University of São Paulo

School of Pharmaceutical Sciences of Ribeirão Preto

Graduate Program in Biosciences Applied to Pharmacy

Abstract collection

III WORKSHOP OF BIOSCIENCES AND BIOTECHNOLOGY

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III WORKSHOP

OF THE BIOSCIENCES AND BIOTECHNOLOGY GRADUATE PROGRAM

THURSDAY

November • 28 • 2019

2:00pm POSTER SESSION

FRIDAY

November • 29 • 2019

9:00am ORAL PRESENTATIONS
9:45am COFFEE-BREAK
10:10am CONFERENCE:
EVOLUÇÃO DA TERAPIA CELULAR NO BRASIL
DR. DIMAS TADEU COVAS
11:10AM CONFERENCE:
TECNOLOGIAS PARA PRODUÇÃO DE CÉLULAS PARA TERAPIA
DR AMANDA MIZUKAMI

2:00pm ORAL PRESENTATIONS
3:40pm COFFEE-BREAK
4:00pm CONFERENCE:
INTEGRIDADE NA CONDUÇÃO DE PESQUISA CIENTÍFICA
DR HAMILTON BRANDÃO
5:30pm AWARDS ANNOUNCEMENT AND CLOSING REMARKS

USP

EFFECTS OF TESTOSTERONE SUPPLEMENTATION ON BONE MARROW-DERIVED MACROPHAGES DURING *IN VITRO TRYPANOSOMA CRUZI* INFECTION

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Despite all the scientific progress in recent decades to unravel the host immune processes and the way the parasite evades the immune system, Chagas disease is still a major public health problem, affecting about 3.5 million people, only in Brazil. Several papers describe the influencer of the immune response triggered by steroid gonadal hormones, especially testosterone (TS). TS seems to play an immunosuppressive role on the thymus increasing the differentiation and proliferation of regulatory T cells (Treg) as well as increased Foxp3 expression. The aim of this study was to evaluate the influence of testosterone on the immune response of bone marrow derived macrophages after *Trypanosoma cruzi* infection. Bone marrow from male rats was extracted and cultured with RMPI medium containing 30% L929 cell supernatant for macrophage differentiation. The cells were incubated for 10 days and after this period they were plated into 96 wells at the amount of 1 x 10^5 cells per well. Testosterone (TS) was added at different concentrations: 20 µM, 10 µM, 5 µM and 1 µM, leaving overnight. Cells were washed and added (TS) at the same concentrations and then infected with the Y strain of *T. cruzi* at a rate of 10 parasites per cell. Afterwards the plate was incubated for 6 and 12 hours. The supernatant was harvested and nitric oxide (NO) and Tumor Necrosis Factor (TNF) production was evaluated. The number of parasite inside the cells was measured by 4'-6'-diamino-2-phenylindole (DAPI) staining and rated by High Content Screening (HSC). DAPI staining revealed a significant increase in the number of parasites in TS-containing cells except at the concentration of 1µM hormone when compared to TS untreated control infected cells. After 6 hours of incubation, at the concentrations of 20 and 10 µM of TS, a significant reduction in TNF production was detected. But, at the concentrations of 5 and 1 µM of TS increased concentrations of TNF were noticed at the same levels of the control infected cells. No significant alterations were observed concerning to NO production. However, after 12 hours, increased TNF and NO concentrations were noted being equal or superior when compared to those found in control infected cells. Our data points in the direction that, TS may contribute to cell immunosuppression by increasing cellular parasite infection as well as inflammatory mediators, consequently impairing cellular and tissue architecture in infected individuals.

Palavras-Chave: *Trypanosoma cruzi;* Testosterone; Immunossuppression; Inflamation;

EVALUATION OF CELL DEATH, OXIDATIVE STRESS AND ESTROGEN RECEPTORS EXPRESSION OF BREAST CANCER CELL LINES TREATED WITH COMBINED THERAPY OF RESVERATROL AND CHEMOTHERAPEUTIC DRUGS

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Introduction: According to the World Health Organization, breast cancer is the neoplasm with both highest incidence and mortality rates among women worldwide. Breast cancer is a heterogeneous disease and is classified into three main histologic subtypes according to the presence or absence of molecular biomarkers: i) estrogen receptor (ER)-positive breast cancer; ii) human epidermal growth factor receptor type 2 (HER2)-positive breast cancer; and iii) triple-negative breast cancer. The knowledge of histologic subtypes is important not only for diagnosis and prognosis, but also as a predictive response to chemotherapeutic drugs. However, chemotherapeutic drugs are frequently associated to evident side effects, high toxicity and tumor resistance, that have limited breast cancer therapy. Therefore, novel therapeutic agents with antitumor potential are under investigation. Resveratrol, a natural compound, has previously presented cytotoxicity against several tumor types. Besides, a lot of studies have showed that Resveratrol can improve cytotoxic profile of chemotherapeutic drugs, and combined therapy has reduced the effective dosage of these drugs, leading to decreased toxicity. Thus, Resveratrol has been cited in literature as potentially useful for antitumor therapy, especially when combined to other chemotherapeutic drugs. **Aims:** In this study, we will investigate effects of Resveratrol when combined to chemotherapeutic drugs on cell viability, apoptosis, necrosis, autophagy, oxidative stress and estrogen receptors expression of ER-positive (MCF-7), HER2-positive (SK-BR-3) and triple-negative (MDA-MB-231) breast cancer cell lines, with benign mammary cells (MCF-10A) as control. **Methods:** Cytotoxicity of Resveratrol, chemotherapeutic drugs and combined therapy will be determined via Neutral Red assay. Cell death will be evaluated by flow cytometric analysis with annexin-V-FITC/propidium iodide staining, lactate dehydrogenase quantification via colorimetric method, expression of genes apoptotic/anti-apoptotic via Real Time Quantitative Polymerase Chain Reaction, and expression of proteins apoptotic/anti-apoptotic/autophagic via Western Blot. Oxidative stress will be evaluated by measuring oxidative stress markers, such as malondialdehyde for lipoperoxidation and 8-OHdG for DNA damage. Expression of estrogen receptors will be evaluated by protein levels of estrogen receptors via Western Blot. Statistical analysis of data will be performed using GraphPad Prism 6 software. Data will be expressed as the mean ± standard deviation. Two groups will be compared using the Student's *t* test, while three or more groups will be compared two by two using one-way ANOVA combined to the Tukey *post-hoc* analysis. Statistical significance will be indicated by $p < 0.05$.

Keywords: breast cancer; Resveratrol; chemotherapeutic drugs; cell death; estrogen receptors

Financial Support: CAPES

STUDY OF NEW THERAPEUTIC ALTERNATIVES IN ORAL SQUAMOUS CELL CARCINOMA: CANCER STEM CELLS AND CISPLATIN RESISTANCE

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Introduction: Oral cancer is one of the most common types with an unfavorable prognosis. Late diagnosis, inadequate therapeutic response, recurrence at the primary site, metastasis or development of second primary tumors are the main points associated with short survival. Chemotherapy represents the conventional postoperative treatment for oral squamous cell carcinoma (OSCC) and cisplatin is one of the most commonly used chemotherapeutic agents. However, many patients experience unresponsiveness and subsequent relapse due to chemoresistance acquisition leading to therapy failure. In this context, recurrence and treatment resistance has been associated with the presence of cancer stem cells (CSC). On the other hand, conventional therapies directed at cells in constant cell division do not act on CSC, observing an increase in CSC subpopulation after drug administration. Consequently, there is a need to develop new and more effective therapeutic strategies with fewer side effects that may reverse the resistance of the currently used therapy. Because of this, combination of drugs is proposed as a new pharmacological approach in OSCC. **Objectives:** The aim of the present study is to investigate the anti-tumor effects of new therapeutic combinations in OSCC and to evaluate their action on CSC. **Methodology:** For this purpose, wild type and cisplatin-resistant OSCC cell lines (Cal27, Cal27CisR, SCC9, and SCC9CisR) will be used for cell viability tests and to determine synergism with selected drugs (Cis, FTY720, CQ, and PTX) alone and combined. Cells under the selected drug combination will be analyzed for cancer stem cells subpopulation by using ALDH1 and CD44 staining and flow cytometry, spheroid formation (3D culture) and clonogenic potential analyzed by microscopy. The autophagy markers will be determined by Western blot and resistance (MDR1, BCRP, MRP1, ABC) and stem cell genes (such as b-catenin, Nanog, SOX) by quantitative real-time PCR. **Expected results:** At the end of this study, at least one alternative combined therapy is expected to result in the reduction or elimination of CSC subpopulation, thereby reducing the resistance observed with conventional therapy and disease relapse, significantly increasing patient survival in the future. Financial support: FAPESP, CNPq, and CAPES.

Keywords: OSCC, cancer stem cells, drug combination, cisplatin resistance, recurrence.

EVALUATION OF BIOFILM FORMATION BY *LISTERIA MONOCYTOGENES* IN THE PRESENCE OF *LACTOBACILLUS SAKEI* 1 AND INULIN.

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Financial support: Coordination for the Improvement of Higher Education Personnel (CAPES) – code 001.

Listeria monocytogenes is a bacterium transmitted mainly via food and it is capable of causing serious infections in immunosuppressed people due to several virulence factors (including listeriolysin O, phospholipases, and internalins). Moreover, the ability of biofilm formation by *L. monocytogenes* contributes to its persistence in harsh environments, which is of special concern for food safety. An eco-friendly approach for listerial control in biofilms is the use of antimicrobial peptides (bacteriocins) from commensal or beneficial bacteria (mainly Lactic Acid Bacteria - LAB), combined with selective substrates, such as the prebiotics. In this context, the objective of this work was to evaluate the anti-biofilm activity of *Lactobacillus sakei* 1 (a LAB well established as bacteriocin-producer) combined with the prebiotic inulin, targeting *Listeria monocytogenes* ATCC 19115. To reach this aim, first a crude extract of the bacteriocin of *Lactobacillus sakei* 1 was prepared by growing the bacterium overnight in MRS broth (Oxoid, UK). The culture was centrifuged, the supernatant was filter-sterilized (0.22 µm, GVWP Millipore, USA) and the pH was adjusted with 4 mol/L NaOH. The crude extracted presented an activity of 400 AU/mL (according to critical dilution assay) and it was tested against *L. monocytogenes* biofilms in combination or not with the prebiotic inulin 1% (w/v). For biofilm assays, the microplate technique was used, with violet crystal (Synth, Brazil) 1% (w/v). The results showed that biofilm formation by *Listeria monocytogenes* was inhibited by the crude extract of the bacteriocin of *Lactobacillus sakei* 1, with greater antagonism in the presence of inulin ($P<0.05$). These data demonstrated *Lactobacillus sakei* 1 and inulin presents potential in biofilm control, and suggests that combined use of probiotics and prebiotics can increase the anti-biofilm effect.

Keywords: *Listeria monocytogenes*; Biofilm; Prebiotic; lactic acid bacteria.

GENERATION OF HUMAN RECOMBINANT ANTIBODIES AGAINST ALTERED SELF-GLYCANS RELATED TO MUSCULAR DYSTROGLYCANOPATHIES AND EPITHELIAL CANCERS

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Dystroglycanopathies and epithelial cancers are severe diseases associated with altered-self molecular patterns, such as the hypoglycosylation of α-dystroglycan (α-DG) and transmembrane glycoprotein Mucin 1 (MUC1) associated to the Tn antigen, respectively. Dystroglycanopathies are characterized by progressive muscular degeneration and impairment of patient's quality of life. The diagnosis of these diseases is currently based on the observation of clinical manifestations, muscle biopsies and enzymatic measures, and the available monoclonal antibodies are not specific for the dystrophic hypoglycosylated muscle condition. Regarding MUC1, the Tn antigen has been extensively studied, however, the development of theraupetic strategies targeting this tumor marker posses several challenges. Thus, new strategies based on specific immunodiagnosis of dystroglycanopathies, and the development of immunoliposome-based epithelial cancer drug delivery systems, are suitable approaches to better cope with the diagnosis and therapeutics of these diseases. Here, synthetic GpαDG and GpMuc glycopeptides bearing hypoglycosylated α-DG and MUC1 mucins were employed as targets for the development of novel recombinant antibodies against these molecular targets. Phage display technology, Next Generation Sequence (NGS), and in silico analysis were used to describe and characterize the selected variable heavy (VH) and variable light (VL) domains of human immunoglobulins. The best characterized VH and VL sequences were used to construct the HAbDG (anti-GpαDG) and HAbMuc (anti-GpMuc) single chain fragment variables (scFvs) DNA sequences, which were cloned and expressed in *E. coli* Shuffle T7 cells. ScFvs proteins were able to recognize the respective synthetic glycopeptide or tumor cell extracts. Molecular modeling (docking and molecular dynamics) were performed for HAbDG and showed carbohydrate and peptide recognition, as described and validated by biological assays. Future analysis is needed to improve purification and determine the molecular pattern recognition by HAbMuc scFv. Finally, we have developed human recombinants antibodies against glycans biomarkers of dystroglycanopathies and epithelial cancer with potential theranostic application to face these diseases.

Keywords
α-Dystroglycan; MUC1; Antibodies; Phage Display, Molecular Modelling

STUDY OF GALECTIN-1 PARTICIPATION ON NEUTROPHILS'S HOMEOSTASE

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Introduction: The phagocytic removal of neutrophils in the inflamed tissues is a critical event of leucocytes homeostasis and its failure is associated with several illness such as cancer and autoimmunity. Currently, the process of neutrophil homeostasis is not yet fully understood. Galectin-1 (Gal-1) is a multifunctional glycan-binding protein that can induce exposure of phosphatidylserine (PS) on neutrophil surface favoring its phagocytic recognition by macrophage *in vitro*. However, studies are needed to better understand the role of Gal-1 on neutrophil turnover. **Objective:** We aim to evaluate the impact of mouse Gal-1 (endogenous and exogenous) on neutrophil phagocytosis by macrophages *in vitro* (efferocytosis) and on neutrophil PS exposure and phagocytosis *in vivo*. **Methods**: HL-60 cells and neutrophils derived from wild type ($Lgals1^{+/+}$) or Gal-1 gene deficient ($Lgals1^{-/-}$) C57BL/6 mice will be treated with recombinant mouse Gal-1 (rmGal-1) and analyzed for PS exposure by flow cytometry and confocal microscopy using annexin-V-GFP. Efferocytosis rates will be determined using HL-60 cells and neutrophils from mice treated or not with mouse Gal-1 and incubated with 5-(- 6)-carboxyfluorescein succinimidyl ester fluorochrome (CFSE). Both neutrophils and macrophages will be obtained from $Lgals1^{-/-}$ or $Lgals1^{+/+}$ mice. Then, efferocytosis will be evaluated by flow cytometry using F4/80 antibody (mouse macrophage-specific marker) and CFSE. The participation of endogenous Gal-1 on neutrophil PS exposure and its involvement on *in vivo* granulocyte turnover will be assessed using a sterile inflammation model. A neutrophil chemotactic agent (fMLP - 1 μM) will be injected concomitantly in the subcutaneous region of the ears and into an artificial subcutaneous air pocket. Six or twenty-four hours after fMLP injection, animals will be intravenously injected with a technesium-labeled annexin V solution (Annexin V-HYNIC-99mTc) and animal images will be acquired by micro SPECT / CT. Neutrophil and macrophage migration and phagocytic index in inflammatory exudates will be determined by global and differential accounts. The presence of neutrophils and macrophages in the ears will be determined by the myeloperoxidase and N-acetyl-b-D-glucosaminidase assays, respectively. We believe this work should clarify the role of Gal-1 on neutrophil turnover.

Keywords: Galectin-1; exposure of FS; phagocytosis of neutrophils; neutrophils homeostasis.

Financial Support.
Capes.

