10: PAP Program

10.01 Third introductory course to the "Programa de Aprimoramento Profissional (PAP) da Secretaria de Estado da Saúde no Instituto Butantan"

Batista IFC[1], Horton DSPQ[2], Moraes RHP[3], Rodrigues UP[4], Santos EM[5], Spadacci-Morena DD[6]

[1]Laboratório de Bioquímica e Biofísica, [2]Laboratório de Bacteriologia, [3]Laboratório de Parasitologia, [4]Divisão de Biotério Central, [5]Núcleo de Ensino e Difusão Cultural and [6]Laboratório de Fisiopatologia, Instituto Butantan, SP, Brasil

Introduction: The "Programa de Aprimoramento Profissional (PAP)" was created in 1979 aiming to complement the formation of just-graduated people involved in the health area. In the Instituto Butantan (IBu), this program lasts 2 years and consists of 40 hours/weekly of activities under the direct guidance and supervision of specialized professionals. Since 2007, before the beginning of their laboratory activities, the students have to attend a course which is organized by a commission composed of research scientists from different laboratories of the Institute. In this course, pertinent themes are administered by research scientists, specialists and also 2nd year PAP students. In addition, the course focuses on the integration of grant recipients with the different areas of the Institute (Production, Development, Research and Museums). At the end of the course, the students have to answer an anonymous questionnaire which gives them the opportunity to express their point of view about this activity. **Objectives**: The aim of this work was to describe and evaluate the planning, organization, and application of the third course offered in 2009. **Methods:** The activities lasted 2 weeks and were divided into participating and theoretical classes distributed into an 8-h day. In this period, all of the Divisions of the IBu were presented, the Museums and the Collections were visited. Theoretical classes concerning several topics including routine equipment operation, first aid and laboratory security, animal care and ethics, and preparing solutions were presented. At the end of the course, a representative was chosen by his/her colleagues and a questionnaire was answered by the grant recipients with the objective of learning their opinion about the course. **Results and Discussion:** In 2009, 40 just-graduated students with different trainings (63% biologists, 17% biomedical researchers, 7% pharmacists and 13% other training) were received by the program, and among them 63% did not belong to IBu. The questionnaire was answered by 35 students and the average of the course grade was 8.5. The program was considered totally satisfactory to all of the grant recipients, as well as the content and workload. The helpfulness and attention of teachers and coordination staff were totally satisfactory to 77%. The main subjects of interest were museums (63%), biosafety guidelines (63%), pharmacologic assays (62%), routine equipment operation (62%), animal facility (60%), sterilization (52%), laboratory techniques (52%), bioethics (48%) and historical aspects (49%). Moreover, organization chart (46%), SUS (40%) and reagents and solutions preparation (35%) were pointed out as areas of medium interest. The feedback received at the end of the course can play an important role in the organization and improvement of the course in future years. In general, the grant recipients evaluated the course positively. Although satisfaction does not assure the learning process, certainly it stimulates the performance of the grant recipients in their laboratory activities.

All authors equally contributed to this work

10.02 Immunogenicity evaluation of porcine SP-A

Alves RF[1], Santos F L[2], Sakauchi D[4], Iourtov D[3], Raw I[1], Kubrusly FS[1], Dias SC[1]
[1]Centro de Biotecnologia, [2]Serviço de Bacteriologia, Seção Vacinas Aeróbicas, [3]Seção de Surfactante Pulmonar and [4]Serviço de Bacteriologia, Laboratório Botulínico, Instituto Butantan, SP, Brasil

Introduction: Livestock animals have made a significant contribution to human health and well-being throughout humankind's history. The chronic problem of animal-derived therapeutics, especially those of high molecular weight, is the immunogenicity induction in addition to their biosafety. Surfactant protein, SP-A, is a (calcium-dependent) lectin called collectin, which contributes significantly to surfactant homeostasis and pulmonary immunity. This highly versatile innate immune molecule is involved in a range of immune functions including viral neutralization, clearance of bacteria, fungi and apoptotic and necrotic cells, downregulation of allergic reaction and resolution of inflammation. Because of its ability to prevent or treat lung infections, SP-A appears attractive as a new therapeutic candidate to develop drugs. However, there are few studies about its immunogenicity. The immune response, in many cases, does not have clinically relevant consequences. However, in some cases the consequences can be severe and potentially lethal, causing a loss of drug efficacy or even worse, leading to autoimmunity to endogenous molecules. We have obtained pure porcine SP-A as a by-product of porcine surfactant, and now, we are determining its immunogenicity. **Objective:** In this work, the main goal was to determine the immunogenicity of porcine SP-A. **Methods:** The rejected extract of porcine lung was submitted to acid precipitation before affinity chromatography. The fractions were eluted with 0.15 M NaCl and characterized by SDS-PAGE and Western blotting. Afterward, porcine SP-A was applied to Detoxi-Gel™ to remove endotoxin. Female Swiss mice were divided into three groups. The first (20 animals) and the second (10 animals) groups received subcutaneously 100 μg SP-A and saline, respectively, on 0 and 8 days, and the third group (13 animals) received no treatment. The animals were bled one and two months after the injection. The animal's serum was assayed individually by indirect ELISA. ELISA flat-bottom plates were coated with 1 μg/mL porcine SPA in carbonate/bicarbonate buffer and incubated overnight at 4°C. Afterward, the plates were washed and blocked with PBS-T / 10% fetal bovine serum. The plates were then incubated with serum of the mice treated with SP-A, saline or control. The dilutions used were 1:10, 1:100 and 1:1000. After new washings the plates were incubated with anti-mouse peroxidase conjugated diluted 1:5000 in PBS-T and the color then developed with OPD. **Results and Discussion:** The SP-A group's serum obtained in the first and second bleeding was significantly different only with 1:10 dilution when compared with the saline or control group. Now, we are investigating the positive animal sera (1:10) to see if they contain the neutralizing antibody against native and exogenous SP-A that could preclude porcine SP-A usage in human therapy.

Supported by: PAP/SES, Fundação Butantan, FAPESP and Sadia.

10.03 Placenta transfer of anti-rotavirus antibodies from mothers to their newborns

Ampessan F, Ferreira TL, Nunes SP, Carbonare SB, Tino De Franco M
Laboratório de Imunogenética, Instituto Butantan, SP, Brasil

Introduction: Rotavirus is a major threat to children's health causing severe diarrhea in children up to five years old. Newborns (NB) are more vulnerable to disease because they dehydrate easily. The transplacental transfer of maternal immunoglobulins to the NB, together with breast-feeding, mitigates in part the deficiencies of NB antibody production. The presence of anti-rotavirus serum antibodies has already been detected but little is known about the transfer of these antibodies to the newborn including the involvement of the IgG subclasses. **Objective:** The present work was aimed to analyze the presence of IgG antibodies in serum samples from mothers and their newborns' cord blood reactive with rotavirus serotype G9 and to estimate the percentage of antibody transfer. **Methods:** Fifty pairs of serum samples were collected at the Hospital Israelita Albert Einstein (HIAE) at the time of delivery. Rotavirus and control antigens used in ELISA assay to detect IgG anti-rotavirus G9, circulating in Brazil, were obtained by ultracentrifugation. The detection of anti-rotavirus antibodies was performed by ELISA using a human serum pool as positive control. The titer was determined as the reciprocal of the dilution giving 0.5 absorbance. The ELISA titers were used to calculate the percentage of transfer of serum antibodies from mother to NB. **Results and Discussion:** Preliminary ELISA assay results performed with some of the sample pairs of showed similar titers for mother (from 31.4 to 100.4, mean of 51.0) and NB (from 31.6 to 111.5, mean of 52.0). The percentages of transfer of serum antibodies from mother to newborn were almost 100% for every sample pairs. Although our preliminary results suggest that there is maximal antibody transfer, the analysis of a greater number of samples will allow us to better evaluate the phenomena. The detection of transplacental transfer of serum antibodies directed against the rotavirus is the first step to study of the biological activity of these antibodies in rotavirus neutralization, which is essential for the planning of strategies to improve the protection of newborns.

Supported by: FAPESP, CAPES and PAP/SES.

10.04 Cellular targets of Hpv16 E6 end E7 oncoproteins in cervical cancer

Andrade FB, Kavati EA, Valadão TM, Armbruster-Moraes E, Cianciarullo AM
Laboratório de Genética, Instituto Butantan, SP, Brasil

Introduction: Today, around 120 different types of human papillomavirus (HPV) are well characterized, and the HPV16 and HPV18 are responsible for approximately 90% of all cervical cancers in humans. HPV are classified as low-risk and high-risk, the first is associated with genital warts and non-malignant lesions while most cervical carcinomas express high-risk HPV. A more significant role for malignant transformation can be assigned to the E6 and E7 genes and their respective proteins, which neutralize cellular tumor suppressor function, through degradation of p53 and pRB (retinoblastoma protein), respectively, contributing to tumor genesis. The E6 oncoprotein encoded by the virus is multifunctional showing numerous cellular targets; however, it is not clear if all these activities are related to cellular malignancy. **Objective:** In this study, we evaluated the distribution in HPV-transformed and non-transformed mammalian cells of: E6 and E7 oncoproteins, mitochondria, cytochrome *c*, transferrin receptors (TfR), transferrin (Tf) and ferritin (Fe) for the iron endocytic pathway in human and animal cells, as alternative pathway for HPV infection. **Methods:** HPV-negative cell lines were transfected with the pLXSN vectors, containing the complete sequence of E6 and E7 gene. Cells transformed by HPV were used as positive controls. The cells were plated on glass slides for adhesion, fixed with 2% paraformaldehyde in PBS, for immunofluorescence assays. Primary antibodies anti-ferritin, anti-transferrin, anti-E6 and anti-E7 and cytochrome *c* and their respective secondary antibodies were applied. The analyses were made using a laser scanning confocal microscopy (LSCM). Western blotting as control of protein expression was used. **Results and Discussion:** The antibodies recognized the E6 and E7 oncoproteins in HPV-transformed cells and in pLXSN vector transfected cells. TfR were detected in abundance at the plasma membrane of cells, while Fe was labeled in the cytoplasm, nucleus and mitochondria, and the cytochrome *c* preferentially in the mitochondria. The great amount of iron suggests the participation of this element in the HPV cell transformation, maintaining mitochondrial cytochrome *c* levels. Co-localizations of E6 in mitochondria were detected in HPV-transformed cells, suggesting its involvement in the process of apoptosis inhibition, which makes E6 a promising therapeutic target. Our results are in accordance with the findings that E6 proteins degrade Bak, an apoptogenic mitochondrial factor that undergoes a conformational change, leading to the pore formation in the mitochondrial membrane through which cytochrome *c* is released and that prevents apoptosis in keratinocytes.

Supported by: FAPESP, PAP/SES and Fundação Butantan.

10.05 Characterization of two membrane proteins of *Leptospira interrogans*

Andrade DM[1], Atzingen MV[1], Gonçales AP[2], Morais ZM[2], Vasconcellos SA[2], Nascimento ALTO[1]

[1]Centro de Biotecnologia, Instituto Butantan, SP, Brasil; [2]Laboratório de Zoonoses Bacterianas, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, SP, Brasil

Introduction: Leptospirosis is a worldwide zoonosis of human and veterinary concern. Caused by pathogenic spirochaetes of the genus *Leptospira*, the disease shows greater incidence in tropical and subtropical regions. Infection of animals or humans occurs from direct contact with urine or indirectly from contaminated water or soil. An important focus of the current leptospiral research is the identification of outer membrane proteins (OMPs). Due to their location, leptospiral OMPs are likely to be important in host-pathogen interactions, hence their potential ability to stimulate heterologous immunity. **Objectives:** The aim of this project was to express, purify and characterize two leptospiral proteins, Lp29 and Lp49, using *E. coli* as a host expression system, and to evaluate their reactivity with antibodies present in serum from hamsters experimentally infected with *Leptospira*. **Methods:** The gene sequences LIC10793 (Lp49) and LIC12892 (Lp29) were amplified by PCR using complementary sequence primers and genomic DNA of *L. interrogans* as template. The genes were cloned into the expression vector pAE, an *E. coli* vector, and the recombinant proteins were expressed in fusion with 6xHis-tag at the N-terminus, thus facilitating protein purification by metal-affinity chromatography. Protein expression was analyzed in several conditions, such as NaCl concentration induction. Gene conservation among *L. interrogans* serovars Canicola, Icterohaemorrhagiae, Grippotyphosa, Hardjoprajtino, Pomona and *L. biflexa* serovar Patoc was performed by PCR. Cellular localization was confirmed by liquid-phase immunofluorescence assay with living organisms. **Results and Discussion:** The genes, LIC10793 (Lp49) and LIC12892 (Lp29), were successfully cloned into the *E. coli* expression vector pAE, without their signal peptide sequences. The expected protein bands of 49 and 29.4 kDa, corresponding to the genes LIC10793 (Lp49) and LIC12892 (Lp29), respectively, were detected by 10% SDS-PAGE after Coomassie blue staining. The protein Lp49 is expressed in a soluble form while Lp29 is expressed in its insoluble form. Gene amplification of LIC10793 (Lp49) was detected in all serovars tested but *L. biflexa* serovar Patoc, whereas LIC12892 (Lp29) amplification was only observed in serovar Icterohaemorrhagiae and Copenhageni. The proteins were recognized in live *L. interrogans* isolates by antibodies against Lp29 and Lp49 under a confocal immunoflorescence microscope, suggesting that these proteins are surface-exposed. The reactivity of the recombinant proteins against serum samples of hamsters experimentally infected with *Leptospira* (n = 56) was evaluated by ELISA. The protein Lp29 was not recognized by antibodies, while Lp49 showed a prevalence for IgG antibodies. Further evaluation of these proteins with serum samples of confirmed cases of leptopirosis will indicate their importance in a diagnostic kit development.

