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INVESTIGATION OF THE ANTIMALARIAL EFFECT OF THE ATOMIZED EXTRACT OBTAINED FROM THE PLANT *AMPELOZIZYPHUS AMAZONICUS* IN THE MURINE *PLASMODIUM CHABAUDI* INFECTION MODEL

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Introduction: The infusion obtained from the plant *Ampelozizyphus amazonicus* Ducke (Rhamnaceae) is popularly used in the north region of Brazil in the treatment of malarial infection. This plant is popularly known as Saracura-mirá (SAR). In the present work we evaluated experimentally whether the aqueous extract obtained from SAR would modify B lymphocyte response in *P. chabaudi*-infected mice.

Methods and Results: BALB/c mice were infected by intraperitoneal injection of 10^6 parasitized red blood cells. Some animals were additionally treated with a saline solution containing a spray-dryer atomized aqueous extract obtained from the barks of SAR. The dose of 10mg/kg/day of SAR was used. This dose is popularly used in the treatment of malaria. All experimental procedures were approved by an official animal use ethical committee. Infection levels were measured by determination of parasitemia and hematocrit. Total serum levels of IgM and IgG were measured by ELISA. A spleen cell suspension was labeled with fluorochrome labeled antibodies for subsequent flow cytometry analysis (CD21 FITC, CD23 PE, B220 FITC, CD138 PE and CD86 Cy5). Parasitemia peaked around the 7th and 8th day of infection and survival of most animals did not extend beyond the 10th day of infection. Based on these findings, analysis of splenic cell populations was performed at days 5 and 8 of infection. Treatment with SAR did no modify spleen weight of infected animals. There was no statistically significative modification in parasitemia levels of SAR-treated animals even though parasitemia levels tended to be higher in this group. SAR

treatment induced an increase in circulating total IgM and IgG levels compared to infected only animals. SAR treatment did not modify the number of CD86+ splenic B cells. There was a significative decrease in marginal zone B cells (CD21hi/ CD23lo) in *P. chabaudi*-infected animals and SAR treatment reverted this decrease. Finally, SAR treatment deeply decreased the number of splenic CD138+ antibody producing B cells induced after infection.

Conclusion: Taken together, our data suggest that SAR treatment did not modify the progression of infection but decreased infection-associated exacerbated B cell activation which may be important to control infection-associated polyclonal B cell activation.

F.F. Barboza and M.V.A. Pereira contributed equally in the development of the research.

Financial support: FAPERJ, CPNq

B-1 CELLS PARTICIPATION IN THE IMMUNE RESPONSE INDUCED BY ANTIGEN ENCAPSULATED INTO PHOSPHATIDYLCHOLINE-CONTAINING LIPOSOMES

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Introduction: B-1 lymphocytes comprise a unique subset of B cells that differ phenotypically, ontogenetically and functionally from conventional B-2 cells. The most frequent specificities of the antibody repertoire of peritoneal B-1 cells are phosphatidylcholine (PtC) and phosphorylcholine. Liposomes containing phosphatidylcholine have been studied as adjuvants and their interaction with dendritic cells and macrophages has been demonstrated. However, their interaction with B-1 cells has not been explored. The aim of this work is to study the contribution of B-1 cells to the humoral response induced by ovalbumin (OVA) encapsulated into dipalmitoylphosphatidylcholine (DPPC) - and cholesterol (Chol) -containing liposomes and its possible relation to lipid composition. **Methods and Results:** BALB/c and BALB/xid mice, a strain almost deprived of B-1 cells, were immunized with OVA encapsulated in liposomes of DPPC:Chol/OVA or liposomes of dipalmythoylphosphatidylglycerol and cholesterol (DPPG:Chol/OVA). To determine the contribution of antibodies specific for DPPC, we evaluated the antibody response induced by DPPC:Chol/OVA in BALB/xid mice reconstituted with antibodies DPPC-specific. The ability of B-1 cells to internalize antigens encapsulated into DPPC:Chol liposomes was also evaluated. BALB*/xid* mice showed quantitative and qualitative differences with respect to wild type animals in the anti-OVA antibody response induced with DPPC:Chol/OVA. Transfer of antibodies DPPC-specific to BALB*/xid* mice partially restored the response induced by the liposomal preparation. The OVA-specific immune response was further enhanced in BALB/*xid* mice when reconstituted with B-1 cells. These cells were able to internalize DPPC-containing liposomes and to migrate from the peritoneal cavity to spleen. The adjuvanticity of liposomes was further demonstrated to be dependent on phosphatidylcholine. **Conclusions:** A cognate interaction has

been proven between B-1 cells and DPPC-containing liposomes, which modulates the immune response to encapsulated-antigens, providing a novel targeting approach to assess the role of B-1 cells in acquired immunity.

