EDITORIAL

The 66th volume of "Memórias do Instituto Butantan" presents the proceedings of the XI Annual Scientific Meeting of Butantan Institute, organized by the Division for Scientific Development. The theme of this 11th edition of the Instituto Butantan´s meeting was "Scientific Research and Public Health Challenges".

The scientific program addressed important issues regarding emerging and reemerging problems of public health, and the directions of the scientific research in order to triumph over these challenges. Three round-table, discussions and conferences were planned in order to instigate productive discussions on our current public health problems and innovative research approaches suitable for this matter.

Also, the meeting included three poster sessions, Young Scientist Awards in four categories (scientific initiation, PAP program, master and doctoral degrees) and two special sessions about the memories of Instituto Butantan, also linked to this year´s theme.

This 66th volume brings together all the abstracts of the posters presented in the Poster Sessions, organized in ten different areas: Venoms and Envenomations, Biochemistry, Immunology and Vaccines, Microorganisms, Cellular Biology and Genetics, Animal Biology, Education and Scientific Diffusion, Others, Scientific Initiation (PIBIC) and PAP Program. The impressive number of submitted posters indicates the involvement of our scientific community with the Annual Scientific Meeting. These abstracts are representative of the scientific research that was carried out at Instituto Butantan in the last year. Therefore, this "Memórias do Instituto Butantan" electronic issue is an important source of scientific information and dissemination.

Waldir Pereira Elias Junior
Editor-in-Chief

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1. Venoms and Envenomations

1.01 Characterization and validation of a cDNA library from venom glands of *Bothrops jararaca* for the analysis of differential gene expression

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Introduction: The venom gland of the Brazilian venomous snake *Bothrops jararaca* (Crotalinae, Viperidae) is an exocrine gland related to the salivary gland. Its characteristics include a central lumen where the venom produced by secretory cells is stored. When the venom is lost from the gland, the secretory cells are activated and new venom is produced. The production of new venom is triggered by the activation of noradrenaline on both α_1- and β-adrenoceptors in venom gland. Synthetic activity of the secretory cells and mRNA concentration peak at 4-8 days after venom is lost. Afterward, synthetic activity decreases and venom gradually accumulates in the gland lumen. Expressed sequence tags (ESTs) are informative fragments from DNA sequences, obtained from the complementary DNA (cDNA) derived from reverse transcription of messenger RNA (mRNA) of an organism, tissue or even a single cell type. The construction of a cDNA library from the venom glands of snakes provides a collection of specific transcripts and allows inferences about the gene expression pattern of this high specialized tissue. The aim of this work was to construct a cDNA library of the venom gland of *Bothrops jararaca* and validate this library for use in the construction of cDNA nylon arrays. **Methods:** Two adults of *Bothrops jararaca,* one male and one female, were anesthetized and decapitated. The venom gland was removed and frozen in liquid nitrogen. The RNA was extract using TRIZOL ^{R} (Invitrogen). The poly A^+ RNAs were obtained by purification with oligo dT-cellulose. The cDNA library was constructed using *SuperScript^{TM} Plasmid System for cDNA Synthesis and Plasmid Cloning* (Invitrogen). The cDNA library was sequenced, processed and analyzed using specific programs. **Results and Discussion:** We sequenced 384 clones, and after bioinformatics treatments, 297 sequences with high quality were analyzed using BlastX and BlastN, 263 sequences were indentified, 70,72% toxins clones and 29,28% cellular clones (normalized data). Among the toxin clones, we found the major toxin classes (metaloproteinases, 14.52%; PLA2, 11.29%; serine proteinases, 17.2%; C-type lectins, 33.87%), and as expected most of the cellular clones are involved in transcription and translation process (35.06%), which should be important for the further characterization of cell regulation. This library proved to be representative and was used for the construction of the cDNA nylon arrays containing 4500 clones. These arrays are now being probed against messenger RNAs from different times of the venom gland cell cycle in order to define which genes are differentially expressed.

Supported by: FAPESP and CNPq.

1.02 Sex-related differences in the antinociceptive effect of crotalphine, a peptide activating κ and δ opioid receptors

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Introduction: Sex differences in pain sensation and in opioid analgesia have been observed in human and rodents. Crotalphine (CRP), a peptide obtained from *Crotalus durissus terrificus* venom, induces analgesia by activation of opioid receptors. Due to its analgesic properties, pre-clinical trials with CRP are now in progress. However, studies have been always developed in male animals, with no information about its effectiveness in female rodents. **Objectives:** In the present study, differences in nociception and in the analgesic effect of CRP between male and female Wistar rats were evaluated. **Methods:** Differences in nociception and in the analgesic effect of CRP were evaluated using acute and chronic experimental pain models. Acute hyperalgesia was induced by intraplantar (i.pl.) injection of prostaglandin E_2 (PGE_2) into one of the hind paws. Neuropathic pain was induced by chronic constriction of the sciatic nerve (CCI) and characterized by the presence of hyperalgesia and allodynia, 14 days after surgery. Mechanical hyperalgesia and allodynia were determined using the rat paw pressure test or von Frey filaments, respectively. CRP (p.o.) was administered immediately before the hyperalgesic agent, or on day 14 after surgery. To determine the influence of the estrous cycle, vaginal smears were examined using 10x and 40x objectives. To determine whether the sex-related differences in nociception and crotalphine-induced antinociception were the result of the effects of gonadal hormones, female rats were submitted to ovariectomy. **Results and Discussion:** Female rats responded to lower hyperalgesic doses of PGE_2 than did males. In PGE_2-induced hyperalgesia, females responded to lower analgesic doses of CRP (p.o.) than did males. In females, the peptide, at 0.008 or 5 μg/kg, suppressed PGE_2-induced hyperalgesia for up to 3 or 6 days, respectively, whereas in males, CRP inhibited hyperalgesia for up to 3 h (0.2 μg/k) or 5 days (5 μg/kg). CRP was also more effective in inhibiting neuropathic pain in females than in males; however, in the CCI model, there were no sex differences in the duration of the analgesic action of the peptide. The antinociceptive action of CRP is mediated, in males and females, by activation of κ- and δ-opioid receptors. To determine the influence of gonadal hormones, females were ovariectomized (OVX) 11 days before the tests. The nociceptive behavior of ovariectomized rats was similar to that of male rats. In contrast, the analgesic effect of CRP was more pronounced in intact females than in the OVX group. Hormonal replacement restored the pain threshold and the effectiveness of CRP in females. The estrous cycle phase did not interfere with pain threshold and with CRP effect. These data indicate that sex differences could be observed between male and female rats in relation to pain threshold. Despite displaying opioid activity, CRP is more effective in females than males. The higher effectiveness of CRP in females is related to the presence of ovarian hormones.

Supported by: FAPESP.

1.03 Relaxation effects of *Tityus bahiensis* scorpion venom on rat isolated aorta

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Introduction: The study of scorpion venoms of the *Buthidae* family is of interest not only to better understand the pathophysiology of envenomation, but also because these venoms contain neurotoxins, which act mainly on Na^+ and K^+ ion channels, modifying their gating properties. In this way, scorpion venoms have an important role in the study and characterization of ion channels and neurotransmission mechanisms. **Objectives:** We have previously observed that *Tityus bahiensis* scorpion venom induces a relaxation of pre-contracted rat aorta, so the aim of the present study was to better characterize this relaxation. **Methods**: Different concentrations of venom (3; 30; 300; 500 µg/ml) were added to rat aorta rings pre-contracted with noradrenaline (0.3 µM). Venom effect (300 µg/ml) was also analyzed in the absence or presence of different antagonists and blockers [tetrodotoxin (0.3 µM); atropine (1 µM); suramin (0.1 mM); caffeine (0.1 mM); L-NAME (0.1 mM)] and in rings where the endothelium was removed (n=6). **Results and Discussion:** The venom promoted intense relaxation of pre-contracted rings only when higher concentrations of 300 µg/ml (52.66±5.00%) and 500 µg/ml (51.56±7.40%) were tested. The action of 300 µg/ml of the venom was inhibited in the presence of L-NAME and suramin, or in rings without endothelium ($p<0.05$, ANOVA followed by Tukey's test). On the other hand, tetrodotoxin, atropine or caffeine was unable to abolish the relaxation caused by the venom, excluding the participation of tetrodotoxin-sensitive Na^+-channels, acetylcholine or adenosine. These data indicate that *Tityus bahiensis* scorpion venom causes relaxation of rat aorta by an action mediated by endothelium and that ATP and nitric oxide release could be involved in venom actions.

Supported by: CAPES, FAPESP and Fundação Butantan.

1.04 Metalloproteinase inhibitors can control damage induced by sphingomyelinases from *Loxosceles laeta* spider venom in human keratinocytes

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Introduction: Sphingomyelinases D (SMases D) are considered the main toxic component present in *Loxosceles* spider venom. The best-known features of the local and systemic reactions induced by *Loxosceles* venoms are local pain, swelling and necrosis, sometimes followed by bleeding from persistent wounds, intravascular hemolysis and renal failure. More specific information about the molecular mechanisms operating during envenomation is needed to develop measures aimed at improving treatment of loxoscelism victms. **Objective:** In this study, the possible association of cell death and matrix metalloproteinase (MMP) expression was investigated. **Methods:** In order to analyze the toxic effects, $2x10^4$ HaCat cells were incubated with increasing concentrations of *L. laeta* venom or recombinant SMase D in the presence or absence of tetracycline and galardin. On the third day, cell viability was analyzed by MTT. Supernatants recovered from HaCat cells, during the period of treatment with venom or SMase D, in the presence or absence inhibitors, were analyzed by gelatin zymography. The action of venom or toxin, on the expression of keratinocyte cell surface molecules was determined after two hours of incubation in the presence of inhibitors, by flow cytometry. **Results and Discussion:** Results show that venom and the recombinant SMase D were able to induce morphologic alterations and loss of viability of human keratinocytes, in a dose-dependent manner. SMase D induces the activation of endogenous metalloproteinases, such as MMP-2, MMP-7 and MMP-9, which were positively associated with reduced cell viability. Besides, induction of EGFR, EPCR, MCP and MHCI cleavage was detected after treatment with the toxins, by action of proteases of the ADAM family. Damage to keratinocytes, induced by SMase D of *L. laeta*, could be controlled by the use of metalloproteinase inhibitors, such as tetracycline and galardin. Our results suggest that endogenous metalloproteinases, from MMP and ADAM families, are induced by the action of SMase D on human keratinocytes; these proteases may play an important role in the development of the dermonecrotic reaction induced by *Loxosceles* venom.

Supported by: FAPESP and CNPq.

1.05 Review of accidents caused by snakes of the tribe Tachymenini Bailey, 1967 (Dipsadidae, Xenodontinae) at Hospital Vital Brazil, Instituto Butantan

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Introduction: Snakebites reach 20,000 cases a year in Brazil. Most of these accidents are caused by venomous snakes, represented by: *Bothrops*, *Crotalus*, *Lachesis* and *Micrurus*. However, 40% of all accidents are caused by non-venomous snakes, most of them of Dipsadidae family. Among these non-venomous snakes, some species can cause substantial local effects in humans. We point out the green snake (*Philodryas olfersii*), *Elapomorphus quinquelineatus* (Dipsadidae, Elapomorphini), *Clelia*, *Boiruna*, and some Tachymenini species. Accidents caused by members of the tribe Tachymenini are a neglected subject. **Objectives:** A survey of snakebites caused by Tachymenini snakes that were treated at Hospital Vital Brazil, Instituto Butantan, São Paulo, Brazil. **Methods:** Between 1990 and 2006 48 cases of snakebites were recorded with the following information: sex, age, site of the bite, and symptoms of the victim, and time of the accident. When available, data on snake biology were gathered. **Results and Discussion:** We recorded forty-eight accidents caused by *Tomodon dorsatus* (n= 34), *Thamnodynastes* spp (n= 12), *Ptychophis flavovirgatus* (n= 1), and *Gomesophis brasiliensis* (n= 1). The accidents were more frequent in the spring (n= 18) and summer (n= 14), probably because of intense reproductive and feeding activity during these seasons, although 10 accidents occurred in the autumn and winter seasons. Accidents were prevalent in males, aged from 15 to 40 years, and the regions of the body most affected were feet and hands, including fingers. Usually, these accidents cause pain and local/discrete edema, which regressed in a few hours. However, in one case caused by *Thamnodynastes strigatus* on the index finger of an adult male, there was formation of a hemorrhagic bubble similar to bothropic bite. The species of the genera *Thamnodynastes* and *Tachymenis* can cause substantial local symptomatology in humans, and these accidents deserve not only the attention of health services but also research into the composition and action of its venom.

1.06 Characterization of isolated domains generated by P-I/P-II SVMP precursors

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Introduction: Snake venom metalloproteinases (SVMPs) are zinc-dependent proteolytic enzymes that cause hemostatic alterations in humans, following snake bite accidents. According to their domain structure, SVMPs are classified into three major classes, P-I (metalloproteinase domain only), P-II (metalloproteinase and disintegrin domains) and P-III (metalloproteinase, disintegrin-like and cysteine-rich domains). Proteolytic functions are described mainly for P-I and P-III SVMPs and only a few P-II class metalloproteinases have been studied. We have partially isolated and sequenced a SVMP from *Bothrops neuwiedi* venom, called BnP1, whose partial sequence showed similarity to SVMPs derived from P-II precursors. In this regard, BnP1 could be an interesting model to study the biosynthesis and function(s) of P-II SVMPs. **Objectives:** To purify BnP1 and its corresponding disintegrin, evaluating their capacity in inhibiting platelet aggregation, and to identify the complete cDNA sequence of this putative P-II SVMP by cloning and sequencing cDNAs encoding SVMPs from a *B. neuwiedi* venom gland. **Methods:** BnP1 was purified by FPLC molecular exclusion chromatography (Superdex 75 HR 10/30, pH 7.8) followed by anion exchange chromatography (Mono-Q HR 5/5, pH 7.8). Purification was monitored by relative molecular mass (SDS-PAGE) and fibrinolytic activity (fibrin-agarose plates). Disintegrins were obtained by reverse phase chromatography (Vydac C18 column-HPLC) monitored by inhibition of platelet aggregation. Partial protein sequences were obtained by MS/MS. The inhibition of platelet-aggregation was determined by challenging human platelets with ADP, collagen, thrombin and ristocetin. For cDNA cloning and sequencing, the RNA was extracted from a *B. neuwiedi* snake venom gland. cDNAs were amplified by PCR using primers based on conserved regions of SVMPs, cloned into pGEM–T Easy plasmid. The DH5α transfected colonies were selected by PCR using SVMP-specific primers and the purified plasmids were sequenced. **Results and Discussion:** BnP1 was obtained as a single 25-kDa band, and two new disintegrins were isolated and called D2 and D4, which inhibited platelet aggregation induced by all agonists. BnP1 did not show any significant inhibition of platelet-aggregation. Two distinct cDNAs of each class of SVMP were cloned representing the P-I, P-II and P-III complete sequences. BnP1 partial sequence aligned with high similarity both with P-I (69 out of 73 residues sequenced by MS) and P-II (67 out of 73 residues sequenced by MS) cDNAs. A previous disintegrin sequence identified by MS aligned (100%) with a P-II cDNA, which also encodes BnP1 sequences. Despite sequence similarity with P-II catalytic SVMPs, BnP1 did not show any significant effect on platelet aggregation. Only the isolated disintegrins were able to inhibit platelet aggregation by all tested agonists. The sequence analysis indicated that BnP1 may be coded either by P-I or P-II mRNAs since they encode very similar catalytic domains. This suggests that biosynthesis of low-molecular mass SVMPs is still unclear and may involve rearrangements and processing in different biosynthetic steps rather than the selection of different genes for each SVMP subclass.

Supported by: CNPq/INCTTOX, FAPESP.

1.07 Antiviral activity of the hemolymph of *Lonomia obliqua* (Lepidoptera: Saturniidae)

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Introduction: Insects are regularly exposed to environmental contaminants. Several studies have detected the presence of peptides, enzymes and metabolites in the hemolymph of insects that curb the proliferation of bacteria and fungi. The immune response of insects to bacterial or fungal infections and to filarial or parasitoid infestations is well documented. However, unlike vertebrates, insects have none of the well-characterized mechanisms for recognizing viruses or virus-infected cells. **Objectives:** The objectives of this work were the identification, isolation, characterization of the hemolymph protein from *L. obliqua* and the evaluation of its antiviral activity against human viruses. **Methods:** The protein responsible for antiviral activity was isolated, purified by gel filtration chromatography using a gel filtration column system (Superdex 75) and further fractionated using a Resource-Q ion exchange column system. Influenza (H1N1), polio (Sabin 1) and measles (Edmonston) viruses were used to determine the antiviral activity of the hemolymph. VERO cells were infected with the measles and polio viruses and MDCK cells with the the influenza virus on the 3rd day post-inoculation at an MOI of 0.1. End-point dilution assays were performed in 96-well microtiter plates to measure virus titers. **Results and Discussion:** Potent antiviral activity against measles, influenza and polio viruses was observed in the hemolymph of *Lonomia obliqua*. Experiments with the purified protein led to a 157-fold reduction (from $3.3 \pm 1.25 \times 10^7$ to $2.1 \pm 1.5 \times 10^5$ $TCID_{50}$ mL^{-1}) in measles virus production and a 61-fold reduction (from $2.8 \pm 1.08 \times 10^9$ to $4.58 \pm 1.42 \times 10^7$ mL^{-1}) in polio virus production. Heating and freezing seemed to have no influence on its antiviral activity. The protein did not display virucidal activity and did not act on receptors on the cell membrane. The observations suggest an intracellular mechanism of action and that the protein may act as a constitutive agent that affects the innate antiviral immune response.

Supported by: FAPESP.

1.08 A rat model of bone cancer pain induced by intrafemoral inoculation of Walker 256 carcinoma cells – analgesic effect of crotalphine

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Introduction: Crotalphine, a peptide first identified and isolated from the South American rattlesnake *Crotalus durissus terrificus*, induces analgesia mediated by the activation of δ- and κ-opioid receptors. **Objective:** The aim of this work was to characterize the analgesic effect of crotalphine in a new rat model of bone cancer pain induced by intrafemoral inoculation of Walker 256 carcinoma cells. **Methods:** Rats were injected with carcinoma cells into the femoral cavity. Bone metabolic alterations were determined by scintigraphy, using ^{99m}Tc-MDP, which is significantly concentrated in areas of osteogenesis. Femoral images were obtained before and 7 and 14 days after tumor cell inoculation. Bone cancer pain was characterized by the presence of hyperalgesia and allodynia, determined using the rat paw pressure test or von Frey filaments, respectively. **Results and Discussion:** Incorporation ^{99m}Tc-MDP was significant 7, 14, 21 days after tumor cell injection, suggesting tumor development in the femoral cavity. Hyperalgesia and allodynia were detected on days 1, 3, 7, 14 and 21 after cell inoculation. Interestingly, we observed that paw withdrawal threshold in von Frey test was reduced not only in the ipsilateral hind paw inoculated with the tumor, but also in the contralateral one, demonstrating the existence of bilateral allodynia (mirror-image pain). Crotalphine administered on day 21, blocked hyperalgesia and allodynia. These results indicate that intrafemoral injection of Walker 256 cells causes bone cancer and pain. Crotalphine induces potent antinociception in this model of cancer pain.

Supported by: FAPESP.

1.09 **Purification and characterization of hyaluronidases from *Otostigmus pradoi* centipede venom**

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Introduction: Centipedes are terrestrial arthropods distributed worldwide, except in Antarctica, existing in great abundance in tropical areas. These animals have a long segmented body, which ranges from 4 to 300 mm of length and contains one pair of legs per segment. These arthropods reside in underground galleries, under rocks and tree bark, and also between trash and rubble in urban areas. Accidents occur when centipedes are touched and, as defensive behavior, they inject venom through a pair of forceps - appendages on the first segment behind the head. Centipede envenomation is characterized by pain, erythema and edema. Previous studies showed the presence of hyaluronidases in centipede venoms. **Objectives:** The aim of this work was to purify and characterize hyaluronidases isolated from the venom of the *Otostigmus pradoi* centipede, the species accounting for 27 % of centipede envenomation in the metropolitan area of São Paulo city, Brazil. **Methods:** Animals were captured around the Butantan Institute and kept in captivity. Venom was milked once a month by electric stimulation. Gel filtration (Superdex 75 GL column) and ion exchange (Mono S 5/50 column) chromatography were used to fractionate the venom. SDS-PAGE (10 %) and zymographic analysis, using 170 μg/mL of hyaluronic acid as substrate, were performed to characterize proteins fractionated by chromatography. **Results and Discussion:** Four fractions were eluted after gel filtration chromatography. Hyaluronidase activity was found mainly in fraction 3. This fraction was purified by ion exchange chromatography and eleven peaks were obtained, three of which possessed hyaluronidase activity. The main components with hyaluronidase activity had molecular masses of approximately 53-41 and 32 kDa, which were eluted with 0.14 to 0.27 M NaCl. This result indicates the presence of at least two different enzymes able to hydrolyze hyaluronic acid in *O. pradoi* centipede venom. Hyaluronidases are enzymes that have a biotechnological potential due to their ability to spread substances under the skin. Herein, two hyaluronidases were partially purified from *O. pradoi* centipede venom. Further studies are in progress to determine the optimal conditions of these enzymes, such as pH and temperature.

Supported by: FAPESP.

1.10 Effect of insularin, a disintegrin from *Bothrops insularis* venom, on endothelial cells

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Introduction: Endothelial integrins play an essential role in angiogenesis and cell survival. Disintegrins are a family of low-molecular-weight and cysteine-rich proteins derived from viper venom. They bind to Arg-Gly-Asp (RGD)-responsive integrins with high affinity and block integrin function. We isolated insularin, a RGD-disintegrin from *Bothrops insularis* snake venom, which inhibits platelet aggregation by glycoprotein (GP) IIb-IIa with an IC_{50} of 0.80 μM. **Objectives**: The aim of this study was to evaluate the effects of insularin on human umbilical vein endothelial cell (HUVEC) adhesion and the inhibition of capillary-like tube formation by the Matrigel assay. **Methods:** The adhesion assay was performed after preincubation of insularin with HUVECs (10^6 cells/mL), for 1 h at 37° C. The cells were added to fibrinogen-coated plates, and after 1 h of incubation, adhesion was determined using the MTT method. For HUVEC tube formation assay, Matrigel was added to the *chamber slide system* in a volume of 50 μL in each well. After gel formation (30 min at 37° C), HUVECs, in the presence of various concentrations of insularin or PBS (control samples), were applied to each well and incubated at 37° C for 24 h in 5% CO_2. **Results and Discussion:** Insularin inhibits the HUVEC adhesion to immobilized fibrinogen with an IC_{50} of 36 nM in a dose-dependent manner and significantly inhibits (1μM) tube formation on Matrigel. Several lines of experimental evidence suggest the potential use of RGD-disintegrins for the development of therapeutic anti-angiogenic and/or anti-tumorigenic agents. These results provide further evidence that insularin may act as an anti-angiogenic agent, in *in vitro* assays, by interaction with integrin αvβ3 present in the endothelial cells. Furthermore, the antiangiogenic activity of insularin could be confirmed by *in vivo* assays.

Supported by: FAPESP.

1.11 Effects of PA-BJ and gyroxin, two serine proteinases isolated from snake venom, on endothelial cells *in vitro*

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Introduction: The disturbances in blood clotting are among the most dramatic effects of envenomation by viperid snakes. Venom serine proteinases are involved in proteolytic events, causing disturbances in hemostasis observed upon envenomation. However, their effects on endothelium are still unknown. PA-BJ and gyroxin are serine proteinases isolated from *Bothrops jararaca* and *Crotalus durissus terrificus* snake venom, respectively. PA-BJ induces platelet aggregation mediated by proteinase activated receptors -1 and -4 (PAR -1 and -4), which are also activated by thrombin. Gyroxin, in turn, acts on the central nervous system, causing temporary episodes in mice, characterized by opisthotonos and rotation around the long axis of the animals, and produces clotting on fibrinogen. **Objective:** To investigate the effects of PA-BJ and gyroxin on endothelial cells in culture, evaluating: a) viability and integrity of endothelial cells. b) release of prostacyclin (PGI_2) and c) protein expression of cyclooxygenase-1 and -2 (COX-1 and -2). **Methods:** Endothelial cells (ECs) from the murine endothelioma cell line (tEnd) were grown in RPMI-1640 medium with 10% FBS and seeded on 96 well microplates for formation of monolayers. After reaching confluence (48 h), ECs monolayers were incubated with PA-BJ (0.01, 0.1, 0.5 and 1 μM) or gyroxin (1 μM) or RPMI (control) or Triton X-100 0.1% for selected periods of incubation (3 or 24 h). ECs viability was evaluated by determination of lactate dehydrogenase (LDH) activity and tetrazolium salt reduction assay (MTT). The integrity of monolayers was determined by crystal violet staining. Prostacyclin production was evaluated by enzyme immunoassay and COX-1 and -2 protein expression by Western blotting. **Results and Discussion:** Incubation of ECs monolayers with PA-BJ and gyroxin neither affected the integrity of monolayers nor modified ECs viability at all concentrations and periods of incubation tested. At the highest concentration, these serine proteinases induced release of PGI_2 after 24 h of incubation, but did not affect basal levels of COX expression. The data show the ability of PA-BJ and gyroxin to stimulate the production of prostacyclin by the endothelium in culture. This effect is not related to increased protein expression of COX-2. Since PGI_2 is a potent vasodilator, this mediator may contribute to interference in hemostasis induced by both PA-BJ and gyroxin.

Supported by: FAPESP and CNPq.

1.12 Effect of *Crotalus durissus terrificus* snake venom and crotoxin on neutrophil functions *in vitro*

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Introduction: Previous works showed that *Crotalus durissus terrificus* snake venom (CdtV) modulates macrophage function, inhibiting spreading and phagocytic activity but increasing the oxidative burst of these cells. In addition, crotoxin (CTX), the main component of the venom, was reported to inhibit this phagocytic activity. Recently, CdtV was shown to inhibit carrageenan-induced inflammatory response and phagocytosis by neutrophils. Despite these findings, the component of CdtV responsible for the inhibition of phagocytosis is still unknown. Moreover, the effect of crude CdtV in other important functions of neutrophils, such as reactive oxygen species production, has not yet been investigated. **Objectives:** The aim of this study was to investigate the effect of CTX on phagocytosis activity, via the C3b receptor, and the effect of crude CdtV on hydrogen peroxide (H_2O_2) production, both by neutrophils obtained by carrageenan-induced peritonitis. **Methods:** Neutrophils were obtained from the peritoneal cavity of male Wistar rats (170g) (Institutional Animal Care Committee at Butantan Institute, protocol number 407/07) 4 h after the intraperitoneal (i.p.) administration of carrageenan (cg) (4.5 mg/kg). Phagocytosis of opsonized zymosan was evaluated after in vitro treatment with CTX. For this treatment, cells (1.2×10^6 cells/mL) were incubated (1 h) with RPMI 1640 medium (control) or with RPMI 1640 medium containing CTX (0.02, 0.04, 0.08, 0.16 and 0.32 µg/mL). Reactive oxygen species production was determined by H_2O_2 production. For this assay, cells (4×10^5 cells/mL) were incubated (1 h) with RPMI 1640 medium (control) or with RPMI 1640 medium containing CdtV (0.25, 0.5 and 1.0 µg/mL), and H_2O_2 production was evaluated by the phenol red oxidation method. **Results and Discussion:** In vitro, CTX significantly reduced the phagocytic activity of neutrophils at the following concentrations: 0.02 µg/mL: 24% (cg+CTX: 86.6±7.7; $p<0.05$), 0.04 µg/mL: 26% (83.3±11.7; $p<0.01$), 0.08 µg/mL: 27% (87.2±8.4; $p<0.05$). However, CTX at 0.16 and 0.32 µg/mL did not alter the phagocytic activity of neutrophils. For reactive oxygen species production, crude CdtV, at all concentrations, did not alter H_2O_2 production by neutrophils. These results show that CTX inhibits phagocytosis in neutrophils, as has been described for macrophages. However, CdtV did not stimulate H_2O_2 production in neutrophils, unlike in macrophages. Considering the important role of neutrophils in inflammation, the data presented herein contribute to the characterization of the anti-inflammatory effect of the CdtV, particularly of the CTX, recently described. These data reinforce the role of CTX as a new approach to control inflammatory diseases.

Supported by: FAPESP and CAPES.

1.13 Seroneutralization and pharmacological inhibition of the main toxic activities induced by venom of the freshwater stingray *Potamotrygon falkneri*

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Introduction: Pain and edema are the most conspicuous symptoms observed in patients wounded by stingrays. Moreover, skin necrosis is commonly observed in injuries caused by freshwater stingrays. There is no commercially available antivenom, and the treatment of patients is exclusively symptomatic, but not entirely satisfactory. **Objectives:** The objective of this work was to evaluate the effectiveness of anti-*P. falkneri* serum as well as anti-inflammatory drugs (cyclooxygenase inhibitors) to block toxic activities induced by *P. falkneri* venom. **Methods:** IgG was isolated by affinity chromatography (Protein A-Sepharose) from sera of rabbits immunized against *P. falkneri* venom. ELISA and Western blotting were used to verify the immunogenicity of the venom. Three doses of venom (16, 32 and 64 µg) were administrated to the hind paw of mice for evaluation of edema (measured at 30 min and 1, 2, 24 and 48 h) and nociception. Local tissue injury – characterized by necrosis, ecchymosis and ischemia – was observed 72 h after venom injection (200, 400 and 600 µg). Myotoxicity was evaluated by measuring the levels of creatine kinase (CK) in serum of mice injected i.m. with venom (50, 100 and 200 µg). In order to investigate the ability of specific antibodies to neutralize *P. falkneri* venom activities, groups of mice were inoculated with a mixture of different doses of venom that had been preincubated with a fixed volume of antibodies (30 min, 37° C). To verify the action of anti-inflammatory drugs, indomethacin (4 mg/kg), dipyrone (200 mg/kg) or etoricoxib (10 mg/kg) was administered (i.p.) 30 min before *P. falkneri* venom injection, and the edematogenic and nociceptive activities were evaluated. **Results and Discussion:** By ELISA, *P. falkneri* venom was found to induce high levels of antibodies (titer 2,048,000) in rabbits. Many venom components, mainly above 20 kDa, were recognized by antibodies using Western blotting. Antibodies were effective in neutralizing necrosis (above 68%) and nociception (above 56%), and partially neutralized the edema from 30 min to 24 h. However, antibodies failed to neutralize the myotoxicity elicited by venom. The results demonstrate the efficacy of specific anti-*P. falkneri* antibodies in neutralizing the main toxic activities induced by crude venom, except for myotoxicity. Dipyrone and etoricoxib were effective in reducing nociception in 85% and 59%, respectively. In contrast, dipyrone, but not etoricoxib, was effective in abrogating nociception. However, indomethacin failed to inhibit this venom toxicity. All drugs partially reversed venom-induced edema. Our results demonstrate that eicosanoids are involved in both nociception and edema induced by *P. falkneri* venom, and that anti-inflammatory drugs may be useful to treat pain in patients wounded by this stingray. However, more studies are necessary to demonstrate that the specific antivenom as well as cyclooxygenase inhibitors may be effective in treating patients wounded by *P. falkneri*.

Supported by: FAPESP.

1.14 *Tityus serrulatus* venom downregulates ENaC and AQP5, as well as upregulating NKCC1, in rat lungs

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Introduction: *Tityus serrulatus* envenomation can cause pulmonary edema of cardiogenic or noncardiogenic origin. Pulmonary edema clearance is largely related to active Na+ transport out of the alveoli, rather than the reversal of Starling forces. Pulmonary edema is resolved through active Na transport across the alveolar epithelium via apical amiloride-sensitive Na channels and via basolateral alpha-Na-K-ATPase. This active vectorial sodium flow produces a transepithelial osmotic gradient that results in passive movement of water from the air spaces into the alveolar interstitium. Found in virtually all cells, NKCC1 mediates the Na-/K-/Cl-coupled influx and regulates cell volume. In the lung, AQP5 provides the principal route for osmotically driven water transport. **Objectives:** The aim of the present study was to determine the effects that *T. serrulatus* envenomation has on the mechanisms of pulmonary edema. **Methods:** Rats were inoculated intraperitoneally with *T. serrulatus* venom (3.8 mg/kg BW; V group, n = 13). Control rats were injected with saline (C group, n = 9). Serum urea, creatinine, Na, K, creatine kinase (CK), lactate dehydrogenase (LDH), troponin and amylase were measured one hour after inoculation. In lung tissue, protein expression of the epithelial Na channel, alpha subunit (α-ENaC), Na-K-ATPase, NKCC1 and AQP-5 was determined by Western blotting. Data are expressed as mean ± SEM. **Results and Discussion:** In the V group, 9 rats developed clinical signs of severe envenomation, and serum urea was higher than in the controls (39.4 ± 2.4 vs. 51.8 ± 3.5, P < 0.036). There were no differences between the groups in terms of the serum levels of Na, K and creatinine, amylase, CK or LDH. However, envenomation led to marked downregulation of pulmonary expression of α-ENaC (100.7 ± 6.5 vs. 48.8 ± 5.4, P < 0.0061) and AQP-5 (98.7 ± 2.2 vs. 61 ± 7.3, P < 0.012), whereas NKCC1 protein expression increased (102.1 ± 6.2 vs. 141.2 ± 2.0, P < 0.0061) and Na-K-ATPase protein expression was unaffected. We hypothesize that *T. serrulatus* venom decreases α-ENaC protein abundance, reducing the transport of Na from the lumen to the interstitium, as well as decreasing the movement of water from the lumen to the interstitium, thereby lowering the osmotic gradient and impairing alveolar clearance. The resulting cell shrinkage can stimulate basolateral NKCC1. The decreased influx of Na from the lumen into the cells (induced by the lower α-ENaC levels), together with the increased influx of Na from the interstitium into the cells (induced by the higher NKCC levels), can block the net influx of Na and water from the alveoli. Our data show that *T. serrulatus* venom has a profound effect on the Na+ and water transport capacity of alveolar epithelial cells. Inadequate pulmonary fluid handling can impair lung function and increase susceptibility to lung injury.

Supported by: FAPESP.

1.15 Melittin-S: a novel melittin isoform from *Apis mellifera* venom

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Introduction: Melittin, the main component of *Apis mellifera* venom (~50% by dry weight), is a peptide with 26 residues and a characteristic structure of membrane bound cytolytic, channel-forming peptides and trans-membrane protein helices. This peptide shows hemolytic activity, other than being the primary allergen in bee venom. **Objectives:** To analyze the composition of *A. mellifera* venom over one year, in one specific hive, and to identify the main components. **Methods:** Venom from Africanized honeybee workers was obtained by electric stimulation of the animals. The venom of each month was analyzed and purified by RP-HPLC (C8 column), and the major peaks were analyzed by mass spectrometry and/or Edman degradation. A hemolytic assay was carried out as well. **Results and Discussion:** It was possible to perceive a seasonal variation in the venom contents of melittin. Moreover, we have been able to identify a novel melittin isoform, named melittin-S, produced during the southern winter. Melittin-S levels are 1-2% throughout the year, but abruptly rise to 10% during the southern winter months, thus becoming a 'new' major component. This isoform has the same amino acid sequence of melittin, but has a Ser residue instead of Thr at the tenth position. The hemolytic activity level of melittin-S is equivalent to melittin. Although, a melittin from *A. dorsata* has already been described showing a Ser at this tenth position, it was one amino acid shorter than melittin and melittin-S described here. For *A. mellifera*, this is the first description of a melittin isoform. Moreover, it was observed that the total melittin content remains rather constant throughout the year, indicating that the bee responds to some environmental change by synthesizing melittin-S. The hemolytic activity present in melittin-S indicates that the amino acid modification should retain the other physiological roles of melittin.

Supported by: FAPESP and CNPq.

1.16 Mechanisms involved in prostacyclin production induced by CB, a crotoxin subunit with phospholipase A_2 activty, isolated from *Crotalus durissus terrificus* snake venom

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Introduction: Crotoxin (CTX), the major component of *Crotalus durissus terrificus* snake venom (CdtV) contains two subunits: crotapotin (CA) and phospholipase A_2 (CB). The CB subunit exerts neurotoxic and myotoxic effects and inhibits macrophage functions. The phospholipase A_2 (PLA_2) enzymes catalyze the cleavage of arachidonic acid (AA) from the *sn*-2 position of phospholipids with subsequent conversion of free AA into prostaglandin H_2 by two distinct isoforms of cyclooxygenases (COX-1 and COX-2); newly formed PGH_2 is then converted into distinct prostaglandins by terminal synthases. Prostacyclin (PGI_2), a potent vasodilator and inhibitor of platelet aggregation, is the predominant prostaglandin synthesized by vascular endothelial cells (ECs). Recent data from our laboratory demonstrated the ability of CB to release PGI_2 from endothelial cells *in vitro*. **Objective:** In this study, the role of COX-1 and -2 enzyme systems on CB-induced prostacyclin production by endothelial cells was evaluated. **Methods:** ECs from a murine endothelioma cell line (tEnd) were grown in RPMI-1640 medium with 10% FBS and seeded on 96 well microplates for formation of monolayers. After reaching confluence, ECs monolayers were incubated with CB (0.4 µM) or RPMI-1640 (control) for selected periods of incubation. Inhibition of COX activities was performed by the pre-incubation of ECs with indomethacin (non-selective COX inhibitor) or valeryl salicylate (selective COX-1 inhibitor) or etoricoxib (selective COX-2 inhibitor). Production of PGI_2 was measured using enzyme immunoassay (EIA) and COX protein expression evaluated by *Western blotting* analysis. **Results and Discussion:** Pre-incubation of ECs with indomethacin or valeryl salicylate or etoricoxib significantly decreased the prostacyclin production induced by CB subunit (78%, 42% and 61%, respectively) ($p \leq 0.05$). This toxin also up-regulated COX-2 protein expression (53%), whereas 4-bromophenacyl bromide (BPB), an inhibitor of phospholipase A_2 enzyme activity, abrogated this effect. However, the COX-1 pattern of protein expression was not modified by CB. These data indicate that COX-1 and COX-2 activities are involved in CB-induced prostacyclin production from endothelial cells. In addition, up-regulation of COX-2 protein expression is important for the effect induced by CB. Moreover, the catalytic activity of CB is essential for the stimulatory effect of this phospholipase A_2 on the biosynthesis of PGI_2. Finally, these findings indicate novel regulatory mechanisms for venom secretory PLA_2 in endothelial cells.

Supported by: FAPESP and CNPq.

1.17 *Loxosceles intermedia* sphingomyelinase D induces the activation of proteases on the human erythrocyte membrane, able to hydrolyze fluorescence substrates

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Introduction: Spiders of the *Loxosceles* genus, also known as "brown spiders," are distributed worldwide. Accidents caused by these spiders are associated with severe clinical symptoms, including dermonecrotic lesions, thrombosis, vascular leakage and, eventually, intravascular hemolysis and renal failure, which can be lethal. In Brazil, three major species, implicated with human envenoming, are responsible for more than 6000 cases of envenomation each year. The main toxic component present in the venom is a sphingomyelinase D (SMase D). *Loxosceles* SMase D promotes the activation of membrane bound proteinases on erythrocytes and nucleated cells. In the case of erythrocytes, this leads to an increased susceptibility to activation of complement (C), *via* the alternative pathway, because of proteinase-induced cleavage of glycophorins, which are important regulators of C-activation. **Objectives:** The aim of the present study was to further investigate the activation mechanism of the membrane-bound proteinase(s), responsible for the cleavage of the erythrocyte glycophorins, induced by SMases D. **Methods:** We used the fluorescence resonance energy transfer (FRET) substrate Abz-FRSSRQ- EDDnp as a tool to explore the proteolytic activity of human erythrocyte membranes. Membranes were incubated with 5 mM substrate, in the presence or absence of a recombinant SMase D from *L. intermedia* (referred to as P2 - 5 µg) at 37ºC, in a 96-well plate. The hydrolysis reaction, of the fluorogenic peptide, was monitored during 40 min in a spectrofluorimeter. In addition, inhibition assays were performed in order to determine the class of the activated proteinase(s). **Results and Discussion:** Data obtained show that the erythrocyte membrane preparation have a basal proteolytic activity on the fluorogenic peptide. However, treatment with SMase P2 induces an increase of this enzymatic activity by approximately 20%. Moreover, the use of an elastase inhibitor was able to reduce the membrane proteolytic activity, induced by treatment with P2, by about 40%. Our results suggest that SMase P2 is able to activate proteinase(s) on erythrocyte membrane preparations and that elastase may be involved in the process.

Supported by: FAPESP and CNPq.

1.18 Purification and partial characterization of blood coagulation Factor X activator from *Bothrops jararaca* snake venom

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Introduction: Snake venoms of the viperidae family contain several proteins that cause hemostatic disturbances. Envenomation by this family is characterized by hemorrhage, edema, local tissue damage, myonecrosis, and fibrinolytic and kinin releasing activities. *Bothrops jararaca* envenomation causes local and systemic hemorrhage, together with blood clotting disorders, mainly because of the presence of thrombin-like, factor X and prothrombin-activating enzymes. Although thrombin-like and prothrombin activators have already been isolated from this venom, factor X activators have not yet been purified. Considering the importance of these proteases in the coagulation process, these enzymes have been largely studied as drugs in the clinical therapeutic area. Thus, this work shows the isolation of the factor X activator from *Bothrops jararaca* snake. **Objectives:** Purification and partial characterization of blood coagulation factor X activator from the venom of *Bothrops jararaca*. **Methods:** An amount of 100 mg venom was dissolved in 1 mL 20 mM Tris-HCl (pH 7.5) containing 5 mM benzamidine and 200 mM NaCl, and centrifuged at 5400 g for 10 min. The clear supernatant was filtered through a 0.22 μM membrane and applied to a Superdex 75 column, followed by a benzamidine Sepharose column and finally a SP Hitrap XL column. Along all the purification steps, protein concentration was determined by absorbance at A_{280}. Protein activity was measured using a specific chromogenic substrate. The fractions were analyzed by SDS-PAGE (10%). **Results and Discussion:** The factor X activator was isolated from the *Bothrops jararaca* snake venom using three chromatography steps: gel filtration, affinity and cation-exchange columns. The purified protein was analyzed by SDS-PAGE showing a molecular mass of 67 kDa. This is the first report on the isolation and partial characterization of a factor X activator from *Bothrops jararaca* snake venom. The perspectives for this work are to obtain a larger amount of protein for biological and biochemical characterization.

Supported by: FAPESP and CNPq.

1.19 Identification of novel compounds from solitary wasp venom

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Introduction: Bradykinin (BK) is one of the most important compounds generated following tissue injury. This nonapeptide is released by the action of endogenous or exogenous kininogenases on plasma kininogen, and is a potent algesic, hypotensive, and proinflammatory endogenous substance. It is rapidly hydrolyzed by the action of angiotensin-converting enzyme (ACE). BK and BK-related peptides (BRPs) are also widely distributed in venomous animals, including frogs and insects such as wasps. In a survey of the solitary wasp *Cyphononyx fulvognathus* venom, three novel BRPs, besides the well-known Thr^6-BK, were identified. Herein, we report the chemical, biochemical and pharmacological characterization of Thr^6-BK and of these novel BRPs, named *Cf*-32, *Cf*-46, and *Cf*-146. **Objective:** Aiming for a better understanding of the wasp envenomation process, the extract of the solitary wasp *Cyphononyx fulvognathus* venom was analyzed. The identified compounds were both biochemically and pharmacologically characterized. **Methods:** The venom extracts of *C. fulvognathus* were subjected to reverse-phase HPLC, and the purity and complexity of each fraction was examined by MALDI-TOF MS. The primary sequence of the observed peptides was determined by Edman degradation and ladder sequencing. Enzyme activity assay and determination of kinetic parameters of ACE for these wasp peptides were performed using the synthetic analogs obtained by synthesis on an automated PSSM-8 peptide synthesizer. These same analogs were employed for the BK-potentiation assays on isolated guinea pig ileum and pain threshold evaluations (CEUAIB no. 532/2008). **Results and Discussion:** Interestingly, we found that these peptides showing the highest structural similarity to BK, which was the case with Thr^6-BK and *Cf*-46, were shown to be able to contract smooth muscle preparation, while the other two peptides studied herein, namely *Cf*-32 and *Cf*-146, could not. On the other hand, all these peptides were able to inhibit ACE as well as to induce the hyperalgesic effect in living rats after intraplantar injection. The use of specific BK-receptors antagonists also allowed the identification of BK-receptors as the target of these wasp peptides. We believe that this knowledge will contribute to a better understanding of the wasp envenomation process, which could be a good support in proposing the inclusion of novel elements to be considered for the treatment of current wasp accident symptoms.

Supported by: FAPESP and CNPq.

1.20 Human anti-crotoxin single-chain variable-fragment (scFv) expressed in bacteria and refolded

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Introduction: Nowadays, single chain variable fragments, scFv, are becoming therapeutic alternatives to whole monoclonal antibodies (AcMos), since these are smaller, have different advantages in certain medical applications and are easily genetically manipulated. Recently, an antibody isolated by phage display technique has just been approved for therapeutic use in humans (adalimumab, anti-TNF, form Humira; Abbott Laboratories). At least 14 additional antibodies derived from recombinant libraries are in clinical trial. ScFv contains the variable domain of heavy (VH) and light (VL) chains linked by a flexible polypeptide $(G_4S)_3$ and may be useful as auxiliary therapy to envenoming by snake bite. The South American rattlesnake *Crotalus durissus terrificus* is responsible for about 8% of envenoming in Brazil. Its venom contains many toxins, such as gyroxin, convulxin, crotamin and crotoxin (CTX). CTX is the main toxic component of the venom and responsible for most of its toxic effects, such as neurological disorders, myotoxicity, and renal failure. It is composed of two subunits, an acidic, nontoxic, and nonenzymatic component (crotoxin A, CA, or crotapotin) and the toxic molecule, a basic Asp49-PLA2 (crotoxin B, CB, or Cdt PLA2). ScFv6 anti-crotoxin was isolated by phage display technology from a naive library of more than 10^{10} scFv clones with *in vivo* CTX and crude venom neutralizing activities. Many attempts have been tried to improve its expression in *E. coli* system such as the use of a synthetic scFv6 gene devoid of rare codons with a MBP-linker. However, this strategy resulted in the expected complete protein, in which MBP could not be removed. **Objective:** To express the scFv6 in the cytoplasm of bacteria and optimize refolding conditions to obtain the molecule in its soluble and functional form. **Methods:** ScFv6 coding sequence was cloned into a pAE vector that contains His-tag and used to transform BL21 (DE3) bacteria. The production of scFv was accomplished using 0.5 mM IPTG and growth condition at 37°C for 4 h. The insoluble ScFv was extensively washed and incubated in the reducing buffer (8 M urea solution). Renaturation of reduced ScFV was achieved by dialysis with buffer containing urea. The urea concentration of the dialyzing bottle was gradually diluted with buffer without urea at a flow rate of 0.1 ml/min by high-pressure pump. The purity of the sample was evaluated by SDS-PAGE and Western blotting using mouse anti-His antibody followed by anti-mouse-peroxidase. **Results and Discussion:** The construct expressed a 30 kDa expected protein and the refolding resulted in a 40 μg/mL yield. DNA sequencing of scFv6 revealed identity to the reported sequence (Genbank: AJ132608). The refolding system used had some drawbacks and to solve these problems we are now trying to refold using hydrostatic pressure. Reproducible refolded scFv will be biochemically characterized regarding its affinity and ability to neutralize CTX and venom toxic activities. Satisfactory results will lead us to the next step: improvement of the affinity and specificity of scFv6 using suggestions of the Antibody Evolution (AbEvo) software that predicts advantageous mutations.

Supported by: FAPESP/PIPE and CNPq.

1.21 Structure-activity studies of different peptide-like BPP-10c. Evaluation of cardiovascular effects in SHR

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Introduction: The bradykinin-potentiating peptides (BPPs) from *Bothrops jararaca* snake venom were the first angiotensin I converting enzyme (ACE) inhibitors described, providing the basis for the development of captopril. *In vivo* studies showed that the BPP-10c can cause a potent and long-lasting decrease in mean arterial pressure (MAP) and heart rate (HR) of spontaneously hypertensive rats (SHR) independent of ACE activity. Recently, argininosuccinate synthetase (AsS) was identified as a novel target for BPP-10c, suggesting that AsS could be a new mechanism for the antihypertensive effect of the decapeptide in SHR. **Objectives:** To evaluate the cardiovascular effects of peptide-like BPP-10c in SHR and to identify the responsible amino acid(s) for the BPP-10c effects. **Methods:** Twenty hours before the experiment, polyethylene catheters were introduced into abdominal aorta and femoral vein for measurements of cardiovascular parameters and intravenous (i.v.) injection, respectively. Before drug administration, the cardiovascular parameters were monitored for 30 min (baseline period). Following i.v. injection of the BPP-10c, peptide-like BPP-10c (71 nmol/kg) or vehicle (0.9% NaCl) was given in a total volume of 0.5 ml. The cardiovascular parameters were monitored for 6 h after drug administration. Six peptide-like BPP-10c were evaluated: BPP-10c des<ENW, BPP-10c desPro^{10}, BPP-10c E^{1}, BPP-10c Scrambled, BPP-10c Ala^{5} and BPP-10c Ala^{3}. Data were compared by Student's *t* test or ANOVA followed by Newman-Keuls post-test when appropriate. **Results and Discussion:** The BPP-10c des<ENW, BPP-10c E^{1}, BPP-10c Scrambled and BPP-10c Ala^{5} were able to cause a significant decrease in MAP. The maximal change of MAP ranged from -21±3 mmHg to -25±4 mmHg *vs* -11± 2 mmHg (control group), $p<0.05$. The MAP changes caused by the modified peptides were smaller than that described for BPP-10c (-36±3 mmHg, $p<0.001$). The four peptide-like BPP-10c caused maximal MAP changes from 58 to 70% of the BPP-10c maximal effect. Similar to BPP-10c, bradycardia was observed after BPP-10c des <ENW, BPP-10c desPro^{1}, BPP-10c Ala^{5} and BPP-10c Ala^{3} injection. BPP-10c Scrambled and BPP-10c E^{1} did not cause changes in HR. The BPP-10c des<ENW, BPP-10c E^{1} and BPP-10c Scrambled and BPP-10c Ala^{5} were able to decrease blood pressure in SHR. The absence of tripeptide (<ENW) at N-terminal portion, such as the change in the pyroglutamic acid for glutamic acid at the N-terminal position or replacement of the amino acids at positions 2, 5 and 9 or change of the amino acid at position 6 (proline for alanine) did not make BPP-10c inactive. On the other hand, the BPP-10c desPro^{10} was not able to decrease arterial pressure. It suggests that the presence of dipeptide (proline-proline) at C-terminal position is required to evoke the antihypertensive effect in SHR. Further studies are needed to reveal which amino acid(s) is/are essential(s) to evoke the antihypertensive effect. This approach is important to develop stable molecules to be used to treat hypertension and other cardiovascular diseases.

