Immunization with Enolase of *Trypanosoma cruzi* confers protective immunity against acute phase of Chagas disease in mice

A. Carabarín-Lima[1], G. Cortés-Cortés[2], M.C. González-Vázquez[3], O. Rodríguez-Morales[1], P.A. Reyes[1], M. Arce-Fonseca[1], J. L. Rosales-Encina[4].

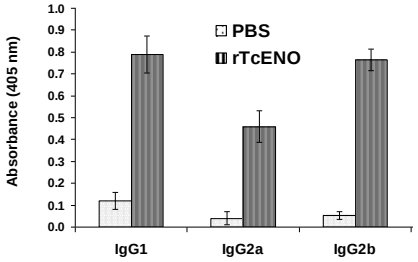
[1] Laboratorio de Inmunología Molecular y Proteómica. Instituto Nacional de Cardiología Ignacio Chávez, México D. F. [2] Posgrado en Microbiología. Benemérita Universidad Autónoma de Puebla. Centro de Investigaciones en Ciencias Microbiológicas, Puebla, México. [3] Biología Celular. CINVESTAV-IPN. [4] Infectómica y Patogénesis Molecular. CINVESTAV-IPN. México. D.F. **ecoli_75@hotmail.com**

Introduction and objectives: Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, is a major public health burden in Latin America and a potentially serious emerging threat to a number of countries throughout the world. Currently, there is no vaccine. For this reason, the aim of the present study was to determine the humoral and cellular immune response generated by the immunization with the *T. cruzi* enolase in a murine model during the experimental acute phase of Chagas disease.

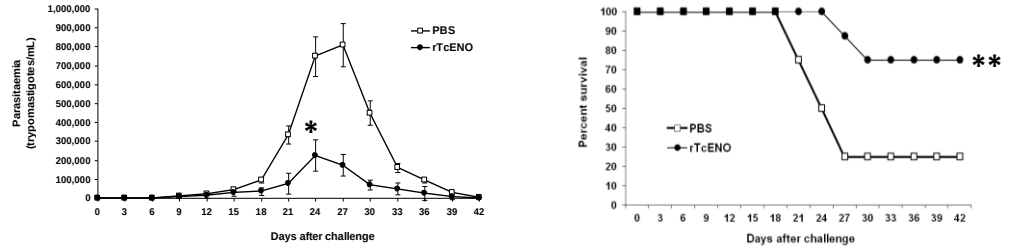
Materials and Methods

T. cruzi enolase gen was obtained from cDNA by RT-PCR, the PCR product was cloned in PCR TOPO TA cloning and then sub-cloned into pRSETB plasmid to obtain pRSETB::TcENO. The recombinant protein (rTcENO) was obtained by affinity chromatography. BALB/c female mice were immunized intraperitoneally with rTcENO with Freund adjuvant (complete and incomplete), each mouse received four doses in total every seven days. A second control group received only PBS. Preimmune and immune sera were obtained. The isotype of antibodies was determined by ELISA. After the immunization, mice were infected with 8 X 10^4 blood trypomastigotes; bled every three days and parasitemia was assessed by direct observation in a light microscope. The survival was monitored every day. Cytokines were analyzed by flow cytometry at the peak of parasitemia. Finally, hearts were isolated aseptically, rinsed with sterile PBS, and fixed for 24 h in 4% paraformaldehyde in PBS (pH 7.4). Fixed hearts were embedded in paraffin, sectioned (5μm), stained with hematoxylin & eosin, and examined by light microscopy.

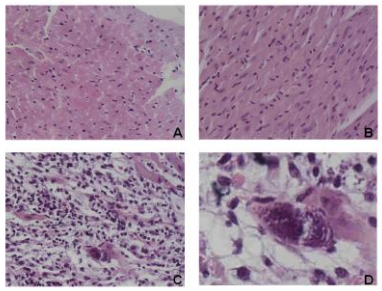
Results



Antibody isotype level generated against rTcENO protein in mice immunized. The antibody level was determined by ELISA with sera from BALB/c i. p. immunized with recombinant protein, using 2 µg of recombinant protein/well. Data are optical density (OD) values of sera from four mice per group. These data show representative results of at least three independent experiments.



Parasitemia and survival



Effect of the immunization with rTcENO on cardiac tissue. Representatives micrographs of tissue sections control mice (A); rTcENO immunized mice (B); and *T. cruzi* infected mice (C, D), are shown. Abundant inflammatory cells and a nest of amastigotes are shown (D).

Conclusions: The findings of this study indicate that immunization with the TcENO recombinant protein induces protection against infection with *Trypanosoma cruzi* in the mouse model.
Therefore, TcENO recombinant protein may be an excellent candidate for further vaccine development.