IMMUNOPHENOTYPIC AND MOLECULAR CHARACTERIZATION OF MONOCYTE SUBPOPULATIONS IN MYELOPROLIFERATIVE NEOPLASMS

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Introduction: Myeloproliferative Neoplasms (MPNs) are clonal hematological disorders resulting from genetic mutations in hematopoietic stem cells that promote cellular hyperproliferation and accumulation of precursor and mature myeloid cells in bone marrow and peripheral blood. The classic negative BCR-ABL1 MPNs are represented by the neoplasms Polycythemia Vera (PV), Essential Thrombocythemia (ET) and Primary Myelofibrosis (PMF), whose pathophysiology is associated with alterations involving Janus Kinases (JAK), CALR and MPL proteins and cell proliferation epigenetic regulator complexes such as TET2 and DNMT enzymes. The JAK2V617F mutation is the most common alteration, giving to the stem cells the ability to autonomously proliferate, resulting in overproduction of one of the myeloid hematopoietic lineages. Although dysregulated hematopoiesis is mainly attributed to genetic mutations in stem cells, abnormalities in the immune system and cells that compound the medullary microenvironment appear to contribute to the pathophysiology of these diseases. MPNs have recently been considered pre-leukemic oncoinflammatory diseases, with elevated serum cytokine levels and exacerbated inflammatory activity, which appear to contribute to genetic instability and increased risk of thrombosis, hemorrhage and other cardiovascular alterations. Human monocyte populations, subdivided into classical, intermediate and non-classical, have been involved in the genesis and modulation of various chronic, autoimmune and neoplastic inflammatory conditions. Although studies show the presence of monocytosis in patients with MPNs, the phenotypic and molecular profile of these cells during these conditions has not yet been elucidated. **Objectives:** to characterize the immunophenotypic, secretory and molecular profile of peripheral blood monocytes in patients with PV, ET, PMF and healthy individuals. **Methodology:** monocytes will be immunophenotyped to determine circulating populations, as well will be done the quantification of cytokine expression related to different monocyte phenotypes (IL-1β, IL-6, IL-8, IL-12, TNF-α, TGF-β) and epigenetic regulation enzymes (DNMT3A, DNMT3B, HDAC1, HDAC2) by real time PCR. In addition, phagocytosis assay and treatment of monocytes with hypomethylating agent (Decitabin) and JAK2 inhibitor (Ruxolitinib) will be performed in order to clarify their influence on monocytes effector activity. **Results:** The results will help to understand the participation of immune cells in the pathophysiology of MPNs and to elucidate the contribution of monocytes to the oncoinflammatory profile of these diseases, as well the possibility of these cells to be aims of new pharmacological therapies.

Keywords: Hematological diseases; Inflammation; JAK2V617F mutation; Monocytes; Myeloproliferative Neoplasms.

TITLE: Molecular and phenotypic characterization of *Shigella flexneri* strains isolated for 34 years in Brazil

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Abstract: Shigellosis caused by *Shigella flexneri* has been a major public health problem in developing countries such as Brazil. However, there are few studies that molecularly and phenotypically characterized *S. flexneri* strains isolated in this country. The aims of this work were to analyze the frequency of 16 important virulence genes by PCR, the genotypic diversity by ERIC-PCR, the antimicrobial resistance profiles by disc diffusion technique of 130 *S. flexneri* strains isolated from 1983 to 2017 in different States of Brazil. It was observed a high frequency of some virulence genes, *ipaH* was found in all strains; *ial*, *sigA*, *iuc*, *virA* and *pic* genes were found in more than 70% of the strains; *sat* and *virF* genes were found in 48.5% and 57.7% of the strains, respectively. The *ipgD*, *icsA*, *virB*, *sepA*, *set1B*, *set1A* and *sen* genes were found in less than 40% of the strains studied. A total of 57 (43.8%) strains were multidrug-resistant (MDR). By ERIC-PCR a total of 96 (73.8%) strains were grouped in a single cluster with ≥ 70.4% of genetic similarity and specifically 75 of these strains presented a genetic similarity ≥ 80.9%. In conclusion, the high frequency of some virulence genes reinforces the pathogenic potential of the strains studied. The high rates of MDR strains are alarming once it may lead to failure when antimicrobial treatment is necessary. The ERIC-PCR results suggest the presence of a prevalent subtype of *S. flexneri* circulating in different States of Brazil.

Keywords: *Shigella flexneri*; virulence genes; antimicrobial resistance; ERIC-PCR.

Financial Support: CAPES e FAPESP

ROLE OF DOPA-MELANIN PATHWAY IN THE DEVELOPMENT AND VIRULENCE OF THE HUMAN PATHOGENIC FUNGUS *ASPERGILLUS FUMIGATUS.*

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Abstract: *Aspergillus fumigatus* is an important human pathogen, responsible for approximately 90% of invasive aspergillosis diagnosed in patients, especially in immunosuppressed patients, which results in high mortality rates. During infection, the fungus needs to adapt to changes in CO_2 concentration, which in the environment is about 0.033% and inside the host reaches up to 6%. In previous studies, the fungus showed an increase in Afu3g01070 gene expression, which encodes the tyrosinase enzyme, in response to an increase in CO_2 concentration, suggesting that this enzyme may be important for the adaptation of the fungus to this new condition. Tyrosinase enzyme is involved in melanin production and there are several studies suggesting the importance of melanin in protecting organisms against different types of stress. In an *Aspergillus* database analysis, it was found that *A. fumigatus* has another gene encoding the tyrosinase enzyme, the Afu1g17430 gene, and a third gene, Afu4g13780, which encodes a monooxygenase, also involved in melanin production. The production of melanin via DHN-melanin and piomelanine has been well studied, however, the DOPA-melanin route, where tyrosinases and monooxygenase are involved, has not been studied. The deletion of each of the genes encoding the tyrosinase enzyme (Afu3g01070-Δtyr1 and Afu1g17430-Δtyr2) was performed in previous studies and the mutants showed no phenotype (unpublished data). Therefore, the present study aims to delete the Afu4g13780 gene, responsible for the encoding of the monooxygenase enzyme, in the wild-type strain $\Delta akuB_{KU80}$ of *A. fumigatus*, besides obtaining the double mutants Δtyr1Δtyr2, Δtyr1Δmoo, Δtyr2Δmoo and the triple mutant Δtyr1Δtyr2Δmoo. For the development of this study will be used the wild-type strain, $\Delta akuB_{KU80}$ (Ferreira et al., 2006), as well as the mutant strains Δtyr1 and Δtyr2 (unpublished data). The deletion cassette of the described genes will be constructed using the homologous “in vivo” recombination system in *Saccharomyces cerevisiae* (Colot et al., 2006) and confirmation of deletion will be through PCR. The phenotypic analysis will include microscopic analysis, macroscopic analysis, sensitivity tests against different stressors and virulence tests, using the larvae model of *Galleria mellonella*. The present study proposes to clarify the role of the DOPA-melanin pathway on the growing mechanisms and virulence of this important human pathogen.

Keywords: *Aspergillus fumigatus*; tyrosinases; melanin; gene deletion; virulence.

DEVELOPMENT AND VALIDATION OF A COMBINED DIAGNOSTIC DEVICE FOR DENGUE, ZIKA AND CHIKUNGUNYA VIRUSES

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Recombinant protein; Arboviruses; Diagnostic; *Escherichia coli*.

The arboviruses dengue, zika and chikungunya represent a serious public health problem in Brazil and in the world. They are responsible for thousands of annual reports of infections in Brazil with serious consequences, leading to the death of many individuals. These arboviruses circulate simultaneously due to vectors of Aedes class, the common transmitter. They have similar symptoms which hinders the exact clinical diagnosis. Specific diagnosis is necessary to avoid aggravation of consequences that each one could bring and also provide an adequate care to the patients. Serological diagnostic tests available on the market identify a single virus at time. The discriminatory tests of these viruses usually employ molecular techniques using real-time PCR. Such methods are excellent but unsuitable for mass diagnosis due to cost and high technological sophistication. Because of the risks of death or sequels that these viruses impose on individuals, a discriminatory diagnosis is necessary, and the improvement of tests is desired. In a previous project of the group recombinant proteins of Dengue, Zika and Chikungunya viruses were expressed in *E. coli* system, Rosetta (DE3) strain. The main objective of this project is the development and validation of a rapid immunochromatographic kit for discriminatory diagnosis of dengue, zika and chikungunya arboviruses using heterologous proteins produced in *E. coli* system. For this, the first step is the scale up of the expression and characterization of the bioprocess to produce larger amounts of proteins. Then, recombinant proteins will be purified by immobilized metal affinity chromatography under denaturing conditions. The isolated proteins will be dialyzed in phosphate-buffered saline (PBS) and identified by SDS-PAGE analysis. Then, these proteins will be incorporate into membranes or paper and this system will be validated for sensitivity, specificity, reproducibility, repeatability and expiring date. The expected results are to development of a fast, discriminatory, unified diagnostic device for the three arboviruses, produced with national technology, that can reduce the costs of importing these products and also can broad the diagnostic cover.

PROFILE OF SPHINGOLIPIDS IN HEAD AND NECK SQUAMOUS CELL CARCINOMA

FAEDO, R. R.[1]; FUGIO, L. B.[1];GARCIA, C. B.[1]; USHIDA, T. R.[1]; MOREIRA, R.[1], FREITAS, L. C. C.[2], KULCSAR, M.[3], MATOS, L. L.[3], LOPES, N. P.[1], LEOPOLDINO, A. M.[1]

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Sphingolipids are active biomolecules involved in the regulation of many cellular processes and signaling pathways such as proliferation, apoptosis, senescence, angiogenesis, endocytosis, transport, migration, and inflammation. The imbalance of these molecules has been associated with the development of cancer and other conditions. Besides that, Fingolimod, a sphingosine analog, has been used as an immunosuppressant to treat multiple sclerosis patients and has been studied as an antitumoral agent. Recently, studies reported that alterations in ceramide levels are related to poor prognosis in breast cancer. However, the sphingolipids profile is not fully known in HNSCC patients. In the present study, our objective was to determine the profile of sphingolipids in samples from HNSCC patients and analyze potential associations between sphingolipids and clinical/pathological parameters. The research protocol was approved by the Ethical Research Committee for Humans of the FCFRP-USP, São Paulo State Cancer Institute (ICESP-FM-USP) and Hospital das Clínicas of Ribeirão Preto-FMRP-USP. Plasma samples from HNSCC patients were provided by Biobanco of São Paulo State Cancer Institute (ICESP-FM-USP) and analyzed by mass spectrometry (LC-MS, triple quadrupole) to obtain the profile of sphingolipids. We analyzed fifty-seven plasma samples until now. The HNSCC patients group (n=57) was composed of forty-six males and the most frequent anatomical sites were the floor of mouth and tongue (n= 41). Most of them were smokers and/or alcohol consumers; only seven cases did not use tobacco and/or alcohol. The pathologic data showed forty-two cases classified as stage IV; thirty-nine were moderately differentiated. The next step will include the analyses of three different biological samples from HNSCC patients (tumor and paired margin tissue fragments) and plasma from a control group. After that, data will be submitted to statistical analyses to identify significant associations between the different clinic-pathological parameters and sphingolipids, as well as differences between HNSCC and control groups. We believe this study will contribute to understanding the role of sphingolipids in HNSCC, with the potential to be used as biomarkers and potential therapeutic targets. Financial support: FAPESP, CEPID, CAPES, and CNPq.

Keywords: head and neck cancer, sphingolipids, mass spectrometry, oral cancer.

COMPARATIVE ANALYSIS OF THE TRANSCRIPTIONAL PROFILE OF TOTAL LIVER AND LUNG CELLS OF *Mycobacterium tuberculosis* INFECTED MICE

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- **PÔSTER**

ABSTRACT

Keywords: *Mycobacterium tuberculosis*; transcriptome; immune response; liver; lung

Introduction. Tuberculosis (TB), caused by *Mycobacterium tuberculosis (Mtb)* infection, is one of the main causes of morbidity and mortality in the world. The ability of the pathogen to infect practically all the organs of the human body is one of the factors that explains those rates. In a previous project, our research group developed a study in which the kinetics of extra pulmonary tuberculosis infection where observed using an animal model, and in the same study a more effective response to the infectious process was observed in the liver, when compared to other organs such as lung and spleen. **Methods.** Seeking to explain how the liver responded more efficiently to the process of *M. tuberculosis* infection, we worked on the data generated by the RNAseq technique of total cells of the liver and lung. **Results.** Comparing both signatures, we identify 2190 differentially expressed genes. Pathway enrichment has allowed us to observe that the inflammation helps to modulate the response profile of each organ, and as it is a biological response of the immune system, consequently it may induce the activation of several signaling pathways in different organs causing different responses to the infectious agent. In our study we have this difference in signaling pathways as the main difference among the organs, shows pathways such as NOTCH, TRAF, and WNT being upregulated, besides several mechanisms having

members of the RUNX family as the transcription factor responsible for transcription activation, pathways that were down regulated in the liver as compared to the lung, indicating to be involved in *Mtb* infection control. Still about the up regulated pathways, we also highlight the fact that epigenetics is shown acting as part of the transcriptional signature through enriched pathways such as “histones deacetylate (HDACs)”. **Conclusion.** We concluded that the better response of liver is directed related to the activation of some pathways that characterize a less inflammatory and more homeostatic profile.

EXPRESSION OF RECOMBINANT PROTEIN CHIMERA FROM HUMAN PAPILOMAVIRUS FOR THERAPEUTIC VACCINES DEVELOPMENT

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Keywords: HPV; recombinant protein; immunoinformatic.

The Human Papillomavirus is capable of inducing precancerous lesions in cervical epithelium which can evolve to cervical cancer, considered the world's second most common cancer among women. Although there are prophylactic vaccines available on Sistema Único de Saúde (SUS), brazil's public health-care system, the effectiveness has been proven only in girls who were not previously exposed to the virus. The oncoproteins epitopes of E6, E7, proteins responsible for the cellular malignancy, and the L2 capsid protein have been presented as a possible target for therapeutic vaccines, with possibility of activating the cytotoxic immune response mediated by T cells and, consequently, eliminating the infected cells. This project intends to develop and produce a chimeric fusion protein combining bind epitopes of HPV16 proteins.

Using an immunoinformatic approach, the protein's amino acids sequences will be selected from a data bank and its structure predict. There is going to be an epitopes selection based on bind-affinity for Major Histocompatibility Complex (MHC) II and MHC I. These epitopes will have their independent structure predicted with biotechnology tools and the best predictions will be selected for computational docking and molecular dynamic, evaluating the epitopes binding efficiency with MHC and the interaction between them, respectively. To assist the protein folding physicochemical characteristics, a sequence of amino acids linkers is going to be designed, creating a protein sequence composed by the epitopes from HPV protein and the linkers. The final sequence is going to be synthetized as a unique protein, inserted in a host expression system, expressed, purified and sequenced.

Even though the selection of epitopes capable of activating cytotoxic response through MHC I and a methodology which could effectively measure the human response are obstacles to overcome in the project development, either by complexity or high cost, the success of chimeric protein expression may result, in the future, in a therapeutic vaccine able to reduce the precancerous lesions and help the elimination of HPV infection.