Financial support. We thank the UNU-BIOLAC and CAPES for providing funding for scientific exchange and development of this work.

DETECTION OF ANTIBODIES TO MAMMALIAN CELL ENTRY (MCE1A) PROTEIN FROM MYCOBACTERIUM TUBERCULOSIS IN PULMONARY TUBERCULOSIS PATIENTS

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Introduction: Tuberculosis (TB), chronic disease caused by *Mycobacterium tuberculosis* (*Mtb*), is an infectious disease that affects most the brazilian population, causing 2.832 deaths in 2010. In *Mtb* infection, the interaction of T cells with infected macrophages (MØs) is a central factor of protective immunity against the bacillus. Mce1A is a *Mtb* cell wall protein with large capacity of adhesion, invasion and survival in MØs. Antibodies anti-*Mtb* are unable to penetrate into the infected MØs and destroy the bacilli, so the humoral immune response is less effective against TB. However, identification and characterization of the protein Mce1A can lead to production of specific antibodies against the bacillus. Our goal is to evaluate the production of IgG to Mce1A protein in pulmonary TB patients. **Material and methods**: Patients diagnosed with pulmonary TB and their household contacts (HHC) were subjected to blood collection by venipuncture at 6º Centro de Saúde Rodrigo Argolo. The diagnosis of TB was based on clinical presentation and/or suggestive radiograph and/or positive sputum smear. In HHC, the infection was determined from the reactivity of the tuberculin test (TST). Serum was collected and stored at -20 ° C until determination of levels of anti-Mce1A IgG by an enzyme immunoassay ELISA. **Results:** Between January and July 2012 were identified 20 pulmonary TB patients and 26 HHC. Of the 26 HHC, 17 were positive TST (TST+) and nine were negative TST (TST-). The average age was 29 years (SD±18.7) and males prevailed among TB patients (63%), however in HHC women prevailed (64%). Most of individuals included were BCG vaccinated (73%). Only 20% of TST- (4%) and TST+ (16%) individuals reported that had prior contact with a TB ill. TB patients had levels of IgG (1.062±0.2913) statistically higher ($p<0.0001$) than TST- individuals (IgG: 0.3213±0.2928). The difference between the TST- and TST+ groups (IgG: 1,011±0,4070) also had statistical difference ($p=0.0005$). There was no statistical difference between TB patients and TST+ individuals. **Conclusion:** It was possible to quantify the production of anti-Mce1A IgG in TB patients and TST+, although the humoral immune response does not have a protective role against *Mtb*. It was found statistically significant differences between the TB and TST- groups and

between TST- and TST+ groups, relative to production of anti-Mce1A IgG, suggesting a potential role as biomarker of disease and TB infection.

Financial support: Centro de Pesquisa Gonçalo Moniz / Fiocruz; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES; Secretaria Municipal de Saúde / Programa Municipal de Controle a Tuberculose; UC Berkeley.

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ASSESSMENT OF POTENTIAL BIOMARKERS FOR THE DIAGNOSIS OF PULMONARY TUBERCULOSIS

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INTRODUCTION: Tuberculosis (TB), disease caused *by Mycobacterium tuberculosis*, has remained a serious public health problem.
Early diagnosis and appropriate treatment are the most effective approaches to disease control. The limitations of current diagnostic methods have driven the development and improve of diagnostic techniques. The aim of the present study was to evaluate antibodies anti-lipids as biomarkers for diagnosis of TB. **METHODS:** Patients newly diagnosed with active TB by sputum smear microscopy positive and healthy individuals with a negative tuberculin skin test (TST) were recruited of the Hospital Especializado Octávio Mangabeira (HEOM) and Instituto Brasileiro para Investigação da Tuberculose (IBIT). About 5 mL of venous blood were collected, serum was separated and stored at 4°C until measurement of antibody by ELISA. The IgM levels were evaluated against five phospholipids found in cell wall of *Mycobacterium tuberculosis*. Lipid antigens included cardiolipin (CL), phosphatidylinositol (PI), phosphatidylethanolamine (PE), phosphatidylcholine (PtC), and sphingolipid (SL). **RESULTS:** Serum samples from 10 TB patients and 12 health donors were evaluated. Of the five lipids used for measurement anti-phospholipid antibody response, mean serum levels of IgM against PTC in TB patients was significantly higher ($p=0.0076$) than health individuals. In contrast, there was no statistical difference between other lipids assessed ($p>0.05$). **CONCLUSION:** The results of this study indicate that a significantly increased of anti-PtC IgM levels were observed in TB patients. This lipid may be a useful biomarker to diagnostic patients with pulmonary TB. Therefore, it is necessary to increase the sample population for the validation of results and investigate other relationships with clinical, epidemiological and laboratory aspects.