Supported by: FAPESP and CNPq.

1.22 Snake venoms of the *Bothrops* genus activate complement by cleaving C1-inhibitor, C3 and C4

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Introduction: Snake venoms are a complex mixture of components, which have a wide range of actions both on prey and human victims. Many of these components are biologically active proteins that function to kill or immobilize the prey as well as assist in the digestion process. The genus *Bothrops* inflicts the vast majority of snakebites in Central and South America, being responsible for 90% of snake envenomations in Brazil. Envenomations are characterized by prominent local effects, including edema, hemorrhage and necrosis, which can lead to permanent disability. Systemic manifestations such as hemorrhage, coagulopathy, shock and acute renal failure may also occur. **Objective:** In the present study, we investigated the action of venoms from 19 species of snakes from the genus *Bothrops* occurring in Brazil on the complement system in *in vitro* studies. **Results and Discussion:** All venoms were able to activate the classical complement pathway, in the absence of sensitizing antibody, in a dose dependent manner. This activation was in part associated with the cleavage of C1-inhibitor by proteases present in these venoms, which disrupt complement activation control. No modification of the membrane bound complement regulators, such as DAF, CR1 and CD59 was detected, after treatment of human erythrocytes with the snake venoms. Moreover, some of the *Bothrops* venoms were also able to activate alternative and lectin pathways, as measured in hemolytic and ELISA assays. C3a, C4a and C5a were generated in sera treated with the venoms, not only by C-activation, but also by the direct cleavage of complement components, as determined using purified C3 and C4. Metalloproteinase and/or serineprotease inhibitors prevented cleavage of C3 and C4. These results suggest that *Bothrops* venoms can activate the complement system, generating a large amount of anaphylatoxins, which may play an important role in the inflammatory processes shown in humans after snake envenomations.

Supported by: CNPq and FAPESP.

1.23 Channel formation in lipid bilayers induced by toxins of the marine sponge *Amphimedon viridis*

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Introduction: Recently, membrane pore-forming antimicrobial peptides have attracted attention as a new type of antibiotic, mainly because their antimicrobial activity is induced through interaction with the cell membrane and thus drug resistance would not be developed. **Objective:** The aim of this work was to investigate channel incorporation in artificial lipid bilayers (LBs) in the presence of the methanol-water extract of the marine sponge *Amphimedon viridis* (AvM) as a possible mechanism of action of the antimicrobial and hemolytic properties of the extract. **Methods:** AvM was prepared from *A. viridis* specimens collected (in Maceio, Alagoas, Brazil) and identified by Profs. Drs. Monica D. Correia and Hilda H. Sovierzoski, of the Biomedical Sciences and Health Institute of Federal University of Alagoas. The LBs were formed by giant unilamellar vesicles (GUVs). The experiments were performed with the patch clamp setup "Port-a-Patch" (Nanion Technologies), using borosilicate glass chips NPC-1. Current signals were recorded and amplified (Heka EPC-10, A/D interface ITC-1600, Patch Control™ software). **Results and Discussion:** AvM (~0.1 to 0.2 µg/ml) induced channel formation in DPhPC/cholesterol (80:20) and asolectin LBs, using both positive and negative holding potentials, and with two electrolyte solutions (150 mM KCl and 100 mM HCl). Conductance varied from 16 to 600 pS (V_{hold} = -100mV, asolectin), and from 10 to 734 (V_{hold} = -100mV, DPhPC/cholesterol). Within a few minutes, the great number of incorporated channels induced the LB break. Channel opening showed varied conductance, which may be due to the presence of more than one pore-forming substance in the extract, or by the polymerization of the substance during the experiment. Previous studies with sponges of the same genus/family demonstrated that alkylpyridinium polymers, which can assemble forming pores in biological and artificial membranes, are responsible for the biological activities of the extracts. Chemical fractionation and isolation of the active compounds are under investigation and will corroborate (or not) the presence of this type of polymer in AvM.

Supported by: FAPESP and CNPq.

1.24 Participation of mast cells and histamine receptors in leukocyte recruitment induced by *Bothrops moojeni* venom (BmV)

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Introduction: *Bothrops moojeni* snake bite causes a marked local inflammatory reaction, but the events involved in this reaction are still unclear. Mast cells (MC) are central elements of innate immune responses releasing a vast array of inflammatory mediators. However, their role in *B. moojeni*-induced envenomation is unknown. **Objectives:** To investigate the inflammatory response to BmV analyzing: a) leukocyte (LK) infiltration and release of chemotactic mediators into mouse peritoneum, b) the number of LKs in peripheral blood, c) the role of MC and histamine receptors in LK infiltration and d) the ability of BmV to induce MC degranulation both *in vivo* and *in vitro*. **Methods:** Male Swiss mice were used. LK numbers were evaluated in both blood (24 h before and at 1-6 h) and in peritoneal washes (1-48 h) after intraperitoneal (ip) injection of BmV (0.25 μg/g) or saline (control). Total and differential LK counts were determined in a Neubauer chamber after dilution in Turk solution and in Hema[3] stained smears, respectively. Groups of mice were treated with an inhibitor of MC degranulation or distinct histamine receptor antagonists or their vehicles before BmV ip injection, and LK influx was evaluated. Levels of chemotactic mediators (LTB_4, TXA_2, MCP-1 and KC) were determined by enzyme immunoassay (EIA). MC degranulation was evaluated by light microscopy after histological processing of mesentery of mice from 5 up to 30 min after BmV (0.025 – 0.5 μg/g, ip) or saline (control) injection. MC activation was evaluated by measuring PGD_2 release by EIA at 5-60 min after injection of either BmV (0.25 μg/g) or saline. *In vitro* MC degranulation was determined by measuring β-hexosaminidase release from MC of cultured PT18 line incubated with BmV (1 – 10 μg/mL) or Tyrode buffer (control). **Results and Discussion:** BmV ip injection markedly increased the peritoneal number of total LK from 3 up to 24 h with polymorphonuclear cells at 3 - 6 h and mononuclear cells from 3 up to 24 h. The number of blood neutrophils were increased at 3 h. Inhibition of MC degranulation by cromoglycate abolished infiltration of LK, whereas the histamine antagonists, diphenhydramine, ranitidine or thioperamide reduced PMN influx by 54, 47 and 78%, respectively. Moreover, BmV significantly increased peritoneal levels of TXA_2 at 1 h, LTB_4 at 6 h, MCP-1 at 3 h and KC at 30 min, and caused a dose-related increase in the number of degranulated MCs at 10 min, in comparison with controls. In addition, BmV caused a significant increase in PGD_2 levels from 5 up to 30 min, indicating activation of MCs. Incubation of PT18 MCs with non-cytotoxic concentrations of BmV increased β-hexosaminidase release compared with controls. BmV was able to recruit leukocytes into the site of its injection, which was dependent on MC degranulation and activation of H_1, H_2 and H_4 histamine receptors. BmV-induced both MCs activation and degranulation in mice may be due at least in part to a direct action of venom on these cells. Moreover, the ability of BmV to release chemotactic mediators such as LTB_4, TXA_2, MCP-1 and KC, and mobilize neutrophils from bone marrow compartments is important for the recruitment of leukocytes to the site of its injection.

Supported by: CNPq and FAPESP.

1.25 Autolysis of patagonfibrase, a metalloproteinase isolated from *Philodryas patagoniensis* venom (Serpentes: Colubridae)

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Introduction: Patagonfibrase is a 57.5-kDa hemorrhagic metalloproteinase isolated from the venom of the South American colubrid snake *Philodryas patagoniensis*. Metalloproteinases isolated from *Bothrops* venoms with molecular mass similar to patagonfibrase are known to undergo autolysis under different conditions, giving rise mainly to one fragment lacking the metalloproteinase domain, that is, containing only disintegrin-like/cysteine-rich domains. **Objectives:** Taking into consideration that patagonfibrase is the only metalloproteinase isolated to date in native form from a colubrid snake venom that inhibits platelet aggregation, and that this enzyme exhibits various biological activities similar to those exhibited by *Bothrops* venom metalloproteinases with similar molecular mass, in this study we aimed to evaluate the autoproteolytic activity of patagonfibrase. **Methods:** Patagonfibrase was incubated at 37°C at a concentration of 0.5 mg/mL in 50 mM Tris-HCl buffer, pH 7.4, for different time intervals (0, 5, 15, 30, 60 and 120 min; 18 h), in the presence and absence of 1 mM $CaCl_2$. Immediately after incubation, autolysis was interrupted by addition of SDS-PAGE sample buffer containing β-mercaptoethanol. Autolysis was visualized by SDS-PAGE (12% running gel) and silver staining. Residual proteolytic activity of patagonfibrase (30 µg/mL), was also evaluated using azocasein as substrate. **Results and Discussion:** Patagonfibrase was able to undergo autolysis at 37°C. The analysis of electrophoretic migration pattern showed a progressive fainting of the proteinase band and an increase in the staining density of bands below 45 kDa over incubation up to 18 h. In this period of incubation, the enzyme was almost totally autolysed, and thus it failed to hydrolyze azocasein. However, in the presence of 1 mM $CaCl_2$, patagonfibrase was only partially autolysed, even after 18 h of incubation, giving rise mainly to one fragment of 52.2 kDa. In addition, preincubation of patagonfibrase at 37°C for 18 h in the presence of 1 mM $CaCl_2$ caused an increase in its azocaseinolytic activity by about 50%. Taken together, these results imply that the calcium ions are important for the structural stabilization of patagonfibrase, as demonstrated for other snake venom metalloproteinases found in different ophidian taxa. Moreover, this work demonstrates for the first time the autoproteolytic processing of a colubrid snake venom metalloproteinase, which will contribute to a better understanding of the structural and mechanistic basis of this type of proteins that are widely distributed among snake venoms.

Supported by: FAPESP, Fundação Butantan and CONICET.

1.26 The peristomial coelomic fluid of the sea urchin *Echinometra lucunter* contains antibiotic molecules

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Introduction: The immune function of the sea urchins has already been described and it is carried out by coelomocytes, the cells present in the coelomic fluid, which are able to neutralize bacteria. However, few molecules in sea urchin coelomic fluid have been described, so far, that are active against these pathogens. **Objectives:** To identify and characterize antibacterial molecules from the peristomial coeloma of *E. lucunter* sea urchin, a common species found in the Brazilian coastal waters. **Methods:** Peristomial coelomic fluid was collected by puncturing the *E. lucunter* sea urchin. The fluid was centrifuged and the supernatant was processed by solid phase extraction (C18 cartridges) with step gradients of 25, 50, 75, 100 % acetonitrile containing 0.1% TFA. The fractions were assessed on bacterial cultures (*E. coli*, *S. aureus*, *P. aeruginosa* and *M. luteus*), in order to determine possible growth inhibition effects. The active fractions were then purified by RP-HPLC (C18 column) and the peaks obtained were re-tested and analyzed by mass spectrometry (MALDI-TOF/MS and GC-MS). **Results and Discussion:** SPE 50% was active in the growth inhibition of *E. coli*. This fraction, under RP-HPLC-C18 chromatography, yielded 8 peaks, where one of them was active. This peak was able to cause 100% growth inhibition of *E. coli*; furthermore, this peak was not active against other bacteria. MALDI-TOF/MS analyses of selected peaks showed molecular masses of 582,029 and 657,055 Da. Moreover, GC-MS analyses confirmed the same masses. Although theses mass spectrometric analyses indicate that the sample is not yet 100% pure, it was possible to correlate the biological activity to the 657,055 Da moiety. This work reports the identification of one specific antibiotic isolated from sea urchin. Although its molecular structure is not unveiled, several pieces of information sum up to the understanding of this molecule's physicochemical characteristics: it is a non-polar (small) organic molecule (higher ACN% in SPE, low RT in CG) that contains one bromine atom, as indicated by GC-MS. Altogether, these data indicate that the peristomial coelomic fluid of *Echinometra lucunter* sea urchin contains biological active molecules, one of them being active against bacterial growth.

Supported by: FAPESP and CNPq.

1.27 Involvement of factor II and X activators of *Bothrops jararaca* venom in the pathogenesis of hemostatic disturbances and intravascular hemolysis during envenomation

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Introduction: Vitamin-K dependent coagulation factors (VKDCF, factors II, VII, IX, X, and proteins C and S) are essential to maintain hemostasis. To attain fully functional biological activity, they are γ-carboxylated prior to secretion from hepatocytes, and this process is dependent on vitamin K. The anticoagulant warfarin limits the γ-carboxylation of VKDCF. *Bothrops jararaca* (Bj) snake venom possesses several activators and inhibitors of coagulation, especially procoagulating enzymes (factor II and X activators) and thrombin-like enzymes. **Objective:** To clarify the participation of factor II and X activators in evoking hemostatic disturbances and intravascular hemolysis during experimental Bj envenomation. **Methods:** Male Wistar rats, weighing 180 to 200 g, were administered warfarin (5 mg/kg, i.v.) or saline for 3 days. To evaluate the effect of *in vivo* VKDCF depletion on the severity of hemostatic disturbances induced by Bj venom, rats were envenomed (1.6 mg/kg, s.c) after the aforementioned treatment with warfarin (warfarin group, WG, n= 6). The positive control group (PCG, n=6) received saline i.v., instead of warfarin, prior to the administration of Bj venom s.c., and the negative control group (NCG, n= 6) received only saline. Blood samples were used for performing complete blood cell counts and differential blood counts and for determining whole blood platelet aggregation induced by collagen; fibrinogen, factors II and X, and plasma hemoglobin were determined in citrated plasma. **Results and Discussion:** The platelet counts of the PCG and WG groups were respectively 14 and 5 times lower than that of NCG (1156 ± 80.0 x 10^9/L), indicating that platelet consumption is partially due to intravascular thrombin generation during Bj envenomation. Platelet aggregation in NCG (15.6 ± 3.8 Ω) was normal, while in the PCG and WG groups it was completely inhibited (0.0 ± 0.0 Ω, and 1.5 ± 1.4 Ω, respectively). No statistical difference was noted between the three groups regarding the red blood cell and white blood cell counts. Plasma fibrinogen levels dropped abruptly after venom administration in PCG (34.5 mg/dL ± 2.0) in relation to NCG (244.0 mg/dL ± 7.0), and fibrinogen consumption was partially reversed by previous warfarin treatment in WG (169.0 mg/dL ± 20.0). Plasma levels of factor II and X decreased in PCG (64.0 ± 3.0%, and 36.0 ± 2.0%, respectively) and WG (2.8 ± 1.5%, and 0.2 ± 0.2%, respectively) in comparison with NCG (107.0 ± 7.0%, and 66.0 ± 6.0%, respectively). Schistocytes and microcytes were observed in blood smears of the PCG group, likely due to intravascular thrombin generation, which forms cross-linked fibrin and causes microangiopathic anemia; these cells were absent in the blood of NCG and WG animals. In agreement with previous observations, there was a five-fold increase in plasma hemoglobin levels in PCG group in comparison with the NCG and WG groups, demonstrating that VKDCF depletion abrogated the lysis of red blood cells in the blood stream. These data indicate that thrombin production is of major importance to in provoking coagulopathy and hemolysis during *B. jararaca* envenomation in rats, and that the activation of factors II and X by procoagulating enzymes of Bj venom has been underestimated so far.

Supported by: CNPq/INCTTOX and FAPESP.

1.28 Leukocyte accumulation and release of inflammatory mediators at the site of *Bothrops insularis* venom injection

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Introduction: *Bothrops insularis* snake is an endemic species restricted to the island of Queimada Grande, coast of the State of São Paulo, Brazil. Previous studies showed the ability of *B. insularis* venom (BiV) to induce local edema. However, the subsequent events involved in the local inflammatory response to this snake venom are still unclear. **Objectives:** To investigate the effect of BiV on the number of leukocytes in the blood and release of TXB_2, LTB_4, MCP-1 and KC into the peritoneal cavity. In addition, the effect of venom on the number of peritoneal leukocytes was evaluated. **Methods:** Male Swiss mice (18-20 g) (protocol no. 463/08) were injected either with BiV (0.05 µg/g) or saline (control) by the intraperitoneal (i.p.) route. At selected time intervals after these injections, peritoneal exudates were harvested and centrifuged. Supernatant concentrations of mediators were measured by a specific enzyme immunoassay. Total and differential blood leukocyte numbers were evaluated at 24 h before or 1, 3 or 6 h after injection of BiV or saline and peritoneal leukocytes at 1 h up to 72 h after these injections. Total cell counts were determined in a Neubauer chamber after dilution in Turk solution (1:20 v/v) and differential cell counts performed in $HEMA^3$ stained smears. **Results and Discussion:** BiV significantly increased the number of total leukocytes from 3 h up to 72 h in the peritoneal cavity of animals (mean BiV= 20.8 ± 2.1 cells/mL; control= 9.4 ± 1 cells/mL). Moreover, BiV significantly increased the peritoneal levels of TXB_2 (2.9 ± 0.3 ng/mL) and LTB_4 (2.6 ± 0.5 ng/mL) at 6 h after injection, compared with respective controls (1.7 ± 0.2 and 0.5 ± 0.2 ng/mL). Levels of MCP-1 were significantly increased from 0.5 up to 48 h (mean: 9.2 ± 0.2 ng/mL; control: 3.3 ± 0.2 ng/mL) whereas levels of KC were not modified by venom injection. In addition, BiV increased total leukocyte (146 ± 16.4 $x10^5$ cells/mL), MN (102.5 ± 11.05 $x10^5$ MN/mL) and PMN (43.5 ± 7 $x10^5$ PMN/mL) numbers in the blood at 3 h after injection, compared to control (64.6 ± 5.4, 54.25 ± 5 and 10.4 ± $0.6x10^5$ cells/mL, respectively). BiV induces a marked leukocyte infiltration at the site of its injection. The ability of BiV to release chemotactic mediators such as LTB_4, TXA_2 and MCP-1 but not KC, as well as to mobilize leukocytes from bone marrow compartments is important for the recruitment of leukocytes to the site of its injection.

Supported by: FAPESP and CNPq.

1.29 Toxic properties of venoms from Micrurus genus: neutralization potential of Brazilian anti-elapidic serum

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Introduction: *Micrurus* bites can cause death by muscle paralysis and further respiratory arrest few hours after envenomation. The specific treatment for *Micrurus* envenomation is the application of heterologous antivenoms. **Objectives:** The aim of this study was to compare the toxic potential of venoms from nine species of coral snakes (*M. corallinus, M. frontalis, M. fulvius, M. surinamensis, M. lemniscatus, M. altirostris, M. spixii, M. hemprichii and M. ibiboboca*) and analyze the ability of the Brazilian anti-elapidic serum to neutralize their main toxic enzymes, *i.e.*, phospholipases and hyaluronidases. **Methods:** The lethal potential of the *Micrurus* spp venoms was assessed in outbred mice by intraperitoneal injection of different amounts of venoms. Phospholipase A_2 activity was determined venom samples incubated with a mixture containing: 5 mM Triton X-100, 5 mM phosphatidylcholine (Sigma), 2 mM HEPES, 10 mM calcium chloride and 0.124% (wt/vol) bromothymol blue. The hyaluronidase activity of *Micrurus* venoms was measured with the substrate hyaluronic acid for 15 min at 37ºC. The ability of the anti-elapidic serum to neutralize venom phospholipase A_2 and hyaluronidase was estimated by incubating *Micrurus* spp venoms with the antivenom. **Results and Discussion:** The LD_{50} values, calculated by probit analysis at 95% confidence, were variable among *Micrurus* venoms, with the more lethal being the ones from *M. corallinus, M. spixii, M. altirostris* and *M. lemniscatus*. High phospholipase activity was detected in *M. hemprichii*, *M. frontalis*, *M. fulvius*, *M. lemniscatus*, *M. altirostris*, *M. ibiboboca*, and *M. spixii* venoms. On the other hand, *M. lemniscatus*, *M. corallinus*, *M. hemprichii* showed higher hyaluronidase activity when compared with the other venoms. In contrast to the high phospholipase and hyaluronidase activities, these *Micrurus* venoms exhibited low proteolytic activity. Neutralization studies showed that the Brazilian anti-elapidc serum was unable to fully block the phospholipase activity of *M. spixii*, *M. frontalis* and *M. fulvius* venoms and the hyaluronidase action of *M. lemniscatus* and *M. hemprichii* poisons. These results suggest that other coral snakes venoms should be included in the immunization pool, in order to produce a fully neutralizing antiserum for the Brazilian coral snake venoms.

Supported by: FAPESP and CNPq.

1.30 Comparative peptidomics of *Bothrops cotiara*, *Bothrops fonsecai* and *Bothrops jararaca* snake venoms

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Introduction: Snake venoms are rich sources of bioactive proteins and peptides of interest in clinical, biochemical and drug discovery research. In this work we analyzed the venom peptidomes of three Bothrops species by mass spectrometric techniques. We found new peptides from the bradykinin-potentiating peptide/C-type natriuretic peptide precursor (BPP/CNP) in the venom of *B. jararaca* and, interestingly, fragments of L-amino acid oxidases (LAAO) in the venoms of the three snake species. **Objective:** The objective of this work was to compare the venom peptidomes of three Bothrops species. **Methods:** The venom peptidomes were analyzed by LC-MS/MS and peptide sequences were determined by de novo sequencing or by automated database searches using the software MASCOT. **Results and Discussion:** The LAAO peptides identified in the venoms provided sequence coverages of up to 56%, 52% and 48% and MOWSE scores of 3914, 1991 and 488 for the protein in the venoms of *B. cotiara*, *B. fonsecai* and *B. jararaca*, respectively. As expected, most of the known BPPs were identified in the venom of *B. jararaca* along with a new glycine-rich peptide derived from the BPP/CNP precursor: HHDHHAAVGGGGGGGGGGA. A similar peptide was also found in the venoms of *B. cotiara* and *B. fonsecai* containing nine glycine residues instead of ten. None of the known *B. jararaca* BPPs was identified in the venoms of *B. cotiara* and *B. fonsecai*; however, new BPP sequences were found in these venoms.

Supported by: CAPES and FAPESP.

1.31 Maintenance of blood-brain barrier integrity and decreased severity of experimental multiple sclerosis in mice treated with tempol

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Introduction: Multiple sclerosis (MS) is a prevalent disease of the central nervous system (CNS). Nitric oxide-derived oxidants have been implicated in the pathogenesis of MS. **Objective:** Here, we studied the effect of tempol, a stable nitroxyl antioxidant in the C57BL/6. strain of mice intracerebrally inoculated with A59-strain coronavirus mouse hepatitis virus. **Methods**: Tempol was administered by i.p. injection (24 mg/Kg, 9 doses and in drinking water, 2 mM, ad libitum). Mice were assayed for: clinical score; spinal cord viral titration by plaque assay; spinal cord BBB permeability using sodium fluorescein as a tracer molecule; and analysis of spinal cord protein nitration by immunoblot. **Results and Discussion:** Our results showed that the effect of tempol in 60% of immunized mice (6-7d.p.i) is associated with the maintenance of spinal cord tissue BBB integrity by a mechanism that inhibits protein nitration. The permeability in tempol-treated mice was about 2 times lower than in vehicle-treated mice. Tempol treatment significantly inhibited spinal cord protein nitration from apparently healthy or mildly sick mice. In contrast, high levels of 3-nitrotyrosine residues were detected in spinal cord proteins from severely sick mice We also showed inhibition of virus proliferation and a survival advantage in tempol-treated mice that extended beyond 60 days in about 60 % of immunized mice. Using this model of MS, we also observed that females are more resistant than male mice. In addition, we performed a pharmacokinetics assay of tempol detection by paramagnetic resonance spectroscopy of mouse brains and demonstrated that the CNS is highly permeable to this drug, reaching the maximal concentration early (5-10 min.) after ip treatment. In conclusion, our results indicate that tempol readily penetrates de blood brain-barrier and can, at least partially, preserve BBB integrity by a mechanism that inhibits protein nitration, indirectly inhibits virus proliferation and prevents and/or delays the onset of multiple sclerosis clinical signs.

Supported by: FAPESP and CNPq/REDOXOMA.

1.32 Toxin cross-reactivity after oral tolerance induction with *Bothrops jararaca* venom

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Introduction: Oral tolerance is classically defined as specific suppression of cellular and humoral immune responses to a determined antigen with previous oral administration of antigen. It has a unique immunological importance because it is a natural and continuous event driven by external antigen. It is characterized by low IgG levels in the serum of animals after immunization with the antigen. Some groups reported low IgE levels in the serum of animals with induction of tolerance. As for the main immunobiological characters, genetic and environmental factors are involved in the immunological tolerance by oral administration, demonstrating that this trait is a process under the influence of multiple factors. Autoimmune processes are an example of this phenomenon. There is no report of oral tolerance induction to serpent venoms. We propose a methodology for knowledge of the major common tolerogenic epitopes and the correlation analysis with the main toxic components among snake venoms pertaining to a given genus. **Objectives:** To induce oral tolerance to *B. jararaca* venom in mice and to evaluate specific tolerance and the cross reactivity to other *Bothrops* species toxins. **Methods:** BALB/c mice received oral administration of *B. jararaca* venom [Group 1: high concentration; Group 2: low concentration on alternate days]. After 7 days, the animals were immunized with *B. jararaca* venom adsorbed/encapsulated into nanostructured SBA-15 silica. Two other groups that received low concentration of venom orally were immunized with *B. atrox* or *B. jararacussu* venom. As a control, a group of animals was only immunized with *B. jararaca*. The antibody titers were determined by ELISA. **Results and Discussion:** Animals orally receiving high concentration of *B. jararaca* venom were responders, showing antibody titers similar to those of immunized animals. On the other hand, mice orally tolerized with low concentration of venom showed low antibody titers after immunization with *B. jararaca* venom. In animals that received low concentration of *B. jararaca* and then immunized with *B. atrox* venom, tolerance was not observed. At least in part, these preliminary results demonstrate that the tolerance to toxins seems specific for the majority of the venom constituents. Immunoblotting analysis of distinct *Bothrops* venom species will provide details about the main tolerogenic epitopes.

Supported by: CNPq/INCTTOX.

1.33 Production of native Fab from an anti-jararhagin monoclonal antibody - MAJar3 - and sequencing of immunoglobulin variable regions for structural studies

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Introduction: Snake venom metalloproteinases (SVMPs) are zinc-dependent proteolytic enzymes that play an important role in hemostatic disturbances and local lesions in bothropic envenomings. Jararhagin is a hemorrhagic PIII metalloproteinase isolated from *Bothrops jararaca* venom, comprised of metalloproteinase, disintegrin-like and cysteine-rich domains. The monoclonal antibody MAJar3 neutralizes the hemorrhagic effect of *Bothrops* venoms and the binding of jararhagin to collagen, suggesting that collagen-binding is a key factor for SVMPs hemorrhagic activity. The binding site of jararhagin to collagen was indirectly analyzed through the molecule region that was recognized by MaJar3. Studies of molecular modeling revealed that it was located in the desintegrin-like domain, and further crystallographic studies are required to confirm the exact interaction between collagen and jararhagin. **Objectives:** To isolate the Fab fragment from MAJar3 and to sequence the variable regions of its heavy and light chains in order to obtain the jararhagin-FabMAJar3 immunocomplex in appropriate conditions for structural studies. **Methods:** MAJar3 was purified from the hybridoma culture supernatant by affinity chromatography using protein-G Sepharose. Purified IgG was digested with 1mg/mL immobilized papain and the Fab was purified by affinity chromatography (HiTrap protein A HP/1mL) in FPLC system. The immunocomplex FabMAJar3/Jararhagin was assembled by incubation at 37°C for 30 minutes, tested for neutralization of hemorrhagic activity on mouse skin and isolated from free antigen or antibody by size exclusion chromatography. To sequence MAJar3 variable regions, total mRNA was isolated from $5x10^6$ mouse hybridoma cells and purified on oligo (dT)-cellulose columns (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) chains of the antibody was carried out using the light and heavy chain primers from Amersham Biosciences. These cDNAs were cloned into pGEM-T Easy vector and sequenced. **Results and Discussion:** The purification of MAJar3 from hybridoma culture supernatant had a yield of 25 mg IgG/L of supernatant. After the digestion with papain, the antibody was submitted to a protein A chromatography which effectively retained the Fc fragments and undigested IgG, being the Fab portion obtained in the flow-through fraction, resulting in 1.9 mg Fab/L of supernatant. The SDS-PAGE showed a double band of mol. mass ~25 kDa. The preliminar assay of hemorrhagic activity using the FabMAJar3/Jararhagin immunocomplex revealed a neutralization in mouse skin tests. Size exclusion in HPLC system demonstrated the retention time of Fab, jararhagin and immunocomplex were 32.1, 29.0, 28.0, respectively. The sequence of variable light and heavy chain were obtained and confirm an identity with mouse immunoglobulins. We are currently improving the protocols of Fab purification in order to get enough yields of jararhagin-FabMajar3 immunocomplexes, in the appropriate conditions for crystallization assays with the objective to elucidate the preliminary results for characterization of jararaghin epitope.

Supported by: CNPq/INCTTOX and FAPESP.

1.34 Venom of the caterpillar *Premolis semirufa* induces the generation of anaphylatoxins by activating the complement system in human serum

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Introduction: The Brazilian caterpillar of *Premolis semirufa*, commonly called *pararama*, belongs to the *Arctiidae* family of the order *Lepidoptera*. This caterpillar is native to the Amazonian rubber tree plantations and the accidental contact with its bristles causes local symptoms such as edema, intense itching and pain, lasting three to seven days, where after multiple accidents it can lead to joint-space narrowing and bone alteration, as well as degeneration of the articular cartilage and immobilization of the affected articulations. Specific treatment for this disease does not exist, but corticosteroids are frequently administered. Despite the public health hazard of *Premolis semirufa* poisoning, little is known about the pathological alterations of poisoned victims and the biochemical and biological properties of the extract of the caterpillar bristles are poorly understood. **Objectives:** Considering that the complement system is an important component of innate immunity and that it may be involved in the pathogenesis of the disease, the present study was carried out to evaluate the *in vitro* effects of the crude extract of the caterpillar's bristles on the complement system. **Methods:** For this purpose, normal human serum was incubated with increasing concentrations of bristles crude extract and the remained complement activity assessed in hemolytic assays, using conditions to develop alternative and classical pathways. Furthermore, activation of the lectin pathway was also determined in microtiter plates coated with mannan (10 μg/mL) and the production of anaphylatoxins C3a/C3a desArg, C4a/C4a desArg, and C5a/C5a desArg was measured using the Cytometric Bead Array (BD Biosciences PharMingen, USA). In addition, direct proteolytic activity of the bristles crude extract on components of the complement system, such as C3, C4 and C1esterase inhibitor (C1inh) was also evaluated. **Results and Discussion:** Results show that the bristles extract was able to induce a dose-dependent inhibition of the alternative complement pathway and a low inhibition of the lectin pathway; however, the extract had no influence on the classical pathway. Moreover, the caterpillar extract induced anaphylatoxin production and caused cleavage of C3, C4 and C1inh components. These results suggest that *Premolis semirufa* venom can activate the complement system, generating a large amount of anaphylatoxins, which may play an important role in the inflammatory processes seen in humans after envenomation.

Supported by: FAPESP and CNPq/INCTTOX.

1.35 Peripheral opioid receptor expression is distinctly regulated by the presence of acute or chronic tissue injury

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Introduction: The main challenge in the therapy of pain is providing relief without causing adverse effects. While opioids efficiently alleviate pain, they induce serious side effects. Besides their central mechanisms of action, opioids also exert analgesia through peripheral mechanisms. This alternative mechanism allows for antinociception after the application of systemically inactive doses of opioids directly into injured peripheral tissue, minimizing adverse central actions. Interestingly, clinical and experimental data have shown that the local efficacy of opioid drugs is enhanced in the presence of tissue injury/inflammation, but the mechanisms involved in this phenomenon are not well characterized. Previous data of our group demonstrated that, in rats, prostaglandin E_2 (PGE_2, intraplantar/i.pl.) and chronic constriction injury (CCI) of the sciatic nerve increase the peripheral analgesic efficacy of opioid agonists and of crotalphine (CRP), a peptide obtained from *C. d. terrificus* snake venom. CRP has a local antinociceptive effect mediated by the activation of κ-opioid receptor in PGE_2-induced hyperalgesia model or κ- and δ-opioid receptor in the CCI model. **Objectives:** The aim of this study was to characterize some of the mechanisms involved in the increase of the analgesic efficacy of opioids caused by inflammation and tissue injury. For this purpose the effect of PGE_2-induced hyperalgesia and CCI on opioid receptor expression in the dorsal root ganglia (DRG) and nerve paw (NP) was evaluated. **Methods:** The expression of μ–, κ– and δ-opioid receptors was evaluated by immunoblotting, in DRG or NP (ipsilateral and contralateral to injury), of male Wistar rats, 3 h after i.pl. injection of PGE2 (100 ng/paw) or 14 days after CCI. **Results and Discussion:** PGE_2 increases the expression of μ- and κ-opioid receptors in NP (43% and 71%, respectively) and decreases (30%) the expression of δ-opioid receptors, when compared to naïve rats. μ-Opioid receptor expression is also increased in the ipsilateral and contralateral DRG (79 and 27%, respectively), while κ-opioid receptor expression is increased only in the ipsilateral DRG (168%), when compared to naïve rats. CCI up-regulates m-opioid receptors in NP (27%) and DRG (ipsilaterally and contralaterally, 49 and 20%, respectively) and δ-opioid receptors in the ipsilateral DRG (35%). On the other hand, κ-opioid receptors are down-regulated by CCI in both NP (51%) and DRG (21%), when compared to naïve rats. Thus, the results obtained indicate that peripheral opioid receptor expression is distinctly regulated by the presence of acute or chronic tissue injury and provide evidence regarding the effectiveness of peripheral opioids in both acute and chronic pain. The different expression patterns of κ- and δ-opioid receptors caused by acute and chronic injury may contribute to the comprehension of the mechanisms involved in the activation of opioid receptors by CRF in PGE_2-induced hyperalgesia and CCI models. In addition, these data also point out that drugs that activate peripheral opioid receptors, including substances derived from animal toxins, could have therapeutic potential as peripherally active analgesics.

Supported by: FAPESP.

1.36 Proteomics profiling of age- and sex-based variability in *Bothrops jararaca* snake venom

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Introduction: Snake venom composition is a result of multiple factors and its inherent variability is often related to environmental and ecological traits, which can change from species to species. **Objectives:** The aim of this work was to explore the age- and sex-based variability of the *B. jararaca* venom proteome. **Methods:** Venoms from 694 two-week-old newborns and 110 adults (49 males and 61 females, older than 3 years) from São Paulo state were milked and the venom lyophilized. Venoms were analyzed by two-dimensional electrophoresis (2D-PAGE), and spot identification was performed by in-gel trypsin digestion followed by liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) analysis. **Results and Discussion:** 2D-PAGE showed striking differences among the samples analyzed. Newborn venom showed a complex profile of high-molecular-weight acidic proteins, identified as P-III class snake venom metalloproteinases (SVMPs). Conversely, adult venom showed several spots identified as P-I class SVMPs, almost absent in newborn venom. L-Amino acid oxidase (LAAO) and nerve growth factor (NGF) are among the highly abundant spots identified in adult male venoms. On the other hand, adult female venom showed several isoforms of C-type lectins (CTLs) and abundant spots identified as P-III class SVMPs. *N*- and *O*-glycosylation showed distinct profiles among the samples, being markedly different between newborn and adult venoms. For a quantitative comparative analysis, we employed isobaric tag peptide labeling (iTRAQ) coupled to 2D LC-MS/MS. Analysis by the iTRAQ approach identified over 70 proteins in the venoms. The major quantitative differences were detected in newborn and adult venoms, where SVMPs, serine proteinases, CTLs, phospholipase A2 and NGF were found in higher abundance in adult venom. However, the presence of unknown toxins in newborn venom may have influenced these results, since identification and quantification are dependent on the availability of the protein sequence in databanks.

Supported by: FAPESP.

2: Biochemistry

2.01 Circulating activities of APN and DPPIV in monosodium glutamate-obese and food-deprived rats

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Introduction: Several peptides related to energy balance are susceptible to hydrolysis by neutral aminopeptidase (APN) and dipeptidyl peptidase IV (DDPIV). **Objectives:** To evaluate the involvement of glycemia, biometry and activity levels of plasma APN and DPPIV in obesity and food deprivation. **Methods:** APN, DPPIV, protein, glycemia, body mass, mass of retroperitoneal and periepididymal fat pads, and Lee index were measured in monosodium glutamate obese (MSG) and food-deprived (FD). **Results and Discussion:** Plasma APN was distinguished as sensitive (PSA) and predominantly insensitive (APM) to puromycin, while DPPIV was sensitive (DPPIV-DS) and predominantly insensitive (DPPIV-DI) to diprotin A. Although unchanged in MSG and FD, APM activity levels were closely correlated with body mass, Lee index and mass of retroperitoneal fat pad in FD but not in MSG. Plasma levels of DPPIV-DI activity decreased in MSG-FD and correlated with body mass, Lee index and mass of periepididymal fat pad. The negative correlation between plasma APM and retroperitoneal fat pad is suggestive of downregulation of somatostatin in food deprivation. Reduction of plasma DPPIV-DI levels may be a homeostatic response associated with food deprivation for recovering energy balance in MSG obese rats.

Supported by: FAPESP and CNPq.

2.02 Hypothalamic and hippocampal DPPIV activity and CD26 in monosodium glutamate and food-deprived rats

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Introduction: Dipeptidyl peptidases (DPPs) are emerging as a protease family with important roles in the regulation of signaling by peptide hormones. **Objectives:** To study DPPIV/CD26 in soluble (SF) and solubilized membrane-bound (MF) fractions from hypothalamus (HT) and hippocampus (HC) of fasted (FD) and non-fasted monosodium glutamate obese (MSG) and normal rats. **Methods:** Catalytic activity of DPPIV by fluorometry and monoclonal protein expression of CD26 by ELISA. **Results and Discussion:** Compared to controls, MSG and/or FD induced the reduction of diprotin A insensitive (DI) DPPIV activity in SF and MF from HT, as well as in diprotin A-sensitive (DS) DPPIV activity in MF from HC. MSG and/or FD induced an opposite response (increase) of DPPIV-DI activity in MF from HC. The monoclonal protein expression of CD26 in MF by ELISA decreased in HT and increased in HC of MSG and/or FD relative to controls. The existence of DDPIV-like activity with different sensitivities to diprotin A and the identity of the less sensitive as CD26 were demonstrated for the first time in the central nervous system. DPPIV-DI/CD26 activity seems to play a role in the endocrine regulation of energy balance and anxiety, respectively, in the HT and HC.

Supported by: FAPESP and CNPq.

2.03 Isolation of bioactive peptides by the action of serinoproteases in venom of *Bothrops jararaca* on endogenous substrates and actions in cell culture and bioassays

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Introduction: Venoms are a rich source of proteolytic enzymes; from the *Bothrops jararaca* venom serinoproteases and metalloproteases are the main enzymes that act on various tissues and proteins present in the victim. Besides the action on tissues, these proteases could generate some compounds that could have specific actions in cells or show other mechanisms towards the generation of bioactive peptides. There are protein precursors of bioactive peptides, but nowadays there is a new class of proteins that in some conditions may generate bioactive peptides; they are called crypteins. **Objective:** Identification of bioactive peptides by the action of the serinoproteases, trypsin and from the venom of *Bothrops jararaca* on endogenous substrates (casein, IgG, hemoglobin, collagen and myoglobin). To biochemically isolate and characterize the peptides obtained and determine the possible effects and biological properties of these peptides through several biological tests "in vitro" and "in vivo." **Methods:** Serinoprotease from *Bothrops jararaca* venom was separated from whole venom using a Sephadex G-100 gel filtration column. The serinoproteases were incubated with the endogenous substrates chosen for a preset time. The endogenous substrates were also incubated with trypsin, as well the serinoproteases from the venom of *Bothrops jararaca*. Directly from the incubations, the remaining proteins and resulting products were observed by gel electrophoresis SDS-PAGE with silver staining and also by means of HPLC (high performance liquid chromatography) profile. The hydrolysates were tested in fibroblast cell cultures. After the test in cell culture, the active hydrolysates were purified by HPLC, and the peaks would then be tested by the same methods in order to identify the active peptides. **Results and Discussion:** In fibroblast cell cultures, a low cell proliferation rate was obtained with hemoglobin and collagen hydrolysates and a significant cytotoxic effect with immunoglobin (IgG) using trypsin as proteolytic enzyme. The results obtained in HPLC showed us that profiles of hydrolysates differed for each type of substrate used. The study suggests that these serinoproteases (trypsin or those from Bothrops jararaca venom) were able to generate peptides with important biological activity; specifically with snake venom, it could be another component of the bite response.

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

2.04 Heat shock protein responses in thermally stressed freshwater snails (*Biomphalaria glabrata*)

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Introduction: The synthesis of a major stress protein class (HSP70) is induced by extremes of a wide variety of environmental variables, both physical and chemical. This protein of approximately 70,000 Da is a widespread heat shock protein and is present in various isomorphs in many species. Nevertheless, it is highly conserved among organisms from bacteria to man. Many researchers have tried to institute HSPs as biomarkers of environmental damage, investigating a broad range of organisms. **Objectives:** This work was designed to identify HSP70 stimulus by increases of environmental temperature in snails *Biomphalaria glabrata* reared in the laboratory, as an initial step to establish HSP70 as a biomarker of environmental stress. This species was chosen because of its increasing importance as an experimental model. **Methods:** Snails were maintained at temperatures of 5-10 °C above its environmental temperature for one week and dissected for protein analysis of digestive gland tissue. The proteins were extracted by RIPA buffer and centrifugation at 14,000 x g, and the supernatants were analyzed using sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting, using the HSP70-directed antibody. The samples were applied to the gels at 200 μg per lane. Protein concentrations were determined by the Lowry method. A 10% SDS-PAGE gel was used to separate the proteins on a BioRad minigel apparatus using Laemmli buffer. The gels were transferred to nitrocellulose membranes using a transfer system (BioRad). Blots were blocked with PBS-milk (5%) and incubated for 1 h with rabbit HSP70 antibody (1:1000 in PBS-milk), rinsed with PBS-milk and incubated with goat anti-rabbit horseradish peroxidase conjugated antibody (1:2000 in PBS-milk) for 1 h. The nitrocellulose membrane was then rinsed and incubated with a developing kit and kept in contact with photographic film to allow visualization. **Results and Discussion:** There was an induction of HSP70 in snails submitted to increased environmental temperature. The stimulus for HSP70 in thermally exposed snails was confirmed by the presence of protein, visualized in the film; no protein was detected in control samples. These results suggest that the stress protein response may be a useful environmental stress indicator.

2.05 A lipocalin sequence signature modulating cell survival, extracellular matrix remodeling and wound healing

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Introduction: Lopap is 20-kDa prothrombin activator with serine protease-like activity found in the bristles of the *Lonomia obliqua* caterpillar. Interestingly, this protein does not have sequence similarity with other proteases, but is considered a member of the lipocalin family. Lipocalins are β-barrel proteins recognized as carriers of small hydrophobic molecules. Some members have been described as modulating cell survival, developmental process and homeostasis. **Objectives:** The aims of this study were to identify the region in Lopap molecule involved in modulation of cell responses, to survey this sequence as a lipocalin signature and to evaluate its potential in extracellular matrix (ECM) remodeling and tissue repair. **Methods:** Synthetic peptides were obtained based on characteristic conserved motifs of lipocalins found in the Lopap primary sequence. The peptides were tested in serum-deprived human umbilical vein endothelial cells (HUVECs) for a screening of their antiapoptotic activity. The peptide that was able to promote cell survival (AP) was analyzed using bioinformatics tools. Primary cultures of human fibroblasts were stimulated with AP and the synthesis of ECM molecules was evaluated by immunocytochemistry. To evaluate the effect of AP *in vivo*, mice were treated in the dorsal area with intradermal injections of AP (0.04-25μg) on one side and saline on the opposite side. Skin fragments of 1 cm^2 were subjected to histological preparation and Picrosirius red coloration to measure the percentage of collagen in the dermis. The potential of AP as a wound-healing agent was evaluated in a rat skin lesion-induced model. The lesions were treated with local applications of AP or saline and the wound closure was monitored. The regenerating tissue was analyzed by histological techniques. In addition, the content of collagen and metalloproteinases (MMPs) were evaluated. **Results and Discussion:** The active peptide had 11 amino acid residues and was located in a β-sheet in the Lopap tertiary structure. Similar sequences were found among other lipocalins and putative proteins. HUVECs treated with AP showed 100% cell viability, in contrast to 65% of non-treated cells. AP-treated fibroblast cultures showed an increase from 2.6±0.1, 4.9±0.5 and 22.7±1.3 to 3.9±0.3, 9.4±0.9 and 36.8±1.9 percent procollagen. tenascin and fibronectin, respectively, in relation to non-treated cultures. Similarly, treatment of mice with AP resulted in a significant increase in collagen in the mouse dermis. A cumulative dose of 0.6 μg induced an increase of 15% in collagen content, which persisted after 12 weeks. Mouse skin lesions treated with the peptide showed an improvement of tissue repair, with a better organization of collagen fibers and the absence of keloid scars. The collagen content was substantially increased in the regenerating tissue of lesions treated with AP. A significant increase in MMP-2 was also observed. All these data reveal the potential of AP in aiding tissue repair. The Lopap-derived sequence identified seems to be a conserved property among lipocalins, promoting cell survival and possibly playing an important role in homeostasis. It should be important in the regulation of the metamorphosis process in *Lonomia*.

Supported by: FAPESP, FINEP and CNPq.

2.06 Purification of coagulation factor VIII using chromatographic methods

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Introduction: Factor VIII (FVIII) is a plasma glycoprotein essential in the coagulation process, whose deficiency causes hemophilia A. Treatment consists in infusions of FVIII recombinant protein or concentrates obtained from plasma, none of them produced in Brazil. Here, we continue the studies to develop a process to purify FVIII employing solely chromatographic methods. We propose the use of ion-exchange as the first step of FVIII purification because it is inexpensive and suitable for a scaling up method. Besides, based on previous results, ion-exchange can be used by direct application of plasma. We have previously used the Q-Sepharose resins, which are strong anion-exchange matrices made from 6% agarose. To improve the recovery of FVIII activity, we have tested ANX-Sepharose Fast Flow (FF). This resin is composed of 4% agarose and, therefore, has high capacity for binding large molecules. Being a weak anion-exchange, this resin also shows an alternative selectivity. Another aspect of this project is the analysis of the FVIII fraction collected from chromatographic separations by immunoblotting. In order to prepare an in-house FVIII standard, we have used a modified method from the GE Healthcare validated process, consisting of gel filtration, cation-exchange and finally gel filtration. **Objectives:** 1. To test the ANX-Sepharose FF as the first step for FVIII purification. 2. To prepare an in-house FVIII standard. **Methods:** 1. We studied the ANX-Sepharose FF capacity of binding FVIII as well as the profile of the collected samples. We also analyzed the FVIII containing fraction in a Sepharose 6 FF gel filtration column. 2. FVIII was purified by direct application of plasma to Sepharose 4FF column, followed by the application of the FVIII-containing fraction to SP-Sepharose FF and desalting in the G25 fine column. All collected fractions were analyzed for protein content (Bradford), FVIII and protein C activity (chromogenic methods), and presence of factors IX and X (Western blotting). **Results and Discussion:** The FVIII binding capacity of ANX-Sepharose FF is much higher than that observed for Q-Sepharose. We applied up to 15 column volumes (CV) of plasma to the ANX-Sepharose FF column and no FVIII was detected in the flow-through fraction, while with Q-Sepharose resins the amount of plasma applied was 5 CV. After the application of 15 CV of plasma, solid residues were clearly visible on the top of the column. FVIII coeluted with protein C and coagulation factors IX and X. The recovery of the FVIII activity in the eluate was between 50 to 60% and was comparable with that found for Q-Sepharose columns. Finally, application of the ANX column eluate to Sepharose 6FF also gave a similar profile compared to that observed using Q-Sepharose column eluate. Two peaks were eluted containing FVIII activity, one in the void volume and a second peak coeluting with lower-molecular-mass proteins.

