ÁREA IMUNOBIOLÓGICOS

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EVALUATION OF CYTOKINE PRODUCTION AND IMMUNODOMINANCE PROFILE OF T CELL RESPONSE TO ZIKA VIRUS

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Resumo

The ZIKV genome encodes a polyprotein with three structural proteins: capsid, pre-membrane, envelope, and seven nonstructural proteins: NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5. The detailed understanding of cellular immunity to ZIKV and its association to antigenic regions of the virus remains unclear. Thus, this study aimed to evaluate the profile of the immune response and the role of multifunctional T cells in individuals exposed to ZIKV. We used a library of 671 synthetic peptides expanding the whole polyprotein of ZIKV, in which pools corresponding to each viral proteins were used to stimulate PBMCs from individuals previously exposed or not exposed to ZIKV. Investigation of cytokine production by $CD4^+$ and $CD8^+$ T cells was performed by intracellular cytokine staining combined with flow cytometry. We observed production of IFN-γ and TNF-α by $CD8^+$ T cells stimulated with prM (IFN-γ, p=0.0267; TNF-α, p=0.0033), capsid (IFN-γ, p=0.0011; TNF-α, p^+ T cells. In addition, there was an increase in the frequency of $CD8^+IL\text{-}10^+$ T cells after stimulation with prM (p=0.0116), capsid (p=0.0017), NS1 (p^+ T cells with capsid (p=0.0017) and NS1 (p=0.0448). Multifunctional cytokine production was observed in ZIKV infected individuals, with triple production of: IFN-γ^+ TNF-α^+ IL-10^+ in cells stimulated with prM ($CD8^+$, p=0.0078), capsid ($CD8^+$, p=0.0020; $CD4^+$ p=0.0117), NS1 ($CD8^+$, p=0.0234) and NS3 (CD8+, p=0.0156), and double production of IFN-γ^+TNF-α^+ in cells stimulated with envelope ($CD8^+$, p=0.0156, CD4+, p=0.0391), prM ($CD8^+$, p=0.0195; $CD4^+$ p=0.0195), capsid ($CD8^+$, p=0.0078, $CD4^+$ p=0.0078), NS1 ($CD8^+$, p=0.0156; $CD4^+$, p=0.0039) and NS3 ($CD8^+$, p=0.0313; $CD4^+$, p=0.0078). In addition, a double production of IFN-$\gamma^+IL\text{-}10^+$ cells stimulated with prM ($CD8^+$, p=0.0195), capsid ($CD8^+$, p=0.0020; $CD4^+$, p=0.0020) and NS1 ($CD4^+$, p=0.0078) was detected. These data indicate a prominent and multifunctional T $CD8^+$ response targeting mainly structural proteins, while the CD4+ T cell response was distributed to structural and non-structural proteins similarly.

Palavras-chaves: Zika Virus, T cells, cytokine, immunodominance, immunity

STUDY OF THE INTERACTIONS BETWEEN PEPTIDES DERIVED FROM THE YELLOW FEVER VIRUS AND THE YF-17D VACCINE WITH HLA CLASSES I AND II: AN IN SILICO APPROACH

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Resumo

The attenuated virus YF-17D is used as a vaccine against yellow fever virus (YFV) and confers protective immunity in 90 to 98% of vaccinated populations. The mechanisms of 2 to 10% in vaccination failures are still not well understood. A possible reason of vaccination failures might be defective antigenic presentation by HLA repertoire of the vaccine. We believe that knowing the amplitude and strength of the interactions between HLA class I and II with the YF-17 and YFV peptides can help us understand cases of vaccine failure. The objective of this research was to predict the amplitude and strength of the interactions between the proteins of the vaccine virus YF-17D and the HLAs of class I and II.

We used bioinformatic tools at EIDB to predict the IC50 values of the interactions between the most common HLA class I and II alleles and polyprotein peptides and viral proteins such as capsid (C), pre-membrane (PrM), envelope (E), NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5 of the YFV and YF -17D. Predicted IC50 values 50 nM and 500 nM were considered low affinity and non-significant interaction.

The proteins with the highest number of significant interactions for both, class I and II HLAs were NS5, which showed 977 and 4462 interactions respectively, NS3 (568, 3118), E (517, 5250) and NS4a (480, 1774), probably associated to their bigger sizes when compared to other virus proteins.