IMMUNOPHENOTYPIC AND MOLECULAR EVALUATION OF ERYTHROID PRECURSORS IN MYELOPROLIFERATIVE NEOPLASMS

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Introduction: Myeloproliferative neoplasms (MPNs) are hematological disorders characterized by clonal expansion and exacerbated proliferation of precursor and mature cells belonging to the myeloid lineage. The pathophysiology is linked to mutations called primers in the Janus Kinase 2 (JAK2), Calreticulin (CARL) and Thrombopoietin Receptor (MPL) genes. These mutations stimulate constitutive activation of the JAK2-STAT pathway and contribute to high proliferation by generating myeloid cell accumulation in the bone marrow and peripheral blood, causing: high blood viscosity, and increased risk for thrombotic, hemorrhagic, inflammatory, and cardiovascular damage. This project will aims on Ph-negative MPN Polycythemia Vera (PV), Essential Thrombocythemia (TE) and Primary Myelofibrosis (PM), and will investigate the frequency and molecular markers of erythroid terminal precursors of these diseases in JAK2 positive patients. Despite the knowledge gained in the last two decades about the pathophysiology of PV, TE and PM, many studies are still needed to clarify how the same mutations originate different hematological disorders. **Objectives:** To evaluate the immunophenotype and the expression of cell cycle and death regulatory genes in terminal erythroid precursors of patients with PV, TE and PM. **Methodology:** Bone marrow aspirates from a total of 15 adult patients with PV, ET and PM will be studied. All selected individuals must consent to participate by signing the informed consent form. Bone marrow samples will be labeled with glycophorin A, band 3, α4-integrin and CD45 antibodies, and will be immunophenotyped and raffled in flow cytometry, according to the strategy established by Xiuli An and Lixiang Chen in 2017. Isolated erythroid precursors will undergo the Cytospin process, to evaluate cell populations. These cells will be subjected to RNA extraction and this mRNA will be quantification for utilization . The detection, identification of mRNA in the samples will be performed using the nCounter® platform (NanoString Technologies®), using the PanCancer CodeSet panel, capable of detecting up to 40 pre-established targets, which are relevant in NMPs. **Results**: This study will promote a better understanding of the pathophysiology of PV, ET and PM and will reproduce a description of the biomarkers used in the differential diagnosis and new therapeutic targets for these hematological malignancies.

Keywords: Hematological diseases; Erythroid precursors; JAK2 mutation; Myeloproliferative Neoplasms.

MOLECULAR CHARACTERIZATION OF MULTIDRUG-RESISTANT *Escherichia coli* ISOLATED FROM SOIL AND WATER

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Escherichia coli are commonly reported causing different types of infections, including urinary tract infections. The environment has been acting as reservoir and disseminator of multidrug-resistant (MDR) bacteria, including *E. coli*, as well as the acquired antimicrobial resistance genes (ARGs). Therefore, this study aimed to characterize molecularly *E. coli* isolates obtained from soil and water samples in Brazil. After bacterial isolation, the isolates were identified by sequencing of the 16S rDNA gene. The antimicrobial resistance profile was determined by the disk diffusion method and ARGs were researched by PCR. The complete genome sequencing was performed using the Illumina MiSeq Platform and then, bioinformatics analyzes were performed for detection of ARGs, plasmids and determination of *Multilocus Sequence Typing* (MLST). A total of 100 *E. coli* isolates were obtained, being 43 from soil and 57 from water. These isolates were obtained in environmental samples from different cities from São Paulo State and Minas Gerais State. Most isolates (60% to 96%) were resistant to penicillin, cefazolin, cefuroxime, streptomycin, sulfonamides, trimethoprim and, trimethoprim-sulfamethoxazole. Among these isolates, 83 were classified as MDR. The *tet*A gene was the most prevalent, being detected in 30 isolates, followed by *sul1* (23), *tetB* (22), bla_{CMY} (19), bla_{SHV} (18), *aadA* (17), *tetC* (13), *sul2* (12), $bla_{\text{OXA-1-}like}$ (7), bla_{TEM} (6), *sul3* (6), *dfrA* (6), *mdf*(A) (6), *aac(6')-Ib* (5), *floR* (5), *aph(3')-Ia* (4), $bla_{\text{OXA-48-}like}$ (2), *qnrB* (2), *qnrS* (2), *ant(2")-Ia* (2), *Inu*(F) (2), *tet(34)* (2), *cmlA* (1), bla_{VEB} (2), $bla_{\text{CTX-M-Gp1}}$ (4), *aac(3)-Ia* (1), *aph(3")-Ib* (1), *aac(3)-IId* (1), *aph(6)-Id* (1), $bla_{\text{CTX-M-14}}$ (1), $bla_{\text{CTX-M-15}}$ (1), and *mphA* (1). Seven MDR *E. coli* isolates (two from soil and five from water) were submitted to WGS analyses. Mutations in quinolone resistance-determining regions of GyrA (Ser83Leu; Asp87Asn; Asp87Tyr), ParC (Ser80Ile; Glu62Lys; Glu84Val; Glu84Gly) and ParE (Ile355Thr; Ser458Ala; Ile529Leu) were also detected. Different plasmids were detected, such as IncR, IncN3, IncX1, IncP1, IncF (-2, -18, -24, -48, -57, and -113), IncFIA (-1 and -8), IncFIB (-1, -20, -49, and -54), IncN, Col440l, ColRNAl, ColpVC, and p0111. MLST typing revealed seven different sequence types (STs), such as ST131, ST189, ST223, ST354, ST648, ST906, and ST1665. Among the detected STs, *E. coli* ST131 and *E. coli* ST648 are classified as international high-risk clones. To date, it is possible to observe the presence of MDR ARGs-producing *E. coli* in the environment, including international high-risk clones in water sources.

Keywords: *Escherichia coli*; Antimicrobial resistance; Soil; Water.

SPHINGOSINE KINASE 2 IN ORAL SQUAMOUS CELL CARCINOMA

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Background and aim: sphingosine kinase 2 (SK2) is one of the enzymes responsible for producing sphingosine-1-phosphate (S1P)[1]. Recently, SK2 has been associated with protective autophagy and survival, and regulation of p21 in breast and colon cancer cells[2; 3]. However, the role of SK2 in oral squamous cell carcinoma (OSCC) is still unclear. Thus, our study aims to investigate the involvement of SK2 in autophagy and proliferation in OSCC cells.

Experimental procedure: HN13 and HN12 (OSCC) cell lines were transduced with short hairpin RNA interference against SK2 and a lentiviral vector containing cDNA for SK2, respectively. Cell cycle analyses were performed by propidium iodide staining and flow cytometry. Western blotting and immunofluorescence assays were adopted to analyze protein levels and cellular distribution.

Results: HN13 cells with SK2 knockdown showed a decrease of pAkt, c-MYC, and LC3 levels (an autophagy marker) while p21 was increased. Besides that, the SK2 knockdown in HN13 cells caused cell arrest in S phase with reduction of the cells in G2/M. SK2 overexpression in HN12 cells leads to an increase of pAkt, c-Myc, and LC3 levels.

Conclusion: Our work is the first to demonstrate the role of SK2 in proliferation and autophagy in OSCC cells. Other studies are in progress to understand the molecular mechanism underlying the role of SK2 and its potential as a target. Financial support: FAPESP (grant: 2016/19103-2; scholarship: 2018/14225-8, CAPES, CNPq- Brazil.

Keywords: sphingosine-1-phosphate, autophagy, proliferation, oral cancer, sphingosine kinase.

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Molecular studies of *Aspergillus* spp. clinical isolates from different anatomical sites and lung of cystic fibrosis patients

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Aspergillus spp. are saprophytic fungi belonging to the order *Eurotiales* and the phylum *Ascomycota,* and commonly found in soil and decaying vegetation. They can be found in household dust, building materials, ornamental plants, flower arrangements, tobacco, food, and water. This genus has naturally distributed filamentous fungi species that are capable of generating several types of infections such as invasive aspergillosis, chronic pulmonary aspergillosis, simple aspergilloma, *Aspergillus* bronchitis, allergic bronchopulmonary aspergillosis or severe asthma with fungal sensitization - especially in immunosuppressed or immunocompromised individuals. Several sections within the genus *Aspergillus*, like *A. fumigatus, A. flavus, A. niger* and *A. terreus* have shown resistance to commercially available antifungals. Thus, it is necessary to study alternative techniques for the treatment of infections, such as antimicrobial photodynamic therapy. This project aims to study the clinical isolates of *Aspergillus* spp. from different anatomical sites of patients affected by aspergillosis and/or affected by cystic fibrosis from Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto - USP (HC - USP). The clinical isolates will be identified by molecular methods (sequencing of Internal Transcription Spacer - ITS, β-tubulin, calmodulin, and Restriction Fragment Lenght Polymorphism - RFLP). The macro (giant colony) and micromorphology of clinical isolates will be characterized as well the thermotolerance and biofilm formation. The *in vitro* susceptibility to commercial antifungals (voriconazole, itraconazole, posaconazole and amphotericin B) and antimicrobial photodynamic therapy with phenothiazine photosensitizers (methylene blue and new methylene blue N) will be tested. The DNA fingerprinting discrimination of clinical isolates from the sputum of cystic fibrosis patients will be performed through Random Amplified Polymorphic DNA (RAPD) technique. The antifungal resistance genes and the virulence of clinical isolates in an alternative animal model *Galleria mellonella* will be evaluated. So, this detailed investigation of *Aspergillus* spp. clinical isolates will provide data to improve the treatment of infections caused by the species of *Aspergillus* genus.

Key words: *Aspergillus* sp., antimicrobial photodynamic therapy, molecular identification, antifungal resistance, biofilm.

STUDY OF INTERACTION BETWEEN GALECTIN-1/SARCOMERIC ACTIN AND ITS POTENTIAL ROLE IN MUSCLE CONTRACTION

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INTRODUCTION - Muscle contraction is a vital and complex process. The molecular mechanisms involved in the physiology of muscle contraction are not yet fully understood. Our group demonstrated that Galectin-1 (Gal-1), a multifunctional carbohydrate-binding protein, is expressed in the musculature showing a striated pattern and co-localization with sarcomeric actin. However, the role of Gal-1/sarcomeric actin interaction in striated muscle physiology is not known. **OBJECTIVES** - We aim to characterize the interaction between Gal-1 and sarcomeric actin and investigate its impact on the contraction of skeletal muscle. **METHODOLOGY** – The analysis of the interaction between recombinant dimeric Gal-1 (rhdGal-1) and purified actin from rabbit muscle was accomplished by co-sedimentation and viscosimetric assays. Also, these assays will be applied to evaluate the molecular mechanism involved in the interaction Gal-1/actin using different forms of Gal-1 [monomeric; oxidized dimeric (does not bind carbohydrates); dimeric Gal-1 (in the presence or absence of lactose)]. The physiological relevance of Gal-1/actin interaction will be further investigated by analyzing the mouse performance in repetitive tests of muscular contraction testing C57BL/6 wild-type ($Lgals1^{+/+}$) or genetically deficient in Gal-1 ($Lgals1^{-/-}$) mice. Also, muscle samples from these animals will be analyzed by western blot and immunofluorescence to investigate the expression and localization of Gal-1. **RESULTS** - rhdGal-1 was able to increase (1.25 to 5.0 µM) or reduce (20 µM) the viscosity of an actin solution. Besides, rhdGal-1 (1.25 µM) caused the highest increase in viscosity (73%), while 20 µM of this lectin promoted a 78% reduction in viscosity compared to the actin control solution. Sedimentation results showed that rhdGal-1 (5 µM) reduced actin solubility and 10 µM favored the solubility of this contractile protein. These findings indicate that rhdGal-1 concentrations between 1.25 and 5.0 µM induce actin polymerization in solution. Furthermore, rhdGal-1 concentrations between 10 and 20 µM favor actin depolymerization. **CONCLUSION** - We suggest that the Gal-1/actin interaction may play important roles in the physiological process of actin polymerization/depolymerization during muscle contraction, depending on the concentration of Gal-1 in this tissue.

Keywords: Galectin-1, actin, muscle contraction.

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BYOSYNTHESIZED SILVER NANOPARTICLES FOR USING IN PHOTODYNAMIC THERAPY WITH PHENOTIAZINUM PHOTOSENSITIZERS AGAINST PATHOGENIC FUNGI

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The emergence of new species of pathogenic and resistant fungi to commercial drugs leads to the need to develop new strategies for treating fungal infections. The antimicrobial photodynamic therapy (APDT) is a promising technique to control resistant microorganism. The aim in the association of photosensitizer (PS) (e.g. methylene blue – MB, new methylene blue N – NMBN and new methylene blue N zinc – NMBN Zn) with nanoparticle is to optimize the efficiency of the process. In this study, silver nanoparticles (AgNPs) were biosynthesized by *Fusarium oxysporum*. The AgNPs and AgNPs+PS were characterized by using Uv-Vis Spectroscopy to measure surface plasmon peak, and dynamic light scattering method to obtain diameter and zeta potential. The microdilution broth method was used to determine the minimal inhibitory concentration (MIC) of PS, AgNPs and AgNPs+PS in APDT (15 J cm^{-2}). The association of AgNPs and PS was based on MIC. Toxicity of compounds was tested on invertebrate model *Galleria mellonella*. AgNPs presented a surface plasmon peak around 420 nm, hydrodynamic diameter of 86,72 nm and zeta potential of -28,6 mV. In the absorbances of complexes (AgNPs+PS), the surface plasmon band of AgNPs appeared larger and dislocated by the PS ligands on the surface. Zeta potencial of complexes were -13,9 mV, -17 mV and -9,25 mV for AgNPs+MB/NMBN/NMBN Zn, respectively. For *Candida albicans,* the MIC of AgNPs was 1,56 µg/mL. MB, NMBN and NMBN-Zn presented the MICs 1,59, 0,43, 0,53 µg/mL, respectively. The complexes AgNPs+MB, AgNPs+NMBN and AgNPs+NMBN-Zn presented the MICs 0,39 and 1,59, 0,04 and 0,21, and 0,04 and 0,13 µg/mL, respectively. For *Fusarium keratoplasticum*, the MIC of AgNPs was 6,25 µg/mL. The MICs of MB, NMBN and NMBN-Zn were 1,59, 0,21 and 0,26 µg/mL, respectively. The complexes AgNPs+MB, AgNPs+NMBN and AgNPs+NMBN-Zn presented the MICs 0,78 and 0,79, 0,39 and 0,1, and 0,39 and 0,26 µg/mL. In the toxicity experiment, the compound that caused more larvae mortality was $AgNO_3$ (positive control), it caused 60% of death of larvae. The other treatments didn't kill any larvae until day 5. Thus, AgNPs+PS have antifungal effects and present a high potential to optimize photodynamic therapy.

Key words: silver nanoparticles; antimicrobial photodynamic therapy; phenotiazinum; *Fusarium keratoplasticum*; *Candida albicans*

Financial support: Capes, FAPESP

DETERMINATION OF THE MINIMUM INHIBITORY CONCENTRATION (MIC) OF *Fusarium oxysporum* TO DIFFERENT ANTIFUNGALS

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Fusarium oxysporum is a filamentous fungus widely known as phytopathogen, and has been emerging as a human pathogen, being isolated even from severe disseminated infections. *Fusarium oxysporum*, may present intrinsic resistance to azol antifungals, which is alarming, due to the low diversity of this class of drugs. Thus, the study of the resistance mechanisms may contribute to the use of new drug targets. In this sense, the omic techniques can be of great value, since they can correlate genome with differential expression and therefore use a complete arsenal of transcripts, proteins and metabolites when fungi are grown under specific conditions. The purpose of this study is to determine the MIC of three antifungals tested for *Fusarium oxysporum* and the concentration that allows 50% fungal growth. Primarily, the MIC test was performed to select the antifungals that will be used in the farther experiments and the concentration of these drugs that enable 50% of fungal growth. The MIC test consists of serial dilutions of the antifungals tested (Amphotericin B, Fluconazole and Itraconazole) in 96-well microplates, the addition of a culture medium (RPMI-1640) and the spore inoculum. Amphotericin B and Itraconazole was tested in the concentration range of 0,031 to 16 µg/mL. Fluconazole was tested in the range of 0,125 to 64 µg/mL. The microplates were incubated for 48 h, at 35 ºC and then, read at 530 nm in plate reader. A visual analysis of the plates was also performed to determine in which wells the fungus grew. For azoles fungal growth was observed at all concentrations tested. For Amphotericin B, MIC was observed in the range of 0.5 to 1.0 µg / mL. The concentrations that allowed around 50% growth of the fungus were 0.25, 16 and 0.125 µg / mL for Amphotericin B, Fluconazole and Itraconazole, respectively. Itraconazole was also found to be poorly soluble in the diluent used (DMSO), which may result in precipitates that interfere with the experiments. For this reason, Amphotericin B and Fluconazole were selected to continue the experiments. These two antifungals will be applied in growth to induce fungal resistance, which will be analyzed by omic techniques (proteomics, transcriptomics and metabolomics). The data will be analyzed using statistical and bioinformatics tools, allowing us to delineate what is being differentially expressed by *Fusarium oxysporum* under specific conditions, and is promoting the resistance and the mechanism used.