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

2.07 Purification and characterization of a hypothermic component from the venom of *Phoneutria nigriventer*

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Introduction: The neural control of body temperature can be modulated by several toxic substances. Arthropod venoms, from scorpions and spiders for example, are a rich source of potent toxins responsible for a significant number of non-lethal human envenomations. The evenomation by the spider "*Phoneutria nigriventer*" shows several systemic manifestations including vomiting, sweating, hyperthermic or hypothermic state, mainly in children. **Objectives:** The biochemical characterization of "*Phoneutria nigriventer*" venom profile on temperature regulation of young anesthetized rats. **Methods:** The crude venom lyophilized and its "pool II" purified using gel filtration (Sephadex G-50) and reverse phase HPLC; was administered intraperitoneally in young rats, anesthetized with a solution of ketamine hydrochloride (40 mg/kg)/ diazepam (5 mg/kg). The body temperature was recorded by rectal probe acquisition system (3x20mm) and registered on software Contemp® (mod.IPM94) every 0.5 min up to 3 h. **Results and Discussion:** The data of the crude venom indicated a marked hypothermic state in young rats in the first hour of the assay. In the second hour, the venom induced a reduction in hypothermic effect to compensate for the hemodynamic changes, and in the 3rd hour the temperature fell significantly to above the control level. The preliminary data obtained from pool II demonstrated similar effects mainly in the 3rd hour. The results suggest that the hypothermic effect involves a neurotoxin activity on the thermoregulation of young rats. Purification of the other pools is under way to determine their activity on the temperature regulation of young rats.

Supported by: CNPq/INCTTOX.

2.08 Anti-*leishmania* molecule in the extract of the body of *Otostigmus* sp.

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Introduction: Arthropods constitute one of the oldest groups of organisms, and also show a wide distribution in different ecosystems and habitats. This fact leads to the question about which factors allowed such an ecological success in these organisms. Knowing that most of the environments where they are found show a high presence of microorganisms and pathogens, it can be affirmed that part of the success of arthropods in colonizing these environments are their defense mechanisms and immune systems. One of the main components of the defense mechanism in vertebrates and invertebrates are the peptides with immune functions, which control the growth and invasion of the different pathogens. The defensive role of a variety of antibiotic peptides in multicellular organisms is increasingly recognized, but the characterization of the peptides with anti-parasitic function, had not begun until recent times. Also, the majority of this research has focused on the hydrophobic peptides, ignoring the hydrophilic compounds. For all these reasons, it is important to study this subject, not only to understand the success of these invertebrates and their defense mechanisms, but also to find alternatives to fight infectious and also parasitic diseases that affect humans. This is why the purification and characterization of these peptides and the knowledge of the function of their immune systems are even more interesting. **Objectives:** The aim of this study was the separation and analysis of highly hydrophilic compounds to identify the antimicrobial factors in the extract of the body of the Brazilian myriapod *Otostigmus* sp. **Methods:** The bodies of the animals were first subjected to maceration and acid extraction and then fractionated in two steps. First, using C18 Sep Pak column cartridge, the hydrophilic and hydrophobic fractions were separated. The hydrophilic fraction was concentrated in a vacuum centrifuge, reconstituted in ammonium acetate (pH 6.7) and loaded onto an Asahipak GS-320 column, using an isocratic gradient of ammonium acetate (pH 6.7) for the second purification step. The column effluent was monitored by absorbance at 225 nm and the anti-parasitic and antimicrobial activity was determined by liquid growth inhibition assay. **Results and Discussion:** After the HPLC separation, antimicrobial activity was detected against *Escherichia coli*. The same fraction also showed anti-parasite activity against *Leshmania* spp. The first analysis with mass spectrometry showed that the fraction that showed that anti-parasitic and antimicrobial activity had a low molecular weight. As a hydrophilic fraction, its mechanism of action must be different from the mechanisms of action of hydrophobic molecules, which will be important in understanding the mechanism of action of these substances and learning how they interact with the membrane. The purification and characterization of these fractions is still in progress.

Supported by: FAPESP and CNPq.

2.09 The effect of a recombinant Kunitz type inhibitor (r-KTI) from *Amblyomma cajennense* on cell survival and its molecular target

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Introduction: A recombinant protein, showing factor-Xa inhibitor activity and characterized as a Kunitz-type inhibitor was obtained from a cDNA library of salivary glands of the tick *Amblyomma cajennense*. The protein did not show cytotoxicity against normal cells (fibroblasts and HUVECs), but it was able to induce death of tumor cells (human melanoma SK Mel-28, pancreatic adenocarcinoma Mia Paca-2) *in vitro*. At a later stage, we also investigated the influence of this rKTI on the control of cell cycle phases and gene expression in tumor cells. **Objectives:** The aim of this study was to evaluate the mechanism of the action of the rKTI inducing tumor cell death. **Methods:** Cell cycle and cytotoxicity studies were performed by flow cytometry. Changes in gene expression of Sk Mel-2 and Mia-Paca-2 were evaluated by DNA microarray using the CodeLink platform Whole Human Genome Bioarray. Proteasomic catalytic activity was assessed by fluorimetry and poly-ubiquitinylated tumor cell proteins by immunoassay. **Results and Discussion:** rKTI induces cell cycle arrest at G0/G1 and apoptosis in tumor cells. Analysis of gene expression by micorarray showed several genes altered in Mia Paca-2 and SK Mel-28, among them 24 common genes for both cell lines. The results showed that the rKTI inhibits the trypsin and chymotrypsin-like catalytic activity of proteasome. The total poly-ubiquitinylated cancer cell proteins were increased after treatment. Altogether, our results suggest that among the common altered genes after rKTI treatment, the majority are related to cell cycle control and cell death by the apoptotic route. The alteration in the *PSMB2* gene (which codes for a proteasome β-2 subunit) corroborated the alterations observed in the inhibition of catalytic activity and consequent increase in poly-ubiquitinylated proteins, indicating that the proteasome is a cellular target of the rKTI in inducing cell death.

Supported by: FAPESP and CNPq.

2.10 Characterization of digestive lipase from hematophagous vectors

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Introduction: Lipases (EC 3.1.1.3) catalyze the hydrolysis of triacylglycerols resulting in the release of fatty acids. Lipases are enzymes found in animals, plants, fungi and bacteria. Several Arthropoda such as mosquitoes and ticks are vectors of plant, animal and human diseases, and they are public health problems. The mosquito *Aedes aegypti* (Insecta) and the tick *Amblyomma cajennense* (Arachnida) are important Arthropoda vector in Brazil of dengue fever and Brazillian spotted fever, respectively. **Objectives**: To characterize the lipid digestion in *A. aegypti* (adult and larva) and *A. cajennense*. In order to do that, we measured the lipase activity in midgut homogenates from these animals. Properties such as pH effect, the effect of Ca^{+2}, acid precipitation and chromatographic separation were determined for these lipases. **Methods**: Midgut from *A. aegypti* (adult and larva) and *A. cajennense* females were isolated and homogenized in Milli Q water or homogenized in 100 mM sodium acetate pH 3.5 buffer in a Potter-Elvehjem homogenizer. Lipase activity was measured using 4-methylumbelliferyl oleate (MUO) as substrate. Homogenate samples were submitted to different chromatographic steps (cation and anion exchange chromatographu). *A. aegypti* adult and larva homogenates were initially applied to a Hitrap Q column equilibrated in 20 mM Tris buffer, pH 7.0. Proteins were eluted in a NaCl gradient (0-1 M) in the same buffer. Lipase-active fractions from *A. aegypti* adult chromatography were pooled, desalted and applied to a Resource Q column. Protein was eluted using a linear gradient of 0-0.5 M NaCl in 20 mM Tris-HCl buffer, pH 7.0. **Results and Discussion**: Lipase activity was measured in midgut homogenate samples from *A. aegypti* (adult and larva) and *A. cajennense*. Absolute and specific activities were as follows: *A. aegypti* (adult) showed 2.1 mU/animal (4.5 mU/mg); *A. aegypti* (larva) showed 6.1 mU (100 mU/mg) and *A. cajennense* 112 mU (8.9 mU/mg). Acidic precipitation tested to *A. aegypti* adult midgut homogenate was not efficient in order to purify lipase due to low activity recoveries differently from what was observed to *A. cajennense* (1.5 x purification). Lipase activities from these samples were not affected by Ca^{+2}. Optimum pH of lipase activities were: *A. aegypti* (adult), 9.0; and *A. cajennense,* a range of 8 to 9.5). Separation after a cation-exchange chromatography (Hitrap S column) indicated only one activity of lipase in samples of midgut homogenate from *A. cajennense*. Lipase assay of anion-exchange chromatography (Hitrap Q column) fractions from *A. aegypti* adult sample indicated one activity of lipase eluted with 0.36 M NaCl, and from *A. aegypti* larva two activities of lipase were eluted with 0.12 M and 0.2 M NaCl. Lipase-active fractions from *A. aegypti* adult were pooled, desalted and applied to a Resource Q column and two distinct activities were eluted (0.22 M NaCl and 0.26 M NaCl). It was observed that there were important differences between lipase from *A. aegypti* (adult and larva) indicating different enzymes involved in lipid digestion in larva and adult probably due to different feeding habits. Optimum pH from lipases studied are in the range of 8.0 to 9.5. *A. cajennense* and *A. aegypti* (adult) had distinct interaction with ion exchange chromatography, and the isolation of these enzymes is being tested.

Supported by: CNPq and CAPES.

2.11 rLosac, the factor X activator from *Lonomia obliqua*: comparative studies between normal and Gla-domainless factor X

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Introduction: Losac is the first factor X (FX) activator purified from *Lonomia obliqua* bristles. The mechanism of recognition and cleavage of FX by Losac is still unknown. On the other hand, this mechanism is well known for RVV-X, the factor X activator from *Daboia russelli* venom. RVV-X activates FX through the recognition of the Gla domain and then by the cleavage of the FX heavy chain by the RVV-X metalloprotease domain, and this interaction is Ca^{+2}-dependent. **Objectives:** To obtain the recombinant Losac with enzymatic activity on FX and to compare its activity with RVV-X. **Methods:** The *E. coli* BL21 (DE3) strain was transformed with the recombinant plasmid (pAE-Losac). This system is designed for expression of rLosac with a 6x-His tail in the N-terminus. We evaluated the activation of human FX (345 nM) using different concentrations of rLosac and RVV-X in chromogenic assays (0.2 mM S-2765) and also through the degradation of normal and deglycosyled FX by SDS-PAGE. The specificity of Losac activity on FX was evaluated assaying its activity on several other substrates such as prothrombin (chromogenic assays), fibrinogen and fibrin (SDS-PAGE and fibrin plates, respectively). The capacity of rLosac to activate factor X in plasma was assayed by plasma recalcification time. **Results and Discussion:** rLosac and RVV-X activated FX in a dose-dependent manner. Only Losac was able to activate deglycosylated FX although this activation was less when normal FX was used. Apparently, the protein is specific for FX since it does not activate prothrombin, does not cleave fibrinogen or fibrin. The normal plasma recalcification time (195.6s ± 37.67) was shortened after 2 min of incubation with rLosac (70.08s ± 4.80) or RVV-X (60.9s ± 2.04). Moreover, when we incubated Losac plus plasma for 10 min, the plasma recalcification time induced by rLosac was faster than that induced by RVV-X (11.6s ± 4.27 and 54.06s ± 6.15, respectively), compared with the control (148.6s ± 25.9). All these results demonstrate that the FX activation by rLosac is different than that induced by RVV-X. The exact mechanism of FX recognition by rLosac is yet to be determined. We are currently working to understand the mechanism by which rLosac recognizes FX.

Supported by: CNPq and FAPESP.

2.12 Acid protein digestion in the spider *Nephilengys cruentata*

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Introduction: Peptidases form the largest enzyme gene family in vertebrates. According to the Merops database, they comprise 641 genes in humans and 677 in the mouse. Spiders are efficient predators of insects. Protein digestion in spiders has been sporadically studied and there are few reports in the literature. Our group characterized protein digestion in the hepatopancreas of the spider *Nephilengys cruentata*. Studies with natural protease substrates and specific inhibitors indicated two distinct activities: an alkaline activity already characterized as an astacin-like enzyme and an acidic activity. **Objectives:** Classification, isolation and characterization of the digestive acidic peptidases in the spider *Nephilengys cruentata*. **Methods:** Adult females were collected and maintained fasting for two weeks. Afterward, cannibalism among spiders was favored. Fed females were dissected and the isolated hepatopancreas was homogenized in Milli-Q water. Partial isolation was obtained with a combination of two cation-exchange chromatography steps, a Hitrap S and a Resource S both equilibrated in 0.05 M citrate-phosphate buffer, pH 5.0, and eluted with a linear (0 – 1 M) NaCl gradient. The activity was measured at 30° C with 10 µM Z-Phe-Arg-MCA as substrate diluted in 0.1 M citrate-phosphate buffer containing 3 mM cysteine and EDTA. Activity was measured after native PAGE using Z-FR-MCA as substrate. **Results and Discussion:** Chromatographic separation and specific assay using Z-FR-MCA as substrate showed two different peptidases in acidic conditions: an aspartic endopeptidase, inhibited by pepstatin and a cysteine-endopeptidase inhibited by E-64, MMTS and stabilized in the presence of cysteine. Activity measured after native electrophoresis on a 7.5% polyacrylamide gel resulted in one activity band on Z-FR-MCA and gelatin as substrates. Assays using quenched fluorescent substrates showed highest activity on the substrate Abz-F-R-Q-EDDnp, indicating that cysteine-endopeptidase is the major acidic peptidase, probably related to a cathepsin-L-like enzyme hydrolyzing preferentially substrates with a Phe residue at P2. Thus, this cathepsin-L-like activity was characterized. This enzyme showed a pH optimum of 3.6 and a molecular weight of 30.4 kDa determined by SDS-PAGE from partially isolated samples. It was mainly stable in acidic conditions (pH 3.0 to 6.0) at 4°C and 30°C and showed a half-life of 13 min at 60°C. E-64 showed a K_D of 55 nM, which is in agreement of data for other arthropod cathepsin-L-like enzymes. This enzyme will be isolated to homogeneity for detailed specificity studies, in order to characterize its ability to hydrolyze spider silk.

Supported by: FAPESP.

2.13 Batch mode cultivation of CHO-EPO cells in suspension to establish purification process

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Introduction: Natural erythropoietin (EPO) is present in picomolar amounts in biological samples, making its purification very difficult. In 1977, Miyake et al. succeeded in isolating and purifying the human hormone EPO from the urine of patients with aplastic anemia. This small amount of EPO obtained after a 7-step procedure was the starting point for the full characterization of the molecule. Currently, recombinant DNA techniques allows the hormone be produced through the cultivation of genetically modified cells. It is a glycosylated protein of 165 amino acids with a molecular weight of 34 to 39 kDa; approximately 40% of its weight consists of neutral sugars and sialic acid. Responsible for the maturation of erythrocytes, it is used in the correction of anemia in patients with chronic renal failure. Regarding its production processes, recombinant EPO is obtained from mammalian cells preferentially cultivated with serum-free medium. Thus, the proteins from the cells are the main source of contaminants to be eliminated, besides hydrolysates present in the medium and degradation products of the product itself. **Objectives**: To establish the steps for EPO purification from supernatants obtained with cells adapted to growth in suspension and serum-free medium in batch mode cultivation. **Methods:** CHO cells transfected with the human gene coding for EPO were grown in suspension with EX-CELL™ 302 medium (JRH Biosciences) in a 8 L spinner-flask in a batch mode. Dissolved oxygen, pH and temperature were monitored online. The controller (Corning Life Science) was used to regulate the level of DO (60%) through injection of gas mixture in the headspace of the spinner-flask. The supernatant of the culture was clarified by centrifugation, and then purified in 3 chromatography steps. The first consisted of an affinity resin. EPO was eluted with a high salt concentration. The eluate was diafiltrated through a 10,000 cutoff cartridge. The second and third chromatography steps consisted of ion-exchange resins. At the end the diafiltration step, buffer exchange was needed. **Results and Discussion:** Affinity chromatography allows the elimination of impurities in addition to concentrating the sample about 10 times with a yield of 78%. In the subsequent chromatography with anion exchanger matrix, an additional removal of contaminants was observed with a yield of 27%. The subsequent column filled with a cation exchanger matrix resulted in the elimination of contaminant molecules larger than EPO and final yield of 20%. The analysis of isoelectric focusing demonstrated the presence of 9 isoforms, showing two bands more acidic when compared with the internal standard used by the laboratory, meaning an improvement in capturing isoforms with higher EPO biological activity. The process did not result in a purification profile necessary for clinical use; however, it must be considered that batch cultivation is not suitable for EPO production, as all the contaminants are concentrated and defective forms of EPO accumulate due to protease action. To cultivate suspension cells is very different from adherent cells and the downstream process must be adjusted for this new environment. We are now starting perfusion mode cultivation of suspension cells expecting to work with fewer contaminants in the supernatant.

Supported by: Fundação Butantan.

2.14 Toxicity of both gallic acid and the free phenolic acid fractions from pomegranate pulp (*Punica granatum, L*): effects on growth and apoptosis in MDCK cells

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Introduction: Antioxidants from natural origins, including phenolic acids, due to their expected potential to prevent a chronic pathologic process that results from a burst of free radical excess, are the targets of this type of study. **Objective:** The present study evaluated the effect of antioxidants on cell viability, by determining both growth and apoptosis in MDCK cells. **Methods**: The extract obtained from pomegranate pulp (*Punica granatum,L)* with phenolic compounds were evaluated by the Folin-Ciocalteau method, and its antioxidant activity was measured by the ß-carotene/linoleic acid and DPPH methods. The cells were treated with gallic acid (GA), as a control of antioxidant activity, and free phenolic acid fractions (FFAp) from pomegranate extract, at concentrations of 150, 75, and 15 µg, for 24 h at 37°C. Also, the TUNEL test (Roche®; T^{d} T- mediated dUTP nick end-labeling) was used to determine the apoptosis level of these cells treated with FFAp (10 µg), with the same cited conditions. It was observed that cell growth was reduced when treated with gallic acid using 150 µg of this antioxidant, compared with control cells. **Results and Discussion**: Cell growth and viability was 31.37%, compared with 65.86% for control cells. These results were better (70.05%) using lower doses such as 75 µg of GA. Also, the cells treated with FFAp fractions revealed that growth increased (196.58%) with a dose of 15 µg, compared with doses of 150 µg. This showed higher (78.56%) cell viability compared with control cells (65.85%). According to the TUNEL test, these cells treated with FFAp (10 µg) showed more protection when compared with the control group that showed a low cell apoptosis level, and the cells treated with 200 ug of gallic acid showed a higher apoptosis level. These data suggest that, at appropriate doses, the pomegranate extract is an antioxidant with protective action on cell growth.

Supported by: CNPq.

2.15 Effect of aqueous extract of pequi (*Caryocar brasiliensis*, Camb) on antioxidant enzymes in liver

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Introduction: The induction of antioxidant enzymes by chemoprotective agents was shown to be an effective strategy for protecting organisms against deleterious effects such as tissue oxidation. The phenolic compounds present in fruits can prevent free radical formation and protect tissues against peroxidation. *Pequi* is a very popular tropical fruit and it is consumed as part of the meals among the population from the central west and northeast areas of Brazil. **Objective:** The objective of this study was to evaluate the antioxidant activity of the aqueous extract of pequi fruit pulp and to determine its effect on the expression of the protective enzymes superoxide dismutase (SOD) and catalase (CAT) in rats. **Methods:** Antioxidant activity was measured by the ß-carotene/linoleic acid and DPPH methods. Rats (eight in each group) were orally administered 100 mg/kg aqueous pequi extract for 30 consecutive days alongside the control group. Afterward, these animals were sacrificed and the livers were extracted for enzyme evaluation. SOD was determined by the McCord and Fridovich (1969) method and CAT by the method reported by Beuter (1975). Enzyme expression was evaluated by RT/PCR techniques using specific primers for SOD and CAT. **Results and Discussion**:The phenolic compounds in dried pequi pulp fruit amounted to 209 mg/100 g with antioxidant activity of 80% (50 ppm) measured by ß-carotene/linoleic acid and 260.37 mg/mL by DPPH. Superoxide dismutase and catalase showed respectively 40% and 20% more activity in the group that received the pequi extract than in the control group. SOD and CAT gene expression, detected by RT/PCR in liver, revealed that there were no changes in SOD, but that CAT gene expression was 50% higher than in control. These results provide a perspective for the contribution of pequi pulp fruit as a functional food.

Supported by: CNPq.

2.16 Aqueous extract of pequi (*Caryocar brasilienses*, Camb): phenolic composition, antioxidant activity and influence on MDCK cell growth

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Introduction: Pequi is a tropical fruit that is an abundant crop in the central west and northeast regions of Brazil. This fruit is very popular in Brazil and is normally consumed with rice and chicken meals. The phenolic compounds present in many foods, including fruits, vegetables and spices, exhibit antioxidant properties. **Objective**: The objective of this study was to evaluate the aqueous extract from the pequi fruit pulp, its phenolic composition, its antioxidant activity and its effect on MDCK cultured cells. **Methods**: The phenolic compounds were determined by the Folin-Ciocateau method. The antioxidant activity was measured by the ß-carotene/linoleic acid and DPPH methods. MDCK cells were grown at 37ºC in L15 medium in six 24-well plates. After seven days, the cell monolayers were exposed to 20 ppm and 40 ppm 30 and 60 min, after which the viable cells were evaluated. The phenolic compounds in dried pequi fruit were totaled 209 mg/100 g. The antioxidant capacity (AC) with 50 ppm was higher than 80% in ß-carotene bleaching and 260.37 mg/mL in the DPPH method. **Results and Discussion:** The percentages of viable MDCK cells after 30 min and 60 min with 20 ppm of pequi aqueous extract were 90% and 97%, respectively in contrast to 84% and 15% of the control cells. With 40 ppm of the extract these results were 73% and 50%, respectively. These data suggest that the antioxidant activity of phenolic compounds in the pequi fruit can influence both the growth and protection of MDCK cells.

Supported by: CNPq.

2.17 Trypsin-inhibitors against insect peptidases in the spider *Nephilengys cruentata*

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Introduction: Some insects are agricultural pests and vectors of several diseases. Development of control strategies is important to increase world food production and reduce the incidence of diseases such as dengue fever. Spiders are efficient predators of insects. Prey digestion starts extraorally by the digestive juice produced in the hepatopancreas. Data in literature indicate that spider digestive juice (SDJ) could have peptidase inhibitors (PIs) to control ingested prey's proteases. **Objectives:** To investigate the presence of PIs in both SDJ and hepatopancreas homogenate (HP) from the spider *Nephilengys cruentata*. **Methods:** SDJ and HP samples were used as a source of PIs. Chromatographic separations in anion exchange (AEX) and gel filtration (GF) columns of these samples were performed and fractions were tested against different insect and mammalian peptidases. *Periplaneta americana*, *Musca domestica*, *Aedes aegypti* female, *Aedes aegypti* larva and *Gryllus sp* midgut homogenates were used as sources of insect trypsins. As mammalian trypsin source, commercial bovine trypsin was tested. SDJ samples were submitted to GF chromatography (SuperdexG-75) in 20 mM Tris-HCl, 0.5 M NaCl buffer, pH 7.0. Identification of inhibitory fractions was done with a mixture of 12.5 µL of chromatographic fractions and 12.5 µL of enzyme source. These mixtures were pre-incubated for 30 min at 30°C. Afterward, 0.83 mM benzoyl-Arg-p-nitroanilide (BApNa) in 0.1 M Tris-HCl, 0.25 M NaCl buffer, pH 8.5, was added to mixtures as trypsin substrate. Fractions with decreased trypsin activity were pooled, desalted and applied to an AEX chromatography, using a Resource Q column equilibrated with 0.02 M Tris-HCl buffer, pH 7.0, and eluted with a linear NaCl gradient (25 mL; 0 - 1 M). Fractions with trypsin-inhibitory activity were pooled, desalted and applied to the same Resource Q column equilibrated in the same conditions and eluted with a linear NaCl gradient (25 ml; 0- 0.5 M). Fractions with trypsin-inhibitory activity were individually submitted to SDS-PAGE. HP was boiled for 5 min and centrifuged for 10 min at 13,000 rpm. The supernatant was submitted to AEX chromatography, using a Hitrap Q column, equilibrated with 0.02 M Tris-HCl buffer, pH 7.0, and eluted with a linear NaCl gradient (0-1 M). Fractions with trypsin-inhibitory activity were pooled, desalted and applied to a Resource Q column equilibrated and eluted in the same conditions described before. Fractions with trypsin-inhibitory activity were individually submitted to SDS-PAGE. **Results and Discussion:** Measures of inhibitory activities from *N. cruentata* SDJ and HP on different insect trypsins indicated that SDJ (30 to 100% inhibition) and HP (76 to 96 % inhibition) were able to inhibit insect trypsins. The isolation of inhibitory activity from SDJ using a combination of GF and AEX chromatography was demonstrated by a SDS-PAGE showing the enrichment of a protein of 13 kDa, corresponding to the inhibitory activity. The isolation of inhibitory activity from HP using a combination of AEX chromatographic steps was shown by SDS-PAGE indicating a partial purification of different molecules with molecular masses of 14 to 30 kDa. SDJ and HP possess efficient trypsin inhibitors with low molecular masses which will be kinetically characterized and used to bioassay against *A. aegypti* being added to their diet.

Supported by: FAPESP, IFS.

2.18 The effect of *Bothrops jararaca* antithrombin on cell migration induced by carrageenan in mice

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Introduction: Antithrombin is an important inhibitor of several coagulation serine proteases, including factors X_a, IX_a, XI_a and thrombin. Besides, a large number of recent studies have shown that human antithrombin has anti-inflammatory actions, which are independent of its effects on coagulation. **Objective:** The aim of this work was to investigate the effects of *B. jararaca* antithrombin (BjAT) on cell migration induced by carrageenan (cg) in mice. **Methods:** Antithrombin was purified from *B. jararaca* plasma using a HiTrap Heparin HP column. BjAT (20 μg/100 μL i.v.) or saline (100 μL) was administered 1 h before intraperitoneal injection of cg (300 μg/200 μL) or saline (sal) (200 μL) in male Swiss mice (18-22 g). After 4 h of cg injection or sal, cell migration to the peritoneal cavity was evaluated. Total peritoneal cell counts were carried out using a Neubauer hemocytometer, and differential counts were preformed with smears stained with panchromatic stain. A total of 100 cells were counted by optical microscopy. **Results and Discussion:** Pre-treatment with BjAT diminished cg-induced cell influx into the peritoneal cavity, when compared with the group pretreated with sal (sal+cg). The decrease in cell migration in animals pretreated with BjAT was 41% (sal+cg: 4.66 ± 0.56, BjAT+cg: 2.74 ± 0.31; $p<0.05$). A significant decrease of 82% was observed for polymorphonuclear cells in animals pretreated with BjAT (sal+cg: 3.50 ± 0.81 , BjAT+cg: 0.60 ± 0.09; $p<0.05$). The results demonstrate that BjAT significantly inhibited the migration of polymorphonuclear cells to the peritoneal cavity. Thus, we suggest that BjAT possesses anti-inflammatory properties.

Supported by FAPESP, CNPq and CAPES.

2.19 Characterization of digestive carbohydrases and isolation of α-fucosidase from *Amblyomma cajennense*

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Introduction: *Amblyomma cajennense* is one of the most important tick species in the Brazilian fauna, and it is an important pest of horses, cattle and dogs. Besides, *A. cajennense* is the main vector of Brazilian spotted fever caused by *Rickettsia rickettsii.* Although it is of economic importance and a vector of human and veterinary diseases, only a few studies on this species are available. Conventional tick control methods have been based mainly on the use of acaricides. However, novel control methods, such as vaccination and biological control, are needed. Although an anti-tick vaccine may be the most promising control method, its development still depends on the identification and characterization of one or more protective tick antigens. α-Fucosidases (EC 3.2.1.51) are glycosyl-hydrolases (GH, family 29) that catalyze the hydrolysis of glycosidic linkages (α-1,2, α-1,3, α-1,4 and α-1,6) present in glycoconjugates (carbohydrates, glycoproteins, glycolipids and glycosaminoglycans) between a fucose and other molecules. The importance of the digestive system as a target of control methods has been already demonstrated and a few studies on carbohydrate processing in ticks are available. **Objectives:** We aimed to characterize carbohydrate digestion in *A. cajennense* and isolate the digestive α-fucosidase. In order to do that, we determined α-amylase, trehalase, α-fucosidase, β-glucosaminidase, chitinase, α-glucosidase, and α-galactosidase activities in the gut of *A. cajennense.* **Methods:** Ticks were fed on rabbits and engorged females were dissected for gut isolation. Guts were homogenized in MilliQ water. In order to characterize carbohydrate digestion in tick, we determined α-amylase (starch), trehalase (trehalose), α-fucosidase (4-methylumbelliferyl-α-L-fucopyranoside), β-glucosaminidase (4-methylumbelliferyl-β-N'-acetylglucosamine), chitinase (4-methylumbelliferyl-β-N',N'',N'''-triacetylchitotrioside), α-glucosidase (4-methylumbelliferyl-α-glucoside), and α-galactosidase (4-methylumbelliferyl-α-D-galactoside) activities. In order to purify digestive α-fucosidase from *A. cajennense*, the midgut was homogenized in acetate buffer pH, 3.5, and centrifuged at 4°C for 30 min at 13,500 rpm. The soluble fraction was desalted using a Hitrap desalting column eluted with MilliQ water and applied to a HiTrap S column in 50 mM citrate-phosphate buffer, pH 5.0. Proteins were eluted in a NaCl gradient (0-1 M) in the same buffer. α-Fucosidase active fractions were pooled and applied to a Resource S column. Protein elution used a linear gradient of 0-0.6 M NaCl in the same buffers used in the previous chromatographic step. **Results and Discussion:** The following enzyme absolute and specific activities were measured: α-amylase 880 mU/gut, (8.3 mU/mg); trehalase: 230 mU/gut (25 mU/mg); chitinase 478 mU/gut (23 mU/mg); α-glucosidase 68.5 mU/gut (2 mU/mg); α-galactosidase 6.9 mU/gut (0.5 mU/mg); β-glucosaminidase 4900 mU/gut (341 mU/mg); and α-fucosidase 416 mU/gut (35 mU/mg). α-Fucosidase activity was purified to homogeneity and demonstrated by a 12% polyacrylamide gel electrophoresis. Main digestive carbohydrase activities were identified in samples of *A. cajennense* gut, and we could identify β-glucosaminidase, α-amylase and chitinase as the most active enzymes.

Supported by: FAPESP.

2.20 Effect of leech salivary gland compounds on hemostasis and cell survival

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Introduction: Salivary molecules of hematophagous animals have been identified and characterized as new targets for the development of therapeutic agents against several diseases. Salivary glands (SG) from leeches are enriched with molecules that display diverse functions acting against the host's hemostatic, inflammatory and immune responses. **Objectives:** To identify and characterize proteins able to degrade fibrinogen/fibrin, to inhibit factor Xa and also to induce tumor cell death. **Methods:** SG from *Haementeria vizottoi* (Vizotto, 1967) leeches were removed by dissection, stored frozen at – 70 ºC and sonicated at 4 ºC in 10 mM Tris-HCl, pH 7.8, 10 mM $CaCl_2$. After that, the salivary complex extract (SCE) was centrifuged at 12,000 *g* for 3 min at 4 °C and the supernatant filtered. SCE was loaded on a Superdex G-75 column equilibrated with 20 mM Tris-HCl buffer pH 8.0. Fractions were evaluated for their ability to inhibit FXa on chromogenic substrate S-2765, and to modify the human plasma recalcification time. Fibrino(geno)lytic activity was measured by zymography assays on fibrin-agarose-plates and also by thrombin time assays (TT). Cell viability of melanoma (SKmell-28) cells was analyzed by the MTT method **Results and Discussion:** Regarding the chromatographic profile (SGE loaded on Superdex G-75), one protein peak (R1) was able to degrade fibrinogen and fibrin. The molecular mass of the protein responsible for this activity was around 70 kDa detected by zymography. The other isolated peak (R2) showed FXa inhibitory activity and caused an increase in the recalcification time assays. The protein band responsible for this activity was revealed by SDS-PAGE and it is about 10 kDa. SKMell-28 cell viability was significantly altered after 48 h treatment with SGE. Previous LC/MS experiments (proteomic analysis) confirmed the presence in the SGE of two major proteins with molecular masses of 42,115 and 56,145 Da. The *H. vizottoi* SGE contains fibrino(geno)lytic and FXa inhibitor components able to promote blood incoagulability during animal feeding (contributing to the animal's survival and possible development of new therapeutic agents). The identification of the compounds responsible for the cell death effects is now in progress in our laboratory.

Supported by: FAPESP and CNPq/INCTTOX.

2.21 Molecular characterization of four nucleotide pyrophosphatase/phosphodiesterases of *Schistosoma mansoni*: cloning, expression and purification

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Introduction: NPPs are ubiquitous membrane-associated or secreted ecto-enzymes that have a role in regulating extracellular nucleotide metabolism and require divalent cations and alkaline pH. They hydrolyze pyrophosphate or phosphodiester bonds in a variety of extracellular compounds including nucleotides, (lyso) phospholipids and choline phosphate esters, releasing nucleoside 5'-monophosphate. Extracellular nucleotides, and in particular ATP and adenosine, elicit a broad range of responses in biological processes. Catalysis by NPPs could affect multiple physiological processes as diverse as platelet aggregation, apoptosis, cell proliferation, differentiation and motility. **Objectives:** The objective of this work was to identify NPP proteins in the *S. mansoni* genome, characterize them by bioinformatic tools and clone them for *E. coli* expression. **Methods:** We searched for proteins with the NPP domain in the *S. mansoni* genome "GeneDB." Next, Blast and PSI-Blast searches against the NCBI non-redundant protein sequence database were used to identify orthologues of SmNPP. For phylogenetic analysis, alignments of protein sequences were performed using the ClustalX 1.83 software. The tree was constructed using Clustal, and the TreeView program was used to visualize the tree. The signal peptide prediction was performed using the SignalP 3.0 server, and transmembrane helices were analyzed by TMHMM, version 2.0. Molecular weight (MW) and isoelectric point (pI) were calculated with the Compute pI/Mw tool. The *smnpp* genes were amplified by RT-PCR from adult worm RNA, and then fragments with the NPP domain, but without putative signal peptides and putative transmembrane domains, were cloned into an *E. coli* expression vector, pAE. The proteins were expressed as inclusion bodies, which were solubilized with 8 M urea, purified under denaturing conditions by nickel affinity chromatography and dialyzed against PBS buffer. **Results and Discussion:** Four genes with NPP domain were found in the *S. mansoni* genome "GeneDB," and their linear sequence was submitted to an initial characterization by bioinformatic tools. The phylogenetic analyses and the multiple alignment of the SmNPPs showed that three of the four were more similar to human NPP-5, while the other was more similar to human NPP-6, so they were named SmNPP-5a, SmNPP-5b, SmNPP-5c and SmNPP-6. It was observed that SmNPP-5a and SmNPP-6 show a putative N-terminal signal peptide and a putative C-terminal transmembrane domain; SmNPP-5b has two putative transmembrane domains, one N- and the other C-terminal; and SmNPP-5c shows only a putative N-terminal signal peptide. All SmNPPs showed six metal-binding conserved sites and in the position of the catalytic center, an amino acid whose side chain is polar but electrically neutral at neutral pH. The recombinant proteins showed good expression levels, with similar predicted molecular weight. Unfortunately, as proteins precipitated during dialysis, we did not detect enzymatic activity. Therefore, we now plan to improve the refolding process to obtain soluble - and possible enzymatically active - proteins.

Supported by: FAPESP.

2.22 N-terminal determination of Mlx-6, a protein isolated from the venom of the snake *Micrurus lemniscatus*

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Introduction: Toxins isolated from venoms of Old World elapids have been extensively studied as sources of presynaptic PLA2s and postsynaptic neurotoxins. These toxins have been shown to be valuable tools for the characterization of the structure and function of muscle cholinergic receptors as well as for identifying subtypes of nicotinic and muscarinic receptors that control specific functions in the brain. Therefore, few investigations have dealt with isolated toxins from venoms of Brazilian snakes of the genus *Micrurus* (coral snakes, family Elapidae) and their effects on the brain. Previously, we reported that the toxin Mlx-6 isolated from the *Micrurus lemniscatus* venom induced a displacement on the H3-QNB binding in hippocampal membrane suggesting an activity on the muscarinic acetylcholine receptors. **Objective:** The aim of this study was to describe the N-terminal of the toxin Mlx-6 isolated from the *Micrurus lemniscatus* venom, and compare the obtained sequence to proteins isolated from the venom of *Micrurus surinamensis.* **Methods:** To isolate Mlx-6, venom of the *M. lemniscatus* snake was loaded onto a C18 RP–HPLC column and eluted with an ACN gradient containing 0.1% TFA. After concentration, the fraction of interest was chromatographed again to avoid eventual contaminants. To determine the N-terminal portion, the obtained fraction was submitted to Edman degradation in a PPSQ-21 Sequencer (Shimadzu). **Results and Discussion:** The 10 amino acids of the N-terminal portion of the Mlx-6 were determined; however, the obtained sequence shows similarity to other proteins isolated from the genus *Micrurus.* Mlx-6 shows similarity to the Three-Finger proteins that act on cholinergic receptors and our protein could be considered a new member of this group not described before. With the aim to understand the functional importance of this toxin, studies have been designed to determine if Mlx-6 acts like an agonist or antagonist on the muscarinic acetylcholine receptors. Therefore, studies using preparations of isolated organs and behavioral studies of learning and memory are in progress in our laboratory.

Supported by: FAPESP.

2.23 *Phoneutria* from Western Amazonia: antimicrobial peptides

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Introduction: Infectious diseases are among the main causes of death in human beings. The main reason is the resistance of microorganisms to different antibiotics. Owing to this, the search for antimicrobial molecules in Brazilian fauna and flora could be important. In arachnid toxins, mainly from spiders and scorpions, some antimicrobial peptides have been identified. **Objectives:** The objective of this study was to identify antimicrobial peptides in the venom of spiders of the genus *Phoneutria* from the Western Amazonian rainforest. In this region, three species of the genus can be found: *P. fera, P. reidyi* and *P. boliviensis*. Contrary to the non-Amazonian species *P. nigriventer*, the *Phoneutria* spp. from the Amazon have been poorly studied with regard to their venom. **Methods:** The venom was milked by electric stimulation and lyophilized. The venom was reconstituted in 0.05% trifluoroacetic acid (TFA) and the soluble part was submitted to HPLC reversed-phase chromatography on a semi- preparative Jupiter C18 column. Elution was performed with a linear gradient of ACN/TFA 0.05% over 60 min at a flow rate of 1.5 mL. The column effluent was monitored by absorbance at 225 nm. The presence of antibacterial activity was determined by a liquid growth inhibition assay against the Gram-negative bacteria *Escherichia coli SBS363*, Gram-positive bacteria *Micrococcus luteus A270* and yeast *Candida albicans*. **Results and Discussion:** Several fractions inhibited the growth of the bacteria *M. luteus* and *E. coli* and the yeast *C. albicans*. Two groups of antimicrobial molecules were found in the venom of *Phoneutria* spiders: hydrophilic and hydrophobic. The same hydrophilic molecules were observed in the venom of the three spiders in this study, a molecule with 410 Da also found in *P. nigriventer* venom, the nigriventrin neurotoxin. Nigriventin is a non-protein low-molecular-mass neurotoxin, isolated from the hydrophilic fraction of *P. nigriventer* venom. This molecule inhibited the growth of *E. coli*. The fractions with hydrophobic molecules are peptides and were not pure on analysis by mass spectrometry (MALDI-TOF). These molecules should be submitted to new stages of purification for their full characterization. The purification and characterization of these antimicrobial factors are in progress.

Supported by: CNPq and FAPESP.

2.24 Is the antifungal rondonin widespread in all Theraphosidae?

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Introduction: Antimicrobial peptides have become recognized as important components of the nonspecific host defense or innate immune system in a variety of organisms ranging from plants and insects to animals, including mollusks, crustaceans, amphibians, birds, fish, mammals, and humans. The widespread occurrence of these antimicrobial substances suggests that they play a role in innate immunity against microorganisms and other pathogens. We found an antimicrobial peptide in the plasma of *Acanthoscurria rondoniae* with antifungal activity, and this molecule was characterized by mass spectrometry as a single molecule with 1,236.405 Da. This peptide has been submitted to "de novo" sequencing, elucidating its primary structure: IIIQYEGHKH, which showed similarity with a hemocyanin fragment and called rondonin. These studies have made it increasingly clear that due to the continuous use of antibiotics multi-resistant bacterial strains have developed all over the world, and as expected, antibacterial and antifungal peptides have attracted attention in recent years in order to find new therapeutic agents. **Objectives:** The objective of this study was to determine if rondonin is widespread among the genera of the family Theraphosidae. **Methods:** The hemolymph was collected from *A.gomesiana*, *Vitalius wacketi, Nhandu coloratovilosum*, *Avicularia juruensis* and *Grammostola pulcra*, by cardiac puncture with an apyrogenic syringe. The hemocytes were removed from plasma by centrifugation at 800 x g for 10 min at 4ºC. The plasma collected was mixed with acidified water (TFA – 0.05% trifluoracetic acid) and kept in agitation on ice for 30 min and centrifuged at 16,000 x g. The soluble part was loaded onto classic Sep–Pak C18 cartridges. The Sep-Pak fractions were concentrated in a vacuum centrifuge and reconstituted with 0, 5% and 40% TFA and submitted to a reverse phase chromatography on a semi-preparative Jupiter C18 column. Elution was performed with a linear gradient of 2% to 60 % ACN/0.05% TFA over 60 min at a flow rate of 1.5 mL/min. The presence of antibacterial activity was determined by a liquid growth inhibition assay against *Candida albicans* MDM8. These fractions were submitted to mass spectrometry to verify the presence of the same molecular mass. **Results and Discussion:** According to our results, we found the same peptide only in *A. gomesiana* and *N. coloratovilosum*; it showed the same molecular mass as that of rondonin and the same activity against *C. albicans.* It is possible because they belong to the same subfamily Theraphosinae. Maybe rondonin is a specific character of the subfamily Theraphosinae since it is not present in *Avicularia* which belongs to the subfamily Aviculariinae. We need to analyze the results for *Grammostola* which now belongs to the subfamily Theraphosinae but that has already been placed in a subfamily, Grammostolinae.

Supported by: FAPESP and CNPq.

2.25 Collagen-induced arthritis and bee venom treatment affect aminopeptidases in synovia, synovium and plasma of rats

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Introduction: Rheumatoid arthritis affects about 1% of the population worldwide. It is characterized by cellular infiltration in the synovium, progressive erosion of cartilage and bone and pannus formation in the affected joints, as well the immune response to cartilage components and the presence of rheumatoid factors. The destruction of cartilage, inflammatory mechanisms and antigenic processing are related to aminopeptidases, such as neutral (APN), basic (APB) and dipeptidyl-peptidase IV. Moreover, bee venom therapy has been used in oriental medicine to treat inflammatory diseases, such as rheumatoid arthritis, due to its property to decrease pain and inflammation. **Objectives:** To analyze: (1) the involvement of basic (APB), neutral (APN) and dipeptidyl IV (DPPIV) aminopeptidases (APs) in the rat synovia, synovium and plasma in collagen-induced arthritis (CIA); and (2) if changes in the activity levels of these APs could be mitigated after treatment with *Apis mellifera* venom (BV). **Methods:** Male Wistar rats (150-160g) received a single dose of type-II collagen, id, into the base of the tail. After 21 days, animals were treated with 0.25 mg BV/ kg body wt, sc, in the back, every other day. On the 41st day after immunization, plasma and synovial fluid and synovial membrane were collected from the groups: control (A1), A1 treated with BV (A2), arthritic (B1), B1 treated with BV (B2), and injected with collagen which did not develop arthritis (B1R). The synovium was homogenized and ultracentrifuged in order to obtain soluble (SF) and solubilized membrane-bound (MF) fractions. APs were measured fluorometrically and expressed as pmoles of hydrolyzed substrate/min/mg protein [mean±SEM (n)]. **Results and Discussion:** DPPIV in the synovial fluid was increased by BV and/or CIA, since A2 [200±25 (6)], B2 [329±48 (3)] and B1R [271±27 (3)] showed higher DPPIV activity, while B1 [215±25 (3)] showed a tendency for an increase, compared to A1 [103±29 (4)]. No alteration of any activities was found in synovium MF, but in SF APB was higher in B1R [3698±896 (3)] than in other groups [A1=1421±403 (4); A2=928±172 (6); B1=1902±903 (3); B2=1183±455 (3)], while DPPIV was higher in B1 [1086±339 (3)] than in B1R [296±60 (4)]. APN in plasma was higher in B1 [506±13 (3)] than in B2 [386±9 (3)]. Alteration of the AP activities examined and their possible functions during arthritis may depend on their localization. In SF, DPPIV could be causal, while APB might be involved in the mechanisms of resistance. BV decreased plasma APN activity in CIA, but it is still not clear what benefits this effect could have in this disease.

Supported by: FAPESP and CNPq.

3: Immunology and Vaccines

3.01 *Lactobacillus casei* expressing the major capsid protein L1 of HPV16 induces systemic and vaginal mucosal immune response in mice after intravaginal immunization

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Introduction: Infections with HPV-16 are closely associated with the development of human cervical carcinoma. Nowadays, the most promising vaccines against HPV16 infection are based on the major capsid protein L1 which self-assembles into structures similar to HPV, called virus-like particles (VLPs). Since the genital mucosa is the host infection site of HPV16, a prophylactic, safe, low-cost mucosal vaccine would constitute an interesting alternative to parenteral vaccines. In this work, we used the constitutive P1 promoter from *Lactococcus lactis* for the expression of HPV-16 L1 in *Lactobacillus casei*. **Objectives:** The objective of this work was to evaluate the constitutive expression of L1 in *L. casei*, the production of VLPs, and the potential of the intravaginal immunization as an effective mucosal route for systemic and vaginal induction of HPV16 VLPs-specific IgA and IgG antibodies in mice immunized with HPV-16 L1-expressing *L. casei* (*L. casei*/L1C). **Methods:** The expression of L1 was evaluated in *L. casei*/L1C extracts carrying the pT1NX/L1C expression plasmid by SDS-PAGE and confirmed by Western blotting using the HPV16 L1-specific antibody Camvir. The production of VLPs was analyzed by electron microscopy. Six- to eight-week-old female C57BL/6 mice were used for intravaginal immunization experiments. Mice were treated with medroxyprogesterone acetate five days before each vaccine dose for estrous cycle synchronization. *L. casei* expressing constitutively L1 were grown until the OD_{600} was 1, and the concentration was then adjusted to 10^{10} CFU per 10 µL (1 dose). Groups of six anesthetized mice were inoculated intravaginally with 10^{10} CFU of *L. casei* or *L. casei*/L1C on three consecutive days. Three administrations, one priming and two boosts, were performed at 2-week intervals, for a total of nine doses. Ten days after the last dose, individual vaginal secretions and serum samples were collected for analysis of HPV-16 VLP-specific IgA and IgG antibodies by ELISA. **Results and Discussion:** The expression of the 56 kDa L1 protein by *L. casei*/L1C was confirmed by Western blotting with Camvir antibody, and ultrastructural analysis showed the production of VLPs. Intravaginal immunization with *L.* casei/L1C elicited serum VLPs-specific IgG antibodies in mice (U-Test, p=0.005; p<0.05). However, vaginal VLP-specific IgA was only induced after an intranasal booster immunization with a yeast-produced HPV-16 VLP sub-optimal dose (U-Test, p=0.04; p<0.05). Mice immunized with *L. casei* (L1-non-expressing) did not show IgA after purified VLP-intranasal booster. The results indicate that T and B lymphocytes of the vaginal mucosa were previously stimulated by *L. casei*/L1C after vaginal immunization. The production of HPV16-VLP by *Lactobacillus* and the induction of VLP-specific systemic and mucosal antibodies open the possibility for the development of new live mucosal prophylactic vaccines.

Supported by: FAPESP, CNPq and Fundação Butantan.

3.02 Purification of capsular polysaccharide produced by *Haemophilus influenzae* type b using ethanol precipitation in combination with detergents and tangential ultrafiltration

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Introduction: The capsular polysaccharide (PSb) produced by *Haemophilus influenzae type* b (Hib) is the most important virulence factor, and it is currently purified, conjugated to a protein and used as a vaccine against Hib, mainly in children under 2 years old. The classical polysaccharide purification process includes many ethanol precipitations steps, organic solvent extraction and several rounds of centrifugation/ultracentrifugation which are inappropriate in large scale process due its complexity and toxicity of some reagents, resulting in a high cost of the final product. **Objectives:** The aim of this work was to optimize the capsular polysaccharide (PSb) purification process by reducing the ethanol precipitation steps, and introducing enzymatic hydrolysis treatment combined with detergents and tangential ultafiltration membrane to make the scale up easy. **Methods:** The supernatant from the fermentor broth was concentrated and diafiltered by tangential ultrafiltration using a 100 kDa cut-off membrane. The concentrated fraction was treated with ethanol at 30% and the precipitated material (waste) was separated by centrifugation from supernatant fraction containing PSb (30% ethanol fraction). The 30% ethanol fraction was submitted to a second stage of precipitation with ethanol at 80%. The insoluble material was separated by centrifugation, and the precipitate containing PSb was solubilized with water. The insoluble material was separated by a third centrifugation and discarded. The supernatant water-soluble fraction containing PSb was concentrated and diafiltered with deoxycholate and betaine buffer using a tangential ultrafiltration membrane, cut-off of 100 kDa. **Results and Discussion:** Relative purity in relation to protein was RP_{prt} =106.3 and 259.6 mg PSb/mg prt, and nucleic acid was RP_{AN} = 734.9 and 384.0 mg PSb/mg NA with 20% recovery. The proposed purification process for polysaccharide achieved the required purity for protein and nucleic acid contaminant; however more improvements should be made in relation to the polysaccharide recovery. This process was shown to be efficient, simple and technically easy for scale up.

