DISRUPTION OF TRYPANOSOMA CRUZI GENES ENCODING ACTIVE TRANS-SIALIDASES RESULTS IN A HIGHLY ATTENUATED STRAIN THAT PRESENTS IMPAIRED PARASITE EGRESS FROM HOST CELL

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Trans-sialidases (TS) are encoded by the largest gene family in the *T. cruz*i genome in which only few sequences encode enzymatically active TS (aTS). aTS roles during infection have not been fully characterized due to multiple copies of the aTS genes, along with repetitive sequences. Using CRISPR/Cas9 technology, we generated aTS knockout parasites (aTS-KØ) that display undetectable levels of TS activity. Although aTS-KØ parasites can infect cell *in vitro*, they are unable to establish infection *in vivo* even in the highly susceptible IFN-γ knockout mice. Moreover, data showed that the highly attenuated aTS mutants are capable of generating protection in immunized mice that were subsequently challenged with a virulent strain. *In vitro* infection assays with wild type (WT) and aTS-KØ parasites in different cell models: HeLa (non-phagocytic), THP1 and peritoneal macrophages (phagocytic) confirmed that the lack of aTS does not limit parasite internalization and amastigote multiplication in these cell types, but drastically affect release of trypomastigotes. Addback cell lines in which the aTS genes were re-expressed, showed that this *in vitro* infection phenotype is partially restored. HeLa cells infected with aTS-KØ have significantly lower mRNA transcript levels of inflammatory cytokines such as IL-1-β, IL-1-α, IL-6 and CXCL8 compared to WT parasites. The differential cytokine induction may be associated with a persistent MEK/ERK protein kinase activation axis during aTS-KØ infection. We also showed that the activation of anti-apoptotic pathways (PI3K/AKT) by *T. cruzi* is independent of aTS. Infection in the insect vector is also independent of aTS activity since no significant differences in total parasite numbers were observed in the digestive tract or urine of infected *Rhodnius prolixus*. These data highlight aTS as an essential virulence factor for *T. cruzi* survival in mammalian that could be used as attenuated vaccine.

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