VAC_11 - Disruption of active trans-sialidase genes impairs the egress from mammalian host cells and generates highly attenuated *Trypanosoma cruzi* parasites

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Introduction: Trans-sialidases (TS) are unusual enzymes present on the surface of *Trypanosoma cruzi*, the causative agent of Chagas disease. Encoded by the largest gene family in the *T. cruzi* genome, only few members of the TS family have catalytic activity. Active trans-sialidases (aTS) are responsible for transferring sialic acid from host glycoconjugates to mucins, also present on the parasite surface. The existence of several copies of TS genes has impaired the use of reverse genetics to study this highly polymorphic gene family.

Objective: Here we used CRISPR/Cas9 technology to generate knockout parasites to aTS genes and investigate the role of those proteins in the *T. cruzi* infection.

Methodology: To generate the knockout cell lines, epimastigotes constitutively expressing a GFP tagged Cas9 (Cas9::GFP) were transfected with two sgRNAs designed to target aTS genes together with an oligonucleotide to be used as a repair template during homologous recombination repair. This oligonucleotide is a single strand oligonucleotide containing the EcoRV restriction site, the M13 reverse primer and three stop codons flanked by 25 nucleotides complementary to aTS sequences.

Results: We generated aTS knockout cell lines displaying undetectable levels of TS activity as shown by sialylation assays and labelling with antibodies that recognize sialic acid-containing mucins. *In vitro* infection assays showed that disruption of aTS genes does not affect the parasite capacity to invade cells or to escape from the parasitophorous vacuole but resulted in impaired differentiation of amastigotes into trypomastigotes and parasite egress from the cell. When inoculated in mice, aTS mutants were unable to establish infection even in the highly susceptible IFN-γ knockout mice. Mice immunized with aTS mutants were fully protected against a challenge infection with the virulent *T. cruzi* Y strain.

Conclusion: Altogether, our results confirmed the role of aTS as a *T. cruzi* virulence factor and indicated that aTS play a major role during the late stages of intracellular development and parasite egress. Notably, mutants lacking TS activity are completely avirulent in animal models of infection and may be used as a live attenuated vaccine against Chagas disease.

Keywords: *Trypanosoma cruzi*; CRISPR/Cas9; Vaccine