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DNA VACCINES ENCODING SM29 OR TSP-2 GENES AND A CHIMERA CONTAINING BOTH MOLECULES CONFER PARTIAL PROTECTION AGAINST *S. MANSONI* IN A MURINE MODEL

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Introduction: Schistosomiasis is an important parasitic disease worldwide that affects more than 207 million people in 76 countries and causes about 250.000 deaths per year. Currently, the main strategy used for the control of schistosomiasis is the use of safe chemotherapy, such as praziquantel. However, the high rate of reinfection after the treatment restricts its use and it requires other forms of control, such as basic sanitation and vaccination. Due to the ability of DNA vaccines to generate humoral and cellular immune responses, such vaccines are considered to be a promising approach against schistosomiasis. Sm29 and tetraspanin-2 (TSP-2) are two proteins located in the *S. mansoni* tegument of adult worms and schistosomula, inducing high levels of protection by recombinant protein immunization. In this study, we evaluated the DNA vaccines containing Sm29 and TSP-2 in a murine model of infection.

Methods and Results: We transfected BHK-21 cells with the plasmids encoding the genes of Sm29, TSP-2 or chimera containing both genes. By western blot and RT-PCR, we confirmed that the DNA vaccine constructs were transcribed and translated in BHK-21 cells. After immunization, we evaluated the reduction in the worm burden. We observed worm burden reduction of 17%, 22%, 31% or 24% in animals immunized with the plasmids pUMVC3/Sm29, pUMVC3/TSP-2, pUMVC3/Chimera or pUMVC3/Sm29 + pUMVC3/TSP-2, respectively. The cytokine profile produced by the spleen cells of immunized mice was then evaluated. We found higher production of Th1 cytokines such as TNF-α and IFN-γ in vaccinated mice, and no significant production of IL-4 and IL-5.

Conclusion: The DNA vaccines, tested in this study, showed the ability to generate a protective immune response against schistosomiasis, probably by

the production of Th1 cytokines. However, future strategies aiming to optimize the protective response need to be developed.

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EXPRESSION AND IMMUNOLOGICAL CHARACTERIZATION OF A RECOMBINANT *Mycoplasma hyopneumoniae* POTENTIAL VACCINE ANTIGEN

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Introduction: *Mycoplasma hyopneumoniae* is the causative agent of porcine enzootic pneumonia (PEP), a chronic respiratory disease that affects swine farms worldwide, causing significant economic losses. Current vaccines against PEP (bacterins) are relatively expensive and confer only partial protection. Therefore, the production of well-characterized recombinant antigens is a promising alternative for the development of alternative recombinant vaccines.
Methods and Results: In this work, the complete coding sequence (CDS) of a *M. hyopneumoniae* surface protein, here identified as MH1, was cloned into a pGEX-4T vector and overexpressed in *Escherichia coli* BL-21 cells. Recombinant MH1 (rMH1) was purified with yields of 5 mg per liter of *E. coli* culture. Contaminant *E. coli* lipopolysaccharides (LPS) were removed by affinity chromatography with polymyxin B. After LPS removal, rMH1 was used to immunize mice and its humoral and cellular immunogenicity is being assessed by ELISA detection of specific IgG in sera, and by cytokine secretion assays in supernatants of cultured splenocytes. The MH1 CDS was also cloned into the pcDNA 3.1(+) vector for expression of rMH1 in mammal cells. After confirmation of rMH1 expression in cultured mammal cells, the pcDNA:MH1 DNA construct will be used for initial immunogenicity assessment in mice.
Conclusion: An antigenic *M. hyopneumoniae* surface protein was expressed in *E. coli* and the produced recombinant antigen was able to induce a high titre specific IgG response in immunized mice. A DNA clone capable to express rMH1 in mammal cells was constructed and can now be tested to assess its potential as a DNA vaccine component.

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DEVELOPMENT OF A MULTI-ANTIGEN VACCINE AGAINST CANINE VISCERAL LEISHMANIASIS USING RECOMBINANT PROTEINS AND AN ADENOVIRAL VECTOR

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Introduction: Brazil is responsible for 90% of reported cases of visceral leishmaniasis (VL) in the American continent. In the epidemiological cycle of this disease, the dog may act as a domestic reservoir of *Leishmania infantum/chagasi* and it is the main responsible for the spread of VL in Brazilian's largest cities. Therefore, induction of a protective immune response in dogs should have a direct impact on the control of human visceral leishmaniasis. In this context, this work aims at the development of a new protective vaccine for dogs.

Methods and Results: We have formulated a multi-antigen vaccine made of antigens that were already described in the literature, such as histones *Li*H2A/H3 and *Li*H2B/H4 of *Leishmania*, as well as new antigens selected *in silico*. These antigens are being used as recombinant proteins or encoded by adenoviral vectors. So far, we selected two hypothetical proteins of *L. infantum/chagasi*, generically known as *prt4* and *prt5*, after they were prospecting *in silico* in the genome and proteome of the parasite, using tools of Computational Immunology. These proteins are amastigote-specific, may be surface proteins and/or secreted ones. They have a high frequency of T-cell epitopes and a low frequency of B-cell epitopes. They also have a high degree of promiscuous binding of their $CD4^+$ T-cell epitopes to MHC class II molecules. The higher frequency of T-cell epitopes in both proteins are distributed outside the regions of similarity between human and canine proteins. Furthermore, four recombinant adenoviruses able to express the selected antigens were constructed: Ad5-*Li*H2A/H3, Ad5-*Li*H2B/H4, Ad5-*prt4* and Ad5-*prt5*.

Perspectives: The next stage of the study will consist of pre-clinical and clinical vaccine trials conducted in murine and canine models of VL. The selected antigens will be administered in heterologous prime/boost protocols (protein/adenovirus) and

induced immunogenicity analysis will be performed before and after experimental infection with *L. infantum/chagasi*.

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IDENTIFICATION OF NEW ANTIGENS FROM *Rhipicephalus microplus* TICKS ASSOCIATED TO INFESTATION PHENOTYPES OF SUSCEPTIBILITY OR RESISTANCE IN BOVINE HOSTS.

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Introduction: *Rhipicephalus microplus* cattle tick causes great economic losses to livestock. Current vaccines have partial and transient effects against infestations. To develop effective anti-tick vaccines, new targets must be identified. Bovines express breed-specific, heritable, contrasting phenotypes during infestation. Composition of tick saliva proteins may be affected by these different levels of host immunity and may be crucial to hematophagy, i.e., potential antigens. **Methods and Results:** With DIGE, MudPIT and 454-based RNA-Seq we investigated the protein expression profile of salivary glands from nymphs (NSG), males (MSG) and females (FSG) from *R. microplus* ticks fed on

resistant or susceptible bovines as well as unfed larvae (UFL) from eggs of females fed on these hosts. We identified 321 different proteins: 68 in samples derived from the ticks fed on susceptible hosts, 17 only on resistant and 236 shared by both groups. DIGE results showed 20 differentially expressed proteins in UFL, 27 in NSG and 35 in FSG. In addition, the sialotranscriptome revealed 11,676 coding sequences (CDS), with 3,590 CDS for putative secreted proteins. Many differentially expressed proteins in the global analyses show similarity with proteases, nucleases, protease inhibitors, antimicrobial peptides and pathogen recognition proteins among others and are associated with the immunity raised in susceptible or resistant hosts. **Conclusion:** This study represents the first attempt to identify protein profiles in developmental stages of *R. microplus* ticks that are affected by the different host immune responses developed by susceptible or resistant bovines. It offers an opportunity to identify new protective antigens for an effective anti-tick vaccine.

Key words: *Rhipicephalus microplus*; saliva; salivary glands; MudPIT; DIGE; RNA-Seq; potential antigens.

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EVALUATION OF IMMUNE RESPONSE TO RECOMBINANT ANTIGENS OF *Mycoplasma hyopneumoniae* WITH POTENTIAL FOR USE IN VACCINATION AGAINST PORCINE ENZOOTIC PNEUMONIA

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Introduction: *Mycoplasma hyopneumoniae* is the etiological agent of porcine enzootic pneumonia (PEP). The vaccines utilized against PEP present a high final cost and partial protection. The development of recombinant vaccines arises as a promising alternative for the control of PEP.
Methods and Results: To provide novel antigenic proteins with vaccinal potential, three *M. hyopneumoniae* gene products were selected for evaluation based on previous antigenicity demonstration in proteomic studies and *in silico* prediction of extracellular domains and epitopes. Portions of the coding sequences (CDSs) corresponding to extracellular domains and devoid of TGA stop codons (that code for Trp in *M. hyopneumoniae*) were cloned and expressed in *Escherichia coli*. The recombinant polypeptides (named rMH2, rMH3 and rMH4) were produced with yields of 7, 10 and 3 mg/L of culture, respectively. BALB/c mice were initially immunized with rMH2 and rMH3, and the humoral and cellular immune responses were evaluated by indirect and capture ELISA, respectively. The presence of significant specific antibodies in the sera was observed in both experimental groups, showing that the proteins were immunogenic for mice. The rMH2 and rMH3 induced also strong Th1-polarized immune responses. The rMH4 is still being assessed. The MH2, MH3 and MH4 CDSs were also cloned into an eukaryotic expression vector for immunization assays with DNA. The *in vitro* expression of rMH2 and rMH3 was confirmed in mammal cells. The immunization assays with DNA constructs of the recombinant antigens MH2 and MH3 are being done.
Conclusion: The rMH2 and rMH3 of *M. hyopneumoniae* were expressed in *E. coli* and the produced antigenic proteins were able to induce a high titre specific IgG response, and preferentially a strong Th1 immune response that can be beneficial to the host. The purified antigens or DNA constructs presenting the best results in mice will be later used for the immunization of pigs and further evaluation of their potential for use in vaccination against PEP.