Supported by: PAP/SES, FAPESP, CNPq and Fundação Butantan.

10.06 Influence of kefir on proliferation, toxin release and adhesion to human epithelial cells of pathogenic bacteria

Anjos MT[1], Andrade GR[1], Melo KCM1, Ruiz RC[1], Suazo CAT [2],Domingos MO[1]
[1]Laboratório de Bacteriologia, Instituto Butantan, SP, Brasil; [2]Laboratório de Engenharia Química, Universidade Federal de São Carlos, SP, Brasil

Introduction: Kefir is an acidic and mildly alcoholic fermented milk product that contains many functional substances. It is the product of several species of bacteria and yeast that are confined in polysaccharide matrixes of "Kefir grains." The metabolic activity of kefir produces lactic acid, acetic acid, ethanol, ethyl acetate, peptides (bacteriocins) and molecules that inhibit the growth of pathogenic bacteria. Kefir has been used empirically for more than 2000 years to combat infectious diseases. However, few studies characterizing its biological activity have been published in the scientific literature. **Objective:** The purpose of this work was to determine the effect of kefir on pathogenic Gram negative and Gram positive bacteria with regard to proliferation, toxin-α release and adhesion to human epithelial cells. **Methods**: To determine the influence of kefir on bacterial proliferation, 40 µl of *Staphylococcus aureus*, *Micrococcus luteus*, *Pseudomonas aeroginosa* or *Escherichia coli* culture previously grown in TSB media at 37° C for 18 hours were added in triplicate to 3 ml of TSB culture diluted 1/2 in kefir and incubated for 18 h at 37 °C. As control, bacterial samples were grown in TSB only. After incubation, the optical density of the cultures was measured at 600 nm and the number of CFUs was determined. The influence of kefir on the release of α toxin was determined by visualization of erythrocyte hemolysis in blood agar plates. The inhibition of bacterial adhesion to human epithelial cells by kefir was determined according to the method of Scaletsky et al. (1984). **Results and Discussion:** The results obtained in this work showed that kefir is capable of inhibiting the proliferation of pathogenic Gram positive and Gram negative bacteria. They also indicate that kefir is able to inhibit the adhesion of pathogenic bacteria to human epithelial cells, but not the release of α toxin by O6:H1 *E. coli*. The results indicate that the supernatant of kefir can be used as an anti-microbial agent in cases where antibiotics are not indicated for treatment.

Supported by: PAP/SES.

10.07 Study of the population of *Crotalus durissus linnaeus*, 1758 (Rattlesnake) in the Vale do Paraiba, São Paulo State, Brazil: distribution and morphometry

Armentano A, Calleffo MEV
Laboratório de Herpetologia, Instituto Butantan, SP, Brasil

Introduction: The genus Crotalus is distributed through the Americas from Mexico down to Argentina and is distinguished from other snakes by the presence of the rattle at the tip of the tail. The species Crotalus durissus, Linnaeus, 1758, in Brazil, recognized in seven subspecies, occurs in open areas and environments modified by humans. In the state of São Paulo, two subspecies predominate, Crotalus durissis collilineatus and C. d. terrificus. It is distributed preferentially in cerrados, fields and caatingas, excluding the littoral. The Vale do Paraíba, Cerrado and Floresta de Planalto have a high incidence of C. d. terrificus. **Objectives:** To determine if rattlesnake populations are extending their limits in the areas modified by humans and if they show a decrease or increase in abundance, based on the records of registration and collection. **Methods:** Forty-four cities had been selected whose individuals had been analyzed and the localities located. The specimens of Crotalus had been analyzed with regard to morphology, biometry and folidosis to observe the peculiarities of these snakes compared to specimens of other regions recorded from different cities of the Valley. ANCOVA was used for evaluation of the results. **Results and Discussion:** The rattlesnakes from the Vale do Paraíba are fewer and possess differences in the coloration pattern and design. The analysis of the patterns of males and females proved that there is a correlation between the number of subcaudal scales and the length of the tail in both; the males possess a bigger tail, data corroborated for test ANCOVA and also which had the presence of the hemipenis. There is an individual and geographic variation in these populations of the Vale do Paraíba. The distribution pattern of the rattlesnakes proved to be influenced by the alteration caused by humans and industrialization in the occupied habitat; therefore, great number of individuals next and inside the Serra do Mar was observed. We suggest that this has been occurring due to deforestations, occupation by humans or even the occasional introduction of specimens. The rattlesnakes of the Vale do Paraíba come showing different morphologic characters compared to the ones found in other populations, inferring that these rattlesnakes, supposedly assigned until then as C. d. terrificus, have suggestive patterns of polymorphism inside the population and thus validate a subspecies. More detailed studies can lead to the characterization of a new species.

Supported by: PAP/SES.

10.08 Partial sequence of a fraction with antimicrobial activity from *Avicularia juruensis* venom and its similarity with cystine knot toxin

Ayroza G, Tashima AK, Klitzke CF, Silva Jr. PI
Laboratório Especial de Toxinologia Aplicada – CAT/CEPID, Instituto Butantan, SP, Brasil

Introduction: Antimicrobial peptides (AMPs) are the key elements of the innate immunity against bacteria and fungi in both the animal and plant kingdoms. AMPs are an extremely diverse group of small proteins that are considered together because of their native antimicrobial activity, and their function is essential to the animal immune response. Natural animal venoms are good sources of potential antimicrobial substances, and their venoms contain a large number of diverse biologically active components of various chemical structures, such as proteins, polypeptides and amines. Cystine knot toxins (CKTs) in spider venoms represent a rich source of novel ligands for varied ion channels, and are among the most extensively studied constituents of spider venoms. They are small, compact molecules cross-linked by three to five disulfide bonds, and have molecular masses ranging from 3.5 to 7 kDa. **Objective:** The objective of this study was to identify new antimicrobial peptides and compare the sequence found in the *Avicularia juruensis* venom with the sequence found in the *Chilobrachys jingzhao* venom. **Methods:** The venom was obtained from glands of three animals, which were macerated with water and centrifuged, and the soluble part was dried by vacuum centrifugation and reconstituted with 1 mL of acidified water (TFA - 0.05% trifluoracetic acid). The soluble part was applied to HPLC reversed-phase chromatography on a semi preparative Jupiter C18 column. Elution was performed with different linear gradients of ACN/TFA 0.05% over 60 min at a flow rate of 1.5 mL. The fractions that showed antimicrobial activity (determined by a liquid growth inhibition assay against Gram-negative bacteria *Escherichia coli SBS363*, Gram-positive bacteria *Micrococcus luteus A270* and yeast *Candida albicans*) were submitted to MALDI-TOF mass spectrometry to determine the purity, and those that were not pure were re-purified by HPLC reversed-phase using a Jupiter C-18 analytical column. The pure ones were submitted to Q-TOF mass spectrometry for their sequencing. The fractions almost pure, were separated by Amicon Ultra-4 and -15 Centrifugal Filter Units – 3,000 NMWL and the material obtained was applied to MALDI-TOF to confirm the separation. **Results and Discussion:** This fraction showed antimicrobial activity against *Candida albicans, Escherichia coli* and *Micrococcus luteus*. The fraction was partially sequenced by Q-TOF mass spectrometry and was found to contain two components of 1,370 and 4,005 Da. BLAST analysis showed 71% similarity with cystine knot toxin from the Chinese Bird spider *Chilobrachys jingzhao*. The sequence obtained is the 35 amino acid sequence CAXSCNXKVNKGPCKGTNEGKCSGGWSCKFNVCVK. Apparently, the fraction submitted to Amicon Ultra-4 and -15 Centrifugal Filter Units – 3,000 NMWL was separated, but we still find some remnants of the 4,005 molecule in the 1,370 molecule. The next step is to obtain the full sequence of the fraction and synthesize it for further experiments.

Supported by: FAPESP, CNPq and PAP/SES.

10.09 Biodiversity of bacteria in *Culex quinquefasciatus* larvae in São Paulo State, Brazil

Bernardino TC[1], Pires ES[2], Esch LVS[2], Viviani A[3], Cavados CFG[2], Araújo-Coutinho CJPC[1]
[1]Laboratório de Imunologia Viral, Instituto Butantan, SP, Brasil;[2]Laboratório de Fisiologia Bacteriana, Instituto Oswaldo Cruz, Brasil; [3]Superintendência de Controle de Endemias, Brasil

Introduction: Mosquito control programs began in 1940s with dichlorodiphenyltrichloroethane, DDT, the first insecticide with residual activity, which is toxic for many insect vectors. Although DDT and other chemical insecticides are widely used, they are toxic to animals, pollute the environment and also contribute to the emergence of resistant individuals. As an alternative to chemical control, biological control of vector insects has a great efficiency, by the fact that it is a more specific method with less impact on the environment. **Objectives**: To determine the occurrence of natural infections by entomopathogenic bacteria in larvae of culicidae in São Paulo State and to establish its seasonality aspects. **Methods:** In the search for the isolation of new suppressor agents of the immature forms of *Culex quinquefasciatus*, larvae were collected every 15 days in Rio Negrinho, located in "Parque Ecológico do Tietê" in São Paulo State and in three other streamlets in the municipal district of Caraguatatuba. During 27 months, 12,221 larvae were collected, from which 441 (3.6%) were analyzed since they showed outward symptoms of infection. To eliminate external contamination, the larvae were sterilized following the method described by Alves (1986). Afterward, a homogenous extract was prepared in sterile water using vortexing. From that extract, 2 ml were removed and submitted to heating at 80°C for 10 min. Next, 100 µL were plated in BHI medium and incubated at 30 °C for 24 h; colonies with morphology similar to *Bacillus* were picked and cytological morphology was observed with a light microscope at 1500 X. **Results and Discussion:** Strains of *Bacillus thuringiensis, B. sphaericus B. amyloliquefaciens, B. subtilis, B. pumilus, B. licheniformis, B. cereus, Brevibacillus brevis, B. megaterium, B. mycoides, B. badius, B. globisporus* and *B. circulans* were identified, showing standard enzootic.

Supported by: FAPESP, FAPERJ, IOC/FIOCRUZ and PAP/SES.

10.10 The anti-aggregation protein dispersin is not involved in the establishment of the diffuse and non-characteristic adherence patterns of *Escherichia coli* on HEp-2 cells

Bevilacqua, LF, Vasconcellos, F.M, Elias, WP
Laboratório de Bacteriologia, Instituto Butantan, SP, Brasil

Introduction: The anti-aggregation protein dispersin is a 10.2-kDa protein encoded by the *aap* gene. This protein is an important virulence factor of enteroaggregative *Escherichia coli* (EAEC) and was demonstrated to be immunogenic in studies with human volunteers. Mutants in the *aap* gene express a very intense autoagglutination, indicating that dispersin acts re-covering the surface of the bacterium, diminishing the autoaggregation and allowing the bacteria to disperse along the intestinal tract. Recent findings of our group demonstrated that the *aap* gene is not exclusive of EAEC, since it was also detected in diffusely adherent *E. coli* (DAEC) and non-pathogenic *E. coli* displaying a non-characteristic adherence pattern to HEp-2 cells. **Objectives:** To determine the role of dispersin in the establishment of the diffuse and non-characteristic patterns of adherence to HEp-2 cells expressed by *E. coli* that harbor the *aap* gene. **Methods:** Three *aap*-positive *E. coli* strains isolated from children with diarrhea were selected. These strains display the aggregative adherence (AA) pattern (strain 268), the diffuse adherence (DA) pattern (strain 84) and a non-characteristic (NC) pattern (strain 901) on HEp-2 cells. The *aap* genes of these strains were mutagenized using the suicide vector pJP5603. An internal fragment of *aap* was amplified by PCR using the EAEC prototype strain 042 as template. This 232-bp fragment was initially cloned in the pGEM-T Easy vector, removed by digestion with *Eco*RI and subsequently subcloned in the suicide vector pJP5603. Conjugation of *E. coli* DH5αλpir harboring this construct and the wild type strains led to the *aap* knockout by homologous recombination. The effect of *aap* inactivation on the adherence ability of the wild type strains was evaluated on HEp-2 cells (3- and 6-h assays). **Results and Discussion:** The cloning of 232-bp internal fragment of *aap* in pGEM-T Easy generated the pLB1 plasmid. The insert of pLB2 was subcloned in pJP5603 generating the pLB2 plasmid. The *E. coli* strain DH5αλpir harboring pLB2 was conjugated with the wild type strains 268, 84 and 901, and transconjugates were selected in LB agar containing kanamycin and ampicillin (strains 84 and 268) and kanamycin and nalidixic acid (strain 901). The correct inactivation of *aap* in the transconjugates obtained was confirmed by PCR and Southern-blot analysis. The mutants selected were named 268::pLB2, 84::pLB2 and 901/Nal::pLB2. These mutants were tested in HEp-2 cells adherence assays with incubation periods of 3 and 6 h. The results demonstrate that the mutation in *aap* abolished the AA pattern of the 268::pLB2 mutant, which did not occur in the other two mutants expressing DA (84::pLB2) and NC (901/Nal::pLB2). We conclude that the product of the *aap* gene (dispersin) is involved in the establishment of the AA phenotype, but not in the DA and NC, which are mediated by uncharacterized adhesins.

