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PREVALENCE AND INCIDENCE DE DENGUE AND ZIKA IN THE PARTICIPANTS OF THE PROSPECTIVE COHORT STUDY IN SAO JOSE DO RIO PRETO, SP.

Autores NATHALIA ZINI [1], CASSIA FERNANDA ESTOFOLETE [1], RAFAEL ALVES DA SILVA [1], GISLAINE CELESTINO DUTRA DA SILVA [1], ANA CAROLINA BERNARDES TERZIAN [1], BRUNO HENRIQUE GONCALVES DE AGUIAR MILHIN [1], FLORA GANDOLFI [1], MARCOS TAYAR AUGUSTO [1], LEONARDO CECILIO da ROCHA [1], RODRIGO SBORGHI [1], PEDRO HENRIQUE CARRILHO [1], ELIANE APARECIDA FAVARO PEREIRA [1], CAMILA SOARES [2], JAQUELINE CRUZ [5], DANIELLE BRUNA LEAL DE OLIVEIRA DURIGON [2], EDISON LUIZ DURIGON [2], LUIS CARLOS DE SOUZA FERREIRA [2], ALBERT ICKSANG KO [6,5], FRANCISCO CHIARAVALLOTI NETO [7], MAURICIO LACERDA NOGUEIRA [1]

Instituição [1] FAMERP - MEDICINE SCHOOL OF THE SÃO JOSÉ DO RIO PRETO (AVENIDA BRIGADEIRO FARIA LIMA, 5416 - SAO PEDRO, SAO JOSE DO RIO PRETO, SAO PAULO, BRASIL), [2] ICB -USP - UNIVERSITY OF SAO PAULO (AVENIDA PROF. LINEU PRESTES, 1374 - BUTANTAN, SAO PAULO, BRASIL), [5] FIOCRUZ - iNSTITUTE GONCALO MUNIZ (RUA WALDEMAR FALCAO,121 CANDEAL - SALVADOR - BA, BRAZIL), [6] YSPH - YALE SCHOOL OF PUBLIC HEALTH (60 COLLEGE STREET, NEW HAVEN, CONNECTICUT, USA.), [7] UPH - USP - UNIVERSITY PUBLIC HEALTH DE SAO PAULO (AVENIDA DR. ARNALDO, 715, SAO PAULO, BRASIL)

Resumo

Dengue and Zika are arboviruses transmitted to humans through the hematophagous vector bite. Dengue has 4 serotypes (DENV1-4), responsible for annual epidemics in Brazil and in many other countries, considered a challenge for the Public Health System. The emergence and spread of Zika virus (ZIKV) in endemic areas for dengue have raised a concern about how this virus would impact in a population exposed to dengue. In Brazil, Zika has caused different clinical syndromes, ranging from asymptomatic, mild symptomatic and severe forms with neurological disorders and fetal malformations. Seroprevalence studies of arboviruses are important to determine the disease severity, co-circulation of viruses and establishing prevention and control measures. The objective of this study was verifying the prevalence and incidence of anti-DENV and anti-ZIKV antibodies in participants from a prospective cohort, who live in an endemic area for dengue in São José do Rio Preto city, São Paulo, Brazil. Paired samples of peripheral blood from 777 individuals were collected in accordance with the eligibility criteria of the project, one at the time of the study entry and the other in the subsequent year. They were analyzed by IgG ELISA serological tests and data obtained through the sociodemographic questionnaire were analyzed by Launch Epi Info software. Of the 777 analyzed samples, 577 (74.3%) was a reagent for DENV and 80 (10.3%) for ZIKV. In the follow-up year, 665 (85.6%) samples were a reagent for DENV and 227 (29.2%) for ZIKV. When the 777 samples were analyzed in groups matching serological status, the result of the previous antibodies prevalence was 64 (8.2%) ZIKV+/DENV+ (both reagent); 16 (2.1%) ZIKV+/DENV-; 513 (66.0%) ZIKV-/DENV+; and 184 (23.7%) ZIKV-/DENV- (double non-reagent). In the year of follow-up, 199 (25.6%) was ZIKV+/DENV+; 28 (3.6%) ZIKV+/DENV-; 466 (60.0%) ZIKV-/DENV+; and 84 (10.8%) ZIKV-/DENV-. The attack rate of ZIKV was 21.1% and for DENV was 44.0%. Our data has shown a high prevalence of anti-Zika and anti-dengue antibodies in the population. After two major dengue outbreaks, in 2015 and 2016, the high prevalence of anti-Zika antibodies in our study groups suggests DENV and ZIKV co-circulation in non-apparent levels. Our data also highlight the importance of additional

studies to verify if the presence of anti-dengue and anti-Zika in the population might provide a protective factor or support more severe manifestations caused by these viruses.

Palavras-chaves: Dengue, Zika, Sero-epidemiological Studyste, Serological diagnosis

HIV-1 TAT MODULATES M1-M2 ACTIVATION PHENOTYPE OF BV-2 MICROGLIAL CELLS.

Autores Douglas Bardini Silveira [1], Monique Ferrary Américo [1], Hegger Machado Fritsch [1], Hernan Francisco Terenzi [1], Aguinaldo Roberto Pinto [1]

Instituição [1] UFSC - Universidade Federal de Santa Catarina (Campus Reitor João David Ferreira Lima, Bairro Trindade, Florianópolis, SC, Brasil.)

Resumo

The HIV-1 transactivator protein (Tat) plays a critical role in viral replication and is related to several pathological features of HIV-1 infection, including a broad spectrum of neurological harms known as HIV-1-Associated Neurocognitive Disorders (HAND). HIV-1 Tat-induced bystander activation of microglia has been documented as a key factor of development and maintenance of HAND. Reactive microglia assumes diverse phenotypes, which are roughly categorized into M1 and alternative M2 activation phenotypes. M1 microglia releases pro-inflammatory cytokines and reactive oxygen species, which are implicated in neuronal damage; M2 microglia acts in clearing debris and repairing neuronal injuries by establishing an anti-inflammatory environment. Therefore, M1-M2 polarization may play a role in determining the potential neurotoxic or neuroprotective activity of microglia in HIV neurodegeneration disorders, although the mechanisms regulating differential microglial activation during the course of HIV-1 infection remain largely unknown. Based on this, the present work aimed to explore the potential M1-M2 phenotypic modulation of microglial cells induced by HIV-1 Tat bystander activation. BV-2 microglial cells were stimulated with recombinant HIV-1 Tat and expression levels of M1-M2 activation markers were assessed through flow citometry and RT-qPCR. The bystander stimulus of Tat on BV-2 cells resulted in a synergistic overexpression of major M1 markers comprising secreted TNF-α, IL-6 and MCP-1 as wells as CD16/32 extracellular receptors and iNOs gene. However, Tat-treated cells did not shown detectable changes in expression levels of the canonical M2 markers IL-10, CD206, and Arg-1. These findings suggest that HIV-1 Tat can drive a M1 microglial activation profiling, directly triggering an inflammogenic and neurotoxic response. Overall, a better understanding of how HIV-1 infection and viral protein exposure modulate microglial function during the course of infection could lead to the identification of novel therapeutic targets for both the eradication of HIV-1 reservoir and treatment of neurocognitive impairments. Financial support: CNPq.

Palavras-chaves: HIV-1, TAT, Microglial Cells, Neurocognitive Disorders

INFECTION OF ENDOTHELIAL CELLS BY DENGUE VIRUS INDUCED ROS PRODUCTION BY DIFFERENT SIGNALING PATHWAYS, AFFECTING VIRUS REPLICATION, CELLULAR ACTIVATION, DEATH AND VASCULAR PERMEABILITY

Autores LANA MONTEIRO MEUREN [1], LUIZA RACHEL PINHEIRO DE CARVALHO [2], YASMIN MUCUNÃ MUSTAFÁ [1], MICHELLE PREMAZZI PAPA [1], ANDREA THOMPSON DA POIAN [2], LUCIANA BARROS DE ARRUDA [1]

Instituição [1] Virologia - UFRJ (Av. Carlos Chagas Filho, 373, CCS - Bloco I, Lab ISS - 048 Rio de Janeiro, RJ - Brasil), [2] Bioquímica Médica - UFRJ (Universidade Federal do Rio de Janeiro, Instituto de Bioquímica Médica. Av. Carlos Chagas Filho, 373, CCS, bloco E, sala 18)

Resumo

Increased vascular permeability has been described as one of the factors for dengue disease complication. Reactive Oxygen Species production induces changes in cell physiology and can act as signaling molecule for cell death. Our group had previously demonstrated that infection of endothelial cells with DENV results in the activation of RNA sensors, production of interferon and proinflammatory cytokines, cell death and permeability. We have also reported that DENV infection promoted ROS production, but the signaling associated to this event and its consequences for virus replication and endothelial cell physiology had not been investigated yet. In the present study, we evaluated the role of mitochondrial function and NADPH oxidase activation for ROS production and investigated how these mediators affected brain microvascular cells (HBMEC) infected by dengue virus (DENV). HBMECs were infected with DENV2, at MOI of 1. The oxygen consumption was measured by high resolution respirometry and ROS production by flow cytometry. Virus replication was evaluated by qRT-PCR, flow cytometry and plaque assay. Cell death was evaluated by flow cytometry. Cytokine production was analyzed by qRT-PCR and ELISA. DENV-infected HBMECs showed a decrease in the maximal respiratory capacity and altered membrane potential, indicating functional mitochondrial alteration, what might be related to mitROS production. Indeed, mitROS was detected at later time points after infection, which was dependent on RIG-I activation. Specific inhibition of mitROS diminished virus replication and cell death but did not affect cytokine production. On the other hand, inhibition of cytoplasmic NADPH oxidase-associated ROS production inhibited virus replication, cell death and permeability, and the secretion of inflammatory cytokines, including IL-8, and CCL5. Importantly, the production of cytoplasmic ROS did not depend on virus RNA sensing. These data indicate that DENV replication in endothelial cells induced ROS production by different pathways. Both RIG-dependent mitROS and NADPHox-dependent ROS were important for virus replication and cell death, affecting endothelial permeability; however only the species triggered by cytoplasmic stress affected inflammatory signals, which could further contribute to endothelial activation and vascular lesion associated to DENV infection.

Palavras-chaves: DENV, Cell endothelial, ROS, Cell death , Vascular permeability

PHENOTYPE OF VIRUS-LIKE PARTICLES (VLP) AND HPV-POSITIVE CARCINOMA CELL LINES BY ELECTRON MICROSCOPY

Autores Rachel Siqueira de Queiroz Simões [1], Ortrud Monika Barth [1]

Instituição [1] Fiocruz - Fundação Oswaldo Cruz (Avenida Brasil 4.365 - Manguinhos, Rio de Janeiro, cep: 21040-900)

Resumo

Papillomavirus express two oncogenes E6 and E7 which are produced in the basal layers of the epithelium under regulation of the E2 gene and disrupts in the differentiation program of the cell cycle. After initial infection, the viral DNA is maintained in episomal form in low copy number. There are several phenotype of antigen-presenting cells (Langerhans cell, Migratory LC, Langerin dendritic-cell populations, dermal macrophages) in the skin which are migratory in the epithelial tissue. Some studies have investigated the ability of the cytokine to inhibit proliferation "*in vitro*" normal keratinocytes and infected with HPV as well as the expression of E6 and E7 oncogenes. The present study reports ultrastructural cell morphology in samples of *bovine papillomavirus* (BPV) virus-like particles (VLP) and describes morphological alterations inside the SiHa and HeLa cell lines by electron microscopy. Few studies have assessed the transmission electron microscopy in different cell lines. For ultrastructural analysis, the specimens were embedded in epoxy resin, fixed in 1% glutaraldehyde and post-fixed in 1% osmium tetroxide. Later steps followed by washes in cacodylate buffer 0.2 M in sodium sucrose 0.7% and distilled water. The dehydration steps were performed. Warts and cells lines were included in epoxy resin and kept at 60^0C to complete polymerization. Ultra-thin sections and semi-thin sections were performed. Morphologically, very electron-dense cells were detected by electron microscopy presenting well developed mitochondria and rough endoplasmic reticulum (rER), many vesicles and ribosomes in HeLa and SiHa cell lines. Cellular modifications similar to antigen-presenting cells, many activated mitochondria and vesicle transport well preserved also were observed. Furthermore, the presence of VLP and cellular junctions like desmosomes were also detected in samples de BPV. These morphological alterations suggest a high cellular activity of SiHa and HeLa cell lines can be possible prognostic markers of cervical cancer. One of the main functions of natural killer cells (NK) are cytotoxic activity with ability to destroy virus-infected cells and the ability of NK cells to distinguish infected cells from uninfected cells is related with the presence of destruction inhibitory receptors on their surface are called killing inhibitory receptors (KIR). So more studies about immunology of viral infection are need.

Palavras-chaves: cell lines, cellular markers, keratinocytes, ultrastucture, virus like particles

IN VITRO EVOLUTION OF ZIKA VIRUS IN INSECT CELLS

Autores Karina Pinheiro Pessoa [1], Valdinete Alves do Nascimento [1], Debora Camila Gomes Duarte [1], Victor Costa de Souza [1], Felipe Gomes Naveca [1]

Instituição [1] ILMD - Fiocruz - Instituto Leônidas e Maria Deane (Rua Terezina, 476 - Adrianópolis, Manaus - AM)

Resumo

The Zika virus (ZIKV) is an arbovirus, genus *Flavivirus*, *Flaviviridae* family, with single-stranded RNA genome, of positive polarity. Just like the other RNA viruses, ZIKV shows variations in their sequences due to the lack of proofreading activity of the viral RNA polymerase and the high replication rate. Considering the occurrence of mutations as an event that may lead to the selection of variants with altered fitness, this study aimed to evaluate the *in vitro* evolution of ZIKV, analyzing the mutations that occurred during the adaptation process in cultured cells. A positive ZIKV sample denominated BR_AM_ILMD_0305JFMB, was isolated in *Aedes albopictus* C6/36 cells and submitted to successive 33 passages in biological replicates. The complete genome of the BR_AM_ILMD_0305JFMB directly from plasma and from passages P1, P33.1, P33.2, was obtained by capillary sequencing using an ABI 3130 automatic sequencer. Sequencing data were analyzed using the Geneious software v10.2.6 showing that seven mutations occurred in the regions coding for the non-structural proteins, two synonyms mutations in the NS1 and NS2A CDS; three non-synonyms mutations occurred in the NS3 CDS other two in NS5 CDS. To evaluate the impact of these mutations on viral competence, P1 and P33 were titrated by RT-qPCR using the delta-delta Ct method and then inoculated for *in vitro* (C6/36) and *in vivo* (*Aedes aegypti*) fitness assays. For the *in vitro* assay, we evaluated the number of viral RNA copies present in both cells supernatant and cells pellets. The results showed a significant increase in the number of copies of viral RNA in P33 infected cells at 72 hours post-infection. The results of the *in vivo* experiment showed a significantly lower viral copy quantity for P33, in eight days (P-value 0,333) and twelve days post-infection (P-value 0,040), suggesting that the mutations observed in P33 adversely affected the viral replication in *Aedes aegypti*. Further studies should be performed to confirm the effects of the mutations observed in other biological systems both *in vivo* and *in vitro*.