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CLONING AND EXPRESSION OF *LEISHMANIA (L.) INFANTUM* VISCERALIZATION-RELATED GENES FOR THE DEVELOPMENT OF RECOMBINANT AND LIVE VACCINES

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Introduction: Several strategies of vaccination have been tested against leishmaniasis, including the use of avirulent organisms. Among the different species of *Leishmania*, *Leishmania (L.) tarentolae* is a parasite that is not pathogenic to humans. It can differentiate to amastigotes, but is unable to persist in macrophages for long time. The A2 gene, first described in *L. (L.) donovani*, is amastigote-specific and it plays an important role in virulence and ability to visceralization of *Leishmania* species. Recent studies have shown that *L. (L.) tarentolae* expressing A2 protein had an increased infectivity and survival in *in vitro* and *in vivo* experiments. Furthermore *L. (L.) tarentolae* expressing A2 was able to protect BALB/c mice against challenge with *L. (L.) infantum* through increased production of IFN-γ before and after challenge. Novel genes of *L. donovani* have been associated with visceralization after transfection into *L. major*, and may contribute to the development of vaccines. However, studies assessing the importance of these genes as virulence genes and/or in the generation of protective immune responses have not been performed. In other to do that, our work describes the cloning and expression of genes of *L. (L.) infantum* associated to visceralization and virulence of parasites in *L. (L.) tarentolae* and *E. coli*. **Methods and Results:** The genes Li40 and Fc were amplified by PCR from genomic DNA of *L. (L.) infantum*. The PCR product containing proper restriction sites was ligated into pGEM, and then transformed into XL1-blue competent bacteria. After selection on LB-Amp with Xgal/IPTG, colonies were evaluated by PCR. The insert was removed from pGEM by digestion with restriction enzymes and religated in the expression vector pLEXSYneo2 (parasite expression) or pET15b (bacteria expression). Cloning was confirmed by PCR amplification for each gene, as well as performing sequencing of the miniprep product. The expression of the proteins in recombinant bacteria was already confirmed by Western blot assays and now they are being purified for *in vivo* assays. We also electroporated the pLEXSYneo2/insert into *L. (L.) tarentolae* to obtain recombinant parasites. **Conclusion:** We have produced recombinant proteins and parasites that will be used as vaccine constituents in the development of vaccines against Leishmaniasis.

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THE APPLICABILITY OF SBA–15 SILICA AS AN ADJUVANT FOR THE DEVELOPMENT OF POLYVALENT VACCINE ADMINISTERED BY THE ORAL ROUTE

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Introduction and aim: The ordered nanostructurated silica SBA-15 is a polymer that due to physical and structural properties displays application potential for design of therapeutic and preventive nanovaccines and for development of oral vaccines delivery systems [International Patent WO 07/030901]. The goal of this study was to assess the potential of SBA-15 to carry, protect and to improve systemic and mucosal immune responses of a polyvalent vaccine consisting of recombinant S antigen of HBV (HBsAg – Butang® – Butantan Institute) and Intimin–β (Int–β), a protein expressed on the enteropathogenic *Escherichia coli* (EPEC) surface. **Methods and Results:** Outbreed NIH mice were immunized by the oral route on days 0, 30 and 60 with 5 µg/animal of HBsAg + 5 µg/animal of Int–β encapsulated/adsorbed or not in SBA-15 at a ratio 1:10 antigens:SBA–15, v/v, in a final volume of 0.25 mL. Other groups of mice were immunized by the intramuscular route with 0.5 µg/animal of HBsAg + 5 µg/animal of Int–β encapsulated/adsorbed in SBA-15 (1:10) or adsorbed in $Al(OH)_3$ (1:20) in a final volume of 0.25 mL; for both routes, control groups received HBsAg + Int–β in PBS. Serum and fecal samples were obtained at different periods after immunizations for specific antibodies titration by ELISA. SBA–15 orally immunized mice produced anti–HBsAg and anti–Int–β IgG and s–IgA levels higher than control group ($p<0.001$). In contrast, there was no difference in the anti–HBsAg and anti–Int–β IgG antibody response elicited by the intramuscular immunization. In both routes, mice immunized with SBA–15 polyvalent vaccine produced similar anti–

Int–β IgG levels; however, intramuscular vaccination induced anti–HBsAg IgG antibodies higher than oral route ($p<0.001$ for control group; $p<0.05$ for SBA–15 group). **Conclusion:** These results agree with the SBA-15 ability to induce successful immunity against combined antigens and seem to prevent the antigen degradation by acid aggression when administered by the oral path. Thus, this new adjuvant may be useful for polyvalent vaccine orally delivery, being able to improve responsiveness in a safety and effective behavior.

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A *BRUCELLA* PROTEASE INHIBITOR IS A USEFULL ADJUVANT IN ORAL VACCINE FORMULATIONS.

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Introduction: Previous results showed that a *Brucella* protein (BP) administered by the oral route and without adjuvants induced protection against oral *Brucella* challenge. These results indicated that BP had self-adjuvant properties and prompted us to study its potential application as adjuvant for other antigens (Ags).
Methods and Results: Our results indicated that BP is a broad spectrum protease inhibitor with a stronger activity against serine proteases secreted by the pancreas to the intestine (elastase, trypsine and alpha chymotrypsin; $p<0.05$, $n=5$). As BP is able to inhibit *in vitro* the activity of many proteases present at the gastrointestinal tract we directed all our efforts to study its potential application in oral vaccine delivery. We showed that in BALB/c mice ($n=5$) oral delivery of OVA in the presence of BP resulted in a significantly increased OVA-specific T cell response (DTH assay; $p<0.05$, $n=5$) and OVA-specific IFN-γ producing CD4+ and CD8+ T cells determined by flow cytometry at the mucosal (MLNs; $p<0.05$, $n=5$) and systemic (spleen; $p<0.05$, $n=5$) level.
The natural route of infection and the need for a Th1-biased response, makes *Salmonella* Typhimurium a strong candidate for oral vaccination with the novel adjuvant BP. Therefore, we fed BALB/c mice ($n=5$) with i) Heat-killed *Salmonella* (HKS), ii) HKS+BP or iii) HKS+CTB and challenged them later with virulent *S*. Typhimurium by the oral route. We also evaluated anti-HKS specific IgA in feces and IgG in serum of immunized animals by ELISA and found that BP induces an increase in HKS-specific antibody production.
Conclusion: Altogether, our results suggest that BP would be an ideal mucosal adjuvant. When co-delivered orally it can bypass the harsh environment of the gastrointestinal tract inhibiting proteases. Besides it can enhance Th1 and CD8+ T Ag-specific immune response and would be a useful adjuvant in oral killed vaccine formulations against *Salmonella*.

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A BRUCELLA MEMBRANE PROTEIN IMPROVES THE INMUNOGENICITY OF THE CO-ADMINISTERED ANTIGEN BY INCREASING ITS HALF LIFE IN ENDOSOMAL COMPARTMENTS.

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Introduction: In previous studies we have shown that a protein from *Brucella* spp. (Bp) co-administered as adjuvant increases antigen (Ag) cross-presentation by dendritic cells (DCs) and induces IFN-g producing $CD8^+$ and cytotoxic T cells. We also found that this protein has significant sequence homology with protease inhibitors from other bacteria, therefore we hypothesized that it would inhibit lysosomal proteases activities in endosomal compartments increasing the half life of the co-administered Ag thus increasing its immunogenicity.

Methods and results: We analyzed the biological activity of Bp against cysteine and aspartyl proteases (cathepsins) that constitute a subset of endocytic proteases using a fluorometric assay kit (Enzcheck) which contains casein as substrate. We found that Bp inhibited partially the activity of cathepsin D, L, C, and B *(p<0.05).* Next, we examined whether Bp would limit the different lysosomal proteolytic capacities of APCs. Bp partially inhibited (30%) the proteolytic activity of crude lysosomal extracts derived from macrophages and DCs. To examine if Bp affects Ag proteolysis in the context of whole cell, purified DCs and macrophages from spleen of mice were incubated *in vitro* with the fluorescent Ag (OVADQ or CASEINBODIPY) and then its intracellular degradation was analyzed by flow cytometry. We have found that Bp limited Ag proteolysis inside DCs or macrophages *in vitro (p<0.05)***.** Moreover, Bp was able to inhibit Ag degradation by APCs from spleen and lymph nodes after subcutaneous immunization with the Ag *(p<0.05, n=5)***.** Finally, we analyzed if DCs incubated with OVA in presence of Bp were able to induce a sustained cross-presentation of OVA in the context of MHCI using an antibody that recognizes OVA257-264 (SIINFEKL) peptide bound to H-2Kb molecules. DCs pulsed with OVA plus Bp facilitates a greater and maintained cross-presentation of OVA along the time compared with DCs incubated with OVA alone *(p<0.05)***.**

Conclusion: These results suggest that Bp increases the half life of the Ag inside the endosomal compartments of APCs by limiting its proteolysis. A controlled degradation of the Ag would promote a sustained cross presentation of the Ag, thus increasing its immunogenicity.

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PRELIMINARY STUDIES OF A *SCHISTOSOMA MANSONI* Y-BOX PROTEIN AS VACCINE AGAINST SCHISTOSOMIASIS

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Introduction: Organisms that have life cycles with alternating morphological and physiological characteristics in response to different environments, such as *Schistosoma mansoni*, use currently regulatory proteins during their development. The studies of gene regulation in *S. mansoni* can reveal essential aspects for the development of new intervention strategies against schistosomiasis. In this context, the Y-box proteins constitute a family of multifunctional regulators of gene expression by participating in a variety of cellular functions, including transcriptional and translational regulation, induction of DNA repair system, cell proliferation, drug resistance and responses to stress. Studies with SMYB-1, a protein *S. mansoni* belonging to the family of Y-box proteins, showed that the protein is capable of binding double or single-stranded DNA. Furthermore, the protein is capable of binding RNA and interacting with other regulatory proteins of gene transcription. In this context, this preliminary study aimed to characterize the immunological and parasitological response induced by recombinant SMYB1 (rSMYB1) in experimental schistosomiasis.