Supported by: CNPq and PAP/SES.

10.11 Clinical and PTCH1 gene mutation studies in patients bearing non-syndromic multiple basal cell carcinomas

Cardoso AO[1], Souza AF[2], Ruiz IGR[2], Neto CF[1]
[1]Faculdade de Medicina, Universidade de São Paulo, SP, Brasil; [2]Laboratório de Genética, Instituto Butantan, SP, Brasil

Introduction: Basal cell carcinomas (BCC) are the most common skin cancers that affect humans. Sporadic BCCs are prevalent, often arising in people chronically exposed to UV radiation from the sun. Eventually, BCCs may be associated with different syndromes such as Bazex-Dupré-Christol, Rambo and Gorlin. Contrary to syndromic BCCs, few studies are found in the literature concerning multiple familiar BCCs. Only three families have been described to date; they showed multiple superficial BCCs but no further accompanying abnormalities. Since sporadic and Gorlin BCCs are associated with many mutations in the PTCH1 gene, we hypothesized that the multiple BCCs phenotype is also associated with mutations in this same gene. The PTCH1 tumor suppressor gene is located in the 9q22.3 chromosomal region, contains 23 exons, and has an important role in embryogenesis. **Objective:** To perform a genetic analysis of PTCH1 exons 9, 10, 11, 16 and 17. **Methods:** Eight individuals belonging to different generations from the same family (II, III, IV) were studied. Three of them bore multiple BCCs. DNA was extracted from blood leukocytes, submitted to PCR, and the PCR products were cloned (*pGEM T Easy Vector, Promega*) and sequenced (*Big Dye Terminator Kit; ABI Prism 3100 sequencer; Applied Biosystems*). The polymorphisms and mutations found were analyzed and compared to PTCH1database (www.cybergene.se/PTCH/). **Results and Discussion:** Alterations found in exon 9 included: Patient II-5 exhibited insertions nt1444(insT) and nt1465(insT), which would cause ORF *frameshift* changes, and one polymorphism 1448(T/C), which did not alter the serine amino acid (TCC and TCT). Patient II-7 showed the mutation 1421(G→T), and Patient IV one polymorphism 1421(G/T) in the same position, causing a putative substitution of serine (TCA) for alanine (GCA) [A474S]. Patient IV-3 showed the insertion nt1448 (insA), causing an ORF *frameshift*, and a polymorphism 1504(A/T), substituting phenylalanine (TTC) for isoleucine (ATC) [I999F]. As to intron 9, Patient IV-1 showed two insertions, namely nt0(insA) and nt1(intA), which would alter the *splicing* process, and one polymorphism, 27(G/A). Intron 10 was altered in Patients II-2, II-4, II-7, III-2, IV-2 and IV-4, which showed the same mutation at position 595(C→G). Besides this, Patients IV-2 and IV-3 also had one mutation 682(T→C); Patient II-2 had the mutation 639(C→G). Concerning intron 16, Patients II-2, II-4, II-7, IV-2 and IV-3 showed the same polymorphisms 9(A/T) and 20(A/T) and Patient II-4 the polymorphism 13(G/C). Exon 17 was altered only in the case of Patient II-4 with one deletion nt2995(delT), which would cause an ORF *frameshift*. In the case of intron 17, Patients II-2 and II-4 showed the same polymorphisms 30(A/T), 31(A/T), 35(A/T), 37 (A/T), and 39(A/T). Exons 9 and 17 encode the final portion of the first and second extra cellular *loops* of the *ptch* protein, respectively. Alterations in those regions may lead to changes in signaling pathways, to activate cell proliferation and tumor progression. All patients showed alterations in introns 9, 10, 16 and 17. Mutations observed in introns are poorly discussed in the literature, but recently a few papers have found that this kind of alteration may favor the development of BCCs.

Supported by: PAP/SES.

10.12 Distribution and expression of α5 integrin and cyclin D1 in actinic cheilitis and human lip squamous cell carcinoma

Carvalho AM[1], Lourenço SV[2], Menta MS[3], Neves AC[1].
[1]Laboratório de Biologia Celular, Instituto Butantan, SP, Brasil; [2] Faculdade de Odontologia da Universidade de São Paulo, SP, Brasil; [3] Faculdade de Medicina, Universidade de São Paulo, SP, Brasil

Introduction: Actinic cheilitis is the most common pre-malignant lesion of the lip and it is directly related to sun exposure. Patients who postpone seeking treatment can develop lip squamous cell carcinoma. The invasion of malignant cells in the tissue requires alterations in the extracellular matrix components and integrins. **Objectives:** To evaluate through immunohistochemical methods the expression and distribution of α5 integrin and cyclin D1 in actinic cheilits and human lip squamous cell carcinoma in different histological grades. **Methods:** Paraffin-embedded tissues of actinic cheilitis and lip squamous cell carcinoma (SCC) provided by Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo were submitted to immunohistochemistry for integrin α5 (Clone: SAM 1- Chemicon) and cyclin D1 (Clone: DCS 6 – Dako). Slides were analyzed by light microscopy. **Results and Discussion:** The majority of actinic cheilitis clinical cases showed no expression of cyclin D1. However, in areas of dysplastic epithelium, nuclear expression in basal and parabasal layers was observed. SCC showed cyclin D1 immunostaining in peripheral layers of tumor islands and strands, with the staining pattern varying according to the histological grade. This finding was expected since cyclin D1 is an activator of the cell cycle, and peripheral cells are supposed to be the most proliferative ones. The majority of actinic cheilitis cases were negative for integrin α5 but superficially invasive carcinomas and some cases of actinic cheilitis expressed this protein mainly in the granular layer and in the cells of the spinous layer which showed a large or clear cytoplasm. It is suggested that such cells are in the synthesis process since in invasive carcinomas these cells are located close to keratinized areas. Nevertheless, further studies are required to understand this process.

Supported by: FAPESP and PAP/SES.

10.13 Hemolytic and mast cell degranulation activities of the marine sponge *Amphimedon viridis* extracts

Garcia AN[1], Campos Jr AG[1], Faquim-Mauro EL[1], Franzolin MR[2], Franzolin TMP[2], Correia MD[3], Sovierzoski HH[3], Carvalho LR[4], Rangel M[1]
[1]Laboratório de Imunopatologia and [2]Laboratório de Bacteriologia, Instituto Butantan, SP, Brasil; [3]Setor de Comunidades Bentônicas, Instituto de Ciências Biológicas e da Saúde, Universidade Federal de Alagoas, AL, Brasil; [4]Seção de Ficologia, Instituto de Botânica de São Paulo, SP, Brasil

Introduction: Marine sponges are a rich source of bioactive compounds, and some of them can be useful as models for the development of new medicines and pharmacological tools. During the prospecting for new antimicrobial peptides from extracts of Brazilian aquatic organisms, *A. viridis* extracts showed strong antibacterial activity. In the present work, we studied some pharmacological activities of these sponge extracts, focusing on pore formation in biological membranes. **Objectives:** To evaluate the role and the possible toxicity of the antimicrobial extracts of *A. viridis* against mammalian mast cells and erythrocytes using assays that indicate membranolytic activity. **Methods:** The water (AvW) and methanol-water (AvM) extracts prepared from *A. viridis* specimens (collected in Maceió, Alagoas, Brazil), showed hemolytic activity against mouse (Swiss, male, 25-30g) erythrocytes prepared in a 4% suspension (v:v) in Krebs-Henseleit physiological solution (ES). The 50% effective concentrations (EC_{50}) of the extracts were: AvM: 481.6 µg/ml ES with 95% confidence intervals (CI) between 406.3 and 570.8 (n=5); AvW: 415.1 µg/ml ES, with 95% CI between 320.5 and 537.7 (n=5). The mast cell degranulation activity *in vitro* assay was carried out using a mouse mast cell line (PT 18) at $4x10^6$ cells/ml. Degranulation was determined by the release of the granule marker, N-acetyl-β-D-glucosaminidase (β-hexosaminidase), measuring the absorbance at 405 nm in a microtiter plate reader. Both extracts induced mast cell degranulation, based on the increase of the spontaneous rate of the enzyme release. At a concentration of 5 µg/ml, the extracts increased the baseline rate 100%, which can be considered a very strong effect. The activity of both extracts was dose-dependent and had similar potency. **Results and Discussion:** Toxic and cytotoxic alkylpyridinium polymers called *halitoxins* were previously identified in sponges of the same genus/family. AvW and AvM may contain a type of halitoxin, which needs to be confirmed. Fractionation and purification of the active components of these extracts were initiated to identify their chemical characteristics.

Supported by: PAP/SES, FAPESP and CNPq.

10.14 Histomorphological comparison of the skin of *Phyllomedusa distincta* and *Phasmahyla cochranae* (Anura; Hylidae; Phyllomedusinae)

Giorgi-Barsotti AM[1], Faivovich J[2], Antoniazzi MM[1], Jared C[1]
[1]Laboratório de Biologia Celular, Instituto Butantan, SP, Brasil; [2]Museu Argentino de Ciências Naturais Bernardino Rivadavia, Argentina

Introduction: Two skin gland types are present in the amphibians, the mucous and the granular (or venom) glands, which are associated with mucous production and passive chemical defense, respectively. The granular glands can form accumulations which, in the case of bufonids (toads) and hylids (tree-frogs) of genus *Phyllomedusa*, are located in the post-orbital region. In this genus it is known that a third type of gland is present associated with lipid secretion, which is supposed to act as a protection against desiccation. The genus *Phasmahyla* was described in 1980, when it was separated from the genus *Phyllomedusa*. The two genera show very similar morphology and behavior. Also, both show bright orange or reddish body sides, in an aposematic pattern. **Objectives:** To compare the skin morphology of *Phyllomedusa distincta* and *Phasmahyla cochranae* using histological and histochemical methods, aiming to determine if these genera have maintained the general pattern of skin and cutaneous glands, despite their taxonomic distance. **Methods:** Samples from the dorsal, ventral, inguinal, lateral and post-orbital skin were fixed and embedded in glycol methacrylate. The sections were stained with toluidine blue-fuchsin. Histochemical reactions were applied for mucous detection (PAS and alcian blue, pH 2.5), proteins (bromophenol blue), lipids (Sudan black B) and calcium (von Kossa). **Results and Discussion:** The chromatophores in both species show a very organized arrangement, with the more superficial layer composed of xanthophores, followed by iridophores and melanophores. The two species show very similar mucous and granular glands from morphological and histochemical viewpoints. Also, none of the species was positive for the von Kossa method. Both species were positive for Sudan black B in a specific type of gland (the lipid gland). In *P. distincta*, the post-orbital glandular accumulation was composed mainly of granular glands positioned side by side, which were much larger than the ones present in the rest of the body skin. In *P. cochranae*, despite that it was not possible to observe anything different on the skin on the outside, the examination of the internal side of the post-orbital skin showed the existence of a gland cluster, which on histology was revealed to be of the granular type. The morphological pattern of the skin in *P. cochranae* is similar to that of *P. distincta*. The chromatophore arrangement is related to the bright green color, which is characteristic of both species, and is also present in other *Phyllomedusa*. Like *P. distincta* and other species of this genus, the existence of a post-orbital gland cluster was also revealed in *P. cochranae*, even being very discrete. The positive result for bromophenol blue in the granular glands of both species indicate that they secrete mainly protein material. The negative result for von Kossa method indicates that there is no calcified dermal layer in these species, which can be related to the existence of lipid glands in the skin. The identification of the same cutaneous pattern for another genus of Phyllomedusinae besides *Phyllomedusa* can be indicative that this pattern may be a synapomorphy for the whole sub-family.

Supported by: CNPq, FAPESP, Fundação Butantan and PAP/SES.