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Palavras-chaves: Zika Virus, Evolution, in vitro

HUMAN INTERFERON-INDUCED PROTEIN WITH TETRATRICOPEPTIDE REPEATS 5 (IFIT5) INHIBIT RABIES VIRUSES

Autores: Camila Mosca Barboza [1], Jaíne Gonçalves Garcia [1], Raphaela Mello Zamudio [1], Lucas Matheus Stangherlin [2], Maria Cristina Carlan da Silva [2], Juliana Galera Castilho [1], Paulo Michel Roehe [3], Ana Claudia Franco [3], Helena Beatriz de Carvalho Ruthner Batista [1]

Instituição: [1] IP - Instituto Pasteur (Av. Paulista, 393 - Cerqueira César, São Paulo), [2] UFABC - Universidade Federal do ABC (Alameda da Universidade, Bairro Anchieta - São Bernardo do Campo), [3] UFRGS - Universidade Federal do Rio Grande do Sul (R. Sarmento Leite, 500 - Farroupilha, Porto Alegre - RS)

Resumo

Rabies is a fatal zoonotic disease caused by *Rabies lyssavirus* (RABV). Although there are effective vaccines for rabies, this disease results in 60,000 human deaths worldwide every year, being an important social and economic problem. Dogs are the main source of infection but in Brazil, bats have developed an important role in RABV transmission. Due to the absence of an effective cure, there is an urgent need for an alternative antiviral compounds. In this scenario, the Interferon-induced proteins with tetratricopeptide repeats (IFITs) can be highlighted. IFITs are innate immune molecules that confer antiviral defense to the host. There are four members in humans (IFIT1, 2, 3 and 5). Amongst the members, IFIT5 is highly sensitive to various cellular stresses, including those caused by dsRNA, lypopolysaccharides and virus infections. Consequently, one major feature of IFIT5 involves inhibition of virus replication by nucleic acid sensing and possibly translation inhibition. The aim of this study was to evaluate the activity of human IFIT5 against RABV from different genetic lineages. Two distinct RABV isolates recovered from central nervous system (CNS) tissues of different species and considered representative of different RABV genetic lineages were used in the present study. Isolate IP4005/10 is a virus genetic lineage whose natural host is the hematophagous bat *Desmodus rotundus* and isolate IP3629/11 was originated from a domestic dog. The samples were titrated in HEK-293T cells and 100 fifty percent tissue culture infectious doses ($TCID_{50}$) were determined and inoculated onto V5-tagged humanIFIT5 transfected cell monolayers. Supernants of transfected and infected cells were collected then titrated and compared with the control cell monolayers (untransfected infected cells). Preliminary results indicate a reduction of virus titres when the bat isolate (4005/10) was inoculated onto humanIFIT5 transiently expressing cells, in comparison to the titres obtained with the dog isolate. These results suggest that human IFIT5 has potential antiviral effect against RABV. Additional studies are necessary to a better understanding of the mode of action of this antiviral protein. Financial support: Instituto Pasteur

Palavras-chaves: Antiviral , Innate immunity, IFIT5, Rabies virus

RABIES VIRUS ISOLATION IN HUMAN EMBRYONIC KIDNEY (HEK-293T) CELL LINE: AN ALTERNATIVE FOR RABIES DIAGNOSIS AND RESEARCH

Autores	Jaíne Gonçalves Garcia [1], Camila Mosca Barboza [1], Adriana Candido Rodrigues [1], Willian de Oliveira Fahl [1], Juliana Galera Castilho [1], Maria Cristina Carlan da Silva [2], Raphaela Mello Zamudio [1], Ana Lee Aparecida Francisco [1], Helena Beatriz de Carvalho Ruthner Batista [1]
Instituição	[1] IP - Instituto Pasteur (Av. Paulista, 393 - Cerqueira César, São Paulo), [2] UFABC - Universidade Federal do ABC (Alameda da Universidade, Bairro Anchieta - São Bernardo do Campo)

Resumo

The Rabies lyssavirus (RABV) is a neurotropic virus that causes encephalitis in all mammalians. Despite the recognized stability of RABV, differences among isolates from different species have been identified. The Gold standard method to detect RABV is direct fluorescent antibody test (DFAT). The rabies tissue culture infection test (RTCIT) can be used as potential complementary test of DFAT, it enables propagation of virus and have been valuable for obtaining large quantities of virus for production of vaccine and other studies. The viruses can infect a several cell lines of neural origin and non-neural cells. Several different cell types have been used for virus propagation. The Murine neuroblastoma (Neuro-2A) and the Baby hamster kidney (BHK-21) cell lines are the most commonly used, however not all rabies strains replicate equally in these lines. Human embryonic kidney cell line (HEK-293T) expresses several neuronal proteins and can be an alternative cell line for RTCIT. This study evaluated the susceptibility of HEK-293T for RTCIT and compare with results obtained with Neuro-2A cell line. Test was performed in 96-well plates, the procedure involved the addition of a brain tissue suspension from different species to a suspension of HEK-293T cells. The plates were incubated in CO2 incubator at 37°C at different times (24h and 48h) adopting two distinct conditions where the virus was allowed to adsorb for 2h and another plate without virus adsorption. At time the plate was fixed, stained with fluorescein isothiocyanate (FITC) anti-rabies conjugate antibody and examined with a fluorescence microscope. The results show that at 48h post infection with virus adsorption the fluorescent focus can be easily noticed. Our preliminary results suggest that HEK-293T can be an alternative cell line for RTCIT.

Palavras-chaves: Cell culture, HEK-293T, Rabies, Virus isolation

ANALYSIS OF THE EFFECT OF ANTIOXIDANT ACTIVITY OF A CARBON-BASED NANOMATERIAL ON ZIKA VIRUS INFECTIONS

Autores	Samille Henriques Pereira [1], marina de souza ladeira [2], leticia trindade de almeida [1], cintia lopes de brito magalhaes [1], luiz orlando ladeira [2], BRENO DE MELLO SILVA [1]
Instituição	[1] ufop - universidade federal de ouro preto (campus morro do cruzeiro - ouro preto - minas gerais), [2] ufmg - universidade federal de minas gerais (belo horizonte - minas gerais)

Resumo

Zika virus (ZIKV) is one of the most important public health arboviruses. The increase of ZIKV cases in recent years has led to a worldwide concern due to the ease of dissemination, difficulty to combat vectors and related serious diseases. It is already known that during the viral infections occurs an increase of reactive species (RS) that leads an imbalance in redox homeostasis that causes biological damage important to viral pathogenesis. Thereby, to control RS may be a strategy to fight the infection. Some Carbon-based nanomaterial (CBNs), has antioxidant activity due to its high capacity for sequestrating the RS. In this sense, this study aimed to evaluate the antioxidant activity of one CBN during ZIKV infection. To evaluate CBN cytotoxicity, a MTT cell viability assay was performed on Vero cells. The antioxidant capacity of CBN was tested from ORAC assay using a standard Trolox curve. The antioxidant potential of CBN was measured by reactive oxygen species assay (ROS), U87MG cells were infected with ZIKV (MOI 1) and treated with CBN for 24 hours, after the incubation the Carboxy-DCFDA probe (Invitrogen™) was added and the reading was performed on the Victor X3 (Perkin Elmer) plate reader with wavelength of 485/535nm. The results showed that for all tested concentrations (up to 12.5 µM) are not cytotoxic. CBN presented an antioxidant capacity in low concentrations 50, 20, 5 and 10 nM, as expected since in higher concentrations occurs the formation of crystals, which could impair its activity. The reduction of ROS production of 17, 44, 43 and 57% was observed at concentrations 50, 25, 12.5 and 6.25 nM, respectively. Also, preliminary results have been shown a reduction of virus multiplication in cell treated with this CBN in lower concentrations. These results suggest that this CBN could be used against ZIKV in the context of infections.

Palavras-chaves: zika, antioxidant, nanomaterial, carbono

HUMAN BETAHERPESVIRUSES 6 AND 7 SALIVARY SHEDDING IN RENAL TRANSPLANTATION RECIPIENTS: LONGITUDINAL STUDY REVEALS ACTIVE REPLICATION

Autores: Jéssica Vasques Raposo [3], Dmitry José de Santana Sarmento [2], Rafaela Barbosa da SIlva Pinto , Amanda de Oliveira Lopes [3], Tânia Regina Tozetto Mendoza Tozetto Mendoza [2], Paulo Henrique Braz da Silva Braz da Silva [1], Vanessa Salete de Paula [3]

Instituição: [1] FOUSP - Departamento de Estomatologia - Faculdade de Odontologia da Universidade de São Paulo (Av. Dr. Enéas de Carvalho Aguiar, 470 - Jardim America, São Paulo), [2] USP - Laboratório de Virologia do Instituto de Medicina Tropica / Universidade de São Paulo (Av. Prof. Lineu Prestes, 2227 - Butantã, São Paulo - SP), [3] IOC/FIOCRUZ - Instituto Oswaldo Cruz – Fundação Oswaldo Cruz (Av Brasil, 4365, Manguinhos. Rio de Janeiro)

Resumo

Infections remain the most common non-cardiovascular causes of death after kidney transplantation, accounting for approximately 15% to 20% of deaths. In pediatric or adult renal transplant recipients, Human Betaherpesvirus 6 (HHV-6) and Human Betaherpesvirus 7 (HHV-7), also called Roseolovirus, often react after transplantation. The reactivation Roseolovirus in immunocompromised patients has been associated with fever, rash, encephalitis and bone marrow suppression. Although it has been reported that Roseolovirus latency and persistence may occur in the salivary glands, there is few information about salivary excretion of these viruses. Therefore, the aim of this study was to evaluate the active Roseolovirus infection in saliva samples from renal transplant recipients. The monitoring of the viral load and detection mRNA of 32 patients were performed in three different moments: T1: before the transplant; T2: 15 to 20 days after transplant, and T3: 40 to 60 days after the transplant. A duplex qPCR was used to quantification of Roseolovirus (gene). The positive samples were tested by nRT-PCR to mRNA detection. HHV-7 showed a significant increase in viral replication during in T3 (72.9%) compared to the pre-transplant period T1 (25%) (McNemar Test, p= 0.001). HHV-6 also showed an increase in replication in T2 and T3, but without statistical significance (p>0.05). Analysis of the ratio of viral replicative to quantitative (DNA copies / mL) showed that positive cases for viral replication had a higher number of DNA copies (> 10^6) when compared to cases without replication for both HHV-6 and for HHV-7 (p

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Palavras-chaves: Herpesviruses, qPCR, Renal Transplantation, Roseolovirus, Saliva

THE ROLE OF P53 PROTEIN IN HCMV REPLICATION IN U138 GBM CELL LINE

Autores GUSTAVO MOCRUHA ALCANTARA [1], LUCAS MATHEUS STANGHERLIN [1], CLARISSA RIBEIRO REILY ROCHA [2], MARIA CRISTINA CARLAN DA SILVA [1]

Instituição [1] UFABC - UNIVERSIDADE FEDERAL DO ABC (São Bernardo do Campo, São Paulo - Brasil), [2] UNIFESP - UNIVERSIDADE FEDERAL DE SÃO PAULO (São Paulo, São Paulo - Brasil)

Resumo

The human cytomegalovirus (HCMV), belongs to the *Herpesviridae*, *Betaherpesvirinae* subfamily and is a high prevalent infectious agent on the worldwide. In immunocompetent hosts, HCMV primary infection is generally asymptomatic, however in immunosuppressed individuals, life threatening diseases can occur. After primary infection HCMV establishes life time latency in some cellular types, such as CD14+ monocytes in the bone marrow, characterized by the presence of the viral genome, limited gene expression and absence of viral particles production. During lytic infection the viral genes are expressed in a coordinate cascade manner and are classified as immediate early (IE), early (E) and late (L). Numerous studies have associated HCMV with cancer and is assumed that the virus can increase tumor malignancy in a process termed as oncomodulation. In the last few decades, many studies have focused in the relation between HCMV and glioblastoma multiforme (GBM), a highly malignant CNS tumor. The virus can modulate the cell cycle, apoptosis, angiogenesis, cell invasion, and host immune response in tumor cells. The P53 protein, expressed by the gene *TP53*, also called tumor suppressor protein, is required for control of the cell cycle and plays an important role in the HCMV replication on permissive cells. Single point mutations that impair the functions of P53 protein are frequently found in diverse tumors, including GBMs. The role of P53, containing mutations that affect its functions, for HCMV replication in tumor cells is not well understood and therefore we decided to investigate HCMV infection and replication in the U138 GBM cell line with P53 mutated ($P53_{mut}$). We have produced a cell line in which expression of $P53_{mut}$ is inhibited by shRNA and analyzed HCMV infection and viral gene expression in these cells comparing to parental U138 cells. Our results demonstrated that in both U138 and U138 knockdown cells all classes of genes are expressed (IE (UL123), E (UL44/UL83) and L (UL99)), however inhibition of P53 leads to an increase in their expression levels. This initial data indicates that $P53_{mut}$ is able to inhibit viral gene expression, which may leads to viral persistence in tumor cells, contrary to the effect of $P53_{wt}$ in permissive cells. Further experiments are underway to better understand the role of P53 in HCMV replication in tumor cells.

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Palavras-chaves: P53, CANCER, GBM, GLIOBLASTOMA, HCMV

THE HOST PROTEIN AP1 IS RELEVANT TO HIV-1 NEF ANTAGONISM AGAINST SERINC5

Autores CRISTINA SANTOS DA COSTA [1], Érika da Silva Czernisz [1], Luis Lamberti Pinto da Silva [1]

Instituição [1] FMRP USP - Faculdade de Medicina de Ribeirão Preto, USP (Av. Bandeirantes 3900, Monte Alegre, Ribeirão Preto- SP)

Resumo

The HIV-1 is a lentivirus that causes one of the largest viral pandemics. One of the first immune system responses to infection is mediated by cell restriction factors, such as Serinc5 – a recently discovery host transmembrane protein incorporated onto viral particles decreasing its infectivity. The viral accessory protein Nef is responsible for the viral antagonism to Serinc5 and acts by removing Serinc5 from the plasma membrane, avoiding its incorporation on viral progeny. It is known that Nef manipulates the endocytic pathway to change the subcellular localization of Serinc5. However, the cellular components involved in this process are not well elucidated. As Nef utilizes the clathrin adaptor complex protein 1 (AP1) on CD4 and MHC-I downregulation, we hypothesized that AP1 could also be involved on Nef-induced Serinc5 redistribution. Thus, the present study aimed to verify the role of AP1 on Nef antagonism to Serinc5. To test the importance of AP1 on Serinc5 redistribution by Nef, the AP1 subunit g-1 was depleted by siRNA in HeLa cells with transient co-expression of Serinc5 and Nef. Indirect immunofluorescence and confocal microscopy analysis confirmed that Nef expression re-localizes Serinc5 from the cell periphery to intracellular structures. Importantly, in the absence of AP1 Nef effect on Serinc5 is abolished, and Serinc5 was localized mostly on cell periphery. In order to get a homogeneous Serinc5 expression, we generate a cell line with doxycycline inducible expression of Serinc5 fused to HA (HEK_SER5-HA). After determining the minimal required concentration of doxycycline, HEK_SER5-HA cells was transfected with either pIRES-GFP or pNef-IRES-GFP plasmids, and the SER5-HA localization accessed by immunofluorescence. In this way, we confirmed that Nef is able to modify the distribution of Serinc5 in this novel cell line system. To verify if Nef antagonizes the SER5-HA incorporation onto HIV-1 particles, HEK_SER5-HA cells was transfected with pNL4-3 WT or ΔNef proviral plasmids. After 48h the virus particles were purified by ultracentrifugation. Western blot analyzes revealed a higher incorporation of SER5-HA on HIV-1 ΔNef than WT, validating the our SER5-HA cell system. We are currently knocking out AP1 in HEK_SER5-HA through CRISPR/Cas9 system to establish the specific function of AP1 in Nef-induced cell surface dowregulation of Serin5 and the consequent impairment incorporation onto budding HIV-1 particles.

