The cattle tick *Rhipicephalus microplus* saliva: a source of serine proteinase inhibitors

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The tick feeding style of lacerating host tissue and sucking host blood from the pool formed at the bite site is expected to strongly trigger host defense responses. These responses are dependent on the action of several proteins, such as pro-coagulant, pro-inflammatory, and complement proteinases. These host defenses are highly regulated by specific endogenous inhibitors, maintaining homeostasis. From this perspective, it has been suggested that ticks secrete peptidase inhibitors to disrupt host defenses. Indeed, proteomic analysis revealed that *Rhipicephalus microplus* saliva is a source of proteinase inhibitors such as serpins, Kunitz-type, Kazal-type, TIL, thyropin, and cystatins. In particular, serpins are endogenous inhibitors of blood coagulation, fibrinolysis, inflammation, and complement activation in mammals. Analyses of two *R. microplus*-transcript databases allowed the identification of 18 full-length ORFs with similarity to tick serpin-encoding sequences. RT-PCR results revealed that *R. microplus* serpins (RmS) are largely transcribed in tick tissues. Transcription in salivary gland and midgut suggests that they play a role in hematophagy. At least three serpins (RmS-3, RmS-6 and RmS-17) were identified as secreted salivary proteins. Inhibitory functions of these three salivary serpins against a panel of proteinases across the mammalian defense pathway validated that they are likely inhibitors of pro-inflammatory and pro-coagulant proteinases, with typical inhibitory mechanism of serpins. In blood clotting assays, rRmS-17 delayed plasma clotting in the recalcification time assay. Consistent with inhibitory function profiling data, rRmS-3 and rRmS-17 inhibited cathepsin G-activated platelet aggregation. Of significant interest, polyclonal antibodies blocked inhibitory functions of the three serpins. Also notable, antibodies to *Amblyomma americanum, Ixodes scapularis,* and *R. sanguineus* tick saliva proteins cross-reacted with the three serpins suggesting the potential for these proteins as candidates for universal anti-tick vaccines. Based on their inhibitory properties, we suggest that these salivary serpins have a role as immunomodulatory and anticoagulant proteins during feeding by *R. microplus*.

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