In addition, we found that alleles HLA-A*02:03, HLA-A*68:02, HLA-A*02:01, HLA-A*02:06 of class I HLAs and the alleles HLA-DRB1*01:01, HLA-DQA1*05:01/DQB1*03:01, HLA-DRB5*01:01, HLA-DRB1*07:01 and HLA-DRB1*11:01 HLA class II display higher number of interaction with different peptides, which can be classified as good presenters of antigens of the YF-17D. On the other hand, HLAs such as HLA-A*01:01, HLA-B*53:01 and HLA-A*26:01 of class I, and HLA-DQA1*05:01/DQB1*02:01, HLA-DQA1*03:01/DQB1*03:02 and HLA-DPA1*02:01/DPB1*05:01 of class II showed a limited number of interactions with the YF-17D peptides, which makes them poor antigen presenting.

There are differences in the capacity of different HLA alleles to present peptide antigens of Yellow Fever vaccine, which may underlie vaccination failure.

Finacial support: CAPES

Palavras-chaves: Human leukocyte antigen, yellow fever vaccine, viral proteins

REFOLDING OF RECOMBINANT EDIII ZIKV PROTEIN GENERATED UNDER HIGH PRESSURE CONDITIONS PRESERVES ANTIGENICITY AND IMMUNOGENICITY IN MICE.

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Resumo

Zika virus (ZIKV) belongs to the *Flavivirus* genus (family Flaviviridae) has a wide geographical distribution and is transmitted to humans by mosquito vectors. Although most infected individuals do not develop symptoms, symptomatic cases cause dramatic consequences to neonates, such as microcephaly and other neurological disorders. Despite efforts, there are no specific and effective preventive or therapeutic methods available against this infection. In this sense, the induction of neutralizing antibodies has been correlated with virus protection. In particular, antibodies targeting the domain III of E ZIKV protein (EDIII ZIKV), responsible for attachment of the virus to the cell, represents an important target for antibodies capable to block virus infection. Thus, the present study aimed to produce and characterize a recombinant form of EDIII ZIKV protein with antigenic and immunogenic features compatible with vaccine applications. The expression of EDIII ZIKV protein was carried out with *E. coli* BL21 (DE3) strain. As strategy to obtain the EDIII protein from insoluble fraction of the bacterial lysate, we optimized an *in vitro* refolding method using High hydrostatic pressure (HHP) associated with alkaline pH. This technique allowed the recovery of EDIII ZIKV at yields up to 27 fold higher (~111 mg/L) than conventional methodologies and the protein was obtained with high purity. In addition, the EDIII ZIKV antigenicity (reactivity against antibodies generated following infection by ZIKV) and its biological function (binding to cellular receptors) were preserved, as demonstrated by ELISA and flow cytometry. To evaluate the EDIII immunogenicity, we carried out immunizations on C57BL/6 mice using the target protein associated with different adjuvants (Alum and a recombinant derivative of the heat-labile toxin (LT) originally produced by enterotoxigenic *E. coli*). After three doses, high of EDIII-specific titers were detected in sera of immunized mice, particularly after immunization with the LT derivative. Thus, this study confirms the antigenic and immunogenic properties of the recombinant EDIII ZIKV protein produced under HHP conditions. Importantly, these results open the possibility of the use of this antigen in the development of new vaccine strategies against ZIKV.

Palavras-chaves: Zika virus, Domain III from E protein, EDIII, Vaccine, Flavivirus

RECOMBINANT EXPRESSION OF ZIKA VIRUS-LIKE PARTICLES (VLPS)

