What doesn't kill you makes you stronger:

Selection of a more resistant and infective population in *Trypanosoma cruzi* by high exposition to normal human serum

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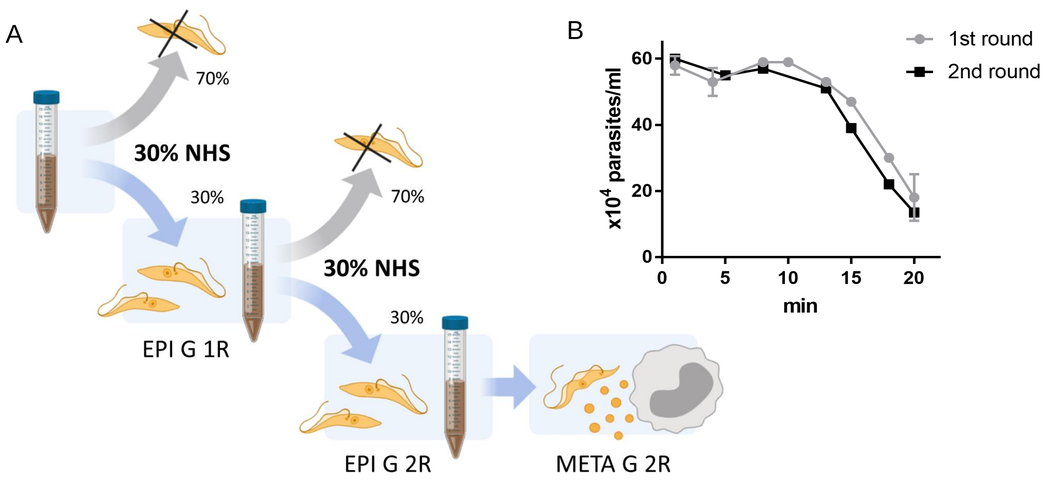
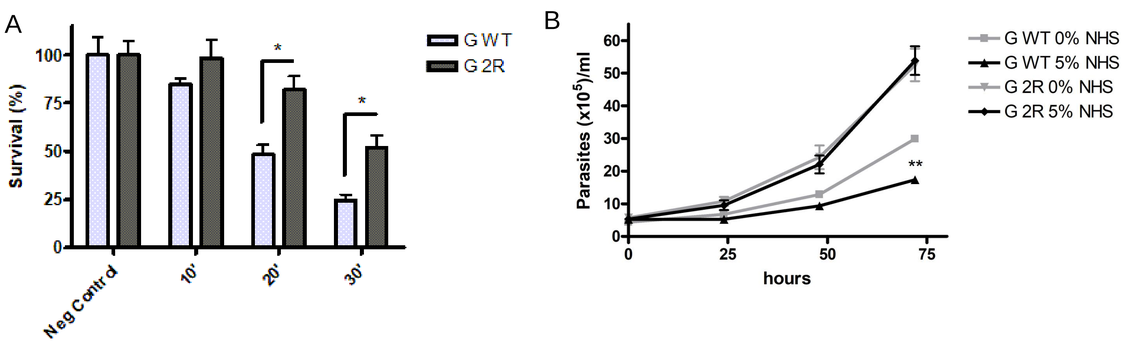
Trypanosoma cruzi, the protozoan that causes Chagas disease, has a complex biological cycle. Upon entering the mammalian host, the parasite needs to effectively evade the attack of the complement system, which consists of the main humoral mechanism of the innate immune system. To guarantee infection, *T. cruzi* expresses different complement system inhibitors on its surface and quickly infects mammalian cells before their lysis. Our group showed that at that moment, extracellular vesicles (EVs) released by metacyclic trypomastigote forms in interaction with host cells participate by inhibiting the complement system and providing greater invasion of mammalian cells. Here, we wondered whether it would be possible to select parasites by exposure to normal human serum (NHS) and characterize the phenotype of this population.

