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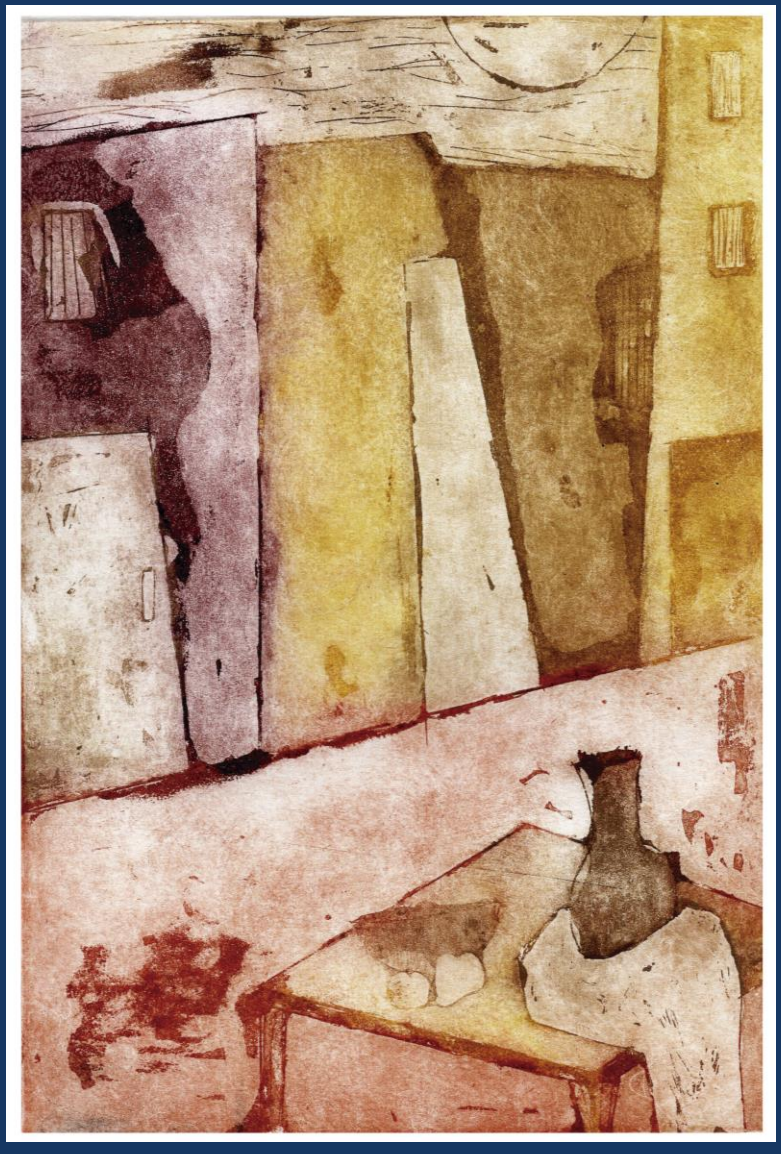

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polymerase together with the tested samples (RNA genomes references). Ranges of temperature, pH, Mg and time were evaluated. The readout of the test was defined as a color change - visible to a naked eye-, by the addition of neutral red dye, or HNB dye (Hydroxy naphthol blue) prior to the amplification; these dyes changes its color as a result of the amplification. The sensitivity and specificity of the RT-LAMP were evaluated. Here we described the achievement of an early and easy method to detect and diferentiate DENV serotypes, ZIKAV and CHIKV. The results showed that our ColorRT-LAMP test is highly sensitive and specific. No cross-reactivity was observed with all other three closely related arboviruses. The COLOR-RT-LAMP method used in our study is specific, sensitive, and suitable for further investigation as a useful alternative to the current methods used for clinical diagnosis and diferentiated of DENV1-4, ZIKAV and CHIKV, especially in hospitals and laboratories that lack sophisticated diagnostic systems.

0745 - BIOGENESIS OF EXTRACELLULAR VESICLES RELEASED BY THE PARASITE TRICHOMONAS VAGINALIS

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T. vaginalis is a parasite that causes trichomoniasis, the most common non-viral sexually disease worldwide. Given it is an extracellular parasite, adhesion to host cells is one of the key processes for the development of infection. In recent years, various factors that influence this process have been identified, including extracellular vesicles (EVs). Previously in our laboratory we demonstrated that both exosomes (vesicles with a size range of 40 - 100 nm) and ectosomes (vesicles with a size of 100 - 1000 nm) are involved in the process of interaction of T. vaginalis with host cells. It is currently known that ESCRT-III subunit of to the ESCRT complex is involved in membrane cleavage being the VPS32 protein the key effector during membrane scission. These antecedents lead to our hypothesis that VPS32 might be regulating the process of extracellular vesicles formation in T. vaginalis. Based on this, we transfected the protein VPS32 and an empty vector as a control (EpNEO) in a poorly-adherent parasite strain and evaluated the amount of EVs released by these parasites by TEM, NTA and Western blot using Evs markers. Our results demonstrated that VPS32 transfected parasites released more EVs than EpNEO parasites. The amount of both EVs populations is affected; suggesting that VPS32 is involved in the biogenesis of exosomes as well as ectosomes. As our previous results demonstrated that EVs are regulating parasites adhesion and now, we observe that VPS32 parasites produces more EVs, we performed an adhesion assay to host cells to evaluate the adherence capacity of VPS32 and EpNEO parasites. Interestingly, our results indicate that parasites transfected with VPS32 are ~40 times more adherents than EpNEO parasites. In summary, our results demonstrated that VPS32 is a key player in the regulation of the adherence process; provably due to its role in the increased biogenesis of extracellular vesicles.