10.15 Localization of OCT3/4 and vimentin proteins in human dental pulp

Gomes CM[1],De Cara SPHM[1], Ferreira LS[3], Marques MM[3], Neves AC[2], Kerkis I[1]
[1]Laboratorio de Genética and [2]Laboratório de Biologia Celular, Instituto Butantan, SP, Brasil; [3]Faculdade de Odontologia, Universidade de São Paulo, SP, Brasil

Introduction: Adult stem cells can be found in different tissues including blood, fat, epithelial, bone marrow and other tissues. Dental pulp is a source of stem cells composed of ectoderm-mesoderm components. It is located inside the pulp chamber and supports the formation of dentin and dentin-pulp complex. According to our knowledge, stem cells have specific anatomic locations known as a stem cell (SC) niches, which allow SC to maintain their undifferentiated state during the life of organism. We reported the isolation of immature dental pulp adult SC, which share hallmarks with embryonic stem (ES) cells (Kerkis et al., 2006). OCT3/4 protein, which is a marker of ES cells and highly expressed in early embryo at the blastocyst stage. It was intriguing to find their expression in cell culture (Kerkis et al., 2006). This finding raised the question if adult SC can really express this marker and where is the niche of these cells? **Objective:** The aim of this study was to investigate the localization of OCT3/4 and vimentin proteins within human dental pulp. **Methods:** Teeth were extracted after clinical evaluation and 5 dental pulps were isolated and submitted to histological procedures. The preserved paraffin-embedded pulps were stained by hematoxylin and eosin procedures, and were processed for immunohistochemical analyses. Two principal markers for human ES cells and mesenchymal stem cells (MSC) were used: OCT3/4 protein which is critically involved in the self-renewal of undifferentiated ES cells and vimentin, which is used as a sarcoma tumor marker to identify mesenchyme. Antibodies were purchased from Santa Cruz and Thermo Fisher Scientific. **Results and Discussion:** All samples studied showed positive immunostaining with anti- Oct3/4 antibody near the pulpal horns, surrounding blood vessels and nerve fibers. Anti-vimentin antibody reacted positively with the cells throughout the pulp tissue. Vimentin protein did not show specific localization in the pulp tissue. Our finding demonstrates the presence of OCT3/4 positive cells in the dental pulp part, which is an object of frequent dental injuries that need to be rapidly recovered.

Supported by: PAP/SES and FAPESP.

10.16 Comparative immune response: *Neisseria lactamica* and *Neisseria meningitidis* B

Gonçalves BI, Rivera HCB, Schenkman RPF
Centro de Biotecnologia, Instituto Butantan, Brasil

Introduction: *Neisseria lactamica* and *Neisseria meningitidis* are gram-negative diplococci that colonize the upper respiratory tract of humans. They are carried in the aerosol of nasopharynx secretions. *Neisseria lactamica* is a commensal microorganism while *Neisseria meningitidis* can be pathogenic causing meningococcal disease. *N. lactamica* is one of the first species from *Neisseria* to colonize the newborn pharynx. It is actually postulated that colonization by *N. lactamica*, that possibly share antigens with *N. meningitidis*, contributes to the natural development of immunity against *N. meningitidis*. During cultivation, *N. lactamica* and *N. meningitidis* release outer membrane vesicles (OMV). Studies have shown that OMV are a good inducer for immune responses against surface antigens in meningococcal disease. **Objectives:** The aim of this study was to purify OMV from both species of *Neisseria*, to obtain sera from mice immunized with OMV prepared from *N. lactamica* and OMV from *N. meningitides*, and to analyze the possibility of cross-reactivity. **Methods:** OMV are released during *Neisseria spp* cultivation. They were purified by centrifugation followed by ultracentrifugation. Groups of 5 female Swiss mice, 3 weeks old, were immunized with OMV from *N. lactamica* or *N. meningitidis* by intra-muscular injection. Each dose contained 2 μg OMV in 0.1 mL of saline. Three doses of vaccine were administered on days 0, 21, and 42. Retro-orbital bleedings were performed seven days after the last immunization. OMV from *N. meningitidis* were transferred to nitrocellulose membrane after SDS-PAGE. Antibodies were evaluated by Western blotting. **Results and Discussion:** Sera from mouse immunized with OMV from each species recognize itself and also react with the other one. The proteins recognized in each situation are different. These data indicate that there is cross-reactivity between the species.

Supported by: PAP/SES and FAPESP.

10.17 Analysis of serum cross-reactivity among PspA family 1 fragments

Goulart C[1], Darrieux M[2], Leite LCC[1].
[1]Centro de Biotecnologia, Instituto Butantan, SP, Brasil; [2]Laboratório de Biologia Molecular de Microrganismos, Universidade São Francisco, SP, Brasil

Introduction: *S. pneumoniae* is a major cause of disease. Among the vaccine candidates against this pathogen is pneumococcal surface protein A, an exposed and protective protein. Due to its structural diversity – there are 3 families and 6 clades - an effective PspA-based vaccine should include at least one fragment from each of the two major families (1 and 2). Also, it has been demonstrated that PspAs from different clades show variable degrees of cross-reactivity. In the present work, we investigated the level of cross-reaction among different PspA molecules within family 1, in order to determine the best candidate to be included in a PspA-based vaccine. Since PspA inhibits complement deposition onto pneumococci, therefore avoiding phagocytic clearance by the immune system, we evaluated the ability of the antibodies produced to abrogate PspA's function, enhancing complement deposition onto pneumococci, as a correlate of their protective efficacy. **Objective:** The aim of this study was to determine, from a panel of Brazilian pneumococcal isolates, which is able to induce the higher level of cross-reactivity within family 1. **Methods:** We produced recombinant PspA fragments from 10 family 1 pneumococci (5 of each clade), containing the whole N-terminal half of the protein. These fragments were used to immunize BALB/c mice and the sera were tested for their ability to recognize diverse pneumococcal strains bearing PspAs of clades 1 and 2 by Western blotting. The most cross-reactive antibodies were tested for their ability to enhance complement deposition on pneumococci. **Results and Discussion:** The analysis of serum cross-reactivity among PspA fragments from clades 1 and 2 revealed a significant variation in the level of recognition. Five sera able to recognize bacteria from both clades were tested for their ability to increase complement deposition on the pneumococcal surface. Of these, two led to an increase in complement deposition on strains bearing PspAs from both clades, in FACS analysis. The results indicate that antibodies made against PspAs of the same clade induce different levels of cross-reactivity. Also, sera from two PspAs, one belonging to clade 1, and another to clade 2, were able to induce greater complement deposition in family 1 pneumococcal strains, suggesting a possible protective effect. Future studies will evaluate whether the protective ability of the antibodies correlates with the cross-reactivity data presented in this work.

Supported by: PAP/SES, FAPESP and Fundação Butantan.

10.18 Synthesis and application of proline analogues in the study of peptidase inhibitors: the restricted conformation-function relationship

Hoga HE, Klitzke CF, Melo RL
Laboratório Especial de Toxinologia Aplicada - CAT/CEPID, Instituto Butantan, SP, Brasil

Introduction: Carboxypeptidases (CPs) are metallopeptidases capable of performing the most diverse functions in the body: they are involved in the digestion of food, regulation of fibrinolysis, processing of intercellular peptide messengers, regulation of peptide hormone-processing, among others. A key member of this enzymatic group is carboxypeptidase B (CPB subfamily), an enzyme that removes lysine or arginine from the C-terminus of proteins and peptides. Members of the CPB subfamily have an important regulatory role in fibrinolysis, in which thrombin activatable fibrinolysis inhibitor (TAFI) is crucial to control blood clot lysis. Because of the involvement of TAFI in fibrinolysis, this enzyme has become a valuable target for developing new drugs. The high specificity of TAFI on the recognition of arginine and lysine can be used for the development and synthesis of new amino acid analogues that combine a positively charged group with a constrained structure. In this strategy, the proline scaffold is an interesting starting point because it can provide resistance to hydrolysis that is necessary for the development of a potential inhibitor. **Objective:** Our purpose was the synthesis of eight proline analogues which have a positively charged side chain and then employ these structures in the synthesis of potential inhibitors for the CPs. **Methods:** 4-*trans*-hydroxyproline was used as the starting material for the synthesis of proline analogues. The strategies of organic chemistry include Mitsunobu, Kolbe and Staudinger reactions. All syntheses involve the SN2 reaction mechanism. The quality of intermediate and final products was obtained by chromatographic methods using TLC and HPLC. The identity of the molecules obtained was confirmed by LC-MS. Kinetics analyses were performed by HPLC. The inhibition constant (K_i) was determined using Hipp-Arg-OH as standard substrate by monitoring the conversion of hippuryl-arginine to hippuric acid and arginine. Commercial porcine CPB was used for hydrolysis assays. **Results and Discussion:** Eight non-natural amino acid analogues of proline were generated, four of them being *cis*-isomers and four *trans*-isomers: 4-aminoproline, 4-amino-methylproline, 4-guanidoproline and 4-guanido-methylproline. These molecules were obtained with purity greater than 95% by HPLC and were protected for use in solid phase peptide synthesis using either Fmoc or Boc groups. Protected amino acids were coupled with hippuric acid with good yields and generated eight inhibitors. Inhibition analyses showed that both the *cis* and *trans* structures of Hipp-4-guanido-methylproline were able to inhibit porcine CPB with K_i values in the micromolar range. This work is the first description of the use of these amino acids with a proteolytic enzyme. The two inhibitors obtained agree with previous molecular superimposition studies. Moreover, the K_i value is similar to that of the best available inhibitors, synthesized by others, based on *in silico* molecular screening. In conclusion, the 4-guanidoproline scaffold synthesized here is an extremely interesting lead moiety for further development of selective inhibitors.

Supported by: FAPESP and PAP/SES.

10.19 Immobilized metal ion affinity chromatography in FVIII and protein C purification

Iwashita C, Rodriguez ES, Oliveira DS, Carvalho RR, Raw I, Martins EAL, Cheng E
Centro de Biotecnologia, Instituto Butantan, SP, Brasil

Introduction: Factor VIII (FVIII) and protein C (PC) are proteins involved in blood coagulation. While FVIII deficiency causes a severe inherited bleeding disorder called hemophilia A, patients deficient in PC are at risk of deep vein thrombosis. PC is a member of the vitamin K-dependent (VKD) family and also presents anti-inflammatory and cytoprotective effects. Purification of these 2 proteins is important from the biotechnological point of view, because the treatment for the above mentioned deficiencies is infusion of the corresponding protein concentrate. Activated protein C is also approved for the treatment of severe septicemia. Most of the licensed plasma-derived factor VIII concentrates are produced from cryoprecipitate, which requires expensive equipments such as centrifuges and cold rooms. Alternatively, direct chromatography of plasma has been found to be particularly advantageous for fine and rapid capture of plasma proteins. In this context, we propose an ion-exchange followed by immobilized metal ion affinity chromatography (IMAC). Ion-exchange is an inexpensive separation technique while IMAC has relatively high specificity and great potential for difficult protein separations. This is essential for development of biotechnological products. **Objectives:** Development of a process for FVIII and protein C purification using immobilized metal ion affinity chromatography (IMAC) with different metal ions. **Methods:** Chromatographies: The eluate of the anion-exchange ANX-Sepharose Fast Flow (FF) column, containing FVIII and vitamin K dependent proteins, is purified by IMAC using Cu^{2+}, Ni^{2+} or Zn^{2+} as metal ligands. Analytical methods: Bradford, for protein content; chromogenic, for FVIII and protein C activities; and Western blotting for factors IX, X and von Willebrand detections. **Results and Discussion:** Analysis with IMAC- Cu^{2+}, Ni^{2+} and Zn^{2+} demonstrate that FVIII and protein C can be well separated by this method, while the separation of protein C from the other vitamin K-dependent proteins are not as efficient as the FVIII and protein C separation. Factor VIII and protein C coelute in the same chromatographic fraction from the ANX-Sepharose FF column. Using IMAC columns, these 2 proteins could be well separated. The efficient separation of FVIII and protein C in IMAC columns can be explained by the high number of histidine residues present on the surface of the FVIII protein, that lead to a tighter binding to the metal ions present in the matrix, in relation to protein C. Factor VIII has 75 and protein C 15 histidine residues. On the other hand, because of the similarity in the primary sequence, vitamin K-dependent coagulation factors share the same identical conformational features and have consequently similar physicochemical properties. Therefore, it is not surprising that the separation of protein C is very difficult.

Supported by: PAP/SES, FAPESP and Fundação Butantan.