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Resumo

In recent years, arboviruses have become a major public health problem in the tropical and subtropical regions of the world. The Zika virus (ZIKV) is an arboviral disease prevalent in the Americas, Africa and Asia and has increased its area of endemicity and it is considered a major public health problem in our country. The diagnosis of the infection is made through molecular techniques and serological tests, but these may be non-specific. Due to this and to the advances of the infections caused by the ZIKV it is of extreme importance the development of tools that allow the adequate combat to the ZIKV. Virus-like particles (VLPs) vaccines appear as an enormous potential for use as extremely effective antiviral vaccines, since they mimic the viral particle, inducing immune response and, as they don't have the genetic material of the virus they won't replicate making them safe as viral particles. In this work, we established a methodology for production and characterization of VLPs containing the structural proteins C, prM and E of ZIKV produced in insect cells and using gene expression system derived from baculovirus. In order to obtain the recombinant baculovirus (BV-ZIKV), vectors containing the sequences of the proteins of interest were constructed. The bacmid was transfected into *Spodoptera frugiperda* (Sf-9) insect cells and stocks of BV-ZIKV were obtained for infection. Infection kinetics were performed to determine the multiplicity of viral infection (MOI) of the inoculum to be used to infect the cells and the best time for BV-ZIKV and VLPs production. Sf-9 cells were infected with different MOIs of BV-ZIKV and were collected at the times 24h to 144h. The best MOI for infection and the best BV-ZIKV collection time was determined by dot blot, western blot and indirect immunofluorencence techniques. The purification step to separate the VLPs from the BV-ZIKV were done by sucrose and iodixanol gradients. The work evidenced the correct expression of ZIKV proteins in this system and were observed by SDS-PAGE and western blot. The VLPs were analyzed by transmission electron microscopy. The results of this project can generate important tools in the development of a vaccine method against the ZIKV.

Financial Support: FAPESP, CAPES and Butantan Foundation

Palavras-chaves: Zika virus, Virus-like particles, Recombinant baculovirus

GENERATION OF A LIBRARY OF HYBRIDOMAS WITH ANTI-MAYARO VIRUS REACTIVITY

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Resumo

Mayaro virus (MAYV) is an *Alphavirus* present in Latin America. MAYV is transmitted by mosquitos and causes an acute febrile disease characterized by rash, retro-orbital pain and polyarthralgia. Chronic clinical manifestations are associated with incapacitating articular pain which may persist for months. There is no treatment or vaccine available against MAYV and also no specific diagnostic test for MAYV, as serological cross-reactivity to more prevalent alphaviruses takes place. By developing a hybridoma-based anti-MAYV library we expect to select a wide range of specific antibodies able to detect MAYV and potentially neutralize it. Female Balb/c mice were immunized with a MAYV strain isolated from a patient at Sao Jose do Rio Preto. Serum samples were collected 30 days p.i. and antibody titer was accessed by Indirect ELISA. All sera seroconverted after immunization. In order to obtain neutralizing or highly specific anti-MAYV monoclonal antibody (Mabs), the splenocytes from the previously immunized mice were fused with myeloma cells (SP2/0). Next, the supernatant was collected and the reactivity against MAYV was determined by indirect ELISA followed by ROC curve analysis ensuring a 100% of sensitivity and 92% of specificity. We identified 42 MAYV antibody-secreting hybridomas (Absorbance (Abs)/ml of serum ≥0,100). Of those, 76% were considered low positive (0,100 - 0,399 Abs) and 24% were considered high positive anti-MAYV (≥0,400 Abs). To characterize the Mabs isotype, the supernatant was collected purified by protein G chromatography affinity. We observed IgG1 subclass was predominantly isotype. IgG2b subclass was also detected, but in lower concentrations. In summary, the fusion of splenocytes from immunized mice with SP2 cells allowed us to create a hybridoma library in which 24% of the cells produce highly reactivity antibodies against MAYV. Further experiments are needed to characterize potential neutralizing Mabs and also, specificity against MAYV. Financial support: FAPESP/Capes

