Título Functional characterization of T. cruzi mucins in the infection of the invertebrate host

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Autores María de los Milagros Cámara, Débora Passos de Matos, Camila Centeno, Marcelo González, Fernando Genta, Patricia Azambuja y Carlos Buscaglia

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FUNCTIONAL CHARACTERIZATION OF *T. CRUZI* MUCINS IN THE INFECTION OF THE INVERTEBRATE HOST

María de los Milagros Cámara [13], Debora Passos de Matos [2], Camila Centeno [1] Marcelo Gonzalez [2], Fernando Genta[2], Patricia Azambuja [2] and Carlos Buscaglia [1]

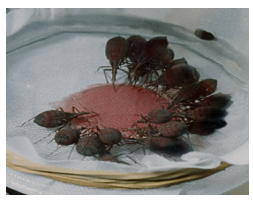
[1] IIB-INTECH-UNSAM, milagritos.camara@gmail.com, [2] Instituto Osvaldo Cruz, Rio de Janeiro Brasil , [3]INTEC UADE

Trypanosoma cruzi, the etiological agent of Chagas diseases is covered with different glycoconjugates that contribute to parasite protection and to the establishment of a persistent infection. TcSMUG (S and L families) is a group of genes coding for *T. cruzi* mucins anchored from the surface of replicative, insect-dwelling developmental forms (i.e. epimastigotes)

In order to characterize epimastigote mucin function we generated *T. cruzi* strains overexpressing TcSMUGL, TcSMUGS and TcTSSA (tripomastigote stage mucin) in two different genetic backgrounds CL Brener (high TcSMUGL levels) and Y strain (low TcSMUGL levels and is unable to infect *Rhodnius prolixus)*.

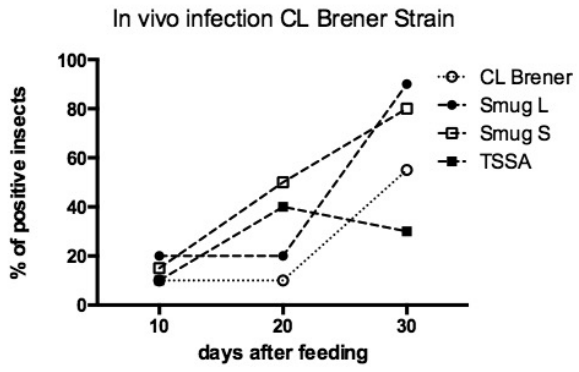
TcSMUGL overexpression enhances *in vivo* infection in *Rhodnius prolixus*

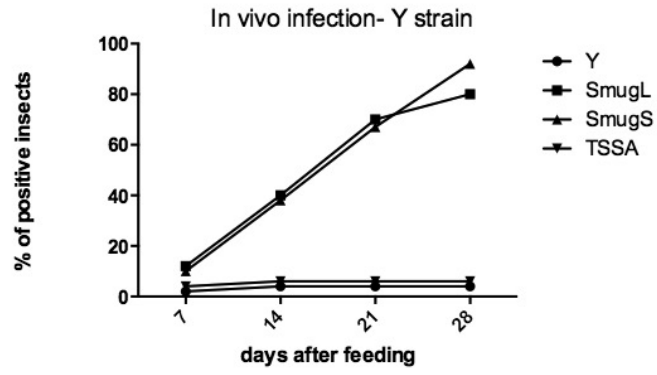
Starved fifth-instar nymphs of *R. prolixus*

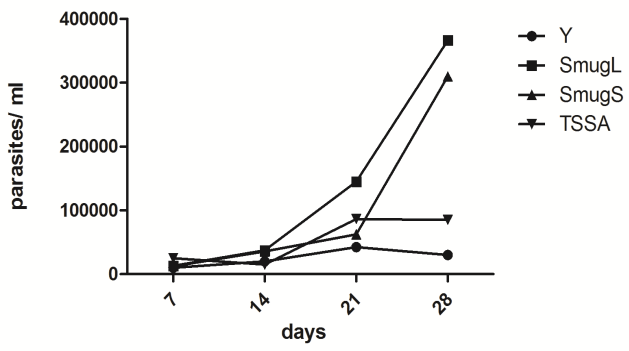


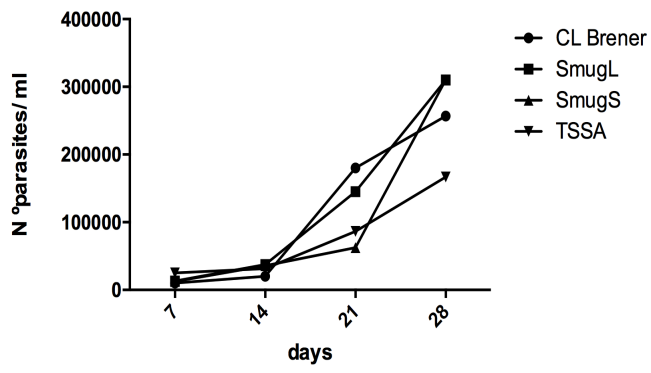
Artificial feeding with human blood added with epimastigotes