Financial support: FAPESB, UC Berkeley, EBMSP.

HUMORAL IMMUNE RESPONSE AGAINST *Haemonchus contortus* PROTEASES IN GOATS NATURALLY INFECTED

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Proteases are essential for the survival of pathogens and their biological functions. These enzymes participate in the evasion of host defense. Parasite proteases can direct the immune response of the host mammal to Th1 or Th2 and, in accordance with this profile, difficult to eliminate of the parasite. *Haemonchus contortus,* most prevalent and pathogenic helminth of small ruminants, uses protease to digest hemoglobin as the primary source of aminoacids. These hemoglobinases belong mainly to the class of cysteine proteases and are related to the induction of a protective immune response.
The objective of the present study was obtain an enriched antigenic fraction of protease *H. contortus* and evaluate the humoral immune response of naturally infected goats against total antigen and enriched fraction. **Methods and Results:** Adult parasites were selected to produce the lysate from which were isolated proteases, through two sequential chromatography, Con A and DEAE-Sephacel. Protease activity was determined by SDS-PAGE with 0.1% gelatin. Serum samples of sixty naturally infected animals were selected for the detection of specific IgG anti-protease and anti-total lysate. Were selected sera from 28 animals with different parasitic loads for the evaluation of immunodominant antigens. For statistical analysis was performed Pearson correlation test ($p < 0.05$). Goats studied showed parasite burden (FEC) between 50 and 4500. We observed a negative correlation between parasite load and specific IgG anti-protease (r=-0,6508 e *p=0,0001) and parasite load and specific IgG anti-total lysate (r= -0.5213 e *p=0.003). The evaluation of the immunodominant antigens revealed that antibodies present in sera of the animals recognized proteins with molecular weights between 45 and 50 kDa. **Conclusions**: Animals that have a higher humoral immune response have lower parasite load, this data is evidence of the important role of the humoral immune response in controlling infection by *H. contortus* in goats.

Finacial support: BNB-FUNDECI; CAPES.

HUMORAL IMMUNE RESPONSE AGAINST RECOMBINANT MPT51-AG85C-HSPX FUSION PROTEIN IN INCARCERATED POLULATION WITH HIGH RISK TO DEVELOP ACTIVE TUBERCULOSIS.

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Introduction: Tuberculosis (TB) is an infectious disease caused by the bacillus *Mycobacterium tuberculosis*. TB is the second leading cause of death from an infectious disease worldwide, after HIV, causing 1.2-1.5 million deaths in 2010. Approximately 5% of all individuals infected with *M. tuberculosis* will develop active tuberculosis in the first two years after infection. Incarcerated individuals in contact with active tuberculosis inmates/patients frequently have a higher risk for developing TB. Rapid diagnosis, prompt initiation of effective treatment, and adequate infection control measures are of particular importance to prevent infection. We have previously developed a recombinant fusion protein (CMX) based upon MPT-51, Ag-85C and HSP-X antigens from *M. tuberculosis* that showed good discriminatory power between active TB and controls individuals. **Objective**: To evaluate the levels of IgG antibodies against CMX fusion protein in a prison population at the State of Goias, Brazil. **Methodology**: Serum was obtained from 196 male individuals. All individuals were submitted to tuberculin skin test (TST) with application of 100 µL of PPD Rt 23. Classification was made according to the WHO guidelines: TST+ (≥10mm) and TST- (<10mm). The serum levels of specific IgG to CMX fusion protein were detected by indirect ELISA (O.D. 492nm). **Results**: Twenty individuals had TST= zero, in 79 of them the TST was negative and nighty seven persons presented a positive TST. Comparing the specific IgG serum levels between individuals with zero TST (average+- sd=0.34±0.09), the TST negative (0.38±0.14), and the individuals TST positive (0.38±0.13), no differences were observed. **Conclusions:** Similar IgG levels specific to CMX were observed among incarcerated population regardless of the TST statuses.

Financial Support: CNPq.