Financial Support: CAPES, CNPq, FAPESP.

Palavras-chaves: HIV-1, SERINC5, Nef, AP1

IMMUNOMODULATION OF MONOCYTES AND LYMPHOCYTES BY HYDROXYPROPYL-BETA-CYCLODEXTRIN (HP-BCD) AS A POTENTIAL STRATEGY TO CONTAIN HIV-ASSOCIATED CRHONIC IMMUNE ACTIVATION

Autores: Bruno Braz Bezzera [1], Flavio Lemos Matassoli [1], Natália Caetana de Souza Santos [1], James E. K. Hildreth [2], Luciana Barros de Arruda [1]

Instituição: [1] UFRJ - Universidade Federal do Rio de Janeiro (Cidade Universitária - Rio de Janeiro), [2] UCD - University of California - Davis (California - EUA)

Resumo

Chronic immune activation is a hallmark of HIV infection and increased frequency of activated monocytes and T lymphocytes are strong predictor of disease progression. Hydroxypropyl-beta cyclodextrin (HP-bCD) is a cholesterol-sequestering drug that inhibit HIV infectivity. Since cholesterol metabolism is also involved in inflammation, we investigated whether treatment of monocytes and $CD4^+$T lymphocytes with HP-bCD impacted their response to inflammatory stimuli as proof of concept for anti-HIV therapeutic strategy. We previously demonstrated that treatment of monocytes from HIV-patients with HP-bCD potently inhibited the expression and secretion of TNF-a and IL-10 induced by LPS, by a p38MAPK dependent pathway. Also, transcriptome analysis indicated altered expression of PPAR-g HP-bCD treated cells. Here, we further investigated the molecular mechanisms associated to inhibition of monocyte activation and evaluated if this drug also impacted $CD4^+$T cells activation. Cells from HIV-patients or healthy donors were treated with HP-bCD and the expression of cholesterol and raft-associated proteins were followed. Cholesterol-associated raft was replenished after 48h post treatment, and we chose this time point to stimulate the cells. Monocytes were stimulated with LPS and we followed the TLR4-mediated signal transduction pathways. Corroborating our previous data, HP-bCD treatment resulted in decreased expression of TNF-a, however, production of IL-8 and IFN-a were unaffected, indicating that MyD88 and TRIF adaptor proteins were functional. HP-bCD-treated cells showed reduced phosphorylation of IκBα, suggesting an inhibition of NF-KB transcription factor. HP-βCD also reduced PPARγ expression. However, addition of PPAR-agonists did not impact TNF-a production, indicating that this may not be the pathway associated to HP-bCD function. Regarding T cell activation, we initially evaluated PHA-induced expression HLA-DR and CD38, since HIV progressor patients present increased frequency of $HLA\text{-}DR^+/CD38^+$ $CD4^+$ T cells. Pretreatment of purified T cells with HP-bCD significantly reduced the expression of those markers, without affecting cell viability, indicating that the drug affects unspecific T cell stimulation. Further assays are needed to determine the impact on T cell specific response and on the monocyte-T cells interaction. Still, our data suggest that HP-bCD has an immunomodulatory effect, which may impact HIV pathogenesis and AIDS progression.

CNPq, FAPRJ e CAPES

Palavras-chaves: HIV 2, HP-BCD , Monocyte , Lymphocytes, Inflammation

DIFFERENTIAL MODULATION OF TYPE I IFN RESPONSE BY DISTINCT ZIKA VIRUS ISOLATES AND ITS ROLE FOR VIRUS REPLICATION AND DISSEMINATION TO THE CENTRAL NERVOUS SYSTEM

Autores: Yasmin Mucunã Mustafá [1], Flavio Lemos Matassoli [1], Michelle Premazzi Papa [1], Sharton Vinícius Antunes Coelho [1], Luan Rocha Lima [1], Lana Monteiro Meuren [1], Ricardo Correia da Silva [1], Pedro Moreno Pimentel Coelho [2], Luciana Barros de Arruda [1]

Instituição: [1] IMPG - Instituto de Microbiologia Paulo de Góes, Universidade Federal do Rio de Janeiro (Avenida Carlos Chagas Filho, 373, CCS. Cidade Universitária. Rio de Janeiro, RJ, Brasil. CEP. 21941-902), [2] IBCCF - Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro (Avenida Carlos Chagas Filho, 373, CCS. Cidade Universitária. Rio de Janeiro, RJ, Brasil. CEP. 21941-902)

Resumo

We have previously demonstrated that ZIKV crosses the blood brain barrier (BBB) and causes neurological syndrome without affecting the barrier integrity. We also developed lethal or nonlethal in vivo experimental models based on infection with distinct ZIKV isolates - $ZIKV_{MR766}$ and $ZIKV_{PE243}$, respectively. In these models, we observed that the BBB was disrupted at late time points only in mice infected with $ZIKV_{MR766}$, which succumb to infection. The relevance of type I interferon response for the control of ZIKV infection was clearly evidenced by higher virus replication efficiency in IFNAR-deficient mice (A129 strain), in comparison to wild type (Sv129) mice, when inoculated systemically. But, the role of IFN response for BBB integrity had not been addressed. Here, we investigated whether $ZIKV_{PE243}$ and $ZIKV_{MR766}$ interfered in the production and response to type I IFN upon infection of human brain microvascular endothelial cell line (HBMEC), as an in vitro BBB model. We observed that infection of HBMEC with $ZIKV_{MR766}$ induced a higher and faster production of IFN-b, concurrent with increased IRF-3 phosphorylation, in comparison to infection with $ZIKV_{PE243}$. Infection with both viruses strains significantly inhibited IFN-mediated response, as evidenced with a luciferase/ISRE-reporter HBMEC (lucHBMEC). Accordingly, addition of IFN-β to the cultures did not affect virus replication, nor cell viability, corroborating that ZIKV escapes from this response. Infection with $ZIKV_{MR766}$ resulted in a more potent inhibition of IFN response, associated to increased degradation of STAT2, and stronger inhibition of STAT1 and STAT2 phosphorylation. On the other hand, when the cells were treated with IFN-β prior to ZIKV infection, ISRE activation was induced and a decrease in viral replication was detected. In vivo assays demonstrated that intracerebral inoculation of ZIKV in A129, but not Sv129 promoted BBB disruption, despite virus replication in both mouse strains, indicating the relevance of IFN response for the integrity of BBB. Importantly, $ZIKV_{MR766}$ showed similar titers in A129 and Sv129, corroborating its higher ability to escape the IFN-mediated response. These data suggest that infected, but not bystander cells are resistant to IFN mediated antiviral response. Therefore, whereas ZIKV-infected BMECs block antiviral IFN responses, allowing virus replication, IFNs produced during infection may restrict virus dissemination to bystander cells and control BBB disruption.

Palavras-chaves: ZIKV, IFN response, Blood-brain barrier, HBMEC

INVESTIGATION OF THE ROLE OF VARIATIONS IN E AND NS1 PROTEINS IN ZIKA VIRUS PATHOGENESIS

Autores Iasmim Silva de Mello [1], Déberli Ruiz Fernandes [1], Nathalia Dias Furtado [1], Alexandre Araújo C. dos Santos [1], Marta Pereira dos Santos [1], Lidiane S. Menezes Souza [1], Myrna C. Bonaldo [1]

Instituição [1] Fiocruz - Fundação Oswaldo Cruz (Rio de Janeiro, RJ, Brasil)

Resumo

Zika virus (ZIKV) is an arthropod-borne virus belonging to the genus *Flavivirus* and in the family Flaviviridae. ZIKV is mainly transmitted by *Aedes* spp. mosquitoes. In the recent epidemic, ZIKV has been associated with severe neurological and congenital syndromes. This study aims to correlate amino acid substitutions in the envelope protein (E) and the nonstructural protein-1 (NS1), detected in post-epidemic strains, with the disease's pathogenesis. Infectious clones based on parental virus Rio-U1 (Genbank: KU926309), and two other clones carrying the changes in E and NS1 proteins were constructed using reverse genetics technology. The construct was based on the cloning of fragments that bear the complete ZIKV genome into a low copy number plasmid followed by PCR reactions. The resulting genomic cDNA was transcribed *in vitro* into viral RNA and transfected in Vero cells. The other two clones with alterations in E and NS1 proteins were obtained by site-directed mutagenesis. The IC Rio-U1, IC. MutE and IC. MutNS1 constructs were able to generate infectious particles. RT-PCR confirmed viral recovery from the RNA extract of the culture supernatant. Furthermore, the detection of the E protein was positive by immunofluorescence with the panflavivirus 4G2 antibody for all the variants. The IC. RioU1 virus genomic sequence is identical to its parental virus, Rio-U1. The replication profile of the parental virus is similar to the infectious clone in both Vero and C6/36 cells. The viruses were analyzed by plaque size assay and the phenotype of the IC. Rio-U1 displayed smaller plaques than the parental vírus Rio-U1. The plaque phenotype of the IC. MutE virus is similar to the IC. Rio-U1 virus. The IC. MutNS1 virus has similar plaque sizes as the isolate in which the mutation in NS1 was described, Rio-BM1. In summary, our preliminary findings indicate that the infectious clone IC. Rio-U1 exhibits similar viral fitness compared to the parental virus. The methodology of synthetic ZIKV recovery was achieved and will be of great value in evaluating the role of the amino acid changes in E and NS1 proteins in viral fitness and virulence. Financial support: MCTIC / FNDCT - CNPq / MEC-CAPES / MS-Decit. (Grants 426767 / 2018-7 and 88881.130684 / 2016-01) and INOVA-Fiocruz (Grant VPPIS-004-FIO18)

Palavras-chaves: Zika Virus, Infectious clone, Reverse genetic, Site-directed mutagenesis

THE PROTEIN COMPLEX MTORC MAY INFLUENCE CHIKV INFECTION IN MURINE DENDRITIC CELLS

Autores	Gabriela Fabiano de Souza [1], Stéfanie Primon Muraro [1], Mariene Ribeiro Amorim [1], Aline Vieira [1], Matheus Cavalheiro Martini [1], Daniel Augusto de Toledo Teixeira [1], Renata Sesti Costa [1], Pedro Manoel Mendes de Moraes Vieira [1], Carla Cristina Judice [1], José Luiz Proença Modena [1]
Instituição	[1] UNICAMP - Universidade Estadual de Campinas (Rua Monteiro Lobato, 255 CEP: 13083-862 Campinas - SP - Brasil.)

Resumo

The Chikungunya virus (CHIKV) is an emerging arbovirus present in tropical and subtropical regions transmitted by the arthropod vector *A. aegypti*. Although CHIKV infection may be asymptomatic, it usually leads to the development of an acute febrile illness, joint pain and swelling. One of the major complications associated with CHIKV are chronic manifestations such as arthralgia and arthritis, these symptoms can last for months to years. The chronic disease resulting from CHIKV infection has similar characteristics to rheumatoid arthritis which there is an imbalance in the function of dendritic cells (DCs). Thus, we believe that the infection, activation and metabolic imbalance of dendritic cells may play an essential role in the pathogenesis of CHIKV and in the development of chronic inflammation in the joints. To analyze the effect of CHIKV on the function and metabolism of DCs in vitro, DCs differentiated from WT C57BL/6's bone marrow were pretreated with rapamycin (5, 10, 100 or 200 ng/mL) and infected with CHIKV (MOI 1) for 3, 6 and 24 hours. We characterize viral replication and expression of genes associated with the immune innate response. Preliminary results suggest that bone marrow-differentiated dendritic cells from wild type mice previously treated with inhibitor of the MTORC 1 and 2 metabolic pathways and infected with CHIKV may contribute to an increase in infection within 24 hours. In addition, we observed changes in the expression of IRF 3, 5 and 7, NFk-B and RNase L at different concentrations of inhibitor, suggesting an important role of these pathways during CHIKV infection.

Financial support: FAPESP

Palavras-chaves: Chikungunya, Dendritic cells, Metabolism, mTOR, Arbovírus

THE ROLE OF INNATE RECOGNITION PATHWAYS IN PLACENTAL CELLS AFTER OROV INFECTION

Autores: Stéfanie Primon Muraro [1], Gabriela Fabiano de Souza [1], Aline Vieira [1], Mariene Ribeiro Amorim [1], Matheus Cavalheiro Martini [1], Daniel Augusto de Toledo Teixeira [1], Eurico de Arruda Neto [3], Jean Pierre Schatzmann Peron [2], Maria Laura Costa do Nascimento [1], José Luiz Proença Modena [1]

Instituição: [1] UNICAMP - Universidade Estadual de Campinas (Rua Monteiro Lobato, 255 CEP: 13083-862 Campinas - SP - Brasil.), [2] USP - Universidade de São Paulo (Av. Prof. Lineu Prestes, 1730 Cidade Universitária - CEP 05508-000 São Paulo - SP - Brasil), [3] FMRP-USP - Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo (Av. Bandeirantes, 3900 CEP 14049-900 Ribeirão Preto – SP- Brasil)

Resumo

The Oropouche virus (OROV) is an arbovirus with potential to cause epidemics in the most populated regions of Brazil. Individuals infected with OROV develop a febrile illness, which can progress to neurological and hemorrhagic complications. Furthermore, an increased incidence of abortion was reported during major OROV epidemics. However, the teratogenic potential of OROV and the pathogenetic mechanisms associated with the hematoplacental barrier breakdown have not yet been investigated. The immunological response and antiviral activity in the placenta are dependent on the expression of pattern recognition receptors (PRRs) and production of type I interferon (IFN), mainly on cytotrophoblast, syncytiotrophoblast cells and placental macrophages. However, some viruses are able to establish long-term placental infections, in part by mechanisms dependent on the antagonism of the type I IFN pathways. Thus, we intend to characterize the mechanisms and pathways by which type I and III IFNs modulate the placental immune response during infection by OROV. Preliminary results show that OROV replicates in human placental lineage and can induce the expression of IFN type I and III genes in addition to interferon regulatory genes such as IRF-1, IRF-3 and IRF-7. These results suggest an involvement of immunological recognition pathways and contribute to the follow-up of the next experiments.

