HA.15 - Recognition of *Trypanosoma cruzi* epitopes by IgG of benznidazole treated Chagas disease patients

Luis Antonio Rodriguez Carnero [1], Jhonatas Sirino Monteiro[1], João Carlos Setubal[1], Ester Cerdeira Sabino[2], Edécio Cunha Neto[3,4,5], Ricardo José Giordano[1,5]
[1]Department of Biochemistry, Institute of Chemistry, [2]Institute of Tropical Medicine, [3]Faculty of Medicine, Heart Institute (InCor), [4]Faculty of Medicine, Division of Clinical Immunology and Allergy, School of Medicine - University of São Paulo (SP, Brazil), [5]INCT, Institute for Investigation in Immunology (iii) (São Paulo, Brazil)

Chagas disease, caused by the protozoan *Trypanosoma cruzi*, affects millions of people worldwide. Treatment is challenging due to lack of effective drugs and assays to monitor parasite persistence. Here, we use our recent develop gPhage platform to analyze and compare antibody response in Chagas patients, before and after benznidazole treatment, to identify epitopes that could be used as molecular marker for disease status. Then, we searched for antigens/epitopes recognized preferentially by patients treated with benznidazole that are responders (PCR-negative for *T. cruzi*) or non-responders (that remain PCR-positive). We used IgG purified from sera of patients (N=20) collected before and after treated with benznidazole. Patients were then classified on responders (N=10) and non-responders (N=10) based on their PCR-status. In order to enrich for antigens recognized by IgG from patients before and after treatment, we devised a two-tier biopanning procedure. One of the IgG samples of the same patient (before or after treatment) was first used to pre-clear the *T. cruzi* genomic phage library (gPhage) before performing the positive selection on the remaining IgG samples. After 4 rounds of selection, phage bound to IgGs were characterized by Next Generation Sequencing. Sequences were assembled and epitopes identified by alignment with *T. cruzi* genome and clustering. We observed a significant and inverse correlation between the number of phage display *T. cruzi*-antigens recovered by IgG from patients and their PCR status. There was a significant enrichment in *T. cruzi*-specific antigens bound to IgG from non-responders compared to responders. The surface antigen 2 (B13) was the most prevalent antigen recovered, although we also identified other molecular marker of interest. Our work corroborates previous studies indicating the *T. cruzi* antibody response is a potential marker for cure. Several antigens, including the well-known B13, are potential molecular markers of disease status.
Keywords: Chagas disease, benznidazole, epitopes. **Supported by:** CNPq, FAPESP, CAPES

HA.16 - Study of the effects of palmitic acid and pregnancy steroids on the survival of insulin-producing cells

Rafael Teixeira do Nascimento [1], Azevedo-Martins, A.K[1]
[1]Bioquímica e Biologia Molecular, Programa de Bioquímica e Biologia Molecular da Escola de Artes, Ciências e Humanidades da Universidade de São Paulo (São Paulo, Brasil)

Gestational diabetes (GD) attacks 3-17% of pregnant women, four-times prevalent between obese women, correlating adiposity and GD. Adiposity interferes in adipokines amount, reflecting adipose tissue alterations what could contribute to metabolic diseases. Study pregnancy steroids and fatty acids effects on viability and DNA fragmentation of insulin-producing-cell line. INS-1E were cultivated in RPMI with 5% fbs, bicarbonate, sodium pyruvate, 2-mercaptoethanol and 5% CO2 in humidified atmosphere. Cells were treated with 50, 100, 200 and 300μM palmitic acid (PA) , for 24h or 48h, alone or combined with mixes of progesterone (P4) and estradiol (E2): mix1 (M1: concentrations of P4 and E2 in healthy pregnant women); mix-2 (M2: P4 and E2 in GD pregnant women). Was performed the growth curve for INS-1E (n = 2) and toxicity of PA (n=4) and steroid hormones (n=5) curves. After incubations, membrane integrity and DNA-fragmentation were evaluated by cytometry. A 3-days folding time for INS-1E was consistent with literature. Treatment with PA caused a slight loss of membrane integrity after 24h (200μM, 8% and 300μM, 10%, regarding to control) and 48h (200μM, 12% and 300μM, 16%, regarding to control). Percentage of DNA-fragmented cells was pronounced: 24h (200μM, 73% and 300μM, 90%, regarding to control) and 48h (200μM, 77% and 300μM, 91%, regarding to control). The toxic effect of PA was dose-dependent without any significant influence of incubation time. Membrane integrity, after treatment with 25μM PA and hormones did not show significant difference in all groups regarding control. Was observed DNA fragmentation in all groups compared to the control (PA: 44%, PA+M1: 44%, PA+M2: 50%, M1: 67% and M2: 68%). Growth curve allowed to establish parameters for experiments with the strain; PA toxicity was dose-dependent, but not time-dependent; only pregnant hormones combinations were more toxic to cells than those with fatty acid.
Keywords: Gestational Diabetes, sex hormones, INS-1E
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