Selection strategy of resistant parasites: Two rounds of exposure to NHS



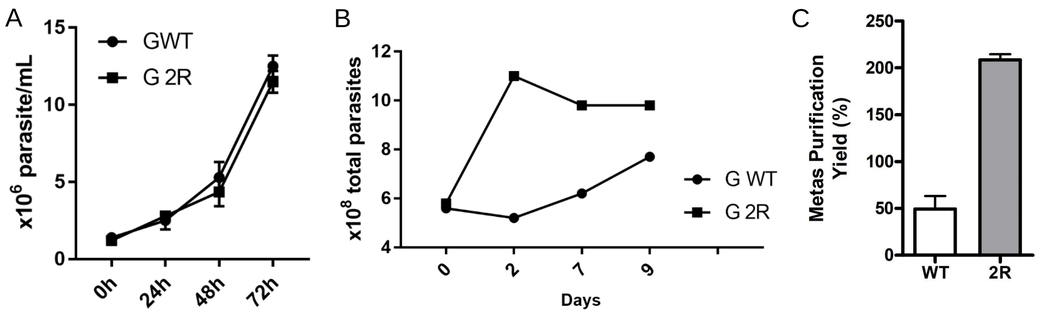
A) Schematic representation of the selection protocol of parasites populations (1R and 2R) resistant to complement lysis. The selection protocol is based on short exposition to high NHS concentrations (30% NHS). B) Parasite survival under 30% NHS exposure during the first and second rounds of selection.

The selected 2R population is more resistant to NHS



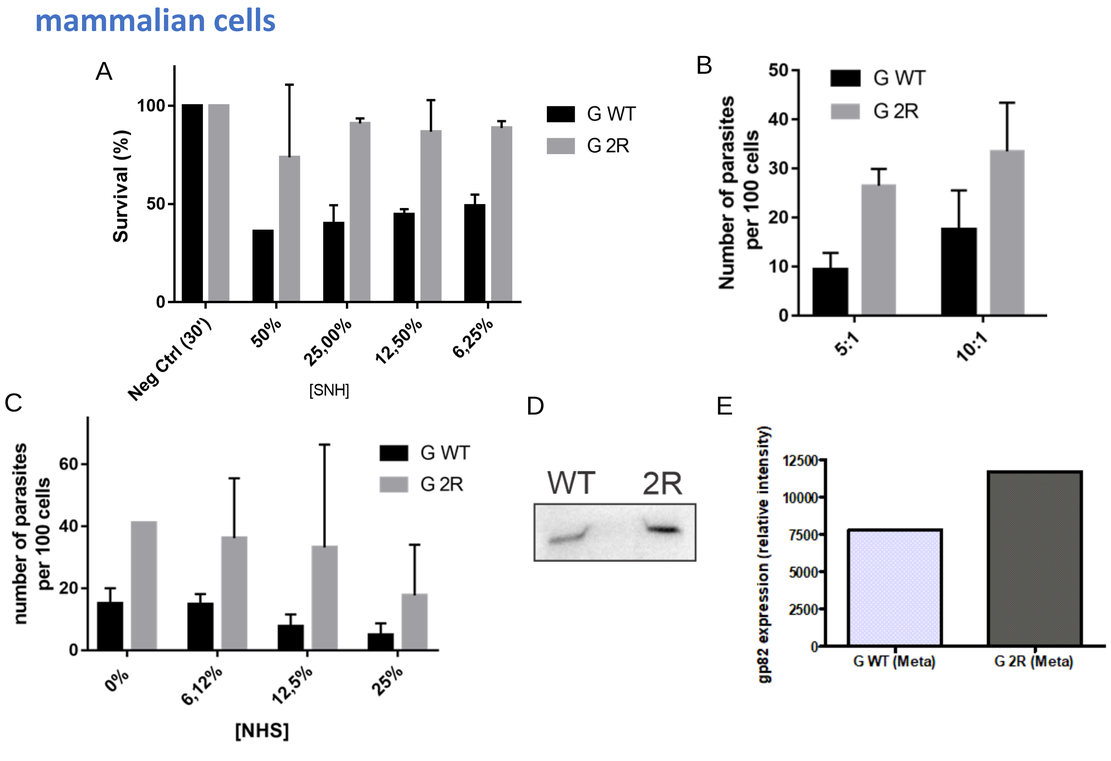
A) EPIs growth curve in LIT medium and with the addition of 5% NHS. B) EPIs complement-mediated lysis kinetics at 6.25% NHS.