Supported by: FAPESP and Fundação Butantan.

3.03 Influence of Vaxcine formulation on humoral immune response against influenza virus

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Introduction: Because it is necessary to produce influenza vaccines yearly, there is only a short time available to start distributing the first doses of vaccine. In the case of an influenza pandemic, a delay in production due to shortage of antigen can have serious consequences. One way to make more doses of influenza vaccine available for rapid distribution is to use an adjuvant in the formulation that is capable of inducing a protective immune response against influenza using a smaller number of virus particles per dose. **Objective:** The objective of this work was to determine whether Vaxcine, an oil-based adjuvant, is able to induce a protective immune response against influenza using, as antigen, three times fewer virus particles than the current influenza vaccine. **Methods:** Swiss female mice were immunized subcutaneously once with 5 or 15 µg/0.2ml of Influenza A (H3N2) virus either in PBS, or incorporated in Vaxcine, or adsorbed on alum. One day before and 100 days after immunization, the animals were bled by tail vein puncture. The blood was used to determine the level of IgG1 and IgG2a against the virus by ELISA. The hemagglutinin inhibition test was used to determine *in vitro* the ability of the IgG1 and IgG2a to inhibit the adherence of influenza A virus to chicken erythrocytes. **Results and Discussion**: The results showed that using 5 µg of virus particles per dose of vaccine in the oil-based formulation (Vaxcine) generates a better antibody response when compared to the responses obtained from the groups immunized with either 5 µg of virus adsorbed on alum or 15 µg of virus in PBS, the latter being the concentration used currently for human vaccination. The results also showed that only the animals immunized with Vaxcine were able to induce both IgG1 and IgG2a antibodies against the virus. The antibodies generated by Vaxcine were also able to inhibit virus adherence to chicken erythrocytes. The results obtained in this work showed that using Vaxcine as an adjuvant in the formulation of a vaccine against influenza can increase the number of doses of influenza vaccine available for distribution. The results also suggest that Vaxcine is able to induce long and lasting humoral and cellular responses against influenza.

Supported by: Proxima Concepts.

3.04 IgG response against O111 polysaccharide in mice immunized with a polysaccharide vaccine using the fragment C recombinant protein as a carrier protein

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Introduction: The serogroup O111 of *E. coli* can be found in three different categories of pathogenic *E. coli*, namely enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC) and enteroaggregative *E. coli* (EAEC). In addition, the O111 serogroup is the one mainly responsible for child diarrhea in endemic areas of Brazil and for outbreaks of bloody diarrhea in developed countries. Previous results obtained in our laboratory demonstrated that O111 detoxified polysaccharide conjugated to proteins using an ADH cross-linker, is able to induce antibodies that can recognize and neutralize the adhesion of live bacteria belonging to EHEC, EPEC and EAEC categories. **Objectives**: To conjugate the polysaccharide O111 to the fragment C recombinant protein of tetanus toxin and determine whether it is capable of inducing an antibody response against the O111 polysaccharide in mice. **Methods**: Detoxified O111 polysaccharide was conjugated either to fragment C recombinant protein of tetanus toxin using adipic acid dihydrazide as cross-linker. The conjugate was incorporated in alum as an adjuvant. Balb/c female mice (6-8 years old) were immunized three times with 10 µg/0.2ml of the conjugate either in PBS or incorporated in alum. Five days after the second immunization and fifteen days after the third immunization, blood samples were collected to determine the titer of IgG antibodies against the polysaccharide O111 by ELISA. **Results and Discussion**: The results showed that IgG antibodies against the O111 polysaccharide were detected in the blood seven days by the second and fifteenth day after the third immunization. O111 detoxified polysaccharide conjugated to fragment C recombinant protein of tetanus toxin incorporated in alum, is able to induce antibodies against O111 polysaccharide in mice.

Supported by: CNPq.

3.05 Analysis of recombinant alkaline phosphatase, carboxypeptidase and sphingomyelinase from *Schistosoma mansoni* as vaccine candidates

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Introduction: Schistosomiasis is a neglected tropical disease affecting more than 200 million people in the world, and the search for a prophylactic vaccine is the main goal established in the fight against the disease and morbidity caused by the blood fluke *Schistosoma mansoni*. Therefore,e we tested three antigens from this parasite in the form of recombinant proteins as potential immunogens aiming to reduce the worm burden in an experimental murine model of infection and disease. **Objectives:** To assess the immunological potential of the three molecules as well as to evaluate the immunological traits associated with the immunization and then challenge against the infective form of the parasite. **Methods:** C57Bl/6 mice were immunized with three doses of 50 μg of each recombinant protein (adjuvant: CFA/IFA) obtained from *E. coli*, in a 14-day interval and challenged with cercariae from *S. mansoni* two weeks after the last dose. The animals were bled before and after challenge, and both the cellular and humoral immune responses were assayed by ELISPOT and ELISA. Forty-five days after challenge, animals were euthanized and the worms perfused in order to analyze the worm burden reduction. **Results and Discussion:** According to our results, the immunization with the three proteins resulted in a higher IgG1/IgG2a ratio for all of them before challenge than after. For mice immunized with alkaline phosphatase, a decrease in IFN-γ secretion was also observed after challenge, when compared to that observed before challenge, and the induction of IL-4 and IL-10 secretion as well. For mice immunized with carboxypeptidase there was a higher production of IFN-γ, IL-4 and IL-10 than for those immunized with sphingomyelinase. There was no significant reduction of worm burden or eggs for any of the three proteins tested. All these results indicate a clear involvement of molecular mechanisms of immune regulation induced by the parasite which results in its survival and no loss of fecundity as observed here.

Supported by: FAPESP.

3.06 qRT-PCR based methodology for quantification/titration of recombinant SFV particles

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Introduction: The Semliki forest virus (SFV) is an enveloped single-strand positive RNA virus, belonging to the *Alphavirus* genus and Togaviridae family. The wild SFV genome was genetically engineered, generating one of the most promising vectors for recombinant expression and vaccine delivery candidate. Although extensively studied and used in the biotechnology field, this expression system shows some difficulties in viral particle quantification (titer) by traditional methods, leading to a lack of reproducibility in the recombinant protein expression. **Objective:** To develop and standardize a quantitative RT-PCR (qRT-PCR) directed to a conserved sequence present in several SFV constructs. **Methods:** A set of primers directed to the NsP3 region of the *SFVgp1* non-structural polyprotein gene of SFV were designed and used for reverse transcription and for amplification of SFV-RNA. The standard curve for absolute quantification was obtained by serial dilution of linearized SFV expression plasmids. For methodology validation, we utilized two approaches: several SFV constructs were submitted to the qRT-PCR or the SFV bearing the rabies virus glycoprotein genetic information (SFV-RVGP) was previously treated at different temperatures before titration and further used for cell infection. **Results and Discussion:** The primers were considered suitable for use, showing no primer-dimer or hairpin structure formation. The qRT-PCR developed was able to amplify the target sequence in all SFV constructs. SFV-RVGP samples treated at different temperatures were efficiently titrated and successfully used for cell culture infection at the desired ratio of infection. The qRT-PCR was successfully developed for recombinant SFV titration, significantly improving the accuracy of the SFV titer determination.

Supported by: FAPESP and CNRS.

3.07 A novel leptospiral protein of 95 kDa binds to extracellular matrix components and activates E-selectin on endothelial cells

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Introduction: Leptospirosis, an emerging infectious disease, is a worldwide zoonosis of human and veterinary concern. *Leptospira* invasiveness is attributed to its ability to disseminate widely within the host during the early stage of infection, but the mechanisms associated with this invasion are poorly understood. Due to their location, surface-associated proteins are likely to be relevant in host-pathogen interactions, hence their potential to elicit several activities, including adhesion. **Objectives:** The aim was to study a predicted outer membrane leptospiral protein encoded by the LIC12690 gene in mediating the adhesion process. **Methods:** The gene was cloned and expressed in the *Escherichia coli* BL21 (SI) strain using the expression vector pAE. The recombinant protein tagged with N-terminal hexahistidine was purified by metal-charged chromatography and used to assess its ability to activate human umbilical vein endothelial cells (HUVECs). **Results and Discussion:** The recombinant leptospiral protein of 95 kDa, named Lp95, activated E-selectin in a dose-dependent fashion but not the intercellular adhesion molecule 1 (ICAM-1). In addition, we showed that pathogenic and non-pathogenic *Leptospira* are both capable of stimulating endothelium E-selectin and ICAM-1, but that the pathogenic *L. interrogans* serovar Copenhageni strain promotes a significantly higher activation than the non-pathogenic *L. biflexa* serovar Patoc ($P<0.01$). Lp95 was identified *in vivo* in the renal tubules of animal during experimental infection with *L. interrogans*. The whole Lp95 as well as its fragments, the C-terminal containing the domain of unknown function (DUF), the N-terminal and the central overlap regions bind laminin and fibronectin ECM molecules, where the binding is stronger with the DUF-containing fragment. This is the first leptospiral protein able to mediate adhesion to ECM components and the activation of HUVECS, thus suggesting its participation in the pathogenesis of *Leptospira*.

Supported by: FAPESP, CNPq and Fundação Butantan.

3.08 The effect of Hsp65 on immune responsiveness in young and old genetically selected mice

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Introduction: The 60-kDa heat shock proteins (Hsp) are highly conserved during evolution, abundant in prokaryotic and eukaryotic cells, and participate in numerous steps of protein assembly and transport. They are over expressed in cells under stress and during autoimmunity or inflammatory processes, mainly in older individuals. **Objectives:** The aim of the study was to evaluate the effect of the passive administration of wild type [WT] and the point mutated $K^{409}A$ recombinant Hsp65 of *Mycobacterium leprae* in the immune response of old mice of two murine lines. **Methods:** Young [3-4 months] and old [12-14 months] mice genetically selected for high [H_{III}] and low [L_{III}] antibody responsiveness were intraperitonially inoculated with 2.5 µg of purified WT or $K^{409}A$ rHsp65. Anti-WT rHsp65 and anti-DNA antibodies were individually measured during the lifetime of the animals, and the mean survival time [MST] was determined. **Results and Discussion:** The treatment with WT shortened MST by 42% [308 days] in old female H_{III} mice, while the MST of control mice was 530 days. Old male H_{III} mice receiving the WT protein had a MST similar to that of control [493 days] and the $K^{409}A$-treated group [506 days]. No effect of WT rHsp65 was observed in young mice. There was an increase in the production of IgG2a anti-rHsp65 antibodies in serum of $K^{409}A$-injected H_{III} mice and a decrease in the anti-DNA IgG2a/IgG1 antibodies ratio in H_{III} female mice treated with WT [non significant] and $K^{409}A$ [$p<0.05$] proteins. This study may contribute to the understanding of the general biological role of Hsp in the immunosenescence process mainly in females, and the results are compared with previous data on Hsp65 effect in autoimmune processes.

Supported by: CNPq and FAPESP.

3.09 A new use of DMT-MM, an activating reagent, in the conjugation of *Haemophilus influenzae* type b capsular polysaccharide and tetanus toxoid

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Introduction: *Haemophilus influenzae* type b (Hib) is a capsulated bacterium that causes invasive infections, where the most frequent is meningitis in infants. The capsular polysaccharides (PS) are the main factor of virulence, and as a consequence, the main antigen for vaccines. However, PS are T-cell-independent antigens and their covalent linkage to a protein carrier converts them into a T-cell-dependent antigen, making this antigen efficient for inducing protection in young children. **Objectives:** To develop a conjugation process between capsular polysaccharide of *H. influenzae* type b (polyribosyl ribitol phosphate - PRP) and tetanus toxoid (TT), which has a high yield and is suitable for scale-up. **Methods:** The synthesis of this conjugate antigen consists of three steps. 1) Oxidation of native polysaccharide (Hib) with the generation of aldehyde groups (PRP-oxi). The reaction mixture was prepared by mixing PRP (10 mg/mL) and $NaIO_4$ (10 mM) in 10 mM phosphate buffer, pH 7, in the dark for 30 min, and the reaction was stopped by adding glycerol (10 eq). 2) Reaction of aldehyde group with adipic acid dihydrazide (ADH) followed by reduction with $NaBH_4$. PRP-oxi (6 mg/mL) was added to a solution with 10 mM phosphate buffer, pH 7, and adipic acid dihydrazide (10 eq). The mixture was stirred for 3 h, and afterward, $NaBH_4$ was added. The solution was then dialyzed. 3) Conjugation of PRP-ADH with tetanus toxoid. In this step the carboxyl groups from tetanus toxoid was activated with a soluble condensing agent DMT-MM (4-[4,6-dimethoxy-1,3,5-triazin-2-yl]-4-methylmorpholinium chloride). It should be noted that in all cases to determine the molar mass distribution, we analyzed all products by liquid chromatography (AKTA Prime system) employing a Sephacryl S-400 resin previously calibrated with dextrans. **Results and Discussion:** 1) Oxidation: the yield of this step was 98%. 2) Formation of PRP-ADH: yield 96%. 3) To obtain a covalent linkage between PRP-ADH and the carboxyl group of the protein, the carboxyl group should be activated, and this activation is usually done with a soluble carbodiimide, EDAC (1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride). In this work we tested a new use for the activating reagent DMT-MM. The advantage of DMT-MM over EDAC is that it is less susceptible to hydrolysis. We studied the reaction conditions such as: concentration of polysaccharide, protein and activation agent, pH, buffer, time, etc. The reaction carried out using EDAC gave the product PRP-TT with a maximum yield of 14% (in polysaccharide content). Reaction conditions: PRP-ADH:TT (14.8 mg:16.8 mg), 0.1 M phosphate buffer, pH 5.5, 3 h. The reaction carried out in 24 h did not show an improved yield as expected. The relation between PRP and TT in mass, in PRP-TT, was 0.65. Until now, using DMT-MM, we have achieved 35% of PRP-TT. This result is very promising. Reaction conditions: PRP-ADH:TT (15.0 mg:30.0 mg), 0.1 M DMT-MM; 5 h. In this case, the relation between PRP and TT (in mass) in PRP-TT, was 0.52. Using PRP-ADH:TT (15.0 mg:15.0 mg) the relation between PRP and TT (in mass) in PRP-TT, was 0.83. We believe that in changing some reaction conditions, as we did in present study, we could improve the yield.

Supported by: Fundação Butantan and FAPESP.

3.10 Involvement of acute inflammatory reaction loci in the sensitivity or resistance to endotoxic shock induced by LPS

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Introduction: Mice selected for maximal (AIRmax) or minimal (AIRmin) acute inflammatory reaction differ in susceptibility to *Salmonella enterica* serotype Typhimurium (*S.* Typhimurium) infection and to LPS-induced endotoxic shock. Different frequencies of *Slc11a1* (formerly *Nramp1*) *R* and *S* alleles, involved in innate resistance or susceptibility to *S.* Typhimurium infection, respectively, were found in AIRmax and AIRmin mice. To study the *Slc11a1* gene interaction with acute inflammatory reaction loci (AIR QTL), AIRmax^{RR}, AIRmax^{SS}, AIRmin^{RR} and AIRmin^{SS} sublines were produced. AIRmax^{RR} mice are extremely susceptible to LPS-induced shock, while AIRmin^{SS} were the most resistant. **Objectives:** The objective of this work was to identify genes in AIR QTL that interact with *Slc11a1* alleles to modulate LPS shock in these mice. **Methods:** Mice were injected i.p. with 20 μg of LPS, and mRNA from bone marrow cells was isolated after 40 min. Global gene expression analysis was performed on Codelink bioarrays (36k genes) using RNA pools (n=4) of LPS treated or control cells from AIRmax^{RR}, AIRmax^{SS}, AIRmin^{RR} and AIRmin^{SS} mice. Differentially expressed genes were detected with the Codelink array expression software and over-represented biological themes were analyzed by the EASE program. qPCR was used to measure gene expression, and ELISA was performed to determine serum levels of inflammatory cytokines. **Results and Discussion:** The highest number of differentially expressed genes ($P<0.001$) after LPS injection was found in AIRmin^{SS} line when compared to the other lines. The inflammation response and cell death biological themes were over-represented in all sublines. AIRmax^{RR} animals had higher serum levels (2- to 5-fold) of inflammatory cytokines as well as higher *Tnf*, *Il6* and *IL1b* gene expression intensities in liver and bone marrow cells. *Il10* expression was higher in AIRmin^{RR} mice than in the other lines. Taken together these results suggest that *Slc11a1* alleles modulate inflammatory genes during LPS shock.

Supported by: FAPESP and CNPq.

3.11 Rabies virus production in Vero cells using different virus concentrations for cell infection

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Introduction: Rabies is a worldwide fatal disease and represents a severe public health problem in developing countries. Rabies vaccine is essential for the control of rabies. Vero cell culture is a good substrate for rabies vaccine production, and the number of viral particles used to infect this cell is very important in this procedure. **Objective:** To study rabies virus production in Vero cells using different concentrations of the virus (MOI= multiplicity of infection). **Methods:** Vero cells were infected with different PV rabies virus (MOI of 0.01, 0.02 and 0.08). After incubation for 1 h at 37°C, the suspension of infected cells was distributed in flasks (5 x 10^7cells/flask), and 200 ml of serum-free medium VP-SFM AGT (GIBCO) were added to each flask. Three days later, the supernatants were harvested. This procedure was repeated every 24 h and samples were taken to determine the viral titer. **Results and Discussion:** The titers of rabies virus found in the samples of the harvests were $10^{4.8}$ – $10^{6.2}$ FFD_{50}/ml for the cultures infected with 0.01 MOI; $10^{4.4}$- $10^{5.8}$ FFD_{50}/ml for 0.02 MOI and $10^{5.0}$- $10^{5.8}$ FFD_{50}/ml for 0.08 MOI. The results showed that all the virus concentrations used to infect Vero cells with rabies virus showed similar viral titers. Thus, the use of 0.01 MOI is more economic than the other concentrations.

Supported by: Fundação Butantan.

3.12 Transfer of maternal antibodies reactive with intimins α, β and γ to the newborns of healthy Brazilian mothers via placenta and breastfeeding

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Intoduction: Intimin is a protein adhesin of enteropathogenic (EPEC) and enterohemorrhagic (EHEC) *Escherichia coli*, capable of inducing attaching and effacing lesion in enterocytes. The main subtypes of intimins of EPEC and EHEC prevalent in Brazil are α, β and γ. **Objectives:** Our aim was to investigate the transfer of maternal anti-intimin antibodies to the newborns of healthy Brazilian mothers. **Methods:** IgG and SIgA antibodies were determined by ELISA in sera and colostrums from 50 healthy women and cord sera from their newborns, using purified recombinant proteins, conserved and variable regions of α, β and γ intimins. **Results and Discussion:** The antibody concentrations of colostrums were higher than in serum for all intimins, and there were no statistical differences between them in colostrums. The concentrations of antibodies reactive with the conserved region of intimin were significantly higher compared to the variable regions in the serum groups, for mothers and newborns. There were high correlation coefficients between all the anti-intimin antibodies in colostrums. In the groups of sera, the coefficients were higher between α and β than all other pairs. Discussion: Our results confirm the transfer of maternal antibodies to the newborns via the placenta and breastfeeding and reinforce the high protective effect of breastfeeding against EPEC infection.

Supported by FAPESP and CNPq.

3.13 The effect of SBA-15 silica adjuvant on phenotype and function of murine lymphoid cells

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Introduction: Adjuvants are important components of vaccine formulations. Their function includes delivery of antigen, recruitment of specific immune cells to the site of immunization, activation of these cells to create an inflammatory microenvironment and maturation of antigen-presenting cells for the enhancement of antigen-uptake and presentation in secondary lymphoid tissues. The SBA-15 silica is a polymer that due to its physical and structural properties proved to be an effective adjuvant, carrying, protecting and delivering antigens [patent obtained]. **Objectives:** The main goal of this work was to evaluate the capacity of SBA-15 in inducing efficient immune responses against hepatitis A vaccine and its effect on antigen–presenting processes such as the increase in the expression of MHC class II and co-stimulatory molecules. **Methods:** Mice genetically selected for low responsiveness or BALB/c were immunized by the subcutaneous route with 0.48 µg/animal of hepatitis A vaccine adsorbed or not on SBA-15. At different periods after immunizations, specific serum IgG antibodies were determined. The expression of CD11c, CD11b, CD4, CD8, B220, CD80, CD86, CD40 and MHC-II molecules in lymph node cells were analyzed by flow cytometry. **Results and Discussion:** Specific IgG antibodies levels were significantly higher in the group immunized with the vaccine on SBA-15 [$p<0.001$], and memory induction was proven by the increased titers of specific antibodies after booster [$p<0.001$]. SBA-15 significantly increased the expression of CD80 and CD86 molecules in lymph node cells, but this nanoparticle did not modulate MHC-II or CD40 expression in these cells, or in CD11c, CD11b, CD4, CD8 or B220 positive cells. These results indicate the ability of SBA-15 to induce successful immunity, recruiting and activation of specific immune cells at the site of immunization. Thus, this new adjuvant may reveal novel therapeutic targets for immune modulation and vaccine design.

Supported by: FAPESP, CNPq and Cristália.

3.14 Quality assurance assays of an unencapsulated *Streptococcus pneumoniae* strain to be used in a whole-cell pneumococcal vaccine

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Introduction: Instituto Butantan is developing a killed whole cell pneumococcal vaccine (WCV) derived from an unencapsulated mutant of *Streptococcus pneumoniae*, the strain Rx1E PdT Δ*lytA*, originally a serotype 2 strain, autolysin-negative, carrying a kanamicin resistance and a pneumolysin defective gene. The deletion of the gene *lytA*, which causes autolysis of the bacteria in the stationary phase, should enhance bacterial growth, and mutants lacking the pneumolysin activity are less virulent. The new whole cell pneumococcal vaccine presents to the immune system more conserved and not serotype-dependent antigens in native configuration, which are normally occluded by the polysaccharide capsule, probably enhancing the coverage and diminishing the limitations for serotype-specific replacement. **Objectives:** In this work we developed specific tests to be included in the Standard Operating Procedures (SOP's) for characterization of the seed lot used for a cGMP pilot production of the WCV. **Methods:** Samples from seed lot were cultured in Todd-Hewitt with yeast extract (THY) medium at 36°C at 3% CO_2 until OD_{600} 1.0 for identity tests. Culture samples were evaluated by Gram staining and colony morphology on BHI/blood agar plates, as well as hemolysis, viability and kanamycin resistance. The deletion of the gene *lytA* in the seed lot of the vaccine strain was evaluated by lysis in the presence of 1.0% deoxycholate (DOC) in purified water, measured by OD_{600} after 10, 20 and 30 min. The absence of pneumolysin activity was checked by the hemolysis of SRBC in dithiothreitol (DTT) buffer (10 mM DTT, 0.1% BSA in PBS, pH 7.4). The test was performed in microplates and recorded as hemolysis or presence of SRBC pellets. As a control of the assays, we used a wild type pneumococcus strain, St322/08 serotype 14 (obtained from Instituto Adolfo Lutz). **Results and Discussion:** The seed lot of the pneumococcal vaccine strain was satisfactorily identified by Gram staining and morphology. The selective marker of the strain (Kan^R) was confirmed by its kanamycin resistance in BHI/blood agar. No lysis of the bacterial suspension was observed in the presence of DOC, confirming the deletion of the gene *lytA*, while the control wild strain showed 95% lysis after 20 min. Hemolytic activity in SRBC was not detected when elicited by the seed lot suspension, suggesting the absence of biologically active pneumolysin. The control strain induced complete SRBC lysis. The production procedures of this whole cell pneumococcal vaccine can be achieved at low cost, since the methodology includes basically fermentation and detoxification processes. However, the seed lot characterization is essential for the approval of the new vaccine. Our results tested the identity of the vaccine strain and reinforced the feasibility of the inclusion of this assay in SOP's of the final product.

Supported by: Fundação Butantan, FAPESP, CNPq and PATH.

3.15 **Interaction of human complement factor H variants Tyr402 and His402 with *Leptospira* spp**

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Introduction: The complement system is a major component of both innate immune and acquired responses. Several pathogens have developed strategies to evade the activation of this system on their surfaces. Factor H (FH), a 150-kDa plasma protein, inhibits the alternative pathway of complement by preventing binding of factor B to C3b, accelerating decay of the C3-convertase C3bBb and acting as a cofactor for the cleavage of C3b by factor I. Recently, it has been demonstrated that pathogenic leptospiral strains are able to bind to this complement regulator, and that surface-bound FH acts as a cofactor for the cleavage of C3b by factor I, thus indicating that acquisition of this complement regulator may contribute to leptospiral serum resistance. Two forms of human FH have been intensively studied: FH Tyr402 and FH His402 since it has been shown that this last variant is highly associated with the development of age-related macular degeneration. **Objectives:** In this study, we assessed the importance of the FH Tyr402His polymorphism with respect to the binding to *Leptospira*. **Methods:** Both FH variants were purified from human plasma by chromatography and incubated with three different species of *Leptospira*. Surface-bound proteins were evaluated by an enzyme immunoassay using whole *Leptospira* and also by Western blotting with overlay. **Results and Discussion**: We observed a marked difference in the interaction between these two FH forms and the bacteria: FH Tyr402 bound more efficiently to *Leptospira* than did FH His402. Despite binding to *Leptospira* with different affinities, both variants exhibited similar cofactor activities against C3b, thus indicating that they are equally efficient in regulating the complement cascade.

Supported by: FAPESP and CNPq.

3.16 Production and purification of human papillomavirus 16 (HPV-16) L1 protein

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Introduction: Human Papillomavirus (HPV) are non-enveloped double-stranded DNA viruses with icosahedral capsid. The viruses are responsible for a wide variety of clinical manifestations ranging from benign warts to cervical cancers. They are classified as high-risk, probably high-risk or low-risk, according to their ability to drive the infection to carcinogenesis. Among the types of papillomavirus, HPV-16 is found in 50% of cervical cancers. The L1 protein is the major capsid protein, 55 kDa in size, and has conformational epitopes that stimulate neutralizing antibodies production against papillomavirus. The L1 protein has the ability to self-assemble into particles similar to virus, known as virus-like particles (VLPs) when expressed in heterologous expression system. VLPs are the basis of current prophylatic vaccines licensed against HPV16 and HPV18, among others. **Objectives:** The aim of this study was to set up the expression and purification protocol for L1 protein of HPV-16 expressed in *Pichia pastoris*, for the development of the Brazilian prophylactic vaccine against HPV-16. **Methods:** The codon optimized sequence of L1 protein was cloned in pPICHOLI episomal vector (MoBiTec) for *Pichia pastoris* expression. Subsequently, yeasts were electroporated with pPICHOLI-L1 vector. The expression of protein was performed by induction with 0.5% methanol for 48 h. The purification of L1 protein was performed by affinity chromatography on a Heparin-Sepharose column. The eluted fractions were analyzed with 12% SDS-PAGE and characterized by Western blotting using anti-L1-CAMVIR monoclonal antibodies. After the purification protocol, eluted fractions with the presence of L1 protein were pooled and concentrated by ultracentrifugation, and resuspended L1 protein was quantified with BSA as standard. **Results and Discussion:** The cloning of L1 protein in pPICHOLI vector was confirmed by sequencing. Through the induction of small-scale cultures, positive recombinant yeasts were selected. The purification of L1 protein was performed from 1 L of culture, and the expression of L1 as a 55-kDa protein band was confirmed, the expected size of L1 protein. The protein was eluted from a Heparin-Sepharose column and all aliquots analyzed, from 0.4 to 2.0 M NaCl elution. The amount of purified protein was 15µg/L of culture. The L1 protein of HPV-16 was expressed and purified. Further studies have to be performed in order to improve the expression and purification of L1 protein for vaccine development.

Supported by: FAPESP, CNPq and Fundação Butantan.

3.17 Contribution of MIP-2 chemokine and TNF-α in the immunological response of C3H/HeJ mice during infection with *Leptospira interrogans*

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Introduction: Leptospirosis is a worldwide zoonosis caused by spirochetes of the genus Leptospira. It has been considered as a re-emerging infectious disease in both industrialized and developing countries. The bacteria are transmitted by direct contact with urine from infected animals or indirect contact with contaminated water. Hematogenous dissemination of *Leptospira* throughout the infected host can result in a wide range of clinical manifestations. The symptoms of leptospirosis vary from sub-clinical infection to a variety of adverse effects. In severe cases, pulmonary hemorrhage, hepatic and renal dysfunction or multi-organ failure can occur and lead to fatality. Although several virulence factors such as lipopolysaccharide, outer membrane proteins, and various secretory proteins have been studied, the mechanisms of pathogenesis, host defense and protective immunity in leptospirosis remain poorly understood. An improved understanding of host immune response in leptospirosis may contribute to the development of more effective treatment and prevention of the disease. **Objectives:** We examined the expression of the chemokine C-X-C motif ligand 2 (CXCL2), also called macrophage inflammatory protein 2 (MIP-2), and tumor necrosis factor alpha (TNF-α) in C3H/HeJ mice, the animal models for susceptibility to leptospirosis. **Methods:** Fifty-two animals (26 female and 26 male) were infected intraperitoneally with $1x10^6$ bacteria of a virulent strain of *L. interrogans* serovar Copenhageni. A group of 5 animals from each sex was kept not infected as control. Infected animals from each sex were sacrificed on different days after inoculation. The presence of DNA from leptospires in tissues was demonstrated by PCR. The cytokines MIP-2 and TNF-α were measured in serum, spleens, livers, kidneys and lungs using immunoenzymatic assay (ELISA). **Results and Discussion:** Infected C3H/HeJ mice started to present leptospirosis symptoms and death around the seventh day of inoculation. Increasing levels of cytokines MIP-2 and TNF-α were observed in the kidneys of the infected mice. MIP-2 and TNF-α concentrations reached higher levels around the third day after infection, reaching averages of 78±10 ng and 35±7 ng (per mg of tissue), respectively. These values were three times higher than the ones found in the control animals. After peaking, the levels of these chemokines decreased and stabilized close to baseline values. Our results suggest that increased levels of MIP-2 and TNF-α in kidney may correlate with severity of leptospirosis in susceptible C3H/HeJ mice. Experiments in process may provide additional information whether there is a correlation between the expression of these cytokines and pathologies found in the affected kidneys.

Supported by: FAPESP, CNPq, FIOCRUZ and Fundação Butantan.

3.18 DLCs as new vaccine candidates against schistosomiasis

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Introduction: Schistosomiasis is one of the most important helminthic diseases in the world. It is present in 76 countries, with more than 200 million people infected, and it is estimated that more than 600 million people live in endemic areas. In 2003, extensive data from the transcriptome of *Schistosoma mansoni* was made available. The information of the encoded proteins allowed the analysis of protein function and improved the search for vaccine candidates. The analysis of the transcriptome allowed the identification of three families of protein homologs to the mammalian dynein light chain (DLC). One of these was the L8 family, with at least 18 members, all proteins with around 10 kDa. These proteins were found to be expressed in the different stages of the *S. mansoni* life cycle. Two DLCs were recognized in the tegument of *S. japonicum*, suggesting that they are exposed to the host immune system. Considering these aspects, we selected two DLCs from the L8 family to be tested as vaccine candidates. **Objectives:** To investigate the immunogenicity and antigenicity of two DLCs from *S. mansoni*; to evaluate the protective profile in challenge assays with cercariae and to analyze the development of hepatic granulomas that are involved in the main pathology of schistosomiasis. **Methods:** Two genes of DLCs were cloned in *E. coli* for protein expression; the purification was carried out by metal affinity chromatography. The purified proteins were used for immunization assays. The sera generated against DLCs were tested by ELISA and Western blot assays. After three immunizations with 10 μg of purified protein plus 0.3% allydrogel, mice were challenged with cercariae. After 45 or 55 days, mice were perfused and the worm burden and hepatic granuloma formation were determined. **Results and Discussion:** Both DLCs were shown to be very immunogenic, increasing the IgG titers in the sera. Besides, the group immunized with DLC1 increased the IgE levels before infection. After infection, both DLCs showed lower IgE levels when compared to the control groups. DLCs were found to be antigenic, since they were recognized by the sera of the control infected mice. In the challenge assays, DLCs showed a significant decrease in the worm burden (between 40 and 60%). The granuloma analysis at 45 days after infection showed that the groups immunized with DLCs had a significant increase of 70% smaller granuloma areas, when compared to the control group. After 55 days, the granulomas from groups immunized with purified proteins were still smaller (between 25 and 35%). The most promising antigens tested as vaccine candidates against schistosomiasis showed a protective immune profile with 30-40% decrease in worm burden when alhydrogel was used as adjuvant. Taken together, the results of decreasing the worm burden and the granuloma size after immunization with purified DLCs, suggest that these proteins could be considered as very interesting vaccine candidates, affecting the main causes of the pathology of schistosomiasis, and they could be included in a vaccine formulation against the disease.

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

3.19 Media hold test for the production process of bulk tetanus anatoxin

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Introduction: Media hold test is a step of process validation and an assessment to ensure the integrity of the process, environment, machinery and facility that demonstrates the product safety, which is the focus of regulatory requirements and official inspections. In the media hold test a suitable microbiological medium is used, in place of the actual product. In the actual process, the toxin, produced by *Clostridium tetani* fermentation, is released into media culture, recovered by tangential flow filtration and concentrated in an enclosed system. After concentration, the toxin is submitted to a sterile filtration and a detoxification process. The production process is carried out in cleanroom (grade D - ISO 8) where the environment is monitored by air sampling for microbiological and non-viable particles. The process is simulated exactly at the same point as that of the product for monitoring the microbiological population present in critical steps. The facility systems (air, pure water and pure steam) are validated and the equipment used in the production process is qualified (Installation Qualification - IQ, Operational Qualification - OQ and Performance Qualification - PQ). **Objective:** To perform the media hold test to provide documented evidence to validate the production process of tetanus anatoxin bulk, ensuring consistency, safety and quality of the product. **Methods:** Three consecutive media hold tests were performed. The medium used in place of actual product was trypticase soy broth (TSB). It was introduced into two fermentors in line, filtered and heated. The inoculum of production is also simulated with TSB incubated and inoculated as if it were an actual inoculum of production. The TSB that simulated the inoculum preparation was done 14 days before the fermentor inoculation, and the medium was tested for fertility and sterility. For each media hold test for production of tetanus anatoxin bulk, twenty samples were taken throughout the entire production process. Seventeen samples were submitted to sterility testing, and three samples were submitted to a bioburden test to determine the microbial load of the non-sterile step of the production process. To perform the growth promotion in the sterility test, incubations were carried out for 14 days at 20 to 25 °C and 30 to 35 °C. For the bioburden test, an incubation was performed for 5 days at 30 to 35 °C. **Results and Discussion:** In every media hold test, the seventeen samples submitted to sterility determination showed the absence of bacterial growth. In the first media hold test, one of the three samples submitted to the bioburden test showed growth of 1 CFU/50 mL (*Staphylococcus sp.*) before the sterile filtration. In the second media hold, the three samples submitted to the bioburden test showed no growth, and in the third media hold test one sample showed 2 CFU/50 mL (Gram positive and negative bacteria with no growth viability) before sterile filtration. After the sterile filtrations, all samples showed absence of bacterial growth. The execution of the simulation of production process (media hold) showed no occurrence of deviations.

Supported by: Fundação Butantan.

3.20 Evaluation of *Schistosoma mansoni* tegumental ectonucleotidases as vaccine antigens

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Introduction: The elimination of schistosomiasis morbidity, transmission, and infection are different battlefronts, and mass chemotherapy will not be enough to reach these goals. Thus, vaccination would contribute to the reduction of the disease burden. Recent proteomic characterization of the tegument, the major parasite-host interface and a source of potential antigens, identified three ectonucleotidases: a nucleotide pyrophosphatase/ phosphosdiesterase (NPP), a diphosphohydrolase (apyrase) and an alkaline phosphatase (AP). Ectonucleotidases are membrane-associated or secreted enzymes involved in extracellular nucleotide metabolism and, therefore, participate in purinergic signaling. **Objectives**: In the current work we determined if the immunization of mice with recombinant tegument ectonucleotidases is capable of reducing the worm burden and/or the number of liver-trapped eggs following challenge with cercariae. We also measured the levels of IgG antibodies and the IgG1/IgG2a ratio. **Methods**: The ectonucleotidases were cloned by RT-PCR, expressed in an *E. coli* system, and purified by nickel affinity chromatography. Female C57BL/6 mice were divided into five groups of ten animals each, a control group, one group for each protein and a group immunized with the three proteins to evaluate a possible synergistic effect. Mice were subcutaneously injected in the nape of the neck with 25 µg of each recombinant protein (NPP, apyrase, FA) or the three proteins together on days 0, 15 and 30. The recombinant proteins were formulated with Freund's adjuvant. Fifteen days after the last boost, mice were challenged through percutaneous exposure of abdominal skin for 1 h with water containing 100 cercariae. Forty-five days after challenge, adult worms were recovered from the portal vein by perfusion. A piece of liver was weighed and digested with 5% KOH, and the trapped eggs were counted. Additionally, serum was collected from mice before cercariae challenge and before perfusion, and used in indirect ELISA to evaluate the level of specific IgG, IgG1 and IgG2a antibodies. **Results and Discussion**: Similar levels of specific anti-ectonucleotidases IgG antibodies were elicited in the animals immunized with the recombinant proteins; a reduction in the antibody levels was observed in the group immunized with the 3 proteins. The apyrase group showed a balanced IgG1/IgG2a ratio, while the other groups displayed higher levels of IgG1. The antibody levels were similar both before the perfusion and before challenge. The experimental groups did not show a reduction in worm burden or in the number of liver-trapped eggs. Therefore, despite inducing a specific immune response against the antigens, the *E. coli* expressed recombinant *S. mansoni* ectonucleotidases were not capable of developing protective immunity, nor could they reduce oviposition.

Supported by: FAPESP.

3.21 High-molecular weight components from *Ascaris suum* exert a suppressive effect on dendritic cells independent of TLR2 and TLR4

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Introduction: We showed that high MW components (PI) from *Ascaris suum* extract modulate the ability of dendritic cells (DCs) to activate OVA-specific T cells. DCs discriminate pathogenic compounds through pattern-recognition receptors (PRRs) and when activated acquire the capacity to induce effector T cells. We also verified that PI inhibits the DCs maturation induced by LPS *in vitro*. **Objective**: We studied the involvement of TLR2 or TLR4 in the suppressive effect of PI on anti-OVA response. Furthermore, we determined the role of these receptors in the ability of PI to down-modulate the DC maturation induced by LPS or pam3CSK4. **Methods**: WT, TLR2$^{-/-}$ or TLR4$^{-/-}$ C57BL/6 mice were immunized with OVA (200 µg/animal) or OVA+PI (200 µg/each antigen/animal) in CFA, and after 5 days, lymph node cell suspensions were prepared. In other experiments, iDCs derived from WT, TLR2$^{-/-}$ or TLR4$^{-/-}$ mouse bone marrow were cultivated in RPMI medium plus GM-CSF/IL-4. On day 7, iDCs were incubated *in vitro* with LPS (1 µg/mL), PI (200 µg/mL), LPS+PI (1 µg+200 µg/mL), pam3 (10 ng/mL) or pam3+PI (10 ng+200 µg/mL) for 18 h. In all experiments, the cells were stained with anti-CD11c, CD11b, CD80, CD86 and CD40 or MHC class II mAb-FITC/PE and analyzed by flow cytometry. **Results and Discussion:** There was a reduction in the expression of CD80 (40, 33 and 65%), CD86 (76, 60 and 10%) and MHC class II (63, 50 and 75%) molecules in cells from WT, TLR2$^{-/-}$ or TLR4$^{-/-}$ OVA+PI-immunized mice when compared with the cells of OVA-immunized group. The cytometric analyses showed high expression of MHC-II and costimulatory molecules on DCs stimulated with LPS or pam3. LPS and pam3 were unable to induce maturation of DCs from TLR4$^{-/-}$ and TLR2$^{-/-}$ mice, respectively. In contrast, PI down-modulated the expression of these molecules in DCs from WT, TLR4$^{-/-}$ or TLR2$^{-/-}$ mice when stimulated with LPS or pam3 ($p< 0.001$). The results indicate that TLR2 and TLR4 are not involved in the suppressive effect of PI on the activity of DCs.

Supported by: FAPESP, CNPq and CAPES.

3.22 Human serum ability to react with and neutralize SA-11 (serotype G3) rotavirus

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Introduction: Rotavirus is well established as the major etiological agent of diarrhea worldwide. The role of serum antibodies in immune protection against natural infection is not fully understood. Some studies have correlated serum antibodies against rotavirus in adults with protection and lower probability of infection and illness. Anti-rotavirus IgA and IgG are candidate markers for rotavirus immunity. **Objectives:** Our aim was to examine the presence of anti-rotavirus IgA and IgG antibodies in human serum samples from healthy donors and to determine their neutralizing ability against rotavirus G3 serotype. **Methods:** Rotavirus and control antigens were obtained by ultracentrifugation purification from the supernatants of SA-11-infected or mock-infected cells, respectively. These antigens were used in ELISA assay to detect anti-rotavirus IgG and IgA antibodies in 50 serum samples. For neutralization assays, serum samples were incubated with 100 $DICT_{50}$ of SA-11 rotavirus, the mixtures were added over a monolayer of MA-104 cells, and the inhibition of cytopathic effect was evaluated after 48 h. **Results and Discussion:** Anti-rotavirus IgG titers varied from 3.6 to 457.6 (mean of 138.2). The IgA titers ranged from 4.1 to 201.4 (mean of 63.1). There was no correlation between IgA and IgG ELISA titers. Preliminary results of neutralization assays performed with some of the samples showed that samples with high titers of anti-rotavirus IgA exhibited high neutralization titers, and samples with low IgA titers exhibited lower neutralizing titers. However, it was not possible to establish a relationship with IgG anti-rotavirus titers in ELISA and neutralization assays. The population studied had varying levels of anti-rotavirus G3 IgG and IgA antibodies, perhaps due to different levels of exposure to the virus. However, our preliminary results suggest that serum IgA are directly correlated with the neutralizing potential of the samples studied. The analysis of a greater number of samples will allow us to establish a better correlation between antibody titer and neutralizing ability of the samples.

Supported by: FAPESP and CNPq.

3.23 High hydrostatic pressure refolding of the recombinant protein OmpA70 from *Leptospira interrogans.* Partial characterization of immunological properties of the protein

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Introduction: *Leptospira* is the etiological agent of leptospirosis, a life-threatening disease that affects populations worldwide. Currently available vaccines have limited effectiveness, and therapeutic interventions are complicated due to the difficulty to establish an early diagnosis of leptospirosis. The genome of pathogenic *Leptospira* strains was recently sequenced, and bioinformatic analysis led to the identification of surface antigens, potential candidates for development of new vaccines and serodiagnosis. **Objectives**: The aim of this work was to study the protein OmpA70 as vaccine and diagnosis candidate. OmpA70 is a putative outer membrane protein from *Leptospira interrogans* serovar Copenhageni that combines structural characteristics from Loa22, the first genetically defined virulence factor in *Leptospira* species, and Lp49, an antigenic protein that reacts with sera from early and convalescent leptospirosis patients. Considering the importance of the structural integrity of a protein to confer immune protection, high hydrostatic pressure (HHP) was used to refold insoluble OmpA70 aggregated as inclusion bodies in *E. coli*. **Methods:** The gene was amplified from the genomic DNA of *Leptospira interrogans* serovar Copenhageni by PCR, cloned in the vector pGEM-T Easy and subcloned in the expression vector pAE. The protein was expressed in *E.coli* BL21(DE3)StarpLysS in inclusion bodies. HHP was applied to refold the insoluble protein. HHP was combined with different compounds: redox-shuffling reagents (reduced and oxidized gluthatione), the chaotropic agent guanidine hydrochloride or the aminoacid L-arginine. The refolded protein was purified by metal affinity chromatography and its secondary structure was analyzed by circular dichroism. In order to evaluate the immunological properties of the protein, mice were immunized three times and the sera collected 14 days after the second and third immunization, were analyzed by ELISA and Western blotting. The recognition of the protein OmpA70 by sera from hamsters infected with *Leptospira interrogans* serovar Pomona and Copenhageni was also tested. **Results and Discussion:** About 40% of the protein OmpA70 expressed as inclusion bodies was refolded by applying a pressure of 200 MPa for 16 h at concentrations of L-arginine above 0.4 M. The refolded OmpA70 was purified by metal affinity chromatography, and circular dichroism analysis revealed the presence of secondary structure. OmpA70 has immunogenic and antigenic properties, since high antibody titers were raised after immunization with the protein, and sera from infected hamsters reacted with soluble OmpA70. The refolding of OmpA70 was only achieved by the combination of HHP with L-arginine. Neither pressurization nor L-arginine alone was able to refold the protein. Preliminary characterization of the immunological properties of OmpA70 showed that the protein is immunogenic and antigenic. Future investigations will focus on the effectiveness of the protein as a recombinant vaccine.

Supported by: FAPESP.

3.24 Distinct inflammatory events during ear tissue regeneration in mice selected for high inflammation bearing *Slc11a1 R* and *S* alleles

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Introduction: Homozygous AIRmax and AIRmin sublines for *Slc11a1 R* and *S* alleles, produced by genotype-assisted breeding, differ in tissue regeneration capacity, which is increased in AIRmax^{SS} when compared to AIRmax^{RR} mice, suggesting that the *Slc11a1* S allele enhances tissue regeneration. Tissue regeneration was not observed in either AIRmin subline. *Slc11a1* is involved in the transport of divalent ions in macrophages and neutrophils, interfering in their activation. **Objectives:** Our aim in this work was to search for distinctive inflammatory events between AIRmax^{RR} and AIRmax^{SS} mice during the initial phase of tissue regeneration. **Methods:** Two-millimeter ear holes were punched in mice of each subline, and the inflammatory reaction was characterized by measuring ear thickness, MPO activity and cellular influx. Global gene expression analysis was used to identify sets of differentially-expressed genes, and quantitative PCR experiments were performed to validate microarray results. **Results and Discussion:** Local inflammation was more intense in AIRmax^{SS} than AIRmax^{RR} mice, with elevated levels of MPO and edema and predominance of neutrophils. Significantly ($P<0.001$) differentially-expressed genes were observed in AIRmax^{SS} and AIRmax^{RR} mice. A total of 794 genes were up- and 674 down-regulated in AIRmax^{RR}, while 735 genes are found to be up- and 1616 down-regulated in AIRmax^{SS} mice. Both AIRmax^{RR} and AIRmax^{SS} mice showed significant over-represented biological themes of genes related to cell proliferation; however, AIRmax^{SS} displayed up-regulation of inflammatory response genes. Interestingly, muscle contraction was the only significant functional category in down-regulated AIRmax^{SS} genes. Microarray results were validated by quantitative PCR. These results suggest that the *Slc11a1 S* allele positively modulates early inflammatory events leading to ear tissue regeneration in mice with an appropriate genetic background.

Supported by: FAPESP, CAPES and CNPq.

3.25 Comparison of two methods for antirabies serum potency assessment: seroneutralization in mice and in BHK21 cells

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Introduction: Nowadays, the method used for equine antirabies serum potency assessment in Brazil is the mice seroneutralization assay (MNT), which is described in the Brazilian Phamacopoeia. Tests using animals are subject to criticism, either due to animal suffering or the difficulties in obtaining good quality animals and qualified staff to perform the intracerebral challenge. In many laboratories, the 3R policy (reduction, refinement, replacement) is being widely used, which promotes the replacement of in vivo techniques by in vitro alternatives, resulting in more reproducible and reliable results. Recently, a validation study was carried out between Instituto Butantan and INCQS (National Control) based on a RFFIT-like assay in BHK21 cells. **Objective:** In this study, 40 lots of antirabies serum produced at Instituto Butantan, either concentrate or formulated products were assayed with both methods. **Methods:** Serial dilutions of serum were incubated with a fixed amount of rabies challenge virus strain (CVS) for 90 min at 37 °C. Residual viral infectivity was then determined by inoculating groups of mice or BHK21 cell cultures in microplates of 96 wells. Mice were observed for 14 days and the animals dead or that showed clinical signs of rabies between the 5th and the 14th days were considered positive for rabies. After 22 h at 37 °C, cell cultures were fixed and stained with anti-rabies nucleocapsid fluorescent conjugate (BIO-RAD) and examined with a fluorescence microscope. For both methods, virus neutralizing antibody titers were determined by comparison of the 50% effective dose (ED_{50}) obtained for the test serum and the reference included in each test. **Results and Discussion:** Although there are some studies showing that MNT assay tends to give higher results, this tendency was not observed. Although difficult to establish a direct correlation between the methods, the statistical analysis showed they were not significantly different ($p>0.05$). The in vitro assay can be considered an interesting alternative to MNT, with a considerable gain of time and reproducibility.

