PNAS
www.pnas.org

Cytosolic Fe-superoxide dismutase safeguards *Trypanosoma cruzi* from macrophage-derived superoxide radical

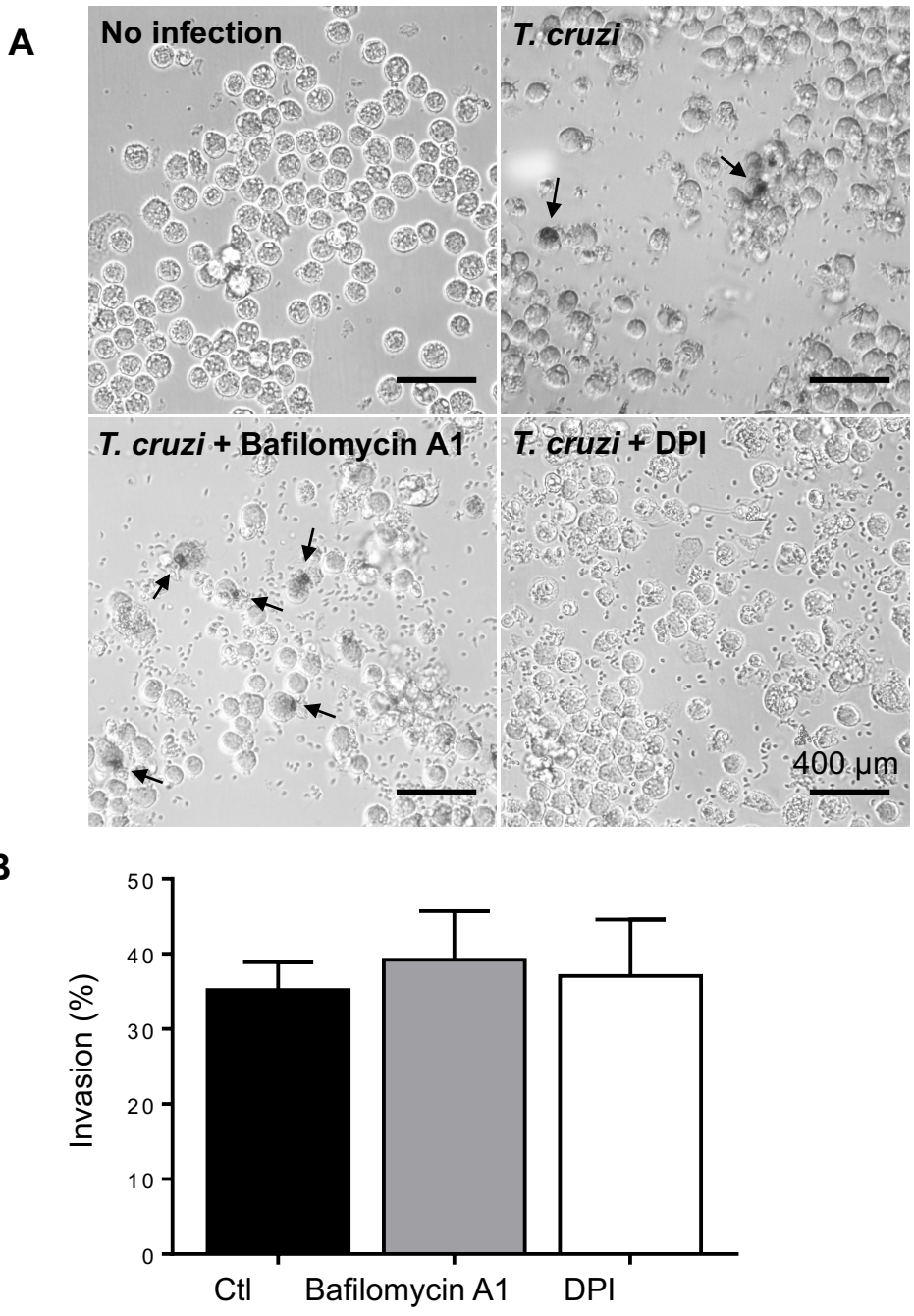
Alejandra Martínez[1], Carolina Prolo[1], Damián Estrada[1], Natalia Rios[1], María Noel Alvarez[1], Dolores Piñeyro[1,2], Carlos Robello[1,2], Rafael Radi[1*] and Lucía Piacenza[1*].
[*] **To whom correspondence should be addressed:** Dr. Lucía Piacenza, e-mail: lpiacenza@fmed.edu.uy and Dr. Rafael Radi, e-mail: rradi@fmed.edu.uy. Departamento de Bioquímica and Center for Free Radical and Biomedical Research, Facultad de Medicina, Universidad de la República, Montevideo 11800, Uruguay.
Paste the full author list here