Key words: Fusariosis; filamentous fungi; antifungals; fungal resistance; omics.

THIOREDOXIN-DEPENDENT PEROXIDASE AS CYTOPLASMATIC REDOX-SENSOR IN *NEOSPORA CANINUM*

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ABSTRACT

The apicomplexan protozoan *Neospora caninum*, the etiological agent of neosporosis, is associated with neurological symptoms in dogs and abortion in cattle. This disease is worldwide spread and has no efficient treatment. The survival and replication of the parasite depends on an enzymatic antioxidant system defense for evading the oxidative stress inside the host cell. Among these enzymes, thioredoxin-dependent peroxide reductase (NcPrx) draw our attention due to a significant relative abundance in the proteome of *Neospora caninum* allied to the absence of literature. Previous results showed that NcPrx act as functional enzyme of antioxidant system defense once it demonstrates antioxidant and peroxidase activity *in vitro*. We investigated the localization and role of Thioredoxin-dependent peroxidase (NcPrx) in the antioxidant system of *N. caninum*. For this purpose, the potential of hydrogen peroxide (H_2O_2) to induce Reactive Oxygen Species (ROS) was measured on cell cultured parasites. Tachyzoites (2 ×105/well in a 24 well plate) were allowed to invade and proliferate in Vero cells for 18 h. Then each well was treated with H_2O_2 (2- 0,5mM) over 1h30min and incubated with 10µM DCFDA (Sigma) for 30 min. The percentage of DCF positive cells (from gates representing cells or tachyzoites) were measured in a BD LSRFortessa™ (BD Biosciences) flow cytometer (Ex.488/Em.535 nm) and indicated an increase of ROS production in *N. caninum* egressed tachyzoite induced by H_2O_2 treatment. Then, the NcPrx oxidation status was evaluated in tachyzoites ($1x10^6$) incubated in H_2O_2 (1-0,25mM) over 1h30min, followed by alkylation and lyses (triton 1%). The samples were analyzed by Western blot (anti-NcPrx polyclonal serum) and showed a significant increase of dimerization/hyperoxidation ($p<0,05$) after H_2O_2 treatment (0,5mM) denoting an H_2O_2 reduction by NcPrx. Finally, the immunolocalization of native NcPrx was performed in cell cultured parasites after fixation and permeabilization and labelling with anti-rNcPrx1 serum (1: 2000) and secondary antibody anti-mouse Alexa Fluor 488. The mitochondrial were detected by MitoTracker™ Red and nucleus by DAPI (4′,6-diamidino-2-phenylindole) and the images were captured by Multiphoton Microscopy and processed in Fiji ImageJ. The images analyses revealed that NcPrx has a cytoplasmic distribution and low occurrence of mitochondrial colocalization. In summary, these results suggest that NcPrx is a cytoplasmic redox sensor in *N. caninum* and presents peroxidase activity in this parasite. Besides, NcPrx oxidative state could provide insight into disturbances of tachyzoite redox homeostasis under drug treatments.

Keywords: *Neospora caninum*, peroxidase, oxidative stress, hydrogen peroxide

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CYTO AND ECOTOXICOLOGICAL EVALUATIONS OF NEW PHENOTHIAZINE PHOTOSENSITIZERS USED IN ANTIMICROBIAL PHOTODYNAMIC TREATMENT

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Antimicrobial photodynamic treatment (APDT) is an interesting alternative to conventional antimicrobials. The approach is based on the use of a photosensitizer (FS), such as phenothiazinium dyes, which preferentially accumulate in the microbial cell. FS exposure to light results in local production of several reactive oxygen species that kill the microorganism without causing significant damage to the animal or plant host. In recent years, APDT has been proposed for the control of phytopathogens. This will imply the application of large amounts of PS in the environment and, consequently, the contamination of soil and water bodies. Therefore, essential prerequisites to the use of APDT in the field are the ecotoxicological and environmental impact assessments of these compounds. The objective of the present study is to evaluate the cytotoxicity and the ecotoxicity of phenothiazinium FS both in the dark and under light. Cytotoxicity was evaluated in human fibroblasts by the XTT assay and ecotoxicity is being evaluated by the determination of the acute toxicity to aquatic microcrustacean *Daphnia similis* and to embryos of the fish *Danio rerio*. To date, four commercial phenothiazinium FSs have been evaluated: Methylene Blue (MB), New Methylene Blue N (NMBN), Toluidine Blue (TBO) and Dimethyl Methylene Blue (DMMB). In the dark, the most toxic FS was NMBN, followed by TBO, MB and DMBN. All FS were more toxic under light than in the dark and, in general, the toxicity increased with increasing the exposure time. All FS were very toxic to *D. similis*, both in the dark and under light. The dark toxicity of DMMB was the highest and differed from the toxicities of the other three FSs (MB, NMBN and TBO). To date, experiments with *D. rerio* were conducted only with MB and NMBN in the dark. The results showed that MB was not toxic to the embryos even at the highest concentration evaluated (100 μM) and that the NMBN was non-toxic up to 1 μM but killed 100% of the embryos at the concentration of 100 μM. Further experiments will be performed using the most appropriate concentration ranges for the determination of the lethal concentration 50 (LC_{50}) of these FS. The combined analysis of the results indicates that *D. similis* is much more sensitive to phenothiazinium FSs than the embryos of *D. rerio*, which reinforces the importance of the use of different organisms in ecotoxicological evaluations.

Keywords: Photodynamic antimicrobial treatment; phenothiazine photosensitizers.

CYTO AND ECOTOXICOLOGICAL EVALUATIONS OF NEW PHENOTHIAZINE PHOTOSENSITIZERS USED IN ANTIMICROBIAL PHOTODYNAMIC TREATMENT

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Antimicrobial photodynamic treatment (APDT) is an interesting alternative to conventional antimicrobials. The approach is based on the use of a photosensitizer (FS), such as phenothiazinium dyes, which preferentially accumulate in the microbial cell. FS exposure to light results in local production of several reactive oxygen species that kill the microorganism without causing significant damage to the animal or plant host. In recent years, APDT has been proposed for the control of phytopathogens. This will imply the application of large amounts of PS in the environment and, consequently, the contamination of soil and water bodies. Therefore, essential prerequisites to the use of APDT in the field are the ecotoxicological and environmental impact assessments of these compounds. The objective of the present study is to evaluate the cytotoxicity and the ecotoxicity of phenothiazinium FS both in the dark and under light. Cytotoxicity was evaluated in human fibroblasts by the XTT assay and ecotoxicity is being evaluated by the determination of the acute toxicity to aquatic microcrustacean *Daphnia similis* and to embryos of the fish *Danio rerio*. To date, four commercial phenothiazinium FSs have been evaluated: Methylene Blue (MB), New Methylene Blue N (NMBN), Toluidine Blue (TBO) and Dimethyl Methylene Blue (DMMB). In the dark, the most toxic FS was NMBN, followed by TBO, MB and DMBN. All FS were more toxic under light than in the dark and, in general, the toxicity increased with increasing the exposure time. All FS were very toxic to *D. similis*, both in the dark and under light. The dark toxicity of DMMB was the highest and differed from the toxicities of the other three FSs (MB, NMBN and TBO). To date, experiments with *D. rerio* were conducted only with MB and NMBN in the dark. The results showed that MB was not toxic to the embryos even at the highest concentration evaluated (100 μM) and that the NMBN was non-toxic up to 1 μM but killed 100% of the embryos at the concentration of 100 μM. Further experiments will be performed using the most appropriate concentration ranges for the determination of the lethal concentration 50 (LC_{50}) of these FS. The combined analysis of the results indicates that *D. similis* is much more sensitive to phenothiazinium FSs than the embryos of *D. rerio*, which reinforces the importance of the use of different organisms in ecotoxicological evaluations.

Keywords: Photodynamic antimicrobial treatment; phenothiazine photosensitizers.

MESENCHYMAL STEM CELL-DERIVED EXOSOMES: PRODUCTION IN BIOREACTORS AND EVALUATION OF *IN VIVO* FUNCTIONALITY

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Recently, the therapeutic effects of human mesenchymal stem cells (MSCs) have been attributed to their paracrine activity, exerted by growth factors, cytokines and chemokines and, mainly, by small secreted extracellular vesicles (EVs), not involving the integration of cells in this process (Free-cells therapy). The extracellular vesicles (EVs) represent a class of small membrane vesicles, typically of 30 to 1000 nm in diameter (exosomes and microvesicles), and formed by a lipid bilayer that contains proteins, lipids and various nucleic acids, including mRNAs and microRNAs (miRNAs). In recent years, EVs have attracted considerable attention due to their ability to stimulate or repress immune system functions through intracellular interaction from the transfer of biologically active molecules. Currently, EVs production is done in traditional static culture systems with limited capacity. The different cultivation conditions of MSCs can directly interfere with production, therefore the control of cultivation parameters such as pH, dissolved oxygen and key nutrients are extremely important to obtain a vesicular content. Considering the development of EVs-based therapies, the *in vitro* expansion of MSCs is required to provide a therapeutic dose. Bioreactors have been used with the purpose of monitoring and controlling the cultivation conditions necessary for efficient cell growth, enabling large-scale cell expansion. Thus, this project aims to produce EVs derived from human umbilical cord-derived mesenchymal stem cells in bioreactors, as well as to characterize and evaluate their functionality in an experimental model. For this purpose, the hollow fiber and stirred tank bioreactors will be used. The agitated tank bioreactor will be supported by microcarrier technology for cell growth. The EVs production in monolayer static flasks will be performed for comparative purposes. After production, EVs will be isolated and characterized according to the minimum experimental requirements for their definition and functions, proposed by the International Society for Extracellular Vesicles. The functionality of the produced EVs will be evaluated in an experimental mouse colitis model.

Keywords: Extracellular vesicle; mesenchymal stem cell; bioreactor; hollow fiber, stirred tank.

CLONING, EXPRESSION, SOLUBILITY AND PURIFICATION OF *Neospora caninum* RECOMBINANT SUPEROXIDE DISMUTASE 2 (SOD2) PROTEIN

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Superoxide dismutases (SODs) are a family of metalloproteins that protect aerobic organisms from toxic radicals or ROS by converting superoxide anions into molecular oxygen and hydrogen peroxide. Among them, there is the antioxidant enzyme SOD2, produced by several obligate intracellular parasites, belonging to the Apicomplexa phylum, such as *Toxoplasma gondii*, *Plasmodium spp* and *Neospora caninum.* SOD2 is one of the antioxidant enzymes that protect these parasites from oxidative stress caused at the moment of invasion and during permanence of these protozoa inside the cell. Based on this information, the aim of this study was to express a recombinant *N.caninum* SOD2 to investigate the importance of this enzyme in the mechanism of invasion and replication. First the *N. caninum* NcSOD2 enzyme gene (NcLIV_058830) was cloned into pET-28 and transformed by electroporation into different strains of competent cells (*Escherichia coli* BL-21, Shuffle, Rosetta-gami 2 and Arctic) and inoculated into plates containing the solid medium Luria Bertani (LB). The isolated colonies were selected with kanamycin and cultured in liquid LB for expression of the enzyme using different conditions, such as incubation time, temperature and varying concentrations of Isopropyl- beta-D-thiogalactopyranoside (IPTG). For the characterization of solubility the bacterial pellet obtained in the previous step was sonicated with 3 different buffers (100 mM Tris-HCl + Phosphate; 1.5% Triton or 8M Urea). To evaluate enzyme expression, solubility and purification, the samples were applied on a 12.5% SDS-PAGE gel and finally purified on a PD-10 column containing a nickel resin. The NcSOD2 fragment (~ 586 pb) was cloned into the pET-28 vector, and all bacteria strains used in the study expressed the recombinant form of *N. caninum* SOD2 (rNcSOD2). The enzyme was more soluble in urea when produced in BL-21 at 37 ° C while in Arctic bacteria solubility was higher in Tris-HCl + Phosphate at 15 ° C. To date, the rNcSOD2 has been successfully purified in urea buffer. The results indicate that SOD2 is expressed in *E.coli*, however differences in solubility and yield were observed between the strains analyzed. This project will contribute to the knowledge about SOD2 in the mechanisms involved in *N. caninum* infections which are poorly understood. The next steps are: production of polyclonal anti-rNcSOD2 antibody and functional enzymatic assays.

Keywords: Recombinant Protein; Superoxide Dismutase 2; *Neospora caninum*

LPS-TARGETING GALECTINS -3 AND -4 ON BLOOD GROUP B POSITIVE E. COLI O86: NEW ASPECTS ON ITS MICROBICIDAL ACTION AND ASSOCIATION WITH ANTIBIOTICS

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Background: Galectin-3 (Gal-3) and galectin-4 (Gal-4) are mammalian lectins that recognize glycans containing β-galactosides. Both galectins recognize the LPS on *Escherichia coli* O86, which contains blood group B epitope (B^+ *E. coli*), but only Gal-4 kills this microorganism. This death is known to be fast, promotes loss of membrane integrity and it is dependent of the C-terminal Carbohydrate Recognition Domain (CRD) of Gal-4. However, little is known about the action mechanism of Gal-4 towards B^+ *E. coli* and how it correlates with known bactericidal antibiotics mechanisms. **Aims:** Contribute to better understanding the Gal-4 killing mechanism on B^+ *E. coli* and its associations with Gal-3 and antibiotics such as Polymyxin-B and Ampicillin. **Materials and methods:** Human recombinant Gal-3 and Gal-4 were produced in plasmid-transfected *E. coli* and purified in sepharose-lactose column by biological affinity chromatography. Viable bacteria count after isolated or concomitantly incubations of Gal-4, Gal-3, Ampicillin and Polymyxin-B in B^+ and B^- *E. coli* was evaluated counting the Culture Forming Units (CFU) of serially diluted suspensions. Evaluation of the impact of Gal-4 and Gal-3 on the membrane of B^+ *E. coli* was determined by Atomic Force Microscopy (AFM) imaging of paraformaldehyde-fixed cells. **Results:** Gal-3 and Gal-4 were successfully produced and purified, with estimated SDS-PAGE bands of ~30 and ~35 kDa, respectively. After 20 minutes of Gal-4 (5 µM and 1 µM) treatment, B^+ *E. coli* viability was reduced to ~5% and ~40%, respectively. AFM evaluation showed loss of membrane integrity and blebs induction for Gal-4 (5 µM) but not for Gal-3 (5 µM) treatments. Surprisingly, a high concentration of Gal-3 (40 µM) reduced B^+ *E. coli* viability to ~50% and Gal-3 (5, 10 and 20 µM) concomitant addition with a non-lethal concentration of Gal-4 (0.2 µM) potentiated its bactericidal effects. Additionally, Gal-4 (0.2 and 1 µM) and Gal-3 (1 to 40 µM) potentiated lipid-A-binding agent Polymyxin-B bactericidal activity towards B^+ *E. coli*. Gal-4 and Gal-3 didn't potentiate the bactericidal effect of β-lactam Ampicillin at these same concentrations. **Conclusions:** Gal-4 presents good bactericidal potential and can acts synergistically with Gal-3 on B^+ *E. coli*. Both galectins can cooperate with Polymyxin-B and could be further explored in order to evaluate the influence of lectin recognition of the LPS at membrane-targeting combination therapy in bacteria.