Methods and results: The protein has been expressed fused to histidine tail in a heterologous system (pET28a/*E. coli* BL21) and purified by affinity column. To characterize the immune response, C57BL/6 mice were immunized with 25µg of rSMYB1/Freund (immune group) or Tris-NaCl/Freund (placebo group). The specific antibody was evaluated in the animal serum by ELISA. Immunization with rSMYB1 induced the production of significant total IgG and IgG1, but not IgG2a. To characterize the parasitological response of animals immunized with

rSMYB1 after the 3rd dose, both groups were challenged with 100 cercariae of *S. mansoni* (percutaneous). Six weeks following infection, animals were sacrificed, and with respect to the recovery of adult worms in the hepatic portal system, we observed 26% reduction in the number of adult worms of the immunized group compared to placebo. The number of eggs retained in the liver was 28% lower in immune group.

Conclusion: These results demonstrate rSMYB1 is an immunogenic protein, which stimulates a Th2 humoral response in mice with decreased worm burden and egg retention in the liver of animals. Complementary studies have been made to evaluate the involvement of SMYB1 in pathologic processes of disease and antigenic response in sera of patients infected with schistosomiasis.

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DEVELOPMENT OF RECOMBINANT BACILLUS CALMETTE GUÉRIN (BCG) EXPRESSING *ESCHERICHIA COLI BFP*A AND *ESCHERICHIA COLI INTIMIN* VIRULENCE GENE.

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Introduction: Enteropathogenic *Escherichia coli* (EPEC) isolates are a leading cause of diarrhea, especially among infants in the developing world. The virulence genes bfpA and intimin are responsible for primary adhesion and intimate adherence of these bacteria to the enterocyte membranes, respectively.

Methods and Results: *The encoding DNA gene for BfpA and Intimin were amplified by PCR and cloned into expression vectors resulting in the constructs pMH-bfpA and pMH-intimin. Expression and secretion of recombinant BfpA and Intimin were observed in M. Smegmatis and BCG, as revealed by western.* Groups of female 4-6 week-old BALB/c mice received orally 6 x10^7 CFU in a final volume of 0.2 mL PBS. Boosters were administered 15 and 30 days after first immunization. Serum and fecal samples were collected for examining antibodies levels and spleen, plate peyer and mesenteric lymph nodes were colleted for cells and performed flow citometry.

Conclusion: Our results suggests that the BfpA and intimin fragment may be good candidates for the composition of a vaccine formulation with a broader coverage among *E. coli* expressing different virulence factors.

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Recombinant BCG-LTA-K63 induced a Th1 immune response in mice

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Introduction: *Mycobacterium bovis* BCG (Bacille Calmette-Guérin) has been widely used as a live anti-tuberculosis vaccine, administered to over 3 billion individuals with a very good safety record, despite showing highly variable efficacy (0-80%) in different trials. The efficacy of BCG in adults is poor in tropical and subtropical regions. BCG is considered a high potential candidate for the presentation of heterologous antigens in the development of new vaccines, since recombinant BCG (rBCG) based vaccines would carry many of the advantages of BCG. The induction of long lasting cellular and/or humoral immune responses with one dose, which can be administered at birth, could greatly increase vaccine coverage. Adjuvant properties were described for the heat-labile toxin (LT) from *Escherichia coli*. The LT is composed of two subunits, a monomeric enzymatically active A and nontoxic B subunit. In the present work, we used a rBCG expressing the mutant of nontoxic subunit A (rBCG-LTA) and analyzed the cellular immune response in BALB/c mice. **Methods and Results:** BALB/c mice were immunized subcutaneously with 10^6 cfu of BCG, rBCG-S1PT or rBCG-LTA. After 60 days, the spleen was removed and the cells were restimulated in culture with PDS. After incubation, the cells were collected for intracellular cytokine staining with FITC-, PE-, or PE-Cy7-conjugated monoclonal antibodies against: CD4, CD8a, IFNg, IL-4, IL-17, TNF-a and IL-2. The cytokine secretion was also evaluated in the supernatant of spleen cell culture. A proportion of CD4 T cells produced IFN-g and TNF-a (47.13% and 34.6%, respectively) was significantly higher in spleen cells of mice immunized with rBCG-LTA than in BCG (21.4% and 18.9%), rBCG-S1PT (17% and 15%) or saline controls (13% and 8.4). IFN-g production was significantly higher in rBCG-LTA cells (1231+/- 119.8 pg/mL) when compared with the other groups; BCG (862.2+/- 258.5 pg/mL), rBCG-S1PT (1084.3+/- 92.2 pg/mL). IL-17, IL-2 showed a similar concentration in immunized and control groups and IL-4 was not detected. **Conclusions:** We demonstrated that immunization with rBCG-LTA can induce a Th1 response characterized by increased production of IFN-g and TNF-a and these responses are significantly higher than BCG and rBCG-S1PT.

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PRODUCTION AND CHARACTERIZATION OF POLYMERIC NANOCAPSULES WITH CD8 T CELL EPITOPE FROM HERPES SIMPLEX VIRUS-1 (SSIEFARL)

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Introduction: Even with the numerous studies to develop a vaccine against *Herpes Simplex Virus*-1 (HSV-1) we still lack an efficient system to prevents the infection. The main immune response to HSV-1 is mediated by cytotoxic T cells, then for vaccines designs this response should be priorized. Actually the primary and memory $CD8^+$ T cell response to HSV-1 is to immunodominat epitope from the glycoprotein B 495-502 (SSIEFARL) in neonatal, adult and old C57BL/6 mice. After infection with HSV-1 in the Ag-specific TCR repertoire more than 90% of $CD8^+$ T cells are specific to SSIEFARL. However the response only reaches that magnitude when infection or immunization with heat killed virus is used. Polymeric nanocapsules (PC) are nanostructured systems protect molecules from degradation in vivo and improve the biodistribution of molecules. The aim of this study is the construction of nanocapsules with aquous core to delivery the HSV-1 SSIEFARL peptide and also tests the immunogenicity of this construction as a vaccine model to HSV-1.

Methods and Results: Polymeric nanocapsules were produced by interfacial polymerization with the monomer Methyl Metacrylate (MMA), Miglyol 812 and Span 80. Briefly, water solution of SSIEFARL peptide was added to an organic fase containing Miglyol and Span-80 under vigorous stirring. The monomer was added slowly to the emulsion which remained under mechanical stirring at 500rpm for 4h. The physic-chemical characterization was done though pH, size, zeta-potential, and infrared. The biocompatibility of PC was determined through MTT assay using splenocytes, lymph node cells and bone marrow cells culture with different quantities of PC (3µL, 6µL and 15µL). The pH of PC was 6.85, the median size was 250nm and the zeta-potential was -28.5mV. The infrared analysis showed that the polymer was formed. When cells were treated with 3 µL of PC the proliferation was increased in all groups (spleen 0,963; Lymph node0,642, bone marrow 0,443; untreated 0,347). The proliferation was the same as the control, when the quantity was increased to 6µL (spleen 0,489; lymph node 0,456, bone marrow 0,364). However the proliferation in all groups of cells decreased when the dose used was 15µL (spleen 0,208; lymph node 0,163; bone marrow 0,128).

Conclusion and Perspectives: The preliminary results indicate that a nanometric structure was obtained and the chemical constitution is polymeric.

Also the pH is compatible with biological system and the zeta-potential is distant from zero, which indicates that the PC is stable. By one hand high doses of the PC are toxic for cells, by the other hand low doses seems to be proliferative. The next steps will be the analysis of the PC by Scanned Electronic Microscopy and the releasing profile of SSIFERAL. PC loaded with SSEFARL-FITC will constructed and used to determine the level of phagocytosis by dendritic cells.

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STUDIES OF VACCINES IN DENGUE-3 MURINE MODEL.

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Dengue is currently one of the most important arboviral diseases that affects humans and constitutes a serious world public health problem, especially in tropical and subtropical regions. The disease is caused by one of four serotypes of dengue virus (DENV) and is transmitted by mosquitoes of the genus *Aedes*. Dengue infections may cause a febrile illness, the classical dengue fever (DF), which may progress to more serious complications of the disease, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) that can lead to death. Several dengue vaccines are in development, including attenuated vaccines, inactivated vaccines, subunit vaccines, chimeric vaccines and DNA vaccines, but currently no vaccine is available. The lack of an animal model for dengue has been an obstacle in the development of vaccines. Our group recently developed an animal model of dengue that mimics many aspects of human disease. In this work, we tested different vaccine candidates in the model of infection for serotype dengue-3 virus adapted to the murine model. For this, BALB / c mice were vaccinated with a DNA vaccine (pVAC3DEN3), an inactivated vaccine DENV-3(INAT3) and an attenuated vaccine (VDV3). After infection of vaccinated animals with the DENV-3, we investigated the protective effects by analyzing the signs of the disease: thrombocytopenia, measurement of hepatic transaminases, increased influx of neutrophils, production of inflammatory mediators, viral load and lethality. Also, we analyze the production of antibodies against DENV-3 after immunization and cellular immune response to antigen *in vitro*. After infection, the vaccine PVAC3DEN3 was not able to generate a satisfactory humoral and cellular immune response and therefore, failed to protect animals. The vaccine INAT 3 was most effective in inducing a response to infection with DENV-3, with of protection of lethality in animals and the generation of humoral and cellular responses against DENV-3. The vaccine

VDV3, despite the poor protection in the model, demonstrated the importance of the number of doses of a vaccine to generate more effective immune response. When analyzed the levels of antibody production, the protocol of three vaccine doses showed a significant increase when compared to two doses. Strategies that enhance immunization vaccines such as the use of adjuvants and different vaccine schedules can result in better protection of these vaccines.