EVALUATION OF CROSS-REACTIVITY AND INHIBITION OF PHOSPHOLIPASIC ACTIVITY BY POLYCLONAL ANTIBODIES

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Introduction Snake venom phospholipase A2 (PLA2) are enzymes capable to induce several alterations of plasmatic membrane, increasing its permeability and leading to cell death (Toxicon 42:827-840,2003). **Objective** The aims of this study are polyclonal antibodies production anti-*Bp* (*Bothropoides pauloensis* venom) and anti-BnSP7 (PLA2 isolated from this same venom); identification the cross-reactivity between anti-BnSP7 and PLA_2s present in others snake venoms; and evaluation the inhibitory potential upon the phospholipasic activity. **Methods and Results** Snake venoms used were purchased from Serpentarium Bioagents (Batatais-SP), while BnSP7 PLA_2 was purified by two chromatographic steps: CM-Sepharose and Reverse-Phase on C2C18 HPLC columns. For immunization, *Bp* or BnSP7 were mixed with Freund's complete adjuvant or Freund's incomplete adjuvant and inoculated by *i.p.* route in Balb/c mice (n=20). After challenge period, the blood obtained by cardiac puncture was centrifuged and the supernatant applied to an affinity column Protein G Sepharose for antibodies purification. The evaluation of cross-reactivity was performed by ELISA and Western Blotting (WB) activities, which showed that the anti-BnSP7 was able to recognize the PLA_2s present in *Bp* and *Bothrops moojeni* venoms at ratios up to 1:6400 and 1:3200 (w/v), respectively. This recognition in both venoms was confirmed by WB. For phospholipasic activity anti-BnSP7 was incubated with *Bp* or *Bm* venom at ratios of 1:1, 1:10 and 1:20 (venom/antibody; w/w) for 30 min at 37°C. The anti-*Bp* was incubated only *Bp* venom at same ratios and conditions. In relation to positive control (venom), our results showed that the anti-*Bp*, at ratio 1:20 (w/w), was able to neutralize the PLA2 activity of crude venom (*Bp*) in 71.6%. In the same ratio, anti-BnSP7 was able to neutralize the activity of *Bp* and *Bm* venoms in 70% and 41.2%, respectively. **Conclusion** Our results showed that the specific polyclonal anti-PLA_2 antibodies were able to recognize PLA_2s isoforms present in different snake venoms. Therefore, these antibodies could be useful for serum enrichment in the treatment of ophidian envenomation. Financial supported: FAPEMIG, UFU.

TITLE: PRODUCTION OF ANTI-LOXOSCELIC SERUM BY IMMUNIZATION OF HORSES WITH RECOMBINANT PROTEIN CONSISTING OF EPITOPES FROM SPHINGOMYELINASE-D OF *LOXOSCELES INTERMEDIA* SPIDER VENOM

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Introduction: Loxoscelism are the most important clinical syndromes resulting from *Loxosceles* spp spider bite and can be observed as two well-defined clinical variants: the cutaneous form manifests as pain and erythema that can develop into a necrotic ulcer whilst systemic loxoscelism is characterised by intravascular haemolysis and occasionally renal failure. *Loxosceles* venom contains several protein toxins but sphingomyelinases family members, also called dermonecrotic toxins, are the major toxic component and play an essential role in the pathogenesis of loxoscelism. Antivenoms prepared from horse anti-sera by immunization with crude venoms are an important treatment for spider envenomation. A novel approach based on synthetic epitopes of sphingomyelinases selected by multiple synthesis technique (Spot synthesis), phage-displayed random peptide libraries and bioinformatics analyses shows potential to production of efficient antivenoms to be used therapeutically.
Methods and results: A recombinant chimeric antigen consisting of B-cell epitopes of a sphingomyelinase-D from *L. intermedia* spider venom was expressed in *Escherichia coli* BL21 and used as immunogen to horses for the production of anti-loxosceles antivenoms. To analyze comparatively, horses were also immunized with crude venoms of the three main species of *Loxosceles*. The immunization program used is the same of the Research and Production Center of Immunobiologicals, Piraquara - Paraná, Brazil, to make the anti-loxoscelic serum. Our previously results shows that the kinetic of antibody production against *L. intermedia* venom was similar in the groups of animals immunized with different immunogens. *In vivo* neutralization assays (in rabbits) of dermonecrotic activity of *L. intermedia* venom by horse immunized sera indicate a reduction in the development of necrosis formation by the new antigens.
Conclusion: These results encourage using recombinant chimera proteins to production of efficient antivenoms to be used therapeutically.

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GENERAL BIOCHEMICAL AND IMMUNOLOGICAL CHARACTERISTICS OF THE VENOM FROM PERUVIAN SCORPION *Hadruroides lunatus*