Keywords: LPS, antibiotics, galectin-3, galectin-4.

Financial support: This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) - Finance Code 001.

EFFECT OF HORMONAL COMPONENTS OF CONTRACEPTIVES ON THE NEUTROPHIL OXIDATIVE METABOLISM

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Introduction: Venous thromboembolism (VTE) is a global health problem and the use of combined hormonal contraceptives (CHC) is associated with changes in hemostasis that increase the risk of VTE. This justifies the constant search for hormone combinations to minimize this risk. Several components of hemostasis are shared with the complement system, regulating a complex network of interaction involving the endothelium, neutrophils and inflammation. However, the relationship among these systems is poorly understood. **Aim:** To evaluate, in vitro, the effect of exposure time to CHC components on neutrophil oxidative metabolism. **Methods:** Using the density difference method in gelatin solution, neutrophils were obtained from venous blood from women who do not use CHC. Then, neutrophils ($2x10^5$) were exposed to the following CHC components: EE (43.5 pg/mL), LNG (4.3 ng/mL), E2 (50.3 pg/mL), NOMAC (4.4 ng/mL) or the combination (EE + LNG or E2 + NOMAC) for 0 or 15, 30, 60, 180 and 360 minutes at 37°C in a 5% CO_2 atmosphere. Thereafter, neutrophils were stimulated with phorbol myristate acetate (PMA, 10^{-7}M) in the presence of luminol (10^{-4}M) as a chemiluminescent probe, and oxidative metabolism was recorded by the production of reactive oxygen species (ROS) at 37°C for 30 minutes using a luminometer. Neutrophils and PMA in the absence of CHC components was the reference control. Cell viability after exposure to CHC components was assessed by the exclusion of Trypan blue dye. **Results:** Neutrophil exposure to hormonal components did not affect cell viability, which remained ≥90%. ROS production decreased when neutrophils were exposed or not to CHC component treatments after 60 minutes of preincubation. Then, ROS production kinetics was analyzed at 0 to 60 minutes of pre-incubation of neutrophils with CHC components. The results showed that there was a significant difference in the kinetic profile of ROS generation by neutrophils ($p<0.05$) when calculating the time for neutrophils to reach the maximum chemiluminescence peak (T_CLmax). Exposure for 30 minutes to the EE + LNG, E2, NOMAC and E2 + NOMAC reduced the T_CLmax of neutrophils. This implies faster generation of ROS by neutrophils compared to control (neutrophils without CHC). This result indicates a regulation of neutrophil oxidative metabolism by these CHC components. The implications of this regulation on interactions with endothelium and neutrophils constitute the objectives of the next steps of this study.

Keywords: neutrophils, endothelium, complement system, hormonal contraceptives, thromboembolism.

Financial Support: CAPES

CANNABIDIOL SYNERGIC ANTIMICROBIAL ACTIVITY COMBINED WITH POLYMYXIN B AGAINST GRAM-NEGATIVE BACILLI

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Introduction: Polymyxins were reintroduced into the clinical practice due to emergency of bacterial resistance to antibiotics and they are now used as agents of last resort in the treatment of bacterial infections by carbapenem-resistant Gram-negative bacilli (GNB) like *Enterobacterales* species, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. These pathogens were recognized by World Health Organization like critical bacteria for the research and development of new antibiotics. Cannabidiol (CBD) is a major phytocannabinoid from *Cannabis sativa* with several biological activities and it has shown antimicrobial activity against Gram-positive bacteria, but not against Gram-negative bacteria, presumable due to impermeability of cell wall. **Objective:** To investigate CBD synergic or antagonistic activity associated to Polymyxin B (PB) against GNB. **Methods:** Minimal inhibitory concentration (MIC) of PB was determined using broth microdilution method, two-fold serial dilution (256 - 0.01 mg/L), according to standard procedure. Synergistic or antagonistic effect of ultrapure CBD (99.9%; BSPG-Pharm, Sandwich, UK) (256 μg/mL) combined with PB (256 - 0.01 mg/L) was investigated. A minimal three-fold difference in the MIC values was considered as synergistic or antagonistic effect. Type-strains, quality control strains and multidrug-resistant clinical strains were evaluated, such as carbapenem-resistant (including carbapenemases GNB producers); PB susceptible and resistant, including acquired (plasmid MCR-1 and chromosomal mutations), and intrinsically resistant GNB. **Results:** CBD combined with PB showed highlighting synergic antimicrobial activity against PB-resistant (non MCR-1) and KPC-producing *Klebsiella pneumoniae* (decrease of 32 to ≤ 0.5 μg/mL), Transconjugant azide-resistant *Escherichia coli* J53 producing KPC (decrease of 2 to ≤ 0.01 μg/mL), *Escherichia coli* ATCC 25922 (decrease of 0.25 to ≤ 0.01 μg/mL), *K. pneumoniae* ATCC 13883 (decrease of 8 to 0.125 μg/mL), *Stenotrophomonas maltophilia* ATCC 13673 (decrease of 4 to 0.06 μg/mL), *Acinetobacter baumannii* ATCC 19606 (decrease of 0.5 to ≤ 0.06 μg/mL), carbapenem-resistant *A. baumannii* (decrease of 0.5 to ≤ 0.01 μg/mL). Surprisingly, CBD association with PB also demonstrated antimicrobial activity against intrinsic PB-resistant *Edwardsiella tarda* ATCC 15947 (decrease of >8 to 0.03 μg/mL) and *Serratia marcerens* ATCC 13880 (decrease of >8 to ≤ 0.01 μg/mL). **Conclusion:** CBD shows synergic antimicrobial activity with PB against several Gram-negative bacilli, showing perspective of combined use in antimicrobial therapy of bacterial infections, minimizing PB inherent neurotoxicity and nephrotoxicity. Moreover, this association also showed activity against intrinsic-PB-resistant bacteria.
Keywords: Antimicrobial Activity; *Cannabis sativa*; Cannabidiol; Polymyxin B; Gram-negative bacilli

HETEROGENEOUS RIBONUCLEOPROTEIN K AND DNA REPAIR IN ORAL SQUAMOUS CELLS

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Heterogeneous ribonucleoprotein K (hnRNPK) has been described as an increased protein in many cancers, including in oral squamous cell carcinoma (OSCC), associated with poor prognosis. This protein binds to RNA and DNA and participates in different regulatory protein-protein interactions, being found in the nucleus, cytosol, and mitochondria. HnRNPK protein is the target of a series of post-translational modifications, such as methylation and phosphorylation, which in turn regulates its different interactions to other proteins and its subcellular location, ensuring that it is associated with different mechanisms of gene regulation, like transcription, RNA processing, translation, and messenger RNA stability. HnRNPK presents interaction with mitochondrial proteins, integrating signals from different signaling pathways in the nucleus, cytoplasm, and mitochondria. Considering: i) its association with cancer; ii) the occurrence of mitochondrial DNA damage; iii) the DNA repair mechanisms present in this organelle; iv) previous data from our research group demonstrating the interaction between YB-1 and hnRNPK; and v) the need of YB-1 for mismatch repair, our hypothesis is that hnRNPK acts in the DNA repair system both in nucleus and/or mitochondria. Thus, our objective is to characterize the potential involvement of hnRNPK protein in DNA repair through its interaction with YB-1, focusing in the mismatch repair and excision repair systems. Molecular biology, biochemistry and molecular genetics strategies will be used, such as cloning of human *YB-1* and *hnRNPK* cDNAs in expression vectors, protein analysis by Western blot, as well as protein/protein/DNA interactions and functional assays by using human cell lines (tumoral and non-tumoral oral squamous cells) with different levels of hnRNPK and chemical inhibitors of signaling pathways regulating hnRNPK, and electrophoretic mobility shift assay (EMSA). The human cell lines will be exposed to UV and methotrexate to induce DNA damage and stimulate hnRNPK protein. Perhaps, our results will provide new insights into the role of hnRNPK protein in the development and progression of cancer, including OSCC.

Financial support: CAPES, CNPq and FAPESP.

Keywords: hnRNPK, mismatch repair, YB-1, mitochondria, oral cancer.

AVALIATION AND COMPARATION OF STROGENIC ACTIVITY OF TRADITIONAL AND TRANSGENIC SOYBEAN EXTRACT BIOTRANSFORMED BY *ASPERGILLUS AWAMORI* IN THE COLLAGEN PRODUCTION

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Currently, treatments that mitigate the effects of hypoestrogenism in menopausal women are steadily increasing. Decreased concentrations of estrogen hormone cause reduced collagen production, leading to tissue regeneration problems in the case of skin breakdown, hydration, blood circulation, superficial burns, scarring and immune balance. An alternative to traditional hormone replacement therapy (HRT) that has lower rates of side effects is the use of phytoestrogens for new formulations that alleviate menopausal problems. Phytoestrogens are a group of compounds that structurally and functionally mimic mammalian estrogen and can be used as an alternative to synthetic hormone therapy. And in addition to it the use of genetically modified soybeans as biotransformed source of extraction of these compounds can result in lower production costs. The objectives of this project will be evaluate and comparate traditional and transgenic soybean extracts biotransformed by the *Aspergillus awamori* fungus to collagen production by activity in fibroblast cell culture. The evaluation of the cellular cytotoxicity of the extracts will be performed by the Neutral Red method. The evaluation of necrosis induction and cell apoptosis of the extracts will be performed by the Annexin-V and Propidium Iodide (PI) assay after the treatments. Collagen production in primary human fibroblast cell culture will be verified by Immunofluorescence assay and Western Blot. The quantification of Daidzein and Genistein present in the formulations of traditional and transgenic biotransformed extracts will be performed by High Performance Liquid Chromatography (HPLC). Vehicle trials for incorporating cosmetic formulations of biotransformed soybean extracts have been previously performed by group collaborators. In this way, cell permeability tests will be performed by incorporating by traditional and transgenic biotransformed extracts with the subsequently established vehicle by Franz Cells in Human Epidermal Tissue that will be donated by women located in the menopause period. Statistical analysis will be performed by analysis of variance (ANOVA) with significance $p < 0.05$.

Keyword: Phytohormone; Glycine max; Estrogen; Menopause; Fibroblast

HEK-293F AS AN ALTERNATIVE CELL FOR THE RECOMBINANT HUMAN GRANULOCYTE COLONY STIMULATING FACTOR (rhG-CSF) EXPRESSION

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The human granulocytic colony stimulating factor (hG-CSF) acts mainly by promoting the maturation of granulocytes and stimulating their phagocytic and chemotactic activity. It has been used in the treatment of many diseases, particularly in neutropenic conditions. In Brazil, this drug is part of the Pharmaceutical Assistance Program of the National Health System (SUS) as an exceptional medicine, but at high acquisition costs. The HEK293 lineage is derived from human embryonic kidney having a post-translational modification system that can guarantees the quality of expressed therapeutic proteins. This lineage has also robust protein synthesis machinery and high replication rates. Our group has already performed the cloning and expression of rhG-CSF in *P. pastoris*. This work aims to produce rhG-CSF in HEK293 cells in serum-free medium. In addition, we intend to compare the glycosylation patterns of the recombinant protein produced in this work and of the rhG-CSF produced in the *Pichia pastoris* system in our laboratory. We will also perform *in vivo* and *in vitro* biological function tests with both proteins to verify the capacity to stimulate neutrophil proliferation of each protein. In this work we assessed cell characteristics important to recombinant protein expression. A growth curve for HEK-293F cells in CD293 medium were constructed and the maximum growth rate was μ_{max} = 0.0198 and the doubling time (t_d) was 35 hours. Metabolism of HEK-293F cells was analyzed by the concentration of glutamate/glutamine and an increase in glutamine concentration was observed up to 72 hours (0.214 g/L), followed by a decline until the last day (216 hours). The concentration of dextrose/lactate was also measured and there was the consumption of glucose in the culture medium until 240 hours and consequently an increase in the lactate, with later consumption of the same. The geneticin killing curve showed that from 120 hours onwards, almost all antibiotic concentration was able to cause cell death of the unmodified cells. The rhG-CSF gene was cloned into three different vectors. A transient transfection with pcDNA3.1_rhG-CSFb vector was done and the recombinant protein was detected 24 hours after transfection. The recombinant protein was identified by Dot Blotting and purified by nickel-affinity chromatography. A protein about 20 kDa was swing at SDS-PAGE gel as expected for rhG-CSF. HEK-293F showed to be a good cell for rhG-CSF production.

Key words: rhG-CSF; biotechnology; HEK293 cells; recombinant protein; transfection

IMPACT OF WNT, INTERFERON AND LSC GENE SIGNATURES ON DISEASE PROGRESSION AND TKI RESISTANCE IN CHRONIC MYELOID LEUKEMIA PATIENTS.

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The chronic myeloid leukemia (CML) is a myeloproliferative neoplasm positive for "Philadelphia (Ph) Chromosome" and *BCR-ABL1* oncogene. The *BCR-ABL1* encodes the BCR-ABL protein that has constitutive tyrosine kinase (TK) activity. Therefore, it disturbs downstream signaling pathways leading to enhanced proliferation and apoptosis resistance. As therapy, the Tyrosine Kinase Inhibitors (TKIs) are the current first-line therapeutic approach that target the BCR-ABL protein for Ph-positive leukemia. Usually, the disease is diagnosed in the chronic phase characterized by uncontrolled clonal expansion and high white blood cell count. Without therapy intervention, CML proceeds to accelerate and blastic phases characterized by increased immature myeloid/lymphoid cells counts in bone marrow and peripheral blood. Nevertheless, disease progression and therapeutic resistance are the current barriers to improve the CML patients' prognosis. Within this context, the WNT signaling and Leukemia Stem Cell (LSCs) signature genes have been related to disease progression and they are more expressed in late phase of CML. On the other hand, Interferon signaling pathway has been related to be downregulated as the disease progress. Therefore, there is the eminent need for identification of new biomarkers that make it possible to avoid or delay CML disease progression. Thus, the present project will evaluate the relationship among LSCs genes signature, WNT and Interferon signaling pathway on the disease progression and TKI resistance. To achieve our goal, a well-known WNT, IFN and LSC signature will be analyzed in a large collection of CML patients' transcriptome datasets from publicly available affymetrix cohorts. Following, the signatures will be evaluated by real-time qPCR from Formalin-Fixed Paraffin Embedded (FFPE) bone marrow

or peripheral blood samples of CML patients in different phases of the disease. In addition, these signatures will be analyzed in BCR-ABL-positive cell lines sensitive or resistant to TKI therapy. Lastly, these cell lines will be treated with DNA demethylating agents in order to upregulate the Interferon signature. These results will contribute to describe new therapeutic targets, biomarkers and enhance efficacy of TKI therapy on CML patients.

Keywords: CML; progression; Wnt, interferon, LSC signature; DNA Demethylating Agents.