Financial support: FAPESP, CNPq

Palavras-chaves: Oropouche, Placenta, Interferon, Arbovirus

CELLULAR ALIX PROTEIN: A HIV INFECTIVITY PROMOTER

Autores	Gustavo Peixoto Duarte da Silva [1], Isadora Alonso Corrêa [1], Pedro Telles Calil [1], Luís Lamberti Pinto da Silva [2], Luciana Jesus da Costa [1]
Instituição	[1] UFRJ - Universidade Federal do Rio de Janeiro (Av. Carlos Chagas Filho, 373, Rio de Janeiro), [2] USP-RP - Universidade de São Paulo - Ribeirão Preto (Av. Bandeirantes, 3900, Monte Alegre, Ribeirão Preto)

Resumo

Cellular Alix protein plays different roles in cell such as regulating basal autophagy, incorporation of LBPA in late endosomes, plasma membrane and lysosome repair. It's well known that Alix plays a central role in ESCRT machinery and both can be coopted for enveloped viruses budding. Our group have previously demonstrated that Alix can interact with HIV accessory protein Nef, but the role of Alix cooption by Nef at the life cycle of HIV-1 was not fully characterized. HIV Nef plays an essential role increasing viral infectivity and progression to Aids. It has been reported that Nef interacts with different cell partners, however the function related to increased viral infectivity wasn't completely understood. Alix-Nef interaction can mediate downregulation of CD4 receptor from the cell surface and targets CD4 to lysosomal degradation. That interaction occurs by a late domain-like motif in Nef. The aim of this study is to investigate the role of Alix on HIV-1 infectivity and the importance of interaction with Nef in this process. In our assays, we observed that silencing of Alix in Hek293T and HeLa cells by transfection of a pool of siRNA against Alix followed by transfection of these cells with pNL 4-3 (HIV-1 infectious clone) leads to a 4-fold decrease in infectivity of viral progeny produced from these, but yet no impact on viral release was observed. Nevertheless, no impact on viral infectivity was observed on a NL 4-3ΔNef upon Alix silencing. A better characterization of viral progeny produced from Alix knock-down cells by analyses of viral protein contents present in iodixanol gradient fractions showed that although viral particles are released from these cells they are partially or not mature, indicating that Nef and Alix together cooperate to virus processing. This data confirms that Alix influences the infectivity of HIV-1 viral progeny and it dependent on Nef by a mechanism related to HIV Protease activity. Interestingly, lymphocytic MOLT4 cells silenced for Alix was shown to be less susceptible to HIV-1 infection spread. Alix silenced MOLT4 cells transfected with pNL 4-3 showed a lower expression of viral proteins and lower virus production after 72h, demonstrating a phenotype of lower permissiveness to HIV-1 infection in cells lacking of Alix. Thus, we confirmed that Alix plays an important role during the replicative cycle of HIV-1. Financial support: CNPq; Faperj; Capes.

Palavras-chaves: HIV, Alix, Infectivity, Nef, Protease

MOLECULAR, BIOLOGY AND CLINICAL CARACTERIZATION OF CHIKUNGUNYA VIRUS STRAIN RJ-IB1 FROM RIO DE JANEIRO, BRAZIL.

Autores	Vinícius Wakoff Pereira Fonseca [1], Romário Mattos de Souza [1], Pedro Antônio Gomes Costa [1], Marcela Sabino Cunha [1], Iranaia Assunção Miranda [1], Luciana Jesus da Costa [1]
Instituição	[1] UFRJ - Departamento de Virologia, Instituto de Microbiologia Paulo de Góes, Universidade Federal do Rio de Janeiro (Av. Carlos Chagas Filho - Cidade Universitária, Rio de Janeiro - RJ, 21941-590)

Resumo

Chikungunya virus (CHIKV), member of alphavirus genus, is the etiological agent of an arthritogenic disease with significant morbidity. Our group isolated and characterized a CHIKV strain (RJ-IB1) from Rio de Janeiro, that clustered with the 1st Brazilian ECSA isolate, from Feira de Santana (BHI3745, 2014). Given the recent introduction of CHIKV in Brazil, little is known about strains viral growth kinetics and pathogenesis. Thus, the aim of this study was to characterize *in vitro* biological behavior and *in vivo* pathogenesis of RJ-IB1. Initially, we performed viral growth kinetics in 4 different cell lines: Hela, HEK293T, HBMEC, and MEF. RJ-IB1 replicated in all cell lines, reaching a replication peak in 24 hpi, ranging between $5x10^5$ and $1.5x10^9$ PFU/ml, depending on the cell line. In general, RJ-IB1 infectious titers were up to 1 log higher than BHI3745. Wild type SV129, 12 (PN12) or 21 days-old (PN21), were inoculated subcutaneously in the left paw with 10^6 PFU/animal with RJ-IB1 or BHI3745. In PN12 mice, we observed for both isolates a pronounced swelling on the left paw as early as 1 dpi that persisted up to 5 dpi, and also a progressive weight loss during this period. We also observed other clinical symptoms: diarrhea, fecal hypocholia, joint pain, hair loss, restless behavior (possible impact on CNS) and skin erythema, with survival rates ranging between 70% and 80%, for both isolates. From RJ-IB1-infected mice we accessed the viremia (peak of $1x10^7$ PFU/ml in 1 dpi) and viral load in brain (peak of $1x10^4$ PFU/ml in 3 dpi); left gastrocnemius muscle (peak of $1x10^4$ PFU/ml in 3 dpi); and liver (peak of $8.2x10^5$ PFU/ml in 1 dpi). Viral load in brain ($4x10^3$ PFU/ml) and muscle ($6x10^4$ PFU/ml) was persistent on 6 dpi. In PN21 mice, the paw swelling persisted up to 10 dpi. Viremia reached a peak on 2 dpi in RJ-IB1-infected mice and 3 dpi in BHI3745-infected mice. The weight gain curve was similar and no other clinical symptoms were observed, for neither of experimental conditions, demonstrating the ability of Brazilian CHIKV isolates to establish infection in 3 weeks-old mice. Histological analysis shows reversible and irreversible cell lesions and presence of mononuclear cell infiltrates in muscles and livers. In conclusion, our results show that infection of SV129 mice with an isolate circulating in Brazil displace an apparently higher pathogenicity in those animals, with possible neurological symptoms and intense viral replication, including in CNS.

Palavras-chaves: CHIKV, Characterization, RJ-IB1, in vivo, in vitro

NOVEL QUINOLONE DERIVATIVE COMPOUNDS: BIOTECHNOLOGICAL APPLICATION AS ANTI-MAYARO AGENTS

Autores: LEONARDO DOS SANTOS CORRÊA AMORIM [1], LETÍCIA VILLAFRANCA FARO [1], ANDREW BARCELOS MONÇÃO MEIRELES , VITOR WON-HELD RABELO [1], LUCIENE SOARES SILVA [1], MARIA LEONISA SANCHEZ NUÑEZ [1], ALDENISE MONT'SERRAT ROSA DA SILVA [1], NATASHA CRISTINA DA ROCHA [1], CAROLINE DE SOUZA BARROS [1], CLÁUDIO CESAR CIRNE DOS SANTOS [1], MARIA CECÍLIA B. V. DE SOUZA [1], IZABEL CHRISTINA NUNES DE PALMER PAIXÃO [1]

Instituição [1] UFF - Universidade Federal Fluminense (Rua Outeiro de São João Batista, S/N - Centro, Niterói - Rio de Janeiro)

Resumo

Mayaro virus (MAYV), causative agent of Mayaro Fever, is an arthropod-borne RNA virus enveloped and is classified in the family *Togaviridae*, genus *Alphavirus*. The MAYV has an endemic epidemic enzootic in pan-Amazonian countries surrounding central South America. Until now, there are no vaccines or drugs available for clinical use, so the search for new therapies is an urgent need. Therefore, this study proposes to evaluate the cytotoxicity and antiviral potential of a quinolone derivatives. We studied 8 new substances from quinolone derivatives for the development of drugs against the MAYV. Vero cells were used to evaluate the effect and mechanism of action of these substances. The cytotoxicity effect was evaluated on Vero cells using the MTT method. Our results showed that the CC_{50} value varied from 659µM to 2815µM. Next, all compounds were screened for their anti-MAYV activity at concentration of 50µM by plaque reduction assay. The most active compound, quinolone derivative 03 (R= *o*-methyl*)*, presented inhibition of plaque formation over than 90% and the value of EC_{50} was 0,83µM. Further, we evaluated the ability of this compound to inactivate the viral particle of MAYV during the replication cycle of the virus (*Time of drug addition assay*). The compound 03 exhibited significant activity in inhibition virus replication in all times of treatment, however the major activity was the beginning of replicative cycle, when cells were infected and treated at the same moment. Moreover, we carried out *in silico* pharmacokinetics and toxicological analysis of this compound using admetSAR2.0 server. Therefore, we identified compounds with high anti-MAYV activity and low cytotoxicity as well as promising theoretical pharmacokinetics, which reinforced their potential to be explored.

Financial support: CNPq , CAPES, FAPERJ, UFF (PROPPI)

Palavras-chaves: Mayaro, Antiviral, Quinolone derivatives

HIV-1 NEF PROTEIN REGULATES VIRAL PROTEASE ACTIVITY TO INCREASE VIRAL INFECTIVITY VIA ALIX.

Autores	Isadora Alonso Corrêa [1], Pedro Telles Calil Pedro Telles Calil [1], Gustavo Peixoto Duarte da Silva Gustavo Peixoto Duarte da Silva [1], Luciana Jesus da Costa Luciana Jesus da Costa [1]
Instituição	[1] UFRJ - Universidade Federal do Rio de Janeiro (Avenida Carlos Chagas Filho, 373, Centro de Ciências da Saúde)

Resumo

The HIV-1 protease (PR) is an enzyme encoded by the HIV-1 *pol* gene that plays a central role in the virus life cycle by cleaving the viral polyproteins $p55^{gag}$ and $p160^{gag\text{-}pol}$ allowing viral maturation. This process is highly regulated and PR activity could be affected by viral polymorphisms and interactions between PR and other viral proteins. The interaction with its substrate, Gag-Pol, is well described and several studies relate the impact of mutations in Gag on PR activity. Other important interaction partner is the viral protein Nef which is cleaved by viral PR and, in a previous study from our group, was associated with the regulation of the enzyme. However, a clear correlation between Nef and PR activity is lacking. In this study, we used PR mutants, that had already been described to increase (K45I) or decrease (T26S) the enzyme processivity and investigated the influence of Nef on viral phenotype. Virus production and infectivity were measured in supernatants of Hek-293T transfected with HIV-1 wild-type and mutant proviral clones. PRT26S and PRK45I had a deleterious impact on production of mature virions and consequently, viral infectivity due to alterations in viral processing. However, specifically for PRT26S mutant, the absence of Nef rescued virus maturation and infectivity to the levels of the wild-type virus. Nef-deletion in PRK45I mutant lead to a partial recovery. This data confirms the importance of Nef for PR activity and demonstrates for the first time that viral infectivity due to Nef is dependent on PR and Nef association. Nef interacts with cellular protein Alix and this interaction is important to promote viral infectivity. A 3-fold reduction in infectivity of the Nef-deleted PRT26S mutant was observed upon Alix knock-down by siRNA, implicating the Nef and Alix interaction in the regulation of PR activity. Phenotypic assays for several PR inhibitors were performed with PRT26S and PRK45I mutants. The K45I mutation conferred a two-fold increase in IC50% concentration for Tipranavir while T26S mutant showed a greater sensitivity to all PIs tested when compared to the WT. Taking together results clearly demonstrate that the absence of Nef could restore the infectivity of viruses harboring mutations that alter PR processing. In addition, these mutations can directly influence the kinetics and replication rate of these viruses and affect the sensitivity to PIs. Financial support: CNPq; Faperj; Capes.

Palavras-chaves: HIV-1, Nef, Protease, Alix

A POSSIBLE ROLE OF RAB27A/B IN OROPOUCHE VIRUS REPLICATION CYCLE.

Autores	Juan Oswaldo Concha Casaverde [1], Cristina Santos da costa [1], Natalia da Silva Barbosa , Luis Lamberti Pinto da Silva [1]
Instituição	[1] FMRP - USP - Faculdade de Medicina de Ribeirão Preto - Universidade de São Paulo (Ribeirão Preto - São Paulo)

Resumo

The Oropouche virus (OROV) belongs to the Peribunyaviridae family, genus Orthobunyavirus, and is the cause of one of the most important arbovirosis in Brazil. OROV is responsible for major outbreaks in different countries of the equatorial region of America, demonstrating a high emerging potential. Despite its relevance, little is known about the molecular aspects of the OROV replicative cycle, and most of the knowledge generated about the genus Orthobunyavirus originates from studies of Bunyamwera viral, the genus prototype virus. The present study aims to elucidate mechanisms used by OROV to release its viral progeny. OROV assembles in virally induced replication compartments derived from Golgi membranes that morphologically resemble multivesicular bodies (MVBs). Because Rab27a and Rab27b are known to be involved in directing MVBs for plasma membrane fusion, we hypothesised that Rab27a and Rab27b may be involved in OROV externalization. To test this hypothesis, confocal immunofluorescence analyses were performed and showed that Rab27a and Rab27b are recruited to intracellular vesicles that are enriched in OROV proteins, which may indicate a possible direct or indirect interaction between these proteins. To further test the involvement of these proteins in OROV production, Rab27a expression was suppressed by siRNA in HeLa cells prior to infection. Expression of OROV proteins was not affected by Rab27a depletion. Interestingly, analysis of the supernatant of these cells 12h post-infection showed a decrease in the percentage of virus released relative to non-silenced control cells, in addition to increasing the amount of infectious virus within cells, suggesting that the presence of Rab27a is important for efficient viral progeny release. To confirm this hypothesis and understand how this possible relationship occurs, more experiments are being performed.

Financial Support: CAPES, FAPESP, CNPq.

Palavras-chaves: Oropouche, Rab27a, Rab27b, Orthobunyaviridae, Peribunyaviridae

ZIKA VIRUS INHIBITION BY COPAIBA (COPAIFERA OFFICINALIS) OIL NANOEMULSION

Autores	Cintia Bittar [1], Tamara Carvalho [1], Marcela Guimarães Landim [2], Paula Rahal [1], Graziella Anselmo Joanitti [2], Marilia de Freitas Calmon [1]
Instituição	[1] UNESP - Universidade Estadual Paulista "Julio de Mesquita Filho" (Rua Cristóvão Colombo, 2265- Jardim Nazareth- São José do Rio Preto-SP), [2] UNB - Universidade de Brasilia (Campus Universitário Darcy Ribeiro, Brasília-DF, CEP 70910-900)

Resumo

Since the 2015 outbreak, Zika virus (ZIKV) has spread all over the world. It has become a major global health issue due to the neurological complications related to ZIKV infection such as Guillain–Barré Syndrome and Zika virus Congenital Syndrome. The virus is transmitted by *Aedes* mosquitoes but also by blood transfusion and sexually which allows the transmission of the virus in vector-free environments. So far, there are no vaccines or specific treatments for ZIKV infection, which makes important to develop specific therapies for its treatment. Here we evaluated the ability of a copaiba oil (*Copaifera officinalis*) nanoemulsion to inhibit ZIKV. Copaiba oil nanoemulsion was prepared by ultrasonication and characterized using Zetasizer®. The obtained nanodroplets were homogeneous (PdI = 0.219) showing hydrodynamic diameter of 120.8 nm and Zeta potential of -29.6 mV. For the *in vitro* assays, first, we defined the highest non-cytotoxic concentration of the copaiba-based nanoemulsion in Vero cells by MTT assay. A concentration of 180 µg/mL was chosen since it maintains 100% cell viability up to 96h after treatment. Vero cells were infected and simultaneously treated with copaiba oil nanoemulsion at the highest non-toxic concentration. After 96h, results were evaluated by plaque assay revealing a viral inhibition of 80%. In order to understand in which steps of the viral life cycle the drug is acting on, we performed time-of-addition experiments and analyzed viral RNA by qPCR after 48h. Preliminary results show that the copaiba oil nanoemulsion has virucidal effect inhibiting 92.5% of virus release. It also showed an effect in post-entry steps inhibiting 99.1% of ZIKV intracellular RNA, when compared to the control. Additional experiments are being performed to confirm the preliminary results and also to understand the mode of action of the copaiba oil nanoemulsion in inhibiting Zika virus infection.