Palavras-chaves: MAYV, hybridomas, immunization, monoclonal antiboies, IgG

AN IMMUNOENZYMATIC ASSAY FOR ZIKA VIRUS INFECTION DIAGNOSIS UTILIZING IMMUNOGLOBULIN Y

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Resumo

The Zika virus (ZIKV) is an arbovirus from family Flaviviridae. Its main form of transmission is through the bite of an infected Aedes aegypti mosquito. ZIKV clinical manifestations are similar to those manifestations caused by other arboviruses, such as fever, headache, arthralgia, myalgia, and rash. Besides that, ZIKV infection is related to microcephaly in newborns and Guillain-Barré syndrome in adults. The similarities between arboviruses lead to cross-reactivity in immunoassays, hindering the differential diagnosis. Therefore, this work aimed to standardize a sandwich ELISA utilizing immunoglobulin Y (IgY), an antibody that may be found in birds serum and eggs. IgY presents several advantages when compared to IgG, such as high concentration in egg yolks, no cross-reactivity with IgG, reduction of background in immunoenzymatic assays and it can be obtained by the egg yolks harvest. Eight Isa Brown hens were selected and divided in two groups. The first group was inoculated with two doses of ZIKV by intramuscular via to produce IgY anti-ZIKV, while the second group was inoculated with PBS. Then, the eggs were collected and submitted to the purification method described by Polson. After that, the purified yolks were submitted to polyacrylamide gel electrophoresis to demonstrate the presence of IgY, and then to Western Blotting, to demonstrate the specificity of these antibodies. IgY was conjugated to peroxidase, and then several tests were done to standardize the ELISA utilizing IgY as conjugated and capture antibody. The electrophoresis showed the presence of IgY in the purified and, through the Western Blotting, was observed that these antibodies are specific to the E protein of the ZIKV. Besides that, there was specific binding between ZIKV and IgY anti-ZIKV on ELISA assay. Results showed be possible the immunization of laying hens against ZIKV and the obtaining of IgY anti-ZIKV. Furthermore, the IgY was able to bind to the ZIKV on ELISA test, demonstrating that IgY anti-ZIKV can be used to diagnose Zika infection.

Financial Support: Oswaldo Cruz Institute

Palavras-chaves: ELISA, IgY, Zika

DEVELOPMENT OF A ELISA BASED ON PEPTIDE ASSAY (ELISA-PEPTIDE) FOR SPECIFIC DETECTION OF ZIKA VIRUS: PRELIMINARY RESULTS

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Resumo

Introduction: ZIKV transmitted by *Aedes* is included in the genus Flavivirus, family *Flaviviridae*, which include other important viruses such as Dengue and Yellow Fever. In acute phase different of Dengue IgM antibodies profile antibodies against ZIKV could detect after 3 days of symptoms. Cross-reactivity in diagnostic tests is common but a major problem to address due to epidemiological importance of this viruses as well as their severity associated with ZIKV as microcephaly in newborns, Guillain Barré syndrome and subclinical infections that may go unnoticed, and even. In this work we have started the standardizing a simple, inexpensive, sensitive ZIKV-specific serological test to perform within 5 days of disease. Alternatives to NS1 protein used commonly in the literature, here we have used a peptide from domain III region selected after silico analysis. Aim: The aim was to develop a diagnostic differential ELISA-peptide assay to detect IgM antibodies against ZIKV in acute phase of infection. Methodology: The work was approved by ethical commit COEP at Federal University. For this study retrospective samples of 2017-2018 were collected and the infection by ZIKV was confirmed by RT-PCR using specific primers. To standardizing the reaction different block titration assays were used starting a range of different concentration of peptide vary from 1 to 15 µg/mL in at least two buffers (carbonate, pH 9,6 and saline buffer, pH 7,4 following the addiction of primary antibodies (MIAF – Mouse Immune ascitic fluid and human positive sera for ZIKV) and conjugate peroxidase antibodies. The analysis for this step was made in triplicate with other sera using mean plus 3 standard deviation (SD). Results. On the preliminary test the best results for these assays indicate buffer carbonate was more sensible for coat and the concentration of peptide that indicate the presence of peptide on the plate against antibodies was 15 µg/mL and the dilution of sera and MIAF was 1:100. Conclusion: The results presented here shows the initial steps for standardization of ELISA-Peptide using part of Domain III of E protein of ZIKV is promising, however additional tests using this peptide is necessary for next steps to discrimination of response to others acute phase samples for others arboviruses.