Supported by: Fundação Butantan.

3.26 Cloning, expression and characterization of gene LIC13435 of *Leptospira interrogans* serovar Copenhageni

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Introduction: Leptospirosis is a re-emergent zoonosis characterized by an acute febrile and systemic illness in humans caused by pathogenic spirochetes belonging to the genus *Lepstospira*. In Brazil, leptospirosis is an important economic and public health problem. The complete genomic sequence of *Leptospira interrogans* offered a new strategy for the identification of new proteins that could be vaccine candidates, since environmental control measures are difficult to implement and there is no available vaccine for human use. **Objectives:** Secreted and surface-exposed molecules are potential targets for inducing immune responses in the host. Thus, we selected the gene LIC13435 as predicted sequence coding for putative outer membrane protein to analyze as vaccine candidate against leptospirosis and for biological characterization. **Methods:** The sequence of gene LIC13435 was selected from the genome of *Leptospira interrogans* serovar Copenhageni using bioinformatics tools. The sequence was cloned by PCR and the expression of the recombinant protein was tested in *Escherichia coli* strains. Purification of the recombinant proteins was done by metal affinity chromatography due to the presence of a 6Xhis tag introduced at the N-terminus of LIC13435. Circular dichroism was performed to characterize the secondary structure, being mainly composed of α helix. The antisera were produced by intraperitoneal immunization of BALB/C mice. **Results and Discussion:** ELISA and Western blotting were done to confirm the titers and specificity of the antiserum. Preliminary Western blot test indicates that this protein is expressed by virulent low-passage forms of pathogenic *Lepitospira* serovar Copenhageni, while its expression is decreased during stationary phase. Preliminary challenge assay against *Leptospira* in the hamster, indicates that LIC13435 is not protective. Further characterizations are underway.

Supported by: FAPESP, CNPq and Fundação Butantan.

3.27 Construction, expression and evaluation of recombinant fusion proteins containing domains of pneumococcal surface proteins A and C (PspA and PspC) for vaccine purposes

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Introduction: *Streptococcus pneumoniae* (pneumococcus) is a Gram-positive bacterium responsible for the majority of pneumonia cases around the world. Available vaccines are based on capsular polysaccharides, but the variability and low cross-reactivity displayed by them require the fermentation of different serotypes for the induction of broad-range coverage immunity. Such formulations are available at high costs and have the potential drawback of inducing serotyping replacement for non-vaccine serotypes in vaccinated population. Protein antigens are interesting alternatives for the constitution of a low-cost formulation that can elicit immunity to the different serotypes. The PspA and PspC antigens play a role in bacterial evasion of the immune system and adhesion to epithelial cells and have already been shown to be good candidates for vaccine formulations. **Objectives:** The aim of the present work was to produce different fusion proteins based on important domains of PspC and PspA for their evaluation as antigen components for a new pneumococcal vaccine. **Methods:** The sequences encoding the N-terminal domain of PspC, responsible for binding to factor H, a known inhibitor of the complement system, was cloned and expressed in *E. coli* either i) alone ($PspC_{104}$), ii) in fusion with the SM1 domain of PspA, responsible for PspA binding to the bactericidal protein lactoferrin (PspC-SM1) or iii) in fusion with the N-terminal domain of PspA (PspC-PspA3AB). A larger N-terminal fragment of PspC (PspC) and the PspA3AB fragment (PspA3AB) were also used in this work. Proteins were purified by affinity chromatography and were used to produce specific antisera against each of them. Cross-reactivity of each serum among the different proteins was tested by ELISA and Western blotting. C57Bl/6 mice were immunized with the different proteins through the nasal route, using whole cell *Bordetella pertussis* vaccine (WCP) as adjuvant, and protection against nasopharyngeal colonization was evaluated. **Results and Discussion:** All proteins were expressed and purified with success. Specific sera produced against $PspC_{104}$ were able to recognize PspC-SM1 and PspC-PspA3AB but not PspA3AB alone. On the other hand, besides recognizing PspC containing proteins, sera against PspC-SM1 showed a low but positive cross-reactivity with PspA3AB, indicating that some antibodies were directed to the SM1 fragment. Antibodies to the fusion PspC-PspA3AB were able to recognize all proteins. Nasopharyngeal colonization analysis of immunized mice indicate that all vaccines containing PspC proteins and WCP were able to protect mice from pneumococcal carriage, with the best results observed for the groups immunized with $PspC_{104}$ +WCP or PspC +WCP. PspA3AB was not able to protect mice in this colonization model, even when administered in the presence of the adjuvant. Analyses of these proteins in other pneumococcal challenge models are under investigation.

Supported by: FAPESP, CNPq and Fundação Butantan.

3.28 Expression of the *Schistosoma mansoni* SLP-2 surface protein in recombinant BCG

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Introduction: Schistosomiasis is a parasitic disease affecting 200-300 million people around the world, being endemic in over 70 countries, characterizing it as a serious public health problem. In spite of the effectiveness of praziquantel – the chemotherapeutic agent of choice – it does not protect against reinfection, and periodic mass administrations are needed, especially in endemic areas. This strategy raises the possibility of the emergence of resistant strains. The consensus is that the induction of sterile immunity by a vaccine is not essential because it would be beneficial if it reduces the morbidity. Vaccines based on live vectors that present heterologous antigens are an attractive idea because they eliminate the necessity of multiple doses to obtain maximum protection against infection. **Objectives:** The aim of this work was to investigate the potential of the bacillus Calmette-Guérin (BCG) to express the *S. mansoni* SmSLP-2 vaccine candidate identified in functional genomic studies, capable of inducing a 32% reduction in worm burden when presented as a recombinant protein in the murine model. **Methods:** The mRNA of the parasites was extracted and cDNA obtained by reverse transcription. Based on the *S. mansoni* transcriptome data, the cDNA of SmSLP-2 was amplified by PCR, cloned in an expression vector for production of the recombinant protein in *E. coli BL21(DE3)pLys*. The protein was purified by nickel affinity chromatography in a Sepharose column. Mice were immunized to produce anti-SmSLP-2 antibodies. The cDNA was amplified again by PCR and cloned in mycobacterial expression vectors: i) in fusion with the β-lactamase signal sequence (pLA71) or ii) in fusion with the whole β-lactamase gene (pLA73), or iii) with a ribosomal binding site (pMIP12); the vectors were named pKL71-SLP2, pKL73-SLP2 and pKL12-SLP2, respectively. The BCG strains were transfected with these vectors and the clones selected by resistance to kanamycin. The expression of the heterologous SmSLP-2 was determined by Western blotting using the total protein extract of the clones and anti-SmSLP-2 antiserum. The localization of the heterologous protein in the BCG clones was also determined. **Results and Discussion:** The Western blotting results showed that in the rBCG-pKL71-SLP2 clones, the protein was expressed and enriched in the cytosolic fraction, and in the rBCG-pKL12-SLP2 clones, the protein was shown to be expressed and enriched in the cell wall associated material. However, it was not possible to observe the expression of the protein in the rBCG-pKL73-SLP2 clones. The preliminary results obtained indicate that the signal sequence was not able to direct the localization of the antigen. On the other hand, the results show that recombinant BCG was able to express the vaccine candidate SmSLP-2 and therefore raises the possibility to investigate if this presentation system – which induces an immune response with a strong Th1 profile – is able to increase the protection levels against this infectious disease.

Supported by: FAPESP.

3.29 Toxicity of 7,12-dimethylbenz(a)anthracene (DMBA) in bone marrow cells from mice genetically selected for low inflammatory response

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Introduction: Polycyclic aromatic hydrocarbons (PAHs), such as DMBA, are genotoxic compounds that react with DNA directly, inducing decrease in bone marrow (BM) cellularity resulting in an immunosuppressive state. This process involves the metabolism of the PAHs that depends on the activation of the aryl hydrocarbon receptor (Ahr). Two lines of mice genetically selected for maximal (AIRmax) or minimal (AIRmin) local acute onflammatory response (AIR) to a non-immunogenic substance (Biogel) showed a complete segregation of the low PAHs affinity *AHR* allele (AHR^{d}) and of the high affinity allele (AHR^{b1}). **Objectives:** Here, we studied the effect of *in vivo* DMBA treatment on the BM of AIRmax and AIRmin mice and on immune response. **Methods:** AIRmax and AIRmin mice were treated with a single i.p. dose of 50 mg/kg DMBA in olive oil. Proliferation index of bone marrow and spleen cells was determined in response to GM-CSF and LPS, respectively, after in vivo treatment with DMBA. Apoptosis levels were observed in BM cells stained with propidium iodide and Annexin V. DNA damage was evaluated by the single-cell gel electrophoresis (Comet) assay, which detects DNA strand breaks. **Results and Discussion:** Hypocellularity was observed in BM from AIRmin ($0.26\pm0.01\times10^{6}$/ml) when compared to controls ($0.51\pm0.02\times10^{6}$/ml) at 24 h post-DMBA treatment, mostly in the myeloid population. On the other hand, AIRmax mice showed normal levels of BM cells ($0.54\pm0.03\times10^{6}$/ml). Myeloid cells from DMBA-treated AIRmin mice showed low proliferation capacity after 1 (0.59×10^{6}/ml) or 7 (0.45×10^{6}/ml) days *in vitro* GM-CSF stimulation, whereas AIRmax BM cells displayed normal proliferation (8.57 ± 0.1/ml). Apoptosis levels were observed in BM cells stained with propidium iodide and Annexin V only in AIRmin ($28.7\pm5.1\%$ versus control mice $1.45\pm0.26\%$). A significant increase in tail moment index (TM) was observed in AIRmin mice after DMBA (2.7 ± 0.2). Spleen cells from DMBA treated AIRmin mice showed impaired proliferation after in vitro LPS stimulation. AIRmin mice were more susceptible to the DMBA toxic effects than AIRmax. DMBA-treatment produces DNA strand breaks due to direct hematotoxic effects with significant increase of apoptotic cells in AIRmin mice. These effects on myeloid BM cells reflect on an impaired immune response.

Supported by: CAPES and CNPq.

3.30 Comparative study between two bioreactor methods for rabies virus production

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Introduction: Rabies represents a severe public health problem in developing countries. Actually, despite of some new protocols for late-stage rabies post-exposure prophylaxis of humans and animals, only vaccination and anti-rabies serum are effective for post-exposure treatment. However, the rabies vaccine is expensive, and thus, the production of a safe and inexpensive vaccine is very important. **Objective:** To evaluate two methodologies of rabies virus production in Vero cells maintained in serum-free medium in a bioreactor. **Methods:** Two strategies of virus production were used in a bioreactor of 150 L: 1) Vero cells (16 cells/microcarrier) in a flask of 14 L together with microcarriers (Cytodex 1) and PV rabies virus (MOI= 0.02) were maintained at 37ºC for 2 h, and after they were introduced in the vessel of the bioreactor and maintained in serum-free medium; 2) in the same conditions, the virus+ cell + cytodex were incubated directly in the vessel. After 70 h harvests were carried out every 24 h for 6 days, and the samples were withdrawn to evaluate adhesion of the cells to the microcarriers, cellular integrity and viral titers. The virus titer was obtained in cell culture test and the result was expressed in FFD_{50}/ml. **Results and Discussion:** In this study, four cycles of virus production in a bioreactor were performed. After 3 days of incubation, the cover of the beads was: 0.4- 1.0% and 2.1- 27.9% of Cytodex without cells attached in the cultures that used the methodology 1 and 2, respectively. The harvest virus titers, obtained in cells, were: $10^{5.1}$- $10^{5.7}$ FFD_{50}/ml to cultures with adsorption were inside the vessel and $10^{5.8}$ – $10^{6.7}$$FFD_{50}$/ml in outside the vessel. The analysis of the results showed that when Vero cell, rabies virus and solid microcarriers were incubated outside of the bioreactor, the cell adhesion and viral titer were optimized.

Supported by: Fundação Butantan.

3.31 Cellular immune response in BALB/c and C57BL/6 mice infected with the nematode *Lagochilascaris minor*

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Introduction: Lagochilascariosis is a human parasitosis caused by the nematode *Lagochilascaris minor* which usually affects the neck region with exsudative abscesses. Mice are considered intermediate hosts of the parasite. We have shown recently that C57BL/6 are more susceptible to *L. minor* than BALB/c mice. **Objectives:** The aim of this study was to analyze the immune response of the two strains of mice infected with *L. minor*. We investigated splenocyte proliferative response and the production of IL-10, IFNγ, TNFα, TGFβ, IL-4 and IL-5 after re-stimulation *in vitro* with parasite antigens. **Methods:** BALB/c and C57BL/6 mice were orally infected with 10^3 eggs of *L. minor* per animal. After 7, 35 and 250 days of infection, groups of 5 mice were sacrificed. The same number of non-infected mice was used as control. Splenocytes were isolated and 5×10^5 cells were plated per well and stimulated *in vitro* with 5 μg/ml of crude extract (CE) or excretory/secretory (ES) products of third stage larvae of *L. minor*. Proliferation of splenocytes was measured by [^{3}H]-thymidine incorporation after 4 days of culture. Cytokine production was measured by sandwich ELISA for IL-10 and TGFβ and by CBA and FACS analysis for IL-4, IL-5, IFNγ and TNFα, in splenocyte supernatant collected 48 h after stimulation with the same antigens. **Results and Discussion:** SE antigen induced a proliferative response at 7 days of infection in infected and controls of both mouse strains and also in C57BL/6 at 35 days. In contrast, CE antigen inhibited the proliferative response at 7 and 250 days in both infected and controls of both strains. SE induced IL-10 and TNFα in infected BALB/c splenocytes at 7 days of infection compared to control group. Moreover, SE induced TNFα in C57 infected mice and IL-10 in the controls at 250 days of infection. CE induced IL-10 in infected BALB/c at 35 days and IFNγ in BALB/c at 250 days of infection. C57BL/6 mice produced TGFβ at 7 days of infection. The two antigens did not induce significant IL-5 or IL-4 in either mouse strain during the infection. Our results suggest that different T cell populations seem to be associated with the response to CE and SE antigens during infection with *L. minor.* The two strains of mice display a different cytokine profile. The greater production of IL-10 in the initial phase of infection in BALB/c mice may be associated with its resistance to the parasite compared to C57BL/6.

Supported by: FAPESP and FAPEG.

3.32 The use of free trypsin in the maintenance of Vero cells

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Introduction: Porcine or bovine trypsin derivates are used in cell culture to remove adherent cell from surfaces. However, manufacturers of human vaccines that use cell cultures and serum free- medium need a non-animal trypsin derivates. **Objective:** To evaluate an animal origin-free trypsin (Trype™ Select- Gibco) in Vero cell culture used for rabies vaccine production. **Methods:** Five different procedures to detach the cells were tested: 1- washing the culture with 3.0 ml of trypsin and afterward adding 1.0 ml of trypsin; 2- washing the culture with 4.0 ml PBS and afterward adding 1.0 ml of trypsin; 3- washing the culture with 4.0 ml of 0.1% EDTA and afterward adding 3.0 ml of trypsin; 4- only 4.0 ml of trypsin and 5- use of 4.0 ml of 0.1% EDTA + trypsin. Vero cells (1.1×10^7) were grown in 75-cm^2 flasks. The parameters evaluated were: time to detach, cellular integrity and growth. **Results and Discussion:** The time of detachment was the same for all the procedures; the cellular integrity was satisfactory in procedures 1, 2, and 3 and unsatisfactory for 4 and 5. After four days the cell concentrations were 1.4×10^7, 1.5×10^7, 1.4×10^7, 8.4×10^6 and 1.2×10^6 respectively for 1, 2, 3, 4 and 5. The results showed that procedures 1, 2 and 3 are adequate for detaching Vero cells. However, procedure 2 (washing the culture with PBS before the use of the trypsin) showed lower costs when compared to the others.

Supported by: Fundação Butantan.

3.33 Cloning strategy of intimin-specific single-chain variable-fragment (scFv) antibody

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Introduction: Adhesion of enteropathogenic (EPEC) and enterohemorrhagic (EHEC) *Escherichia coli* to enterocyte is mediated by intimin, a 94-kDa outer membrane protein. The conserved N-terminal region of intimin molecule consists of a 280-amino acid sequence ($int_{388-667}$), which is immunogenic and, therefore, an excellent target for diagnostic. Despite the excellent specificity by immunoblotting, the anti-intimin N-terminal domain-specific IgG2b monoclonal antibody failed to detect some EPEC isolates expressing different intimin subtypes. Besides, harvest and purification of hybridomas is expensive and time-consuming. An alternative model to hybridomas is the production of recombinant single chain variable fragment (scFv) antibody in heterologous expression systems. **Objectives:** The aim of this study was the cloning of intimin-specific scFv in an amplifying strategy using specific primers to link the antibody variable chains. **Methods:** IgG2b anti-intimin hybridomas were used extract mRNA which was reversely transcribed to cDNA. The light (LC) and heavy chain (HC) from antibody variable fragment were amplified by PCR using a commercial kit and cloned into pGEM-T Easy for sequencing. A four-step cloning strategy of scFv was used. 1st Step: four primers were drawn to amplify the LC and HC; reverse primer of the HC and forward of the LC contained complementary regions to linker ($[Gly_4Ser]_3$-coding DNA sequence that links the LC and HC); forward and reverse primers contained the *BamHI* and *HindIII* restriction sites, respectively. 2nd Step: the LC was re-amplified using the linker as the forward primer (resulting in the linker-LC product). 3rd Step: HC and linker-LC were linked by PCR due to complementarity between the 3' region of the HC and 5' region of the LC. 4th Step: the scFv was amplified using the HC-forward and LC-reverse primers. scFv obtained was cloned into pAE expression plasmid using the *BamHI* and *HindIII* sites. **Results and Discussion:** Using this cloning strategy, HC and LC was amplified, resulting in 330-bp products. The assembling by PCR of the HC and linker-LC resulted in a 720-bp product, corresponding to scFv predicted size. The scFv-cloning process required design of new primers and amplification strategies, since that the first scFv product obtained using a commercial kit resulted in a product containing an untimely stop codon. The specific primers allowed the correct cloning of light and heavy chain and the amplification with the linker sequence. The intimin-specific scFv cloning will allow the expression of recombinant antibody in large scale and with low cost to EPEC and EHEC diagnosis.

Supported by: FAPESP.

3.34 Reactivity of antibodies against the intimin conserved domain: detection of atypical enteropathogenic and Shiga toxin-producing *Escherichia coli*

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Introduction: Diarrheagenic *Escherichia coli* pathotypes are important agents of diarrheal diseases, especially in developing countries. Among these pathotypes, enteropathogenic *E. coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC) have in common the capacity to cause the attaching and effacing (A/E) lesion on intestinal mucosa. A/E lesion is triggered by the intimate bacterial adherence to enterocytes, which is mediated by intimin, an outer membrane adhesin. Several types and subtypes of intimin are defined by its variable C-terminal region. The conserved N-terminal region of intimin is immunogenic and, therefore, an excellent target for EPEC/EHEC diagnostic. **Objectives:** The aim of this study was to evaluate and compare the efficiency of different intimin antibodies for detection of atypical EPEC (*eae*+ EAF- BFP- *stx*-) and EHEC. **Methods:** The conserved region of intimin ($int_{388-667}$) was purified from His-pET28a-intimin. Rabbits, rats and mice were immunized with purified intimin to raise polyclonal and monoclonal antibodies. Antibodies were characterized and their reactivity was evaluated by immunoblotting against 72 aEPEC and 14 EHEC strains showing a wide range of serotypes and intimin subtypes. **Results and Discussion:** Rabbit anti-intimin polyclonal serum reacted with 96% and 100%, and rat anti-intimin polyclonal serum reacted with 91% and 79% of the aEPEC and EHEC strains, respectively. IgG2b monoclonal anti-intimin, which displayed a dissociation constant of 1.3×10^{-8} M, reacted with 74% and 57% of aEPEC and EHEC isolates, respectively. Rabbit polyclonal antisera demonstrated a better performance than the rat polyclonal antisera in the recognition of a wide spectrum of intimin subtypes in different aEPEC and EHEC serotypes. On the other hand, intimin specific IgG2b failed to detect some of these isolates. These results suggest that the manipulation by site-directed mutagenesis of the monoclonal antibody single chain variable fragment (ScFv) may improve its affinity and allow large-scale production of recombinant antibodies with low cost and desirable sensitivity and specificity.

Supported by: FAPESP.

3.35 Characterization of *Leptospira interrogans* Lsa31 protein binding adhesive matrix molecules

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Introduction: Leptospirosis is an infectious disease caused by pathogenic leptospires that are transmitted to humans through direct contact with infected animals, or through indirect contact with water or soil contaminated with the urine from infected animals. Leptospires enter the body by penetrating mucous membranes or broken skin and disseminate via the bloodstream to colonize the renal tubules of hosts. The colonization and survival of leptospires in the host require several types of surface-exposed proteins, such as lipoproteins, porins, adhesins and others. The protein chosen for this work is an outer membrane lipoprotein of *Leptospira interrogans* serovar Copenhageni that displays extracellular matrix binding properties. **Objectives:** The aim of this work was to study the capacity of Lsa31 protein to mediate attachment to extracellular matrix molecules (ECM). **Methods:** The recombinant 6xHis-tagged protein expressed in *Escherichia coli* was purified from the insoluble fraction by nickel affinity chromatography, and characterized by circular dichroism spectroscopy (CD). The capacity of recombinant purified protein to bind to ECM components was evaluated by ELISA and Western blotting-based methods. **Results and Discussion:** The structural integrity of Lsa31 recombinant purified protein was assessed by CD spectroscopy, which revealed a predominant β-sheet secondary structure, and showed a successful refolding process. Furthermore, recombinant Lsa31 was able to bind strongly to laminin, collagen type I, collagen type IV, cellular fibronectin, plasma fibronectin and matrigel. Lsa31 is probably a leptospire antigen involved in adherence to host tissues.

Supported by: CNPq and FAPESP.

3.36 Potency evaluation of rabies vaccine: comparison of three methods (NIH, ABT and SRD)

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Introduction: The NIH (National Institutes of Health) test is the most widely used method to evaluate the efficacy of inactivated rabies vaccines. Although the World Health Organization (WHO) recommends this test to determine the potency of each final lot of vaccine, there are concerns about the method, such as the high variability, the difficulty of obtaining valid results in individual tests, the large number of animals required and the long duration of the test (28 days). During production phases, antigen content may be determined by other methods, such as single radial diffusion (SRD), enzyme immunoassay (EIA) or the antibody binding test (ABT). WHO also encourages the support of the data generated by the NIH test by antigen content determination in order to ensure production consistency. **Objective:** In this study, the potency of ten lots of rabies vaccine, produced at Instituto Butantan, Brazil, were analyzed by three methods: NIH, ABT and SDR. **Methods:** For NIH test, groups of mice were vaccinated twice, 7 days apart, with serial dilutions of vaccine and a reference vaccine. Seven days after the last vaccination, the immunized animals were challenged with the challenge virus strain (CVS) of rabies virus. The mice were observed for 14 days and the median effective dose (ED_{50}) was determined based on the number of survivors. The potency of the vaccine was then determined by comparison of the ED_{50} obtained for the vaccine test and the reference. For ABT, serial dilutions of vaccine and a reference vaccine were incubated with a fixed amount of antirabies serum for 90 min at 37 °C. The antigen completely or partially neutralized by the antibodies was indicated by adding a determined concentration of CVS. After a new incubation for neutralization of the CVS, the mixtures were added to BHK21 cells in a 96-well microplate. After 22 h at 37 °C, cell cultures were fixed and stained with anti-rabies nucleocapsid fluorescent conjugate (BIO-RAD®) and examined in a fluorescence microscope. The potency of the vaccine was then determined by comparison of the test vaccine and the reference ED_{50}. For SRD, serial dilutions of vaccine and a reference vaccine, treated with detergent to release the glycoprotein antigen from the rabies virus particles, were distributed into wells in agarose gels containing antibody to glycoprotein. The potency of the vaccine was then determined by comparing the diameter of the precipitation zone produced by the vaccine test and the reference. **Results and Discussion:** Thirteen of 29 NIH tests performed were rejected by non linearity, parallelism or regression, by unsatisfactory values of the viral titer or the reference vaccine's DE_{50} or due to the large difference between replicate results. The ABT and SRD methods showed high repeatability, without test rejection. Although we did not observe a correlation between the NIH and SRD tests ($p<0.05$) and a weak correlation between NIH and ABT tests, correlation between the ABT and SRD methods was noted. The ABT and SRD tests were shown to be useful in assessing the stability and the consistency of rabies vaccine production.

Supported by: Fundação Butantan.

3.37 Production of recombinant anti-digoxin antibodies using phage display technology

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Introduction: Digoxin is a medicine with very narrow therapeutic range used for the treatment of cardiac disorders. The therapeutic level of digoxin is near the toxic level and cases of intoxication have been reported. The main therapy utilized in these cases is the intravenous administration of anti-digoxin polyclonal antibodies. These antibodies are not produced in Brazil and must be imported. Phage display technology allows the production of recombinant monoclonal antibodies in unlimited amounts after the selection of a clone that produces antibodies with high affinity and specificity for a determined antigen. This technology uses filamentous phages that are able to incorporate exogenous DNA and display the antibody on the surface. The antibody can be selected by binding to the antigen. The construction of an immunoglobulin library is the first step of phage display technology, and it can be prepared from immunized animals or hybridoma producing antibodies of interest. **Objectives:** To amplify the light and heavy chain as Fab fragment from an anti-digoxin hybridoma and construct a combinatorial library of anti-digoxin. **Methods:** The hybridoma producing monoclonal antibody anti-digoxin was generated at the Laboratory of Immunology (InCor/USP). The production of anti-digoxin was confirmed by ELISA using as antigen BSA conjugated to digoxin, previously prepared in our laboratory. Total RNA of the hybridoma was extracted and used for cDNA synthesis. Specific primers for the amplification of mouse immunoglobulin light and heavy chains were synthesized and they were used for the amplification of cDNA. **Results and Discussion:** The conjugate digoxin-BSA (antigen) was recognized by the anti-digoxin produced by the hybridoma and it will be used for subsequent steps of antibody clone selection. After total RNA extraction and RT-PCR, the primer sets tested to amplify the heavy and light chains of the antibody were analyzed by agarose gel electrophoresis. We observed the amplification of the heavy chain IgG1 with primers 2A; 2B; 2C; 3A; 3B+3C and 3D and light chain kappa with primers k1; k3 and k6. The light and heavy chain fragments will be cloned separately and at specific sites into pComb3XTT vector for the construction of a anti-digoxin combinatorial library. After the library construction, clones expressing anti-digoxin will be selected. The clones showing higher affinity will be expressed as soluble Fab fragment for the characterization of the antibody.

Supported by: CNPq.

3.38 Expression of antigens from *Leptospira interrogans in vivo* using attenuated *Salmonella* as carrier

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Introduction: Leptospirosis is an important zoonosis in the world; it is caused by spirochetes of the genus *Leptospira*. In Brazil, the disease represents high costs for the public health system, mainly in the rainy season and in places with lack of sanitation. In 2008, more than 3300 cases were recorded with 234 deaths. The initial symptoms are fever, headache, muscle pain, fatigue and nausea. A small number of patients develop the severe form of the disease, known as Weil's disease. There are vaccines based on bacterins licensed for human use in Cuba, China, Russia and Argentina. This type of vaccine is characterized by the bacterial LPS and is specific for strains used in the preparation. The ideal vaccine must not cause side effects, should protect against most species and produce a long term immunization. Besides, stimulation of both humoral and cellular immune response is desired against infections by most pathogens. Salmonellas have been largely used as heterologous antigen carriers, with advantages of possible administration by the oral route and capacity to elicit a more complete immune response compared to purified antigens. **Objectives:** The goal of our work was to identify antigens based on the genome of the *Leptospira interrogans* serovar Copenhageni and to test their potential to induce protective immunity, comparing the response to antigen presented as purified proteins or carried by recombinant salmonellas. We also tested the possibility of salmonella carrying two different antigens. We constructed a hybrid plasmid with two different genes being expressed simultaneously *in vivo*. **Methods:** The antigens LIC10191 and LIC10793 were cloned in vector pAEsox for protein purification in *E. coli* and for *in vivo* expression using salmonellas as carriers. The sox system can be activated *in vivo* by oxidative stress and *in vitro* by the bipyridyl paraquat. The recombinant proteins fused with a 6xHis tag were purified by metal affinity cromatography. The hybrid plasmid was constructed from available plasmids already sequenced. Balb/C mice were immunized with purified proteins and recombinant salmonellas for analysis of antibody titers by ELISA and western blot. Also, hamsters were immunized for challenge assay and measurement of immune protection. **Results and Discussion:** The recombinant proteins LIC10191 and LIC10793 were successfully purified from soluble fraction of bacterial culture. Vaccine strain *S. typhimurium* SL3261 carrying the pAEsox constructions were obtained. The expression of the antigens in the all recombinant salmonellas was tested *in vitro* before immunization assays. We observed that purified proteins induced higher titers of antibodies in mice while recombinant salmonellas raised low level of antibodies against leptospiral antigens. However, the recognition of purified proteins by the sera of immunized animals showed that both antigens were expressed *in vivo* by the recombinant salmonellas. However, the immunization with the antigens, purified proteins or recombinant salmonellas were not enough to protect hamsters during challenge with leptospira.

Supported by: CAPES, FAPESP and Fundação Butantan.

3.39 Construction of an unmarked recombinant BCG expressing a pertussis antigen by auxotrophic complementation: protection against *Bordetella pertussis* challenge in neonate mice

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Introduction: We have previously described an rBCG-Pertussis strain that confers protection against challenge with a lethal dose of *B. pertussis* in neonate and adult mice.[1,2] However, this rBCG strain contained an antibiotic resistance marker, which makes it unsuitable for use as a human vaccine. **Objectives:** 1. Construction of an unmarked BCG lysine auxotroph on a BCG Moreau background, complemented with a vector expressing the pertussis antigen and the lysA gene without the kanamycin resistance gene; 2. Investigation of the immune response and the protection induced in neonate-immunized mice against challenge with a lethal dose of *B. pertussis*. **Methods:** The auxotrophic BCG Moreau strain was obtained by transduction with specialized mycobacteriophages. The expression vector was constructed by inserting the lysA gene in the previously obtained expression vector pNL71-S1PT[1]. Production of IFN-γ and TNF-α by stimulated splenocytes of immunized mice was measured by ELISA or ELISPOT, respectively. Protection was determined by the percentage of surviving mice following ic challenge with a lethal dose of *B. pertussis*. **Results and Discussion:** 1. Initially, we constructed the unmarked BCG-ΔlysA strain using specialized mycobacteriophages. 2. The complementation vector was constructed, in which the deleted lysA gene was placed in tandem, following the genetically detoxified S1 pertussis toxin gene, both under control of the same promoter, pBlaF*, organized as a synthetic operon; the antibiotic resistance marker was then eliminated. 3. Transformation of the BCG-ΔlysA with this vector allows growth of the bacteria in media without addition of lysine. 4. The complemented auxotrophic rBCG-ΔlysA-S1PT-lysA$^+$ strain maintains the same characteristics of the original rBCG-pNL71S1PT strain, such as the antigen expression level. 5. There was nduction of cellular immune response. 6. Protection against ic challenge with a lethal dose of *B. pertussis* in neonatal immunized mice was demonstrated. Our results show that the unmarked complemented auxotroph rBCG-Pertussis maintains the stable expression of the pertussis antigen, and all the immunological characteristics of the original rBCG-Pertussis strain. This strain is now suitable for evaluation in clinical trials, and is undergoing production under Good Manufacturing Procedures.

Supported by: FAPESP, CNPq and Fundação Butantan.

3.40 Cloning and expression of groEL protein of *Leptospira interrogans*

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Introduction: Leptospirosis is a zoonotic disease caused by infection with pathogenic *Leptospira* spp. The genus *Leptospira* has been classified into at least 12 pathogenic and 4 saprophytic species, with more than 250 serologic variants (serovars). The wide distribution of *Leptospira* spp results from their ability to colonize the renal tubules of mammalian hosts, including humans, wildlife, and many domesticated animals. Transmission to humans involves either direct or indirect contact with the urine from chronically infected animals. **Objectives:** The aim of this project was the cloning and expression of the groEL protein encoded by the gene LIC11335 identified in *L. interrogans* serovar Copenhageni genome. **Methods:** The gene was amplified from genomic DNA by PCR and cloned into the expression vector pAE. Recombinant clones were investigated for the presence of the insert by restriction analysis and DNA sequencing. The pAE vector containing the DNA insert in the correct reading frame was used to transform *E.coli* BL21- C43, and expression was induced with IPTG. **Results and Discussion:** The recombinant protein was expressed in both soluble and insoluble forms and the expected protein band of 60 kDa was observed by 12% SDS-Page. Purification of the recombinant protein is currently underway.

Supported by: CNPq and FAPESP.

3.41 Development of a whole, inactivated influenza (H5N1) vaccine

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Introduction: Effective vaccines against influenza A/H5N1 virus are considered to be the first defense to protect populations in advance of an influenza pandemic. Increasing efforts were made to develop a production process that improves vaccine productivity and shortens the production time. Since 2008, Butantan Institute has produced the split formalin-inactived vaccine against H5N1. The vaccine was produced using the non pathogenic strain, NIBRG-14 (cod. 05/160, NIBISC - National Institute for Biological Standards and Control, Blanche Lane South Mimms Potters Bar Hertfordshire ENG 3QG United Kingdom), injected into embryonated chicken eggs as a substrate for influenza propagation. The virus was purified in a sequence of downstream processing steps comprising previous clarification, purification, split and inactivation. **Objectives:** Here, were report the development and optimization of downstream processing methods for the purification of H5N1 whole vaccine. **Methods**: The steps studied were inactivation and gel filtration. The formol concentration for inactivating the virus ranged from 0.1% to 0.2%. The gel filtration was optimized using 4 different columns: XK26/40, XK50/30, BPG140/500 and BPG200/750. Sample volume ranged from 5% to 7% of the column volume and the flow rate was fixed at 30 cm/h (160 mL/min). Collected fractions were tested by direct hemagglutinagion using guinea pig erythrocytes and optical density (OD280). Final product was analyzed for inactivation, total protein concentration, hemagglutinin and ovalbumim concentration, purity by SDS PAGE, and residual formol. **Results and Discussion:** Both inactivation procedures were effective. All columns showed the same chromatogram. Compared with the split inactivated vaccine, the whole virus vaccine showed better purity with lower concentration of ovalbumim, formol and the yield of dose per egg was two to three times more. The results obtained are promising, opening the possibility to scale up the gel filtration step for industrial production.

Supported by: Fundação Butantan.

3.42 *Salmonella enterica* serovar Typhimurium flagellin FliCi production and purification

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Introduction: Flagellin is the most abundant protein in a bacterium's flagellar structure. Each flagellum has around 20,000 subunits of flagellin (50 kDa) in its propeller. It is known that this protein has an adjuvant potential and there is evidence that flagellin acts as an immunomodulator. As a consequence, the production and purification of flagellin is interesting for vaccine development companies. **Objectives:** The main purpose of this work was to produce and purify native flagellin from *Salmonella enterica* serotype Typhimurium in order to enable a simple scale-up procedure. **Methods:** The bacteria were cultivated in two types of culture media: LB medium (tryptone and yeast extract) and Soytone and yeast extract medium, in a shaker or in a 3-L BioFlo 3000 Bioreactor (New Brunswick Scientifc). Culture conditions such as temperature and agitation were controlled and maintained at desired levels. Purification process of the supernatant was based on tangential flow ultra filtration. Cells were submitted to homogenization prior to the ultra filtration steps. Analysis assays such as SDS-PAGE, Western blotting and Lowry′s total protein quantification were performed for each step of production and purification in order to follow the efficiency of the process. **Results and Discussion:** According to the data obtained, flagellin production is probably associated with bacterial growth. Cells were able to grow in the culture media free of animal components, which is important for vaccine development. During cultivation, long pieces of the flagellar filament are believed to be released into the supernatant. Therefore, small filter cuts such as 10 kDa or 30 kDa are used as pre-purification steps to reduce the working volume, but they are not vital to the purification itself. The material was also submitted to 700 kDa and 300 kDa concentration steps. It was observed that the bigger cut had almost no influence on purification efficiency, and it actually resulted in a significant loss of product; as a result, it should be ignored in future process development. The cellular material, which was homogenized in previous experiments, contained significant amounts of flagellin. On the other hand, a more vigorous agitation during cultivation was used to release more flagellin filaments in the supernatant, which allows a simple scale-up procedure to be followed.

Supported by: FAPESP and Fundação Butantan.

3.43 Production and immunochemical characterization of anti-surface antigen of hepatitis B virus (HBsAg) monoclonal antibodies to be used for in-process control of vaccine production

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Introduction: Hepatitis B virus (HBV) is one of the world's most widespread infectious agents and causes millions of infections each year. Between 500,000 and 1.2 million people die each year from chronic infection-related cirrhosis, hepatocellular carcinoma (HCC) or acute hepatitis B. Hepatitis B vaccine provides protection against infection and its complications including liver cirrhosis and HCC. In response to the demand elicited by the Ministry of Health immunization program, the Butantan Institute started in 1996 the production of recombinant vaccine against HBV, using *Hansenula polimorpha* yeast as expression system of the surface antigen of HBV (HBsAg). **Objective**: To produce a monoclonal antibody against HBsAg to develop a simple, specific and reproducible assay for the in-process control of vaccine production. **Methods**: Hybridomas secreting HBsAg were obtained by fusing murine myeloma cell line (SP2O) to spleen cells of BALB/c mice immunized with recombinant hepatitis B vaccine (purified by ultracentrifugation in cesium chloride gradient) produced by Butantan Institute. They were selected in HAT-containing medium and cloned under limiting dilution conditions. Supernatants from growing hybrids were then screened by ELISA using recombinant HBsAg as coating; the plates were blocked and incubated with hybridoma supernatants. Bound antibodies were detected using anti-mouse IgG-peroxidase conjugate and OPD plus H_2O_2 as enzyme substrates. Clones were considered positive when O.D. was higher than 0.5. The heavy chain isotype of MoAbs was determined by ELISA, using monoclonal antibodies against different mouse immunoglobulin classes and subclasses. The specificity of the MoAb obtained was evaluated by Western blotting. For this, 10 µg HBsAg was resolved by 12% SDS-PAGE under reducing or non-reducing conditions and transferred to nitrocellulose membrane. After blocking unoccupied sites, the membrane was incubated with supernatant of MoAb, followed by rat anti-mouse-IgG peroxidase conjugate. **Results and Discussion**: A total of 2×10^6 lymphocytes were fused with 1×10^6 SP2O giving rise to thirteen clones, from which three hybridomas were positive by ELISA. Two stable clones were obtained and cloned twice. They are of IgG isotype (IgG1 and IgG2a). When analyzed by Western blotting, both monoclonal antibodies recognized non-reduced (dimeric form, 46 kDa) and reduced (monomeric form, 23 kDa) HBsAg. In conclusion, two IgG monoclonal antibodies recognizing linear and conformational epitopes were obtained. These MoAbs may be produced in large scale to ensure sufficient availability and batch-to-batch consistency essential to the in-process control of anti-HBV vaccine.

Supported by: Fundação Butantan.

3.44 Effect of aflatoxin on the immunological system of cut chicken

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Introduction: Aflatoxins are metabolites produced mainly by the fungus Aspergillus flavus and A. parasiticus (Leeson, 1995). The possible existence of aflatoxins in some foods of plant and animal origin, which are substances that are highly toxic and carcinogenetic for humans and animals, has led to an intense investigation in the last years, with regard to their prevention and detection in foods (LOVER, 1999). Several aflatoxins can be found in the food products, the most important and better known being B1, B2, G1, G2. The aflatoxin B1 is the most known, being considered more important in terms of occurrence and toxicity. The aflatoxins in cut chickens, can determine great economical losses causing growth retardation and poor absorption of nutrients due to intestinal lesions, predisposing the animals to infections, besides causing considerable mortality. **Objective**: The objective of this study was to evaluate the immune response of cut chickens fed with contaminated feed with aflatoxin and vaccinated against Newcastle's disease. It is besides, to test a kit ELISA, with antibodies monoclonals, to detect and to quantify the aflatoxin levels in the serum of those birds. **Methods**: Cut chickens of the lineage "Ross," with 41 days of age, fed since the first day with ration contaminated with aflatoxin, and vaccinated up to 21 days of age against the Newcastle's disease (ND) with the sample La Sota through drinking water. The samples were obtained from 5 treatments each with 24 cut chickens, with the following characteristics: treatment 1 - Chickens receiving feed with 39 ppb aflatoxin; treatments 2, 3 and 4 - Chickens receiving feed with unknown levels of aflatoxin; treatment 5 - chickens receiving feed without aflatoxin. Response to Newcastle vaccine was determined by inhibition of the hemaglutination caused by Newcastle's disease (IH/ND); the ELISA technique was used for detection and quantification of the aflatoxin B1; and histological examination of the liver and of the bursa of fabricius was carried out to evaluate the lesions cause by aflatoxin. **Results and Discussion**: The IH/ND test and ELISA showed that the group of chickens given contaminated feed with aflatoxin showed lower levels of antibodies against ND virus and higher serum levels of aflatoxin, when compared with the group that was given feed not contaminated with aflatoxin. In the histological analysis of the liver and of the bursa of Fabricius, no lesions were found compatible with the role of aflatoxin. This study showed that based on the test of IH/VDNC, aflatoxin affected the immunological system of the birds negatively. Moreover, using ELISA it was possible to detect and to quantify aflatoxin B1 in serum.

Supported by: CNPq.

3.45 $CCID_{50}$ test to determine the potency of rotavirus

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Introduction: Rotavirus is the most common cause of severe diarrhea in children around the world. Every year, rotavirus is associated with 600,000 deaths worldwide among children younger than five years of age. The development of rotavirus vaccines and the introduction of these into global immunization programs have been high priorities for many international agencies, including WHO and the Global Alliance for Vaccines and Immunizations. Rotavirus vaccine is produced with a human attenuated virus, bovine-human or rhesus-human reassortant virus. A new rotavirus vaccine in development at Instituto Butantan contains four principal genotypes that occur in the world (G1, G2, G3 and G4) and G9 present in Brazil. **Objectives**: The aim of this study was to assess two variations of the methodology used to determine rotavirus potency, the $CCID_{50}$ (50% cell culture infection dose). **Methods**: a) Virus inoculation in MA-104 cell culture maintained at 37°C in 96- well microplates for 24 h; b) MA-104 cells and rotavirus incubated at 37°C for 1 h and then placed in a microplate. After infection, the microplates were maintained in Minimum Essential Medium (MEM) with 10% calf serum and 1 µg trypsin/ml at 37°C in 5% CO_2. The cultures were observed by microscopy to determine the cytopathic effects during three days, and the viral titer was calculated using the Spearm Karber test. **Results and Discussion**: The results obtained in 14 samples of rotavirus (G2, G3 and G9) utilizing the virus inoculation in 24-h culture were of $10^{1.8}$ to $10^{4.6}$/ml. When the virus was injected at the same time as the cells, the potency achieved was $10^{1.8}$ to $10^{3.8}$ $CCID_{50}$/ml. The results obtained showed that this test was more sensitive when the virus inoculation was performed in MA-104 cells of 24 h.

Supported by: Fundação Butantan.

3.46 *Slc11a1* gene modulates gene expression on arthritic joints of mice submitted to pristane-induced arthritis

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Introduction: AIRmax and AIRmin mice homozygous for *Slc11a1 R* and *S* allele were obtained through genotype-assisted crosses and submitted to pristane-induced arthritis (PIA). AIRmax^{SS} were more susceptible than the other sublines and the presence of *S* allele also increased arthritis severity. **Objective:** The objective of this work was to identify genes in acute inflammatory reaction loci (AIR QTL) that interact with *Slc11a1* alleles to modulate experimental arthritis. **Methods:** Mice received two i.p. injections of 0.5 ml pristane with a 60-day interval, and mRNA from the paws was isolated at day 180. Global gene expression analysis was performed on Codelink bioarrays (36k genes) using RNA pools (n=4) of arthritic or control joints from AIRmax^{RR}, AIRmax^{SS}, AIRmin^{RR} and AIRmin^{SS} mice. Differentially-expressed genes were detected using the Codelink array expression software and the over-represented biological themes were analyzed using the EASE program. qPCR was used to determine gene expression of inflammatory cytokines. In parallel, genome wide association studies were performed to determine arthritis QTL in F2 (AIRmax x AIRmin) population. **Results and discussion:** Highly significant (LOD > 4) arthritis QTL on chromosome 5 and several suggestive ones on chromosomes 1, 7, 8, 10, 17, 19 and X were detected. Global gene expression analysis demonstrated 95, 255, 37 and 27 up- and 26, 270, 48 and 15 down-regulated genes in AIRmax^{RR}, AIRmax^{SS}, AIRmin^{RR} and AIRmin^{SS} mice, respectively. Significant (P<0.001) over-represented genes related to inflammatory response and chemotaxis were observed in AIRmax^{RR} and AIRmax^{SS} mice. Up-regulation of *Cxcl1, Cxcl9, Cxcl5, Cxcl13* genes on chromosome 5 and *Ccl2, Ccl3, Ccl7 and Ccl12* genes on chromosome 11 were observed in AIRmax^{SS}. qPCR showed distinct expression for *Il1b, Tnf, Il6, Il8rb and Il10* genes. These results revealed a significant arthritis QTL on chromosome 5 with a gene expression profile that predisposes AIRmax^{SS} mice to PIA.

Supported by: FAPESP and CNPq.

3.47 Cloning of the highly expressed proteolipid protein 2 found in astrocytomas to produce polyclonal antibody in order to study its correlation with tumor development

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Introduction: Astrocytomas are the most common glioma, a tumor arising from star-shaped glial cells called astrocytes. Differential expression of 10,000 genes between astrocytomas of different degrees of malignancy and normal tissue was carried out by microarray analysis. We found that the proteolipid protein 2, also known as PLP2 or A4, was highly expressed in astrocytomas. **Objectives:** The aim of this work was to clone and express PLP2 to produce specific antibodies to be used in immunohistochemistry analyses in order to identify this protein in astrocytomas of different degrees of malignancy and to find a correlation of this protein in tumor development and study its distribution in the tumor tissue. **Methods:** The sequence of PLP2 was isolated from astrocytomas by RT-PCR, and its external loops were cloned into pSMT3 vector in fusion with sumo protein and 6 histidines for further purification. The production of PLP2 was accomplished in *Escherichia coli* (C43 (DE3)). The PLP2 external loops were purified and their size, integrity and purity were evaluated by SDS-PAGE. This protein was used to raise antibodies in mouse and its specificity was evaluated by Western blotting. **Results and Discussion:** The protein sequence was analyzed with TMHMM Server v. 2.0 program which revealed that this protein possesses 3 regions outside the membrane. The sequence corresponding to these regions was united, cloned and successfully expressed in *E. coli* (C43 (DE3). Polyclonal antibody against recombinant external loops of PLP2 with high titer was obtained in mouse. These antibodies were able to specifically recognize recombinant PLP2 and native PLP2 present in extracts of the cell lines A172 and U87 in Western blot analysis. Studies have shown that the activation of PLP2 after its association with the CCR1 receptor promotes cell migration, suggesting that this protein may have some important role in the process of malignancy. In this work we expressed a small fragment of PLP2 and produced antibodies against this fragment. In order to do so, an extensive study was conducted to choose the most exposed regions of this protein. Our next step will be to evaluate the specificity of this antibody in immunohistochemical tests, so these antibodies might be used as a potential marker for diagnosis and monitoring the status and progression of this disease.

Supported by: FAPESP and CAPES.

3.48 Applicability of tetanus antigen conjugated to derivatives of monomethoxypolyethylene glycol

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Introduction: The schemes of equine immunization to obtain anti-tetanus serum are based on inoculations that stimulate the immunological system in order to achieve high specific antibodies titers. The main difficulties of such production are due to the low antigen immunogenicity and/or to the toxic activity that may lead to local or systemic reactions. **Objectives:** The purpose of this study was to evaluate the monomethoxypolyethylene glycol succinimidyl propionic acid as adjuvant and inhibitor of tetanus toxin neurotoxic activity since this polymer is inert, nontoxic and non-immunogenic. **Methods:** The SPA-mPEG 5 and 20 kDa conjugated to tetanus toxin adsorbed or not by $Al(OH)_3$ gel was analyzed. The pegylation degree was determined by colorimetry using the trinitrobenzenesulfonic acid method. The sample toxicity was evaluated by DL_{50} determination disclosing that the conjugation of tetanus toxin to SPA-mPEG 5 and 20 kDa inhibited the neurotoxic activity of the toxin adsorbed or not by $Al(OH)_3$ gel. The influence of the subcutaneous and intramuscular inoculation route was evaluated. Thirty horses were submitted to a selective scheme of immunization, and eighteen animals were chosen, which were divided into different groups to be immunized with the antigens: tetanus toxin conjugated to SPA-mPEG 5 kDa and SPA-mPEG 5 kDa(2X); and tetanus toxin adsorbed or not by $Al(OH)_3$ gel. **Results and Discussion:** It was observed that the subcutaneous inoculation route was more effective in inducing the response to the toxin treated with SPA-mPEG, while the adjuvant effect of $Al(OH)_3$ gel was demonstrated by the intramuscular method of application. The sera of immunized horses were individually tested for the concentration of antitetanus antibodies by the ToBI test, and the results obtained enabled the evaluation of the immune response development during the immunizations. These sera were also analyzed using the methods of immunodiffusion, electrophoresis and immunoblotting, and the last one is indicative, under the conditions of this study, of a probable antigenic superiority of fluid tetanus toxin in relation to the adjuvants used. The SPA-mPEG conjugation proved to be effective for anti-tetanus human therapeutic serum production.