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A GENETIC AND PATHOLOGIC STUDY OF A DENV2 CLINICAL ISOLATE CAPABLE OF INDUCING ENCEPHALITIS AND HEMATOLOGICAL DISTURBANCES IN IMMUNE COMPETENT MICE

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Introduction: Dengue virus (DENV) is the causative agent of dengue fever, a mosquito-borne illness endemic to tropical and subtropical regions. There is currently no effective drug or vaccine formulation for the prevention of the disease. Two experimental models are available for the study of DENV pathogenicity and for the evaluation of potential vaccine candidates. The first approach is based on non-human primates, which do not develop symptoms but a transient viremia, while in the second one, mouse-adapted virus strains or immune compromised mouse lineages are used. The aim of this study was to develop an infection model based on a DENV clinical isolate and an immune competent mouse lineage. **Methods and Results:** A DENV strain, named JHA1, isolated from a hospitalized patient serum, was shown to be naturally able to infect and kill Balb/c mice and to reproduce some of the symptoms observed in DENV-infected humans. Genetic analysis revealed that JHA1 belongs to the American genotype group and carries polymorphisms previously associated with neurovirulence in mouse-adapted virus strains. The JHA1 strain was lethal to mice following intracranial inoculation with a reduced LD_{50} (approximately 50 PFU). Mice infected with JHA1 strain lost weight and exhibited general tissue damage and hematological disturbances, with similarity to those symptoms observed in infected humans. Moreover, JHA1 strain was demonstrated to share immunological determinants with the DENV2 NGC reference strain, as evaluated by cross-reactivity of anti-envelope glycoprotein

(domain III) antibodies. **Conclusion:** The results indicate that JHA1 isolate may be a useful tool in the study of DENV pathogenicity and may help the evaluation of anti-DENV vaccine formulations as well as potential therapeutic approaches.

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STUDY OF THE SCHISTOSOMA MANSONI PROTEIN ANTIGENS RECOGNITION PROFILE BY THE SERUM FROM INDIVIDUALS OF A SCHISTOSOMIASIS ENDEMIC AREA

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Introduction: Schistosomiasis is one of the most prevalent parasitic diseases in the world, caused by parasites of genus Schistosoma. Even with the attempts to control the disease and the introduction of the treatment with the Praziquantel drug in 1980, the disease persists. Although the chemotherapy has an effect on morbidity of the disease, it does not prevent the reinfection, especially of those people who live in endemic areas. In this way, the development of one long lasting protection, based in vaccine therapy, would be a great benefit for the disease control. Although some antigens potentially candidate to the schistosomiasis vaccine have been suggested, none have shown an effective protection level so far. Among other evidences, the high protection level achieved by the vaccination with irradiated cercariae and the existence of non infected individuals in endemic areas suggest that it is possible to develop a vaccine against schistosomiasis. In this study we intend to select new Schistosoma mansoni (S. mansoni) vaccine candidate antigens by two-dimensional Western-blot (2D-WB) and test them in mice immunization/challenge assays using recombinant proteins and DNA vaccination strategies.

Methods and Results: In attempt to identify new immunoreactive proteins we associated the separation of the adult worms S. mansoni proteins by two-dimensional electrophoresis to the Western-blot methodology using serum from individuals of a schistosomiasis endemic area, as approved by the CPqRR – FIOCRUZ Research Ethics Committee (CAAE: 1.0.245.000-08). We selected the protein SmPM, which was recognized by the pool of serum from infected and non infected individuals of endemic area. We amplified the coding region of the corresponding gene by RT-PCR and the fragment was inserted into a vector for gene expression in mammalian cells, pcDNA 3.1 V5/HIS B, containing the C-terminal 6xHis tag. The protein expression was certified in cultured HEK 293T cells by Western-blot using 6xHis antibody. Another similar DNA construction,

without the 6xHis tag, will be used in DNA immunization of mice. We also amplified the coding region of the same gene to be inserted into a bacterial expression vector, pQE-30, for production and purification of the N-terminal 6xHis tag SmPM fused recombinant protein.

Conclusion: The 2D-WB strategy has allowed us to identify different S. mansoni antigens and to select the SmPM to be tested in mice immunization/challenge assays.

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CO-ADMINISTRATION OF A PLASMID ENCODING IL-2 TO A DNA VACCINE BASED ON HPV-16 E7 PROTEIN ENHANCES CD8+ T CELL RESPONSES AND ANTI-TUMOR EFFECTS IN MICE

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Introduction: Cervical cancer is the second most lethal cancer among women and all cases are associated with high-risk Human papillomavirus (HPV) persistent infections. Cellular malignization is attributed to E6 and E7 HPV oncoproteins, which represent ideal targets to the development of immunotherapeutic strategies against HPV-induced tumors. In this regard, our group has developed a cervical cancer DNA therapeutic vaccine encoding HPV-16 E7 protein genetically fused to the glycoprotein D (gD) of herpes simplex virus type 1 (HSV-1). Previous results showed that the vaccine encoding HPV-16 E7 fused to gD (pgDE7), when administered in a four dose vaccine regimen (100µg of DNA/dose) intramuscularly, induced significant activation of E7-specific $CD8^+$ T cells and 40% of therapeutic anti-tumor effect in mice. **Methods and Results**: As an attempt to enhance the observed therapeutic anti-tumor effects, we tested the co-administration of plasmid encoding IL-2 (pIL-2) to the DNA vaccine administered in saline solution by the intramuscular route. Immune responses and tumor protection monitoring were evaluated in immunized mice previously challenged with tumor cells expressing HPV-16 E6 and E7 proteins (TC-1 cells). Co-administration of plasmid encoding IL-2 to pgDE7 DNA vaccine increased the therapeutic protection to 100% after a single dose administration and augmented the IFN-γ production and cytotoxic effector function of $CD8^+$ T cells. Also, IL-2 expression enhanced the number of total and E7-specific $CD8^+$ T migrating to the tumor site. Moreover, IL-2 concomitant expression with the protein encoded by the DNA vaccine did not enhance the activation of regulatory T cells or myeloid derived suppressor cells in tumor bearing mice. **Conclusion:** In summary, the data presented in this study describes the development of a new and potent therapeutic vaccine against HPV-16-associated tumors and encourages its further evaluation in clinical trials.

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PROTECTION AGAINST THE TOXIC EFFECTS OF LOXOSCELES INTERMEDIA SPIDER VENOM ELICITED BY MIMOTOPE PEPTIDES.

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Introduction: The venom of Loxosceles intermedia (Li) spiders is responsible for cutaneous lesions and other clinical manifestations. We previously reported that the monoclonal antibody LimAb7 can neutralize the der-monecrotic activity of crude Li venom. In this study, we observed that this antibody recognizes several proteins from the venom dermonecrotic fraction (DNF), including LiD1. Identifying the epitope of such a neutralizing antibody could help designing immunogens for producing therapeutic sera or vaccination approaches.
Methods and Results: To this aim, two sets of 25- and 15-mer overlapping peptides that cover the complete amino acid sequence of LiD1 were synthesized using the SPOT technique. None of them was recognized by LimAb7, suggesting that the epitope is discontinuous. Then, the screening of four peptide phage-display libraries yielded four possible epitope mimics that, however, did not show any obvious similarity with the LiD1 sequence. These mimotopes, together with a 3D model of LiD1, were used to predict with the MIMOP bioinformatic tool the putative epitope region (residues C197, Y224, W225, T226, D228, K229, R230, T232 and Y248 of LiD1) recognized by LimAb7. This analysis and the results of alanine-scanning experiments

highlighted a few residues (such as W225 and D228) that are found in the active site of different SMases D and that may be important for LiD1 enzymatic activity. Finally, the only mimotope NCNKNDHLFACW that interacts with LimAb7 by SPOT and its analog NSNKNDHLFASW were used as immunogens in rabbits.

Conclusion: The resulting antibodies could neutralize some of the biological effects induced by crude Li venom, demonstrating a mimotope-induced protection against L. intermedia venom.

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ADJUVANT PROPERTIES OF SBA-15 SILICA

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Introduction: Amorphous silicon oxide particles formed of hexagonally ordered nanopores known as SBA-15 are promising adjuvant vectors and the aim of this study is to explore how they might act in promoting immune responses.
Methods and Results: We first confirmed that subcutaneous immunisation of mice with ovalbumin (OVA) + SBA-15 induced better primary and secondary serum IgG responses than when OVA was used alone. To explore the ability of SBA-15 to activate DC, bone marrow derived DC from C57BL/6 mice were pulsed for 6 hours with different doses (50, 250, 500, 1000 and 5000 µg/mL) of the particles and the expression of MHCII, CD40, CD80 and CD86 and IL-6 levels were assessed by flow cytometry. SBA-15 had no effects on these parameters and did not alter the ability of DC to induce proliferation and γIFN production by TcR transgenic $CD4^+$ and $CD8^+$ T cells after presentation of ovalbumin (OVA) or OVA peptides *in vitro*. To analyse T cell responses *in vivo*, C57BL/6 mice were transferred with CFSE labelled $CD8^+$ OT1 or $CD4^+$ OT2 T cells. One day after transfers, the recipients were immunised footpad (s.c.) with 10 µg OVA or 10 µg OVA: 250 µg SBA-15 and the clonal expansion of donor cells was assessed 4 days later in the popliteal lymph node by flow cytometry. There were no significant differences in the T cell response when SBA-15 was used as adjuvant and SBA-15 also did not allow OVA to induce specific cytotoxic T lymphocyte activity *in vivo*.
Conclusion: These preliminary data confirm that SBA-15 acts as an adjuvant for antibody responses and suggest that its effects may reflect enhanced availability of antigen, rather than direct effects on antigen presenting cells such as DC.

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Prime-boost vaccination protocol using recombinants influenza and adenovirus encoding the amastigote surface protein-2 induces protective immunity against experimental *Trypanosoma cruzi* infection

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Abstract

Introduction: Despite the great advances on medical sciences over the last century, infectious diseases still as a major health problem, especially in developing countries. There is no human vaccine for Chagas' disease, and its treatment is restricted to acute phase, that has almost non specific symptoms, making difficult to treat promptly. Thus studying the development of an optimum vaccination protocol is an interesting research subject. ASP2 protein from *T. cruzi* is a conserved immunogenic protein that reaches high protection levels in susceptible mice by specific $CD8^+$T cells stimulation being an interesting candidate for vaccine antigen. Recombinant viruses emerge as promising tools for testing heterologous prime-boost vaccination protocols. **Methodology and Results:** In the present study, we generated and evaluated recombinant influenza viruses carrying sequences coding for polypeptides corresponding to medial and carboxi-terminal portions of *Trypanosoma cruzi*´s ASP2. Those recombinant viruses were used in vaccination of C57BL/6 and C3H/He mice in sequential immunization with recombinant adenovirus encoding the complete sequence of ASP2 (Ad-ASP2; heterologous prime-boost protocol). This immunization protocol was able to elicit specific anti-ASP2 cellular immune response in vaccinated animals, which was comparable to that observed in mice immunized twice with Ad-ASP2 and more robust than that observed in mice that were immunized once Ad-ASP2. Also, on susceptible mice strain C3H/He, a higher degree of protection could be found in mice primed with

recombinant influenza virus and boosted with Ad-ASP2. By intracellular staining of CD8+ T cells obtained from spleen of vaccinated C3H/He mice, we observed that heterologous prime-boost immunization protocol elicited more CD8+ T cells specific for the immunodominant epitope as well as higher number of specific $CD8^+$ T TNF-α and IFN-γ producers and displaying surface CD107a. **Conclusions:** Overall, these results demonstrated the usefulness of recombinant influenza viruses in immunization protocols against Chagas Disease, Moreover, our results suggest that immunodominant specific CD8+ T cells elicited after immunization could be directly related to degree of protection achieved by different immunization protocols using different viral vectors.