Finantial support: FAPEG, 04/2017

Palavras-chaves: vírus Zika, ELISA-Peptide, Diagnostic, Flavivirus

ANTIGENIC AND PHYSICOCHEMICAL CHARACTERIZATION OF HBSAG VLPS

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Resumo

The main component of the recombinant hepatitis B vaccine is the surface antigen (HBsAg), a protein that self-assembles into 22 nm spherical virus-like particles (VLPs) which contain lipids, derived from the host cell, and around 80-100 monomers of HBsAg in a particle. The effectiveness of this vaccine is cleared related to the maintaining of the native conformation of the main epitopes within the VLP antigen in all stages of its manufacturing process and storage. The aim of the present work was characterized the HBsAg during each step of the production and storage in order to stablished the critical quality attribute of the vaccine (CQA). HBsAg diluted in different solutions used routinely during the manufacturing process were analyzed by biophysical methods [small angle X-ray scattering (SAXS), synchrotron radiation circular dichroism (SCRC), fluorescence spectroscopies and surface plasmon resonance (SPR)] as well as *in vivo* and *in vitro* potency assays. The antigen submitted to different storage temperature (4^{o}C, room temperature and – 20^{o}C) was also studied. Data obtained from SRCD and fluorescence spectroscopies indicate that the HBsAg is a well-ordered globular protein, with a mixed α/β conformation (24% helix, 21% β-strands) in solution, preserving its Trp residues from exposure to water molecules. The native secondary and tertiary structure of the protein is high stable in a large range of pH (from 5.5 to 8.5) and at different temperatures studied. But it can change after treatment at high temperatures, especially after it´s melting point (56^{o}C), where the protein could unfold. When frozen the protein tend to form large aggregates (>200 nm) and precipitates causing a loss of antigen content measure by ELISA. Some level of aggregates and precipitation were also observed in extremes of acid pH. However, these alterations didn´t interfere in the interaction with antibodies, analyzed by SPR instrumentation, and in the in vivo assay. These results showed that the conformational structure of the protein was preserved and it is crucial for the immune response. The high stability of the protein, even at room temperature, could improve the distribution of the vaccine and consequently the efficiency of global vaccination programs.

FINANCIAL SUPPORT: FUNDAÇÃO BUTANTAN; FAPESP; CNPq

Palavras-chaves: Hepatitis B vaccine, HBsAg, pH stability, Thermal stability

NANOMULTILAMELLAR LIPID VESICLES POTENTIALIZE THE IGG ANTIBODY RESPONSES AGAINST ZIKA VIRUS NS1 PROTEIN.

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Resumo

The search for safe vaccines against Zika virus (ZIKV) infection is a worldwide priority due to its association with severe manifestations, such as Guillain-Barré Syndrome, microcephaly and congenital malformations. The lack of effective treatments and vaccines support the development of new approaches that can circumvent the occurrence of cases in endemic regions, such as Brazil. In this sense, the present work aimed to develop a vaccine formulation composed of lipid nanostructured particles (NMVs) combined with a recombinant non-structural protein 1 (ΔNS1) of ZIKV. To this end, the ΔNS1 protein was expressed in a prokaryote system with subsequent standardization of expression and purification conditions. The antigenicity of ΔNS1-ZIKV was confirmed against murine anti-ZIKV sera. The recombinant vesicles (NMVΔNS1-ZIKV) were characterize by charge, size, polydispersion, percentage of incorporated protein and release rates. The cytotoxic effects of the NMVΔNS1-ZIKV also determined under *in vitro* conditions. The immunological effects of the NMVΔNS1-ZIKV combined with monosphosphoryl lipid A adjuvant (MPL-A) were evaluate after intramuscular (i.m.) immunization of female C57/BL6 wild-type mice. The induction of antigen-specific IgG and subclasse responses was measure by ELISA. Under the tested conditions, the NMVΔNS1-ZIKV were capable to induce higher specific IgG ΔNS1-ZIKV titers when compared to protein-only administration, as well as high levels of IgG1 and IgG2c subclasses, with predominance of IgG1. Antibodies generated after the third dose were able to recognize native NS1 protein present in virus-infected VERO cells. Thus, the results obtained so far offer a basis for further evaluation of antibody functionality and induction of protective immunity to ZIKV infection under pre-clinical conditions.

FINANTIAL SUPPORT:

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Palavras-chaves: Nanoparticles, Vaccine, NS1, ZIKV, Protein