PULSED-FIELD GEL ELECTROPHORESIS ANALYSIS AND ANTIMICROBIAL RESISTANCE OF *SALMONELLA* INFANTIS ISOLATED FROM HUMANS AND FOOD SOURCES IN BRAZIL

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Salmonellosis caused by non-typhoidal *Salmonella* serovars has been among the most frequently reported foodborne infections worldwide. *Salmonella enterica* subs. *enterica* serovar Infantis (*S.* Infantis) is a non-specific host serovar, capable to infect multiple animal reservoirs besides humans that has been reported among the most isolated *Salmonella* serovars in this country. The aims of this study were to molecularly type and determine the phenotypic antimicrobial resistance profiles of *S.* Infantis strains isolated from humans and food in Brazil. For this, 73 *S.* Infantis strains isolated from humans (30) and food (43) in Brazil between 2013 and 2018 were studied. The genotypic diversity was accessed by Pulsed-Field Gel Electrophoresis (PFGE) using the enzyme *XbaI*. The antimicrobial susceptibility testing was performed by the disk diffusion method and 18 antimicrobials were selected and tested according to the CLSI (2018) guidelines. PFGE grouped the strains into five groups. Group A clustered 53 strains isolated from humans and food between 2013 and 2018 with a similarity >64.2%, and was subdivided into four subgroups, with a similarity >71.0 among them. Group B clustered six strains isolated from humans and food between 2014 and 2017 with a similarity >63.2%. Group C clustered six strains isolated from humans and food between 2013 and 2018 with a similarity >58.9%. Group D clustered two strains isolated from food in 2015 and 2017 with a similarity >66.7%. Group E clustered five strains isolated from food in 2016 and 2018 with a similarity >51.8%. No correlation regarding the years, sources or location of isolation were observed. Moreover, the strains presented resistance to ampicillin (42.5%), tetracycline (34.2%), piperacillin (31.5%), cefazolin (28.8%), nalidixic acid (27.4%), cefotaxime (24.7%), ceftriaxone (21.9%), chloranphenicol (19.2%), ceftazidime (11.0%), cefepime (11.0%), amoxicillin-clavulanic acid (9.6%), cefoxitin (8.2%), trimethoprim-sulfamethoxazole (6.8%), streptomycin (6.8%), ciprofloxacin (2.7%), imipenem (2.7%), amikacin (1.4%) and gentamicin (1.4%). In addition, 23 strains (31.5%) of the total studied presented a multidrug-resistance (MDR) profile, being 19 strains isolated from food (26.0%) and four from humans (5.5%). In conclusion, the high resistance rates to antimicrobials and the presence of a prevalent subtype among human and food strains is an alert of the potential hazard of *S.* Infantis strains for food safety and public health in Brazil.

Keywords: antimicrobial resistance; molecular epidemiology; multidrug-resistance; PFGE; *Salmonella* Infantis.

CAFETERIA DIET MODIFIES LYMPHOID CELL PROFILE IN VISCERAL ADIPOSE TISSUE OF *TRYPANOSOMA CRUZI* INFECTED RATS

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Chagas disease (CD) is a neglected tropical disease caused by *Trypanosoma cruzi*. CD is still considered neglected and causes about 10,000 deaths. In Brazil, there are between 2 and 3 million people with CD. Obesity is a global problem and affects about 107.7 million children and 603.7 million adults, predisposing people to diseases such as cancer, type 2 diabetes, liver disease, osteoarthritis, and cardiovascular disease. Studies indicate that during chronic *T. cruzi* infection, adipocytes act as reservoirs of the parasite for long periods of time. Thus, adipose tissue is considered an important escape mechanism for the parasite to evade the host's immune response. The aim of this work was to evaluate the influence of cafeteria diet on the immune response in adipose tissue in *T. cruzi*-infected rats. Male Wistar Hannover rats were used and received water and food ad libitum (chow groups). Cafeteria-fed groups received the normal rodent diet with a cafeteria diet. Animals were intraperitoneally (i.p.) infected with a dose of 1 x 105 trypomastigote forms of *T. cruzi* Y strain present in whole blood from a previous infected mouse. Cafeteria-fed rats showed a significant increase in visceral adipose tissue weight when compared to chow-fed rats. A significant reduction in the visceral adipose tissue weight in cafeteria-fed rats was observed after infection. Moreover, our results demonstrated that there is a significant decrease in T lymphocytes (CD3+), Natural Killer (NK) cells (CD161+) and T lymphocytes (CD3+CD8+) present in adipose tissue of infected obese rats compared to control infected rats. No significant differences were found between groups when T helper lymphocytes (CD3+CD4+) or B lymphocytes (CD45Ra+) were analyzed in fat. Besides, we found a significant increase in immature T lymphocytes (CD3+CD4+ CD8+) in obese infected rats when compared to obese animals. In conclusion, the Cafeteria Diet changed the lymphoid cytotoxic cell profile in adipose tissue in rats, altering the effector immune response in the acute phase of CD.

Keywords: Chagas Disease; Cytotoxicity, Immune Response.

Financial Support: CAPES, FAPESP, CNPq

TRANSCRIPTOME AND ENZYMATIC ACTIVITY OVERVIEW OF THE THERMOPHILIC FUNGUS *RHIZOMUCOR MIEHEI*

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Enzymes are widely used in different types of industries and microbial enzymes are the most important class of industrial enzymes. The industrial potential and diversity of the fungus stimulate research into the development of large scale bioprocess and enzyme purification. The thermophilic fungi *Rhizomucor miehei* is already known for the production of hydrolases, mainly lipases and peptidases, including aspartic peptidases used in milk clotting. The functional characterization of new peptidases and the elucidation of the structural factors that contribute and modulate the thermal stability of these enzymes will provide valuable information in the application in different processes. This work overviews enzymatic activity of the *R. miehei* crude extract and the construction and analysis of the transcript library from its extracted RNA, aiming at the production and biochemical characterization of two of its peptidases (aspartic and carboxypeptidase). The submerged cultivation of the fungus was performed with a nutritional medium supplemented with four complex organic sources (microcrystalline cellulose, olive oil, casein and feather flour) act as inducers for enzyme production. The crude extract obtained from the cultivation was used for enzymatic activity and mycelial mass used for RNA extraction. In the caseinolytic assay, the culture supplemented with casein had the highest activity (35 $U.mL^{-1}$ in 24 h of culture). Quantitative reducing sugar assays were performed using starch, xylan and carboxymethylcellulose substrates. Activity was found only for starch substrate in which cultivation with olive oil was the best inducer (25 $U.mL^{-1}$ in 144 h of cultivation). Lipase activity with p-nitrophenyl palmitate substrate was detected only for cultures supplemented with casein and feather flour, with the highest activity in the casein culture (1.5 $U.mL^{-1}$ in 48 h of culture). The transcript library was constructed from DNA sequencing complementary to the RNA extracted from the cultures and the functional annotation was made using *Trinotate* software. 69080 sequences of transcript were obtained, 71% of them with functional annotation in UNIPROT. Among the annotated sequences, 33% are hydrolases, and 25% (418) of these are peptidases. Based on the annotated and sequence features and the number of transcripts we had selected the aspartic peptidase (rhizopuspepsin) and the carboxypeptidase Y for the biochemical analyzes. The high number of sequences of transcripts indicated that the medium supplements was efficient as enzymes inducers for different class.

Keywords: Peptidases; Hydrolytic enzymes; mRNA.

THE ROLE OF CD14 RECEPTOR ON THE METABOLIC REGULATION OF MACROPHAGES STIMULATED WITH THE VENOM FROM *TITYUS SERRULATUS* SCORPION

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Tityus serrulatus (TsV) scorpion venom consists of several compounds that interact with innate immune cells, mainly macrophages. This process is performed by molecular pattern recognition receptors, highlighting the CD14 receptor present in these cell surfaces, and when activated, macrophages produce pro-inflammatory mediators and triggers signaling cascades that culminate on the inflammatory process, induces alterations in several organs that can be fatal. In this work, the role of CD14 in the metabolism of bone marrow derived macrophages and peritoneal macrophages of C57BL/6 and CD14 -/- after stimulation with TsV or LPS is being evaluated. We are evaluating metabolic alterations resulting from the two stimuli, which are being compared between bone marrow-derived macrophages or peritoneal cavity. For this purpose, is being applied high resolution mass spectrometry coupled with liquid chromatography, a state-of-art technology on the field of metabolomics and systems biology. In addition, we performed gene expression analysis and cytokine dosage to elucidate which changes in molecular profile and inflammatory mediators production would be occurring in BMDMs after TsV or LPS stimulation. The results showed that TsV, LPS and the absence of CD14 receptor induce differences in ionized compounds, besides triggering changes in the metabolic profile of these cells. Following the stimuli, BMDMs also show changes in gene expression of CD14 signaling pathway-related components and inflammatory mediators that induce the production of pro-inflammatory cytokines such as IL-1β and IL-6.

Keywords: macrophage, TsV, metabolome.

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BENZNIDAZOLE ASSOCIATED WITH ASCORBIC ACID ATTENUATES THE OXIDATIVE STRESS IN ACUTE CHAGAS DISEASE

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Intense inflammation and high parasitic proliferation define the acute phase of the Chagas Disease (CD). Thereby the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) is intensified. The levels of these reactive intermediates are also increased by the metabolism of nitroheterocyclic compounds such as Benznidazole (BZ) and Nifurtimox, which are the only drugs available for the treatment of the disease. These aspects associated with the intracellular multiplication of *Trypanosoma cruzi* (*T. cruzi*) are pointed out as the key to the pathogenic process that leads to tissue damage. Furthermore, the drugs currently employed have limited efficacy and serious side effects, highlighting the need for alternative therapies. Given the difficulty in the drug discovery, the drug-sparing regimens appear advantageous and due to the close relation between CD and oxidative stress, the association of the therapies to antioxidants as ascorbic acid (AA) would be an alternative method, which could lead to attenuation of the oxidative injury. Our aim was evaluated if the association of a low dose of BZ with two different dosages of AA would attenuate the oxidative stress during the acute phase of CD. Female Swiss mice and Y strain of *T.cruzi* were used in this study. Mice were treated during fifteen days and the parasitemia, cardiac parasitism, ROS levels, lipoperoxidation and the activity of antioxidant enzymes in the heart were analyzed on the 18th day after infection. When a low dose of BZ was administered concomitantly with AA, the trypanocidal activity of the drug was amplified and the generation of superoxide radicals was reduced. Mice treated with the combined therapies had the cardiac catalase activity increased and the lipoperoxidation was greatly reduced in groups treated with both doses of AA (whether or not associated with BZ). These findings reveal an improvement in trypanocidal activity and decrease in oxidative damage, thereby greater cardioprotection could be achieved.

Keywords: Ascorbic acid; Benznidazole; Chagas' disease; Oxidative Stress.

THE IMATINIB RESISTANCE OF BCR-ABL+ CELLS IS REVERSED BY THE KNOCKOUT OF SPECIFIC MICRORNAS

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Introduction: Chronic Myeloid Leukemia (CML) is a myeloproliferative neoplasm and central to the CML pathogenesis is the Bcr-Abl protein with a constitutively tyrosine-kinase (TK) activity, responsible for abnormal cell proliferation and resistance to cell death. The failure of CML patients to the imatinib mesylate (IM) treatment has raised the need for more knowledge about the CML pathogenesis and progression, as well as the description of new therapeutic targets and biomarkers. MicroRNAs are post-transcriptional gene regulators that play important roles in the control cell processes. Changes in the expression levels of microRNAs have been pointed as drivers in the development of malignancies. Aim: to identify the microRNAs related to the IM-resistance phenotype in CML. Methods: to establish the signature of the most relevant microRNAs contributing to the resistance of CML cells to IM treatment, we performed a phenotypic screen based on the pLX-miR Library in LAMA-84 sensitive (S) and resistant (R) Bcr-Abl^{+} cell lines. The pLX-miR Library is a miRNA-focused CRISPR-Cas9 library that contains 8382 sgRNAs, of which 7382 sgRNAs target 1594 annotated human primary miRNAs and 1000 sgRNAs are non-targeting controls, with no target sites in the human genome. LAMA-84S and LAMA-84R cells were transduced with the Cas9 vector and then transduced with the pool of sgRNAs lentivirus in three separate biological replicates. All the replicates were grown for four weeks and genomic DNA was harvested at time zero, after 2 weeks of grown, and by the end of the four weeks of grown. DNA samples were then submitted to Next-Generation Sequencing to quantify the frequency of each sgRNA in the three time-points studied. The algorithm used for counting and analysis was the Model-based Analysis of Genome-wide CRISPR/Cas9 Knockout (MAGeCK). Results: We ranked the sgRNAs which frequency of expression was lost over the three time-points of the screen in the replicates of LAMA-84R cells, but not of LAMA-84S cells. Thus, the five microRNAs most related to the IM-resistance of LAMA-84R cells were miR-181a-1, let-7e, miR-484, miR-616, and miR-96. Future Perspectives: to validate these findings through the individual knockout of the selected microRNAs and to elucidate the biological processes affected. With the results, we hope to contribute to overcome the resistance of CML patients to the IM therapy.

Keywords: Chronic Myeloid Leukemia; microRNAs; imatinib-resistance.

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COMPARATIVE ANALYSIS OF LENTIVIRAL VECTOR PRODUCTION UNDER DIFFERENT CULTURE CONDITIONS FOR CAR-T CELL GENERATION

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The studies using chimeric antigen receptor (CAR) modified T cells has obtained significant results in the treatment of leukemias and lymphomas. Lentivirus are widely used as vectors for gene modification and generation of CAR-T cells due to their stability in cell genome integration, transduction efficiency and safety. Lentivirus production in monolayer culture, generally used in academia, hospitals and research centers, although effective present limitations especially regarding control of the culture conditions and scalability. Suspension cell culture for bioreactor applications is a desirable approach for vector production, nevertheless more variables may influence the process under these conditions. Thus, establishing an appropriate transient transfection protocol is more complex and time consuming. The aim of this project is to compare the production of lentiviral particles coding the expression of anti-CD19 CAR and IL-18 and GFP in monolayer serum-supplemented and suspension serum-free culture. For the last condition HEK293T cells were adapted for serum-free suspension culture. The monolayer serum-supplemented culture yielded $1,7 \times 10^{7}$ lentiviral particles/mL on average, determined by the titration method based on the expression of positive GFP cells. Vector functionality was evaluated by modifying T cells for CAR expression. The results indicated that the vector was able to genetically modify T cells and then induce antitumor response by identifying and killing CD19 positive Burkitt lymphoma cells *in vitro*. The adapted HEK293T cells were grown with and without the addition of 10% Cellboost to evaluate the effect of this compound on cell culture to improve growth and viability. This supplementation was chosen and the adapted cells were able to growth at satisfactory cell densities reaching $9,86 \times 10^{6}$ cells/mL after 216 hours and $\mu_{máx}$ 0,0231 h^{-1}. By using the same protocol employed for lentiviral production in monolayer serum-supplemented conditions, it was not possible to achieve a satisfactory lentiviral production level in serum-free suspension conditions. Novel protocols are being studied to enable this production.

Palavras-chave: vetores lentivirais; células T-CAR; cultivo em suspensão; HEK 293T; imunoterapia.

IMPACT OF GALECTIN-1 GENE DEFICIENCY IN EXPERIMENTAL *DIABETES MELLITUS* INDUCED BY STREPTOZOTOCIN

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ABSTRACT

INTRODUCTION: Type I diabetes mellitus (TD1) is a common multi-genetic disorder characterized by persistent hyperglycemia caused by a deficiency in insulin production. TD1 is associated with an autoimmune reaction against insulin-producing β cells in pancreatic islets. The immunopathological mechanism of TD1 is not entirely understood. Galectin-1 (Gal-1) is an endogenous immunomodulatory molecule and its deficiency is related to several autoimmune diseases. However, in the literature, the role of Gal-1 in TD1 still unclear. Interestingly, preliminary results from our group showed that mice deficient in Gal-1 (*Lgals-1*$^{-/-}$) are resistant to streptozoticin (STZ)-induced TD1. STZ is a selective toxic agent for insulin-producing β cells, as this drug is cell-captured through the glucose transport protein (GLUT2 receptor). Then, we hypothesized that *Lgals-1*$^{-/-}$ mice might have an alteration in STZ biodistribution. **OBJECTIVE**: We aimed to develop an analytical method for STZ measurement in plasma from *Lgals-1*$^{-/-}$ and *Lgals-1*$^{+/+}$ mice and to investigate the level of GLUT-2 expression in the pancreas of these animals. **METHODOLOGY:** To induce the experimental TD1, male 8-12 weeks old C57BL/6 (*Lgals-1*$^{+/+}$ or *Lgals-1*$^{-/-}$) mice were chosen. Fasted animals were intraperitoneally injected or not with STZ using a multiple low-dose approach, which consists of five consecutive injections of STZ (40 mg/Kg/day) and a follow-up of twenty days. After the last drug dose administration, samples of the tail vein blood (~ 400 µL) were collected in different time points and subsequently analyzed by liquid chromatography-mass spectrometry (LC-MS). To LC-MS method validation were used the parameters established in Guideline on Bioanalytical Method Validation from European Medicines Agency. **RESULTS:** STZ was separated on a C18 column using a mixture of water, ammonium acetate 100 mM (pH 5) and acetonitrile (86:10:4, v/v/v) as the mobile phase at a flow rate of 0.6 mL/ min. The method showed linearity in the range from 0,624 µg/mL to 320 µg/mL plasma. The coefficients of variation and the relative standard errors of the accuracy and precision analyses were < 15%. The method allowed us to quantify the plasma concentration of STZ in the dose interval of 90 minutes of drug administration. **CONCLUSION:** We developed the first LC-MS method to determine plasma levels of STZ in mice. This procedure may be a useful approach for pharmacokinetic studies on streptozotocin-induced type 1 diabetes using mice. Finally, this method will be crucial to better understand the impact of murine endogenous Gal-1 using this diabetes model.