Financial Support: CAPES/CNPq , FAPESP

Palavras-chaves: ZIKV, antiviral, OIL NANOEMULSION, copaíba

A NEW (?) PHLEBOVIRUS ISOLATED FROM AMAZONIAN SANDFLIES

Autores Antonio José Leão Cardoso [1], Eric Fabrício Marialva dos Santos [1], Karina Pinheiro Pessoa [1], Dana Cristina da Silva Monteiro [1], Valdinete Alves do Nascimento [1], Débora Camila Gomes Duarte [1], Victor Costa de Souza [1], Felipe Arley Costa Pessoa [1], Felipe Gomes Naveca [1]

Instituição [1] ILMD-FIOCRUZ/AM - Instituto Leônidas e Maria Deane - FIOCRUZ/AM (Rua Terezina, 476)

Resumo

Sandflies (Diptera: Psychodidae) shows worldwide distribution and approximately 10% of the known species are incriminated as vectors of etiological agents of human illness, mainly parasites of the Leishmania genus. Despite the transmission of some medically relevant viruses; sandflies have been treated as neglected vectors of viral diseases. The transmission of arbovirus by sandflies may be observed in urban, periurban, and rural areas, often associated with poverty. The phleboviruses, arboviruses of the *Phlebovirus* genus (family *Phenuiviridae*), may cause from a self-limiting fever (known as sandfly fever) to neurological infections. In the Brazilian Amazon, viruses within this genus were only registered in the state of Pará until now. We conducted an entomological survey in Rio Pardo rural settlement, Presidente Figueiredo municipality, metropolitan area of Manaus, intending to detect viruses in sandflies. Between 2017 and 2018, we use CDC-like light traps, as well as a mechanical aspiration on the base of trees to sandflies capture. Specimens were kept under the cold chain until species identification following entomological keys. A total of 2,468 sandflies were collected, among these, 991 females were distributed in 35 species, generating 460 pools containing from 1 up to 26 sandflies, which were macerated and inoculated into VERO and C6/36 cells. Of those, 35 pools induced cytopathic effect, 21 only in VERO, 11 only in C6/36 and 3 in both. Conventional RT-PCRs were performed for the *Phlebovirus* genus and one pool with 14 female Lutzomyia sp., collected in a forest environment, was positive. Preliminary results of nucleotide sequencing and phylogenetic analysis indicate that it may be a new phlebovirus, related to the Uriurana virus, previously isolated in Pará. We tentative denominated the virus isolated in the present study as Rio Pardo phlebovirus (RIOPV). The isolate induced a cytopathic effect in both C6/36 cells and HepG2 cells; thus, suggesting that RIOPV may infect vertebrate hosts. Further experiments are ongoing to fully characterize the RIOPV isolate. In conclusion, our results strengthen the necessity of continuous surveillance of potential humans' pathogens in vectors of the Amazon rain forest.

Keywords: Sandflies, Phlebovirus, Brazil, Amazon

Financial Support: DECIT-MS, Capes, CNPq, Fiocruz

Palavras-chaves: Sand flies, Phlebovirus, Brazil, Amazon

HUMAN HERPESVIRUS 6 (HHV-6) AND HUMAN HERPESVIRUS 7 (HHV-7) EXCRETION IN ORAL FLUIDS OF PATIENTS WITH CHRONIC HEPATITIS C

Autores Jéssica Vasques Raposo [1], Nathalia Alves Araújo de Almeida [1], Ana Carolina da Fonseca Mendonça [1], Bianca Leires Marques [1], Lívia Melo Villar [1], Vanessa Salete de Paula [1]

Instituição [1] IOC/FIOCCRUZ - Instituto Oswaldo Cruz (Av. Brasil 4365, Rio de Janeiro)

Resumo

Human Herpesvirus 6 (HHV-6A / B) and Human Herpesvirus 7 (HHV-7), also called Roseoloviruses, belong to the Betaherpesvirus subfamily. Like other herpesviruses, the Roseoloviruses persist in the host after primary infection. Moreover, HHV-6 and HHV-7 are considered opportunistic viruses and co-infections with other viruses are often found and associated with several diseases. In a previous study, the herpesvirus reactivation in patients with chronic Hepatitis C virus (HCV) infection was reported and demonstrated that immune changes that follow HCV might lead to reactivation of other viruses, such as herpesviruses. Due to latency and persistence of Roseoloviruses occur in the salivary glands, the objective of this study was to analyze the risk factors associated with the presence of Roseoloviruses in oral fluids of patients with chronic HCV. Thus, the oral 117 fluids samples were of patients with chronic hepatitis C tested by multiplex qPCR and statistical analyses was performed using R Studio software. The average age of the patients was 54.6±12 years and most of them were (59.9%; 70/117) were female. The presence of Roseoloviruses was found in 47.2% (50/117) to HHV-6 with load viral mean 1,25E+05 copies/mL and 36.7% (43/117) to HHV-7 with load mean 2,24E+05 copies/mL. The comparison with co-infections among Roseoloviruses demonstrated that HHV-6 infection is related to HHV-7 infection (p=0.001). Furthermore, in patients with high fibrosis levels (F3-F4) there were twice the chance (95% IC=1,30-6.83) of HHV-7 infection when compared to individuals with lower fibrosis levels (F1-F2). Roseoloviruses prevalence was high in oral fluids of patients with chronic HCV. However, more cases are required to confirm whether high fibrosis levels may be associated with increased incidence of HHV-6 and HHV-7 reactivation.

Financial support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.

Palavras-chaves: Chronic HCV, HHV-6, HHV-7, Roseolovirus, Saliva

POXVIRUS-HOST INTERACTIONS: THE ACTIVATION OF COMPONENTS OF THE HOST'S UNFOLDED PROTEIN RESPONSES (UPR) DURING INFECTIONS BY THE VACCINIA VIRUS STRAINS GUARANI P1 AND PASSATEMPO

Autores Karine Lima Lourenço [1], Thiago Lima Leão [1], Flávio Guimarães da Fonseca [1]

Instituição [1] UFMG - UNIVERSIDADE FEDERAL DE MINAS GERAIS (Av. Pres. Antônio Carlos, 6627 - Pampulha, Belo Horizonte - MG, 31270-901)

Resumo

Vaccinia virus (VACV) is a member of *Poxviridae* family. Poxviruses multiplication and maturation are closely associated to the endoplasmic reticulum (ER) and their membranes. The RE is able to respond to perturbations through the unfolded protein response (UPR) pathway. This work aims to investigate the effects of infections by zoonotic VACV Brazilian strains, *Guarani P1 virus* (GP1V) and *Passatempo virus* (PSTV), on the activation of UPR pathway sensors in comparison to the VACV *Western Reserve* (WR) prototypical strain. We evaluated, by qPCR, the mRNA levels of genes induced by activation of the UPR pathway in mouse embryo's fibroblasts after infection with GP1V, PSTV or WR viruses . We verified increase in the expression levels of the GADD34 PERK inhibitor after infection with the WR virus. However, this was not observed for GP1V and PSTV. Overall, levels of the ATF4, transcription factor induced by PERK did not increase after infections with any virus. One-step growth curves showed no difference in the multiplication of these viruses in PERK-KO cells when compared to MEF-WT. Activation of ATF6, another sensor of the UPR pathway, was previously confirmed by reporter gene assays; however, the mRNA levels of this protein did not increase after infection with any of the tested viruses. CHOP is another redundant component of the UPR and its transcription up regulation was not observed after infection by the different viruses. CHOP induces the expression of Pdia4, an enzyme that has chaperone and EROI1 oxireductase activities. As expected, we detected no increase in Pdia4 transcription after infection. IRE1 is yet another sensor of the UPR, and infected cells treated with the kinase domain inhibitor of this sensor resulted in smaller plaques for all tested VACVs when compared to non-treated cells. XBP1 is a downstream component of the IRE1 UPR arm, and its expression levels increased after infection. As for BIP, a downstream chaperone that is redundant in the UPR pathways, expression levels did not increase after infections and viruses' single cycle curves in the presence of a BIP inhibitor showed no difference in replication compared to the untreated control. Taken together, ours and other results suggest that PERK activation is irrelevant for efficient GP1V, PSTV and WR

replication On the other hand, the ATF6 sensor and the kinase domain of the IRE1 sensor seem to play an important role in the replication of viruses.

Palavras-chaves: VACV, UPR, WR, PSTV, GP1V

IDENTIFICATION OF DIFFERENTIALLY EXPRESSED MIRNAS IN HUMAN PROSTATIC CELLS INFECTED WITH ZIKV

Autores	Francielly Cristina Machado [1], Ricardo R F Campos [1], Paula Rahal [1], Cintia Bittar [1], Marilia Freitas Calmon [1]
Instituição	[1] UNESP - Universidade Estadual Paulista "Julio de Mesquita Filho" (Rua Cristóvão Colombo, 2265- Jardim Nazareth- São José do Rio Preto-SP)

Resumo

Zika virus (ZIKV) is a virus transmitted mainly by *Aedes aegypti* mosquitoes. However, recent evidences indicate the occurrence of sexual transmission. Although some studies have indicated testes and prostate as the main organs that collaborate in sexual transmission, little is known about which cell types in these tissues are more susceptible to this virus. In addition, infection with distinct ZIKV strains in some models *in vitro* and *in vivo* has demonstrated that the host's response to infection is strain-dependent. Recent findings suggest that this virus deregulates host miRNA profile and that this is an important event throughout the course of the infection. Herein, we evaluated the susceptibility, the permissiveness and the cellular miRNA profile of human prostatic epithelial cells (PNT1A) to two different strains of ZIKV, a classical African strain, MR766 ($ZIKV^{MR766}$) and a Brazilian strain, $ZIKV^{BR}$. So, we infected PNT1A cells with ZIKV strains and performed an indirect immunofluorescence assay for protein envelope; monitored infectious viral particles production and RNA viral copies by plate assay and qPCR, respectively, and analyzed the miRNA cellular profile by PCR array. Our results demonstrated that human prostate cells are susceptible and permissive to ZIKV infection and did not present any imposition regarding infection by distinct strains of this virus. The strains did not differ in the kinetics of replication in prostate cells, but presented differences in miRNA's cell expression modulation. After infection, 16 miRNAs were modulated in prostate cells, a small group of 6 miRNAs were modulated by both strains while a set of 10 miRNAs showed to be modulated exclusively by $ZIKV^{BR}$. *In silico* analyses predicted that the miRNA upregulated exclusively by the infection by the Brazilian strain may regulate genes and pathways associated to inflammation, immunity, cell survival and cell proliferation. Taken together, our results indicate that prostate may be an important role in the sexual

transmission of ZIKV and highlights that different strains of ZIKV may induce a differential host miRNA expression which may influence the differences in the physiopathology presented after the infection by different strains.

Financial support: FAPESP

Palavras-chaves: ZIKV, Prostatic cells, miRNA expression.

FULL GENOME CHARACTERIZATION OF GROUP II CONFIRMS THE DICHOTOMY BETWEEN BRAZILIAN VACCINIA VIRUS

Autores	Mauricio Teixeira Lima [1], Felipe Lopes de Assis [2], Giliane de Souza Trindade [1], Jonatas Santos Abrahão [1], ERNA GEESSIEN KROON [1]
Instituição	[1] UFMG - Departamento de Microbiologia , Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais (Av. Antônio Carlos, 6627 Belo Horizonte, Minas Gerais, CEP 30270-901, Brazil), [2] FDA - U.S. Food and Drug Administration (Estados Unidos)

Resumo

Vaccinia virus (VACV), the prototype virus of the genus *Orthopoxvirus* (OPV), shows serological cross-reactivity with other OPV species and was used during the smallpox eradication campaign. Several exanthematic VACV outbreaks have occurred in Asia and South America, affecting mainly the dairy cattle and rural workers. Brazilian VACV (VACV-BR) have been isolated since 1999 and were characterized since then. The VACV-BR belong to at least two distinct clusters, and these groups were referred to as group I (GI) and group II (GII) of the VACV-BR. Here, used VACV isolates Carangola eye 2(CE2) and Serro human 2/2011 (SH2V) previously described as GII members and etiologic agents of outbreaks in humans from Minas Gerais. The viruses were replicated into VERO cells and purified on a sucrose gradient. Viral DNA was extracted and sequenced in a MiSeq-Illumina apparatus with paired-end applications. After sequencing, reads from CE2 and SH2V were *de novo* and read mapping assembled using Geneious software. The gene predictions were performed using Geneious tools. The functional annotations were inferred by BLAST searches against the GenBank NCBI and the genome annotations were then manually curated. The genomes of CE2 and SH2V show 190,567 base pairs (bp) and 185,934 bp encoding 214 and 199 ORFs, respectively. Both genomes showed a very similar G+C content (~33,3%) and 99.62% of identity. Furthermore, we identified 303 single nucleotide polymorphism (SNPs) between CE2 and SH2V genomes. On the other hand, when compared to VACV Cantagalo genome (KT013210) belonging to VACV-BR GI, the isolates show 1.754 SNPs (CE2) and 1.875 SNPs (SH2V). To better understand the evolutionary relationship between VACV-BR and mainly the GII

viruses, we performed phylogenetic analyses. Other GII sequences available in the literature for VACV-BR genes A56R, B5R, C23L and A26L were used. The trees recurrently clustered GII members separately of GI isolates, corroborating all previous analyses. However, the CE2 and SH2V share common ancestor with New York City Board of Health derived isolates in opposite of VACV-BR GI viruses. In summary, our study reported for the first time the full genomic characterization of VACV-BR GII, reinforcing and expanding previous results with these viruses. In addition our study raises new questions and possibilities, to regard VACV-BR evolutionary relationships, origin and the dichotomy of VACV-BR from a new angle.

Financial support: CNPq, CAPES, PRPq-UFMG, FAPEMIG

Palavras-chaves: vaccinia virus, genome characterization, group II VACV-BR, Evolution

ILHEUS VIRUS IDENTIFIED IN THE CEREBROSPINAL FLUID OF A PATIENT WITH CEREBRAL HEMORRHAGE IN AN ARBOVIRUS ENDEMIC AREA

Autores	Bruno Henrique Gonçalves de Aguiar Milhim [1], Cássia Fernanda Estofolete [1], Leonardo Cecílio da Rocha [1], Elisabete Liso [2], Vânia Maria Sabadoto Brienze [2], Nikos Vasilakis [3], Ana Carolina Bernardes Terzian [1], Gislaine Celestino Dutra da Silva [1], Maurício Lacerda Nogueira [1]
Instituição	[1] FAMERP - Faculdade de Medicina de São José do Rio Preto (Av.Brigadeiro Faria Lima, 5416 Vila São Pedro. São José do Rio Preto, São Paulo, 15090-000), [2] HB - Hospital de Base de São José do Rio Preto (Hospital de Base, São José do Rio Preto, São Paulo, Brazil. 5544 Brigadeiro Faria Lima Ave. Vila São Pedro. São José do Rio Preto, São Paulo, 15090-000), [3] UTMB - University of Texas Medical Branch (Galveston, Texas, USA. 301 University Blvd, Galveston, Texas, 77555.)