Supported by: Fundação Butantan.

3.49 Process validation of bulk diphtheria anatoxin

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Introduction: Diphtheria anatoxin bulk is an intermediate product that after purification is a component of DTP (diphtheria, tetanus, pertussis), DTP + hib (DTP and *Haemophilus influenzae* type b), dT (diphtheria - tetanus, adult use) and DT (diphtheria - tetanus, child use) vaccines and is also used as antigen to immunize horses for hyperimmune diphtheria serum production. Instituto Butantan produces diphtheria toxin by growing toxigenic *Corynebacterium diphtheriae* Park Williams 8 strain, in a 500-L bioreactor containing N.Z. amine culture medium. Diphtheria toxin is separated from biomass by tangential flow filtration (0.22 µm), concentrated by molecular ultrafiltration (30 kDa), sterile filtered, detoxified with formaldehyde and heated to convert toxin into anatoxin. Good Manufacturing Practices require the process validation of the production. Process validation is the means of ensuring and providing documentary evidence that the process, within specified design parameters, is capable of consistently producing a product of required quality. Installation qualification (IQ), operational qualification (OQ) and performance qualification (PQ) for equipment and validation of analytical assay and facility systems such as air, pure water and pure steam are pre-set requirements for the process validation. **Objective:** To validate the diphtheria anatoxin bulk production process to ensure that it is reliable, accurate and effective. **Methods:** To evaluate the manufacturing process and to collect data to validate the production process of diphtheria anatoxin bulk, three consecutives batches were analyzed. During the production of diphtheria anatoxin bulk, process controls were perfomed, such as fermentation parameter (pH, air flow, dissolved oxygen, temperature, pressure), purity, pH, flocculation limit (Lf/mL), protein nitrogen, antigenic purity (Lf/mgPN) and specific toxicity. In order to analyze the process, twelve samples were collected and three of them were evaluated by the Bioburden test. **Results and Discussion:** The flocculation limit (Lf/mL) of diphteria toxin in the *Corynebacterium diphtheriae* culture were 150 Lf/mL, 60 Lf/mL and 90 Lf/mL in the 3 batches (minimum requirement $\geq$ 40 Lf/mL). The yield average after concentration was 94.7% compared to the diphtheria toxin titer obtained from the production culture. The Bioburden average before sterile filtration of concentrated diphtheria toxin with formaldehyde added was 42 CFU/50 mL and after sterile filtration, it was 0 UFC/50 mL. After detoxification all baths analyzed showed no toxicity in the specific toxicity test. All control tests were conducted according to the pre-set criteria, showing the consistency of the production process according to the national requirements and WHO recommendations, allowing the validation of diphtheria anatoxin bulk production.

Supported by: Fundação Butantan.

3.50 Evaluation of the role of *SLC11A1* (formerly *NRAMP1*) gene in the activation of LPS- stimulated macrophages

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Introduction: Mouse lines were genetically selected for maximal and minimal acute inflammatory response (AIR). AIRmax mice are more resistant than AIRmin when infected with *S. typhimurium.* Slc11a1 (formerly Nramp1) protein is involved in resistance to this infection; it interferes with macrophage activation, oxidative burst, inflammatory cytokine production and nitric oxide (NO) secretion. Mouse lines homozygous for resistance and susceptibility *Slc11a1* gene alleles (AIRmax^{RR}, AIRmax^{SS}, AIRmin^{RR} and AIRmin^{SS}) were produced. AIRmin^{RR} and AIRmin^{SS} are highly resistant to LPS inoculation, whereas AIRmax^{RR} and AIRmax^{SS} are highly susceptible (LD50 200 and 326 ug; 23 and 46 ug, respectively). **Objective:** To investigate the mechanisms of activation of resident or thioglycolate (TG)-induced peritoneal macrophages (Mϕ) stimulated in vitro with LPS. **Methods:** Mice were inoculated ip with TG or PBS. After 48 h, the peritoneal cells were collected and placed in culture. After 2 h, the non adherent cells were discarded, and the adherent Mϕ were used in all experiments. **Results and discussion:** The total resident cells in peritoneal cavity of AIRmax^{RR}, AIRmin^{RR} and AIRmax^{SS} were similar but approximately 2.5-fold higher than in AIRmin^{SS} mice. TG promotes cell migration in all lines (up to 10.7_x10^6 cells/ml). LPS induced the expression of NO in all lines, especially in TG-induced Mϕ from AIRmax^{RR} (14.9 μM+/-1.03). Total RNA was extracted from cell cultures. Real-time qPCR analysis detected the up regulation by LPS of *Tnf* and *Il6 genes* in resident Mϕ from all lines. *Trem1* and *Dap12* genes were highly expressed in AIRmax^{RR} and AIRmax^{SS} compared to AIRmin^{RR} or AIRmin^{SS} Mϕ (49- to 105- and 61- to 116-fold, respectively). *Ifng* and *Trem2* genes were not detected. In this model, the genetic background relevant to acute inflammatory response regulation is more important than the *Slc11a1 gene* polymorphism in the control of Mϕ activation induced by LPS.

Supported by: CAPES and FAPESP.

3.51 Media hold test for bulk diphtheria anatoxin production

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Introduction: Diphtheria toxin is produced by *Corynebacterium diphtheriae* fermentation, released into culture medium, recovered by tangential flow filtration and concentrated in an enclosed system. Formaldehyde is added to concentrated diphtheria toxin for detoxification, and then it is submitted to a sterile filtration and incubated at 36 ºC ± 1ºC for 30 days. The final product loses toxicity, but immunogenicity remains and is named diphtheria anatoxin bulk. The production process is carried out in a cleanroom (grade D - ISO 8) where the environment is monitored by air sampling in order to determine the quantities of particles and viable microorganisms. The facility systems (air, pure water and pure steam) are validated and the equipment used in production process are qualified (installation qualification - IQ, operational qualification - OQ and performance qualification - PQ). In the aseptic processing of immunologicals, the product quality is ensured by quality control tests as well as by process validation. The media hold test is an instrument of process validation that demonstrates product safety, which is the focus of regulatory requirements and official inspections. Media hold test consists in a production process simulation where the product is replaced by the culture medium (tryptcase soy broth - TSB) which is exposed at the same risk factor as the product. **Objectives:** To perform the media hold test in order to ensure that the process used in the diphtheria anatoxin bulk production is able to yield a product without microbiological contamination. **Methods:** Three consecutive media hold tests were performed with TSB instead of the culture media as well as the strain of *C. diphtheriae* which are usually used in the production. The simulation of inoculum preparation was done 14 days before the fermentor inoculation and TSB media was tested for fertility and sterility. In relation to collecting samples after fermentation, simulating the culture in the fermentor, we introduced a sterile, closed and disposable system for sampling. To monitor the microbiological population in the critical steps of production, 11 samples were submitted to sterility test and 2 samples were performed by the Bioburden test. The incubation for sterility test was 14 days at 20 to 25 °C and 30 to 35 °C. For the Bioburden test, it was 5 days at 30 to 35 °C. The operator and the environmental cleanroom were microbiologically monitored during the operations. **Results and Discussion:** The average of the Bioburden test before filter sterilizing simulation of concentrated diphtheria toxin was 4 CFU/mL, and after filtration all samples passed the sterility test. The results showed that there is no contamination during the aseptic process. An accurate process design in an enclosed system and the introduction of technological innovation as part of a continuous improvement of the process minimize the inherent risk of contamination. Application of the media hold test for diphtheria anatoxin bulk production demonstrated that the process is safe and effective.

Supported by: Fundação Butantan.

3.52 Immunomodulatory cytokines are released by cells of mice injected with crotoxin isolated from *Crotalus durissus terrificus* venom

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Introduction: *Crotalus durissus terrificus* venom and its main fraction (crotoxin-CTX) have the ability to suppress the immune system. Anti-inflammatory cytokines such as IL-10 and TGF-β have the ability to down-modulate the development and maintenance of the adaptive immune response to several antigens. Furthermore, it has been reported that indoleamine 2,3-dioxygenase (IDO), the tryptophan degrading enzyme, is important for immune regulation. Dendritic cells (DCs) expressing functional IDO can inhibit T cells by depleting them of essential tryptophan and/or by producing toxic metabolites. **Objective**: We evaluated the production of IL-10 and TGF-β as well as the inflammatory cytokine IL-12 in cells from mice that received or not CTX. The tryptophan content in cells from these mice was also studied. Furthermore, the effect of the CTX on the cytokine secretion by DCs stimulated with LPS was analyzed. **Methods:** BALB/c mice were immunized with HSA (100 μg/animal) or HSA+CTX (5 μg/ animal) in CFA, and after 7 days, lymph node cell suspensions were prepared and cultured with ConA for 48 h. Cells from these mice were also lysed and the tryptophan content analyzed in an HPLC system. In another experiment, DCs were incubated *in vitro* with LPS (1 μg/mL), CTX (10 μg/mL), LPS+CTX (1+10 μg/mL) for 18 h. In all experiments, cytokine production was analyzed in the supernatants by ELISA. **Results and Discussion:** Higher secretion of IL-10 and TGF-β but lower of IL-12 were verified in supernatants of cells from HSA-CTX immunized mice compared with those obtained in cells from HSA-immunized mice. The tryptophan content was also lower in cells from HSA-CTX immunized mice compared with those obtained in cells from HSA-immunized or non-immunized mice. The results suggest that CTX promotes an anti-inflammatory effect on the immune system inducing the secretion of modulatory cytokines. The analyses of the tryptophan content also suggest that CTX may induce higher IDO expression; however, this hypothesis needs to be further investigated.

Supported by: FAPESP, CNPq and CAPES.

3.53 Murine experimental asthma induced by *Schistosoma mansoni-4* protein

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Introduction: Schistosomiasis is an important parasitic disease, caused by trematode worms of the genus *Schistosoma*, affecting more than 200 million people worldwide, with a further 650 million individuals living at risk of infection. Screening the Schistosoma transcriptome for genes with functions that could indicate surface exposure and interaction with the host immune system, a family of venom allergen-like proteins (VAL) were identified raising the question of what benefits would there be to the parasite in amplifying allergic and other inflammatory responses. It is a large family of genes composed of 28 members with different expression profiles, and probably playing different functions on the parasite host interface. For example, SmVAL-4 is released by cercaria during the invasion of the definitive host, and SmVAL-26 is released by the miracidium and is associated with the invasion of the intermediate host, while SmVAL-5 is released by the egg and could be involved in the development of the disease. There are important and similar aspects between the immune responses in allergic asthma and those observed in response to helminthic infection. Asthma results from an intrapulmonary allergen-driven Th2 response, and is characterized by intermittent airway obstruction, airway hyperreactivity (AHR), and airway inflammation. **Objectives:** In the present work, we used the murine model for asthma, to test whether recombinant SmVAL-4, 5 and 26 expressed in *P. pastoris*, were capable of inducing allergic airway inflammation. **Methods**: BALB/c mice were immunized on days 0, 7 and 14 with 10 µg Val-4 Val-5 or Val-22, using 1.6 mg alum as adjuvant, and challenged on days 21 and 28 with intranasal (i.n.) administration of the proteins (10 µg) as challenge. The control group was only challenged with the different proteins. The experiment was performed on day 29. **Results and Discussion:** The BAL of BALB/c mice immunized and challenged with Val-4 displayed an increase in the number of eosinophils (7 x 10^5/mL) as compared to the control or when compared with mice immunized and challenged with Val-5 (0.79 x 10^5/mL) or Val-22 (0.182 x 10^5/mL). The production of IL-5 (664.4 ± 132 pg/mL) was also increased in animals immunized and challenged with VAL-4 as compared with VAL-5 (200 ± 56 pg/mL) or VAL-22 (39.9 ± 30 pg/mL). IFN-γ and IL-10 was not detected in BAL. Data revealed that only rSmVAL-4 was able to induce a response that resembles allergic airway inflammation, as demonstrated by the increased number of total cells, mainly eosinophils and macrophages in BAL when compared with control mice. This is in agreement with previous studies that have suggested that cercariae of *S. mansoni* (the expression of SmVAL-4 seems to be restricted to the cercariae stage) produces Th2 responses and a mast cell-triggering factor that can release histamine from rat peritoneal mast cells *in vitro*. SmVAL-4 could be the factor or one of the factors contributing to this effect.

Supported by: FAPESP and Fundação Butantan.

3.54 Retrospective process validation of bulk tetanus anatoxin production

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Introduction: Tetanus anatoxin bulk is an intermediate product that after purification is used for formulation of DTP (diphtheria, tetanus, pertussis), DTP + hib (DTP and *Haemophilus influenzae* type b), dT (diphtheria –tetanus adult use) and DT (diphtheria - tetanus child use) vaccines and also used as antigen to immunize horses for hyperimmune tetanus serum production. Tetanus toxin is produced by the growth of *Clostridium tetani* (Harvard Caracas strain) inoculated in two fermentors (360 L and 420 L, respectively) containing IB culture medium. After 88 h of culture, tetanus toxin is separated from biomass by tangential flow filtration (0.22 µm), concentrated by molecular ultrafiltration (30 kDa) and formaldehyde added for detoxification process. Following formaldehyde addition, the product is sterile filtered and incubated at 36 °C ± 1 °C to detoxify the tetanus toxin and transform it into tetanus anatoxin. The aim of production process validation of tetanus anatoxin bulk was to check if the equipment and services involved in the production process were correctly installed, well documented and properly working as well as if the production is done in a repetitive way according to predefined parameters. The validation of facility systems such as air, pure water and pure steam, the installation qualification (IQ), the operational qualification (OQ) and the performance qualification (PQ) for equipment and systems and the analytical assay validation are pre-set requirements for the process validation. **Objective:** To collect retrospective data providing documented evidence to validate the production process of tetanus anatoxin bulk, ensuring consistency, safety and quality of the product. **Methods:** A retrospective validation was done using three batches of tetanus anatoxin bulk. During the production of tetanus anatoxin bulk, parameters such as pH, sterility, temperature, air flow, vibration of culture, pressure, flocculation limit (Lf/mL), protein nitrogen, antigenic purity (Lf/mgPN) and specific toxicity, were checked and analyzed. In order to analyze these parameters, seven samples were collected for each batch during the process. **Results and Discussion:** The Lf/mL of the final step of tetanus anatoxin bulk production was 800 Lf/mL, 750 Lf/mL and 820 Lf/mL, respectively. The average antigenic purity was 978.28 ± 179.28 Lf/mgPN in the three analyzed batches. After detoxification process, all the batches analyzed showed a non-toxic result in the specific toxicity test. All results of control tests performed during the fermentation and toxin production were according to the pre-defined criteria, showing the consistence of the production process. It was concluded that tetanus anatoxin bulk production process was validated showing production consistency within its pre-determined specifications and quality characteristics according to the national requirements and WHO recommendations.

Supported by: Fundação Butantan.

3.55 Pristane-induced arthritis is influenced by number of doses and fails to develop when subcutaneous route is used

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Introduction: Pristane-induced arthritis (PIA) in high (HIII) and low (LIII) antibody producer mice selected for *Salmonella* flagellar antigen responses are characterized by extreme susceptibility divergence. HIII mice are completely resistant, while LIII mice show a 100% incidence of severe lesions. Previous data suggest that the differences between HIII and LIII mice are expressed in the early phase of induction, influencing the late-phase of arthritis development. The classical induction protocol is based on two pristane injections; however, the role of the second dose is unknown. On the other hand, PIA in rats can be induced by a single s.c. injection. This route of administration has not been described for mice. **Objectives**: The aim of this study was to evaluate whether PIA could be efficiently induced by a single pristane injection and also whether the route of injection would influence arthritis induction. **Methods**: HIII and LIII mice were i.p. injected with either one or two 0.5 mL doses of pristane. In another experiment, mice were subcutaneously injected with 0.1 mL pristane, and a control group was injected with two i.p. doses of 0.5 mL pristane with a 60-day interval. Arthritis was evaluated for 160 days by visual scoring of the hind paws. **Results and Discussion**: PIA incidence in mice injected with either one or two i.p. doses reached 100% on day 105. PIA development was delayed in single-injected (max. score on day 156) when compared to twice-injected (max. score on day 119). However, there was no difference in severity for LIII mice on day 156. LIII mice injected with s.c. pristane did not develop arthritis, while i.p. injected control mice developed severe autoimmune arthritis. PIA incidence and severity were not significantly altered in single-injected mice. However, full arthritis onset was delayed when compared to double-injected animals. Moreover, PIA could not be induced using s.c. route, which suggests that the underlying immunopathogenetic mechanisms differ from those of PIA in rats. We conclude that the two-dose i.p. protocol is the most appropriate for PIA induction in mice.

Supported by: FAPESP and CNPq.

3.56 Methodology to determine KDO in the *Bordetella pertussis* LPS, source of a new influenza vaccine adjuvant

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Introduction: The use of an appropriate adjuvant to produce influenza vaccines is crucial to obtain increased immunogenicity at reduced doses and supply those vaccines to developing countries with large populations. In order to contribute to this huge problem, Instituto Butantan has produced a new adjuvant obtained from *Bordetella pertussis* (*Bp*MPLA) by acid hydrolysis of its LPS. The evaluation of its adjuvant capacity showed good results for influenza A vaccines, enabling a 4-fold safety dose reduction of the antigen. **Objective**: The aim of this study was to standardize the measurement of 2-keto-3-deoxyoctonate (KDO) in *Bp*LPS during *Bp*MPLA production and characterization. This task is considered extremely difficult when using the conventional KDO assay not only because of the method itself but also due to the intrinsic characteristics of *Bp*LPS. The Purpald method was investigated as an alternative to make the task easier and to provide more reliability.[2] **Methods**: KDO assay involves H_2SO_4 hydrolysis of the sample at 100°C followed by oxidation with periodic acid at room temperature and colorimetric assay with thiobarbituric acid (TBA) at 100°C. Purpald avoids the steps of boiling for both acid hydrolysis and the TBA reaction and employs the oxidation by periodate of specific glycol groups in KDO and in heptose molecules of LPS to release formaldehyde. The molarity of KDO in each sample was determined by its absorbance, and the standard curve constructed with KDO (FW 255.2) (Sigma) standards (0.01 to 0.5 mM). **Results and Discussion**: Apart from the Purpald method conveniences, the sensitivity of both assays was similar (4 µg/mL of the standard). The sensitivity obtained was considered very important when monitoring each step of *Bp*MPLA production with the aim of obtaining an adjuvant free of LPS.

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

3.57 Construction of a vaccine against diarrheagenic *Escherichia coli* using O111 LPS antigen

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Introduction: The best approach to developing a vaccine against strains of O111 diarrheagenic *E. coli* that exhibit different mechanisms of virulence is to target their LPS. Two parts of the LPS molecule, the O111 polysacccharide and the core, can be used as antigens for vaccination and immunotherapy. However, structural variations found in both parts can make it difficult to formulate an effective vaccine to combat all categories of O111 pathogens. In addition, the ability of antibodies against O111 *E. coli* to recognize, inhibit and stimulate the clearance of O111 bacteria by macrophages was investigated. **Objectives:** The objective of this work was to determine whether the core and the polysaccharide parts of the LPS are good antigen candidates for the construction of a vaccine against EHEC, EPEC and EAEC. **Methods:** Accordingly, in this study, gas chromatography, molecular, electrophoretic and serological analysis were employed in order to determine whether these antigenic variants present on the LPS core and on the O111 polysaccharides had elements in common which could be targets for pan-specific immunotherapy. **Results and Discussion:** The data obtained in this work, from gas chromatography analysis, demonstrated that O111ab polysaccharide derived from EHEC and O111ac polysaccharide derived from atypical EPEC have different sugar molar ratios, suggesting that the amount of repetitive oligosaccharide units present in these strains are different from each other. However, immunoblotting analyses of both O111 subtypes showed that antibodies raised against either O111ab or O111ac polysaccharides can recognize EPEC, EHEC and EAEC regardless their ab or ac subtype. In line with the above observations, it was demonstrated that antibodies generated in rabbits immunized with a capsulated O111:H^- EHEC strain are able to recognize, aggregate and inhibit the adhesion to human epithelial cells of all categories of live O111 bacteria, regardless of their flagellar antigen or mechanism of virulence. In addition, these antibodies were also able to increase the clearance of O111 *E. coli* by macrophages. In the case of O111 LPS core, PCR analyses of the pathways involved in its biosynthesis showed that all EAEC strains from the O111 serogroup have core type R2, whereas, typical EPEC and EHEC have core type R3. In contrast, atypical EPEC have cores type R2 and R3. In summary, the results presented in this work indicate that despite the differences encountered in the LPS O111 polysaccharide, it still is a good candidate antigen for the development of a vaccine that can be used to combat all three categories of O111 diarrheagenic *E. coli*. In addition, the results also indicate that antibodies against the O111 LPS core type R3 can be used to prevent bloody diarrhea and HUS induced by EHEC and severe diarrhea induced by typical EPEC. However, core types R2 and R3 as antigen targets for vaccination can be used to generate antibodies against all categories of the O111 serogroup: EHEC, typical and atypical EPEC and EAEC.

Supported by: CNPq and FAPESP.

3.58 Rotavirus and human milk: presence of SIgA anti serotype G9P[8] and neutralizing activity

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Introduction: Rotaviruses are the most common cause of severe, dehydrating diarrhea in children worldwide. Studies of passive immunity in animals and humans have suggested that rotavirus- specific antibodies present at the mucosal surface of the gastrointestinal tract are effective for protection against rotavirus infection. Some authors have reported that breastfeeding combined with oral vaccination can decrease the immune response against the virus. **Objectives**: Our aim was to verify the presence of the SIgA antibodies reactive with G9P[8] which is one of the five serotypes present in the anti-rotavirus vaccine produced by Butantan. We also determined the ability of human milk to neutralize this serotype. **Methods**: Purified rotavirus antigens were used in ELISA to detect anti-rotavirus IgA antibodies in 30 milk samples from healthy mothers. For neutralization assays, serum samples were incubated with 100 $DICT_{50}$ of G9P[8] rotavirus and placed on a monolayer of MA-104 cells; inhibition of cytopathic effect was evaluated after 48 h. **Results and Discussion**: ELISA titers varied greatly (from <0.1 to 154.66, mean of 32.07). We obtained a wide range of neutralization titers (from 10 to 160, mean of 54.33) indicating the ability of some milk samples to neutralize G9P[8]. We demonstrated a significant correlation between the inhibitory effect on rotavirus and the concentrations of IgA in human milk samples. The IB assays revealed a reaction pattern against rotavirus proteins. The quantitative differences found in ELISA titers and neutralizing capacity of these samples are probably due to the different degrees of exposure to serotype G9P[8], an emerging serotype in Brazil. In addition, differences in neutralizing titers could be attributed not only to the presence of antibodies but to other milk components such as lactoferrin, lactadherin and lyzozyme. This approach may be important in studies concerning the protective effects of breastfeeding and in anti-rotavirus vaccination strategies.

Supported by: FAPESP and CAPES.

3.59 Influence of freezing and thawing on the potency of the pentavalent rotavirus vaccine produced at Instituto Butantan

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Introduction: A pentavalent rotavirus vaccine with the G1, G2, G3, G4 and G9 serotypes was produced at Instituto Butantan. This vaccine is provided in the lyophilized form with 4 doses per vial and one ampoule of diluent. **Objective:** To evaluate the potency of this product reconstituted with water or diluent after freezing and thawing. **Methods:** Vials of three lots of pentavalent rotavirus vaccine with initial potencies of $10^{6.3}$, $10^{6.8}$and $10^{6.2}$ PFU/ml after reconstitution with WFY water and $10^{6.7}$, $10^{6.8}$ and $10^{6.7}$ PFU/ml with diluent (citrate-phosphate buffer) were frozen at -80°C and thawed three times. Samples were taken after each thawing to determine the potency using the PFU (plaque forming units) test. **Results and Discussion:** The potencies obtained after three freezing and thawings, were $10^{6.3}$, $10^{6.7}$and $10^{6.1}$ PFU/ml for pentavalent vaccine reconstituted with water and $10^{6.8}$, $10^{6.9}$and $10^{6.6}$ PFU/ml when the product was reconstituted with citrate-phosphate buffer. The potencies found after freezing and thawing of this vaccine showed that this product has excellent stability. Some vaccines when submitted to freezing and thawing show variable potency.

Supported by: Fundação Butantan.

3.60 The adjuvant effect of the mesoporous nanostructurated SBA-15 silica in immunizations by the oral route

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Introduction: Nanostructured SBA-15 silica is an inorganic material that due to its physicochemical properties shows great potential as a mucosal adjuvant. Studies indicate that SBA-15 is effective in carrying, protecting and delivering antigens. It is non-toxic and induces non-selective IgG isotypes. **Objectives:** To analyze the adjuvant effect of this nanoparticle in immunizations by the oral route and the acute inflammatory response it elicits. **Methods:** BALB/c mice were immunized by the oral route with hepatitis A vaccine or human gamma globulin adsorbed on SBA-15 silica. Flow cytometry assays were performed to determine Peyer´s patches and mesenteric lymph nodes cells after immunizations. Recruitment of inflammatory cells induced by SBA-15 was investigated in the air pouch model of subcutaneous inflammation in mice genetically selected for high [AIR_{MAX}] or low [AIR_{MIN}] acute inflammatory responses. **Results and Discussion:** Oral immunizations with the antigens adsorbed on SBA-15 revealed increases in serum [IgG and IgA] and in secretory [IgA] specific antibody titers and showed that this silica does not interfere in the polarization of T_H1 or T_H2 immune responses. Flow cytometry assays demonstrated that SBA-15 silica was efficient in the recruitment of phagocytes and in increasing the numbers of B and T lymphocytes in Peyer´s patches and mesenteric lymph nodes of immunized mice, promoting the proliferation of immunocompetent cells. Subcutaneous administration of SBA-15 in AIR_{MAX} and AIR_{MIN} mice showed the low inflammatory potential and the non-toxicity of this nanoparticle. The results indicate that SBA-15 silica is an effective and safe adjuvant especially in immunizations by the oral route.

Supported by: CNPq, FAPESP and Cristália.

3.61 Identification of a complement regulator-acquiring protein of *Leptospira interrogans*

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Introduction: Leptospirosis is a spirochetal disease caused by pathogenic members of the genus *Leptospira.* After penetrating the host, leptospires have the ability to disseminate and to trigger a specific immune response. Their capacity to adhere to host cells and to escape the host´s innate immune defense systems contributes to colonization and persistence of these pathogens in the organism. A number of pathogenic microorganisms have evolved strategies to circumvent the immune defense systems of a variety of hosts, notably mechanisms to escape complement activation and/or lytic complement attack. Recently, we have shown that pathogenic leptospiral strains are able to bind C4b binding protein (C4BP). Surface-bound C4BP retains its cofactor activity, indicating that acquisition of this complement regulator may contribute to leptospiral serum resistance. **Objectives:** In the present study, the ability of six leptospiral recombinant proteins to interact with C4BP was evaluated. **Methods:** Ligand affinity blot analyses and ELISA were used to assess the interaction between recombinant proteins and C4BP. Surface localization was achieved after Triton X-114 extraction. The ability of both high- and low-passage *in vitro* cultured leptospires to express the gene coding for LepCRP was assessed by PCR amplification of reversely transcribed total RNA. **Results and Discussion:** One of the six proteins tested, named LepCRP (leptospiral complement regulator protein), interacted with this human complement regulator. Binding of LepCRP to C4BP was further examined by ELISA, and our results indicate that the recombinant protein exhibits a strong and saturable binding to C4BP. Triton X-114-solubilized extract of *L. interrogans* and phase partitioning showed that LepCRP was exclusively in the detergent phase, indicating that it is a component of the leptospiral membrane. Significant levels of LepCRP transcripts could be only detected in low-passage strains. This newly identified membrane protein may play a role in immune evasion of *Leptospira.*

Supported by: FAPESP, CNPq and Fundação Butantan.

3.62 Fed-batch and fed-batch followed by perfusion cultivation to produce capsular polysaccharide by *Haemophilus influenzae* type b

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Introduction: *Haemophilus influenzae* b (Hib), an encapsulated Gram-negative coccobacillus, is one of the most common agents of meningitis worldwide. The capsular polysaccharide type b consists of repeated units of the polymer of ribosylribitol phosphate (PRP) and plays an important role in the virulence of this microorganism. Usually, the conjugate vaccine results in high cost product due low yield from the purification and conjugation steps. The improvement of the cultivation condition is one possibility to enhance the polysaccharide production in order to reduce the final cost of this product. **Objectives:** The Butantan Institute plans to produce, in the near future, the pentavalent vaccine composed of DTP, hepatite B and Hib (all antigens produced at the Institute) by using innovative national technology. The purpose of this work was to increase the capsular polysaccharide produced by *Haemophilus influenzae* b through fed-batch and fed-batch followed by perfusion. **Methods:** *Strain: Haemophilus influenzae* type b GB3291. The fed-batch and the fed-batch followed by perfusion were carried out in Bioflo 2000 bioreactor, at 37°C, pH controlled to 7.5 with 5M NaOH and pO2 controlled at 30%. The main parameters were determined by the Labview system and samples were collected at regular times in order to measure DO_{540nm}, glucose, polysaccharide and metabolites. **Results and Discussion:** The biomass and productivity were 24 g DCW/L and 136 mg PRP/L*h, respectively, in the fed-batch with perfusion, i.e., two times higher than fed-batch with 10 g DCW/L and 74 mg PRP/L*h, respectively. On the other hand, polysaccharide production and acetic acid in the bioreactor were almost the same, around 1700 mg PRP/L and 24 g/L of acetic acid. The productivity in the fed-batch with perfusion was double compared with fed-batch, generating a large volume which can be a bottleneck in the further purification process. However, the fed-batch with perfusion cultivation reduces considerably the cost of polysaccharide production process due to high productivity.

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

3.63 Differential expression of sialyltransferase coding genes in murine B cell producers of anaphylactic and non-anaphylactic IgG1 antibodies

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Introduction: We showed that the ability of murine IgG1 antibodies to mediate anaphylactic reaction is directly dependent on the amount of sialic acid residues attached to the carbohydrate chain *N*-linked to its Fc region. We then hypothesized that differences in the glycan composition mainly the sialylation grade observed between the anaphylactic and non-anaphylactic IgG1 may result from the differential expression genes coding from glycosyltransferase, essentially sialyltransferase, during its synthesis by B cells. **Objective:** To analyze the expression of sialyltransferase genes in the hybridoma producer of these two types of IgG1 Abs as well as B cells isolated from mice. **Methods**: The expression of ST8SiaI-V; ST6GalNAc I-IV, ST3Gal II - V genes was analyzed quantitatively by real time-PCR in the hybridoma producer of anaphylactic and non-anaphylactic IgG1 Abs as well as B cells isolated by CD-19-positive magnetic beads from IL-4$^{-/-}$ mice immunized with PI (suppressive fraction of *Ascaris suum* extract-Asc) or IFN-y$^{-/-}$ mice immunized with PIII (allergenic fraction from Asc) which produce non-anaphylactic and anaphylactic IgG1, respectively. **Results and Discussion**: We observed that the expression of ST3Gal I, III and V coding genes was similar in both hybridomas, while the ST3Gal II and IV genes were less expressed in the hybridoma producer of non-anaphylactic IgG1 ($\Delta\Delta$ct=0.1-0.3) compared with hybridoma producer of anaphylactic IgG1 ($\Delta\Delta$ct=1.0). In addition, the expression levels of ST8Sia and ST6GalNAc genes in the hybridoma producer of anaphylactic IgG1 ($\Delta\Delta$ct=1.8-1.0) were significantly higher compared to those observed in hybridoma producing non-anaphylactic IgG1 ($\Delta\Delta$ct=0.02). Interestingly, the expression of sialyltransferase coding gene, excepting ST6Gal and ST6GalNAc, was higher in B cells from IFN-$\gamma^{-/-}$ mice ($\Delta\Delta$ct=1.0) when compared with B cells from IL-4$^{-/-}$ mice ($\Delta\Delta$ct=0.1-0.4). These data suggest a direct correlation between the sialylation grade of the carbohydrate chains attached to the murine IgG1 Abs and the expression of sialyltransferase enzymes by hybridomas and B cells isolated from immunized mice. Sialyltransferase activity in the sialylation of IgG1 antibodies needs to be further investigated.

Supported by: CNPq, CAPES and FAPESP.

3.64 Validation of purification process of tetanus anatoxin final bulk

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Introduction: Tetanus anatoxin final bulk is one component of the vaccines dT (diphtheria and tetanus adult use), DT (diphtheria and tetanus child use), DTP (diphtheria, tetanus and pertussis) and DTP-Hib (DTP plus *Haemophilus influenzae* type b). Tetanus anatoxin, obtained by detoxification of tetanus toxin, is called tetanus anatoxin final bulk after its purification. Validation of the purification process consists in collecting data during the procedures to ensure and provide documentary evidence that the purification process is reproducible and performed according to quality requirements. **Objective:** To validate the purification process, ensuring and providing in a report that the purification procedure of tetanus anatoxin final bulk is consistent. **Methods:** In order to obtain an elevated purity product according to quality requirements, tetanus anatoxin is purified by diafiltration and concentration using molecular ultrafiltration (50 kDa), and then, by size exclusion chromatography. For the validation of the purification process, three consecutive batche procedures of tetanus anatoxin bulk were performed with previous qualification and validation of all involved equipment and facilities. During the purification process samples were collected at critical stages and submitted to the Bioburden test. After purification, the following process controls were analyzed: microbiological test (bacterial and fungical sterility), physical and chemical tests (flocculation limit, sodium chloride, residual formaldehyde, pH, thimerosal, total nitrogen quantity, protein nitrogen quantity and antigenic purity), and biological tests (specific toxicity, irreversibility, potency and innocuity). **Results and Discussion:** The Bioburden in critical stages was between Ø CFU/50 mL and 26 CFU/50 mL, and after the filter sterilization it was Ø CFU/ 50 mL. The Bioburden results are informative and will be used for reference. The potency average for tetanus anatoxin final bulk was 11.5 IU/mL. The result of antigenic purity average was 1,783.90 Lf/mgPN. All results obtained in the microbiological, physical, chemical and biological tests were in accordance with the quality requirements. The purification procedures of tetanus anatoxin final bulk were validated showing the consistency of the process.

Supported by: Fundação Butantan.

3.65 Validation of purification process of diphtheria anatoxin final bulk

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Introduction: Diphtheria anatoxin final bulk is one component of dT (diphtheria and tetanus adult use), DT (diphtheria and tetanus child use), DTP (diphtheria, tetanus and pertussis) and DTP-Hib (DTP plus *Haemophilus influenzae* type b). Diphtheria anatoxin, obtained by detoxification of diphtheria toxin, is called diphtheria anatoxin final bulk after its purification. Validation of the purification process consists in collecting data during the procedures to ensure and provide documentary evidence that the purification process is reproducible and performed according to quality requirements. **Objective:** To validate the purification process ensuring and providing in the report that the purification procedure of diphtheria anatoxin final bulk is consistent. **Methods:** In order to obtain an elevated purity product in accordance with quality requirements, diphtheria anatoxin is purified by diafiltration and concentration using molecular ultrafiltration (30 kDa), and then, by precipitations with ammonium sulfate. For the validation of the purification process, three consecutive batch procedures of diphtheria anatoxin bulk were performed with previous qualification and validation of all involved equipment and facilities. During the purification process samples were collected at critical stages and submitted to the Bioburden test. After purification, the following process controls were analyzed: microbiological test (bacterial and fungical sterility), physical and chemical tests (flocculation limit, sodium chloride, residual formaldehyde, pH, thimerosal, total nitrogen quantity, protein nitrogen quantity, antigenic purity and ammonium sulfate), and biological tests (specific toxicity, irreversibility, potency and innocuity). **Results and Discussion:** The Bioburden at critical stages was between Ø CFU/50 mL and 90 CFU/50 mL and after the sterile filtration was Ø CFU/50 mL. The Bioburden results are informative and will be used for reference. The potency average for diphtheria anatoxin final Bulk for dT was 4.4 IU/mL; for DT, DTP and DTP-Hib was 4.8 IU/mL. The result of antigenic purity average was 1,593.20 Lf/mgPN. All results obtained in the microbiological, physical, chemical and biological tests were in accordance with the quality requirements. The purification procedures of diphtheria anatoxin final bulk were validated showing the consistency of the process.

Supported by: Fundação Butantan.

3.66 Transcriptome of normal lung distinguishes mouse lines with different susceptibility to inflammation and to lung tumorigenesis

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Introduction: AIRmax and AIRmin mouse lines show a differential lung inflammatory response and differential lung tumor susceptibility after urethane treatment. **Objective:** Thus, these mice constitute a good genetic model to investigate differences in gene expression profiles related to inflammatory response and lung tumor susceptibility. **Methods:** The transcript profile of 24,000 known genes was analyzed in normal lung tissue of untreated and urethane-treated AIRmax and AIRmin mice. The over-represented pathways were identified using the Genecodis program, and qPCR was used to validate microarray experiments. **Results and Discussion:** In lungs of untreated mice, inflammation-associated genes involved in pathways such as "leukocyte transendothelial migration," "cell adhesion" and "cell junctions" were differentially expressed ($P<0.001$) in AIRmax versus AIRmin mice. Moreover, gene expression levels differed significantly (1.5- to 4-fold) in urethane-treated mice even at 21 days after treatment. In AIRmin mice, modulation of expression of genes involved in pathways associated with inflammatory response paralleled the observed persistent infiltration of inflammatory cells in the lung of these mice. A specific gene expression profile in normal lung tissue is associated with mouse line susceptibility or resistance to lung tumorigenesis and with different inflammatory response, and urethane treatment causes a long-lasting alteration of the lung gene expression profile, which correlates with persistent inflammatory response of AIRmin mice.

Supported by: FAPESP, CNPq, UICC and ARC.

3.67 Validation process of pertussis vaccine final bulk product

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Introduction: Pertussis vaccine, one component of DTP (diphtheria, tetanus and pertussis) and DTP + Hib (DTP + *Haemophilus influenza* type b) vaccines, has been part of national childhood immunization programs. Instituto Butantan is the sole Brazilian producer of pertussis vaccine final bulk product. In the production, batch fermentations of *Bordetella pertussis* 137 strain are carried out in 1000-liter bioreactors containing liquid culture medium, in compliance with Good Manufacturing Practices. The suspension of *B. pertussis* was detoxified with formaldehyde, diafiltered and concentrated by a high resolution tangential flow filtration system. The production process validation is to prove and to document the results showing that the process is reproducible according to established criteria. The Installation Qualification (IQ), Operation Qualification (OQ) and Performance Qualification (QP) of the equipment and the systems are prerequisites to initiate the validation process. **Objective:** Pertussis Vaccine production process validation ensures that all critical parameters show reproducible results according to the specifications of the final product. **Methods:** The *B. pertussis* fermentation process for the cultivation preparation takes place under vortex mixing and by introduction of sterilized air onto the culture surface. During the fermentation process the following parameters are observed: mixing, pH, temperature, pressure and dissolved oxygen. At the end of fermentation, the cultivation was detoxified and inactivated by addition of formaldehyde. After 24 h, the suspension was concentrated by tangential flow filtration system and collected in a stainless steel tank. The samples collected in the cultivation were submitted to the following tests: pH, opacity, identity, purity and microscopy. The tests of pertussis vaccine were: sterility, identity, inactivation, microscopy, pH, residual formaldehyde, thimerosal, opacity, toxicity, absence of dermonecrotic toxin and potency. **Results and Discussion** The opacity of the three batches of the cultivation was 30 OU/mL. Pertussis vaccine showed the following average values: pH = 6.86 ± 0.11, residual formaldehyde = 21.17 ± 3.3 ppm; thimerosal = 146.36 ± 20.3 ppm, opacity = 225 ± 8.7 OU/mL. The potency average was 6.2 ± 1.8 IU/dose, higher than WHO requirements (≥ 4 IU/dose). In all batches, the specific toxicity and absence of dermonecrotic toxin tests were demonstrated. The process of pertussis vaccine final bulk product was validated based on the analysis of the sample data during process according to established criteria for acceptance for microbiological, physico-chemical and biological tests following the national requirements and WHO recommendations.

Supported by: Fundação Butantan.

3.68 Expression of recombinant rabies virus glycoprotein (RVGP) by S2 cells grown in a bioreactor

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Introduction: *Drosophila melanogaster* Schneider 2 cells (S2) have been used to express heterologous proteins. The recombinant rabies virus glycoprotein (RVGP) is an interesting biotechnological product, since it is responsible for the induction of protective immune response against rabies infection and for virus entry into cells upon the virus infection. **Objectives:** The aim of this work was to compare two recombinant S2 cell lines cultivated in a bioreactor. The S2AcGPV-2k with RVGP expression by the constitutive promoter (actin) and the S2MtGPVHy (Mc7) with an inducible promoter (metallothionein) were examined. **Methods:** The cell lines were cultivated in a BioFlo110 bioreactor. The culture conditions were: work volume of 1 L, temperature of 28°C, dissolved oxygen at 50% of air saturation, sparging aeration (air, nitrogen and oxygen – 0.1 L/min), 90 rpm agitation, pitched blade impellers, SF-900 II medium and initial cell seeding of $5x10^5$ cells/mL. S2MtGPVHy (Mc7) cells were induced at $3\text{-}5x10^6$ cells/mL with 700 µM of $CuSO_4$. **Results and Discussion:** RVGP expression was three times higher in the inducible S2MtGPVHy (Mc7) cells (1.33 $\mu g/10^7$ cells). The constitutive expression of S2AcGPV-2k showed 0.45 $\mu g/10^7$ cells of specific RVGP expression. The total RVGP produced in a S2MtGPVHy (Mc7) batch was of 1.6 mg, and the maximum cell concentration (Xmax) was $1.4x10^7$ cells/mL. The S2AcGPV-2K cells showed a total RVGP production of 1.1 mg and a Xmax of $3x10^7$ cel/mL. Higher RVGP expression by S2MTGPVHy (Mc7) cells can be related to: a higher affinity of RNA polymerase to the target gene, in the presence of $CuSO_4$; the presence of a higher number of gene copies in S2MtGPVHy (Mc7) cells. An inverse correlation between Xmax and protein expression was observed, since S2AcGPV-2K cells showed a higher Xmax, but the total protein expression was about 50% higher in the S2MtGPVHy (Mc7) cells.

Supported by: FAPESP and TACnet.

3.69 Analysis of *Pycard* as a candidate gene in the *locus* modifier of acute inflammatory reaction and IL-1β production

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Introduction: AIR mouse lines obtained after many generations of selective breedings according to maximal (AIRmax) or minimum (AIRmin) acute inflammatory response (AIR) induced by sc Biogel differ largely in the number of infiltrated leukocytes and exudated protein, as a result of diverse alleles of opposite effect that were fixed during the selection process at various quantitative trait *loci* (QTLs) controlling AIR. By linkage analysis in whole genome, one major effect *locus* modifier of AIR was mapped at the distal portion of chromosome 7 and shown to be co-localized with a QTL regulating the differential IL-1β production observed in AIR mice. **Objectives:** Our goal was to analyze polymorphisms and expression of candidate genes in this AIR QTL. **Methods:** In the confidence interval of AIR and IL-1β QTLs at chromosome 7, we analyzed SNP makers in linkage disequilibrium (LD) between AIR lines, sequenced and evaluated expression by real time-PCR of a candidate gene. **Results and Discussion:** The SNP marker CEL-7_115892950 (135,558,390 bp) showed the larger LD between AIRmax and AIRmin lines and was also the nearest SNP from the peak LOD score of both AIR and IL-1β QTLs. An obvious candidate gene situated in this region is the *Pycard* gene (135,135,148-135,138,250 bp), which encodes an inflammasome component protein involved in pro-IL-1β processing. The genome sequencing of *Pycard* gene obtained from AIRmax and AIRmin lines revealed 2 SNP polymorphisms (at downstream and intron regions). Despite this, neither alternative transcripts nor different levels of *Pycard* expression were observed between AIR lines. In conclusion, LD data obtained in confidence interval region of AIR and IL-1β production QTLs from AIR lines provide evidence for the localization of an AIR modifier gene in the region of SNP marker at 135,558,390 bp in chromosome 7, apparently not involving the *Pycard* gene.

Supported by: FAPESP and CNPq.

3.70 Genetic heterogeneity affects susceptibility to inflammatory response and to skin tumorigenesis in phenotypically selected mice

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Introduction: Non-inbred AIR (AIRmax, AIRmin) and Car (Car-S, Car-R) mouse lines were generated from the same 8 inbred mice through bidirectional selective breeding for acute inflammatory response and for susceptibility to two-stage skin tumorigenesis, respectively. Interestingly, AIR lines also showed a differential predisposition to skin tumorigenesis, and Car lines differed in the extent of inflammatory response. **Objectives:** Our goal was to identify the genetic elements affecting skin tumor susceptibility and inflammatory response to determine if AIR and Car lines share genetic control for these complex phenotypes. **Methods:** We carried out genome-wide association (GWA) studies using a panel of 1449 SNPs arrays in AIR and Car lines, as well as, in intercross populations of (AIRmax x AIRmin)F2 and (Car-R x Car-S)F2 mice. **Results and Discussion:** We found an inverse correlation between susceptibility to skin tumorigenesis and acute inflammatory response in AIR mice, whereas the two phenotypes were directly correlated in Car mice, with Car-S mice highly susceptible to both skin tumorigenesis and acute inflammation and Car-R resistant to both phenotypes. GWA analysis revealed 1224 informative SNPs in AIR mice and 1206 SNPs in Car mice. Statistically significant SNPs, that reached the statistical threshold of P=8.2e-06 determined by Bonferroni's criteria (α=0.01), were identified in either the AIR (n=519) or Car (n=211) mouse population. These markers exhibited a chromosomal distribution along the whole mouse genome, with neither apparent clustering nor shared common regions. GWA analysis also detected unrelated *loci* in AIR and Car intercross populations where none of the SNPs reached the Bonferroni's statistical threshold (α=0.01); at nominal P-value<0.01, 35 SNPs were detected. In Car F2 mice, SNP rs6213083, on chromosome 2, reached the Bonferroni's statistical threshold and at nominal P-value<0.01, 41 SNPs were detected. These markers, although identifying distinct *loci* modulating the two phenotypes in intercross populations of AIR and Car mice, confirmed the results obtained in the parental lines. These findings point to the complexity and the important role of genetic heterogeneity in the modulation of inflammatory response and skin tumor susceptibility in mice.

Supported by: FAPESP and CNPq.

3.71 Immune response induced by recombinant heat-shock protein (Cpn60r) of *Bordetella pertussis*

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Introduction: *Bordetella pertussis* is a pathogenic bacterium that causes whooping cough. The current vaccine consists of whole bacteria cells with added aluminum hydroxide as adjuvant which may cause toxic and undesirable effects. The heat-shock protein Cpn60 is a member of the chaperonin 60 family of highly conserved proteins which are involved in many essential cellular functions. This protein is known to be implicated in immune regulation. **Objectives:** We have been working on the immune response of this protein and we aimed to evaluate the activity of the recombinant protein (Cpn60r) we have produced. **Methods:** Groups of mice (BALB\c) were immunized with 5 or 10 μg of recombinant protein alone or mixed with DPT (diphteria-pertussis-tetanus) vaccine without aluminum hydroxide (NADPT). DPT vaccine produced at Instituto Butantan (DPT) was used as control. Sera were evaluated for antibodies against DPT antigens by ELISA. Spleen cells from immunized mice were evaluated for the production of IL-2, IL-4, IL-6, IL-12 and IFN-γ after *in vitro* stimulation with Cpn60r. Cytokines concentrations were determined by ELISA. Animals were challenged after the immunization protocol. **Results and Discussion:** Cpn60r, 5 μg, mixed with NADPT was able to induce a higher level of antibodies against pertussis antigens than did the DPT vaccine. IgG1 and IgG2a levels against DPT were similar in the groups immunized with Cpn60r and Cpn60r+NADPT. Higher levels of IL-6 were produced in the groups immunized with Cpn60r compared to DPT group, and higher levels of IFN-g were produced only in the groups immunized with 5 μg Cpn60r. IL-2, IL-4 and IL-12 were not detected. Cpn60r+NADPT induced an 80% protection rate, similar to DPTBut. The recombinant protein Cpn60r could stimulate Th1 (IgG2a, IL-6) and Th2 (IgG1, IFN-γ) cells, suggesting that it can induce a balanced immune response. Cpn60r showed similar levels of protection compared to DPT. These results show the immune response of the recombinant protein that could be included in immunization protocols for pertussis.

Supported by: FAPESP and Fundação Butantan.