10.20 Evaluation of *Clostridium histolyticum* growth and proteinase profile in different culture conditions

Kurihara LT[1], Pascoal SV[1], Oliveira JM[2], Souza AS[2], Carneiro PS[1], Trezena AG[2], Higashi HG[3], Prado SMA[1], Riboldi MB[4], Cassago CG[4], Fardelone LC[4] and Alegria MC[4]
[1]Serviço de Bacteriologia, [2]Seção de Vacinas Anaeróbicas and [3]Divisão Bioindustrial, Instituto Butantan, SP, Brasil; [4]Cristália Produtos Químicos Farmacêuticos, SP, Brasil

Introduction: *Clostridium histolyticum* is a facultative anaerobic Gram-positive bacteria found in soil, dust, water and intestinal contents of healthy humans and animals. This bacterium is one of those responsible for gas gangrene (clostridial myonecrosis), an infection of muscle tissue. The genera of *Clostridia* produces proteases with applications in food and pharmaceutical industries. **Objectives:** To evaluate the bacterial growth and synthesis of exogenous protein of *C. histolyticum* in different culture media. **Methods:** Cultures of *C. histolyticum* were performed in fluid thioglycollate, proteose-peptone and casein hydrolyzed, with redox agent, nutrient broth. Bacterial growth and production of proteins were evaluated in static cultures or subjected to constant homogenization. Samples were taken every 24 h. The growth kinetics was determined by optical density (OD_{600nm}) and protein profile by gel electrophoresis (SDS-PAGE). **Results and Discussion:** All cultures kept in constant homogenization showed high rates of growth, and moreover, remained more stable. The static cultures showed a sharp drop in the growth curve after 24 h and furnished a greater number of protein bands when compared to constant homogenization, indicating a supernatant with less degraded product. The cultures carried out with fluid thioglycollate medium were less efficient than proteose-peptone and casein hydrolyzed with redox agent medium, which had higher rates of protein production.

Supported by: PAP/SES, Fundação Butantan and Cristália.

10.21 Combination of whole cell pertussis vaccine and the pneumococcal surface protein A (PspA) antigen: proposal of a combined vaccine against pertussis and pneumococcal diseases

Lima FA, Moreno AT, Ferreira DM, Ferreira PCD, Miyaji EN, Ho PL, Oliveira MLS
Centro de Biotecnologia, Instituto Butantan, SP, Brasil

Introduction: *Streptococcus pneumoniae* (pneumococcus) is one of the major agents of respiratory acute diseases, accounting for about 1 million deaths per year around the world. The situation is worse in developing countries where pneumococcal diseases kill up to 800,000 children every year. PspA is a surface exposed pneumococcal antigen, shown to elicit protection against animal models of pneumococcal infections, in different vaccine formulations. We have previously shown that the whole cell pertussis vaccine (WCP) is able to act as adjuvant in combination with PspA, inducing high titers of anti-PspA antibodies, when administered to mice through the nasal route. Such formulation is also able to elicit protection against a pneumococcal respiratory lethal challenge in mice. **Objectives:** The proposal of the present work was to extend the analysis by testing the efficacy of the WCP-PspA nasal vaccination against a model of pneumococcal nasopharynx colonization in mice as well as new routes of immunization. In addition, we aimed to test the possible effects of PspA in such formulation, on immune responses directed against *Bordetella pertussis* antigens. **Methods:** C57Bl/6 or Balb/C mice were immunized with recombinant PspA in combination with WCP (produced by Instituto Butantan) through nasal or subcutaneous routes. Anti-PspA antibodies or antibodies directed against proteins present in acellular pertussis vaccine (produced by Instituto Butantan) were analyzed by ELISA and Western blotting. Pneumococcal loads in immunized mice were determined by plating nasal washes dilutions in blood-agar, 5 days after a nasal challenge with a non-virulent pneumococcal strain. **Results and Discussion:** Nasal immunization of mice with WCP-PspA was able to elicit higher amounts of anti-PspA antibodies than the immunization with PspA alone. In addition, mice immunized with the combination carried significant lower levels of pneumococci in the nasopharynx, when compared to the control groups that received saline or WCP alone. No inhibition of pneumococcal colonization was observed in mice immunized with PspA alone. As for subcutaneous immunization, 2 doses of the combination WCP-PspA were enough to elicit significant high amounts of anti-PspA antibodies which were not further increased by a third dose. Evaluation of protection against a pneumococcal lethal respiratory challenge by subcutaneous immunization with WCP-PspA is under investigation. Finally, WCP-PspA combination elicited similar levels of anti-pertussis antibodies when compared with WCP alone, as evaluated by antibodies reacting against the acellular pertussis vaccine by ELISA. The same bands were also observed in western blot, with a slightly augmented reactivity for the sera collected from the WCP-PspA vaccinated group, indicating that no deleterious interference of PspA in *B. pertussis* responses is expected.

Supported by: PAP/SES, FAPESP, CNPq and Fundação Butantan.

10.22 Preliminary studies of antistasin family molecules from the leech *H. depressa*

Mambelli NC, Lacerda-Alves J, Faria F
Laboratório de Bioquímica e Biofísica, Instituto Butantan, SP, Brasil

Introduction: Antistasin was originally isolated from the salivary glands of the *Haementeria officinalis* mexican leech. It is a molecule rich in cysteine and is about 15 kDa in size, has 119 residues of amino acids, and apart from anticoagulant activity (FXa inhibition), also has antimetastasic activity. The cDNA of antistasin was cloned and the recombinant protein expressed in baculovirus vector in insect cells. Several inhibitors similar to antistasin have been described from different animals, mainly from leech species, and therefore the antistasin-family was created. Inhibitors from this family have been described with different enzyme targets, for example, FXa (antistasin, ghilanten, therostasin, etc), trypsin (antistasin, ghilianten, hirustasin, therin, etc), elastase (guamerin I), chymotypsin (guamerin II, tessulin), and cathepsin G (jirustasin), among others. Our group has studied several compounds with activity in the hemostatic system, from *H.depressa* leech salivary complex through biochemical, transcriptomic and proteomic analysis. By transcriptomic analysis, 4 clones were detected similar to therostasin, an antistasin-family member. **Objectives:** The purpose of this work was to obtain the complete sequencing, to clone and to start the preliminary studies of expression standardization of the antistasin-family transcripts from the *H.depressa* leech. **Methods:** The selected transcripts previously cloned in pGEM11Zf plasmid were completely sequenced. Two of them were selected for subcloning in expression vector (pAE), and then these clones were expressed in *E.coli* system (BL21DE3 strain) with different IPTG concentrations and also with different induction times. The protein expression was determined by Western Blotting analysis using anti-His tag antibody. **Results and Discussion:** Through the complete sequence of the transcripts, the H01C09 and H05D10 clones were chosen to start the expression studies and showed 67 and 55 % of identity respectively with therostasin. The expression protocol standardizations showed that 0.2 mM IPTG is the best condition for expression in BL21DE3 *E.coli* for both clones. The best time of induction using this IPTG concentration was 2.5 h. The recombinant proteins were expressed in inclusion bodies and their purifications are yet to be done. Here, we described the first antistasin-family molecules from the *H.depressa* leech. In the future, we intend to characterize theses new molecules and to compare with other members of this family.

Supported by: PAP/SES and FAPESP.

10.23 E6 gene cloning of bovine papillomavirus type 1 in *Escherichia coli* for viral antigen production

Mazzuchelli-de-Souza J[1], Tofanello WS[1], Ruiz RM[1,2], Sircili MP[2], Beçak W[1], Stocco RC[1], Carvalho RF[1]
[1]Laboratório de Genética and [2]Laboratório de Bacteriologia, Instituto Butantan, SP, Brasil

Introduction: Papillomaviruses (PVs) are members of the Papovavirus family and consist of double-stranded circular DNA. PVs induce benign epithelial tumors (warts) in the skin and mucous membranes. In some cases, these tumors can undergo malignant degeneration. Among different types of bovine papillomaviruses (BPVs), BPV-1 and -2 are the only papillomaviruses that can infect a host of a different species. Horses, donkeys and mules develop sarcoid tumors as a result of BPV infection. In cattle, BPV-1 infection causes skin warts, papillomatosis in teats and udder and cancer in the urinary bladder. These diseases result in substantial morbidity and cause economic losses with affected animals. BPV-1 early open reading frames (ORFs) have been shown to encode transforming proteins. BPV-1 E6 interacts with cellular proteins changing the function of cellular regulatory proteins. **Objectives:** Cloning of E6 BPV-1 gene in a bacterial system where expression and purification of E6 protein could allow the production of biotechnological inputs. **Methods:** Specific primers were designed and the polymerase chain reaction (PCR) has been used to amplify E6 BPV-1 gene, using the genomic DNA of BPV-1 previously cloned in pAT153 vector as template. The PCR product was sequenced and analyzed to confirm E6 BPV-1 sequence and cloned into pCR4-TOPO vector. The recombinant plasmid was transformed into *E. coli* JM 109 by heat shock method. Colonies with recombinant plasmid were selected for ampicillin resistance and cultured for plasmid purification. Recombinant plasmid preparations were used for subcloning into pET 28a expression vector for *E. coli* BL21 strain transformation. **Results and Discussion:** E6 BPV-1 gene was cloned in transformed *E. coli* cells, verified as an adequate system for papillomavirus gene cloning, viral protein expression and purification by affinity. Consequently, BPV antigen production in a prokaryotic system can be useful for the development of diagnostic devices and vaccines development.

Supported by: CNPq, FAPESP and PAP/SES.

10.24 Isolation, characterization and differentiation of marmoset adult stem cells

Morais BP[1], Santos EJC.[2], Rocca M[1], Kerkis I[1] Valle RR[3]
[1]Laboratório de Genética, Instituto Butantan, SP, Brasil [2]CELLTROVET - Genética Aplicada, Centro Inovador de Empresas Tecnológicas, Brasil; [3]Departamento de Cirurgia, Anatomia dos Animais Domésticos e Silvestres, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, SP, Brasil

Introduction: The future of cell therapy, as well as tissue engineering, depends on a source of multipotent adult stem cells (ASCs). The use of animal models in ASCs research is extremely important for further knowledge in this field, considering therapeutic use or preclinical tests. There are, in the literature, some studies about genetic therapy in large primates; however, it is necessary to have a viable economical model, which is capable of an easier adaptation. These characteristics would accelerate and facilitate basic research. *Callithrix penicillata* is an excellent candidate, since it is not a threatened species and also has some advantages related to management, breeding and reproduction. **Objectives:** In the present study, we aimed to isolate, establish and characterize adult stem cells lineages from marmoset (*Callithrix penicillata*), as well as to test the capacity of these cells to undergo chondrogenic and osteogenic differentiation, *in vitro*. **Methods:** After IBAMA permission, some fragments of adipose tissue and bone marrow were collected during post *mortem* examination of two animals in a wildlife management program. The collected tissues were washed, treated and the cells isolated. These cells, here called CP, were maintained in established conditions previously described for other species, which maintain them in an undifferentiated stage. The proliferative potential of these undifferentiated cells is being tested by the cell doubling method, and immunofluorescence analyses were performed using some specific mesenchymal and epithelial markers, such as vimentin and cytokeratin, respectively. Osteogenic and chondrogenic differentiation are being studied. **Results and Discussion:** After four days of tissue treatment, the first adherent cells were noted, and after ten days of culture we could see a satisfactory quantity of CP. At this point, the cells were trypsinized, expanded and part of them cryopreserved. There were established cultures of adipose tissue and bone marrow-derived stem cells. These cultures showed stem cells characteristic with potential for colony formation. The established cells showed fibroblast-like morphology and a high proliferative potential. These cells were positive for anti-vimetin and negative for anti-cytokeratin, suggesting their mesodermal origin. The osteogenic and chondrogenic differentiation were initiated and during the first day of assays, CP changed their morphology toward the proposed lineages. Although this is a preliminary study still being developed, the cells obtained from marmoset adipose tissue and bone marrow showed relevant characteristics of previously established ASCs, which highlighted the importance of studying *Callithrix penicillata* stem cells. CP will provide important data to understand the factors which affect the establishment of multipotent cell lineages, as well as differentiation mechanism and also the therapeutic potential of each of them.

Supported by: FAPESP, CNPq and PAP/SES.

