Inhibition of PARG activity affects lysosomal function and hampers *T. cruzi* infection in Vero cells

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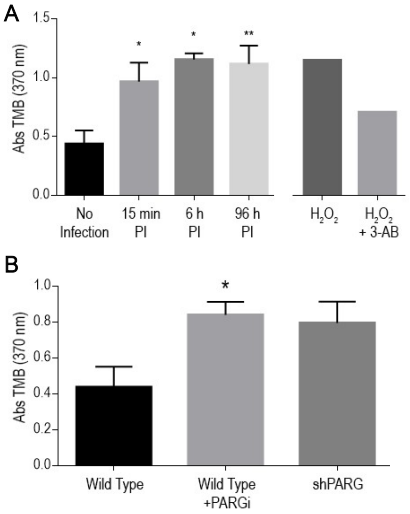
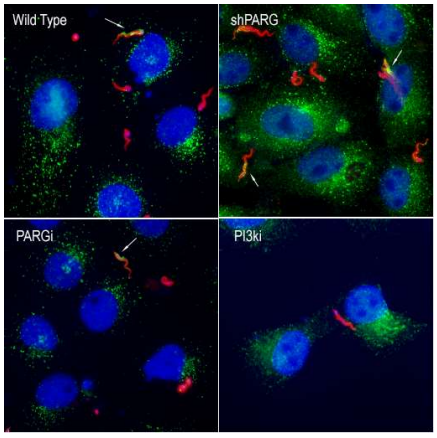
Introduction

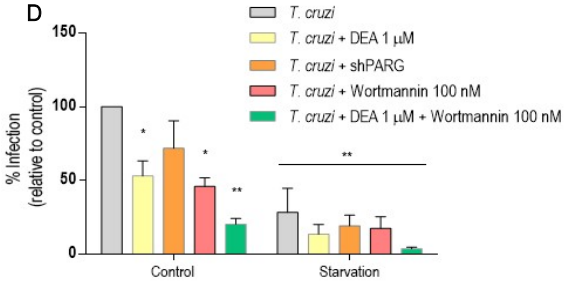
Chagas disease is a potentially life-threatening protozoan infection with little therapeutic alternatives. Since *Trypanosoma cruzi* triggers different host cell signaling pathways, targeting them can be therapeutically valuable. Poly(ADP-ribose) (PAR) participates in host cell response during the infection: Poly(ADP-ribose)Polymease-1 inhibition or silencing decreases *T .cruzi* infection and Poly(ADP-ribose)glycohyrolase (PARG) inhibition or silencing almost completely abrogates it. New results showed PARG might be affecting the mechanism *T. cruzi* exploits to invade the host cell.



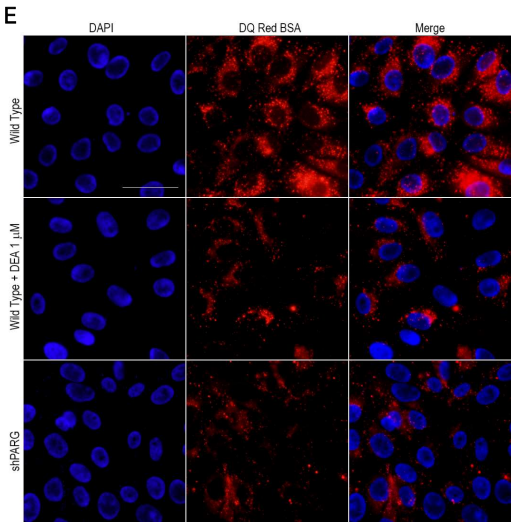
PAR accumulates in response to *T. cruzi* infection in Vero cells. Using an ELISA assay developed by our group, we could detect that PAR levels raised during parasitic invasión (15 min) and remain elevated though the infection. To corroborate that PAR detection was specific, we used H_2O_2 (1 mM – 10 min) to induce PAR synthesis, in the presence or absence of 3-AB 10 mM (PARP inhibitor) **(A)**.