0751 - ESCRTIII COMPLEX IN TRYPANOSOMA BRUCEI: FUNCTIONAL CHARACTERIZATION OF TBVPS32

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INGEBI- CONICET- UBA

The ESCRT (endosomal sorting complex required for transport) drives a diverse collection of membrane remodeling events such as endocytosis,autophagy, release of enveloped viruses, reorganization of the nuclear envelope and cytokinetic abscission.ESCRTIII isthe effector sub-complex for the reason that is capable to form filaments and spirals, which produces membrane constrictions. Vps32 is the most abundant protein in ESCRTIII and its dynamic over membranes is given for its molecular structure that alternates between a monomeric-closed state to polymeric-open state.Here we investigate the conservation of Vps32 in Trypanosoma brucei and we found an orthologue sequence (TbVps32) that shows Snf7 domain and the characteristic secondary structure which gives evidence of a high conservation among eukaryotic cells. For T. brucei procyclic form (PCF) we designed an RNAi strategy where a 405 bp of TbVps32 was cloned into the p2T7 vector (TbVps32-RNAi) allowing a tetracycline inducible downregulation. We confirmed the silencing of TbVps32 by RT-PCR. Moreover, after 72h the viability of the parasites was severely affected with a decrease cell growth and abnormal nucleus-kinetoplast configurations observed by fluorescence microscopy. To further understand the defects in cell cycle progression, knockdown cultures of TbVps32-RNAi were synchronized with HU (hydroxyurea) and then evaluated by propidium iodide (PI) staining showing an increase of cells in G1 phase. On the other hand, we evaluated Vps32 role in vesicular trafficking by receptor mediated endocytosis of transferrin and fluid phase uptake of dextran and the results are under analysis.To perform a functional characterization, TbVps32coding sequence was amplified fused to a hemagglutinin tag at N-terminal (HA-TbVps32) and subcloned into pLew100v5, an inducible overexpression vector. Until now, PCF 29-13 cell line and bloodstream form (BSF) single marker cell line were transfected and are under clonal selection.

0758 - GENETIC DIVERSITY OF NATURAL POPULATIONS OF TRYPANOSOMA CRUZI IN CLINICAL SAMPLES FROM PATIENTS WITH ORAL CHAGAS DISEASE IN VENEZUELA: FOLLOW-UP AFTER TREATMENT WITH TRYPANOCIDAL DRUGS

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INGEBI (1); INSTITUTO DE MEDICINA TROPICAL "DR. FELIX PIFANO" (2)

Oral transmission of Chagas disease (OChD) is an increasingly important aspect in the epidemiology. Venezuela has reported the two largest outbreaks described so far, affecting a total of 192 people mostly children. The long-term impact after treatment on the dynamics of infection in natural populations of T. cruzi is still unclear. In this sense, we proposed a genetic characterization of T. cruzi populations present in peripheral blood in order to differentiate responder or non-responder patients and predict the response to treatment (Tmt). We performed quantification of the parasitic load by qPCR, genetic typing of T. cruzi populations by multiplex qPCR of nuclear genome markers and RFLP-PCR for the hypervariable region of T. cruzi kDNA to demonstrate changes in Minicircle signatures (Ms) of the parasite populations present in 41 clinical samples from 15 patients. To reflect the genetic diversity found, Jaccard distances (Jd) values were compared. This clinical monitoring confirmed the presence of T. cruzi DNA in 26 post-treatment samples up to 9 years after Tmt. These results reveal 100 % therapeutic failure for both outbreaks of OChD, classifying these patients as non-responders to Tmt. All samples showed homogeneity at the DTU level, being typified as TcI. The Ms showed a high degree of polymorphism, with 73 % of total post-Tmt samples with Jd values close to 1. Analyzing the dynamics of each patient`s population separately, in all post-Tmt samples the change in Ms variability respect to pre-Tmt sample was not statistically significant. This variability does not reflect a natural or induced clonal selection process driven by the etiological Tmt; on the contrary, it could be associated with the clonal histotropism process evidenced in natural T. cruzi infections. In conclusion, these strategies of molecular characterization of parasite DNA were useful to detect Tmt failure and find out the lack of parasite population selection with Tmt in these OChD settings.

0778 - FUNCTIONAL ROLES OF AMP-ACTIVATED PROTEIN KINASE (AMPK) COMPLEXES