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SCHISTOSOMA MANSONI AQUAPORIN AS A POTENTIAL VACCINE TARGET

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Introduction: The *Schistosoma mansoni* aquaporin (SmAQP) is one small tegumental membrane protein that transports water and other small solute like glycerol. Besides being expressed in all developmental stages of the parasite, this protein is of crucial importance for osmoregulation and drug uptake. Previous studies have shown that SmAQP is vital for schistosome survival, since the inability to control water movement impairs the parasite biochemistry leading to increased mortality. The present study aims to predict the SmQP epitopes using bioinformatics tools and to design a chimeric soluble protein containing the main epitopes for murine immunization tests. **Methods and Results:** Using the online program BepiPred 1.0 we performed the prediction of linear epitopes and found ten potential epitopes in the native structure of SmAQP. Nine of them are located in the protein loops and only one is inside the transmembrane domain. So, we designed a chimeric protein using only the protein loops fused with a histidine tag, and cloned it in a plasmid for its expression in *Escherichia coli*. This chimera is predicted to be a soluble 18kDa protein and bioinformatics analysis showed that seven of the original epitopes were maintained. We were able to express this protein in bacteria and to purify it with niquel column chromatography. Mice immunized with three doses of the purified protein in Freund adjuvant generated a Th1 immune response, leading to high levels of anti-aquaporin IgG2a antibodies and increased amounts of cytokines TNF-α and IFN-ɤ. By western blot analysis using mouse polyclonal antibodies raised against this chimeric protein, we demonstrated the presence of one band with the predicted size of original SmAQP, around 32kDa. Moreover, in other western blot analysis performed with sera from mouse immunized with whole schistosome tegument, the chimeric protein was recognized probably due to the maintenance of SmAQP epitopes in the chimera. To validate aquaporin as a vaccine candidate, we are about to performe challenge infection experiments in mouse. **Conclusion:**

Bioinformatics studies predicted SmAQP as an immunogenic protein, and a chimeric protein produced with its main epitopes generated a Th1 response in immunized mice. Taken together, these results indicate SmAQP as a potential target for the development of a vaccine against schistomiasis.

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Towards a genetically engineered dendritic cell vaccine against cytomegalovirus reactivation

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High frequencies of patients who undergo hematopoietic stem cell transplantation (HSCT) experience episodes of human cytomegalovirus (HCMV) viral reactivation or become newly infected, which are major causes of morbidity and death for the affected patients. Monitoring studies post-HSCT have demonstrated that the presence and expansion of HCMV-reactive cytotoxic T lymphocytes (CTL) post-reactivation protects the patients against recurrent reactivations. Although several types of novel vaccine modalities are in development, no clinical vaccines are currently available against HCMV in the post-HSCT setting. Thus, we addressed a novel strategy for genetic reprogramming key cells of the immune system for optimized and prolonged antigen presentation to control HCMV infection. Integrase-defective lentiviral vectors (ID-LVs) show several hallmarks of conventional lentiviral vectors such as absence of cytotoxic effects and long-term expression in non-replicating target cells. The integration rate of ID-LVs into the genome of target cells is dramatically reduced, which enhances safety and opens perspectives for their use in vaccine development. We have recently demonstrated that ID-LVs co-expressing GM-CSF and IFN-α used to infect human monocytes in one day of ex vivo culture resulted in "**S**elf-differentiating **my**eloid-derived **le**ntivirus-induced **D**endritic **C**ells, heretofore called "SmyleDCs" (Daenthasanmak et al, Vaccine 2012). Overnight transduction of monocytes with high titer ID-LVs generated highly viable (14 days) and immunophenotypically stable SmyleDCs continuously secreting the transgenic cytokines and additionally endogenously produced inflammatory cytokines (IFN-g, IL-2, -5, -6, and -8). SmyleDCs lentivirally co-transduced for expression of pp65 (a HCMV antigen) used for *in vitro* stimulation of $CD8^+$ T cells resulted in high expansion of central memory and effector memory CTLs reactive against different pp65 epitopes. SmyleDCs also stimulated anti-pp65

CTLs *in vivo* in immune deficient NOD/Rag2$^{(-/-)}$IL2R□c$^{(-/-)}$ (NRG) mice immunized subcutaneously with SmyleDCs co-expressing the full-length pp65**.** NRG mice transplanted with human hematopoieitc stem cells (G-CSF mobilized CD34^{+} cells) and immunized subsequently with SmyleDC/pp65 showed an accelerated expansion of human T cells resulting into faster repopulation of lymphatic tissues with human cells in these mice. We have now validated a tricistronic ID-LV co-expressing simultaneously GM-CSF, IFN-a and codon-optimized pp65. The vector and SmyleDCs will be produced under GMP compliant methods in preparation for a manufacturing authorization and for phase I/IIa clinical trials at the Hannover Medical School.

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CARBON NANOTUBES-BASED FORMULATION AS PROPHYLACTIC VACCINE FOR INDUCING INNATE IMMUNE RESPONSE AND PROTECTIVE IMMUNITY FOR CANCER

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Introduction: The unique physical and chemical properties of carbon nanotubes (CNTs) make them useful for a variety of therapeutic and drug-delivery applications, including their use as nanovaccine scaffolds. In addition, antigen delivery through nanoparticles also can serve as an intracellular depot which improves the specific long-lasting immune response against the delivered molecule. Other attractive aspects of the use of CNTs in biomedicine are their low toxicity, low immunogenicity, improved *in vivo* and *in vitro* stability and reduced side effect profile. In the current study, we propose the use of a carbon nanotube-based delivery vehicle in combination with a recombinant antigen (NY-ESO-1) and a synthetic TLR9 agonist (CpG oligonucleotide) as an adjuvant to provide superior immunogenicity and elicit strong protection against cancer in a mouse model. **Methods and Results:** To demonstrate the biocompatibility of CNTs, MTT cell viability assay was performed and the results showed that solubilized carbon nanotubes alone were nontoxic *in vitro*, and did not have any detectable immunogenicity *in vivo*. Dendritic cells were observed after a phagocytosis assay by confocal microscopy and the images confirmed the presence, in their inner part, of CNTs attached to fluorescence-labeled OVA peptide. CNT constructs were rapidly internalized into professional APCs within 1 hour post-injection. C57BL/6 mice were immunized with formulations containing the tumor-associated antigen NY-ESO-1 and the specific antibody and IFN-γ production was evaluated. Mice that received immunization with NY-ESO-1 were challenged with B16 transgenic melanoma expressing NY-ESO-1. Our findings showed that the formulations containing carbon nanotubes were more effective, both for humoral and cellular immune responses while the antigen alone or mixed with the adjuvant did not induce such response. Furthermore, a therapeutic protocol using carbon nanotubes delayed tumor growth expressing NY-ESO-1 and prolonged the survival in treated mouse. **Conclusion:** The nanovaccine complex developed in the present study, which comprises an efficient delivery of the protein antigen, via CNTs, allied to the co-

stimulatory signal of toll-like receptor agonist, suggests an alternative for the currently used vaccine methods and highlights the potential application of car bon nanotubes as a delivery system to stimulate a highly and long-lasting immune response.

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SELECTION OF BROAD-RANGING SEROCROSS-REACTIVE FAMILY 2 PSPA

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Introduction: *S. pneumoniae* is a major pathogen causing pneumonia, septicemia and meningitis worldwide. The Pneumococcal Surface Protein A is a promising vaccine candidate against this pathogen. It is a protein exposed on the surface and capable of inducing the production of protective antibodies. Because of its structural diversity, an effective PspA-based vaccine should include at least one fragment from each of the two major Families of PspA (1 and 2). According to the literature it is known that PspAs from different clades show variable degrees of cross-reactivity. This study has the major objective to determine, from a panel of Brazilian pneumococcal isolates, which is able to induce the higher level of cross-reactivity within Family 2.
Methods and results: Initially, a set of 15 pneumococcal strains containing family 2 PspA (gently provided by Universidade Federal de Goiás or Institute Adolfo Lutz) were used for PCR amplification of PspA, the sequences were confirmed by sequencing, and inserted in the pAE-6xhis vector. The resulting plasmids were used for transformation into *E.coli* BL21 DE3. Recombinant bacteria were induced with IPTG for expression of the recombinant PspA; the soluble proteins were purified by chelating chromatography providing 6 different rPspA molecules. The rPspA purified proteins were used to immunize BALB/c mice and the sera of these animals were then evaluated for their serocross-reactivity. Initially, the sera were screened by western blot for reactivity against a panel of diverse pneumococcal strains bearing PspAs from family 2: clades 3, 4 and 5, selecting the 4 most broad-ranging sera. The evaluation of these sera for their ability to bind to the pneumococcal wall and to induce the deposition of complement by FACS analysis will allow selection of 2 molecules of PspA family 2. Determination of opsonophagocitic activity with the same panel will confirm sero-crossreactivity.
Conclusion: The analysis showed clearly that antibodies induced against rPspAs of the same clades induce different levels of cross-reactivity and it is possible to select a molecule will broader ranging sero-crossreactivity.

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FORMULATION OF *IN SILICO* PREDICTED DENGUE VIRUS (DENV) INVASION-INHIBITORY PEPTIDES/VIRAL-LIKE PARTICLE CHIMERAS, AND PRE-CLINICAL TRIAL IN A MURINE MODEL.

