STUDY OF PHYSIOLOGICAL FUNCTION AND BIOTECHNOLOGICAL POTENTIAL OF PROTEASE INHIBITORS AND ANTI-HEMOSTATIC MOLECULES PRESENT IN HEMATOPHAGOUS ARTHROPODS

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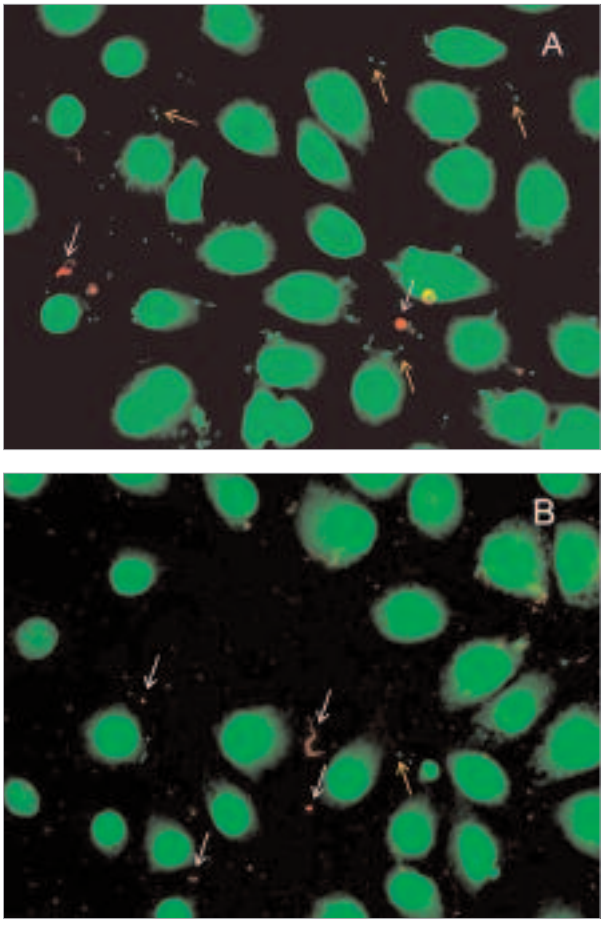

Inhibition of cell culture invasion by T. cruzi *strain strain Y using a* Triatoma infestans *protease inhibitor, Infestin 1R. The LLCMK2 cell line was infected using* T. cruzi *strain Y (1:10 parasites). A) Control experiment. B) Cells pre-incubated with 10 uM Infestin 1R before the infection with* T. cruzi *White arrows indicate the parasite outside the cell. Yellow arrows indicate the parasite inside the cell*

The biological molecule diversity present in hematophagous arthropods, in addition to their importance as disease vectors, has motivated our group to investigate new anti-hemostatic and protease inhibitors present in three different arthropod species, which are responsible for disease transmission to humans and animals in Brazil. They are: *Triatoma infestans* (kissing bug), *Boophilus microplus* (bovine tick), and *Aedes aegypti* (mosquito). In these three cases, the indiscriminate use of pesticide to control those vectors has caused contaminations to the environment and humans. Long term exposition of ectoparasites, to pesticides, has resulted in resistance problems. The World Health Organization has suggested that the main cause of failure in controlling these diseases is our ignorance about the biology of these animals. Therefore, a better understanding of several mechanisms that make these ectoparasites, successfully adapted for hematophagy, would help reveal new targets for developing control strategies, vaccines and also new drugs.

During the last eight years, our group has contributed to a better understanding of the biology of some hematophagous arthropods, among them the *T. infestans* bug, which controls its host's blood coagulation by thrombin and factor XIIa inhibitors that are interestingly coded by the same cDNA precursor molecule. Our studies using factor XIIa inhibitors allowed for a patent process application. We also showed several serine protease inhibitors isolated from *B. microplus* tick and *H. irritans* fly that could present useful physiological functions, such as controlling endogenous proteases and/or microorganisms. We thus aim at characterizing the function and structure of several molecules identified by our group, by using the following tools: RNA interference, real time PCR, proteomics, antifungal activity assays, microorganism protease inhibition, hemolymph coagulation, and activation of the phenoloxidase cascade. With these efforts, we intend not only to understand the role these molecules play in the hematophagous physiology, but also to use them for understanding arthropod protein-protein interaction, for vaccine development, and for biotechnological purposes in human and veterinary medicines and agriculture.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Recently, we have reached some goals including the characterization of three serine protease inhibitors isolated from *Boophilus microplus* tick eggs and larvae. The one named BmSI is a strong subtilisin A inhibitor, and is the first inhibitor which belongs to the TIL (Trypsin Inhibitory Like) inhibitor family to be described; and the others are similar to the Kunitz-BPTI family members (BmTI-6 and BmTI-A) as are practically all other trypsin inhibitors characterized from this tick up to the present time. At the beginning of this project, we finished the characterization of a cysteine protease inhibitor (Bmcystatin) isolated from the fat body of *B. microplus*, along with the characterization of a specific chymotrypsin inhibitor (BmCI) isolated from tick hemocytes. Its behavior after challenge with *Metarhizium anisopliae* fungi and its effects on eukaryotic cell lines suggested a possible role in the apoptosis process. The inhibitors BmTI-6 and BmTI-A are planned to be used in bovine immunization trials.

In addition, we characterized three important molecules from *Triatoma infestans* (kissing bug): (i) a new serine protease inhibitor, the TIPI1 (*Triatoma infestans* Pacifastin Inhibitor 1), the first Pacifastin inhibitor from a hematophagous insect to be described; (ii) a potent platelet aggregation inhibitor induced by collagen, named Tilipo 33, isolated from salivary glands (our results suggest that it targets a new collagen receptor on the platelet); (iii) a Kazal-type inhibitor from midgut, INF1R, which can interfere in the *T. cruzi* cell invasion by a still unknown mechanism.

Finally, the proteins Tilipo 33, Bmcystatin, and Cathepsin L have been used in crystallization experiments for further tridimensional structure determination.

MAIN PUBLICATIONS

Sasaki SD, Lima CA, Lovato DV, Juliano MA, Torquato RJS, Tanaka AS. 2008. BmSI-7, a novel subtilisin inhibitor from *Boophilus microplus*, with activity toward Pr1 proteases from the fungus *Metarhizium anisopliae*. *Experimental Parasitology*. **118**:214-220.

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