10.25 Biofilm formation of enteropathogenic *Escherichia coli* strains by the adhesion assay on glass surface in shaken cultures

Mota CM, Culler HF, Franzolin MR
Laboratório de Bacteriologia, Instituto Butantan, SP, Brasil

Introduction: The association of pathogenic bacteria in biofilms is considered an important factor in causing disease in the human host. Biofilm consists of bacterial communities formed by multiple layers of microorganisms, being of different species or not, and they are a natural barrier that protect the bacteria against the influence of UV light, osmotic stress, heat, starvation, acids, detergents, antibiotics, phagocytes, antibodies, and bacteriophages. A better understanding of biofilm formation would be essential to know their role in pathogenesis, enabling the development of alternative therapies. **Objective:** The aim of this study was to determine the capacity of biofilm formation by typical and atypical enteropathogenic *E. coli* strains through adhesion assay on glass surface in shaken cultures and compare with biofilm formation using the colorimetric assay with crystal violet (OD). **Methods:** We analyzed 10 atypical and 10 typical enteropathogenic *E. coli* strains isolated from children with acute diarrhea. To determine pellicle in standing rich culture interface, bacterial growth was examined visually in 5 ml of the following culture media: Luria-Bertani broth (LB) without sodium chloride, brain heart infusion broth (BHI), and *E. coli* broth at 37°C for 48 h with shaking (210 rpm). The optical density (OD) assay was performed with crystal violet using polystyrene 24-well culture dishes and an enzyme immunosorbent assay plate reader at 595 nm. **Results and Discussion:** The cultures that formed fragile pellicles were characterized by the presence of a pronounced ring at the air-glass interface, which could be easily disrupted into flocks by shaking, whereas rigid pellicles could not be disrupted by shaking. Cell aggregation at the air-solid interface was observed in 60% of the aEPEC strains growing in BHI and 30% in LB without sodium chloride, and in *E. coli* broth there was 100% pellicle formation. With respect to the tEPEC strains, the pellicle formation was higher in the *E. coli* broth, with 80% of pellicle presence. Still with these strains, pellicle formation was observed in 50% in BHI and 40% in LB without sodium chloride. The crystal violet assay for aEPEC strains showed 30% of the strains in the high biofilm formation range (OD = 0.044 – 0.500), and the tEPEC strains showed 80% of strains in the low biofilm formation range (OD = 0 – 0.043). The aEPEC, with only 10% more than tEPEC, showed superior capacity to form biofilm in the air-liquid surface, independent of the medium. The results of the biofilm formation assays indicated that the potential to adhere to glass surfaces in shaken cultures is common in *E. coli* but is obviously influenced by the nutrients, observed in the positive results obtained in *E. coli* broth, which has specific nutrients supporting a better growth of these isolates. However, biofilm production of the pathotypes analyzed (influenced by nutrient status) enables the organism to attach and adhere to glass surface, independent of their OD observed in the colorimetric crystal violet assay, a presumptive test used to select the samples better-producing biofilms. The adhesion assay on glass surface effectively confirm the capacity of biofilm formation of the strains analyzed.

Supported by: PAP/SES.

10.26 Enterotoxigenic *Escherichia coli* (ETEC) expressing-heat-labile toxin (LT) show differences in cellular localization

Ozaki CY; Rocha, LB; Piazza RMF.
Laboratório de Bacteriologia, Instituto Butantan, SP, Brasil

Introduction: Enterotoxigenic *Escherichia coli* (ETEC) is a major cause of travelers' diarrhea which affects tourists that visit endemic areas. ETEC is also responsible for at least 400 million acute diarrhea episodes and 700,000 childhood deaths per year. One of the most important virulence factors of ETEC is the production of heat-labile toxin (LT) and/or heat-stable toxin (ST). These toxins differ in their structure and function and both are used as markers for detection of infection. The heat–stable toxin (ST) is released into the culture medium, but the cellular localization of heat-labile toxins (LT) is uncertain. Some authors report that LT's localization is periplasmic, while others affirm that it is secreted via vesicles. The localization of these toxins will allow a fast and easy diagnosis of ETEC infection. **Objective:** To determine the LT toxin localization in human pathogenic ETEC strains. **Methods:** Sixty-six ETEC strains, 51 that produce only the LT toxin and 15 that produce LT and ST toxins were grown in *E. coli* medium overnight at 37 °C with shaking (250 rev min^{-1}). These strains were centrifuged at 13,200 rpm, 4 °C for 10 min, and then the supernatants were collected. The pellets were treated with 1 mL 8 M urea buffer for 1 h at 37 °C with shaking, centrifuged at 13,200 rpm, 4 °C for 10 min. The supernatant was stored at –20 °C. Both bacterial culture supernatants and pellet-treated supernatants were tested by capture ELISA in plates coated with 30 μg/mL rabbit enriched IgG fraction. The detection was developed with 74 μg/mL anti-LT IgG2b monoclonal antibodies, after incubation with anti-mouse IgG conjugated to horseradish peroxidase and OPD plus hydrogen peroxide; the absorbance was measured at 492 nm. **Results and Discussion:** The LT toxin was detected in the supernatant in 16.6% of the strains; in the pellet the toxin was detected in 65.1%, while 18.2 % of the strains showed it in both fractions. The LT toxin produced by ETEC strains was found mainly in the periplasmic space, but some strains secreted it into the culture medium. For ETEC diagnosis, both fractions should be tested, as the ST toxin is also released into the culture media.

Supported by: FAPESP and PAP/SES.

10.27 Analysis and determination of the prevalence of *Cryptosporidium* (Aplicomplexa: Cryptosporidiidae) oocytes in the feces of colubrids donated to Instituto Butantan

Paiva PRSO[1], Lassen P[1], Alves JAS[1], Fernandes W[1], Catão-Dias JL[2], Grego KF[1]
[1]Laboratório de Herpetologia, Instituto Butantan, SP, Brasil; [2]Departamento de Patologia Animal, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, SP, Brasil

Introduction: Protozoa of the genus *Cryptosporidium* (Aplicomplexa: Cryptosporidiidae) are intracellular parasites that infect a diversity of vertebrate species, and there is transmission between different species of the same order. The oocytes vary in size between species (*C. parvum 5.2x4.6* µm, *C. serpentis 6.2x5.3* µm), and they comprise the infective form where transmission is oral-fecal, and they are resistant to environmental variations and a number of disinfectants. *Cryptosporidium* affects the gastric mucosa of reptiles. Some papers describe the infection in Brazilian snakes (*Boa, Corallus, Epicrates, Crotalus),* confirming the diversity of species that are at risk for infection. In snakes, cryptosporidiosis causes gastroenteritis, gastric hypertrophy, anorexia, regurgitation and weight loss, resulting in death. The protozoa are present in the microvillosities of the gastric epithelia, causing mucosal hyperplasia with areas of necrosis; fibroplasia and collagenization of the submucosa and lamina propria also occur. There are subclinical cases where the infected animal continuously eliminates oocytes in the feces. This fact, in association with the lack of effective treatment against this protozoa, is responsible for euthanasia as a form of control in collections and breeding farms. **Objective:** The objective of this work was to define the incidence of *Cryptosporidium* in recently captured colubrids donated to Instituto Butantan, destined to the feeding of *Micrurus,* utilized in the production of elapid antivenom serum . **Methods:** The feces of 64 young colubrids, distributed in five genera and six species, was analyzed: *Sibynomorphus mikanii* (n=36, 20 males/16 females), *Oxyrhopus guibei* (n=11-3m/8f), *Tomodon dorsatus* (n=11-6m/5f), *Liophis miliaris* (n=4-2m/2f), *Phylodrias patagoniensis* (1m), *Phylodrias olfersi* (1f), from various localities of the state of São Paulo. Samples of the animal feces (5g) were collected and submitted to the Ritchie or the formol-ether methods and afterwards stained using the modified Ziehl-neelsen method. Interpretations were made using a light microscope (40–100x100). **Results and Discussion:** Out of the 64 colubrids that were analyzed, 31% showed *Cryptosporidium ssp* oocytes in their feces, with the following occurrences in the species: *L. miliaris,* 50%; *T. dorsatus,* 36%; and *S. mikanii,* 39%. In the species *O. guibei, P. patagoniensis* and *P. olfersi* the results were negative. In some species, the *Cryptosporidium* oocytes were more frequent in females than in males: *T. dorsatus (*17%m–60%f), *S. mikanii* (25%m–56%f). According to the information obtained, a greater infection rate is observed in the species *L. miliaris.* Perhaps the direct contact of these animals with water can favor contamination since *Cryptosporidium* is largely transmitted through water contaminated with its oocytes and they remain infective for months. Histological examinations of the gastric mucosa will confirm whether there is an established parasite-host relationship or whether the oocytes found originate from other animal orders that contaminate the colubrids in their natural environment.

Supported by: FAPESP, CNPq/INCTTOX and PAP/SES.

10.28 Standardization of the activities of maintenance, safety and sanitation in a semi-intensive snake farm at Instituto Butantan

Paiva PRSO, Lassen P, Alves JAS, Grego KF, Sant'Anna SS, Fernandes W.
Laboratório de Herpetologia, Instituto Butantan, SP, Brasil

Introduction: The standardization of activities in an external area involved in the maintenance of animals to obtain input for the production of immunobiological products, is aimed at establishing procedures to ensure the safety of technicians and the welfare of animals. This reduces the chances of accidents and environmental contamination by external agents, and ensures the quality of the final product. Together with the original purpose of producing venom for the manufacture of antivenom serum and immunobiological products, semi-intensive handling can improve research in the areas of herpetology. **Objectives:** To establish procedures for the maintenance and sanitization of the physical space, materials and equipment; to define protocols for feeding, reproduction, venom extraction, entrance and exit of new animals, disposal of garbage, establishment of prophylactic measures and adequate sanitary barriers in the facilities of the snake farm for the control of environmental conditions of all the species kept. **Methods:** Initially, adequate physical facilities were constructed for each species kept, according to the needs of each group and maintaining the specifications of the environmental laws governing the maintenance of snakes in captivity. Subsequently, the flow of activities and protocols was defined for management, feeding, reproduction, venom extraction, entrance and exit of animals and euthanasia, and for the procedures for the use, cleaning and sterilization of facilities, equipment and materials for semi-intensive breeding, and disposal of garbage, according to the ethics standards of the Institute. **Results and Discussion:** Currently, physical and environmental adaptations were made and 24 protocols were established: 14 for maintenance and eight for cleaning, sterilization and disposal, as well as for the complete equipment, PPE's, materials and products used in the activities of the external area. Nowadays, several genera of Squamata are being kept, including *Pantherophis*, *Crotalus*, *Bothrops*, *Oxyrhopus*, *Sybinomorphus* and amphisbaenids. The protocols and procedures established with this work are being employed in the semi-intensive area and its facilities, and their effectiveness will be evaluated by the number of accidents, the annual number of snake deaths and the amount of poison produced per animal. In the future, these numbers will be compared with the data obtained before initiation of these proposed activities.

Supported by: INCTTOX/CNPq and PAP/SES.

10.29 Cytotoxic effects of crotoxin and its subunits on murine melanoma and fibroblast cells

Paioli FS[1], Guzovsky EM[1], Oliveira JE[2], Spencer PJ[2], Vassão RC[1]
[1]Laboratório de Biologia Celular, Instituto Butantan, SP, Brasil; [2]Centro de Biotecnologia, Instituto de Pesquisas Energéticas e Nucleares, SP, Brasil

Introduction: Crotoxin is the most abundant and active toxin from the venom of the Brazilian rattlesnake *Crotalus durissus terrificus*. It has been described as a neurotoxin involved in pre-synaptic blockade of neuromuscular junctions. It is a heterodimer formed by an acidic subunit (crotapotin) which has no enzymatic activity, bound to a basic protein (PLA2) that displays low toxicity. However, the heterodimer shows high toxicity which is ascribed to the interactions of the acidic subunit, acting as a chaperone and the catalytic counterpart that displays phospholipase A2 activity. Some authors have also attributed a direct cytotoxic effect to this toxin, and have suggested its use as a therapeutic agent against tumor cells. **Objectives**: In the present work, we investigated the cytotoxic effect of crotoxin and its subunits on a murine melanoma cell line and on normal fibroblasts. **Methods:** Cells (5×10^5 /ml) were grown in 96-well microplates. After 24 h, the cells were incubated with increasing concentrations of crotoxin, ranging from 7.8 to 500 μg/ml, previously diluted in RPMI with 10% fetal bovine serum. After 24 or 48 h, the toxin- containing medium was then replaced by MTT in phosphate-buffered saline and incubated for 3 h. The formazan crystals were then solubilized. Similar assays were performed using the isolated subunits, based on the molar ratio of the toxin components, thus ranging from 4.7 to 300 μg/ml for PLA2 and from 3.1 to 200 μg/ml for crotapotin. **Results and Discussion:** Our results for the 48-h assays indicate that crotoxin is highly toxic to the melanoma cells inducing cell death even at the lowest concentration (7.8 μg/ml) while the fibroblasts were only affected with 30-fold higher concentrations (250 μg/ml). The 24-h assays indicate that the cytotoxic effect is not immediate, since no detectable changes were observed for the concentrations that induced cell death in the 48-h experiment. The assays with PLA2 and crotapotin showed that the catalytic subunit displays toxic activity on the tumor cells, but that the concentration of the enzyme used to induce cytotoxicity was much higher than for the whole toxin, and that although dissociated from the targeting peptide, specificity was still preserved, since the control non-tumor cells were not affected.

Supported by: PAP/SES.