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Introduction: Prophylactic measures against Dengue are based on the control of *Aedes* spp. mosquito vectors, since protective vaccines are not yet available. We show here the results of the construction and test in mice of two experimental tetravalent-vaccine formulations based on two peptides present in the dengue virus envelope protein, in an attempt to generate an ultimate solution to prevent this disease.

Methods and Results: Two peptides were selected *in silico* and molecularly engineered to be expressed within a carrier protein that spontaneously assembles into viral-like particles (VLPs). The resulting chimera-enconding expression-optimized genes were subcloned in the expression vector pET-HIS-TEV and transformed in *E. coli* strains BL-21 (DE3) and BL-21 C41 (DE3). Protein expression was induced by IPTG and induction quality was verified by SDS-PAGE analysis, which showed two bands of the expected molecular masses (26kDa and 27kDa, respectively).

Protein purification was performed using Ni^{2+} columns and pure products, formulated in Montanide ISA720, were used to immunize C57BL/6 mice. In order to evaluate the humoral responses that could further show invasion-neutralizing features, pooled murine sera collected at day 30 after immunization were used in ELISA and Western Blot (WB) assays. Preliminary results show that all immunization protocols were capable of inducing intense humoral responses, with one of the two peptides displaying higher immunogenicity. Similar results were visualized in the WB membranes. Precise antibody titers, antibody IgG1/IgG2 profiles and infection-neutralizing activities displayed by these antibodies are underway.

Conclusion: Genetic engineering tetravalent DENV peptides into heterologous VLPs generate functional chimeras, with intense immunogenicity even after the first dose of vaccine, and in case they display invasion-neutralizing properties, they worth being tested in non-human primates, as the next fundamental step to aim at a future human trial.

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PROTECTIVE SPECIFIC EFFECTOR CD8$^+$ T CELLS ELICITED BY HETEROLOGOUS PRIME-BOOST VACCINATION EXPAND AFTER INFECTIOUS CHALLENGE WITH *TRYPANOSOMA CRUZI*.

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Introduction: Heterologous prime-boost strategy using recombinant plasmid DNA followed by replication-defective human recombinant adenovirus 5 is as a powerful strategy to elicit a stable pool of functional long-lived CD8$^+$ T cells with an effector (TE) or effector memory (TEM) phenotype (CD11a^{High} , CD44^{High} , CD127$^{Low/High}$ and CD62L^{Low}). After a challenge with the human protozoan parasite *Trypanosoma cruzi*, specific CD8$^+$ T cells expanded significantly and mediate a strong protective immune response against experimental systemic infection. Because it is not known whether these specific TE or TEM can proliferate *in vivo*, we aimed to characterize the phenotype and functionality of the proliferating cells after challenge.

Methods and Results: We found that the phenotype of specific CD8$^+$ T cells that expanded and expressed the anti-parasitic mediators IFN-γ and/or TNF-α differed from the TE present in immunized mice. The main difference was the presence of a significantly higher frequency of KLRG1 expressing specific CD8$^+$ T-cells. To confirm that specific CD8$^+$ T expanded after the infectious challenge were indeed TE induced by vaccination; we used the gzmBCreERT2/ROSA26EYFP transgenic mouse line. After heterologous prime-

boost vaccination, specific TE $CD8^+$ T lymphocyte became indelible labeled with enhanced yellow fluorescent protein (EYFP). Following an infectious challenge, these EYFP-labeled TE cells expanded at similar rate as the non-labeled cells.

Conclusion: This result strongly argued that cells expanded after an infectious challenge are antigen-experienced TE.

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EVALUATION OF IMMUNE RESPONSES AND PROTECTION INDUCED BY *LEISHMANIA L. INFANTUM* A2 ANTIGEN WHEN EXPRESSED IN A NONVIRULENT STRAIN OF *TRYPANOSOMA CRUZI*

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Introduction: Previous studies have shown that the A2 gene plays an important role in the virulence and visceralization of *Leishmania* species. Also, the characterization of the immune response and ability to induce protection of the A2 protein has already been proven in several models and through different immunization protocols. It is well known that the use of non-virulent organisms as antigen delivery system is very effective, since they can mimetize the natural course of the infection and present a wide set of antigens maintaining a durable immunity. Hence, we expressed the *Leishmania infantum* A2 antigen in a non-pathogenic strain of *Trypanosoma cruzi* for evaluation of the protective efficacy as a live vaccine against *L. infantum* challenge. **Methods and Results:** The CL-14 strain has been transfected with pRockNEO vector containing a 729bp synthetic A2 gene and sites for integration in *T. cruzi* genome. The successful obtention of the CL-14 T.cruzi expressing the A2 antigen has been confirmed with conventional PCR and Western Blot analysis. BALB-C mice were then immunized with 10^7 parasites by intraperitoneal way through homologous prime-boost protocol and checked for IFN-γ production with ELISPOT and ELISA. Mice were then challenged subcutaneously with 10^7 *L. chagasi* stationary promastigotes through their right hind footpad and the parasite burden of liver and spleen will be checked after 30 days. **Conclusion:** With this study we expect to confirm the A2-expressing T. cruzi as a safe live vaccine against visceral leishmaniasis.

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SCREENING OF *LEISHMANIA (LEISHMANIA) CHAGASI* PUTATIVE T CELL ANTIGENS AS NEW VACCINE CANDIDATES

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Introduction: Considering the ability of *Leishmania* to escape of host immune responses, the heterogeneity MHC molecules of the host populations and the limitations of current vaccines, our main goal is to identify and evaluate new *Leishmania (Leishmania) chagasi* antigens to develop multi-component vaccines. The A2 antigen was shown to induce protective responses against visceral leishmaniasis in different formulations. A2 is expressed only by visceral leishmaniasis species, it has potent CD8 epitopes and it is a stress protein associated linked to the ability of the parasite survive in high temperatures in visceral organs. Therefore, using A2 as model antigen we are searching for new molecules that could be associated in a multi-component vaccine. **Methods and Results:** We selected molecules that were previously shown to enhance the ability of *L. (L.) major* parasites to migrate to liver and spleen or that were identified by immunoproteomic analysis using sera of dogs with visceral leishmaniasis, and submitted them to *in silico* analysis for searching of T cell epitopes and peptides containing the identified epitopes were synthesized. Lymph node cells or spleenocytes from chronnically infected or vaccinated mice were stimulated with pool of peptides and submitted to CSFE flow cytometry proliferation assays. Preliminary results indicated that 21 out of 63 peptides induced significant proliferative indexes. **Conclusion:** The selected peptides that have induced significant responses will be further characterized according to determine the cell phenotype that are recognizing them and the cytokine profile that they induce, aiming to select antigens candidate to vaccine development against leishmaniasis.

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ANALYSIS OF THE INNATE IMMUNITY OF AGONIST FLAGELLIN OF *SALMONELLA TYPHIMURIUM* FUSION RECOMBINANT PROTEINS WITH DIFFERENT IMMUNODOMINANTS CS EPITOPES FROM PLASMODIUM VIVAX

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Introduction: In recent studies, we demonstrated the improved immunogenic properties of new malaria vaccine candidates based on the expression of fusion proteins containing merozoite immunodominant epitopes and an innate immunity agonist, the *Salmonella enterica* serovar Typhimurium flagellin (FliC). Herein, we compared the immunogenicity of several recombinant proteins expressing the immunodominant B-cell epitopes of malaria sporozoites of *Plasmodium vivax* and FliC.

Methods and Results: Three recombinant fusion proteins were generated expressing the His_6FliC containing in their C-terminus the repeat regions of each of the three different allelic forms of *P. vivax* circumsporozoite (CS) protein. The immunogenicity of a mixture of these recombinant proteins was compared with a mixture of bacterial recombinant proteins expressing the entire *P. vivax* CS protein alone or in the presence of the adjuvants native FliC or Poly (I:C). C57BL/6 mice subcutaneously immunized with the different mixtures successfully elicited significant antibody titers to bacterial recombinant proteins expressing the different allelic forms of *P. vivax* CS protein. The mixture of bacterial recombinant proteins expressing the entire *P. vivax* CS protein and native FliC or Poly (I:C) as adjuvants generated the highest antibody titers.

Conclusion: These results extend our earlier observations on the immunogenicity of these recombinant fusion proteins and the use of FliC as a potential adjuvant to develop a vaccine against malaria.

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IMMUNOLOGICAL PROPERTIES OF THE HEAT-LABILE TOXIN (LT) AND THE ALPHA-GALACTOSYLCERAMIDE (α-GALCER) MOLECULE IN THE RESPONSE INDUCED AGAINST HUMAN PAPILLOMAVIRUS E7 ONCOPROTEIN

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Introduction: The LT toxin and the α-GalCer molecules represent potent vaccine adjuvants, which are able to induce strong immune response against co-administered antigens. Despite of the extensive knowledge on these compounds in the literature, studies that evaluate their synergism in a new vaccine platform have not been evaluated previously. This project aims to evaluate the immunomodulatory properties of the attenuated LT variant (LT4) in association with the α-GalCer molecule regarding to antigen-specific T and B lymphocyte responses in animals immunized with subunit vaccines by parenteral route. **Methods and Results:** As target antigen, we used the E7 oncoprotein, which is expressed in human papilloma virus (HPV)-induced tumors. After immunization, the α-GalCer molecule induced lower E7-specific IgG titers than LT4, probably due its ability to shift the response towards a Th1-biased pattern. When the LT4 and α-GalCer were co-administered, individual behavior of each adjuvant was partially preserved without the observation of a synergistic effect. The administration of LT4 and/or α-GalCer in combination with purified E7 protein did not improve immunological responses mediated by cytotoxic $CD8^+$ T lymphocytes. Otherwise, following *in vivo* antigen expression by DNA immunization, INF-γ^+ $CD8^+$ T cells were detected in most animals, excepting the LT4-treated mice. Supporting these data, the groups that received LT4 showed increased tumor growth either after immunization with the purified E7 protein or with the DNA vaccine following implantation of TC-1 tumor cells expressing the HPV-16 oncoproteins. **Conclusion:** The results show that co-administration of LT4 and α-GalCer promotes improvement of both humoral and

cellular response mediated by $CD4^+$ T cells in the same formulation, which may be important for the development of different vaccines. However, the use of LT4 protein negatively modulated the protective effects of vaccines containing α-galcer and/or DNA in E7-expressing tumors.