Metacyclogenesis is favored in 2R population



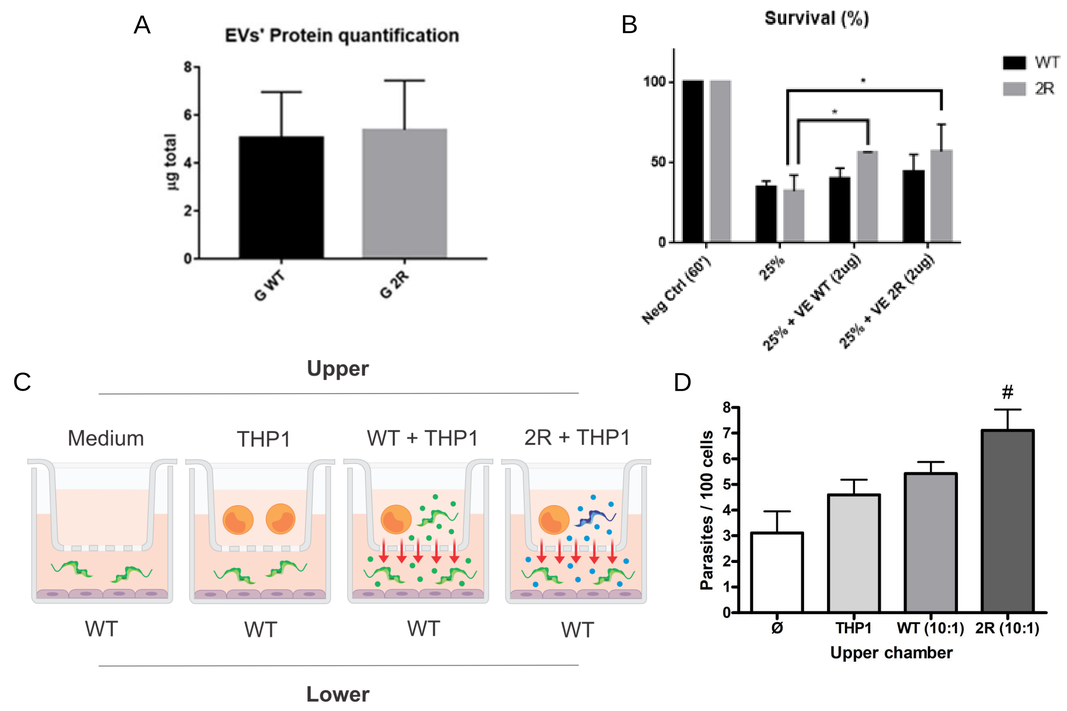
A) Epis growth curve (WT vs 2R). B) Viability of parasites after metacyclogenesis induction by nutritional starvation. C) Yield of METAs purification by ion exchange chromatography.

2R metas were more resistant to complement lysis and had higher infectivity to mammalian cells