In vivo infectivity





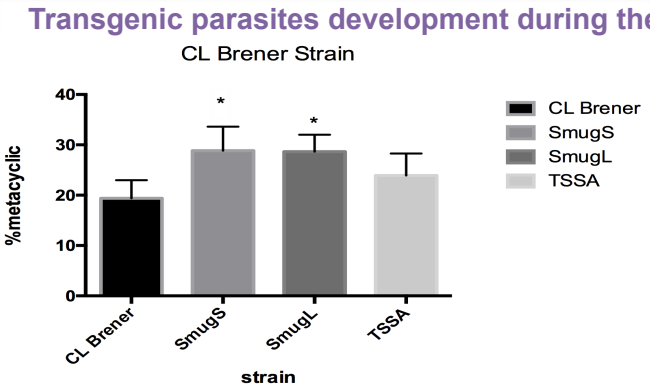


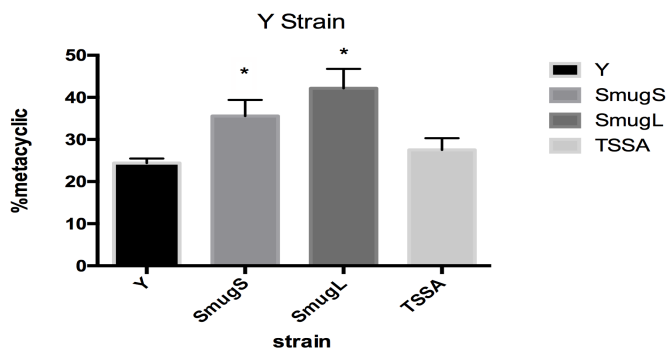


Development of *Trypanosoma cruzi* CL Brener and Y strains overexpressing different surface mucins in whole gut of fifth-instar larvae of *R. prolixus* after infection. Each point represents the mean ± SD of parasites in the gut of 8-10 insects. Control insects and the other three experimental groups were fed on blood containing 3.0 x 10^4 epimastigotes/ml.

TcSMUGL CL overexpressing parasites presented higher infection rates than control lines.
Furthermore TcSMUGL lines belonging to the Y strain were able to establish the infection in the insect host, presenting high infectivity rates.

Transgenic parasites development during the infection in *Rhodnius prolixus*

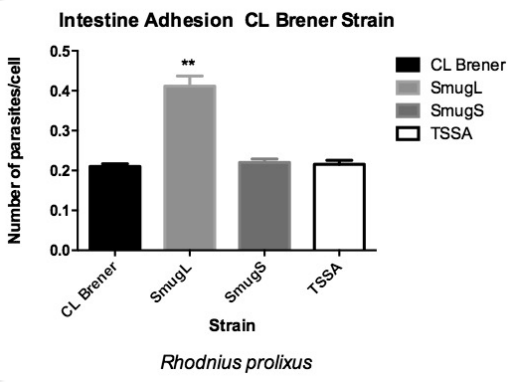


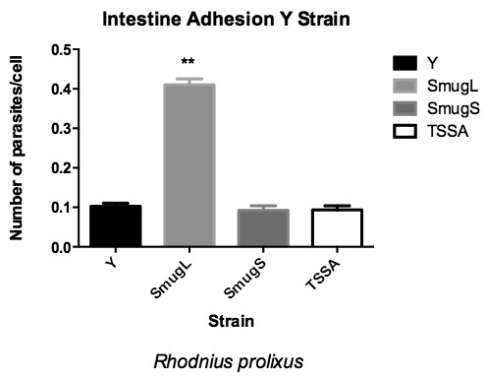


Percentage of the metacyclic forms of the *Trypanosoma cruzi* CL Brener and Y strains overexpressing different surface mucins in whole gut of fifth-instar larvae of *R. prolixus* 30 after infection.
Control insects and the other three experimental groups were fed on blood containing 3.0 x 104 epimastigotes/ml.

TcSMUGL and TcSMUGS transgenic lines in both genetic backgrounds presented more metacyclic forms than control lines

TcSMUGL overexpression enhances epimastigotes attachment to the posterior midgut of *Rhodnius prolixus*

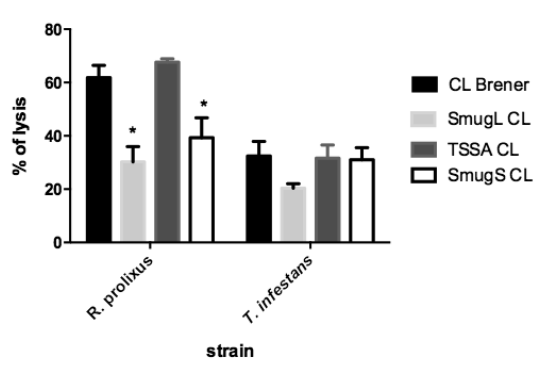


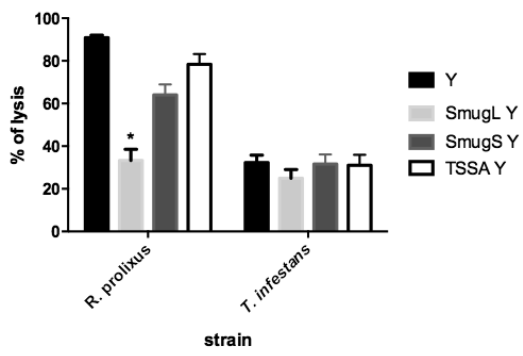


In order to further characterize mucin function we performed in vitro adhesion using different sections of *Rhodnius prolixus* and *Triatoma infestans* nymph's digestive tract.

Our results indicate that TcSMUGL would be involved in parasites adhesion to the posterior midgut, as both transgenic TcSMUGL lines presented 4 times more adhesion rates than control lines.

The TcSMUG family would have a protective role against the insects digestive contents





Parasites need to survive the harsh conditions of the insects digestive system. In order to analyze if mucins have a protective role we performed in vitro lysis assays using insect digestive contents of *T. infestans* and *R. prolixus*

TcSMUGL and TcSMUGS overexpression enhances parasites survival towards stomach contents.
***R. prolixus* and *T. infestans* differences in their digestive contents would have an impact in parasite survival.**

Conclusion

Together, these data indicate that TcSMUG L mucins are key determinants of the infectivity of *T. cruzi* towards the insect population and, hence, on *T. cruzi* epidemiology