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IMMUNOGENICITY OF RECOMBINANT PROTEINS AND ADENOVIRUSES BASED ON THE DIFFERENT ALLELIC FORMS OF THE CIRCUMSPOROZOITE ANTIGEN OF *PLASMODIUM VIVAX* AIMING AT THE DEVELOPMENT OF A UNIVERSAL VACCINE AGAINST MALARIA.

CIBELE APARECIDA TARARAM (1); LAIS HELENA TEIXEIRA (1); ARIANE G. A. CAMACHO (1); MARCIO O. LASARO (2), OSCAR BRUNA-ROMERO (3), IRENE S. SOARES (4); RUTH SONNTAG NUSSENZWEIG (5); HILDEGUND ERTL (2); VICTOR NUSSENZWEIG (5); MAURÍCIO MARTINS RODRIGUES (1).

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Introduction: *Plasmodium vivax* is the second most prevalent and most widespread species causing malaria in the world. Recent data estimated 132-391 million cases annually. The relative inefficiency of the measures currently used for control demands the development of new strategies for prevention such as vaccines, new drugs and insecticides. In the past 15 years, studies aimed at the development of a recombinant vaccine against the human malaria caused by the deadly parasite *Plasmodium falciparum* were based on the circumsporozoite (CS) antigen. In a recent publication, phase III trials in African children reported 50% efficacy of the recombinant vaccine.
Methods and Results: Based on the studies with *P. falciparum*, our studies aimed at the generation of bacterial recombinant proteins and replication defective adenoviruses expressing primary sequences from three different allelic variants of *P. vivax* CS protein. These recombinant proteins in combination with the adjuvant Poly(I:C) and adenoviruses were successfully used in protocols of homologous (recombinant protein/recombinant protein) or heterologous (adenovirus/recombinant protein) vaccination strategies in an experimental mouse model. Most importantly, these different vaccine strategies elicited antibodies which strongly reacted with the immunodominant regions of all three allelic variants of *P. vivax* CS protein.
Conclusions: These recombinants proteins/adenoviruses and the respective protocol of vaccination were object of a patent application to the development of a universal vaccine against *P. vivax* malaria (USPTO IFW61614439).

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ISOLATION AND CHARACTERIZATION OF Vi CAPSULAR ANTIGEN OF Salmonella enterica SEROTYPY TYPHI

Eliana da Silva Nascimento, Raimundo Lopes, Francisco Teixeira Martins, Patrícia Santana Marinho, José Otávio Carrera Silva Júnior, Roseane Maria Ribeiro Costa

Introduction: The Vi antigen is an important virulence factor found in the bacterium Salmonella typhi. It has anti-phagocytic activity and also immunogenic properties. Use of the Vi antigen in vaccines is very promising because it provide a high level of immunity in parenteral vaccines. The need for the search of alternatives to the administration of vaccines against typhoid fever led to the development of methodologies that generate new vaccine formulations as one that uses the controlled release system. For this it is necessary to adopt techniques of isolation of antigen to be used in the construction of these vaccines. This work suggests the isolation and physico-chemical characterization of Vi antigen isolated from Salmonella strain which can be Latter used for the manufacture of vaccines.

Methods and Results: The Vi capsular antigen was extracted from the strain of Salmonella enterica serotype Typhi using an adaptation from the methodology described by Wong and Feeley in 1972. For control, the antigen used was commercially pure Bio-rad Vi antigen. The pure antigen extracted from the strain was characterized by physico-chemical methods of infrared (IR) and nuclear magnetic resonance (^{1} H-NMR) in order to identify the chemical compounds present in the polysaccharide. IR spectra for the commercial antigen showed peaks at 617cm^{1}, 1200-950 cm ^{1}, 1734cm ^{1}, 1650-1540 cm -1 and 1604-1417 cm-1 representing respectively the groups of piranosidic ring, C-O-C stretching, O-acetyl group, N-acetamin group and carboxylate anion. The extracted antigen presented the peaks: 617 cm -1, 1101 cm ^{1}, 1650 -1540 cm -1 and 1604-1417 cm-1 corresponding respectively to the piranosidic ring, the C-O-C stretching, N-acetyl group and the carboxylic acid anion. The O-acetyl groups and N-acetyl antigen present in the structure are important by the immunogenic properties. In analyzes there was a correlation between the NMR spectra obtained from commercial antigen and the extracted antigen demonstrating that the extraction process was efficient.

Conclusion: This analysis shows that the extraction technique adopted was efficient according with the correspondence analysis using the IR and NMR spectra between the extracted antigen and the commercially pure antigen. This method can be adopted for the isolation of the Vi capsular antigen and can be applied in the construction of new vaccines against typhoid fever.

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IMMUNOLOGICAL DIVERSITY AMONG HEAT-LABILE TOXINS (LT) PRODUCED BY NATURAL ISOLATES OF ENTEROTOXIGENIC *ESCHERICHIA COLI* (ETEC)

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Introduction. Heat-labile toxins (LT) produced by enterotoxigenic *Escherichia coli* (ETEC) act as adjuvants following delivery via mucosal, parenteral or tanscutaneous routes. Previously we have shown that LT produced by human-derived ETEC strains encompass a group of 16 polymorphic variants, including the reference toxin (LT1) produced by the H10407 strain and one variant that is found mainly among bacterial strains isolated from pigs (LT4). Indeed, we show that LT4 [with six polymorphic sites in the A (K4R, K213E, N238D) and B subunits (S4T, A46E, E102K)] displays differential toxicity and adjuvant activities compared to LT1. In the present work we evaluated the influence of LT4 polymorphic sites on its immunological properties following intranasal and intrarectal immunization using HIV p24 protein as model antigen. **Method and Results**. Two in vitro-generated LT mutants containing one (LTK4R) and five (LTHybrid) amino acid changes in the LT1 background were studied in comparison with LT1 and LT4 natural variants. LT1, LT4, LTK4R and LTHybrid showed preserved adjuvant activities following intranasal and intrarectal administrations to mice, particularly concerning production of serum and mucosal antigen-specific antibodies and activation of p24-specific cytotoxic $CD8^+$ T lymphocytes derived from spleen, inguinal and mesenteric lymph nodes. Noticeably, LT4 and LTK4R showed a stronger Th1-biased adjuvant activity with regard to LT1 and LTHybrid following immunization via both intranasal and intrarectal routes. **Conclusion.** Based on above results we suggest that the LT4 immunomodulatory behavior is mainly attributed to the K4R polymorphic site, which is also responsible for LT4 attenuation. Our results further emphasize the relevance of LT polymorphism among human-derived ETEC strains that may impact both the pathogenicity of the bacterial strain and the use of these toxins as potential vaccine adjuvants.

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Title: MIXTURE OF RECOMBINANT YELLOW FEVER VIRUSES VACCINES ELICITS SUPERIOR PROTECTIVE IMMUNE RESPONSES AGAINST EXPERIMENTAL INFECTION WITH THE HUMAN PROTOZOAN PARASITE *TRYPANOSOMA CRUZI*

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Introduction: The attenuated Yellow Fever (YF) 17D vaccine virus is one of the safest and most effective viral vaccines administered to humans. Through the infectious clone technology, we used the YF 17D backbone to express a $CD8^+$ T cell epitope, TEWETGQI, in the protease cleavage site between the NS2B and NS3 and an immunogenic fragment from the Amastigote Surface Protein 2 (ASP-2) of the *Trypanosoma cruzi* parasite between E and NS1 of YF 17D. This last strategy involved the construction of a stable heterologous cassette. By

using these approaches we aimed to investigate whether the site of expression had any influence on foreign antigen immunogenicity.

Methods and Results: The genomic sequence of an ASP-2 fragment was inserted between E and NS1 genes of YF 17D virus through the construction of a recombinant heterologous cassette. We generated virus that replicated in cell culture and remained genetically stable after six or more serial passages in Vero cells. Vaccination of a highly susceptible mouse model with a single mixture containing two recombinant YF 17D viruses (viral formulation) induced a superior degree of protection than the recombinant viruses alone after a *T. cruzi* challenge. All recombinant viruses were similarly capable to prime and induce the expansion of gamma interferon (IFN-γ) producing T cells specific to YF 17DD peptides after two doses in immunized mice despite low levels of neutralizing antibodies against the YF virus. However, the T cell response specific to the *T. cruzi* TEWETGQI epitope differed between the immunized mice groups since the YF viral formulation was capable to induce a higher number of TEWETGQI specific IFN-γ-producing T cells after only one dose in comparison to the other viral groups. Based on this finding we suggest that the protection induced by the viral formulation might be due to an earlier priming of epitope-specific T cells. Moreover, after a *T. cruzi* challenge, all mice immunized with the YF recombinant virus or the viral formulation elicited a 6-fold higher T cell magnitude.

Conclusion: We conclude that the use of new YF 17D viral formulations may be a promising strategy to elicit a protective immune response against protozoa.

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SAG2A PROTEIN OR SOLUBLE TACHYZOITE ANTIGEN (STAG) ENTRAPPED WITH CATIONIC LIPOSOMES ENHANCES MICE PROTECTION AGAINST *TOXOPLASMA GONDII* INFECTION.