Supplementary Information Text, Figure 1

NOX-2 activity and infection rate in bafilomicyn A1 or DPI treated macrophages. A. DPI and Bafilomycin A1 effect on NOX-2 activity was evaluated by the NBT assay. Treated or untreated J774A.1 macrophages were infected with *T. cruzi* (5:1, parasite:macrophage ratio) in dPBS containing NBT (1 mg/mL). Slides were incubated at 37 ºC for 30 min and cells were observed by differential interference contrast (DIC) microscopy (x400 magnification, 400 µm scale bar). Arrows indicate formazan-stained inclusions within phagocytic vacuoles. **B.** Treated or untreated J774A.1 macrophages were infected with *T. cruzi* (5:1, parasite:macrophage ratio) for 2 h at 37ºC, rinsed with dPBS, fixed for 10 min in formaldehyde (4 %v/v) at room temperature and stained with DAPI (5 µg/mL). Invasion rate was calculated as the number of intracellular amastigotes per 100 macrophages counted by fluorescence microscopy. Data represent the mean ± SEM of 2 samples.

www.pnas.org/cgi/doi/10.1073/pnas.1821487116

Fig. S1.


A
No infection
T. cruzi
T. cruzi + Bafilomycin A1
T. cruzi + DPI
400 µm
B
Invasion (%)
50
40
30
20
10
0
Ctl
Bafilomycin A1
DPI

Supplementary Information Text

Materials and Methods

Generation of Fe-SODB-Overexpressing Parasites and Enzyme Concentration

For transfection experiments, midlog-phase Dm28c epimastigotes (5 x 10^7 cells per mL) were resuspended in 400 μL of Hepes buffer (Hepes, 21 mM; NaCl, 137 mM; KCl, 5 mM; glucose, 6 mM, pH 7.4) containing pRIBOTEX–Fe-SODB DNA (100 μg). Transfection was performed by two pulses of 450 V, 50 μF, and 25 Ω (ECM 630 Electro Cell Manipulator; BTX). Electroporated cells were cultured in Brain Heart Infusion media (10 mL) and G418 was gradually added until 500 μg/mL was reached in the media.

Exposure of Parasites to External $O_2^{\cdot -}$ Fluxes

Parasites extracts. Parasites were lysed in PBS plus Triton X-100 (0.1% vol/vol) by passing 50 times by syringe needle [27G x 0.5 in. (0.4 x 13 mm)]. Organic extraction of DHE-derived products and protein precipitation were performed with acetonitrile (ACN) (100 μL, 30 min at 4 °C). Samples were centrifuged (20,000 g, 30 min at 4 °C), and organic phase was removed and dried in a vacuum evaporator at 40 °C, 100 mbar, and 40 rpm. Samples were resuspended in 20 μL of sample buffer [90% water; 10% ACN; and 0.1% trifluoroacetic acid (TFA)].

pH Determination of *T. cruzi*-containing Macrophage phagosomes

Generation of *T. cruzi* polyclonal antibodies. Epimastigotes (1 x 10^9) were lysed in PBS containing digitonin (1 mg/mL) and protease inhibitors (Sigma) for 15 min on ice. The sample was washed by centrifugation (20,000 g, 15 min at 4 °C), and the pellet (membrane enriched) was resuspended in solubilization buffer (NaCl, 150 mM; Triton X-100, 1% vol/vol; deoxycholate, 0.5% vol/vol; and SDS, 0.1% wt/vol in 50 mM Tris pH 7.4 containing protease inhibitors; Sigma) for 20 min at room temperature. The extract was centrifuged (20,000 g, 30 min at 4 °C), and the supernatant was used for rabbit immunization.