4: Microorganisms

4.01 Role of intimin and type III secretion system in the ability of an atypical enteropathogenic *Escherichia coli* strain to induce mucus production *in vivo*

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Introduction: Typical and atypical enteropathogenic *Escherichia coli* (EPEC) are major causes of infantile diarrhea in developing countries. The main virulence mechanism of these pathogens is the formation of attaching and effacing lesion (A/E). Two among the various proteins responsible for this lesion are intimin encoded by the *eae* gene and *EscN*, a component of type three secretion system (T3SS), encoded by the *escN* gene. An atypical EPEC strain (aEPEC 3991-1/89) previously isolated in an epidemiological study from a child presenting with diarrheal disease showed the ability of inducing mucus production *in vivo* when tested in the rabbit ileal loop assay. **Objectives:** The objective of this study was to evaluate the role of intimin and the T3SS in the ability of aEPEC 3991-1/89 to induce mucus production *in vivo* in the rabbit ileum. **Methods:** Insertional mutagenesis was used to construct the *eae* and *escN* mutants. To verify the effect of these mutations, we performed the rabbit ileal loop assay, which was analyzed by means of traditional histological and transmission electron microscopy (TEM) techniques. **Results and Discussion:** 1. Individual mutations of the *eae* and *escN* genes abolished completely the ability of aEPEC 3991-1/89 to induce mucus production *in vivo*. 2. Histological and TEM analyses showed that mutation in these genes also abolishes the adherence characteristics of these strains as well as the ability of causing A/E. We conclude that the adherence of aEPEC 3991-1/89 to the enterocyte and/or the formation of A/E lesion are essential processes in triggering mucus production in the *in vivo* assay used. These results corroborate the hypothesis that other pathogenic processes may also be triggered by these initial processes.

Supported by: CNPq and FAPESP.

4.02 Biofilm formation among O157:H7 Shiga toxin–producing *Escherichia coli* strains and its correlation with the presence of curli fimbria

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) is an important food-borne pathogen worldwide. Bacterial attachment, colonization, and ability to form biofilm may be important for bacterial persistence in the animal reservoir and food, as well as in food contact surfaces which can serve as a source for biotransfer and cross-contamination of products. **Objectives:** In this study the ability to form biofilm was analyzed in 18 O157:H7 STEC strains isolated from different origins. A correlation between biofilm formation and presence of curli and type 1 fimbria was also investigated. **Methods:** Gene sequences related to curli (*csgA* and *crl*) and type 1 fimbria (*fimH*) were searched by PCR assays. Curli production and the expression of type 1 fimbriae were respectively characterized on Congo red-binding agar plates after 48 h incubation at 28ºC, and by hemagglutination assays. Quantitation of biofilm formation was performed by the crystal violet method in 96-well polystyrene microtiter plates at 28ºC and 37ºC for 48 h. Strains were also analyzed under negative staining by transmission electron microscopy (TEM). **Results and Discussion:** PCR assays showed that *csgA*, *crl* and *fimH* were identified in all O157:H7 strains. Curli production, as judged by characteristic red-colored colonies formed on Congo red-binding agar, was observed in 27.8% (5/18) of the isolates. Phase variant strains, showing both red and white colonies, were identified and re-isolated. Expression of type 1 fimbriae was not observed in any of the strains by hemagglutination assays. The ability to form biofilm at 28ºC was only identified in the five O157 curli-producing strains. However, when assays were performed at 37ºC, biofilm was observed in 44.4% (8/18) of the isolates, and besides, the five curli-producing strains biofilm occurred in three other curli-negative strains. Quantification of biofilm formation was higher at 28ºC when compared to 37ºC. The ability to form biofilm was only observed in red colonies among phase variant strains, both at 28ºC and 37ºC. TEM analysis of red and white colonies of phase variant strains showed the presence of hair-like fimbrial structures similar to curli only in red colonies. The O157 strains able to form biofilm were isolated from cattle. The results obtained suggest that the ability of O157 STEC to form biofilm at 28ºC is closely related to curli production; however, at 37ºC, in addition to curli, other factors may help in the process of biofilm formation.

Supported by: CNPq and FAPESP.

4.03 A newly identified protein of *Leptospira interrogans* mediates binding to laminin

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Introduction: Pathogenic *Leptospira* is the etiological agent of leptospirosis, a life-threatening disease that affects populations worldwide. The search for novel antigens that could be relevant in host-pathogen interactions is being pursued. These antigens have the potential to elicit several activities, including adhesion. **Objectives:** This study focused on a hypothetical predicted lipoprotein of *Leptospira*, encoded by the gene LIC12895, thought to mediate attachment to extracellular matrix (ECM) components. **Methods:** The gene was cloned and expressed in *Escherichia coli* BL21 Star (DE3)pLys using the expression vector pAE. The recombinant protein tagged with N-terminal hexahistidine was purified by metal-charged chromatography and characterized by circular dichroism spectroscopy. The capacity of the protein to mediate attachment to ECM components was evaluated by binding assays. **Results and Discussion:** The leptospiral protein encoded by LIC12895, named Lsa27 (leptospiral surface adhesin, 27 kDa), bound strongly to laminin in a dose-dependent and saturable fashion. Moreover, Lsa27 was recognized by antibodies from serum samples of confirmed leptospirosis specimens in both the initial and the convalescent phases of the disease. Lsa27 is most likely a surface protein of *Leptospira* as revealed in liquid-phase immunofluorescence assays with living organisms. Taken together, these data indicate that this newly identified membrane protein is expressed during natural infection and may play a role in mediating adhesion of *L. interrogans* to its host.

Supported by: FAPESP, CNPq and Fundação Butantan.

4.04 Scaling up unencapsulated *Streptococcus pneumoniae* cellular vaccine

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Introduction: *Streptococcus pneumoniae* is responsible for 1.6 millions deaths of children annually worldwide. This pathogen causes otitis media, sinusitis, pneumonia, meningitis and sepsis. There are more than 90 different pneumococcal serotypes, each one corresponding to a capsular polysaccharide. The current vaccines are based on these polysaccharides and require laborious and expensive purification processes to obtain the antigens; as a consequence, the costs of pneumococcal vaccines are high, making it difficult to implement mass vaccination campaigns in developing countries. In order to solve this problem, Malley and collaborators suggested a new vaccine consisting of inactivated whole-cells of unencapsulated *S. pneumoniae*, which would involve lower production costs and provide serotype-independent protection. **Objectives:** To establish a fed-batch fermentation strategy for pneumococcal cultivation and scale-up the production process from 10 to 60 liters. **Methods:** The cultures of the unencapsulated *S. pneumoniae* strain Rx1 Pl^- Al^- kan^R (RM200) have been performed in Bioflo2000 (10L) and Bioflo5000 (60L) bioreactors using enzymatically hydrolyzed soybean meal medium at 2.0% for the inoculum and 0.5% in the bioreactor. A pre-culture was used to inoculate the bioreactor in order to obtain an optical density of ~0.1 at 600 nm (OD_{600}). A fourfold concentrated medium was used for feeding when the OD_{600} reached ~4.5 at 0.5 L/h. The cultures were carried out at 36°C, 150 rpm, 0.5 L/min N_2, 0.1 bar and the pH was controlled at 7.0 by addition of 5 M NaOH. Samples were taken from the bioreactors every 30 min and cell growth was monitored by OD_{600}. After centrifugation of culture broth samples at 20,000 g and 4 °C for 10 min, glucose, lactate and acetate were determined in the supernatant using high-performance liquid chromatography (HPLC, Shimazdu) with an Aminex HPX 87H column (300 x 7.8 mm, BioRad) at 60 °C, and 5 mM H_2SO_4 was used as solvent with a flow rate of 0.6 mL/min. **Results and Discussion:** The fermentation process was reproducible, showing similar results at a 10 L and 60 L scale. Cell growth was satisfactory in both reactors, reaching OD_{600} ~9.5 after 5-6 h. The maximum specific growth rate was 0.96-0.98 h^{-1} in the exponential phase and 0.54-0.59 h^{-1} in the fed-batch phase. The main organic acid produced was lactate, whose concentration reached ~20 g/L at the end of the culture. Although acetate was produced in the batch phase, the concentration dropped from ~4.0 g/L at the beginning of feeding to ~2.7 g/L at the end. More variable data were obtained for glucose consumption, ranging from 3.6 $g/L.h^{-1}$ to 6.2 $g/L.h^{-1}$, which could be explained by the utilization of other carbon sources from the complex medium used. In conclusion, a pneumococcal fed-batch culture was established at 10 L and successfully scaled up to 60 L. Some adjustments should be performed in order to identify the ideal moment for the start of feeding and improve glucose to biomass conversion yield. The developed process would allow vaccine production according to cGMP requirements.

Supported by: PATH and Fundação Butantan.

4.05 Comparison of LD_{50} for botulinum toxin type A in different animal species

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Introduction: *Clostridium botulinum* produces toxins that have been classified into 7 serotypes, A, B, C, D, E, F and G, according to their immunological characteristics. Toxin type A, and sometimes type B, have been used to treat dystonia and various other muscle hyperactivity syndromes in humans. The increase of botulinum toxins in medical use has raised the need for precise analysis of the biological activity contained in the toxin preparations for both clinical use and laboratory investigation. One of the most important parameters that must be observed in pharmaceutical products used in humans is the LD_{50} since it is directly related to safety. **Objectives**: The aim of this study was to compare the value of LD_{50} for botulinum toxin type A in different animal species. **Methods**: According to international convention the fundamental unit (IU) of botulinum toxin biological activity is defined as the LD_{50} for the toxin in a population of mice (1 LD_{50} = 1 IU). We conducted two LD_{50} experiments with guinea pigs and rabbits (25 animals/species). The doses varied between 2 and 4 IU for guinea pigs and 15 and 75 IU for rabbits. The administration of botulinum toxin type A in guinea pigs was performed by intraperitoneal injection, whereas in rabbits by intravenous injection. The LD_{50} was determined using a computerized PROBIT-LOG program. **Results and Discussion**: To compare the LD_{50} between species, the results were converted into IU/kg. The value for guinea pigs was 10.33 ± 1.95 IU/kg, and for rabbits it was 17.31 ± 0.21 IU/kg. Considering that the value for mice is stipulated according international convention at 50 IU/Kg, it is concluded that both these values showed a statistical difference ($p<0.001$). There are significant differences of sensitivity to lethal effects of botulinum toxin type A in rodents, since mice are more resistant than guinea pigs. In the same way, mice are more resistant than rabbits. These data suggest that there are important differences in susceptibility to botulinum toxin type A among animal species, even in the same order (Rodentia) or between different orders (Rodentia x Lagomorpha).

Supported by: Fundação Butantan.

4.06 Effects of serum on leptospiral binding and on its hemolytic activity

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Introduction: Pathogenic leptospires live both in aquatic environments and inside their hosts. To infect and grow inside hosts, leptospires need nutrients and adhesion capacity to survive in this adverse environment. To obtain iron, an essential nutrient for growth, leptospires secrete hemolytic proteins to release erythrocyte hemoglobin, which contain iron as their prosthetic group. On the other hand, the ability to bind to host molecules, especially to extracellular matrix (ECMs) components, is also a vital feature of these bacteria, since they leave the bloodstream and penetrate several organs, in which they keep multiplying. **Objectives:** The objective of this work was to examine the effects of immune and non-immune serum on leptospiral binding and on its hemolytic activity. **Methods:** Virulent leptospires were cultured in EMJH medium for 10 days at 29° C. They were pelleted and washed three times with PBS. The bacteria were treated with non-immune serum, immune serum or with PBS. Next, each group was allowed to bind to an ECM-like substrate (Matrigel). After several washes and an ELISA-like procedure, the total bacteria bound to the substrate were determined by spectrophotometry. The supernatant of leptospire culture was also treated with non-immune serum, immune serum or with PBS. Afterward, a 5% erythrocyte suspension was added to each group, and total erythrocyte lysis was determined by spectrophotometry. The immune sera were obtained from leptospirosis patients. **Results and Discussion:** Both binding and hemolytic abilities were significantly reduced in the presence of serum, although neither of them was fully inhibited. The reduction levels were not different between groups treated with non-immune and immune serum, indicating that the binding and hemolytic abilities were not significantly affected by the presence of specific antibodies in this condition. The reduction in the hemolysis level was previously described as being a consequence of the competition between the hemolysin targets (the phospholipids and sphingomyelins of the erythrocyte membrane) and the free phospholipids present in serum (especially with the sphingomyelins and phosphatidylcholines). Since leptospires have about 10 proteins that are involved in the hemolytic process, the lack of a full inhibition may be related to the presence of hemolysins with different specializations, such as the SphH protein, described as a pore-forming protein. The addition of an immune serum did not increase the inhibition of leptospire-mediated hemolysis, possibly because not all leptospire hemolysins were recognized by the immune serum. We can hypothesize that a similar process of inhibition may occur in leptospiral binding when in the presence of non-immune and immune serum. Indeed, several proteins have already been described to be involved in leptospiral binding, and some of them can bind to serum components, such as the plasma fibronectin. In conclusion, it seems that the action of some serum components can produce an adverse environment inside hosts, although leptospires seem to be able to overcome it, as a probable consequence of the use of multiple proteins for each function.

Supported by: FAPESP, CNPq and Fundação Butantan.

4.07 Purification of a recombinant fragment of pneumococcal surface protein A produced in high cell density culture of *Escherichia coli*

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Introduction: *Streptococcus pneumoniae* is a pathogenic bacterium responsible for millions of deaths in children, elderly and immune-compromised people worldwide. Pneumococcal vaccines have demonstrated a good efficacy, but their coverage is limited due to the large number of serotypes. The polysaccharide vaccines, besides not covering all serotypes, do not protect children under two years old, because the immune response is T-independent. The strategy of Instituto Butantan is to develop a new conjugated vaccine using as carriers pneumococcal surface proteins, which are much more conserved than pneumococcal polysaccharides and would offer a broader coverage. The pneumococcal surface protein A (PspA) was chosen, as it has been demonstrated to be one of the most important virulence factors of *S. pneumoniae*. **Objectives:** To develop an industrial production and purification process of a recombinant fragment of PspA from clade 3 (rfPspA3) in high cell density culture of *E. coli*. **Methods:** *E. coli* BL21(DE3) harboring pET37b+/rf*pspA3* was evaluated for gene expression. Growth kinetics, carbon source consumption, and acetate and protein production were evaluated in shaker flasks and in 5-L bioreactor using high cell density medium (HCD) to compare glucose and glycerol as carbon source. The kinetic parameters were applied to control fed-batch cultures with exponential feeding of the carbon source in order to achieve high cell density. The induction was performed with 0.1 mM IPTG+20 g/L lactose for batch cultures and with 0.5 mM IPTG+20 g/L lactose for fed-batch cultures. The cells were disrupted in a continuous high pressure homogenizer. For clarification, three methods were tested: centrifugation and tangential micro- and ultrafiltration. Two sequences of chromatographic steps were evaluated for purification: anion exchange in Q-Sepharose followed by metal affinity in IMAC-Sepharose loaded with Ni^{+2} and IMAC followed by Q-Sepharose. **Results and Discussion:** Glycerol cultures resulted in less acetate formation ($<$ 1.0 g/L) and lower specific growth rate (0.4 h^{-1}) than glucose cultures (up to 5 g/L acetate and ~0.5h^{-1} specific growth rate). The cell and rfPspA3 production were similar in both glucose and glycerol cultures, reaching ~62 g/L of cell dry weight and ~3 g/L of soluble protein in high cell density fed-batch cultures. Although micro- and ultrafiltration were more effective for clarification than centrifugation, they led to higher loss of rfPspA3 (58% and 29%, respectively). The sequence Q followed by IMAC showed higher yield (77%) and purity (81%) than did the inverse sequence (56% of yield and 76% of purity).

Supported by: FAPESP and CNPq.

4.08 Relationship between median paralysis dose (PD_{50}) and median lethal dose (LD_{50}) of botulinum toxin type A in mice

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Introduction: Botulinum toxins are paralytic neurotoxins known for their therapeutic and cosmetic potential. Intramuscular injection of purified botulinum toxin, mainly type A, is the treatment of choice for facial wrinkles and a number of muscle hyperactivity syndromes. The international Unit (IU) of botulinum toxin has been defined as the 50% lethal dose (LD_{50}) in mice which reflects the toxic activity of botulinum toxin but does not reflect its pharmacological properties. On the other hand, regional flaccid paralysis reflects the mechanism of action of botulinum toxin in the clinical setting. **Objective:** This study was performed to compare an assay to assess the paralyzing activity (50% paralysis dose - PD_{50}) with the assay to measure the LD_{50}. **Methods:** Three independent experiments were conducted for lyophilized preparations of 100 IU or 50 IU. In all experiments, the botulinum toxin type A produced at Instituto Butantan was used. For PD_{50} different dilutions of botulinum toxin were injected into the gastrocnemius muscle of NIH mice, whereas for LD_{50} they were injected in NIH mice intraperitoneally. In both experiments the mice were observed for 96 h, and the percentage of paralyzed and dead animals was determined at each dose. The PD_{50} was defined as the inverse of the toxin dilution that caused complete local paralysis in 50% of injected animals, while the LD_{50} was determined as the inverse of the toxin dilution that caused death in 50% of injected animals. Probit analysis was performed to calculate the PD_{50} and LD_{50}. **Results and Discussion:** Botulinum type A toxin preparations were analyzed at different times for up to 180 days after the lyophilization process. During the observation time, LD_{50} ranged from 19.2% (100 IU) to 26.6% (50 IU), while the PD_{50} ranged from 13.9% (100 IU) to 15.2% (50 IU). These results indicate that the PD_{50} activity is more stable than the LD_{50} activity. The more concentrated preparations showed less variation in LD_{50} and PD_{50} during the time studied. The linear regression plots showed high correlation coefficient (r=0.98) between LD_{50} and PD_{50} assays.

Supported by: Fundação Butantan.

4.09 Analysis of biofilm formation of atypical enteropathogenic *Escherichia coli* strains by CFU/cm^2 counting and confocal fluorescence microscopy

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Introduction: Microorganisms can live and proliferate as individual cells swimming freely in the environment, or they possess the capacity to adhere forming biofilms in close association with different types of surfaces and interfaces. This type of formation represents colonization mechanisms. Biofilms confer resistance to some antibiotics in the associated bacteria and it is related to bacterial persistence. Strains of typical EPEC and their isogenic mutants in *bfpA* and *espA* form better biofilms in relation to the wild type, indicating the hypothesis that atypical EPEC strains have a greater capacity for biofilm formation. **Objective**: The aim of this study was to verify the capacity of biofilm formation by atypical EPEC strains isolated from children with diarrhea in Salvador (Bahia) on an abiotic surface (polystyrene) and pre-fixed cellular surface (HEp-2) during prolonged periods of incubation at 37°C. **Methods**: One strain of atypical EPEC, isolated from children with acute diarrhea, of each adhesion pattern (localized adhesion, localized-like adhesion, diffuse adhesion, non adherent, undetermined adhesion and aggregative adhesion) was chosen to carry out the experiments. The test was performed for periods of 6, 12 and 18 days. The strains were tested through the direct method of CFU/cm^2 counting attached to the biofilm after disrupting with Triton X-100. After disruption, serial dilutions were made and plated onto agar Luria-Bertani for counting of CFU/cm^2 and the strains were also visualized with a confocal fluorescence microscope. The bacteria were stained with propidium iodide (appearing red), and the cells (when present) were stained with phalloidin-FITC (appearing green). **Results and Discussion:** Through the analysis with confocal fluorescence microscopy, it was possible to visualize the strains that were capable of forming biofilm in pre-fixed cellular surface and abiotic surface, and some formed better biofilms in pre-fixed cells (HEp-2) and others in polystyrene (abiotic surface). Some strains formed a great amount of biofilm in both surfaces studied; in other words, they were not substrate dependent, at least in the substrates and conditions tested in this work. Other strains of atypical EPEC used as control did not form mature structures of biofilm. By the method of counting CFU/cm^2, the strains showed constant values of CFU/cm^2 in the range of 10^8 UFC/cm^2, and the sample that showed a more highlighted aggregative pattern reached a count of 10^9 CFU/cm^2**.** The strains of atypical EPEC showed great heterogeneity in relation to biofilm formation. Counting CFU/cm^2 should not be used alone to determine biofilm formation; this should be used together with a qualitative method such as confocal fluorescence microscopy which allows the visualization of biofilm structures.

Supported by: FAPESP.

4.10 Validation of sterility test in isolator of hepatitis B vaccine (rDNA) (VRHB) produced at Instituto Butantan by means of bacteriostatic and fungistatic effect

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Introduction: Verification of hepatitis B vaccine (rDNA) sterility produced by Instituto Butantan is done by filtering the product with 0.45 µm porosity membrane, rinsing this membrane with a neutralizing fluid and subsequent incubation in fluid thioglycollate and soybean-casein digest media. The current standards require that all operational procedures used in quality control must be validated according Good Laboratory Practices (GLP). **Objectives:** The aim of this study was to determine the sensitivity of membrane filtration methodology applied to bacterial and fungal sterility test in hepatitis B vaccine (rDNA) in isolators and to validate this technique under Microbiological Control Section conditions. **Methods:** This study was performed using three batches of hepatitis B vaccine (rDNA) produced by Instituto Butantan, previously evaluated for thimerosal concentration by spectrophotometric method. These product batches were tested according to standard methodology and membranes were rinsed with Diluent Neutralizing Pharmacopoeic solution, DNP. After transferring the content of the container to be tested to the membrane, an inoculum of a small number of viable ATCC microorganisms (not more than 100 cfu) was added to the final portion of DNP used to rinse the membrane. Fluid thioglycollate medium (LTM) was challenged with *Clostridium sporogenes, Pseudomonas aeruginosa* and *Staphylococcus aureus,* and soybean-casein digest medium (SCM) was challenged with *Aspergillus niger, Bacillus subtilis* and *Candida albicans*. Culture media were incubated for not more than 5 days at 20 °C - 25 °C and 30 °C –35 °C (SCM and LTM, respectively). **Results and Discussion:** Clearly visible growth of all microorganisms was obtained after the incubation period. The methodology applied in isolator to test the bacterial and fungal sterility of hepatitis B vaccine (rDNA) is effective, and thimerosal present in the product formulation as preservative was completely inactivated by rinsing the membrane with DNP solution, allowing the detection of low levels of microbial contamination, ensuring product quality. The sterility test may be carried out without further modifications.

Supported by: Fundação Butantan.

4.11 Cloning and expression of two predicted surface proteins of *Leptospira interrogans*

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Introduction: Leptospirosis is a zoonosis of global importance that, in recent years, has been considered a major emerging infectious disease. The geographical distribution of leptospirosis is mainly cosmopolitan, where it is associated with inefficiency or lack of infrastructure and poor sanitary conditions. In Brazil, as in other developing countries, most infections occur through contact with water contaminated with rodents' urine, which constitute the main reservoir of these bacteria. In addition, to implement conventional control, new strategies are required to deal with outbreaks. Currently, it is believed that the development of an effective vaccine, with minimum adverse effects, could be an important strategy to prevent the disease. Accordingly, several studies have been conducted to identify and characterize relevant antigens involved in host-pathogen interactions. **Objectives:** Selection, amplification, cloning, expression and purification of two new predicted outer membrane proteins identified in the genome of *L. interrogans* serovar Copenhageni, using *Escherichia coli* as host expression system. **Methods:** Bioinformatics analysis of the sequences encoded by LIC11834 and LIC12253; design of appropriate primers and PCR amplification from genomic DNA; cloning of PCR products in pGEM-T vector; digestion removal of DNA inserts and subcloning in pAE expression vector; sequencing analysis of the cloned inserts; transformation and expression of recombinant proteins in *E. coli* strain BL21 SI; analysis of expression and solubility of the recombinant proteins; purification of the recombinant proteins by metal-affinity chromatography; production of polyclonal antibodies by mouse immunization and immunogenicity tests by ELISA. **Results and Discussion:** After cloning in pAE vector, the proteins could be expressed with the presence of an N-terminal 6xHis tag which makes them suitable for metal-affinity chromatography purification. The recombinant proteins rLIC11834 and rLIC12253 were expressed from induced BL21 (SI) *E. coli* strain in the soluble and insoluble form, respectively. The purification of the proteins was effective, yielding bands with the expected molecular mass of 33 kDa (rLIC11834) and 24 kDa (rLIC12253), as assessed by 12% SDS-PAGE. Mice immunized with the recombinant proteins produced serum with high titers of antibodies against both proteins. These recombinants will be further characterized in other immunoassays to evaluate their participation in leptospiral pathogenesis.

Supported by: FAPESP, CNPq and Fundação Butantan.

4.12 Type I fimbriae is the most prevalent fimbrial adhesin in atypical enteropathogenic *Escherichia coli* isolates

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Introduction: Pathogenic *Escherichia coli* strains are classified as diarrheagenic and extra-intestinal *E. coli*, and besides diarrheagenic, they are differentiated into the following pathotypes: enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC) and enteroinvasive *E. coli* (EIEC). Among these pathotypes, EHEC is a subgroup of Shiga toxin-producing *E. coli* (STEC), and EPEC and the EAEC are subdivided into typical and atypical. EPEC is identified by the presence of *eae* and *bfpA* genes which encode the adhesin intimin and the type IV pili BFP, respectively. Both genes are used to classify EPEC into two groups: typical (*eae*+/*bfpA*+) and atypical (*eae*+/*bfpA*-). Current data demonstrate that aEPEC are more prevalent than tEPEC as the main cause of diarrhea in both industrialized and developing countries. Adhesion, an essential first step in bacterial pathogenesis, is mediated by fimbrial adhesins, which are critical for successful *E. coli* colonization in the host's mucosa. **Objective:** In this study, we investigated the prevalence of genes that encode fimbrial components described in some *E. coli* pathotypes among 72 isolates of aEPEC. **Methods:** The PCR technique was employed to search for the presence of the following gene sequences in 72 strains of aEPEC isolated from cases of acute diarrhea: *fimA* and *fimH* which correspond to the type I fimbriae of Enterobacteriaceae*; papA* to P-fimbriae of uropathogenic *E. coli* (UPEC)*; aggA, aafA, agg3A* to aggregative adherence fimbriae types I, II and III of EAEC*; pilS* encoding a functional type IV pilus related to AA expression*;* $lpfa_{O113}$ a long polar fimbriae of Shiga toxin-producing *E. coli* (STEC) of serogroup O113*; sfpA,* a sorbitol-fermenting (SF) EHEC O157:H-*;* and *lngA* to the longus type IV pilus of ETEC and genes that encode antigens CFA/I, CS1, CS3, CS4 and CS6. The PCR reactions were developed employing specific primers based on published sequences in GenBank. **Results and Discussion:** The *fimA* and *fimH* genes were found in 68 (94.4%) and 70 (97.2%) isolates, respectively. The *pilS* gene was detected in 11 (15.2%) isolates. Regarding the $lpfA_{O113}$ gene sequence, 9 (12.5%) isolates harbored that sequence. The *papA* gene was detected in two (2.7%) isolates and *sfpA* was detected in only in one (1.3%) strain. The other gene sequences were not detected in any isolates. The detection of $lpfa_{O113}$ gene in aEPEC strains corroborates the proposal of a phylogenetic relationship between aEPEC and STEC. The *pilS* gene was described in one particular EAEC strain, where it encoded a functional type IV pilus related to AA expression, and the occurrence of this gene in other *E. coli* pathotypes has yet to be determined. The presence of genes in some strains of aEPEC shows the horizontal transfer of genes between pathotypes of *E. coli*. Despite the fact that the presence of type I fimbriae structures in aEPEC pathogenesis is unclear, the high prevalence of *fimA* and *fimH* genes suggest that they may play a role in the adhesion of aEPEC in the absence of BFP.

Supported by: FAPESP and CNPq.

4.13 Screening of *Streptococcus pneumoniae* serotype 14 for capsular polysaccharide production and effect of vitamins on cell growth

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Introduction: The pneumococci are classified into 91 immunologically distinct capsular polysaccharide (CP) types, and their worldwide distribution is variable. CP is the major virulence factor of this microorganism and is the antigen of the available pneumococcal vaccines. In order to produce pneumococcal vaccines, it is essential to: choose serotypes most commonly isolated, select good producer strains, amass knowledge about their metabolism and establish parameters for their cultivation and CP production. In Brazil, the serotype 14 is the most commonly isolated serotype of *S. pneumoniae* in children. **Objectives:** To evaluate the behavior of cell growth and CP production of *S. pneumoniae* serotype 14 strains, selecting the best CP14 producer strain, and to determine which vitamins are essential for growth of the selected strain. **Methods:** Eight strains (731, 322, 334, 366, 941, 1871, 2721 and 5287) were kindly donated by Institute Adolfo Lutz. The inocula were grown from the -70°C stocks in 50 mL of complex medium, in an atmosphere of ~3% CO_2, 37°C and static cultivation. When these cultures were in the exponential phase, the inocula were transferred to flasks containing 500 mL of fresh medium to obtain an initial optical density of ~0.1 at 600 nm (OD_{600}). Cell growth was monitored by OD_{600}, samples were centrifuged and the supernatants were analyzed for CP14 concentration by inhibition ELISA (Malley *et al*, 2006), using CP14 ATCC as standard. To evaluate the vitamins and cofactors that are essential for cell growth, an inoculum of the best CP14 producer strain was prepared as above, then centrifuged, washed with saline and transferred to flasks that contained 50 mL of chemically defined medium - CDM (van de Rijn & Kessler, 1980). The strain was evaluated in complete CDM (control) and in CDM without each vitamin/cofactor to be tested: riboflavin, nicotinamide, pantothenic acid, thiamine, p-aminobenzoic acid, biotin, folic acid, pyridoxamine, pyridoxal, β-NAD, pyridoxamine + pyridoxal and p-aminobenzoic acid + folic acid. All cultures were started with OD_{600} ~0.1 and the growth was monitored by OD_{600}. **Results and Discussion:** All strains analyzed were able to grow in the complex medium; however, 2 strains were withdrawn because their lag phase was >13 h, using the same inoculum ratio as the other strains (0.2% v/v). The six other strains produced CP14 ranging from 74 to 302 mg/L. The highest CP14 concentrations were 302 and 240 mg/L, obtained with strains 1871 and 5287, respectively. Although the highest CP14 concentration was produced by the strain 1871, the strain 5287 was chosen as the best producer because, unlike 1871, it showed a typical stationary phase, a very important feature for bioreactor cultivation. In the assay for essential vitamins/cofactors, only the absence of the vitamins nicotinamide and pantothenic acid affected the cell growth in comparison with the complete CDM, decreasing the maximum OD_{600} by 25% and 73%, respectively. As a result, the number of vitamins/cofactor used in the CDM for the strain 5287 can be reduced from 10 to 2 without decreasing cell growth.

Supported by: FAPESP and CAPES.

4.14 Occurrence of *Giardia lamblia* assemblages AII and B, but not AI, in human isolates from São Paulo State, Brazil

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Introduction: Public Health Services in Brazil lack molecular techniques for the investigation of *Giardia lamblia* diarrhea outbreaks, and consequently, the source of infection and routes of transmission cannot be reliably determined. **Objective:** Human *G. lamblia* isolates from various regions of the state of São Paulo, Brazil were genotyped by *gdh* gene sequencing to establish this technique as a tool for investigating *Giardia* diarrheal outbreaks. **Methods:** Cysts of 20 individual isolates obtained after routine stool examination and 7 additional isolates representing four *Giardia* diarrheal outbreaks were partially purified by zinc sulfate flotation, lysed by proteinase K and freeze-thawed; DNA was then extracted with phenol:chloroform. Fragments of ~660 bp of the *gdh* gene were nested-PCR amplified, both strands were sequenced, and the corresponding assemblage determined by alignment with reference sequences. **Results and Discussion:** Individual samples were determined as Assemblage AII (13/20) and B (7/20). One outbreak was caused by Assemblage B and 3 by Assemblage AII. Assemblage AI was not found. These were the first *Giardia lamblia* diarrheal outbreaks investigated by molecular techniques in Brazil, and further samples from these outbreaks will be sequenced. This technique is now available as a tool for investigations carried out by the Epidemiological Surveillance Services.

Supported by: FAPESP.

4.15 Plasmid profile of atypical enteropathogenic *Escherichia coli*

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Introduction: Diarrhea continues to be one of the most common causes of morbidity and mortality among infants and children, especially in developing countries. Among the bacterial pathogens, diarrheagenic *Escherichia coli* (DEC) is an important agent of endemic and epidemic diarrhea worldwide. The diarrheagenic *E. coli* strains can be classified into six main pathotypes, based on specific virulence properties, clinical features, association with serotypes O:H, epidemiological aspects, and patterns of interaction with cellular strains. Enteropathogenic *Escherichia coli* (EPEC) cause a histopathological lesion known as "attaching and effacing" (A/E). Typical EPEC differ from atypical EPEC by the presence of a plasmid called EPEC adherence factor (EAF) which encodes the bundle-forming pilus (BFP). Atypical EPEC comprise a very heterogeneous group. **Objective:** To study the plasmid profile in a 72-sample atypical EPEC collection. **Methods:** DNA extraction was performed using the Wizard plus midprep DNA purification system, and the results were obtained using 1% agarose gel electrophoresis in Tris-borate-EDTA buffer and ethidium bromide staining. **Results and Discussion:** Besides the fact that atypical EPEC comprise a very heterogeneous group, we found high molecular-weight plasmids ranging from 60 to 80 MDa in most of the strains. We decided to study most specifically plasmids common to strains belonging to the 055:H7 serotype. Using RFLP patterns, we obtained preliminary results indicating that this high molecular-weight plasmid seems to be conserved in these strains. Based on these results, we are working on the characterization of these plasmids to establish their possible involvement in virulence.

Supported by: FAPESP and CNPq.

4.16 Cloning and expression of membrane proteins of *Leptospira interrogans*

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Introduction: Leptospirosis is an important global disease of human and veterinary concern, caused by pathogenic spirochaetes of the genus *Leptospira.* Humans are accidental hosts that can be infected by exposure to chronically infected animals and their environment. Bacterial outer membrane proteins (OMPs), particularly those with surface-exposed regions, play a crucial role in the virulence of pathogens and in adaptation to several environmental conditions. Functional genomic studies, including transcription profiles, gene cloning, protein expression and characterization, complement *in silico* analysis and should help our understanding of bacterial pathogenesis. The genome of *L. interrogans* serovar Copenhageni has been sequenced and *in silico* analysis identified more than 200 predicted outer membrane proteins.

Objectives: In this work, our goal was to clone and to study the expression of four genes encoding conserved hypothetical proteins (LIC10411, LIC12891, LIC13305 and LIC11030) with different *Escherichia coli* expression host, inducer concentration and temperature. **Methods:** The genes were amplified by PCR from genomic DNA of *Leptospira interrogans* serovar Copenhageni strain Fiocruz L1-130, using the complementary sequence primers. The genes were cloned into the *E. coli* expression vector pAE at Xho I and Hind III restriction sites. All cloned sequences were confirmed by DNA sequencing in an ABI 3100 automated sequencer. The pAE constructs containing the cloned DNA inserts were employed to transform BL21 SI, BL21 (DE3) Star pLys and BL21 (DE3) *E. coli* strains. Protein expression was analyzed under several conditions, including different concentrations of IPTG /NaCl, protein expression inducers and temperatures. **Results and Discussion:** The choice of predicted proteins was mostly based on their cellular localization. According to the PSORT program, all proteins chosen are predicted to be outer membrane proteins (70% for LIC12891, LIC13305 and LIC11030, and 29.6% for LIC10411). The genes were amplified, without the signal peptide sequence, and the DNA insert cloned and expressed as a full-length protein in *E. coli.* Recombinant proteins were expressed with 6 x His-tag at the N-terminus, facilitating protein purification by metal-affinity chromatography. The recombinant protein LIC10411 was expressed in *E. coli* BL21 (DE3) Star pLys culture with the expected size of 14 kDa. The recombinant protein expressed with 6XHis tag at the N-terminus, was purified by metal chelation chromatography, and an aliquot of each step of the process was analyzed by SDS-PAGE. No expression was observed in cultures containing the pAE LIC12891 construct. Evaluations of the expression of recombinant proteins LIC13305 and LIC11030 are currently underway.

Supported by: FAPESP, CNPq and Fundação Butantan.

4.17 Evaluation of the variability of the candidate vaccine PspC (pneumococcal surface protein C) in isolates of *Streptococcus pneumoniae* from University of São Paulo Hospital

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Introduction: *Streptococcus pneumoniae* is part of the normal microflora of the human nasopharynx, being one of the most common causes of respiratory tract infections. The vaccine composed of different capsular polysaccharides (PS) purified from pneumococci has low efficacy in children and the elderly, besides not being able to induce immunological memory. Although the 7-valent PS vaccine conjugated to CRM197 was an advance, its production cost is still a major barrier for its use by the Brazilian public health system. A proposal to increase the vaccine coverage at a low cost consists in the identification of an antigen common to the majority of strains. As a result, protein antigens present in all isolates of pneumococci are being investigated as possible vaccine candidates. PspC (pneumococcal surface protein C) is described as having a role both in the colonization of the nasopharynx and in invasive infection. PspC is highly polymorphic, where it can be divided into 11 groups. Thus, the evaluation of the variability of the antigen in clinical samples is of great importance to determine the ideal vaccine formulation. **Objectives:** The proposal of the present work was to evaluate the variability of *pspC* in Brazilian pneumococcal isolates. We also proposed the expression of variants of PspC and production of anti-PspC antisera to determine the cross-reactivity with different strains of pneumococcus, so that we can assess the potential of vaccination coverage of the expressed proteins. **Methods:** Pneumococcal strains were obtained from the University Hospital of the University of São Paulo. Strains were serotyped by PCR and 13 isolates were chosen based on the serotypes present in the new 13-valent conjugate vaccine (serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F). The complete *pspC* locus was cloned and the gene sequenced for each isolate. BALB/c mice were immunized with two different recombinant PspC variants for the production of antibodies that were used for Western blot analysis. **Results and Discussion:** Of the 13 pneumococcal isolates analyzed, 6 were found to be from group 3, 3 isolates from group 6, 1 isolate from group 5, 1 isolate from group 8 and 1 isolate from group 9. A duplication containing PspC from group 4 and from group 10 was also found. An antiserum raised against PspC3 was able to recognize the majority of pneumococcal extracts by Western blot analysis, showing a broad cross-reactivity. On the other hand, an antiserum raised against PspC8 was able to recognize only the isolate expressing PspC from group 8. These preliminary results suggest that immunization with PspC3 would be a promising strategy capable of inducing antibodies with broad cross-reactivity.

Supported by: FAPESP, Fundação Butantan and CNPq.

4.18 Proteomic approach for analysis of fimbrial adhesins of atypical enteropathogenic *Escherichia coli*

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Introduction: Enteropathogenic *Escherichia coli* (EPEC) is considered an emerging bacterial agent associated with child endemic diarrhea worldwide and is classified as typical and atypical. Adherence of typical EPEC to the intestinal mucosa is mediated by the outer membrane protein intimin and BFP fimbria. Since aEPEC is devoid of BFP, another fimbriae other than BFP may be involved in aEPEC adherence to the intestine. **Objectives:** To identify by proteomic analysis the fimbrial structures in aEPEC isolates. **Methods:** Three aEPEC strains with different patterns of adherence (localized-like/LAL, aggregative/AA and diffuse/DA) as well as one nonadherent/NA strain were studied. Strains were grown in TSB and fimbriae were extracted and analyzed by two-dimensional gel electrophoresis (2DE). The MW and pI parameters were used for spot selection in a comparative analysis of the isolate LAL with data from SwissProt database. **Results:** The analysis of the fimbrial extracts of the DA aEPEC by 2DE using a pH 3-10 strip demonstrated that fimbrial proteins were concentrated between pH 4.5-6.5 with PI of 5.09 - 6.21 for proteins with MW of 14-16 kDa. Taking this result into consideration, 2DE gels with pH 4-7 strips were performed with extracts of LAL, AA and NA aEPEC strains and several spots were observed. The comparative study of selected spots of the LAL strain with data from SwissProt showed 11 spots with MW and pI similar to that of fimbrial proteins of non-pathogenic *E. coli* K12, UPEC, tEPEC and ETEC isolated from pig and human. **Discussion:** The comparative analysis of data from the isolate LAL with fimbrial proteins deposited on SwissProt showed that 11 fimbrial structures have MW and pI identical to those of described proteins. In contrast, 16 other selected spots between 14 and 22 kDa had no matching, indicating that these proteins have so far not been identified or characterized. Thus, further mass spectrometry analyses (MALDI-TOF peptide mass fingerprint and/or complementary MS/MS analyses) of the selected spots will be performed and the protein nature of these spots may reveal fimbriae structures involved in aEPEC pathogenesis.

Supported by: FAPESP and CNPq.

4.19 Characterization of predicted lipoproteins of *Leptospira interrogans* expressed in *Escherichia coli*

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Introduction: Leptospirosis is a worldwide zoonotic disease caused by pathogenic spirochaetes of the genus *Leptospira.* In urban settings, rodents are the most important carriers of the disease because they continuously shed live leptospires in their urine. Humans can be infected through contact with soil or water contaminated with urine containing leptospires. Since the control of the rodents and sanitation measures are not easily implemented, the development of reliable vaccine is necessary to combat leptospirosis. **Objectives:** The aim of this project was to study three genes that encode predicted lipoproteins selected from the genome sequences of *Leptospira interrogans* serovar Copenhageni. **Methods:** The gene sequences of LIC10258, LIC12880 and LIC12238 were amplified by PCR methodology from genomic DNA of *L. interrogans* serovar Copenhageni and the DNA inserts cloned into the *E. coli* expression vector pAE. The pAE constructs were inserted into BL21 SI *E. coli* strain for protein expression. Subsequently, the recombinant proteins were purified using affinity chromatography. The secondary structure content of the purified proteins was evaluated by circular dichroism (CD) spectroscopy. The cellular localization was performed by liquid-phase immunofluorescence assay (L-IFA). The reactivity of recombinant antigens with human sera of patients diagnosed with leptospirosis was analyzed by ELISA. **Results and Discussion:** Structural integrity of the recombinant proteins was assessed by CD spectroscopy. All proteins showed secondary structures as none of the spectra showed a flat line, characteristic of the denatured non-structured form. Polyclonal serum against rLIC12880 showed positive green fluorescence, which suggests that this protein is surface exposed. The reactivity of the recombinant proteins against paired serum from early and convalescent phase of confirmed leptospirosis patients was evaluated by ELISA. The data showed that all proteins tested were reactive with IgG and IgM antibodies present in the convalescent phase (MAT+). The recombinant protein rLIC12880 did not recognize antibodies in the early phase of the disease (MAT-). However, the rLIC10258 showed a significant frequency of responders (~30%) against IgG antibodies in the early phase. This is important because overall the sensitivity of the tests to diagnose the disease in the first week of illness has been reported to be less than 25%. Therefore, further evaluation of the recombinant protein rLIC10258 with larger samples will indicate its appropriateness for diagnostic purposes.

Supported by: FAPESP, CNPq and Fundação Butantan.

4.20 Characterization of a thermoregulated adhesin of *Leptospira interrogans*

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Introduction: Extensive studies aimed at understanding the pathogenesis of *Leptospira* have provided important knowledge about virulence factors. Thus, the expression of genes involved in virulence that are regulated by temperature, as well as the production of proteins capable of interacting with extracellular matrix, are considered critical events during the infection process. **Objectives:** In this study, we set out to characterize a protein, LipL53, from *L. interrogans* previously identified in screening studies to react with a serum sample of an individual diagnosed with leptospirosis (Gamberini et al., 2005). **Methods:** The recombinant protein was expressed as an insoluble form in *E. coli* and refolded by decreasing concentrations of urea during the purification throughout Ni^{2+}-charged chromatography. The presence of the LipL53 transcripts among pathogenic serovars of *Leptospira* and the effect of temperature shift on LipL53 transcription profile were evaluated by RT-PCR. The binding ability of this protein with extracellular matrix components was analyzed by ELISA. **Results and Discussion:** The secondary structure content of recombinant LipL53 as assessed by circular dichroism showed a mixture of β-strands and α-helix. The presence of LipL53 transcripts at 30°C were only detected within the virulent strains. However, upon shifting the attenuated cultures of pathogenic strains from 30 to 37 and 39°C these transcripts could also be observed. The attachment of LipL53 to laminin, collagen I and cellular and plasma fibronectin was specific and dose-dependent. Our results suggest that LipL53 is a novel adhesin of *L. interrogans* that could have an important role in the pathogenesis of the disease.

Supported by: FAPESP, CNPq and Fundação Butantan.

4.21 Application of BacT/ALERT 3D system in pulmonary surfactant production control

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Introduction: BacT/ALERT 3D (B/A 3D) system is an automatic equipment used for early detection of bacterial and fungal contamination, and the flasks containing culture media provides nutritional and environmental conditions suitable for microbial growth. This system uses a colorimetric sensor and reflected light to monitor the presence and production of carbon dioxide dissolved in the culture medium, produced as a result of microbial metabolism. Pulmonary surfactant (PS) is a biopharmaceutical extracted from the lungs of pigs used in respiratory distress syndrome which affects newborns. Traditional evaluation of bacterial and fungal sterility of this product is made by direct inoculation (ID) and visual analysis over 14 days. **Objectives:** To implement the B/A 3D system during PS production control by evaluating sensitivity and ability to detect microbial contaminants, reducing the retention time of the product being tested. **Methods:** We used three batches of SP production previously approved by bacterial and fungal sterility tests using traditional methods. We used the Bact/ALERT FA media, with sample volume of 1.0 mL, injected directly into the culture medium and kept under observation for 14 days. At the end of the observation period, there was a new inoculum of ATCC microorganism dilution. Each batch was challenged with an inoculum of less than 100 CFU/mL of the following microbial strains: *Aspergillus niger*, *Bacillus subtilis Candida albicans*, *Clostridium sporogenes*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Sensitivity of culture media was tested with negative control (sterile water) and positive controls (ATCC microorganisms diluted without product in test). **Results and Discussion:** The three lots tested did not reveal the presence of contamination, as with the traditional method. Bact/ALERT FA tested with the product and the inoculum of microorganisms detected ATCC strains with the following average time recovery: *A. niger* 38.2 h, *B. subtilis* 12.7 h. *C. albicans* 35.8 h, *P. aeruginosa* 13.9 h and *S. aureus* 18.3 h. The negative control showed no microbial growth and the positive pure cultures revealed the following recovery times: *A. niger* 41.0 h, *B. subtilis* 12.7 h, *C. albicans* 49.7 h, *P. aeruginosa* 14.4 h and *S. aureus* 20.6 h. The results showed that the B/A 3D system can be used to control the production process, reducing significantly product retention time. Inhibitory components of microbial growth incorporated in the product composition are completely neutralized by BacT/ALERT FA allowing low levels of contamination detection.

Supported by: Fundação Butantan.

4.22 Quorum sensing in atypical enteropathogenic *Escherichia coli*

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Introduction: Quorum sensing is used to designate a mechanism of gene regulation depending on cell density. The bacteria produce substances that accumulate in growth media, and when these substances reach a threshold concentration, a variety of responses can occur. These molecules are called autoinducers and the phenomena called quorum sensing. At least, four quorum sensing systems were described: two of these systems, which use autoinducer-1 (AI-1) and autoinducer-3 (AI-3), are found in Gram-negative cells while the Gram-positive cells use an autoinducing polypeptide (AIP) system. The fourth system, using autoinducer-2 (AI-2), is found in Gram- positive and Gram-negative cells and might represent a generalized signaling system. It was demonstrated that quorum sensing is involved in type III secretion system regulation, flagellation, and motility in enteropathogenic and enterohemorragic (EPEC and EHEC, respectively) *Escherichia coli* strains. **Objectives:** In this study, we developed the possibility of crosstalk *in vitro* among atypical EPEC and commensal *E. coli.* **Methods:** Pre conditioned medium: bacterial strains were grown to 37°C with aeration until reaching an OD_{600} of 1.0. The growth was centrifuged, and the supernatant was filtered through a 0.22-µm membrane. B-Galactosidase assay: a reporter TEVS232 strain containing the *LEE1:lacZ* fusion was grown in pre conditioned medium until reaching an $OD_{600} \leq 0.2$, and β-galactosidase activity was measured in Miller units. Adherence assays: bacterial strains were grown for 18h in LB medium at 37°C. For the non-induced overnight cultures, 10^5 CFU were added to HEp-2 cells, which were then incubated for 6 h at 37°C with 5% CO_2 , washed with PBS, fixed with methanol and stained with Giemsa stain. PCR: DNA templates for PCR were obtained from overnight *E. coli* cultures that were pelleted, resuspended in 500 µl of sterile deionized water and boiled for 10 min. The gene analyzed in this study was *cif.* Amplified samples were detected by 1% agarose gel electrophoresis in Tris-borate-EDTA buffer and ethidium bromide staining. **Results and Discussion:** It was possible to verify the production of autoinducer AI-3 in the strains tested, and also to quantify their ability of induction. Of the 72 samples studied, 29 tested positive for the gene. The fragment obtained by PCR was submitted to DNA sequencing and showed 98% similarity with the gene described in the literature. Apparently, the inoculation of cell cultures with enterobacteria influences the cellular responses to EPEC strains.

Supported by: FAPESP.