CROSS-REACTIVE NEUTRALIZING HUMAN SURVIVOR MONOCLONAL ANTIBODY BDBV223 TARGETS THE EBOLAVIRUS STALK

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Resumo

Introduction: Three viruses in the Ebolavirus genus cause lethal disease and lack targeted therapeutics: Ebola virus, Sudan virus and Bundibugyo virus. Cocktails of monoclonal antibodies (mAbs) present a potential therapeutic strategy. BDBV223 IgG, which was identified in a human survivor of Bundibugyo virus disease, neutralizes both Bundibugyo virus and Ebola virus. Here we report two crystal structures of the antibody BDBV223, alone and complexed with its epitope on the glycoprotein (GP) stalk. This site is particularly attractive for therapeutic/vaccine design due to its high sequence conservation among ebolaviruses. **Materials and Methods:** BDBV223 IgG was expressed in hybridoma cells generated from a survivor of Bundibugyo virus disease. Purified BDBV223 Fab was co-crystallized with a synthetic peptide representing residues 620–635 of the BDBV GP stalk epitope. Crystals of the BDBV223-GP2 stalk complex were diffracted at SSRL Beamline 12–2 to 3.7 Å and 2 Å resolution, respectively; phases were solved using molecular replacement. Based on structural findings, mutant BDBV223 antibodies were generated to broaden cross-reactivity. Mutants were tested for binding via ELISA and via viral neutralization assays against live filovirus in a biosafety level 4 facility. **Results and Conclusions:** The structures of the BDBV223-GP stalk complex as well as the apo-BDBV223 Fab reveal an unexpected binding orientation of the antibody. The structure suggests that BDBV223 binding may interfere with both the trimeric bundle assembly and the viral membrane. This indicates that BDBV223 likely stabilizes a conformation in which the monomers are separated by lifting or bending of the glycoprotein. Interestingly, mutagenesis of BDBV223 against SUDV indicates that additional determinants of antibody binding likely lie outside the visualized interactions, perhaps involving quaternary assembly or membrane-interacting regions. These quaternary movements are akin to dynamics seen in stalk binding antibodies of HIV and are important to consider in the context of immunogen design. These results reveal new information about the filovirus stalk and can be used to better design or elicit antibodies against this conserved epitope. **Financial Support:** The Scripps Research Institute Graduate Program, NIH F30AI136410, and NIH R01AI089498

Palavras-chaves: Antibody, Crystallography, Ebola, Filovirus, Structure

GUANOSINE IS NOT PROTECTIVE IN THE CONTEXT OF USUTU VIRUS INFECTION

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Resumo

Usutu virus (USUV) is an arbovirus capable of causing encephalitis in humans and other vertebrates. This pathogen demands greater attention due to increased detection in mosquitos and birds throughout Africa and Central Europe. The lack of specific treatments or vaccines only heightens this need. Guanosine is a neuroprotective compound which has been used in the treatment of various neuropathologies. We hypothesized that the neuroprotective properties of guanosine may be beneficial in the context of USUV infection. C57BL/6 mice (8-12 weeks old) intracranially infected with 10^1 (LD_{80}) and 10^3 PFU (LD_{100}) of USUV presented signs of neurological disease including hunched back, paralysis, and conjunctivitis. Mice succumbed to these doses of virus after 10 and 7 days, respectively. Increased viral loads and Inflammatory cytokines such as CXCL1, CCL5, IFN-Y, TNF-a were only observed in the brain, indicating USUV is neurovirulent but not viscerotropic. Guanosine treatment to C57 WT mice infected with 10^1 PFU of USUV is not protective. We therefore evaluated whether guanosine treatment would be protective *in vitro*. USUV infection at a multiplicity of infection (MOI) of 0,1 was cytotoxic to human neuroblastoma cells (SH-SY5Y), leading to 10^7 viral PFU, 48h post infection (p.i.). Treatment with guanosine in SH-SY5Y cells did not improve cell viability or interfered in the viral load. Diversely, we were not able to recover virus from induced pluripotent stem cell-*derived microglia* infected with USUV. When we treated USUV-infected microglia with guanosine, we recovered 10^4 viral units, which indicates that guanosine treatment potentiates USUV replication in these cells. This finding correlates to our previous result, that guanosine is not protective *in vivo*. Overall summary, our data indicates the intracranial infection with USUV causes encephalitis in adult wild type mice. Further, we observed that guanosine does not prevent mortality *in vivo*. In accordance, guanosine does not improve cell viability or interferes in USUV viral load in neuroblastoma cells. However, the treatment led to increased susceptibility to infection in *iPSC*-MGLCs.