10.30 Gelatinase production by *Clostridium histolyticum* in different culture conditions

Pascoal SV[1], Kurihara LT[1], Oliveira JM[2], Souza AS[2], Carneiro PS[1], Trezena AG[2], Higashi HG[3], Prado SMA[1], Riboldi MB[4], Cassago CG[4], Fardelone LC[4], Alegria MC[4]
[1]Serviço de Bacteriologia, [2]Seção de Vacinas Anaeróbicas and [3]Divisão Bioindustrial, Instituto Butantan, SP, Brasil; [4]Cristália Produtos Químicos Farmacêuticos, SP, Brasil

Introduction: *Clostridium histolyticum* produces proteases of medical and industrial interest, such as gelatinases, which are enzymes that act in the process of degradation and synthesis of extracellular matrix. The usual gel electrophoresis technique (SDS-PAGE) detects protein levels but not enzyme activity. Enzymatic activity, including gelatinase, can be detected through a specific method called zymography, an electrophoretic technique used for assessing specific proteolytic activity under non-reducing conditions. **Objectives:** To evaluate the gelatinases produced by *C. histolyticum* using different times and culture media. **Methods:** Gelatinase production by *C. histolyticum* grown under anaerobic conditions was analyzed in three culture media, fluid thioglycollate, proteose peptone and trypticase-soy broth, and for 24, 48 and 72 hours. Gelatinolytic activity was analyzed by zymography by enzyme renaturation in SDS-PAGE, incubation with gelatin solution and staining with Coomassie Brilliant Blue. **Results and Discussion:** Samples collected from *C. histolyticum* supernatants revealed four bands with gelatinolytic activity in gelatin zymography. Preliminary results showed that supernatants from proteose-peptone medium and trypticase-soy broth have higher gelatinolytic activity than with fluid thioglycolate. It was observed that gelatinolytic activity in high molecular weight bands, produced in trypticase-soy broth and proteose-peptone medium, has similar intensity. The lower molecular weight bands were more active in trypticase-soy broth. These results suggest that the protease expression profile can depend on the media used for *C. histolyticum* growth. Furthermore, these different gelatinases can result in new industrial applications.

Supported by: PAP/SES, Fundação Butantan and Cristália.

10.31 Morphological analysis of the inguinal gland clusters of genus *Zachaenus* (Anura, Cycloramphidae)

Pinto EG[1], Antoniazzi MM[1], Verdade VK[2], Carvalho, RMH[3], Rodrigues MT[2], Jared C[1]
[1]Laboratório de Biologia Celular, Instituto Butantan, SP, Brasil; [2]Departamento de Zoologia, Instituto de Biociências, Universidade de São Paulo, SP, Brasil; [3]Instituto de Ciências Biológicas, Universidade Federal de Juiz de Fora, MG, Brasil

Introduction: Amphibian skin is characterized by the presence of glands spread over their whole body which are basically of two types: mucous glands, primarily related to respiration, water balance and reproduction, and granular glands, related to defense by venom production. The frog family Cycloramphidae presently comprises 14 genera, including *Cycloramphus* (with 26 species) and *Zachaenus* (with 2 species). The two genera are very similar and considered a monophyletic group. These anurans are restricted to Atlantic rainforest remnant areas, mainly in southeast and south Brazil and can be recognized by the roundish and robust body, wide and flat head and non-visible tympanum. Their biology is poorly known, but one of the most conspicuous characters in the genus *Cycloramphus* is the presence in males of a pair of discoidal gland clusters in the inguinal region which could be related with the production of pheromones. In a recent morphological analysis of the two species of *Zachaenus*, we identified in the males clusters of glands in the inguinal region which are much smaller and more fragile than those of *Cycloramphus* and visible only when the inner side of the skin is examined. Surprisingly, when examining the same region in *Zachaenus* females, gland clusters were also apparent, similar to those observed in males. **Objective:** The aim of this work was the morphological and histochemical analysis of the inguinal gland clusters of males and females of *Zachaenus*. After comparing the glands in both genders, for phylogenetic purposes, we determined if they were morphologically comparable to the inguinal gland clusters of *Cycloramphus* males. **Methods:** The inguinal skin containing the gland clusters were carefully removed from males and females of *Zachaenus parvulus*, fixed in Karnowsky fixative and embedded in glycol methacrylate. The histological sections were stained with toluidine blue-fuchsin and submitted to PAS, alcian blue pH 2.5, bromophenol blue and von Kossa histochemical methods. **Results and Discussion:** Although present in females, these inguinal gland clusters are much smaller than those of males. These clusters, in both genders are mainly composed by a type of gland, larger and with a different aspect when compared to the glands present in the rest of the body skin. These differentiated glands are roundish, syncytial and full of a homogeneous secretion, which is positive with bromophenol blue staining and negative to PAS and alcian blue. The positive result for von Kossa method revealed the presence of a thin and continuous calcified dermal layer underlining these glands. The results demonstrated that the gland clusters of *Zachaenus* are very similar in both genders and show a histological and histochemical similarity with the inguinal clusters of *Cycloramphus*, although being much smaller. These results give support to recent phylogenetic analysis indicating that the genus *Zachaenus* should be synonymized with the genus *Cycloramphus*. A biochemical analysis of the secretion present in both genera could confirm these results and give more evidence of their use as semiochemicals.

Supported by: FAPESP, CNPq and PAP/SES.

10.32 Characterization of gelatinases from *Scolopendra viridicornis* centipede venom

Prezotto Neto JP[1], Guizze S[2], Knysak I[2], Barbaro KC[1].
[1]Laboratório de Imunopatologia and [2]Laboratório de Artrópodes, Instituto Butantan, SP, Brasil

Introduction: Centipedes are arthropods belonging to the class Chilopoda. About 3,300 species of these animals are known, which can be found on all continents, except Antarctica. Their body is segmented and elongated, adapted to penetrate narrow spaces. In the natural environment, centipedes live under rocks, decomposing tree trunks and underground galleries. On the other hand, they are also found in urban areas probably due to the abundance of prey and sites to hide, rendering human beings susceptible to their bites. The main symptoms of envenomation by centipedes are pain, erythema and edema. It is described in the literature that *Scolopendra* spp. venoms have many enzymes that degrade components of extracellular matrix. **Objectives:** The objective of this work was to characterize gelatinases from *Scolopendra viridicornis* centipede venom. **Methods:** Specimens of *S. viridicornis* were collected in Tocantins State (Brazil), and animals were maintained in captivity and milked by electrical stimulation in order to obtain the venom. To purify gelatinases from this venom, the first step was size-exclusion chromatography (Superdex 75 GL column) followed by ion exchange chromatography (Mono S 5/50 column). To analyze the enzymatic activity, zymography was employed using gelatin (2 mg/mL) as substrate in a polyacrylamide gel (10%). SDS-PAGE (12%) was used to evaluate the protein profile of the peaks eluted after chromatography. **Results and Discussion:** *S. viridicornis* venom was fractionated in a Superdex 75 colunm, and three peaks (A, B and C) were obtained. These peaks were chromatographed on Mono S 5/50 column. Peak A contained an enzyme with gelatinase activity, showing a molecular weight around 190 kDa, eluted with approximately 0.31 M NaCl. Two gelatinases were detected in peak B, with about 200 and 35 kDa, eluted with 0.045 and 0.10 M NaCl respectively. In peak C, another gelatinase was observed with approximately 56 kDa, eluted with 0.13 M NaCl. Other enzymes with weak gelatinolytic activity were also detected, and their molecular masses were around 80 and 117 kDa. These data confirm that *S. viridicornis* venom has many gelatinases that degrade components of extracellular matrix, which may contribute to local symptoms observed in human envenomation.

Supported by: INCTTOX/CNPq, FAPESP and PAP/SES.

10.33 Construction of pseudoparticles expressing GPV rabies protein

Ribeiro LC, Lemos MAN, Pereira CA, Jorge SAC
Laboratório de Imunologia Viral, Instituto Butantan, SP, Brasil

Introduction: Rabies virus glycoprotein (GPV) has been recognized as an antigen able to induce neutralizing antibodies, conferring protective immunity against rabies. Gene expression in cells has been a powerful tool in biotechnology and several biological products have been generated through the construction of gene vectors that upon cell transfection can be expressed and give rise to active proteins. Some viral particles (viral like particles-VLP or pseudoparticles-pp) have been produced in cell culture to express different viral proteins of different viruses. In this work we used ppHCV-GPV to study the GPV expression in human cells. **Objectives:** To establish an expression system using pseudoparticles (pp) with E1 and E2 HCV glycoproteins, GAG and POL proteins of MLV (murine leukemia virus) and mRNA of rabies virus glycoprotein. **Methods:** The pTGGPV vector was constructed by digestion of GPV DNA fragment extracted from pMtiGPV and by ligation with pTG13077. Three vectors (pTGGPV, pGagPol, pE1E2) were co-transfected in HEK 293 cells, producing ppHCV-GPV. **Results and Discussion:** We obtained the pTGGPV vector. This vector has the GPV gene under control of the cytomegalovirus promoter and the MLV encapsidation signal. We produced pseudoparticles containing GPV mRNA in HEK 293 cells after co-transfection procedure. Samples of pseudoparticles will be used to infect hepatocarcinoma cells (Huh7.0). The GPV produced in infected cells will be measured by ELISA essays. The preliminary results with EGFP expression showed the system to be efficient, but there is a need for better results. Therefore, we constructed the pTGGPV vector containing GPV mRNA. This system will help to analyze its efficiency and can be used to infect model animals to determine the immunological responses. We can construct another vector, containing the NS3 HCV gene. This vector will express the NS3 protein, helping to enhance our knowledge of the immunologic mechanisms involved with HCV infection.

Supported by: PAP/SES.

10.34 Functional studies on the toxin-antitoxin system VapBC from *Leptospira interrogans*

Sanson AL, Lopes LM, Nakajima E, Fraga TR, Cainelli Gebara VCB, Martins EAL, Lopes APY.
Centro de Biotecnologia, Instituto Butantan, SP, Brasil

Introduction: Toxin-antitoxin (TA) modules are bicistronic operons that encode a stable toxin and an unstable antitoxin. The TA *loci* are ubiquitous in prokaryotes including archaea. Their general function remains controversial but the most probable hypothesis is the cessation of growth under conditions of nutritional or environmental stress. The *vapBC* operons constitute the largest family of bacterial TA modules and they are grouped together due to homology of a PIN domain of the toxin which is thought to act as ribonuclease. In fact, ribonuclease activity was observed in VapC from *Haemophylus influenzae,* and this activity was inhibited by VapB. On the contrary, it was shown that VapC from *Mycobacterium smegmatis* controls bacterial growth via inhibition of translation and that it does not show any ribonuclease activity. We have cloned the *vapBC locus* of *L. interrogans* and expressed the cognate proteins in order to study their properties. **Objective:** To biochemically and functionally characterize the TA system VapBC from *L. interrogans.* **Methods:** The genes LIC12659 (VapB), LIC12660 (VapC) and the locus *vapBC* were amplified from leptospira genomic DNA and cloned into pAE and pAEsox expression vectors for protein expression in *E. coli* BL21 (DE3). Proteins were purified by Ni^{+2}-Sepharose chromatography. VapC was refolded by dialysis or pressurization. Recombinant proteins were analyzed by SDS-PAGE. Antibodies anti-VapB and anti-VapC are being raised in mice. The growth of clones of *E. coli* containing the constructs with *vapB, vapC* and *vapBC* were followed by OD_{600nm}. Ribonuclease activity was tested by incubating protein samples with approximately 1 μg of purified total RNA from *E. coli* in Tris buffer pH 8.0 and analyzed by agarose electrophoresis. **Results and Discussion:** Approximately 15 mg of purified VapB and VapC were recovered per liter of culture. Growth curves of clones of *E. coli* containing the plasmid constructs were compared. The expression of the toxin VapC reduced severely the growth rate in comparison to the clone expressing the antitoxin VapB. Also, the co-expression of VapB and VapC restored the growth rates, suggesting that VapC is toxic to the bacteria and that VapB neutralizes its toxicity. The affinity between the toxin and its cognate antitoxin was tested by an affinity chromatography (Ni^{+2}-Sepharose) after applying the extract of *E. coli* co-expressing VapB and VapC. The co-elution of VapB+VapC (VapBC) was analyzed by SDS-PAGE indicating that VapB binds to Ni^{+2} through its His tail and that VapC might be tightly bound to VapB. Unexpectedly, our assays demonstrated that ribonuclease activity was present in VapB and VapBC samples, but not in VapC. Control assays were performed using other recombinant proteins obtained the same way as VapB and VapC, showing that this activity was not due to the presence of contaminant ribonucleases from the process. Our present hypothesis is that the inhibitory effect of VapC on the growth of *E. coli* is not related to the ribonuclease activity as previously supposed but is due to an inhibition of translation as proposed by other authors. At present, we are raising specific antisera against VapB and VapC to be used in ribonuclease tests, in order to confirm the activity of VapB and to better understand the properties of the leptospira TA module.

Supported by: FAPESP, PAP/SES and Fundação Butantan.