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Introduction: This communication describes the general biochemical and immunological properties of the venom from the Peruvian scorpion *Hadruroides lunatus*, which is the most medically relevant species in Peru.
Methods and Results: The soluble venom of this scorpion is toxic to mice, the LD_{50} determined was 0.1 mg/ Kg and 21.55 mg/Kg when the venom was injected intracranial or intraperitoneally, respectively. The soluble venom displayed proteolytic, hyaluronidasic, phospholipasic and cardiotoxic activities. High performance liquid chromatography of the soluble venom resulted in the separation of 20 fractions which ranged in size from 1.5 and 80 kDa, as determined by SDS-PAGE. Anti-*H.lunatus* venom sera were produced in rabbits. Western blotting analysis showed that most of the protein content of this venom is immunogenic. Western blotting also showed cross-reactivity between *H. lunatus* sera and others scorpions venoms, such as *Tityus serrulatus, Centruroides sculpturatus* and *Androctonus australis.* This cross-reactivity was better quantified by ELISA assay, and it was observed that *H. lunatus* anti-venom displayed consistent cross-reactivity with venom antigens from the “New World” scorpions *T. serrulatus* and *C. sculpturatus.* However, a weaker reactivity was observed against the venom antigens from the “Old World” scorpion *A. australis.* Cross-reactivity between *H. lunatus* venom antigens and sera developed in rabbits against the neurotoxins from *T. serrulatus* was verified by ELISA. In human scorpionism due to *T. serrulatus* scorpion sting, the neurotoxins are responsible for the main clinical symptoms of envenomation.
Conclusion: The Peruvian scorpion *H. lunatus* is toxic to mice and its venom shown proteolytic, hyaluronidasic, phospholipasic and cardiotoxic activities. *H. lunatus* venom antibodies displayed consistent cross-reactivity with venom antigens from the new World-scorpions. The cross-reactivity

demonstrated between venoms and antivenoms of theses scorpion species, suggested the presence of common epitopes in both venoms.

Financial support: CNPq, CAPES and FAPEMIG

DEVELOPMENT *IN VITRO* METHOD TO EVALUATE THE NEUTRALIZING POTENCY OF ANTIVENOMS FOR THERAPEUTIC USE.

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Introduction: Loxoscelism is a necrotic-hemolytic syndrome caused by accidents with small arachnids belonging to the genus *Loxosceles*. The accidents represent a major public health problem in South America. Therapy has several protocols and the antivenom serum is the only specific treatment. Its effectiveness is determined by the capacity of neutralizing the dermonecrotic effect of the venom which is evidenced through tests in rabbits, an essential process to release the serum for therapeutic use. These procedures are laborious, time consuming and a large number of animals are sacrificed. Thus, the aims of this study are to propose and develop *in vitro* methods to assist in qualifying anti-loxoscelic equine hyperimmune sera. **Methods and Results:** The ELISA technique was employed, ten sera of different potency were tested against various antigens (mix of venoms, *L. intermedia* venom alone and rLiD1-recombinant dermonecrotic protein). Mapping and study of the epitopes of dermonecrotic toxins using the *Spot* method, in which membranes containing peptides covering the entire amino acid sequence of the major toxin (phospholipase-D) representative of each of the venoms of the three species *Loxosceles* (*L. intermedia*, *L. gaucho* and *L. laeta*) were tested against sera of different potencies. It was not possible to establish a direct relation between titers obtained by the ELISA and the neutralizing potency of the sera. However, the sera recognized epitopes from different regions of the proteins and the recognition of some was recurring only in high potency serum when tested in high dilution. Based on these results, three peptides were selected, synthesized, coupled to bovine serum albumin and used in an *in vitro* assay with an indirect ELISA format. Different conditions were tested, in which the composition, the antigen concentration in the plates, as well as the dilution of the sera were varied. **Conclusion:** The results obtained allow us to suggest

that the ELISA test using synthetic epitopes as antigens can be used in the evaluation of the neutralizing potency of antivenoms, because the use of synthetic peptides has allowed the correlation of the reactivity of sera presented in the immunoassay with its neutralizing potential.

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BLIMP-1 EXPRESSION BY B-1 CELLS IS INDUCED BY INFLAMMATORY STIMULUS

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Introduction: The inflammatory response is driven by signals that recruit and elicit immune cells to areas of tissue damage or infection. We previously demonstrated that B-1 cells spontaneously undergo differentiation to acquire a mononuclear phagocyte phenotype *in vitro* (Immunology.126:114-22.2009). B-1 cell differentiation into phagocytes also occurs *in vivo* in response to LPS-induced inflammation (PlosOne.7:e34570.2012). Further, LPS stimulus also induced an increase in “undifferentiated” B-1 cell population. To further characterize the B-1 cell response to inflammatory stimulus, herein we investigated the expression of surface markers and transcription factors by peritoneal B-1 cells after LPS stimulus *in vivo*.

Methods and results: BALB/c mice were inoculated (ip) with 100ul LPS (100ng/mL) or with PBS alone. After 7 days peritoneal cells were collected and submitted to flow cytometry analysis. We observed that LPS increase the total number of peritoneal cells, such as macrophages ($CD19^{-}CD11b^{+}F4/80^{+}$) and B-1 cells ($CD19^{+}CD23^{-}CD11b^{+}$). Peritoneal B cells ($CD19^{+}CD23^{+}CD11b^{-}$) were not affected. Further, B-1 cells were sorted by FACS Aria III and submitted to mRNA extraction to gene expression analysis by RT-PCR. Inflammatory stimulus by LPS decreased Pax-5, E2A and EBF expression by B-1 cells, concomitantly to augment of Pu.1 levels. LPS stimulus also increased expression of Blimp-1 by B-1 cells. Ig secretion profile by these cells is under investigation.