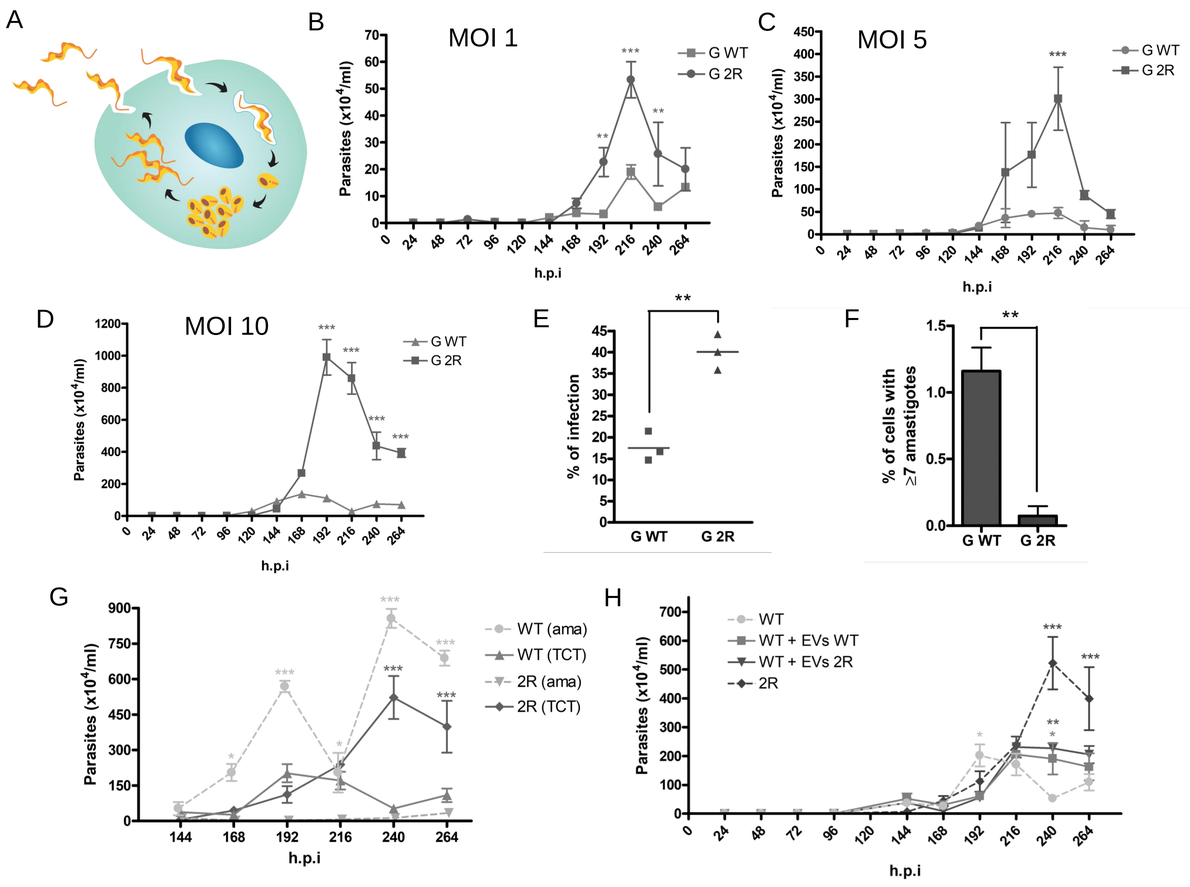
A) Complement-mediated lysis resistance at different NHS concentrations for 30 min for METAs WT and 2R. B) Invasion assays of WT and 2R METAs in VERO cells in MOI 5 and 10 for 3 hours. C) Invasion assays of WT and 2R METAs in the presence of different concentrations of NHS for 3 hours (MOI 10:1). D) Western blot of METAs lysate probed with antibodies against gp82. E) Relative intensity of protein bands calculated using image J software.

The production of EVs is similar among populations, but 2R parasites presents higher resistance in the presence of EVs and WT invasion is increased by 2R EVs



A) Protein dosage of EVs secreted by METAs in the interaction with THP1 cells (5 parasites for each cell). B) Survival of METAs during NHS exposition with addition of Evs. C) Schematic representation of the transwell assay. Two chambers are separated by a membrane with 0.45 µm porosity. In the lower chamber is performed an invasion test that receives EVs from the upper layer. D) Invasion rates of WT METAs to Vero cells during transwell assay. x-Axis labels indicate what was placed in the upper chamber of Transwell plate.

The virulence / infectivity phenotype of the selected population remains in the trypomastigote form derived from cell culture



A) Schematic representation of the intracellular cycle of *T. cruzi*. B) Parasite count in the supernatant after infection of VERO cells with TCTs in MOI 1 (B), 5 (C), and 10 (D). E) Percentage of infected VERO cells after 24 hours of infection and another 24 hours of incubation (MOI 5). F) Percentage of VERO cells containing more than 7 amastigotes after 24 hours of infection and another 24 hours of incubation (MOI 5). G) Comparison between the release of extracellular amastigotes (ama) and TCTs in the supernatant of cells infected in MOI 10. H) Release of TCTs in the supernatant of infected cells in MOI 10 in the presence of 30 µg of EVs during the first 24h of infection.

Future experiments

- Evaluation of Complement Resistance-related genes (CRIT and Calreticulin) and meta stage -specific genes (gp82 and gp90) by qPCR.
- Expression of Trans-sialidases by TCTs
- Effect of EVs during invasion in TCTs
- Analyze the resistance of WT and 2R populations to Benzonidazol

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