10.35 Preliminary scorpion catalog of Pará State, Brazil

Silva APS[1,2,3], Souza CAR[1], Chalkidis HM[3], Silva JM[4], Pardal PPO[5], Dorce VAC[2], Candido DM[1]

[1]Laboratório de Artrópodes and [2]Laboratório de Farmacologia, Instituto Butantan, SP, Brasil; [3]Laboratório de Pesquisas Zoológicas, Faculdades Integradas do Tapajós, PA, Brasil; [4]FUNASA, Laboratório Entomologia Santarém, PA, Brasil; [5]Centro de Informações Toxicológicas de Belém, PA, Brasil

Introduction: Studies about arachnological composition indicates about 1600 known species of scorpions are deposited in museums and scientific collections in the world, of which 150 occur in Brazil. The organization of a consistent information base of the scorpion's diversity is found in scientific collections and can be available in manuals, taxonomic keys and/or catalogs. The world's recent compilation of scorpion taxonomy is the "Catalog of the Scorpions of the World" published in 1998, which includes Brazilian species. Later, many species were described, especially from the Amazonian region, which unites the greatest diversity of scorpions of the country, but this region still needs more studies since specific projects about Brazilian scorpion fauna are concentrated in southern and southeastern regions. In addition, knowledge about northern region scorpions is poor, for example, the scorpion fauna from Pará. **Objectives:** To catalogue the species of scorpions from Pará. **Methods:** The work was based on neotropical scorpion fauna database from Laboratório de Artrópodes of Instituto Butantan, São Paulo and Arachnological Collection of Laboratório de Pesquisas Zoológicas, Faculdades Integradas of Tapajós, Santarém, Pará. **Results and Discussion:** The research showed four families, 10 genera and 30 known species, which are listed below: Bothriuridae: *Bothriurus araguayae* Vellard; Buthidae: *Ananteris balzanii* Thorell, *A. luciae* Lourenço, *A. pydanieli* Lourenço, *A. cachimboensis* Lourenço, Mota & Al; *Isometrus maculatus* (De Geer); *Rhopalurus amazonicus* Lourenço, *R. crassicauda* Di Caporiacco, *R. laticauda* Thorell; *Tityus carvalhoi* Melo-Leitão, *T. clathratus* C. L. Koch, *T. evandroi* Mello-Leitão, *T. gasci* Lourenço, *T. metuendus* Pocock, *T. obscurus* (Gervais), species responsible for the greatest number of accidents in Pará, *T. paraguayensis* Kraepelin, *T. raquelae* Lourenço, *T. silvestris* Pocock, *T. strandi* Werner, *T. tucurui* Lourenço; Chacthidae: *Broteochactas parvulus Pocock; Brotheas amazonicus* Lourenço, *Brotheas gervaisi*, Pocock, *Brotheas overali* Lourenço, *Brotheas paraensis* Simom, *Brotheas silvestris* Lourenço, *Guyanochactas goujei* (Vellard), *Guyanochactas mascarenhasi* (Lourenço); *Hadrurochactas mapuera* (Lourenço) and Liochelidae: *Opisthacanthus cayaporum* Vellard. Besides contributing to the knowledge of North Brazil's scorpion biodiversity, this study enabled us to prepare a scorpion catalog for Pará, increasing the studies in this group, which will be helpful in zoological research and benefit the local, academic and scientific community, with historical and current information about nomenclature and geographical distribution of scorpions from Pará.

Supported by: PAP/SES.

10.36 Distribution and expression of α3, β1 and β4 integrins in actinic cheilitis and human lip squamous cell carcinoma

Silva FH[1], Lourenço SV[2], Menta MS[3], Neves AC[1]
[1]Laboratório de Biologia Celular, Instituto Butantan, SP, Brasil; [2]Faculdade de Odontologia and [3]Faculdade de Medicina, Universidade de São Paulo, SP, Brasil

Introduction: Actinic cheilitis represents an early clinical stage of a continuum that ultimately may progress to become squamous cell carcinoma of the lip. Integrins are a family of heterodimeric cell membrane adhesion molecules which mediate cell-cell and cell-matrix interactions. They play a fundamental role in the maintenance of tissue integrity and in the regulation of cell proliferation, growth, differentiation and migration. It is not surprising; therefore, that integrins have been implicated in tumor progression and metastasis. **Objectives:** To evaluate using immunohistochemical technique the expression and distribution of α3, β1 and β4 integrins in actinic cheilits and human lip squamous cell carcinoma in different histological grades. **Methods:** Paraffin-embedded tissues of actinic cheilits and lip squamous cell carcinoma provided by Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo were submitted to immunohistochemistry for integrin α3 (Clone: P1B5- Chemicon), integrin β1(Clone: P4G11- Chemicon) and integrin β4 (Clone: ASC3- Chemicon). Slides were examined with a light microscope. **Results and Discussion:** The majority of cases of actinic cheilitis and superficially invasive squamous cell carcinoma showed lack of expression of α3, β1 and β4 integrins in basal and parabasal layers of epithelium. In areas of dysplastic epithelium, a loss of expression in the cells of the granular and spinous layers was also observed. An analysis of invasive squamous cell carcinoma slides showed a loss of immunoexpression in peripheral layers of tumor islands and strands. This study suggests that loss of integrin expression may be related to proliferation and migration of tumor cells, during the early stages of progression of human lip squamous cell carcinomas.

Supported by: FAPESP and PAP/SES.

10.37 A monoclonal antibody against BaP1, a metalloproteinase from *Bothrops asper* venom, provides a rapid and efficient purification of BaP1 from total venom

Silveira CRF[1], Faquim-Mauro EL[1], Moura-da-Silva AM[1], Gutiérrez JM[2], Rucavado A[2], Fernandes I[1]

[1]Laboratório de Imunopatologia, Instituto Butantan, SP, Brasil; [2]Faculdade de Microbiologia, Instituto Clodomiro Picado, Universidade da Costa Rica, San José, Costa Rica

Introduction: BaP1, isolated from the venom of the snake *Bothrops asper,* a medically important species in Central America, is a 22.7-kD P-I class of snake venom metalloproteinases (SVMP). It may play an important role in the local tissue damage associated with *B. asper* envenomations. This enzyme exerts multiple tissue-damaging activities, including hemorrhage, myonecrosis, dermonecrosis, blistering, and edema. Its isolation is achieved by a combination of chromatographic procedures. Our group produced and characterized 4 different IgG MoAbs against BaP1 (MABaP1) recognizing only conformational epitopes. **Objective**: To obtain a method to purify BaP1 from total venom using a single method using anti-BaP1 monoclonal antibody. **Methods:** CNBR-activated Sepharose-4B was coupled with MABaP1-8 as suggested by manufacturer. A total of 5 mg of crude *B. asper* venom were added to MABaP1-8-Sepharose and incubated for one hour at room temperature. After successive washes, the bound fraction was eluted with glycine/HCl buffer, pH 2.8. The purity of the eluted fraction was analyzed by SDS-PAGE. **Results and Discussion:** SDS-PAGE of eluted fraction obtained by batch using MABaP1-8-Sepharose showed only one band with molecular mass of 22 kDa corresponding to BaP1. Presently, we are using BaP1 isolated by the technique described here to investigate the role of monoclonal antibodies in neutralizing toxic effects of BaP1.

Supported by: CNPq/INCTTOX and PAP/SES.

10.38 Cloning and sequencing of V_H and V_L immunoglobulin chains from a hybridoma against the metalloproteinase BaP1, isolated from *Bothrops asper* venom, in order to produce a recombinant antibody

Silveira CRF[1], Faquim-Mauro EL[1], Clissa PB[1], Rucavado A[2], Moura-da-Silva AM[1], Gutiérrez JM[2], Magalhães GS[1], Fernandes I[1]

[1]Laboratório de Imunopatologia, Instituto Butantan, SP, Brasil; [2]Faculdade de Microbiologia, Instituto Clodomiro Picado, Universidade da Costa Rica, San José, Costa Rica

Introduction: Poisoning by snake venoms is a major health hazard in tropical and subtropical regions and serum therapy remains the only specific treatment for decades. Although whole antibody is successfully used for specific treatment of the snakebite envenomation, many recipients may develop adverse reactions. Therefore, a better therapeutic antibody has been sought, and recombinant single-chain variable fragment antibodies (*scFv)* may be a promising alternative due to their advantages since these are smaller, have higher specificity and are easily genetically manipulated. ScFv contains the variable domain of heavy (VH) and light (VL) chains linked by a flexible $(G_4S)_3$ polypeptide. Fragments are expressed in bacteria and purified using regular protein tags; they show lower cross-reactivity due to the absence of invariable regions, consistency and reproducibility. Hybridomas producing monoclonal antibodies constitute a valuable source to produce scFv antibodies. **Objective**: To clone V_H and V_L immunoglobulin chains from hybridomas against BaP1, a metalloproteinase isolated from *Bothrops asper* venom, showing an important role in local tissue damage. **Methods:** Total mRNA was isolated from $5x10^6$ MABaP1 mouse hybridoma cells and purified by affinity chromatography on oligo (dT)-cellulose (QuickPrep *Micro* mRNA Purification Kit, Pharmacia Biotech). The purified mRNA was transcribed into cDNA using the reverse transcriptase (Superscript III) and the amplification of variable light (V_L) and heavy (V_H) chain of the antibody was performed using the Light and Heavy primers from Amersham Biosciences. These amplicons were cloned into pGEM-T Easy vector and sequenced. **Results and Discussion:** The cDNA sequence of V_H and V_L chains was amplified and cloned, and their sequence showed high homology with *Mus musculus* immunoglobulin. We are now constructing the complete scFv by joining V_H and V_L sequences to be expressed in a bacterial system.

Supported by: CNPq/INCTTOX and PAP/SES.

10.39 Circulating platelets: are they involved in the local injury induced by *Bothrops jararaca* snake venom?

Yamashita KM, Senise LV, Nogueira TO, Giorgi R, Gonçalves LRC, Cirillo MC, Sano-Martins IS, Santoro ML
Laboratório de Fisiopatologia, Instituto Butantan, SP, Brasil

Introduction: Besides their major role in hemostasis, blood platelets also participate in the inflammatory response. Individuals bitten by adult *B. jararaca* (*Bj*) may manifest hemostatic disturbances and/or intense edema at the site of the bite. Furthermore, the higher the edema intensity is, the lower the platelet count will be in individuals bitten by *Bj* (Santoro et al., *Toxicon* 51:1440, 2008). **Objective:** To analyze the possible importance of circulating platelets in the local injury caused by *Bj* venom. **Methods:** Male Wistar rats were rendered thrombocytopenic by two experimental models, by (1) i.v. administration of polyclonal antibodies to rat platelets (generation of immune complexes) or (2) i.p. administration of busulfan, a drug used to inhibit megakaryocyte growth (20 mg/kg). Control animals received control IgG or polyethylene glycol 400, respectively. Thereafter, *Bj* venom (7 μg/100 μL) was i.pl. administered in the right hind paw, and edema, local hemorrhage, hyperalgesia and albumin leakage were evaluated; the left hind paw received saline. Edema was measured by measuring paw thickness using a caliper at 0.5, 1, 2, 4, 6 and 24 h after venom administration. Local hemorrhage was evaluated at 2 and 24 h by detection of cyanmethemoglobin. Rats were also evaluated by pain threshold after i.pl. injection of *Bj* venom, using paw pressure method. Vascular permeability was evaluated by Evan's blue method. Complete blood count was also determined in blood samples of animals. To determine platelet function in *Bj*-induced edema in non-thrombocytopenic animals, using pharmacological procedures, edema was evaluated in rats injected with aspirin (300 mg/kg, p.o., 18 h prior to *Bj* injection) or the platelet specific $P2Y_{12}$ receptor antagonist clopidogrel (10 mg/kg, p.o., 3 h prior to *Bj* injection). **Results and Discussion:** Platelet counts were statistically diminished in rats administered anti-platelet IgG (± 75%) or busulfan (± 89%) in comparison with control rats. Edema intensity was statistically diminished in animals treated with anti-platelet IgG (around 26% at 1 h, persisting up to 24 h). Vascular permeability was similar in rats treated with anti-platelet or control IgG at 1 h. Edema intensity was similar in animals treated with busulfan, clopidogrel or aspirin in comparison with animals treated with the respective vehicles. Hemorrhage at 2 h after venom injection was statistically higher in the hind paws of thrombocytopenic rats compared to controls. Remarkably, the characteristic *Bj*-induced hyperalgesia was completely blocked in animals treated with anti-platelet IgG 1, 2 and 4 h after venom administration. Our data showed that decreased edema intensity was noticed after generation of immune complexes, when animals were previously treated with anti-platelet IgG. However, when animals were previously treated with busulfan, clopidogrel or aspirin, platelets played no role in the pathophysiology of edema induced by *Bj* venom. The involvement of circulating platelets in the hyperalgesic response induced by *Bj* venom is still under investigation.

Supported by: FAPESP, CNPq/INCTTOX, Fundação Butantan and PAP/SES.