Resumo

Ilheus virus (ILHV) is a arbovirus first described in 1944 and isolated from *Aedes* and *Psorophora spp*. mosquitoes during an epidemiological investigation of yellow fever in the city of Ilheus, Bahia State, Brazil. The clinical spectrum of human infections may range from asymptomatic to central nervous system (SNC) involvement, suggestive of encephalitis. Since 2006, we have established an arbovirus surveillance for encephalitis-suggestive cases in the city of São José do Rio Preto, State of São Paulo, Brazil, located in a hyper-endemic area for dengue and Saint Louis encephalitis, Zika, and documented co-infection among various flaviviruses. In September of 2017, a 68-year-old-man was admitted with right hemiplegia, aphasia, dysarthria and deviation of left lip rhyme. During his hospitalization, brain computed angiotomography showed intraparenchymal hemorrhage, with surrounding brain edema, and intraventricular bleeding. Cerebrospinal fluid (CSF) was collected at the onset of symptoms and after 9 and 15 days to analyze the patterns presented.The patient was hospitalized 24 days in UCI, assisted by mechanic ventilation, sedation and vasopressor drugs. Subsequently, the patient developed an urinary infection by multi-drug resistant bacteria in day 20 of hospitalization and died 96 hours later. CSF sample, bacteria culture negative, were submitted to RNA extraction and tested for dengue, Zika and Chikungunya by Trioplex Real-Time reverse transcriptase-polymerase chain reaction (RT-PCR) and other Brazilian arboviruses by Multiplex-nested-RT-

PCR primer sets specific for the nonstructural protein 5 gene. The CSF sample tested negative for typical neurotropic viruses such as Rotavirus by Enzyme-Linked Immunosorbent Assay, Enterovirus and Norovirus by the RT-PCR. ILHV was the only arbovirus identified by Multiplex-nested-PCR. The amplicons obtained were sequenced by the Sanger method and Phylogeny of the ILHV isolate was made using a dataset comprised of 401-long nucleotide sequences mapping on the NS5 gene. Our isolate is clustered with isolates sampled in Venezuela in 1997, suggesting the widespread distribution of the virus throughout Latin America. Our observations do not conclusively demonstrate that ILHV infection led to brain hemorrhage and death, however our surveillance program allowed the detection of ILHV circulation in an arbovirus endemic area, suggesting that the population may be at risk for ILHV infection.

Palavras-chaves: arboviruses, flavivirus, Ilheus virus, neurologic disorders

IN-DEPTH ANALYSES OF THE REPLICATION CYCLE OF ORPHEOVIRUS EVIDENCED MORPHOLOGICAL CHANGES IN VERMAMOEBA VERMIFORMES

Autores: Fernanda Gil de Souza [1], Rodrigo Araújo Lima Rodrigues [1], Erik Vinícius de Souza Reis [1], Maurício Teixeira Lima [1], Jônatas Santos Abrahão [1]

Instituição: [1] UFMG - Universidade Federal de Minas Gerais (Av. Antônio Carlos, 6627, Pampulha - Belo Horizonte - MG - CEP 31270-901)

Resumo

After the isolation of Acanthamoeba polyphaga mimivirus (APMV), the study and search for new giant viruses were intensified, thus these viruses have been uncovered in different samples and environments. Most giant viruses are associated with free-living amoebae of the genus *Acanthamoeba*, however others have been isolated, such Faustovirus, Kaumoebavirus and Orpheovirus in *Vermamoeba vermiformes*. Due to this, our knowledge about the diversity, structure, genomics, and evolution of this group of viruses have been expanding. In the present study, we present an in-depth investigation of the replication cycle of Orpheovirus using different microscopy techniques and biological assays with pharmacological inhibitors. We observed through optical and immunofluorescence microscopy morphological changes in *V. vermiformes* during Orpheovirus infection, as well as increased motility. The viral factory formation and viral particles morphogenesis were analyzed by electron microscopy, and we demonstrate mitochondria and membranes recruitment into and around the electron-lucent viral factories. Microscopy analysis coupled with pharmacological inhibitors of membrane traffic revealed that membrane recruitment decrease and affects the viral morphogenesis. The first structure observed during the particles morphogenesis is a crescent-shaped, which extends and is filled by the internal content until the formation of a mature particle. Mature particles are constituted by a layer of fibrils, outer membrane, inner shell, inner membrane and core. We also demonstrate by electron microscopy the formation of defective particles with different formats. Analyses performed under a light microscope revealed that at 12 hours post infection these viruses started their release from the host by exocytosis, as well as increased of viral titer. The results obtained in

this study contribute to the understanding of biology, structure and important steps in the replication cycle of Orpheovirus.

Financial support: CNPq, CAPES, FAPEMIG, Ministério da Saúde

Palavras-chaves: Orpheovirus, Vermamoeba, Giant viruses, Replication cycle, Microscopy

MOLECULAR CHARACTERIZATION AND PHYLOGEOGRAPHIC ANALYSIS OF THE FIRST COMPLETE GENOMES OF SUBTYPE 2B HEPATITIS C VIRUS IN LATIN AMERICA

Autores: Natália Spitz [1], José Júnior França de Barros [1], Kycia Maria Rodrigues do Ó [3], Carlos Eduardo Brandão Mello [2], Natalia Motta de Araujo [1]

Instituição: [1] IOC/FIOCRUZ - Laboratório de Virologia Molecular, Instituto Oswaldo Cruz/FIOCRUZ (Avenida Brasil, 4365 Manguinhos - Rio de Janeiro - RJ), [2] HUGG - UNIRIO - Hospital Universitário Gaffrée e Guinle - Universidade Federal do Estado do Rio de Janeiro (R. Mariz e Barros, 775 - Maracanã, Rio de Janeiro - RJ), [3] Membro do comitê técnico assessor das Hepatites Vi - Membro do comitê técnico assessor das Hepatites Virais do Ministério da Saúde (Brasília-DF)

Resumo

The Hepatitis C Virus (HCV) has a high genetic diversity, and eight genotypes (1 to 8) with distinct geographic distributions have been described. While HCV genotype 1 is the most prevalent genotype in Latin America, genotype 2 isolates were successful in becoming established and disseminated in different American countries. However, no complete genome sequence of HCV subtype 2b is available from these regions, limiting the contribution of Latin American isolates to phylogenetic and phylogeographic studies. The aim of this study was to determine the first HCV subtype 2b full-length genomes from the Latin America by amplification of large PCR fragments and to reconstruct HCV-2b spatial and temporal dispersion in Brazil. HCV isolates were obtained from serum of two patients from Rio de Janeiro, Brazil. First, the total viral RNA extracted was precipitated and resuspended in a volume of 9.5 µL. The cDNA was synthetized using SuperScript IV Reverse Transcriptase and a nested PCR was done with Platinum Taq DNA Polymerase High Fidelity. Sequencing was performed using the Sanger method. With this approach, two overlapping amplicons (approximately 5.4 kb and 4.8 kb each) spanning the complete HCV genome sequence were generated. The complete genomes of two isolates named PAT1 (extracted from patient 1) and PAT 2 (patient 2), consisting of 9,318 nt were obtained. Surprisingly, patient 2 had the co-circulation of viral variants containing a 2,022 nt deletion covering most of the E1, E2, p7 and the 5' end of

NS2. This deletion was previously associated with advanced age and increased necroinflammatory activity in the liver. In fact, patient 2 is 81 years old and had cirrhosis. Phylogenetic reconstructions of the NS5B region and the complete genome confirmed the classification of PAT1 and PAT2 as HCV-2b. PAT1 and PAT2 clustered into a single phylogenetic cluster along with all Brazilian HCV-2b NS5B sequences, suggesting a single introduction of this subtype into the country. Phylogeographic analysis showed a single entry of HCV-2b in Brazil from the Netherlands between 1977 and 1981. This study provides the first molecular characterization of complete HCV-2b genomes from Latin America and suggests a plausible route of introduction of this subtype in Brazil. These sequences may be used as reference in the diagnosis of HCV-2b, as well as in investigations of the evolution of this subtype on the continent.

Financial Support: CNPq, FAPERJ, FIOCRUZ

Palavras-chaves: complete genome, HCV, Phylogeography

GENETIC DIVERSITY AND MOLECULAR EPIDEMIOLOGY OF HIV-1 AMONG THERAPEUTIC FAILURE PATIENTS FROM SANTA CATARINA STATE, SOUTHERN BRAZIL.

Autores HEGGER MACHADO FRITSCH [1], AMILCAR TANURI [2], AGUINALDO ROBERTO PINTO [1], TIAGO GRÄF [3]

Instituição [1] UFSC - Universidade Federal de Santa Catarina (Campus Reitor João David Ferreira Lima, Bairro Trindade, Florianópolis, SC, Brasil.), [2] UFRJ - Universidade Federal do Rio de Janeiro (Instituto de Biologia – UFRJ, Av Carlos Chagas Filho, 373, Prédio CCS, Ilha do Fundão, Rio de Janeiro, RJ, Brasil..), [3] IGM - Instituto Gonçalo Moniz (Instituto Gonçalo Moniz - IGM. Rua Waldemar Falcão, 121, Candeal - Salvador, BA, Brasil.)

Resumo

The HIV-1 epidemic in southern Brazil is mostly caused by HIV-1C, with co-circulation of subtype B, F1 and recombinant forms. The Santa Catarina state presents high detection, mortality and incidence rates, with Florianópolis being the second national capital with the worst epidemiological scenario. Although previous reports have aimed to describe the molecular diversity, data collection has focused on the major cities, not describing the population as a whole. In this study we analyzed 3.070 PR/RT gene sequences of patients with therapeutic failure that underwent HIV resistance genotyping tests through the Brazilian Network for HIV-1 Genotyping (RENAGENO) between 2008 to 2017. Sequences were submitted to online subtyping tools and when presenting disagreement between these tools, classification was performed by the construction of phylogenetic trees and the use of bootscanning method. Association between subtypes and discrete variables were measured by Pearson's qui-squared test. Out of 3.070 HIV-1 sequences analyzed, 1.998 (65.1%) were classified as subtype C, 597 (19.4%) as B and 149 (4.8%) as F1. CRF31_BC-like recombination profile was identified in 112 (3.6%) sequences. Unique recombinant forms and CRF with a prevalence ≤1% were classified as "others" (N = 214, 7.4% of the total). HIV-1B infection was observed in 23% of the males, while HIV-1C was identified in 69% of the female individuals (p

Palavras-chaves: HIV-1, Subtype C, Molecular diversity, Geographical distribution

VERTICAL NATURAL INFECTION IN CULICIDAE FROM MATO GROSSO, BRAZIL

Autores	Raquel da Silva Ferreira [1], Lucinéia Claudia De Toni aquino da Cruz [1], Nilvanei Aparecido da Silva Neves [1], Laura Marina Siqueira Maia [1], Fabio Assis de Campos Junior [1], Luciano Chaves Franco Filho [2], Poliana da Silva Lemos [2], Clayton Pereira Silva de Lima [2], Marcio Roberto Teixeira Nunes [2], Marina Atanaka [1], Renata Dezengrini Slhessarenko [1]
Instituição	[1] UFMT - Universidade Federal de Mato Grosso (Av. Fernando Corrêa da Costa, 2367 - Boa Esperança, Cuiabá - MT, 78060-900), [2] IEC - Instituto Evandro Chagas (67030-000 - ANANINDEUA / PARÁ / BRASIL)

Resumo

Vertical infection represents the primary way of insect-specific viruses transmission.This mechanism is responsible for arbovirus maintenance in nature during interepidemic periods.Mato Grosso State (MT)Brazil, presents tropical equatorial climates,which associated to other factors may favor the circulation of several viruses.This study proposed to identify viral natural vertical infection in adult male culicids captured in Cáceres,Cuiabá, Rondonópolis and Sinop,between February 2017 and January 2018.A total of 10,569 especimens were collected and identified as Stegomyia(Ae.)aegypti (n=1.139),Culex(Cx.)quinquefasciatus(n=9.426),Culex sp(n=3)and Psorophora albigenu(n=1).Specimens were pooled in 267 groups according to species, place and date of collection.These pools were subjected to viral RNA extraction and RT-PCR protocols for 10 flaviviruses,5 alphaviruses and Simbu serogroup (orthobunyavirus).Positive pools were subjected to 3 passages in VERO cells(alphavirus and orthobunyavirus)or C6/36 cells (flavivirus).Partial results indicate 8 pools of Cx.quinquefasciatus positive for Chikungunya(CHIKV),13 for Mayaro(MAYV), 1 forOropouche(OROV) and 9 for Zika(ZIKV). Of these, 2 ZIKV were isolated at passage 1 (p1), 1 CHIKV and 2 ZIKV at p2 and 7 CHIKV, 13 MAYV, 1 OROV and 5 ZIKV at p3. 5 pools of Ae.aegypti were positive for CHIKV, 1 for Ilheus virus(ILHV), 1 for ZIKV, 1 for dengue 4(DENV-4) and 1 for Yellow Fever virus(YFV). ILHV was

isolated at p1, 1 CHIKV and 1 YFV at p2 and 4 CHIKV, 1 ZIKV and 1 DENV-4 at p3.Also, the extracted RNA of some pools was sequenced on a MinION plataform,resulting in the identification of 2 putative novel viruses in pools of Cx.quinquefasciatus:one sequence of 461 bp with 53.75% similarity with Negev virus and another sequence of 592 bp with 55.67% similarity with Cordoba virus.Both sequences encode a region between the methyltransferase and the ribosomal methyltransferase RNA of Negevirus taxon, genus Nelorpivirus.Sequencing also resulted in the identification of a putative novel Phasivirus genus, Phenuiviridae family member in an Aedes aegypti pool.The segments L(1,047 bp)and S(450 bp)presented 34%and 45.5% similarity, respectively, with those of Phasi charoen-like virus.Insect-specific viruses and arboviruses are frequently classified in the same family of viruses, sharing common ancestors.Metagenomic studies have demonstrated that ISVs comprise a group of surprisingly diverse and ancient viruses.