Conclusion: We have previously demonstrated that LPS-stimulus *in vivo* induces a differentiation of B-1 cells into a phagocyte, as well as an increase in the “undifferentiated” B-1 cell population. Interestingly, the inflammatory stimulus by LPS changes the gene expression by B-1 cells, reducing expression of B-cell transcription factors, as previously observed in B-1 cell derived phagocytes. However, we have also observed that after LPS stimulus, B-1 cells increased the expression of Blimp-1. In the B cell lineage, Blimp-1 is required for development of immunoglobulin-secreting cells and for maintenance of long-lived plasma cells. Based on these data, we suggest that inflammatory stimulus evoke a B-1 cell differentiation, not only to mononuclear phagocyte, but also into a plasma cell. These data confirm the plasticity/promiscutiy of B-1 cells in respond to inflammatory stimulus.

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MIMOTOPES OF MUTALYSIN-II FROM *LACHESIS MUTA* SNAKE VENOM INDUCE HEMORRHAGE INHIBITORY ANTIBODIES UPON VACCINATION OF RABBITS

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Introduction: Mutalysin-II (mut-II) from *Lachesis muta* snake venom is an endopeptidase with hemorrhagic activity. A mAb against mutalysin-II that neutralized the hemorrhagic effect was produced previously.
Methods and Results: To identify the mAb epitopes, sets of 15-mer overlapping peptides covering the mut-II amino acid sequence were synthesized using the SPOT method and tested but failed to react with the mAb. Using a phage-display approach seventeen clones reactive with mAb were identified. Additional immunoassays with the pep- tides and mAb identified the QCTMDQGRLRCR, TCATDQGRLRCT, HCFHDQGRVRCA, HCTMDQGRLRCR and SCMLDQGRSRCR sequences as possible epitopes.
Conclusion: Immunization of rabbits with these peptides induced antibodies that recognize mut-II and protected against the hemorrhagic effects of Lachesis venom.

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Engineered antibody against Stx1 toxin as tools for Shiga toxin-producing *Escherichia coli* immunodiagnosis

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) are recognized agents of hemorrhagic colitis/hemolytic uremic syndrome. The gold standard test for diarrheal disease caused by STEC is the Vero cells assay, in which the production of Stx1/Stx2 toxins are detected, however it requires specialized work, takes time and it is expensive, thus, unsuitable for laboratories routine diagnostic. Therefore, the development of cheap and fast diagnostic methods for these important enteric pathogens, such as immunodetection, is necessary for our reality. This work aimed the production of recombinant antibodies against the Stx1 toxin to be used in the immunodiagnosis of STEC strains. **Methods and Results:** The variable heavy and light chains sequences were obtained from cDNA of the hybridomas secreting antibodies against the Stx1 RNAm. These sequences were analyzed for CDRs and confirmed by sequencing and alignment with mouse germinal IgG. The sequence obtained was used as template for synthetic gene design. The gene was cloned in pAE expression vector for it purification. The CDRs of the chains were identified by aminoacids analysis and confirmed it is possible functional chains after the alignment with mouse germinal IgG. The cloning was successfully obtained and confirmed by restriction analysis and sequencing. Expression tests was made in 2YT broth and the recombinant antibody was detected in the soluble fraction as well as in the insoluble fraction, but more in the insoluble fraction, the denaturing condition was required to obtain and purify the antibody. **Conclusion**: Obtaining these recombinant antibodies is a promising tool for the rapid diagnosis of that pathogenic strains, as these tend to be more sensitive

and specific than monoclonal antibodies and, in addition, their production are faster and cheaper.

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PRODUCTION OF THREE MUTANTS DERIVED FROM A HUMAN ANTIBODY ANTI-CROTOXIN (scFv) WITH IMPROVED AFFINITY SUGGESTED IN SILICO

