Blocking *Rhipicephalus microplus* SERPIN inhibitory activity by antibodies

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**Introduction and objective:** Serpins (serine protease inhibitors) are proteins with a role on the control of different physiological systems, e.g. regulating blood coagulation, fibrinolysis, inflammation and activation of the complement system. Three serpins have been identified in the saliva of the cattle tick *Rhipicephalus microplus* (RmS-3, RmS-6 and RmS-17). Considering the role of serine peptidases in host immune response it is suggestive that ticks use salivary serpins as a means to modulate host defense responses. The aim of this study was to evaluate the effect of antibodies on serpin activity. **Material and methods:** Polyclonal sera were raised in rabbits against RmS-3, RmS-6 or RmS-17. Monoclonal antibodies were generated from the fusion of spleen cells from RmS-6 or RmS-17 immunized mice and SP2/O cells. Anti-RmS-6 and anti-RmS-17 antibodies producing cells were screened by ELISA. Both polyclonal and monoclonal antibodies were tested as their ability to block serpin inhibitory activity. **Results and conclusion:** A cross-reactivity assay revealed that from the six monoclonal antibodies obtained three specifically bound to RmS-6, one reacts with all three proteins (RmS-3, RmS-6 and RmS-17), and two monoclonal antibodies react with two of the recombinant serpins (either RmS-3 and RmS-6 or RmS-17 and RmS-6). One of each group of cross reactivity was chosen to test further dissociation constant (Kd) characterization which ranged from 253 nM to 986 nM. Only anti-RmS-17-polyclonal antibody was able to block RmS-17 inhibitory activity on chymotrypsin. The results reveal that it is possible to interfere with the serpin function using antibodies and the theses are potential target for immunologic control.

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