Key words: Streptozotocin; galectin-1; *Diabetes mellitus*

THE ROLE OF MELATONIN AS A THERAPEUTIC AGENT IN AN EXPERIMENTAL MODEL OF VISCERAL LEISHMANIASIS

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Leishmaniasis is included in the neglected tropical diseases (NTDs) and represents a serious public health problem, affecting mainly poor and less favored populations. It is caused by protozoa of the order Kinetoplastida, family Tripanosomatidae and genus *Leishmania*. Visceral leishmaniasis (VL), a more serious form of the disease and with high lethality, is caused by *Leishmania infantum* and induces the systemic form, causing mainly hepatosplenomegaly, anemia and fever as symptoms. The current therapeutic strategies for this disease form display low effective, high cost of treatment and the emergence of cases of resistance to some drugs, being necessary the search for new ways of treatment. Melatonin, a hormone produced by the pineal gland, plays an immunomodulatory role and its receptor is found in various organs and tissues, including the immune system cells, where it is also produced. Melatonin treatment showed activity in acute phase of experimental Chagas disease model, which is caused by a protozoan belonging to the same family of the genus *Leishmania*. In this way, the study aimed to evaluate the action of melatonin in *in vitro* and *in vivo* model of *L. infantum* infection, targeting the therapeutic alternatives for the treatment of VL. The promastigote form of the parasite and BALB/C bone marrow-derived macrophages infected with *L. infantum* will be treated with melatonin. Then, leishmanicidal activity and immunological parameters will be evaluated. In *in vivo* experiments, males of BALB/C mice will be inoculated intraperitoneally with *L. infantum* and 30 days after infection they will be treated with melatonin (10mg / kg / day) or amphotericin B (positive control-1mg / kg / day) for 15 days. Physical parameters (weight, body aspect) will be monitored weekly. Subsequently, the animals will be euthanized and blood, spleen, liver and thymus will be collected and parasitic load, immunological, biochemical and hematological parameters will be evaluated.

Key words: *Leishmania infantum*, melatonin, immune response

DETECTION OF BETA-LACTAMASES AND HEAVY METAL TOLERANCE GENES IN GRAM-NEGATIVE BACTERIA FROM A UNIVERSITY HOSPITAL

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Bacteria have been exposed to biocides and heavy metals for decades, either by the consequences of industrialization, such as the disposal of pollutants in water or soil, or even by effluent from hospitals. The current scenario in which multiresistant bacteria are widely disseminated worldwide in health care settings, such as hospitals, is alarming and of extreme concern to public health. Tolerance to biocides and heavy metals has been gaining ground in hospital pathogens, and may be contributing to the silent co-selection of antibiotic resistance and to the evolution of hospital bacteria. The objective of this study is to know the epidemiological distribution and correlation among antibiotic resistance, biocides and heavy metal tolerance genes as well as to evaluate the biocide and heavy metal tolerance phenotype in Gram-negative bacilli isolated from blood culture from a university hospital. One hundred and seventeen Gram-negative bacilli (90 *Enterobacteria* and 27 non-fermenting Gram-negative bacilli) have been studied. Of this total, 59/117 (50.4%) of the bacteria are resistant and/or intermediate to broad spectrum beta-lactam antibiotics (3rd and 4th generation cephalosporins and carbapenems), and 58/117 (49.5%) of bacteria, are susceptible to broad-spectrum beta-lactam antibiotics. $bla_{\text{CTX-M group 1}}$ (n = 32), $bla_{\text{CTX-M group 8}}$ (n = 7), bla_{KPC} (n = 7) and $bla_{\text{OXA-23-like}}$ (n = 5) genes were detected in no susceptible bacteria to broad spectrum beta-lactam antibiotics. Silver (*silA)* and copper (*pcoD)* metal tolerance genes were also detected, 52/117 (44.4%) of the isolates presented *silA* or *pcoD* genes, and in 49/52 (41.8%) of these isolates the presence of both genes was detected. 37.2% (22/59) of the isolates co-harbouring beta-lactamases coding genes and *silA* and/or *pcoD* genes. The presence of *silA* and *pcoD* genes was observed in similar percentages in isolates susceptible and not susceptible to broad spectrum beta-lactam antibiotics. The study is in the initial stage of execution, so preliminary results are presented.

Key words: tolerance genes; resistance genes; hospital infection

EFFECTS OF EXTRACT AND FRACTIONS OF *Phaeoacremonium viticola* ON THE PRODUCTION OF REACTIVE OXYGEN SPECIES BY NEUTROPHILS

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Introduction: Neutrophils are important cells for the immune response, but they are also involved in the pathophysiology of some inflammatory diseases due to the establishment of oxidative stress. Therefore, the search for new molecules/substances that can control pro and anti-inflammatory responses of neutrophils has been the subject of studies. In this context, the biological potential of extracts, fractions and isolated compounds of seaweed and fungal species is being studied, as these natural products represent a source of new metabolites, especially antioxidants. In particular, marine fungi are a potential source for the research of substances with biological/therapeutic activity due to their ability to nutrient reuse and substrate decomposition. Considering oxidative stress as a therapeutic target, this study evaluated the effect of the endophytic fungus *Phaeoacremonium viticola* on the production of reactive oxygen species (ROS) by neutrophils. **Methods:** Extracts and fractions of *Pm. viticola* were obtained by addition of solvent ethyl acetate, filtrated and concentrated under reduced pressure. Neutrophils were isolated from the whole blood of healthy subjects using the gelatin solution (2.5%), then, $1x10^6$ neutrophils/mL were treated with the fungus' extract and fractions D and F in five different concentrations (12.5 to 200 µg/mL) during 30 minutes at 37°C. After that, neutrophils were submitted to the chemiluminescence (CL) assay, using luminol as probe and phorbol myristate acetate as stimulus. The CL assay was used for measurement of the production of the ROS by neutrophils for 20 minutes at 37°C. To evaluate the cytotoxicity of the fungus' extract and fractions, neutrophils ($1x10^6$cells/mL) were stained with Trypan blue and examined at optical microscopy. This study was approved by the local research ethics committee on human experimentation of FCFRP-USP (CAAE 91124918.7.0000.5403). **Results:** The treatment with extract and all the fractions of the *Pm. viticola* resulted in a decrease of the neutrophils' ROS production. However, the extract and D fraction reduced the ROS production in all the tested concentrations (70 to 99%). The cytotoxicity assay showed that the viability of neutrophils was higher than 90% for all extract and fractions. **Conclusion:** These results suggest the potential to obtain molecules with biological activity on the oxidative metabolism of neutrophils from the fungus *Pm. viticola*.

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CONDITIONED MEDIUM OF HYPOXIA PRIMED-MSC EXERTS IN VITRO PROANGIOGENIC EFFECTS

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Multipotent Mesenchymal Stromal Cells (MSC) have been widely studied regarding therapeutic potential for a variety of immune, inflammatory and degenerative disorders. However, some limitations, such as cell senescence by excessive *in vitro* expansion, reduction or inconsistency of the therapeutic potential and low survival of transplanted cells, require immediate search for priming strategies and new approaches GMP-compliant expansion to produce robust and functional MSC for therapies. In this study, MSC from human umbilical cord (UC-MSC) were expanded under xenoantigen-free conditions primed with hypoxia (dissolved oxygen concentration maintained to 5%) in a small-scale stirred-tank bioreactor. Subsequently, the harvested cells were characterized by immunophenotyping and differentiation and immunosuppressive potentials. To access *in vitro* immunosuppressive function, expanded/ primed UC-MSC were co-cultured, in different concentrations, with peripheral blood mononuclear cells stained with CFSE (3uM) and stimulated with Phytohemagglutinin (PHA, 2ug/mL). The paracrine angiogenic potential of expanded/primed UC-MSC upon human umbilical vein endothelial cells (HUVEC) was evaluated by the capillary-like structure assay performed in Matrigel-Growth Factor Reduced Membrane and scratch/ gap closure assay, using conditioned medium (CM) from expanded/primed UC-MSC. Regarding immunophenotypic analysis, decreased CD105 expression post-expansion was observed. Multipotential capacity to differentiate into adipogenic, osteogenic and chondrogenic lineages was retained. Expanded/ hypoxia primed-MSC showed immunosuppressive potential, able to inhibit T cell proliferation in the different ratios. CM from hypoxia primed-MSC induced HUVEC migration more efficiently and higher formation of capillary-like structures in Matrigel. These results represent an important step toward the establishment of a GMP-compliant large-scale production system for functional hypoxia-primed hUC-MSC, and confirm higher angiogenic capacity of these cells *in vitro*. New experiments are needed to evaluate the effect of priming with hypoxia to immunomodulatory and angiogenic *in vivo* potential.

Key Words: Hypoxia; Mesenchymal stem/stromal cells; Microcarriers; Priming; Xenogeneic-free culture; Stirred-tank bioreactor; angiogenic potential.

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TRANSCRIPTOME ANALYSIS REVEALS ALTERATION OF B CELL PATHWAYS IN SICKLE CELL ANEMIA PATIENTS

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Sickle cell disease (SCD) is a group of inherited disorders caused by mutations in the HBB gene, which encodes the haemoglobin subunit β. The incidence is estimated to be between 300,000 and 400,000 neonates globally each year, the majority in developing countries. Pathophysiological mechanisms that contribute to the SCD phenotype are chronic hemolytic anemia, vaso-occlusions, chronic inflammation status, activation of the immune system, and increased susceptibility to infections and autoimmune disorders. Despite detailed characterization of the inflammatory and innate immune responses observed in SCD, the involvement of the adaptive immune responses in the pathogenesis of this complex disease has yet to be described. In this work, we hypothesize that SCA patients who have transcriptional alterations of B-cell related genes could present defective humoral immune responses. To unravel possible transcriptional changes in B-cell pathways in SCA patients, we revisited some microarray datasets available in a public repository. Analyses of microarray datasets from public repositories represent a different strategy for identifying new mediators in complex diseases, such as SCD. We performed transcriptome analysis of peripheral blood mononuclear cell samples from SCA patients and healthy controls retrieved from the public repository, with a focus on B-cell related genes and pathways. Our analysis revealed an altered B-cell gene signature in SCA patients, which may have an important role in disease pathogenesis. Dysregulation of genes involved in B-cell development and B-cell receptor signaling pathways may be responsible for increased plasma cell numbers, susceptibity to infections and occurrence of autoimmune disorders in SCA patients. We also generated a large database of candidate genes and pathways not previously associated with SCA that should be helpful in future studies about the pathogenesis of this complex disease in animal models and humans.

Keywords: sickle cell disease; transcriptome analysis; B-cells; infection susceptibility; autoimmune disease.

Financial support: CAPES; FAPESP.

EVALUATION OF ACID AND OXIDATIVE STRESS IN *SALMONELLA* TYPHIMURIUM STRAINS ISOLATED FROM HUMANS AND FOOD IN BRAZIL

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ABSTRACT:

Salmonella enterica subsp. *enterica* serovar Typhimurium (*S.* Typhimurium) is an important cause of gastroenteritis worldwide and one of the main bacterial genus isolated from foodborne outbreaks in Brazil. The gastroenteritis is usually self-limiting, mainly causing diarrhea and inflammation of the intestinal epithelium. However, systemic infections by *S.* Typhimurium can occur in children and immunocompromised patients. The adaptation of this bacterium is related to several factors, including its ability to survive to the acidic pH of the stomach and to the alkaline pH of the intestine. Therefore, the aim of this work was to assess the survival capability to acid and oxidative stresses of *S.* Typhimurium strains isolated from humans and food in Brazil during 30 years. A total of 40 *S.* Typhimurium strains isolated from humans (20) and food (20) between 1983 to 2013 in different States in Brazil were studied. For the assays, cultures of *S.* Typhimurium in Luria Bertani (LB) were adjusted to the optical density of $O.D._{600nm}$ 0.2 (approximately $1x10^8$ CFU/mL). For the acid stress tolerance assay, the pellets were resuspended in 1 mL of sodium citrate buffer 100 mM pH 7.0 (control) and sodium citrate buffer 100 mM pH 4.5 (stress). For the oxidative stress tolerance assay, the pellets were resuspended in 1 mL of saline 0.8% (v/v) (control) and saline 0.8% (v/v) supplemented with H_2O_2 15mM (stress). Control and stress aliquots were taken after 10 min and 1 hour. Three experiments were conducted on different days. All the 40 *Salmonella* Typhimurium strains isolated from humans (20) and food (20) survived to acid stress after 10 minutes and 1 hour with a survival rate of 34 to 100%. Thirty six *S.* Typhimurium strains isolated from humans (17) and food (19) survived to oxidative stress after 10 minutes and 1 hour with a survival rate of 1 to 73%. In conclusion, the survival rates of the majority of the *Salmonella* Typhimurium strains isolated from humans and food studied under acid and oxidative stress reinforce the ability of this pathogen to tolerate unfavorable conditions and suggest that more rigorous control measures may be needed, given the importance of contaminated food with *Salmonella* Typhimurium.

Keywords: *Salmonella* Typhimurium; acid stress; oxidative stress; humans and food strains

ISOLATION AND CHARACTERIZATION OF THE RESISTANCE PROFILE OF ENVIRONMENTAL ISOLATES *Enterococcus faecalis* AND *Enterococcus faecium*

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Bacteria belonging to the genus *Enterococcus* are Gram-positive cocci, arranged in pairs or chains, which can grow in scarce nutrient environments and at varying temperatures. Although *Enterococcus* sp. are commensal bacteria, these pathogens have been increasingly detected in different types of infections, being considered the third most common cause of nosocomial bacterial infection. *Enterococcus* sp. have several genetic characteristics that contribute to their spread in some environments, such as soil and water, which can act as a reservoir for multidrug-resistant (MDR) *Enterococcus* sp. Several species belonging to the genus *Enterococcus* have been described in the literature, being *Enterococcus faecium* and *Enterococcus faecalis* the most important in human infections. Different antimicrobial resistance genes (ARGs) have already been reported in *Enterococcus* sp. and the resistance genes to vancomycin and oxazolidinones are the most important. The objective of the present study was to isolate *E. faecium* and *E. faecalis* from water and soil samples and to determine the antimicrobial resistance profile. A total of 81 samples were used, including 35 water samples and 46 soil samples. These samples were obtained from different cities, such as Montes Claros, Brumadinho, Rio de Janeiro and Ribeirão Preto. The bacterial isolation was performed on a differential selective culture medium, and the obtained isolates were identified using specific primers for the genus *Enterococcus* sp. (*tuf*), *E. faecalis* (*ddl*) and *E. faecium* (*ddl*). For confirmation, the isolates were identified by the sequencing of the 16S rDNA. The most detect species were *Enterococcus faecium* and *Enterococcus faecalis*, respectively. All isolates were characterized for the antimicrobial resistance profile by the disk diffusion method and they showed resistance to several antimicrobial agents, including ciprofloxacin (93,83%), rifampicin (78,60%), erythromycin (70,37%) and tetracycline (27,16%). Furthermore, (13,58%) of the isolates were resistant to vancomycin. These results demonstrate the presence of resistant *Enterococcus* sp. isolated from environmental samples, highlighting the importance of the environment as a reservoir of multidrug-resistant bacteria.

