest that anticomplement and anticoagulant proteins in tick saliva are potential vaccine candidates for reducing tick populations and reducing the transmission of tick-borne illnesses.

In the current issue of Circulation, Schuijt et al7 have now extended their work by characterizing the biological target of the anticoagulant protein P23 (now called tick inhibitor of factor Xa toward factor V [TIX-5]), revealing additional features in this already intriguing story. Recombinant TIX-5 (rTIX-5) delays thrombin generation in human plasma in which clotting is initiated via intrinsic or extrinsic activators and in factor VIII– or factor XI–deficient plasmas, suggesting that its molecular target lies within the common pathway. Accordingly, rTIX-5 is unable to inhibit thrombin generation in the presence of preactivated factor V (FV), indicating that rTIX-5 inhibits clotting by delaying FV activation. Unexpectedly, however, the inhibitory effects of rTIX-5 on FV activation were not observed in reactions triggered by thrombin or meizothrombin, the hypothesized activators of FV. Rather, rTIX-5 inhibits factor Xa/phospholipid–mediated generation of factor Va (FVa). Schuijt et al7 further interrogated the nature of this mechanism and showed that rTIX-5 does not inhibit factor Xa substrate cleavage or even bind directly to factor Xa but instead specifically blocks factor Xa–mediated activation of FV in a FV B domain–dependent fashion. Thus, not only has this study identified a novel anticoagulant protein in ticks, but also, importantly, the identification of the biological target of TIX-5 reveals the physiological importance of a pathway not previously described during mammalian coagulation in vivo.

This work has important implications for several hot topics in coagulation research. First, the identification of the molecular target of TIX-5 sheds light on a long-standing question about the origin of FVa during the initiation of coagulation. Although several proteases, including α-thrombin,8 meizothrombin,8 calpain,10 plasmin,11 elastase and cathepsin G,12 and factor Xa,13 can cleave FV to generate the active cofactor (FVa) in purified systems, the primary activator of

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FV during the initiation of coagulation has remained elusive. This activity has been attributed primarily to trace amounts of meizothrombin and/or α-thrombin generated by the extrinsic activation of factor Xa. Because rTIX-5 specifically delays factor Xa– but not thrombin- or meizothrombin-mediated activation of FV, this protein provides a unique tool to study the role of factor Xa–mediated activation of FV during coagulation. Schuijt et al have now described the function of TIX-5 as an inhibitor of factor Xa–mediated factor V activation during the initiation of coagulation. TF indicates tissue factor.

Identification of TIX-5 anticoagulant activity and procoagulant pathway gives rise to exciting questions about the role of this pathway in coagulation and future studies to evaluate its potential as a therapeutic target. First, in contrast to ticks fed on TIX-5–immunized rabbits, the mean weight of ticks fed on TIX-5-immunized mice is not reduced, suggesting that mice do not make antibodies against TIX-5 or that the coagulation pathway inhibited by TIX-5 is less important in mice than in humans or rabbits. Because mice are a primary go-to model for human coagulation studies, these data suggest caution in the interpretation of murine studies evaluating initiating events in coagulation. Second, recent studies have shown that in contrast to platelets, when prothrombinase is assembled on the surface of erythrocytes, thrombin generation proceeds via the meizothrombin intermediate, indicating that procoagulant pathways are determined by both plasma protein composition and the nature of the cell surface. The observation that rTIX-5 can bind directly to phospholipids suggests that the composition of the cell surface could also influence the ability of TIX-5 to block factor Xa activation of FV. It will be interesting to determine the role of the factor Xa/FV pathway on different cells and under different initiating circumstances.

Finally, and perhaps most attractive, by demonstrating the physiological relevance of factor Xa–mediated activation of FV in vivo and the ability of rTIX-5 to inhibit this reaction, this work has yielded a potential new antithrombotic approach. Both hemovores and clinicians share a goal with
regard to anticoagulation: reducing clotting in a highly controlled way that does not cause excessive bleeding. Indeed, anticoagulant mechanisms developed by nature have previously been exploited for the development of other drugs, including hirudin and analogs from leeches and defibrinating enzymes from snakes.1 In this case, ticks have identified and inhibited a procoagulant pathway that researchers had not yet characterized in vivo. Can we once again exploit nature’s efforts to regulate coagulation in the clinic? This exciting possibility warrants studies to test the antithrombotic potential of TIX-5 and similar molecules in “traditional” thrombosis models that have shown clinical relevance during the development of other antithrombotic drugs. Because the inhibitory effect of rTIX-5 is reduced in plasmas with reduced anticoagulant levels, targeting this pathway may be less effective in certain types of coagulopathies stemming from deficiency in anticoagu- lant pathways (eg, protein C deficiency). It will be critical to explicitly test the ability of TIX-5 and similar molecules to prevent thrombosis in specific models of plasma hypercoagulability and vascular dysfunction in future studies.

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Disclosures
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References
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