Palavras-chaves: Encephalitis, Guanosine, Neuroprotection, Usutu vírus

THE MARBURGVIRUS-NEUTRALIZING HUMAN MONOCLONAL ANTIBODY MR191 TARGETS A CONSERVED SITE TO BLOCK VIRUS RECEPTOR BINDING

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Resumo

Introduction: Since its first identification 50 years ago, marburgviruses have emerged several times, with 83-90% lethality in the largest outbreaks. Although no vaccines or therapeutics are available for human use, the human IgG antibody MR191 provides complete protection in nonhuman primates when delivered several days after inoculation of a lethal marburgvirus dose. The detailed neutralization mechanism of MR191 remains poorly understood. This study analyzes the structural biology, biochemistry, and immunology behind MR191 mediated viral neutralization and protection. **Materials and Methods:** Marburgvirus glycoprotein (GP) was expressed in Drosophila S2 cells. MR191 IgG was expressed in hybridoma cells generated using B-cells collected from a Marburg virus disease survivor; IgG was subsequently cleaved to yield Fab fragments. Purified GP and MR191 Fab were complexed and subsequently crystallized by hanging drop crystallization. Crystals were diffracted at the Advanced Photon Source to a resolution of 3.2 Å, with phases solved using molecular replacement. To generate escape mutants to MR191, a chimeric vesicular stomatitis virus displaying GP was incubated with serial dilutions of MR191, and any antibody-resistant viruses were subsequently sequenced. **Results and Conclusions:** Here we present a 3.2 Å crystal structure of MR191 complexed with a trimeric marburgvirus surface glycoprotein (GP). MR191 neutralizes by occupying the conserved receptor-binding site and competing with the host receptor Niemann-Pick C1. The structure illuminates several previously disordered regions of GP including part of the critical marburgvirus-specific “wing” antibody epitope. Virus escape mutations mapped far outside the MR191 receptor-binding site footprint suggest a role for these other regions in the GP quaternary structure and antibody recognition. Fc-immune effector function assays revealed a role for monocytes in MR191 mediated phagocytosis. Finally, MR191 escape mutants were generated and provided insight into the role of unresolved regions of the protein in the protection of the receptor binding site. The combined biochemical and structural data suggest that MR191 is an appropriate first immunotherapeutic for development against marburgvirus disease. **Financial Support:** The Scripps Research Institute Graduate Program, NIH F30AI136410, and NIH R01AI089498

Palavras-chaves: Antibody, Crystallography, Ebola, Filovirus, Structure

UsE OF ECO DIAGNOSTICA® RAPID TESTS FOR THE DETECTION OF RESPIRATORY SYNCYTIAL VIRUS ECO F ADENOVIRUS AG (ADV) AS A DIAGNOSTIC TOOL FOR USE IN PEDIATRICS.

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Resumo

INTRODUCTION: Human Adenovirus (AdV) is an important pathogen responsible for many types of diseases, such as respiratory, gastrointestinal, ophthalmological, genitourinary and neurological diseases. Present, there are 79 known types of HAdVs divided into seven species (HVV-A to HAdV-G) and / or after subspecies B1, we find serotypes 3,7, 16, 21 and 50 in many cases associated with respiratory respiration. ECO Diagnostics has the ECO F Adenovirus Fluorescence Detection (FIA) kit for detecting Adenovirus infection using EUROPIO core technology that enables detection of most target analytes. OBJECTIVES: To determine the sensitivity and specificity of the ECO F Adenovirus Ag rapid test, compared with Immunofluorescence (IF), to be used as a diagnosis of AdV in hospitals and pediatric clinics. MATERIAL AND METHOD: Forty-five samples from the University Hospital of USP (HU) and Santa Casa were used in this study from children who had symptoms of respiratory diseases and were hospitalized in these places. The samples were divided into 4 groups: AdV positive (+) (20 samples); Negative (-) for AdV / + for other viruses (10); - for respiratory virus (9 samples); + for Adv / + for other viruses (6 samples). The tests were performed strictly following the instructions for use of the product, with a maximum time of 15 min and the reading was performed on the ECO F200 equipment. CONCLUSION: Of the forty-five samples tested, 4 were divergent between the ECO rapid test and the IFI, the qPCR was performed for the tiebreaker and all samples were positive for AdV, in agreement with the IFI. Thus, the ECO F Adenovirus Kit analyzed, promotes the detection of the viral target in a very specific way, presenting sensitivity of 80% and specificity of 100% when compared to IFI. KEYWORD: Adenovirus, diseases, immunofluorescence, test, virus, diagnosis.

Palavras-chaves: Adenovirus , Doenças, Imunofluorescencia, teste, virus