Keywords: *Enterococcus faecium; Enterococcus faecalis*; Resistance; Soil; Water.

HETEROLOGOUS EXPRESSION IN SACCHAROMYCES CEREVISIAE OF AN EXTERNAL MITOCHONDRIAL MEMBRANE TRANSLOCASE GENE (*TOM20*) FROM ASPERGILLUS FUMIGATUS

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In recent years, *Aspergillus fumigatus* has moved from being just a low importance saprophytic fungus to becoming one of the main opportunistic pathogens in immunocompromised patients. The evolution of infection of this fungus to invasive aspergillosis requires conidial germination processes, which is directly related to mitochondrial activity. Most mitochondrial proteins are encoded by nuclear DNA, which after their synthesis in cytosol are addressed to mitochondria. These proteins are transferred to the different mitochondrial compartments through protein complexes that recognize and address proteins, namely the TOM complex (translocase of the outer mitochondrial membrane) and the TIM complexes (translocase of the inner mitochondrial membrane). One of the components of the TOM complex is the TOM20 subunit, its function has not yet been described in *A. fumigatus*. Such protein plays an important role in other organisms involved in the recognition of electron carrier chain proteins. The objective of this project is to study the function of the *A. fumigatus tom20* gene through its heterologous expression in *Saccharomyces cerevisiae tom20* $^{+/-}$. For this, this gene will be cloned into pYES2/CT vector and *S. cerevisiae tom20* $^{+/-}$ cells will be transformed with the *pYES2/Aftom20* construct. The expression of *A. fumigatus* protein TOM20 will be induced in this yeast, and some parameters such as protein expression level, growth rate, oxygen consumption and mitochondrial membrane potential will be evaluated. Up to the present moment, CPEC was performed and later, the yeasts were transformed with the product of the same. In addition, heterologous expression was induced at times 0, 4, 8, 12, 16 and 24 hours, and Western blotting using nitrocellulose membrane was performed. Regarding the limitations faced, we highlight the strategy previously adopted to study the function of the *Aftom20* gene. The strategy in question was to obtain an *A. fumigatus Δtom20* mutant by deleting this gene. After constructing the deletion cassette and performing 8 transformations to obtain the mutant, in none of the obtained colonies the gene in question had been deleted. This gene is possibly an essential gene for *Aspergillus fumigatus*. Then, we adopted as an alternative method to study the function of this gene the heterologous expression.

Keywords: *Mitochondria; Aspergillus fumigatus; TOM20; heterologous expression; Saccharomyces cerevisiae.*

“FUNCTION AND METABOLIC PROFILE OF PLATELETS ON THE EXPERIMENTAL INFECTION BY *ACHROMOBACTER XYLOSOXIDANS*”

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Platelets are anucleate blood elements that have considerable role in modulating hemostasis. Recent studies indicate that platelets are essential regulators of the immune system and exhibit a fundamental role in infections. Platelets store high amounts of molecules that promote direct pathogen elimination or modulate effector activities of leukocytes. *Achromobacter xylosoxidans* are normally found in the environment and are opportunistic pathogens, infecting mostly persons with cystic fibrosis (CF), tumors and immunodeficiencies. Recently, we have characterized lung experimental acute infection by *Achromobacter xylosoxidans*, in which we showed the essential function of the innate immune system for infection control. However,the molecular mechanisms that drive the host response against *A. xylosoxidans* infections are unknown. The aim of this study was to investigate the role of platelets in the immune response against *A. xylosoxidans.* We first determined the ability of platelets to kill *A. xylosoxidans* directly by bacterial killing assay. Platelets were able to killed *A. xylosoxidans* (>50% in 10 minutes). We further investigated the possible interaction between *A. xylosoxidans* and platelets. Bacteria were able to triggered platelet activation, measured as P-selectin expression by Flow Cytometry. These results demonstrate that *A.* xylosoxidans activate platelets, which also exhibit significant antimicrobial activity when exposed to this bacteria.

Keywords: platelets, *Achromobacter xylosoxidans*, immune system

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EXPRESSION OF RECOMBINANT PROTEINS OF HTLV-1 AND HTLV-2 IN HETEROLOGOUS SYSTEM – *PICHIA PASTORIS* AND *ESCHERICHIA COLI*

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Keywords: *recombinant proteins*; *HTLV*; *diagnosis*

The infection by HTLV compose a serious public health problem. The existence of accurate, fast and accessible diagnostic methods is important, since identifying the presence of this retrovirus in the patients would allow a better approach in the treatment and control of infections. In Brazil, serological screening in blood donor candidates and in pregnant women is mandatory, since the whole country compose an endemic area. Despite de advances of researches, in Brazil the kits used with diagnostic purposes in the national blood banks have, in most, foreign origin. The gp21, p24 and gp46 from HTLV are viral proteins used as capture antigens in diagnostics tests, since the firsts antibodies produced by the infected organism are specific to these proteins. Thereby, the purpose of this work is to obtain the recombinant proteins gp21, p24 and gp46 from HTLV-1, and gp46 from HTLV-2 for further inclusion in rapid diagnostic tests. The HTLV-1 proteins genes were previously obtained from blood donor candidates from Ribeirão Preto Blood Center, cloned in pET28a vectors and used to transform *E. coli* BL21(DE3) cells. The proteins expression was initiated by the addition of IPTG. After expression, the cells were lysed by ultrasound pulses. Soluble and insoluble fractions were separated and submitted to analyzes by SDS-PAGE and Anti-His dot blotting assays, such as quantification of total proteins by the method of Bradford. Different expression conditions were tested aiming to optimize the expression process for each protein. The gp46 from HTLV-2 gene sequence was chosen from analyzes of the retrovirus genome in The Universal Protein Resource (UniProt). The gene sequence was optimizated for *P. pastoris* expression, synthesized and cloned in pPICzαA vector. This construction was used to transform *P. pastoris* cells. The recombinant protein expression was induced by the addition of methanol. Several recombinant expression assays were performed with no success in confirming the expression. Then, primers were designed and synthesized to obtain the gp46 from HTLV-2 synthetic gene from pPICzαA vector. This gene will be cloned in pET28a_1081 vector, which allows the recombinant expression by the addition of IPTG in *E. coli* in the soluble portion of the cell lysate. This construction will be used to transform *E. coli* Rosetta cells and the recombinant expression will be induced by the addition of IPTG. We present the results of recombinant proteins expression experiments from the retroviruses HTLV-1 and HTLV-2. It was detected the expression of all the proteins by electrophoretic analysis in polyacrylamide gel (SDS-PAGE). The detection of the gp21, p24 and gp46 proteins from HTLV-1 was also successfully performed by Dot blotting.

EXPRESSION OF RECOMBINANT PROTEINS OF HTLV-1 AND HTLV-2 IN HETEROLOGOUS SYSTEM – *PICHIA PASTORIS* AND *ESCHERICHIA COLI*

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Keywords: *recombinant proteins*; *HTLV*; *diagnosis*

The infection by HTLV represents a serious public health problem. The existence of accurate, fast and accessible diagnostic methods is important, since identifying these retrovirus infections in the patients would allow a better approach in the treatment and control. In Brazil, serological screening in blood donor candidates and in pregnant women is mandatory, since the whole country compose an endemic area. Despite de advances of researches, in Brazil the kits used with diagnostic purposes in the national blood banks and laboratories have, in most, foreign origin. The gp21, p24 and gp46 from HTLV are viral proteins used as capture antigens in diagnostics tests, since the firsts antibodies produced by the infected organism are specific to these proteins. Thereby, the purpose of this work is to obtain the recombinant proteins gp21, p24 and gp46 from HTLV-1, and gp46 from HTLV-2 for further inclusion in rapid diagnostic tests. The HTLV-1 proteins genes were previously obtained from blood donor candidates from Ribeirão Preto Blood Center, cloned in pET28a vectors and used to transform *E. coli* BL21(DE3) cells. The proteins expression was initiated by the addition of IPTG. After expression, the cells were lysed by ultrasound pulses. Soluble and insoluble fractions were separated and submitted to analyzes by SDS-PAGE and Anti-His dot blotting assays, as well as quantification of total proteins by the method of Bradford. Different expression conditions were tested aiming to optimize the expression process for each protein. The gp46 from HTLV-2 gene sequence was chosen from analyzes of the retrovirus genome in The Universal Protein Resource (UniProt). The gene sequence was optimized for *P. pastoris* expression, synthesized and cloned in pPICzαA vector. This construction was used to transform *P. pastoris* cells. The recombinant protein expression was induced by the addition of methanol. Several recombinant expression assays were performed with no success in confirming the expression. . There will be made an expression system change. The gene will be cloned in pET28a_1081 vector, which allows the recombinant expression by the addition of IPTG in *E. coli* Rosetta cells in the soluble portion of the cell lysate. We present the results of recombinant proteins expression experiments from the retroviruses HTLV-1 and HTLV-2. It was detected the expression of all the proteins by electrophoretic analysis in polyacrylamide gel (SDS-PAGE). The detection of the gp21, p24 and gp46 proteins from HTLV-1 was also successfully performed by Dot blotting.

FUNCTIONAL EVALUATION OF EXTRACELLULAR VESICLES IN HUMAN T-CELL LYMPHOTROPIC VIRUS 1 (HTLV-1) INFECTION

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Human T-cell lymphotropic virus 1 (HTLV-1) is associated with two main clinical manifestations: HTLV-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP) and adult T-cell leukemia/lymphoma (ATLL). About 5-10 million people are infected by HTLV-1 around the world, and its infection is considered to be endemic in Japan, sub-Saharan Africa, South America and the Caribbean. Brazil is considered one of the countries with the highest number of infected individuals (about 2.5 million people), even though only 3-4% of them will develop HTLV-1 associated diseases. One of the gaps in this subject is related to the comprehension of causes that lead to different clinical manifestations. In this context, the study of extracellular vesicles becomes a relevant issue, once extracellular vesicles represent an important mechanism of paracrine and endocrine communication between cells, especially in the immune system. Many studies involving extracellular vesicles and viral infections evaluate the effect of extracellular vesicles on the modulation of the immune system and their role in disease development. Regarding HTLV-1 infection, there is evidence that infected cells can release extracellular vesicles involved in mechanisms of viral particle dissemination, such as transcripts and proteins. However, there are few *in vivo* studies. Therefore, this project aims to evaluate the expression levels of tax and HBZ viral genes in extracellular vesicles isolated from serum of HTLV-1-infected individuals and to evaluate the role of these vesicles in the modulation of immune responses against this infection. We hypothesized that the presence of tax and HBZ genes in circulating extracellular vesicles modulates the immune response against HTLV-1 infection and it is related to the associated diseases progression. The functional study of circulating extracellular vesicles carrying viral contents may help understanding mechanisms of pathogenesis in the development of associated diseases in HTLV-1 infection, as well as in prognosis and diagnosis of this viral disease.

Key words: HTLV-1; Extracellular vesicles; HAM/TSP; ATLL.

PHENOTYPIC CHARACTERIZATION and EPIGENETIC MODIFICATIONS OF PRO AND ANTI-INFLAMMATORY HUMAN NEUTROPHILS POLARIZED *in vitro*

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The cellular plasticity of the innate and adaptive immune system cells is under the control of several factors present in infectious or inflammatory microenvironments, defining a variety of cell differentiation or polarization profiles. In the tumor environment, polarized neutrophils were identified, been associated with control and tumor susceptibility. Neutrophils have also been assigned controversial roles in tuberculosis and other infectious or inflammatory diseases. Previous results from our research group showed that neutrophils can acquire the polarized phenotype according to the cytokines present in the culture. Here we characterized the morphology, pattern of production of reactive oxygen species, production and expression of cytokines and cell surface receptors of pro and anti-inflammatory polarized neutrophils, correlating with epigenetic changes induced after polarization. Thus, neutrophils were isolated from peripheral blood of 20 healthy participants. These cells were polarized *in vitro* with GMCSF and IFN-γ (inflammatory profile) or with IL-4, IL-13 and TGF-β (anti-inflammatory). After 2 h of polarization, the inflammatory neutrophils were characterized by nuclear morphological modifications, increase of size and MHC II and CD86 expression, besides the greater production of reactive species of oxygen, when compared to anti-inflammatory neutrophils. In addition, inflammatory neutrophils have increased of IL-8 production, as well as increased expression of *TNF-α* and *IL-10* cytokines and *TLR2* and *TLR4* receptors when compared to the other profile. By analyzing the epigenetic markers after cell polarization, it was observed that anti-inflammatory neutrophils have a higher expression of DNMT3A when compared to proinflammatory neutrophils, and when evaluating the global methylation profile, proinflammatory neutrophils have a lower degree of genomic DNA methylation. These results demonstrated that the pro-and anti-inflammatory microenvironment enriched by the different conditioning cytokines can modulate the response of neutrophils, characterized by specific functions and gene expression profile. Further, we would suggest that epigenetic modifications can regulate these processes.

Keywords: innate immunity; neutrophils; cell plasticity; epigenetic

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EVALUATION OF BONE MARROW ENDOTHELIAL CELLS IN THE PATHOPHYSIOLOGY AND PROGRESSION OF POLYCYTHEMIA VERA

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Introduction: Polycythemia vera (PV) is a clonal hematopoietic disease characterized by enlargement of mature and precursor cells of one or more myeloid lineages, which has in its natural course the possibility of progression to bone marrow fibrosis or acute myeloid leukemia (AML) in some patients. PV is an oncoinflammatory characterized by abnormal immune system activity. Bone marrow microenviroment cells (BMMC) appears to act as immunomodulators on hematopoiesis and may contribute to the pathophysiology and progression of PV to myelofibrosis (MF) and leukemogenesis. Bone marrow microenviroment (BMM) is composed of extracellular matrix and specialized cells, especially endothelial cells. Besides its participation in physiological processes, the progression of several pathophysiological processes is directly related to the abnormal increase of vascular networks. Altered angiogenesis participates in the pathophysiology of numerous hematologic malignancies and has been described in patients with multiple myeloma (MM), myelodysplastic syndromes (MDS), MF and AML. In myeloproliferative neoplasms (MPNs), neoangiogenesis is less pronounced in PV and Essential Thrombocytemia (ET), however, it is essential in the pathogenesis of MF. However, it is not still elucidated whether increased angiogenesis is a consequence of inflammation or acts on processes related to the pathophysiology and progression of MPNs. Endothelial cells (ECs) are both influenced and release immunomodulatory stimuli such as cytokines, lipid and angiogenic mediators capable of altering BMMC. These changes favor neoangiogenesis, malignant transformation of hematopoietic stem cells (HSC) and exacerbate proliferation of myeloid neoplastic cells. **Objective:** To evaluate the potential immunomodulatory of endothelial bone marrow cells in the development and progression of polycythemia vera. **Methodology:** Eight to twelve weeks old male mice C57/BL6 pepboys will be irradiated with sublethal dose (700 cyG) and transplanted with $1x10^6$ bone marrow cells from C57/BL6 mice JAK2V617F-mutated. After chimerism analysis in 8 weeks, the ECs will be obtained from bone marrow of Pepboy male mice by flushing technique and isolated in culture in specific medium. ECs will be treated separately with Ruxolutinib and hydroxyurea and then proliferation and viability will be evaluated. In addition, the expression of cellular markers associated with leukocyte and platelet recruitment and adhesion by real time PCR will be quantified, as well as, the quantification of cytokines, chemokines and eicosanoids from the supernatant by ELISA.

Keywords: Bone Marrow Microviroment; Endothelial Cells; Polycythemia Vera.