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Introduction: Crotoxin is the main toxic component of *Crotalus durissus terrificus*. It is a heterodimeric β-neurotoxin that consists of a weakly toxic basic phospholipase A_2 and a non-enzymatic, non-toxic acidic component (crotapotin). The human neutralizing recombinant anti-crotoxin scFv6 was isolated by phage display technology from a naive library of more than 10^{10} single-chain Fv (scFv). Single-chain variable fragments (scFv) consist of VH and VL domains joined by short flexible linker and may be useful as auxiliary therapy to envenoming by snake bite. In order to clarify the mechanisms of neutralization, docking and energy minimization calculations of the antibody-CTX were also conducted. From these simulations, three single changes were chosen to be mutated. Mutant (S^{30} => A^{30}) and (Y^{31} => F^{31}) have a mutation in CDR H1 and (R^{103} => H^{103}) in CDR H3. **Objective:** To produce scFvs antibodies with improved affinity viewing a possible therapeutic alternative for the local effects caused by envenoming. **Methodology:** The first mutant (S^{30} => A^{30}) was obtained by site-directed mutagenesis, while the others (Y^{31} => F^{31} and R^{103} => H^{103}) were obtained from synthetic genes with codons optimized for bacteria expression. ScFv original and mutants were cloned into pET20b+ vector and the constructions were used to transform C43 bacteria. The production of scFvs was accomplished by induction with IPTG. The mutated proteins and the original scFv were all expressed in soluble form. Periplasmic fractions were isolated through osmotic shock and further purified by Ni(2+)-immobilized metal affinity chromatography and the purity of scFvs was analysed by SDS-PAGE. Circular dichroism was performed to analyse the secondary structure of original scFv. **Results and Discussion:** Sequencing confirmed the desired mutations. Preliminary results show that all scFvs mutants presented similar expression levels. The circular dichroism of scFv revealed preserved secondary structure. ScFvs will be now analysed regarding their affinity to CTX by surface plasmon resonance (Biacore) assay.

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EXPRESSION AND BIOLOGICAL CHARACTERIZATION OF A RECOMBINANT MONOCLONAL ANTIBODY, scFv, AGAINST BaP1, A P-I METALLOPROTEINASE FROM *Bothrops asper* SNAKE VENOM

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Introduction: BaP1 is a P-I class of snake venom metalloproteinase relevant in the local tissue damage associated with envenomations by *Bothrops asper*, a medically important species in Central and parts of South America. We constructed a recombinant single chain fragment variable (scFv) monoclonal antibody against BaP1 (scFvBaP1). It contains VH and VL domains linked by a flexible $(G_4S)_3$ polypeptide. **Aim:** To express the scFvBaP1 and evaluate its capacity to neutralize important actions of BaP1. **Methods:** ScFvBaP1 was cloned into pMST3 vector in fusion with SUMO protein. Citoplasmic expression of this construction was sucessfully active in C43 (DE3) bacteria. Both scFv and SUMO (control) were analyzed by SDS-PAGE to confirm their purity. The ability of monoclonal antibody (MaBaP1) and the scFv to recognize total venom from *Bothrops asper* and BaP1 was assessed by ELISA. The capacity of scFv to neutralize fibrin degradation induced by BaP1 was evaluated using agarose gel substrate containing fibrin. The ability of scFv to neutralize BaP1-induced hemorrhage in skin mice was estimated by incubating one Minimum Hemorrhagic Dose (35 μg) of BaP1 with scFv (10:1 molar ratio). **Results:** Samples of scFv and SUMO presented bands of 38.9 and 13.6 kDa, respectively. ELISA showed that scFv was able to recognize BaP1 as well as whole venom, while SUMO did not. BaP1-induced fibrinolysis was significantly neutralized by scFv, but not SUMO, in a concentration-dependent manner (ratio 20:1 and 10:1 resulted in 73.8% and 46.7% of inhibition of the fibrin degradation, respectively). ScFv, as well as MaBaP1, completely neutralized hemorrhage induced by BaP1. **Conclusion:** Our data showed that scFv specifically recognized and neutralized biological effects of BaP1 and the whole venom of *B. asper*, while SUMO did not interfere with this ability.

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Epitope mapping of recombinant dermonecrotic protein LiD1 from *Loxosceles intermedia* spider venom using mouse, rabbit and horse antibodies reveals N-terminal antigenic region

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Introduction: Loxoscelism is a necrotic–hemolytic syndrome caused by bites of brown spiders belonging to the genus *Loxosceles.* The spiders *Loxosceles intermedia*, *L. laeta* and *L. gaucho* are a group of arachnids known as “brown spider”, with medical importance in the South and South-East of Brazil. In the state of Paraná, more than 6300 human cases of spider bite were registered in hospital centers in 2007. Many approaches for the treatment of *Loxosceles* poisoning have already been proposed, among which administration of specific antivenom is thought to be the more specific. This work aimed epitope mapping of LiD1, the major toxic component of *Loxosceles intermedia* venom using different animal sera. In the post genomic era where a large number of pathogens have been completely sequenced, it is crucial to identify B-cell epitope in an antigen for the design of subunit vaccines or create diagnosis tools against these pathogens.

Methods and Results: To characterize the recombinant LiD1 antigen, we have evaluated its immunogenicity in mice, rabbits and horses. The recombinant antigen was highly immunogenic in all three animal species, inducing antibodies that recognized the antigen and also the three medical importance *Loxosceles* species. Differences among anti-LiD1 antibodies raised in mouse, rabbit and horse were analyzed using sets of overlapping pentadecapeptides of the LiD1 amino acid sequence to identify epitopic regions recognized by the different antibodies. Despite variation in the exact location of continuous epitopes, defined by different anti-LiD1 antibodies, we found that the N-terminal portion of the protein was more antigenic than other regions.