Financial support: Capes,FAPEMAT

Palavras-chaves: Aedes aegypti, arbovirus, Culex quinquefasciatus, insect-specifics, mosquitões

GENE EXPRESSION MODULATION INDUCED BY OROPOUCHE VIRUS INFECTION IN ENDOTELIAL CELLS

Autores	Pierina Lorencini Parise [1], Daniel Augusto de Toledo Teixeira [1], Aline Vieira [1], Mariene Ribeiro Amorim [1], Fabio Trindade Maranhão Costa [1], Carla Cristina Judice [1], José Luiz Proença Modena [1]
Instituição	[1] IB-Unicamp - Departamento de Genética, Evolução, Microbiologia e Imunologia, Instituto de Biologia, Universidade Estadual de Campinas (R. Monteiro Lobato, 255 - Cidade Universitária, Campinas - SP, 13083-862)

Resumo

Oropouche orthobunyavirus (OROV) is an emerging arbovirus associated with a fever illness called Oropouche fever in the Amazon region of South and Central America. Moreover, OROV can cross the blood-brain barrier and cause central nervous system infection in humans and others vertebrates, such as golden hamster and neonate mice. However, the pathogenic mechanisms associated with the blood-brain barrier breakdown is not fully understood. Thus, to characterize the OROV infection and gene modulation in endothelial cells, we infected human (HBMEC) and murine (BEND3) immortalized endothelial cells with different Multiplicity of Infections (MOIs) and determined the viral load, cell viability and the level of expression of genes genes related with innate immune response and endothelial adhesion during 1, 4, 12, 24, 48 and 72 hours post infection by focus forming assay, MTT and qRT-PCR, respectively. Immunofluorescence was also performed to analyze the structure of endothelial tight junctions using antibodies against ZO-1 and OROV. Interestingly, although OROV is able to replicate in both endothelial cells, the morphological alterations induced by this infection are different in HBMEC and BEND3 cells. While HBMEC cells are lysed during OROV infection, no death was observed in BEND3 cells until 72 hpi. The OROV infection induced a strong antiviral response in BEND3, with increased expression of TLR7, IRF5, IFN-b, IFIT-1, OAS1L and MX-1. In addition, the OROV replication in BEND3 cells induced a expression of adhesion endothelial factors such as ICAM-1. In contrast, nor Interferon Induced Genes (ISGs), TLR7 and IRF5 nor adhesion

endothelial factors were induced in HBMEC cells after OROV infection. Consequently, endothelial Tight-Junctions were not disrupted during OROV infection as seen by anti-ZO-1 staining assay. Thus, we can speculated that OROV is able to antagonize antiviral response in human cells but not in mouse endothelial cells. Financial Support: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Palavras-chaves: Innate immunity, Virus replication, Peribunyavirus, Tight-Junctions, Endothelial cells

THE ANTIBODY PRODUCTION AND INNATE IMMUNE RESPONSE BY B CELLS ARE ESSENTIAL FOR RESTRICTION OF OROPOUCHE VIRUS PRIME-INFECTION

Autores: Daniel Augusto de Toledo Teixeira [1], Matheus Cavalheiro Martini [1], Mariene Ribeiro Amorim [1], Pierina Lorencini Parise [1], Julia Forato [1], Gabriela Fabiano de Souza [1], Stefanie Primon Muraro [1], Karina Bispo dos Santos [1], Aline Vieira [1], Alessandro dos Santos Farias [1], José Luiz Proença Módena [1]

Instituição [1] Unicamp - Universidade Estadual de Campinas (Rua Monteiro Lobato 255)

Resumo

Several arboviruses have emerged or reemerged and dispersed globally in the last years, such as Zika, West Nile and Chikungunya viruses. Brazil has the largest virus diversity in the world and Oropouche (OROV) and Mayaro (MAYV) viruses are pointed as possibly responsible for new outbreaks of arthropod-borne viral diseases in other regions of the world. Oropouche infection is associated with a febrile illness, characterized by symptoms such as rash, photophobia, myalgia, and polyuria. Moreover, some patients have neurologic complications after OROV infection, as encephalitis and meningitis. However, the pathogenic determinants associated with neurological involvement is not fully understood. Thus, the aim of this study was to investigate the role of B cells for protection against neuroinvasion by OROV in a mouse model. For this, we determined morbidity, mortality and viral tropism of 4-5 weeks old C57BL/6 WT mice (n=10), Rag1 KO mice (lacking both mature B and T cells, n=10), µMT mice (lacking only mature B cells, n=10), and TCRbd mice (lacking only mature T cells, n=10) after infection of OROV by subcutaneous route. Interestingly, while WT and TCRbd mice were resistant to infection, Rag1 KO and µMT mice were vulnerable and died with signs of neurologic involvement. The viral load determined by focus forming assay showed a liver and brain tropism as soon as three days after infection. In addition, sera harvested from day 6 post-infection could prevent Rag1 KO mice from neurologic disease, while sera from WT uninfected mice were not able to protect Rag1 KO mice. In the end, $CD19^{Cre}$-$MyD88^{flox/flox}$ mice were partially vulnerable to OROV infection. In

short, our results suggest that antibody production within 6 days, possibly IgM isotype, and the innate immune response by B cells are essential to restrict OROV replication and neuroinvasion in mice.

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Palavras-chaves: Oropouche, B cell, innate immune response, antibody

QUANTITATIVE COMPARISON BETWEEN VERO-76, C6/36 AND BHK-21 CELL LINES USED AS FLUORESCENT FOCUS ASSAY SUBSTRATE FOR FLAVIVIRUS TITRATION.

Autores	EGMA MARCELIN MAYT HUATUCO [1], Enrique Walter Mamani Zapana [1], Sandra Liliana Landazabal Castillo [1], Juan Sulca Herencia [1], Rubén Junior Arancibia Gonzáles [1]
Instituição	[1] UNMSM - UNIVERSIDAD NACIONAL MAYOR DE SAN MARCOS (Av. Universitaria /Calle Germán Amézaga 375. Edificio Jorge Basadre Ciudad Universitaria, Lima 1.)

Resumo

Among research activities in Virology, robust and reliable viral detection and quantification assays are an essential part of the virologist's toolkit. In the case of Flaviviruses, these can be detected and quantified using a variety of virological methods such as Plaque Assays (PA), Reverse Transcription Polymerase Chain Reaction (RT-PCR), Transmission Electron Microscopy (TEM) and 50% tissue culture infective dose (TCID50), each with its own limitations for the detection and quantification of viral genomic particles, viral proteins or intact infectious particles. In this study, we analyzed and compared three different Cell Lines as substrate (Vero-76, C6/36 and BHK-21) for Flavivirus (Dengue Virus, Yellow Fever Virus and Zika Virus) titration in a Fluorescent Focus Assay.

Viruses were adapted independently to each cell line by a certain number of viral passages, each viral strain was subsequently titrated in the three cell lines analyzed by Fluorescent Focus Assay. Titres obtained from the Dengue-2 Virus titration on C6/36, Vero-76 and BHK-21 cell lines were: 6.85x10E5, 6.26x10E5 and 5.95x10E5 respectively. While the Zika Virus titration on Vero-76, C6/36 and BHK-21 cell lines and were 5.61x10E6, 5.13x10E6 and 5.01x10E6 respectively. Finally, titers obtained from the Yellow Fever Virus titration on C6/36, Vero-76 and BHK-21 cell lines were 7.08x10E7, 639x10E7 and 6.88x10E7 respectively. The statistical comparative analysis between the flaviviral titration results demonstrated a trend of higher viral titers on C6/36 and Vero-76

cell lines. These results demonstrate that the quantification of flavivirus by immunofluorescence obtains greater results when using mosquito and kidney cells of green monkey, recommending them as the optimal substrate for flavivirus infection and immunofluorescence detection.

Financed by VRIP-UNMSM

Palavras-chaves: Immunofluorescence, Flaviviral Titration, Cell Line, Dengue Virus, Zika Virus

STANDARDIZATION OF FLUORESCENT FOCUS ASSAY AND COMPARISON WITH THE "GOLD STANDARD" PLAQUE ASSAY FOR DENGUE, YELLOW FEVER AND ZIKA VIRUS TITRATION USING VERO-76 AND BHK-21 CELL LINES.

Autores	Rubén Junior Arancibia Gonzáles [1], Harumy Rojas Rojas Chuchón [1], Katherine Estefany Paz Torres [1], Egma Marcelina Mayta Huatuco [1]
Instituição	[1] UNMSM - UNIVERSIDAD NACIONAL MAYOR DE SAN MARCOS (Av. Universitaria /Calle Germán Amézaga 375. Edificio Jorge Basadre Ciudad Universitaria, Lima 1.)

Resumo

Flavivirus is a genus of arthropod-borne viruses belonging to the family Flaviviridae. The flavivirus genome consists of nonsegmented single-stranded positive-sense ribonucleic acid and have enveloped and spherical virus particles that are between 40 and 60 nm in diameter. Flavivirus that emerge globally and cause significant human diseases like encephalitis or hemorrhagic fever. At laboratory level, Plaque Assay is a "Gold Standard" method for Flavivirus titer quantification, which is based on the formation of plaques in a cell monolayer after viral infection. In this investigation a related technique was proposed, the Fluorescent Focus Assay which is performed very similar to Plaque Assay, and is based on the detection of viral proteins expressed by infected cells through fluorescent-labeled antibodies, which does not require agar overlap, and uses only 24-72 hours of infection time to generate results. Three Flavivirus (Dengue-2; Yellow Fever and Zika) were selected in three cell lines used as substrate (Vero-76, C6/36 and BHK-21). Thawing, propagation and maintenance of virus and cell lines was successfully carried out obtaining optimal viral seeds for the quantification tests. Quantitative comparisons between FFA and PA were performed: Means of viral titers of dengue-2, Yellow Fever and Zika viruses using Vero-76 cell line by Plaque Assay were: 5.54x10E5, 6.79x10E6 and 7.04 x10E7 respectively; while

the results obtained by FFA were 6.22x10E5, 7.1x10E6 and 7.49 x10E7 respectively. Statistically, the quantification results of three flaviviruses analyzed by FFA were greater (p

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Palavras-chaves: Fluorescent Focus Assay (FFA), Plaque Assay (PA), Flavivirus, Viral Titration, Cell Culture

COMPARISON OF IMMUNE RESPONSE IN MICE INTRACRANIALLY INFECTED WITH DIFFERENT ZIKA VIRUS ISOLATES.

Autores	Natália Natália Lima Pessoa [2,1], Beatriz Beatriz Senra Santos [1,2], Thais Barbara Thais Barbara Souza Silva [1], Richard Richard JS Barbosa [1,2], Erna Geessien Erna Geessien Kroon [2], Marco Antônio Campos [1]
Instituição	[1] Fiocruz Minas - Fundação Oswaldo Cruz (Avenida Augusto de Lima 1715), [2] UFMG - Universidade Federal de Minas Gerais (Avenida Antonio Carlos 6627)

Resumo

Zika virus (ZIKV) is an arbovirus transmitted by mosquitoes of the genus *Aedes*, but vertical and possible sexual transmissions are also reported. Phylogenetic analyses of ZIKV reveal the existence of two major lineages: one includes the African isolates, and the other the Asian and American isolates. ZIKV infection was characterized by causing a mild disease presented with fever, headache, rash, arthralgia, and conjunctivitis, with reports of an association with Guillain-Barré syndrome (GBS), microcephaly and meningoencephalitis. Our objective was to compare the immune response triggered by two Brazilian not mice adapted isolates of ZIKV (PE243 and SPH) when the central nervous system was exposed. For this, 8 week age C57BL/6 wild-type mice and TLR2/9 and iNOS knockout mice were infected intracranially with 400 p.f.u. of PE243, SPH and MR766, an African mice adapted isolate, as positive control. Negative control mice were injected intracranially with C6/36 cell culture supernatant (mock). Mice were observed and weighed daily. C57BL/6 mice infected with MR766 lost weight, had conjunctivitis, paralysis, and died 8 to 12 days after infection and only one mouse survived as described in literature. C57BL/6 mice infected with Brazilian isolates did not die nor showed signs, but mice infected with PE243 gained less weight than control mice, with statistical difference, and mice infected with SPH showed no statistical difference

compared to mock. TLR2/9 KO mice infected with MR766 showed the same signs as C57BL/6 mice and died 7 to 9 days after infection, and also only one mouse survived. TLR2/9 KO mice infected with Brazilian isolates did not die nor showed signs but gained less weight than control mice. iNOS KO mice infected with MR766 showed the same signs compared to wild type mice and died at 7 days after infection and no animals survived. iNOS KO mice infected with Brazilian isolates did not die, nor showed signs or lost of weight. We conclude that there was a reduction of weight gain in mice when infected with different Brazilian Zika isolates and TLR2/9 and iNOS in the model used do not impact on infection with ZIKV.

Palavras-chaves: zika virus, animal model, brazilian isolates, reduction of weight gain

PRODUCTION OF HUMAN CYTOMEGALOVIRUS UL111A TRANSCRIPTS IN FIBROBLASTS AND GLIOBLASTOMA CELL LINES: IDENTIFICATION OF A NEW TRANSCRIPT

Autores: Tainan Cerqueira Neves [1], Lucas Matheus Stangherlin [1], Isabela de Godoy Menezes [1], Maria Cristina Carlan da Silva [1,2]

Instituição: [1] UFABC - Universidade Federal do ABC (São Bernardo do campo, SP, Brasil), [2] CAM - University of Cambridge (Cambridge, United Kingdon)

Resumo

The Human Cytomegalovirus (HCMV) belongs to the Herpesviridae family, subfamily *Betaherpesvirinae*, which includes neurotropic viruses with a long replicative cycle and restrict specie specificity. Primary HCMV infection in immunocompetent hosts is commonly asymptomatic due to robust antiviral immune response. On the other hand, the virus can cause devastating diseases in immunocompromised individuals, such as transplant recipients and AIDS patients. In addition, HCMV has been linked to cancers, cardiovascular disease and immune dysfunction. One of the key intriguing features of HCMV biology is the exceptionally large arsenal of virus-encoded proteins capable of counteracting the innate and adaptive host immune defences, allowing the virus to persist throughout the host's life. One group of immunomodulatory proteins expressed by HCMV are host cytokine and chemokine homologues, in particular the human interleukin-10 homologue (hIL-10), called viral IL10 (vIL-10). The vIL-10 coding gene UL111A undergoes alternative splicing producing different isoforms. Classically, the UL111A gene is described as been composed of three exons and two introns, resulting in two transcripts, the cmvIL10, produced by the removal of two introns, coding a 175aa protein which retains the ability to bind and signal through the hIL-10 receptor and LAcmv-IL10, produced by the removal of the first intron, coding a c-terminus truncated protein of 139aa, impairing its ability to bind to the hIL10-receptor. Five new spliced transcripts were described, but their

biological functions during viral infection are not well elucidated. This study aimed to detect the production of UL111A transcripts during HCMV infection in permissive MRC5 primary fibroblasts and semi permissive U138 and U251 glioblastoma (GBM) strains infected with a low passage clinical HCMV strain, TB40e. We report the production of four transcripts previously described in the literature and the identification of a new transcript produced by the UL111A gene. In addition, for the first time, we show the differential expression of UL111A transcripts in permissive and semi permissive infection. Studies of protein expression and analysis of their biological functions in immune cells are under way. These studies can contribute for the understanding of the immunomodulatory role of HCMV IL10 in different states of infection.