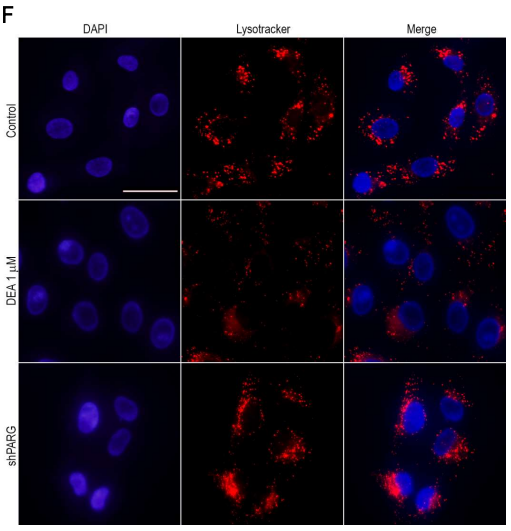
In Vero cells treated with the PARG inhibitor DEA 1 μM (PARGi) or PARG-silenced cells, PAR accumulates even in the absence of an external stimulus. **(B)**

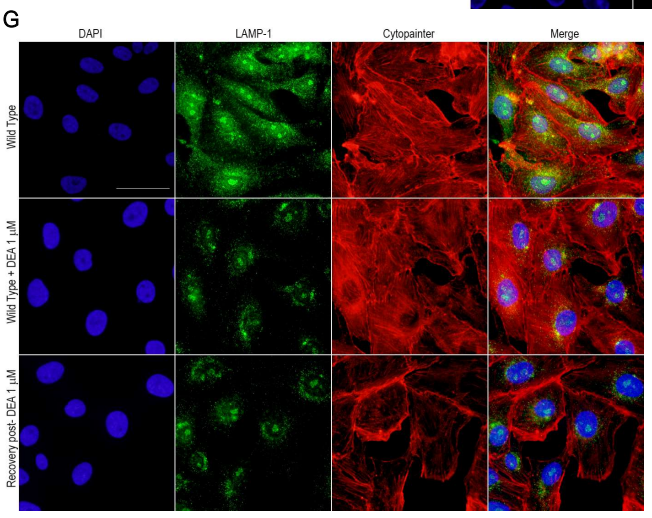




PARG activity does not seem to affect the lysosome-independent or autophagic pathways. Lysosome-independent pathway is characterized by the presence of the early endosomal marker EEA (green) in the parasitophorous vacuole (TcPV) aroung the parasite (red). Inhibiting or silencing PARG did not impede the formation of these vacuoles **(C)**. Although PARG inhibition during parasitic invasion led to a diminished infection, we could not detect any significant difference in infection between fed and starved cells **(D)**.







Lysosomes are crucial for *T. cruzi* trypomastigotes to free from the TcPV and PARG inhibition or silencing in Vero cells alters lysosomal function. DQ-BSA is a quenched substrate that becomes fluorescent when it is cleaved by lysosomal proteases (red). Inhibiting PARG with DEA 1 μM or silencing it decreases DQ-BSA proteolytic processing, indicating lisosomal function is not adequate **(E)**.

We also evaluated if acidity of lysosomes was altered by using a Lysotracker dye (red) and found that pH in PARG-inhibited or PARG silenced Vero cells was also altered **(F)**.

LAMP-1 is a lysosomal protein located in the membrane of this organelles. LAMP-1 signal (green) decreases markedly when PARG is inhibited (DEA 1 μM) and is not quickly recovered after withdrawing the inhibitor **(G)**.

Conclusion

Trypanosoma cruzi invades the host cell by lysosome-independent, lysosome-dependent or autophagic pathways. However, they must all culminate in the fusion of the trypomastigotebearing parasitophorous vacuole (TcPV) to lysosomes.

In PARG inhibited or silenced Vero cells, proteolytic activity, pH and presence of associated processes in lysosomes is altered. These results suggest PARG activity is important for the maintenance of lysosomal activity, and, therefore, for the initial steps of *T. cruzi* infection.

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