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Introduction: *Toxoplasma gondii* is an obligate intracellular parasite that infects various animals endothermic and causes the toxoplasmosis. Several efforts have been undertaken with the purpose of finding a vaccine against the parasite, although there is no effective preparation available nowadays. In this way, the aim of present study was to evaluate the immune response of C57BL/6 mice to immunization with recombinant antigens (SAG2A and BSR4) and soluble antigen from *Toxoplasma gondii* (STAg) incorporated into cationic liposomes. **Methods and Results**: Cationic liposomes (Lip) consisting of phosphatidylethanolamine/ phosphatidylcholine/cholesterol were prepared with proteins (rSAG2A; rBSR4; rSAG2A plus rBSR4; STAg and PBS). C57BL/6 mice were immunized subcutaneously three times every 15 days with Lip-rSAG2A; Lip-rBSR4; Lip-rSAG2A plus rBSR4; Lip-STAg; Lip-PBS (control without antigen); Lip (adjuvant control). The levels of total IgG were higher in the group immunized with: Lip-BSR4 and Lip-SAG2A/ BSR4. The highest levels of IgG1 and IgG2a were found in the group immunized with Lip-BSR4, although higher levels have been also observed in the group immunized with Lip-BSR4/SAG2A. After challenge, mice immunized with Lip-STAg, Lip and Lip-SAG2A had lower mortality in comparison to the other groups. Furthermore, animals from Lip-STAg and Lip did not succumb during acute infection, suggesting an immunoregulatory role of cationic liposome adjuvant since early phase of *T. gondii* infection. Interestingly, animals from group immunized with Lip-BSR4, a major antigen expressed in the bradizoite phase, showed an evident reduction of mice protection. Additionally, animals immunized with Lip-SAG2A or Lip-

SAG2A/BSR4 had lower parasitism scores in brain tissue sections, whereas it was observed that the Lip-BSR4 group showed the greatest parasitism score. **Conclusion**: Cationic liposomes carrying SAG2A or STAg exhibit an immunostimulatory effect enhancing mice protection, while the vaccination with liposome-BSR4 apparently produces an impairment of immune response against *Toxoplasma gondii*.

Keywords: *Toxoplasma gondii*; immune response; liposome, vaccine.

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Intra peritoneal Injection with Trypanosoma cruzi expressing NY-ESO-1 causes changes in local cellular population and in lymphoid organs

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Introduction: Exploring the adjuvant characteristics of *Trypanosoma cruzi* and immunogenic characteristics of the Cancer/Testis antigens, our group constructed a transgenic parasite expressing the protein NY-ESO-1. This parasite was used in prime/boost vaccination protocols and showed great efficacy to rise a specific TH1 immune response to NY-ESO-1 as well as to protect C57/BL6 mice after tumor challenge with cells expressing NY-ESO-1. **Methods and Results:** To elucidate the innate immune response driven by this transgenic parasites, we investigated now the changes that happen in the peritoneum, mesenteric lymphonodes and spleen after peritoneal injection using parasites labeled with CFSE aiming to understand how the parasites migrate to peripheral lymphoid organs and initiate the immune responses. We observed that B cells and macrophages are the main subpopulations in peritoneal cavity of uninfected controls. However, after vaccination with transgenic parasites we notice an increase in the inflammatory monocytes and dendritic cell populations. The assays performed by flow cytometry indicated that the parasites were primarely found associated in B lymphocytes, dendritic cells and macrophages, which rapidly migrated to the lymphoid organs. **Conclusion:** We are currently evaluating the importance of each of those cells in the primary activation of T lymphocytes and development on an effective polarized protective Th1 as well as CD8+ T cell responses.

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ESCHERICHIA COLI HEAT-LABILE TOXIN (LT1) EFFECT ON THE MAGNITUDE AND GLYCOSILATION PATTERN OF MUCOSAL AND SERUM ANTIBODY RESPONSES DIRECTED TO THE E GLYCOPROTEIN DOMAIN III OF TYPE 2 DENGUE VIRUS (DENV2)

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Introduction: The heat-labile toxins (LT) produced by enterotoxigenic *Escherichia coli* (ETEC) strains have been intensively investigated as potential vaccine adjuvants. LT toxins show ability to augment systemic and mucosal antibody responses against co-administered antigens and modulate IgG subclass production in mice vaccinated by mucosal or parenteral routes. However, little is known about the role of these adjuvants on the IgG glycosylation pattern under experimental conditions. In this work we characterized the magnitude of LT adjuvant effects regarding the induced humoral response, IgG glycosylation pattern and virus neutralizing activity using the reference toxin (LT1) and one atoxic derivative (LT1-K63) as adjuvant and the domain III of dengue vírus E glycoprotein (EIII) as a model antigen. **Methods and Results:** Both LT1 and LT1-K63 enhanced the EIII-specific serum IgG response in mice subcutaneous (s.c.) immunized with purified proteins. The adjuvant effects were also measured by *in vitro* virus neutralization activity and IgG glycosylation a lectin enzyme-linked immunosorbent assay. Although the LT1-K63 had induced lower anti-EIII IgG titers than LT1, these induced antibodies showed better virus neutralization activity. Purified anti-EIII IgG antibody from serum of mice immunized with LT1 and LT 1-K63 are more sialylated than IgG obtained of serum of mice it received antigen alone. In addition, evaluation of IgG subclass and secreted cytokine profiles indicated that LT1-K63 modulated the immune responses to a biased Th1 profile. **Conclusion:** Such results suggest that enzymatic activity of LT1 is important to the magnitude of humoral response against the target

antigen following s.c. immunization. However, production of neutralizing antibodies was favored in mice immunized with the atoxic LT derivative suggesting that the differential immune modulatory behavior of LT1-K63 may affect the epitope specificity of the antibodies. Despite of the immunological differences observed in mice immunized with LT1 or LT1-K63, the present results indicate that both adjuvants show good perspectives as adjuvants for anti-dengue vaccines.

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IMMUNOGENICITY OF A FUSION PROTEIN CONTAINING MULTI-STAGE ANTIGENS (AG85C, MPT51, HSPX) OF *M. TUBERCULOSIS* IN MICE.

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Introduction: Tuberculosis (TB) is a worldwide public health problem and an approach to prevent it, is the vaccination *with M. bovis Bacillus Calmette-Guerin* (BCG), but the efficacy of this vaccine to protect adults is questionable. The use of antigens expressed at different stages of *M. tuberculosis* (Mtb) natural infection arise the possibility of modulating the immune response and also of memory cells. Therefore we aimed to construct a fusion protein containing antigens expressed during the replication and latency of the Mtb to be used as a subunit vaccine for TB.

Methods and Results: We constructed Mtb fusion protein containing immunodominant epitopes of the antigens Ag85C, MPT51 and HspX. The recombinant fusion protein Ag85C-MPT51-HspX (CMX) was cloned into pET23a vector for expression in *E. coli* BL21 (DE3), and purified by affinity chromatography using Ni-NTA affinity columns. The vaccine was liposome-encapsulated with CpG-DNA. Mice (n=5 per group) were immunized three times with an interval of 15 days between immunizations subcutaneously (sc) with 100 µL of vaccine formulation, or the respective controls: CpG-DNA encapsulated in liposome, liposome alone or saline. The specific immune response to CMX protein was evaluated by ELISA measuring IgG1 and IgG2a at fifteen days after the vaccination. Higher titers of IgG1 (3.08±0.04) and IgG2a (3.10±0.03) were observed in mice vaccinated with CMX formulation, while the controls had insignificant levels of these antibodies: (CPG formulation: IgG1= 0,16±0,02; IgG2a= 0,16±0,01; liposome: IgG1= 0,127±0,02; IgG2a= 0,16±0,04; or saline: IgG1= 0,16±0,01; IgG2a= 0,16±0,02). To answer whether a Th1 specific immune response was induced, $TCD4^+$ splenocytes were analyzed by flow cytometry. The percentage of $TCD4^+$ cells expressing IFN-γ in the group of mice immunized with CMX (4.16%±0.83) was greater than the percentage of these cells in the group of mice immunized with CpG-DNA liposome-encapsulated (2.20%±0.54, p=0.01), liposomes alone (1.70%± 0.58, p= 0.009) or saline (1.62%±0.19, p= 0.001). Similarly, higher percentages of $TCD4^+$ cells expressing TNF-α were observed in the group of mice immunized with CMX (3.32%±0.70) when compared to the percentage of $TCD4^+TNF\text{-}\alpha^+$ cells in the groups of mice immunized with CpG-DNA liposome-encapsulated (1.98%±0.46, p=0.01), liposomes alone (1.62%±0.78, p=0.01) or saline (1.36%±0.27, p=0.0008). **Conclusion**: The fusion protein constructed in this study has potential for use as subunit vaccine for TB.

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ADJUVANT AND VACCINE DELIVERY INFLUENCIATE THE IMMUNOGENECITY OF A HSP-X VACCINE FOR TUBERCULOSIS.

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Introduction: Tuberculosis kills more then 1.5 million of people a year, although a vaccine exist and have been used for more than a century. This fact prompted the development of new vaccines. *Mycobacterium tuberculosis* resides in granulomatous lesions, deprived of nutrients that induces the expression of stress related protein such as Hsp-X. The objective is compare the immunogenicity of two Hsp-X vaccines liposome encapsulated formulations containing CpG-DNA or MPL. **Methods and Results:** Groups of 6 BALB/c mice per group were immunized trice in fifteen days interval with liposome alone, CpG-DNA liposome encapsulated, MPL liposome encapsulated, Hsp-X liposome encapsulated, Hsp-X and CpG-DNA liposome encapsulated, or Hsp-X and MPL liposome encapsulated. After thirty days of vaccination blood was obtained and an ELISA for IgG1 and IgG2a was performed. Sixty days after of infection with *M. tuberculosis* (intranasal, 10^7CFU per animal) blood and spleen cells were obtained to measure specific humoral and cellular immune responses. All vaccines formulations that contained Hsp-X were able to induce specific IgG1 responses to Hsp-X after vaccination with range from 2.1 to 2.4 OD. While specific IgG2a was highly induced only when CpG-DNA or MPL was used on the vaccine formulation i.e. Hsp-X+CpG-DNA (2.38±0.5); Hsp-X + MPL (2.15±0.2); Hsp-X+CpG-DNA liposome encapsulated (2.35±0.0); or Hsp-X+MPL liposome encapsulated (2.09±0.1). Sixty days post vaccination; both antibodies were maintained at constant titers. Moreover, formulations containing the recombinant protein Hsp-X induced higher levels of CD4+IFN-γ+ and CD8+IFN-γ+ cells than controls groups. **Conclusion:** According to preliminary results of the humoral and cellular immune response, the vaccine formulation Hsp-X + CpG-DNA liposome encapsulated showed higher immunogenic potential and could offer protective efficacy against *M. tuberculosis* infection.

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