Palavras-chaves: HCMV, interleukyn 10, cytomegalovirus, cloning, glioblastoma

QUANTIFICATION OF THE UL111A HUMAN CYTOMEGALOVIRUS TRANSCRIPTS IN PRODUCTIVE AND LATENT INFECTED CELLS

Autores	Lucas Matheus Stangherlin [1], Isabela de Godoy Menezes [1], Ian Groves [3], Helena Beatriz de Carvalho Ruthner Batista [2,1], Emma Poole [3], John Sinclair [3], Maria Cristina Carlan da Silva [1,3]
Instituição	[1] UFABC - Universidade Federal do ABC (São Bernardo do Campo - SP/Brasil), [2] Pasteur - Instituto Pasteur (São Paulo - SP/Brasil), [3] CAM - University of Cambridge (Cambridge/United Kingdom)

Resumo

The Human Cytomegalovirus (HCMV) is a highly prevalent member of the Herpesviridae family, Betaherpesvirinae subfamily. The virus establishes asymptomatic lifelong latent infection in the immunocompetent host. However, in immunosuppressed individuals, the virus can reactivate and enter the lytic cycle, causing severe diseases. Many cell types can be infected in the host, however permissiveness to viral replication virus varies according the cell type. During productive or lytic infection, the virus replicates mainly in epithelial cells, endothelial cells, fibroblasts, smooth muscle cells and macrophages. After the control of replication by the immune system the virus persists in a chronic state with low levels of replication and shedding, for long periods of time, in absence of cytopathic effect, mainly in endothelial and epithelial cells. Ultimately, latency is established and is a mode of persistence associated with a profound restricted lytic gene expression in myeloid progenitor cells in the bone marrow. It is well known that the HCMV has a vast arsenal of genes which encodes proteins capable of modulate diverse cell pathways. One of these genes is UL111A which encodes a human interleukin 10 (hIL-10) homologue, termed vIL10. Several transcripts are produced by alternative splicing and denominated from A to G. So far there are no reports regarding the differential expression of UL111A transcripts in cells in different states of infection. Thus, to clarify

this matter is of great importance to comprehend the dynamics of viral cycle states transition and the balance between host immune system and the HCMV. Here we investigated by quantitative RT-PCR, the expression of transcripts A, B, E and H, a newfound transcript detected by our group in MRC5 infected cells. The data shows that UL111A transcripts are significantly less expressed in CD14 monocytes when compared to HFF cells. Transcripts E and H were not detected in latent infection, which could indicate that these transcripts are absent or far less expressed during viral latency. Also, in order to lead to viral reactivation, CD14 monocytes were treated with PMA, resulting in a significant increase of all UL111A transcripts. Although the A transcript has been almost exclusively related to productive infection, we detected it at levels as high as B in monocytes Therefore, this work supports the idea of differential UL111A alternate splicing during different phases of HCMV cycle.

Palavras-chaves: HCMV, Cytomegalovirus, Alternative splicing, Latency, Interleukin 10

OROPOUCHE VIRUS INFECTION OF HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS IN VITRO

Autores	Mariene Ribeiro Amorim [1], Daniel Augusto de Toledo Teixeira [1], Stefanie Primon Muraro [1], Gabriela Fabiano de Souza [1], Pierina Lorencini Parise [1], Matheus Cavalheiro Martini [1], Karina Bispo dos Santos [1], Júlia Forato [1], Aline Vieira [1], Alessandro dos Santos Farias [1], Renata Sesti Costa [1], José Luiz Proença Módena [1]
Instituição	[1] Unicamp - Universidade Estadual de Campinas (R. Monteiro Lobato, 255 - Barão Geraldo, Campinas - SP, 13083-862)

Resumo

Oropouche orthobunyavirus (OROV) is an Amazonian emerging virus with high potential of dissemination for several regions of the world, mainly due to deforestation, climate changes and expansion of population density in the Amazon. The major symptoms associated with OROV infection are headache, myalgia, arthralgia and exanthema. Moreover, hemorrhagic and neurological complications are also frequently associated with OROV infection in humans and other animals. As the infection of monocytes and dendritic cells are usually key events during arboviral infections, we decided to evaluate the viral replication and gene expression modulation in human peripheral blood mononuclear cells (PBMCs) during OROV infection. For this, PBMCs from healthy donors, THP-1 and Jurkat lineages were infected with OROV. Genome and antigenome of OROV were assessed by RT-qPCR and RNA PrimeFlow assay by flow cytometry and immunofluorescence using specific RNA hybridization probes. Productive infection was also evaluated by focus forming units (FFU) assay and the expression of antiviral innate immunity genes was evaluated by RT-qPCR. Interestingly, although OROV was not able to establish a productive

infection in human PBMCs, significant levels of viral genome were maintained in a small proportion of these cells (mainly monocytes and B lymphocytes), as demonstrated by RT-qPCR and RNA PrimeFlow. The OROV infection in PBMCs were followed by increased expression of type I and II IFNs and Interferon-stimulated genes (ISGs). Thus, the data indicate that human PBMCs cells are not normally permissive to OROV infection. However, the maintenance of viral genome in lymphocytes and monocytes points that these cells may act as a Trojan horse in specific situations or microenvironments, as observed during immunosuppression in the central nervous system.

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Palavras-chaves: Oropouche orthobunyavirus, innate immunity, PBMCs

THE CRISPR/CAS9 COMPLEX AS A NEW ANTIVIRAL THERAPY AGAINST HERPES SIMPLEX TYPE 1

Autores	Rafaela Barbosa da Silva Pinto [1], Jéssica Vasques Raposo [1], Lauana Ribas Torres [1], Luciana Rodrigues Carvalho Barros [2], Martin Hernan Bonamino [2], Elen Mello de Souza [1], Vanessa Salete de Paula [1]
Instituição	[1] IOC - Fiocruz - Instituto Oswaldo Cruz (Avenida Brasil, 4365, Manguinhos - Rio de Janeiro - RJ), [2] INCA - Instituto Nacional do Câncer (Rua André Cavalcanti, 37, Lapa, Rio de Janeiro, RJ)

Resumo

Herpes Simplex Type 1 is a member of *Herpesviridae* family that affects about 67% of the population of worldwide. The major infection route is the direct contact with lesions and contaminated secretions. The primary infection can be asymptomatic followed by a latency infection in the sensory ganglia. In latency, HSV-1 can be reactivated by factors such as stress, immunosuppression and heat and migrated through the nerves until the peripheric tissue, as corneal tissue. Currently the treatment is the antiviral acyclovir, limited to viral replication only; it can cause renal toxicity and, in some cases, viral resistance. This indicates the need for new antiviral therapies that prevent virus reactivation. The power of CRISPR lies in its simplicity and ease of use, its flexibility to be targeted to any given nucleotide sequence by the choice of an easily synthesized guide RNA, and its ready ability to continue to undergo technical improvements. The aim of this study is to use the CRISPR/Cas9 complex as a new antiviral therapy against HSV-1. The target region chosen, UL39, expresses ribonucleotide reductase protein, essential for virus replication. The CRISPOR platform was used to obtain the RNA for the target sequence. The sequence was inserted in the plasmid px 459 from *Streptococcus pyogenes* and into competent bacteria *E. coli*. These bacteria were cloned and the plasmidial DNA isolated. After that, the complex was transfected with lipofectamine to Vero

cells in two different moments: before and after the HSV-1 infection, 24h and 48h. Plaque assay, immunofluorescence assay and qPCR were performed to evaluated inhibition of the viral replication. To verify the consequences of the complex in the cells, an MTT assay were made and the cell viability was not affected. The immunofluorescence showed the intracellular CRISPR complex and qPCR showed a decrease in viral load > 90% in cells transfected with CRISPR/Cas 9 anti-HSV-1. Our data demonstrate the utility of using CRISPR/Cas9 system for inhibition of HSV-1 infection.

Financial Support: CNPq

Palavras-chaves: Antiviral, CRISPR/Cas9, Herpes, HSV-1

PHYLOGENETIC AND STRUCTURAL ANALYSIS OF THE 5' AND 3' UNTRANSLATED REGIONS OF THE CHIKUNGUNYA VIRUS GENOME

Autores: Gabriela Fabiano de Almeida [1], Maria Eduarda Correa Fonseca de Souza [1], Ana Carolina Gomes Jardim [2], Diego Pandeló José [1]

Instituição: [1] UFTM - Universidade Federal do Triângulo Mineiro - Campus Universitário de Iturama (Avenida Rio Paranaíba, 1229, Centro - Iturama, MG), [2] UFU - Universidade Federal de Uberlândia (Avenida Pará , 1720 - Uberlândia, MG)

Resumo

The genus Alphavirus includes etiological agents responsible for serious human diseases, as the Chikungunya virus (CHIKV). CHIKV is transmitted by Aedes aegypti mosquito and can trigger outbreaks of febrile illness showing specific symptoms such as body and joint pains. To date, there is no vaccine against this arbovirus, which has been becoming a serious public health problem in Brazil and other South American countries. Research into the genomic and structural constitution of this virus is becoming increasingly important for the future disease eradication. The present work aimed to perform phylogenetic and structural analysis of the 5' and 3' untranslated regions (UTRs) of the CHIKV genome. For this, fifty sequences of CHIKV complete genome obtained from NCBI were submitted to the phylogenetic reconstructions in the MEGA X software, using the maximum likelihood analysis method with Tamura-Nei substitution model. A thousand replicates were used to test reliability of the tree topology and bootstrap values >60 were considered significant. Bayesian analysis were performed using BEAST v.1.10.1 software package. The prediction of the formation of secondary structures of the 5' and 3' UTR regions were performed using RNAfold WebServer. Phylogenetic analysis of the CHIKV complete genome demonstrated that

sequences were grouped according to the virus genotypes West African, East-Central-South African (ECSA), Asian and Indian Ocean, as expected. Sequences from Brazilian CHIKV-infected patients were grouped only in Asian and ECSA clades. Phylogenetic Tree of UTR sequences has shown a similar topography. At least five sequences grouped in the same clade showed differences in the secondary structures of the UTRs. The major secondary structural diversity of these regions was noted inside the Asiatic group. Thus, it was observed a genetic diversity in the UTR regions of sequences grouped in the same clade, resulting in different secondary structures of these regions. It could indicate changes in the virus replicative cycle due to the increase or loss of affinity by some cell factors. However, new in vitro approaches are needed to investigate more details about these interactions. The results present here may, in the future, be useful to elucidate more details about the influence of untranslated regions on the viral replicative cycle.

Financial Support: FAPEMIG

Palavras-chaves: Chikungunya, Molecular phylogeny, Mutation, Secundary structure, UTRs

PHYLOGENETIC AND STRUCTURAL ANALYSIS OF THE 5' AND 3' UNTRANSLATED REGIONS OF THE MAYARO VIRUS GENOME

Autores	Gabriela Fabiano de Almeida [1], Maria Eduarda Correa Fonseca de Souza [1], Ana Carolina Gomes Jardim [2], Diego Pandeló José [1]
Instituição	[1] UFTM - Universidade Federal do Triângulo Mineiro - Campus Universitário de Iturama (Avenida Rio Paranaíba, 1229, Centro - Iturama, MG), [2] UFU - Universidade Federal de Uberlândia (Avenida Pará, 1720 - Uberlândia, MG)

Resumo

The genus Alphavirus belongs to Togaviridade family and includes etiological agents responsible for serious human diseases, as the Mayaro virus (MAYV). MAYVV is transmitted primordially by Haemagogus genus mosquito and can trigger outbreaks of febrile illness showing specific symptoms such as myalgia, muscle and joint pains. To date, there is no vaccine against this arbovirus, which has been becoming an emergent public health problem in Brazil and other countries from South and Central America. Research into the genomic and structural constitution of this virus is becoming increasingly important for the future disease eradication. The present work aimed to perform phylogenetic and structural analysis of the 5' and 3' untranslated regions (UTRs) of the MAYV genome. Therefore, twenty-eight sequences of MAYV complete genome obtained from NCBI were submitted to the phylogenetic reconstructions in the MEGA X software, using the maximum likelihood analysis method with Tamura-Nei substitution model. A thousand replicates were used to test reliability of the tree topology and bootstrap values >60 were considered significant. Bayesian analysis were performed using BEAST v.1.10.1 software package. The prediction of the 5’ and 3’ UTR secondary structures formation were performed using RNAfold WebServer.

Phylogenetic analysis of the MAYV complete genome demonstrated that sequences were grouped according to the virus genotypes - genotype D, genotype L and genotype N, as expected. Genotype N presents a unique sequence from Peru. Phylogenetic analyzes of UTRs showed some variations in tree topology compared to the complete genome. Nevertheless, some positions remained the same one, such as the N genotype sequence, which remained isolated in all topologies. UTRs secondary structure analysis have demonstrated close similarity among sequences belonging to the same clade. It could suggest a high degree of conservation of these regions, probably due to their importance in viral replicative cycle. However, sequences belonging to different genotypes have shown greater structural divergence of their UTRs. The results present here may, in the future, be useful to elucidate more details about the influence of untranslated regions on the viral replicative cycle.

Financial Support: FAPEMIG

Palavras-chaves: Mayaro, Molecular phylogeny, Mutation, Secondary structure, UTRs

MOLECULAR ANALYSIS OF DENGUE VIRUS TYPE 4 INTRODUCED IN 2012 IN MATO GROSSO, BRAZIL

Autores	Janeth Aracely Ramirez Pavon [1], Nilvanei Aparecido da Silva Neves [1], Nayara Zuchi [1], Marcelo A. M. dos Santos [3], Leticia B. S. Heinen [1], Steven G. Widen [2], Sandra V. Mayer [2], Thomas G. Wood [2], Nikos Vasilakis [2], Renata Dezengrini Slhessarenko [1]
Instituição	[1] UFMT - Post-Graduation Program in Health Sciences, Medicine Faculty. Federal University of Mato Grosso, Cuiabá, Mato Grosso, Brazil. (Cuiabá, Mato Grosso, Brazil), [2] UTMB - The University of Texas Medical Branch (Galveston, Texas, USA), [3] LACEN-MT - Central Laboratory of Mato Grosso, State Secretary of Health (Cuiabá, Mato Grosso, Brazil)

Resumo

Dengue virus (DENV-1,-2,-3,-4) is responsible for Dengue Fever, a major public-health concern worldwide. Despite the efforts to control the disease, Dengue incidence increased approximately 30-fold in the past decades. Brazil is the leading American country in annual reports. During 1981 and 1982, DENV-1 and DENV-4 were reported in Boa Vista, Roraima State. DENV-1 outbreaks disseminated cross the country later, in 1986; DENV-2 introduction in Brazil was evidenced in 1990, when the first hemorrhagic febrile cases were detected; DENV-3 authoctonus transmission were reported from 2000 and, after 25 years undetectable, genotypes I and II of DENV-4 were reintroduced in the country in 2008 and 2010, respectively. Between 2010-2018, DENV-1 and DENV-4 were responsible for dengue outbreaks across the country. Brazil reported 1.5 million cases, 1,032 deaths, reaching a incidence rate of 126,7 per 100.000 habitants by the year of 2018. In 2012, the highest incidence rates were recorded in the Northeast (548,2) and Midwest Brazil (483,4); for the state of Mato Grosso (MT) was of 1.609,1. Between October 2011 and July 2012, we sampled 604 patients with acute febrile illness for up to five days

suspected of dengue fever in public health institutions of MT. These samples were processed for viral isolation of the four dengue serotypes and yellow fever at Lacen MT. Additionally, serum aliquots were subjected to viral RNA extraction and multiplex semi-nested RT-PCR for flaviviruses, alphaviruses and orthobunyaviruses. In total, from 331 samples positive for DENV in 17 municipalities, 315 were for DENV-4; 217 from patients of the metropolitan area of Cuiabá. We sequenced 26 DENV-4 isolates in an Illumina HiSeq 1000 plataform. Phylogenetic analysis based on Envelope gene sequences with another 119 human isolates form previous studies suggested our isolates formed a monophyletic group composed by two distinct lineages within genotype II, closely related with two 2010 isolates from a geographically close region, Boa Vista, Roraima State, which indicates local transmission and spread after initial introduction in North Brazil, and sharing proximity with strains circulating in Venezuela, 2007. Further confirmation of the co-circulation of DENV-4 distinct linages was made with intrahost variation analysis, which demonstrated a high degree of similarity in the consensus amino acids across samples without insertions or deletions.

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Palavras-chaves: Arbovirus, dengue outbreak, molecular epidemiology, phylogeny