Conclusion: The application of peptide/epitope based diagnostics and therapeutics mimicking part of protein antigen is experiencing renewed interest. In this work, we observed that antibodies produced in different species of experimental animals, obtained under the same conditions, reacted with distinct overlapping peptides in the same antigen, the major toxic component of *Loxosceles intermedia,* LiD1 protein. The characterization of the B-cell response elicited by the protein is an important factor for the identification of

candidate regions in it, which could be used as a frame to express foreign epitopes in hybrid vaccine/serum candidates. The present study is a contribution to such an approach.

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Introduction: Enterotoxigenic *Escherichia coli* (ETEC), one of the pathotypes of diarrheagenic *E. coli,* that produces heat-labile toxin (LT) and heat-stable (ST), is an important pathogen involved in diarrhea of children under five years old and tourists traveling in endemic areas. The quick and effective identification of the diarrhea contributes to the correct treatment of the infected patients. Genetic engineering has been used to obtain recombinant single chain antibodies (ScFv) on a large scale with low cost, and maintaining their functional properties. To use these constructions as tools for the immunodiagnosis it must be standardized conditions that favor the expression of ScFv. This work aims to optimize the expression of the scFv – LT fragment from ETEC cloned into the vector pSMT3, which fuses an ubiquitin (SUMO) at the cloned protein in order to improve its solubility. **Methods and Results:** The construction used in this study was obtained in a previous work and sequenced to identify the CDRs. To optimize the expression, different bacterial host cells were cultivated in LB, M9 and 2YT medium, with or without addition of 1% glucose at temperatures of 20, 30 and 37 °C. For induction, we used different concentrations of IPTG, without shaking, or ranging from 150 to 200 rpm for 2, 4 and 16-18 hours. Bacterial cells were disrupted using ultrasonic homogenizer SonoPuls Bandelin and the soluble fractions were analyzed by SDS/PAGE. The CDRs of the heavy and light chain were successfully identified. No chain mutations were found after cloning. The highest expression of the recombinant antibody was obtained in *E. coli* BL21 (DE3) cultivated in M9 medium with 1% glucose induced with 1 mM of IPTG at 30 °C for 16-18 hours at 150 rpm, and a part expressed in its soluble form. **Conclusion:** Our results are encouraging, since the consecutive changes of media and growth conditions favored the induction and expression of ScFv – LT on the soluble fraction, which can be obtained in higher yield, increasing its potential use as a tool for the diagnosis of this important group of pathogens.

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A NEW TOOL FOR ENTEROTOXIGENIC *Escherichia coli* (ETEC) DIAGNOSIS: PREPARATION AND CHARACTERIZATION OF RECOMBINANT MONOCLONAL ANTIBODIES AGAINST THE HEAT-STABLE TOXIN (scFv – ST)

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Escherichia coli associated with gastroenteritis are known as diarrheagenic *E. coli*. Amongst them, the enterotoxigenic *E. coli* (ETEC) is responsible annually for c.a. of 400 million diarrhea episodes and 700,000 of children deaths under five years and also the main cause of the "traveler's diarrhea". The identification of ETEC has been done by detection of its major virulence factors: the heat-labile enterotoxin (LT) and heat-stable (ST) using molecular biology techniques or immunoassays. When compared to other detection methods, immunoassays have several advantages they are rapid and easy tests to be performed, always showing high specificity and sensitivity. Although the monoclonal antibodies present excellent characteristics as consistency and specificity, and can be produced without limit, its production requires the use of cell culture and specialized extensive involvement of time and labor. As alternative, recombinant antibody can be engineered, which is the aim of this study. For this approach, we start from a synthetic gene, codon-optimized for expression in *Escherichia coli*. This gene was amplified in the cloning vector pGEM-T Easy and subcloned into expression vector pET28a. BL21(DE3) *E. coli* cells were transformed with the recombinant plasmid and induced with IPTG in 2YT medium for its expression. By SDS/PAGE and immunoblotting we observed that the insoluble fraction contained a large amount of antibody fragment. Therefore it was purified in affinity nickel-chromatography column in a high-pressure system (AKTA) in urea presence, followed by a refolding step. The concentration of antibody was measured and purity was analyzed by SDS/PAGE. Further the characterization was performed through functionality analysis testing by ELISA and immunofluorescence. Our results showed that the molecule is functional and no reactivity with the negative controls was observed. Moreover, it presented stability when stored at 4 °C, thus showing it as promising tool for use in the diagnosis of ETEC trough ST detection.