4.23 Effects of recombinant β subunit phycocyanin in HEp-2 cells

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Introduction: C-phycocyanin (C-PC) is a water-soluble pigment. It is found in some blue-green microalgae such as *Spirulina platensis*, which are used in many countries as dietary supplements. It consists of two subunits, α and β, with molecular masses of 16 and 17 kDa, respectively. One recent study demonstrated that the β subunit of Anabaena C-PC (C-PC) has anti-tumor activity, since the C-PC inhibits cell proliferation and promotes apoptosis in cancer cells. **Objectives:** The aim of this study was the cloning and expression of the β subunit of *A. platensis* C-PC in *Escherichia coli* and to check if the recombinant protein is able to induce apoptosis in the cell line HEp-2. **Methods**: The subunit was cloned in pGEMT- easy plasmid vector producing pTMP-01 plasmid and subcloned in pET28a plasmid vector giving rise to pTMP-02 plasmid. When the cells transformed with the plasmid pTMP-02 reached a growth of OD_{600} between 0.5 and 0.6, they were induced with 1 mM IPTG for 4 h. The gene expression was confirmed by RT-PCR and immunoblotting. The recombinant proteins were purified with Ni-NTA column and were dialyzed and refolded. HEp-2 cells were submitted to incubation with 50 µg of the recombinant protein. The cells were stained with 0.025 % toluidine blue solution in McIlvaine buffer pH 4.0 for 15 min and followed by treatment with 0.05 M aqueous $MgCl_2$ solution for 15 min (critical electrolyte concentration method – CEC). **Results and Discussion:** The β subunit was cloned and expressed in *E. coli* BL21. The CEC method showed apoptosis in cells treated with the recombinant protein. As CEC method is used only as a sign of apoptosis, other tests are needed to quantify apoptosis.

Supported by: FAPESP.

4.24 Expression of heat-labile (LT) and heat-stable (ST) toxins produced by enterotoxigenic *Escherichia coli* in different enrichment media

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Introduction: Among the diarrhea-associated *Escherichia coli* pathotypes, the enterotoxigenic *Escherichia coli* (ETEC) have been shown to cause up to 10 % of diarrhea in Brazil. These strains produce the enterotoxins, heat-labile (LT) and/or heat-stable toxins (ST) and colonization factors, which allow the organisms to readily colonize the small intestine, and in this way cause diarrhea. Since ETEC can be detected by enterotoxin production, diagnosis must depend upon identifying either LT and/or ST. A prerequisite for successful detection is the production and secretion of the protein in sufficient amounts. There are several conditions and media described to increase toxin production and/or release, including the presence of bile salts or antibiotics such as lincomycin. **Objectives**: Evaluation of ETEC growth and LT/ST production in different broth media. **Methods:** LT- and ST- producing ETEC strain (H10407) was grown at 37 °C (250 rev min^{-1}) for 24 h in five different broth media: Evans, Syncase, tryptic soy broth, *E. coli* (EC) and Dulbecco's Modified Eagle Medium. Bacterial growth was determined at 588 nm, and toxin production was measured in the supernatant by enzyme-linked immunosorbent assay (ELISA). Afterward, the same assay was performed using the adequate medium containing ciprofloxacin or lincomycin. Besides, a collection of 31 ETEC strains was also evaluated in EC broth in the presence or absence of both antibiotics. ST and LT production was detected in the supernatants and in urea-treated pellets of bacterial growth after 7 and 24 h incubation. **Results and Discussion:** The highest level of both toxins in the supernatant was obtained when the H10407 strain was grown in EC broth. Despite the fact that the reference strain showed low growth in the presence of lincomycin, LT and ST production was higher in the medium containing this antibiotic. When the collection of LT-producing ETEC isolates was tested, for some of them LT production increased in the presence of ciprofloxacin, and for other isolates LT production increased in the presence of lincomycin. On the other hand, 85% of the ST-producing ETEC isolates showed considerable increase in toxin when they were grown in the presence of ciprofloxacin. Previous work in our laboratory has demonstrated that EC broth is a suitable medium for Shiga toxin production of STEC isolates and that it is increased with use of ciprofloxacin. In this study, we confirmed that the EC medium can also be employed to enhance ETEC toxin production and that the use of antibiotics could improve toxin production/release. The medium containing lincomycin has been shown to increase LT expression for the H10407 strain, but our results indicate that not all ETEC isolates respond in the same way. Therefore, it is not yet possible to establish a standard condition for LT expression using only lincomycin or ciprofloxacin. Further studies are necessary to optimize LT expression.

Supported by: FAPESP.

4.25 **Analysis of the LEE region of atypical enteropathogenic *Escherichia coli* strains**

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Introduction: Enteropathogenic *Escherichia coli* (EPEC) cause the attaching-effacing (A/E) lesion on the intestinal mucosa. A/E is triggered by proteins encoded by the LEE region, which is organized into 5 operons (LEE 1-5). EPEC has been classified as typical and atypical (aEPEC), based on the presence or absence of the EAF plasmid, respectively. In cultured epithelial cells, aEPEC mainly displays the localized-like adherence (LAL) pattern, although the aggregative (AA) or diffuse (DA) adherence may be expressed. **Objectives:** Structural and functional analysis of the LEE region of aEPEC strains. **Methods:** Four aEPEC strains were studied: LAL (O55:H7), DA (O55:H7), AA (O125ac:H6) and nonadherent/NA (O88:HNM). Adherence and capacity to cause A/E (FAS assay) were investigated in HeLa, HEp-2, Caco, T84 and HT29 cells, and Tir phosphorylation in HEp-2 cells. The *tccP (espFu)* gene was searched by PCR. The presence of 31 LEE genes was searched by PCR and slot blot. Transcription of LEE operons was measured by real time PCR (qRT-PCR) and microarray, after the bacterial growth in DMEM (microarray and qRT-PCR) and after bacterial incubation with HeLa cells (qRT-PCR). Expression of intimin, Tir, EspA, EspB and EspD was detected by immunoblotting. **Results and Discussion:** The adherence patterns observed in HEp-2 and HeLa cells were maintained in all cell lines of intestinal origin. The capacity to cause A/E, to phosphorylate Tir and the presence of *tccP* was detected only in the LAL-expressing strain. Among the LEE genes tested by PCR, 11 were not detected in different strains, but these genes were detected by slot-blot. Therefore, the genetic structure of LEE is intact in all aEPEC strains. Transcriptional profiles of LEE as measured by qRT-PCR were analyzed in comparison to the atypical EPEC strain BA320 (LAL/FAS+). LEE 1-5 transcription levels were decreased in the AA, DA and NA strains in both culture conditions (DMEM and HeLa cells), except for LEE 4 (*espA*) which showed higher transcription level in the DA strain in DMEM. Microarray analysis demonstrated that the transcription levels were decreased in comparison to BA320 in the AA, DA and NA strains. All four aEPEC strains studied expressed intimin, Tir, EspA, EspB and EspD. Despite the incapacity to cause A/E, all LEE genes were detected in the AA, DA and NA strains, and the transcription and expression of LEE 1-5 demonstrated that LEE is functional in these strains. The incapacity to cause A/E in these strains could be due to the absence of *perABC* regulators and/or to the inability of Tir phosphorylation and/or the absence of *tccP* expression.

Supported by: FAPESP.

4.26 The adhesion patterns to epithelial cells of atypical enteropathogenic *Escherichia coli* is modified by secreted proteins that bind to extracellular matrix components

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Introduction: Atypical enteropathogenic *Escherichia coli* (aEPEC) have been a leading cause of childhood diarrhea in developing countries. The main mechanism of atypical EPEC pathogenesis is a lesion called attaching and effacing (A/E), which is characterized by intimate adherence of the bacteria to the intestinal epithelium and destruction of microvillus. Nevertheless, it represents a heterogeneous group and other virulence factors may be involved in atypical EPEC pathogenesis. Previously, we have identified one isolate of atypical EPEC, serotype O26:H11, which secretes proteins that interact with ECM macromolecules. The interactions between pathogenic bacteria and ECM molecules such as fibronectin, laminin and collagen may play an important role in bacterial adherence to and invasion of host cells. Adhesion is critical for successful *E. coli* colonization of the gastrointestinal tract and is mediated by adhesins. **Objective:** The aim of this study was to identify putative adhesins that may contribute to the binding of the isolate of atypical EPEC to extracellular matrix components and to characterize the influence of this interaction in the adhesion to epithelial cells. **Methods:** The supernatant of the atypical EPEC isolate was submitted to a solid phase binding assay with matrigel (a mouse basement membrane composed mainly of laminin, collagen IV and fibronectin), the adhered proteins were stripped from the wells, separated by SDS-PAGE, transferred to nitrocellulose membrane and submitted to immunoblotting assay. **Results and Discussion:** Three major proteins with apparent molecular weights of 107 kDa, 44 kDa and 35 kDa were recognized by the anti-protein polyclonal serum (produced in rabbit immunized with the isolate's supernatant) through immunoblotting assay. In addition, we observed that in the presence of ECM components the isolate clearly changes its adherence pattern to HEp-2 cells, and the number of adhered bacteria to these cells. The atypical EPEC do not share a unique pattern of virulence, suggesting that many virulence factors may contribute to the pathogenesis. The identification of proteins involved in the adhesion with extracellular matrix components in one isolate of this category of diarrheagenic *E. coli* confirms the heterogeneity among the atypical EPEC.

Supported by: FAPESP.

4.27 Milk, an alternative food source for *Paramecium caudatum* culture

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Introduction: Free-living protozoa are of fundamental importance for the equilibrium of the ecosystem. They are also used in the area of biotechnology for the development of remedies, cosmetics and bio-insecticides. In ecology, apart from contributing to the biodiversity of the microbial fauna, they recycle nutrients and control bacterial populations. The standard methods of culturing protozoa are laborious, time-consuming and costly, since they utilize *Enterobacter aerogenes* as a source of food for the protozoa, and boiled rice to feed the *Enterobacter*. For this reason, the development of quicker and more practical methods to culture protozoa is essential to reduce cost and time and to facilitate their manipulation. **Objective**: The objective of this work was to test an alternative method for the culture of *P. caudatum* using whole cow's milk as food source. **Methods**: For the traditional culture of *Paramecium caudatum*, one grain of boiled rice and 1 ml of sterile mineral water containing 10^6 cells of *Enterobacter aerogenes* were added to 10 ml of sterile mineral water and incubated at 30° C for 120 h. During this period, *Paramecium* growth was determined every 6 h by counting visually the number of cells with the help of a magnifying glass. For the alternative culture of *Paramecium caudatum*, the same procedure as described for the traditional method was used, except that rice and *Enterobacter aerogenes* were replaced by 20 µl of whole milk. **Results and Discussion**: The results showed that *P. caudatum* grows faster on milk than when cultured using the traditional method. Utilizing milk as a source of food in the culture of *P. caudatum* is an easier, less expensive and faster way to grow *Paramecium* ssp.

4.28 Plasmid-encoded toxin (Pet) expressed by enteroaggregative *Escherichia coli* and by atypical enteropathogenic *Escherichia coli*: a comparative study

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Introduction: Among diarrheagenic *Escherichia coli* pathotypes, enteroaggregative *Escherichia coli* (EAEC) and atypical enteropathogenic *Escherichia coli* (aEPEC) have emerged as important pathogens causing diarrheal disease in multiple epidemiologic and clinical settings. It is known that EAEC adhere to the intestinal mucosa and produce enterotoxins and cytotoxins, which lead to a secretory diarrhea and mucosal inflammation. One of the toxins believed to contribute to the pathogenesis of EAEC is the plasmid-encoded toxin (Pet), classified as a serine protease auto-transporter. A role of this protein in mediating enterotoxic activity, is the development of cell exfoliation. EPEC adheres to intestinal epithelial cells and subverts cytoskeletal processes through a histopathological activity on intestinal epithelial cells termed “attaching and effacing” (A/E) lesion. While typical EPEC is homogeneous regarding virulence factors, aEPECs constitute a very heterogeneous group, which can show virulence factors common to other *E. coli* pathotypes. So far, little is known about the repertoire of toxins that aEPEC express. **Objectives:** i) To investigate Pet toxin expression in different aEPEC serotype isolates; ii) To compare Pet toxin expression of aEPEC with EAEC. **Methods**: Cytotoxicity assays in HEp-2 cells were performed with the bacterial culture or the supernatant of the bacterial culture from aEPEC isolate 2275 (O113:H19) *pet/sat*, 3160 (O110:H-) *pet/sat/ Ehly*, 2923 (O34:H6) *pet/sat*, and 2991 (O34:H) *pet/sat,* whose genes were amplified by multiplex PCR, isolate 3170 (O145:H2) PCR negative, and EAEC isolates 91A5; 91A9; 215 A2; 215A3; 215A4; 219A4; 252A1. Purified Pet toxin (200 µg/mL) and 042 (O44:H18), were used as positive controls. **Results and Discussion**: All the isolates tested, except 3170, caused cellular damage when the cells were incubated with the bacterial culture. These cytotoxic effects were neutralized when the bacterial isolates were incubated with PMSF, a serine protease inhibitor, or with the IgG enriched fraction from the anti-Pet polyclonal serum. However, no cytotoxic effects were detected in assays performed with the culture supernatants from the same isolates. The Pet toxin was identified, by immunoblotting, in the culture supernatants from all aEPEC isolates, except 3170. This work demonstrates that several aEPEC isolates may express the Pet toxin, like the EAEC isolates tested; where the *pet* gene was amplified these were able to produce cellular damages such as rounding, elongation and detachment, all typically caused by the Pet toxin. Furthermore, Pet is secreted in the supernatant of the bacteria grown in TSB, but remains inactive in the supernatants from both aEPEC and EAEC. These results demonstrate for the first time that isolates of atypical EPEC may express the Pet toxin like EAEC, attesting to the elevated heterogeneity observed among aEPEC. The lack of Pet toxin activity in the culture medium must be further investigated, but this result suggests that an accessory molecule may be necessary for its activity, which is probably absent or inactive in the supernatant.

Supported by: FAPESP.

4.29 Analysis of virulence genes in atypical enteropathogenic *Escherichia coli*

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Introduction: Diarrhea continues to be one of the most common causes of morbidity and mortality among infants and children, especially in developing countries. Among the bacterial pathogens, diarrheagenic *Escherichia coli* (DEC) is an important agent of endemic and epidemic diarrhea worldwide. The diarrheagenic *E. coli* strains can be classified into six main pathotypes, based on specific virulence properties, clinical features, association with serotypes O:H, epidemiological aspects, and patterns of interaction with cellular strains. Enteropathogenic *Escherichia coli* (EPEC) cause a histopathological lesion known as "attaching and effacing" (A/E). Typical EPEC differs from atypical EPEC by the presence of a plasmid called EPEC adherence factor (EAF) which encodes the bundle-forming pilus (BFP). Atypical EPEC comprises a very heterogeneous group. **Objective:** We developed multiplex PCR reactions in order to identify virulence genes present in other DEC pathotypes in a 72-sample atypical EPEC collection. **Methods:** DNA templates for PCR were obtained from overnight *E. coli* cultures that were pelleted, resuspended in 500 µl of sterile deionized water and boiled for 10 min. The PCR was developed by combining specific primers for *efa1/lifA*, *pic, pet, astA, hly, sat, toxB, ldaH, ehly1, ehly2, sheA, cdt and saa.* Amplified fragments were detected by 1% agarose gel electrophoresis in Tris-borate-EDTA buffer and ethidium bromide staining. **Results and Discussion:** The results obtained by PCR were confirmed by *Southern blotting* and DNA sequencing. The following were among the most prevalent genes found: *efa* 14.7%, *pic* 1.67%, *pet* 4.17%, *astA* 32.5%, *hly* 0.83%, *sat* 18.83%, *toxB* 2.5%, *ldaH* 6.67%, *ehly*1 5%, *she*A 56.9%, and *cdt* and *saa* 0%. Based on the results, we can affirm that most atypical EPEC strains carry virulence factors common to those of other DEC.

Supported by: FAPESP and CNPq.

4.30 Prevalence and antimicrobial profile of uropathogens isolated from the urine of children with urinary tract infection (UTI) from Darcy Vargas Children´s Hospital

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Introduction: The most common bacterial infection recorded in clinical medicine worldwide is urinary tract infection (UTI). *Escherichia coli* is the principal etiological agent isolated in cases of UTI. Recently, an increase has been observed in the antibiotic resistance of pathogens responsible for UTI. This is a serious problem, especially for pediatric patients, to whom treatment is given without the help of an antibiogram analysis. Therefore, it is essential to have prior knowledge of the uropathogens and their antibacterial resistance profile in the population of different regions, since antibiotic resistance of uropathogens varies according to the treatment received. However, few studies have been published about the prevalence and antibiotic resistance of uropathogens especially in children. **Objective:** The objective of this work was to characterize the prevalence of uropathogens, and to determine their resistance to antibiotics in children with clinical diagnosis of UTI. **Methods**: Two hundred and six patients of both sexes between 0 to 15 years of age with clinical diagnosis of UTI from Darcy Vargas Children´s Hospital were analyzed in this work. Bacterial samples isolated from their urine were identified by growth in IAL medium (Instituo Aldolfo Lutz). The antibiogram analysis of the bacterial isolates was determined by the disc diffusion method described by Kirby & Bauer. **Results and Discussion**. The bacterium most prevalent in the urine samples was *Escherichia coli* (54.81%), followed by *Proteus ssp* (18.27 %), *Enterobacter* sp. (5.77%), *Pseudomonas sp.* (6.73%), *Klebsiella* sp. (3.85%), *Enterococcus faecium* (2.88%) and *Candida* spp (6.73%). These results are in accordance with the data obtained from studies performed in different Brazilian age groups, except for the high level of *Candida* spp encountered. The antibiogram results obtained in this work showed that 63.55% of the samples tested are resistant to ampicillin, 37.44% to nitrofurantoin and 29.56% to sulphametrin. Ampicillin is rarely used to treat UTI, but sulfamethoprim is a sulfanomide, a group of antibiotics considered first choice for treatment of UTI. Out of all samples tested, 26% were sensitive to all antibiotics tested, of which 80% were *Escherichia coli.* It is interesting to note that 66.35% of all patients tested were boys whereas only 33.65% were girls. These results are in contrast to the data described in the literature, which indicates that most cases of UTI are recorded in women independent of their age group. These results indicate that the use of antibiotic for treatment of urinary infection can delay the recovery of pediatric patients, which can lead to chronic infection and later renal complications. The results also suggest that the use of antibiotics can induce bacterial resistance in the intestinal commensal bacteria, since 76 % of uropathogens are resistant to at least one of the antibiotics used for urinary infection. In addition the higher number of urinary infection detected in boys can be related to lack of hygiene and the presence of phimosis.

Supported by: CAPES.

4.31 New biological function/feature of crotamine

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Introduction: Nowadays, different functional classes of biologically active peptides and toxins isolated from many organisms are known. These compounds can be directly used in medicine or may serve as models for the generation of molecules of medical interest. Crotamine is the most abundant toxin found in the venom of the South American rattlesnake *Crotalus durissus terrificus* and it has been shown to be one of these active peptides with potential pharmaceutical application. This toxin is a strongly basic 42-amino acid residue polypeptide with a molecular weight of about 4.9 kDa. Injection of crotamine in mice induces skeletal muscle spasms, leading to spastic paralysis of the hind limbs, which leads to its inclusion in the small basic myotoxin family. The presence of three disulfide bridges in the crotamine structure gives a high conformational stability to this compound. Interestingly, this same disulfide bridge pattern is also found in antimicrobial peptides from mammals, which usually also show a positively charged surface like crotamine. However, up to now, crotamine was never consistently evaluated as an antimicrobial compound. **Objective:** This study aimed to characterize the antimicrobial activity of crotamine against fungi and bacteria (Gram-negative and positive). **Methods:** The aim of the study was to determine the antimicrobial activity of crotamine purified from the rattlesnake venom, against 10 microorganisms. A colorimetric broth microdilution method was employed for MIC (minimum inhibitory concentration) determination. Microdilution testing was performed according to National Committee for Clinical Laboratory Standards (NCCLS) recommendations (NCCLS document M27-P). Using control growth for comparison, reference microdilution MIC endpoints for crotamine were scored (+) as the lowest concentration at which an absence of growth was observed, and those in which a prominent decrease in turbidity was observed were scored as (++). **Results and Discussion:** The antimicrobial assay demonstrated that crotamine is mainly able to inhibit the growth of fungus, either from reference strains from American Type Culture Collection (ATCC) as well as from clinical isolates. The growth of *Candida krusei, Trichosporon klebahnii, Candida guilliermondii, Candida glabrata, Candida albicans, Candida parapsilosis,* and *Candida tropicalis* was clearly inhibited by crotamine under the conditions used. The antimicrobial activity of crotamine was characterized and the data obtained suggest that this natural compound is a potential candidate for the development of a novel class of antimicrobial compound to treat clinical infections. **CEP:** 1474/07.

Supported by: FAPESP and CNPq.

4.32 Validation of sterility test in isolator of adsorbed diphtheria, tetanus and pertussis vaccine (DTP) produced at Instituto Butantan by means of bacteriostatic and fungistatic effect

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Introduction: The verification of sterility of adsorbed diphtheria, tetanus and pertussis vaccine (DTP) produced by Instituto Butantan is done by filtering the product with 0.45 µm porosity membrane, rinsing this membrane with a neutralizing fluid and subsequent incubation in thioglycollate and soybean-casein digest media. The current standards require that all operational procedures used in quality control must be validated according to Good Laboratory Practice (GLP). **Objectives:** The aim of this study was to determine the sensitivity of membrane filtration methodology applied to bacterial and fungal sterility test in DTP vaccines in isolators and to validate this technique under Microbiological Control Section conditions. **Methods:** This study was performed using three batches of DTP vaccine produced by Instituto Butantan, previously evaluated for thimerosal concentration by a spectrophotometric method. These product batches were tested according to standard methods and membranes were rinsing with Diluent Neutralizing Pharmacopoeic liquid, DNP. After transferring the content of the container to be tested to the membrane, an inoculum of a small number of viable ATCC microorganisms (not more than 100 cfu) was added to the final portion of DNP fluid used to rinse the membrane. Fluid thioglycollate medium (LTM) was challenged with *Clostridium sporogenes, Pseudomonas aeruginosa* and *Staphylococcus aureus,* and soybean-casein digest medium (SCM) was challenged with *Aspergillus niger, Bacillus subtilis* and *Candida albicans*. Culture media were incubated for not more than 5 days at 20 °C - 25 °C and 30 °C –35 °C (SCM and FTM, respectively). **Results and Discussion:** Clearly visible growth of all microorganisms was obtained after the incubation period. The methods applied in the isolator to test the bacterial and fungal sterility of DTP vaccine were effective, and thimerosal present in the product formulation as preservative was completely inactivated, raising the membrane with DNP fluid, allowing the detection of low levels of microbial contamination, ensuring product quality. The sterility test may be carried out without further modifications.

Supported by: Fundação Butantan.

4.33 *Leptospira interrogans* interacts with human plasminogen leading to fibronectin degradation and immune evasion

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Introduction: Leptospirosis is one of the most spread zoonosis worldwide, caused by spirochetes of genus *Leptospira.* Although genomic sequences are available, molecular aspects of pathogenesis, virulence and invasion processes by which these bacteria infect the hosts are poorly characterized. It is well documented that interaction of pathogens with the extracellular matrix (ECM) could play a primary role in the adherence and colonization of host tissues. Indeed, the ability of the leptospires to adhere to ECM has been recently shown. For tissue penetration, proteolytic activity achieved by subversion of host proteases by pathogens, such as plasmin, has been demonstrated to be important in various bacterial infections. Plasmin is a broad-spectrum serine protease component of the fibrinolytic system, composed by the zymogen plasminogen (PLG). **Objectives:** Based on these assertions, we investigated the ability of *Leptospira* to bind PLG and the possible implications in pathogenesis. **Methods:** Bacterial binding to plasmin(ogen) was evaluated by indirect immunofluorescence and by measuring the degradation of specific plasmin substrate. Affinity immunoblotting or modified ELISA was performed to assess the binding of PLG to leptospiral proteins. Human plasma was used to examine immune evasion by modified ELISA. **Results and Discussion:** We demonstrated that leptospires bind purified or plasma PLG *in vitro*, and that binding seems to occur via lysine residues. The binding of PLG to the outer surface of living leptospires was confirmed by confocal microscopy. The PLG-bound bacteria did not exhibit impaired growth and acquired proteolytic activity after addition of exogenous plasmin activator, as evaluated by the degradation of specific plasmin substrate. Plasmin activation was also detected in several species of *Leptospira* but a significantly higher level was observed in a low-passage, virulent strain of *L. interrogans* serovar Copenhageni compared to high-passage non-virulent strain, suggesting the role of this interaction in virulence. Supporting this observation, we demonstrated that several proteins bind PLG in virulent and non-virulent leptospires. We also showed evidence for the participation of fractioned outer membrane proteins in the PLG interaction and showed that neither temperature nor osmolarity shifts in host conditions seemed to influence the binding. Plasmin-coated virulent leptospires were capable of degrading purified ECM fibronectin, an activity that could be important during tissue penetration. We also suggest the role of PLG-binding in the leptospires immune evasion by demonstrating that bacteria bound to plasmin(ogen) are capable of diminishing human C3b complement and human IgG deposition. Our data provide for the first time evidence for the generation of active plasmin on the surface of *Leptospira*, as well as the implications of this phenomenon in infection, tissue penetration and immune evasion. These results give new insights into the understanding of the leptospiral infectious process and molecular pathogenesis of the disease.

Supported by: FAPESP, CNPq and Fundação Butantan.

4.34 Antimicrobial susceptibility profiles and biofilm formation of *Staphylococcus aureus* strains

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Introduction: *Staphylococcus aureus* is one of the most important pathogenic bacteria, where it is frequently associated with nosocomial infections. The use of invasive procedures (sera, catheters and surgeries) in patients can also cause an infectious process. *S. aureus* has shown resistance to multiple antimicrobial agents, making it difficult to treat infections. Biofilm is a sessile microbial community characterized by cells that are adhered to a substrate or interface, enclosed in a self-produced polymeric matrix and adherent to an inert or living surface. Biofilm formation is associated with bacterial persistence and resistance to antibiotics. *S. aureus* is capable of biofilm formation, which increases its persistence and boosts its levels of antimicrobial resistance. The importance of studying the biofilm formation in *S. aureus* strains is essential for understanding its role in pathogenesis making it possible to develop therapeutic alternatives. **Objectives:** In this study we evaluated the susceptibility rates of 12 antibiotics and the capacity of biofilm formation on abiotic surfaces in *S. aureus* strains that were isolated from cutaneous abscesses of patients seen at Vital Brazil Hospital and strains of the bacteriological collection of Bacteriology Laboratory of the Butantan Institute. **Methods:** The bacteria were tested by the method of Kirby-Bauer for the antimicrobial susceptibility, utilizing commercially available sensitivity discs and Muller-Hinton agar. The quantitative analysis of the biofilm formation was carried out in polystyrene plates using the crystal violet colorimetric assay, for an incubation period of 24 h at 37°C, after which absorbance was determined at 595 nm in an ELISA plate reader. **Results and Discussion:** The strains of *S. aureus* were sensitive to the following antibiotics: amoxicillin and vancomycin (100%), oxacillin (96%), chloramphenicol (81%), cotrimoxazole (81%), gentamicin (81%) and ciprofloxacin (78 %). Resistance was observed for penicillin (93%), clindamycin and tetracycline (22%) and amikacin (19%). Regarding the antibiotic erythromycin, strains were observed with intermediate sensitivity (41%) and resistant strains (33%). These results demonstrate a variable antimicrobial sensitivity. The high number of resistant strains (mainly for penicillin) shows the necessity of controlling the spread of antibiotic-resistant *S. aureus* strains. Routine monitoring of antibiotic resistance provides data for adequate antibiotic therapy and resistance control of *S. aureus* strains. Based on the colorimetric test with crystal violet, 33% of the strains showed low biofilm formation (OD = 0 – 1.200), 53% showed intermediate formation (OD = 1.201 – 2.400), and only 13% showed high capacity of biofilm formation (OD> 2.401). The majority of samples showed an intermediate level of biofilm formation. This capacity can be explained by the adherence of bacterial cells to a surface and accumulation depending on the growth of bacteria in the multilayer. The capacity of biofilm formation may be important to the existence of bacterial persistence and the resistance to antibiotics in patients.

5: Cellular Biology and Genetic

5.01 Effects of a Kunitz-type inhibitor (Amblyomin-X) on cell cultures of human renal carcinoma

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Introduction: The incidence of renal carcinoma (RCC) has been detected more frequently nowadays, which now represents 50% of newly diagnosed cases. Approximately 30% of patients with RCC have metastatic disease. Being a disease that highly resistant to chemotherapy and radiotherapy, surgical treatment in early stages, where it is organ-confined, is common. However, after nephrectomy, 60% of patients develop metastasis within 10 years. Amblyomin-X is a recombinant protein produced from *Amblyomma cajennense* cDNA library. This protein is able to inhibit the coagulation factor Xa and also induce apoptosis in different tumor cell lines. **Objectives:** To evaluate the response of renal tumor cells (Caki-1 and Renca) treated with Amblyomin-X and to analyze the presence of inflammation molecules in the microenvironment of treated cells. **Methods:** The morphological characteristics and death of the cells (Renca and Caki-1) were determined by optical microscopy and by MTT assay. DNA content and the phases of the cell cycle were analyzed by flow cytometry (guava system GE), and the levels of interleukin-6 determined by ELISA. **Results and Discussion:** Amblyomin-X induced cytotoxicity in both analyzed cell lines, causing morphological changes. The responses were found to be dose- and time-dependent. Decreases in all stages of the cell cycle and levels of IL-6 were observed after 24 h of treatment. All together, our results suggest that Amblyomin-X exerts a strong cytotoxic effect in renal cells lines.

Supported by: FAPESP.

5.02 Genetic polymorphism of IL-3Rα confers differential proliferation and greater differentiation to neutrophils in bone marrow cells

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Introduction: Hemopoiesis is a dynamic and gradual process on cell development that occurs by the sequential differentiation of hematopoietic stem cells (HSCs) into multipotential progenitors and terminally differentiated blood cells under the action of hematopoietic cytokines and transcription factors. Mice genetically selected for high (AIRmax) or low (AIRmin) acute inflammatory response show interline differences in the number of infiltrated cells in inflammatory exudate produced by s.c. injection of polyacrylamide beads (biogel). **Objectives:** We analyzed the proliferative capacity of bone marrow cells and neutrophil differentiation in AIRmax and AIRmin mice. **Methods:** To evaluate the myeloid cells proliferation, we used a five-day suspension culture method with IL-3+SCF combined with all-trans retinoic acid (ATRA) as *in vitro* stimulus. **Results and Discussion:** AIRmax mice showed a higher response to synergic SCF+IL-3+ATRA action ($6.31 \pm 1.63 \times 10^5$ cells/ml) with accelerated neutrophil differentiation ($5.9 \pm 0.1 \times 10^4$ $CD38^-/GR1^+$ cells) compared to AIRmin mice ($2.4 \pm 0.1 \times 10^4$ $CD38^-/GR1^+$ cells). The study of genetic polymorphism at the IL-3Rα locus showed that the frequency of the allele that codes for a normal protein was 100% in AIRmax mice. On the other hand, AIRmin mice showed preferential fixation of the alelle related to abnormal protein, present in some inbred strains of mice such as A/J, AKR, A.TH or A.TL which were found to be hyporesponsive to IL-3. The identification of functional polymorphism in the IL-3Rα gene between AIRmax and AIRmin mouse lines, which differ largely in the degree of cell differentiation and in inflammatory response, indicates that this gene is an important regulator of the high and low inflammatory reactivity in both AIRmax and AIRmin mouse lines.

Support by: FAPESP and CNPq.

5.03 Canine amnion-derived stem cells causes tumor in mice

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Introduction: Fetal stem cells are isolated from tissues normally discarded at birth. They are attractive for clinical applications because their use avoids ethical concerns that plague the isolation embryonic stem cells. The amniotic membrane (AM), or amnion, delineates the gestational sac, a highly resilient, transparent, fluid-filled cavity that encompasses a developing fetus during gestation. The amnion is an avascular structure consisting of three discrete layers: an inner epithelial layer, an interposing, acellular basement membrane, and an outer layer of mesodermal cells. A mix of multipotent cell populations, including amniotic epithelial and amniotic mesenchymal cells known as amnion-derived stem cells (ADSC), can be obtained from the amnion (Miki et al., 2005). **Objectives:** The goal of present work was the isolation and characterization of ADSC from canine fetuses (C-ADSC). **Methods:** AM was obtained from a dog fetus at 35 days of gestation. The cells were isolated from the amnion using a tissue explant methodology and cultured according to Marcus *et al.* (2008). Differentiation of ADSC towards mesodermal lineage was performed following routine protocols. Analysis of C-ADSC morphology was performed by transmission electron microscopy (TEM). Before injection into mice, C-ADSC were transduced with retrovirus vectors carrying reporting genes LacZ in order to facilitate cell tracking after implantation. A total of $1x10^6$ cells of normal and Lac Z - cells were injected into right limb of each nude mice (n=8) and Swiss mice (n=2) of both strains. Histological analyses were then performed. **Results and Discussion** C-ADSC showed high proliferative rate after isolation and displayed both embryonic stem (ES) cell-like and epithelial-like cell phenotypes. They were positive with both anti-vimentin and anti-nestin antibodies, suggesting that isolated mixed C-ADSC population was composed of both amniotic epithelial and mesenchymal stem cells. TEM analysis showed the cells, which have ES cells – like morphology, with a large nucleus and with cytoplasm poor in organelles. The cells demonstrated tight contact and gap junction formation. Tumor formation was observed in the right limb of all animals one month after cell implantation. Histological analysis confirms the formation of teratocarcinomas composed of undifferentiated and differentiated cells. Our data suggest that mixed C-ADSC population was composed mainly of cells of the epithelial phenotype, which were able to produce teratocarcinomas in mice. Although culture conditions could promote the isolation of cells with teratogenic potential, caution is needed with respect to fetal stem cell use in the cell therapy. On the other hand, C-ADSC could be an interesting model for cancer research.

Supported by: FAPESP.

5.04 Analysis of the signaling pathways involved in vasoconstriction induced by angiotensin II (AngII) in the snake *Bothrops jararaca*

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Introduction: The renin-angiotensin system produces its effects through the interaction of AngII with the classical angiotensin receptors, AT_1 and AT_2. In some vertebrates, such as the Brazilian snake *Bothrops jararaca* (Bj), AngII interacts with an atypical AT receptor. The majority of AngII responses are due to activation of the AT_1 receptor that is coupled to several intracellular signaling pathways, such as phospholipases C, D and A_2, adenylylcyclase and tyrosine kinases. We have demonstrated that the phospholipase C (PLC)/IP_3 and adenylylcyclase/AMPc pathways in cardiac tissue, and L-type calcium channel in the vascular tissue of Bj, are not involved in AngII response in this animal. **Objectives**: The aim of this study was to evaluate the role of calcium and kinase as second messengers in the AngII response in Bj. **Methods:** A functional assay was used to obtain cumulative AngII (10^{-10}-10^{-6}M) curve with isolated aorta rings, in the absence and the presence of specific inhibitors to examine: 1) the activation of PLC pathway, using U73122 and GF109203X, a PLC and a PKC inhibitor, respectively; 2) the participation of tyrosine kinase and phosphatase, using genistein and sodium orthovanadate; and 3) the role of extra and intracellular Ca^{+2} by removal of external calcium from the medium, or pre-treatment of the tissue with either cyclopiazonic acid (CPA), an inhibitor of the Ca^{+2}-ATPase from sarcoplasmic reticulum, or with caffeine (agonist of the ryanodine receptor in the sarcoplasmic reticulum), or with SK&F96365 (a store-operated calcium receptor inhibitor). **Results and Discussion:** U73122 (10^{-5}M, n=7), genistein (1-30x10^{-6}M, n=8) and sodium orthovanadate (10^{-4}M, n=6) failed to modify AngII response, while GF109203X (10^{-5}M, n=6) reduced Ang II maximum response by 52%. These results indicate that phospholipase C, tyrosine kinase and phosphatase are not involved in AngII response of Bj aorta, but PKC activation is involved. Activation of PKC seems to occur through a phospholipase C-independent pathway. There was an 82% reduction of the Ang II response in calcium-free medium, and a recovery after restoring the normal calcium concentration in the medium. In addition, SK&F96365 (3.10^{-5}M, n=6) caused a 68% reduction in AngII response. All together, these data support the participation of external calcium and store-operated calcium receptor in the Ang II response. CPA (1-30x10^{-6}M, n=7) failed to modify AngII response, but caffeine (10^{-3}M, n=4) reduced the Ang II maximal response by 92%. Therefore, a possible role of the sarcoplasmic reticulum as an intracellular source of calcium for AngII response cannot be completely discarded. Taken together, our results suggest the participation of PKC and calcium as signal transducers of snake AT receptor activation by AngII, in the aorta of Bj.

Supported by: CNPq and FAPESP.

5.05 Primary cell lines obtained from BPV-related lesions: cytogenetics and immunochemical analysis

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Introduction: The bovine papillomavirus belongs to a DNA oncovirus group, which is characterized as infecting the host epithelium. In cattle, BPV commonly causes only benign skin lesions, but in some cases can lead to neoplasic processes such as enzootic hematuria and upper digestive tract carcinoma. The integration of papillomavirus in host chromatin has not been reported in cattle, but several studies have reported the presence of increased levels of chromosomal aberrations in animals infected by the virus. **Objective:** We present a study of cytogenetic and morphological characterization of cells from primary cultures from skin papilloma, esophagus papilloma and bladder mucosa of animals infected with BPV. **Methods:** For culture establishment, fragments were collected from lesions detected in animals affected by papillomatosis and a sample of skin without visible lesions used as control. The fragments were incubated in DMEM (Cultilab ™), supplemented with 10% fetal bovine serum and maintained at 37 ° C in an atmosphere of 5% of CO ². All animals were tested for the presence of viral DNA in the lesions and in different passages of the culture by PCR using generic and specific primers. In early passages, cytogenetic preparations were performed with hypotonic treatment with 0.075 M KCl solution at 37°C for 30 min and fixed in a 3:1 methanol and acetic acid solution. Slides were stained in 2% Giemsa and examined with a photomicroscope. The cultured cells were also stained with anti-vimentin and anti-pan-cytokeratin for cytological characterization. **Results and Discussion:** All animals showing lesions were confirmed as BPV positive for BPV-1, BPV-2, and some for BPV-4. There was a higher frequency of chromosomal aberrations in affected animals compared to control animals. A greater number of aberrations were visualized in affected animals, particularly those with sequences of BPV4. All cell lines were stained as vimentin positive, which is the intermediate filament that characterizes mesenchymal cells. The same cells were positive for pan-cytokeratin, a marker of cells of epithelial origin, and the most evident expression were found in the esophagus papilloma and bladder mucosa cells. This double labelling feature demonstrated in the cells lines has been already reported in certain types of metastatic cells, characterizing a process called epithelial-mesenchymal transition (EMT): cells show simultaneous cytokeratin and vimentin characteristics. Another point to consider is that cytokeratin genes in cells with malignant growth have been reported with an altered expression, which could indicate the expression of this filament as a possible element for diagnosis in cancer.

Supported by: CNPq and FAPESP.

5.06 Identification of suitable loci for discrimination of pathogenic *Leptospira* serovars

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Introduction: Leptospirosis is an infectious disease caused by pathogenic *Leptospira* species. To date, almost 300 serovars have been identified and distributed among 26 different serogroups. Definite serovar identification is performed by the cross agglutinin absorption test (CAAT), employing monoclonal antibodies. However, this technique is considerably expensive. Molecular tools have been described for serovar discrimination, but they have limitations. Multi-locus sequence typing (MLST) is a simple PCR-sequencing approach, which has the ability to discriminate *Leptospira* species. Traditionally, the loci chosen for MLST analyses are housekeeping genes. However, these genes may be highly conserved and, thus, exhibit low discriminatory power. Virulence factor sequences are expected to contain more polymorphic sites. **Objectives:** This study aimed at evaluating the candidate loci *ligB*, *secY*, *rpoB* and *lipL41* (concatenated in this order) in the differentiation of *Leptospira* reference serovars. **Methods:** The sequences were aligned by the ClustalW program and the phylogenetic analyses were performed by the Mega 4.1 software. **Results and Discussion:** This study involved 37 reference strains and employed a 1884-bp locus. The species were resolved into major clusters and the serovars appeared to occupy individual branches within them. Through this analysis, it was possible to obtain separation between serovars Icterohaemorrhagiae strain RGA and Copenhageni strain Fiocruz L1-130. Yet, it was possible to differentiate between strains 56601 and Lai (serovar Lai) and strains M20 and Fiocruz L1-130 (serovar Copenhageni). This suggests that this candidate scheme may be helpful to identify clonal isolates in epidemiological studies. The candidate loci can be amplified from different strains, irrespective of the species to which they belong. Several molecular tools have been described for the characterization of *Leptospira* isolates, but they demonstrated a number of limitations. Contrarily, MLST was demonstrated to be simple, suitable for worldwide application and inexpensive and to generate unambiguous and exchangeable data. The proposal scheme is suitable for discriminating pathogenic serovars of *Leptospira*, per species, as demonstrated in our phylogenetic analyses. Although this approach should, in the future, be extended to a larger number of serovars and clinical isolates, it represents the first step towards serovar molecular discrimination.

Supported by: FAPESP, CNPq and Fundação Butantan.

5.07 HPV16 L1L2 gene expressions, protein synthesis and interaction in culture human cells

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Introduction: Of the nearly 200 genotypes identified, at least 15 distinct human papillomavirus (HPV) types are described to be involved in genital, mouth, throat, and skin cancers. Cervical cancer is the second most prevalent cancer in women worldwide, and HPV16 is the most prevalent high-risk HPV type associated with cancer of the cervix. HPV has an 8,000-base pair, circular, double-stranded DNA, containing the E6 and E7 viral oncogenes. L1 is the major and L2 is the minor HPV capsid protein. Together, they compose the small naked icosahedra with a capsid of about 55 nm in diameter, which without viral DNA is known as VLP (virus-like particle). In the worldwide HPV16, VLP L1 has been utilized in prophylactic vaccine development due to their capacity to induce high immunological response, although L2 can induce a low-titer of antibodies to a wide-range of divergent papillomavirus types and species. L2 confer more stability to the VLP and it is also necessary for HPV infection. **Objectives:** We are producing HPV16 L1L2 VLPs to investigate the mechanisms by which virus-cell infection causes cancer. **Methods:** Cultures of the 293T human embryonic kidney (HEK) cell line were transfected with the DNA constructs encoding humanized L1 (L1h) and L2h antigen of HPV16, subcloned into the mammalian expression vectors pUF3L1h and pUF3L2h. Western blotting to control protein expression, immunofluorescence in laser scanning confocal microscopy (LSCM), negative staining and gold immunolabeling for VLPs analyses by transmission electron microscopy (TEM) were used. Pathogen-host cell interaction assays using HPV16 L1L2 VLPs and HEK 293T cells were performed. **Results and Discussion:** Recombinant L1L2 DNA was expressed in HEK 293T cells with high efficiency. At least 85% of cells expressed intracellular L1L2 and VLPs, detected by LSCM and TEM. The HPV16 L1L2 VLPs produced in this study with about 55 nm in diameter interacted with non-transfected HEK 293T cell line, confirmed by LSCM. We are establishing a methodology for an efficient system of recombinant protein expression. The production of HPV16 L1L2 VLPs by transfected HEK 293T cells opens the possibility for new strategies to study HPV-cell interactions and carcinogenesis mechanisms.

Supported by: FAPESP, DKFZ-ATV and Fundação Butantan.

5.08 Renin angiotensin system (RAS) and vascular reactivity in a non-poisonous snake *Oxyrhopus guibei* (Colubridae family)

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Introduction: Snakes are interesting vertebrates to study cardiovascular function because they have an elongated blood column, and have to adapt its blood circulation to different gravitational influences. RAS is important in cardiovascular control, and its study in Brazilian snakes from Viperidae family points to the presence of a RAS relatively conserved compared with other vertebrates species. The angiotensin converting enzyme (ACE) plays a significant role in RAS by removing two amino acids from the inactive angiotensin I (Ang I), converting this peptide into its active form angiotensin II (Ang II). **Objectives:** The aim of this study was to investigate the presence of a functional RAS in a vascular tissue, aorta, of the snake *Oxyrhopus guibei*, which belongs to a family of non-poisonous snakes, namely the Colubridae. We analyzed the presence of an active ACE and used pharmacological tools to characterize the Ang II receptor. **Methods:** Using an *in vitro* assay with vascular smooth muscle from *Oxyrhopus guibei*, we obtained cumulative concentration-effect curves for AngI and Ang II (10^{-10} - 10^{-6}M) in the absence and presence of an inhibitor of ACE, captopril (10^{-6}M). Cumulative concentration-effect curves for Ang II (10^{-10} - 10^{-6}M) were also constructed in the absence and presence of the non-selective antagonist of the Ang II receptor, [Sar^1, Ile^8] Ang II (10^{-7}-10^{-5}M), or an agent that reduces disulfide bridges in the receptor structure, dithiothreitol – DTT ($3x10^{-3}$M). **Results and Discussion:** Pre-treatment with the ACE inhibitor, captopril, shifted the Ang I curves to the right (pD_2 6.9 to 5.9, n=6), but was not able to displace the Ang II curves (pD_2 7.1 to 7.1, n=5). Taken together, these results indicate the existence of angiotensin converting enzyme in the vascular tissue of the snake, which is functionally active and responsible for converting Ang I into Ang II. The initial pharmacological characterization of receptor for Ang II in the snake aorta using three different concentrations of the non-selective antagonist [Sar^1, Ile^8] Ang II (10^{-7}-10^{-5}, n=3) showed a shift to the right in the Ang II curve and a reduction in the maximum effect. These data suggest the presence of an Ang II receptor in this snake, but selective antagonists should be used to identify the subtype of the Ang II receptor. Cumulative concentration-effect curves for Ang II were reduced after DTT treatment (n= 2), suggesting the presence of at least one disulfide bridge functionally important in the Ang II receptor structure. DTT is also reported to produce similar reduction of Ang II response in mammalian species and in two Brazilian snakes from the Viperidae family. These results contribute to the knowledge of RAS in vertebrate species.

Supported by: FAPESP.

5.09 Identification and evaluation of the bovine papillomavirus (BPV) in blood and peripheral lymphocyte cultures from dairy cows in Pernambuco State, Brazil

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Introduction: Bovine papillomaviruses (BPVs), family *Papillomaviridae*, act as causal agent of warts and bladder and digestive tract cancers, causing significant economic losses to the cattle industry. Ten types of BPVs have been well characterized (BPV-1-10). **Objectives**: The main objective of this study was to evaluate sequences of BPV genomes in blood samples collected on a dairy farm in Pernambuco and in respective peripheral lymphocyte cultures, to determine the presence of BPV 1, 2 or 4 and their simultaneous presence. **Methods**: The polymerase chain reaction (PCR) technique was used for viral diagnosis, using specific primers for types 1, 2 and 4, targeted to genes L1, L2 and E7, respectively. Confirmation of the amplified products was performed with enzymatic digestion and sequencing. **Results and Discussion**: Viral sequences were detected in all the animals, regardless of the apparent presence of papillomas. Types 1 and 2 were detected directly from blood samples and correlated with lymphocyte cultures, and type 4 was not detected in any samples. The positive results were confirmed by enzymatic digestion and sequencing results were compared with published sequences available in *GenBank*. The viral presence in blood corroborates studies that argue BPV dissemination through blood while the detection in culture suggests viral maintenance in this system. The sequencing of some positive samples for BPV-1 suggests the occurrence of a new viral variant. Taken together, the results suggest the role of lymphocytes as sites of viral latency, in addition to the presence of a variant of BPV-1 circulating among the animals studied.

Supported by: CNPq, FAPESP and FACEPE.

5.10 Cytogenetics and molecular phylogeny of the genus *Oligoryzomys* (Sigmodontinae, Rodentia) from new Brazilian localities

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Introduction: Pygmy rice rats of *Oligoryzomys* are widespread from Mexico to Tierra del Fuego in a variety of habitats and climates. Recently, 18 species were recognized for the genus, 10 being recorded in Brazil, inhabiting the Atlantic and Amazonian rainforests, Cerrado, Caatinga and Pampa. Some of them are agricultural pests or reservoirs of Hantavirus. They exhibit species-specific karyotypes and the diploid numbers vary from 44 to 70. **Objectives:** The aim of the present study was to characterize karyotypes of several *Oligoryzomys* species and to establish the phylogenetic relationships between them. **Methods:** We used samples of *Oligoryzomys* from 46 localities of 11 Brazilian states: Piauí, Ceará, Bahia, Minas Gerais, Espírito Santo, São Paulo, Paraná, Rio Grande do Sul, Mato Grosso, Goiás, and Tocantins. Cytogenetic data were obtained from bone marrow, spleen and fibroblast culture, and DNA was extracted from liver and muscle. **Results and Discussion:** Conventional and differential staining and fluorescence *in situ* hybridization (FISH) for cytogenetic study evinced *Oligoryzomys nigripes* with 2n=62 and FN=78, 80, 81, 82; *O. flavescens* with 2n=64, 65, 66 and FN=66, 67, 68; *Oligoryzomys microtis* with 2n=64 and FN=64; *Oligoryzomys moojeni* with 2n=70 and FN=74; and *Oligoryzomys fornesi* with 2n=62 and FN=64. FISH with telomeric probes showed exclusively telomeric signals even in rearranged pairs and supernumerary chromosomes. Sequences of 750 bp of the mitochondrial cytochrome-b gene were used for phylogenetic reconstruction. Parsimony and Bayesian analyses recovered the genus as monophyletic and the clades were related to the biomes where animals were trapped: Amazonian, Atlantic forest, Cerrado areas, and a clade composed of samples with 2n=62 from Ceará - from a transitional area between Atlantic and Amazonian rainforests called "Brejos" - and Bahia. The monophyly of *Oligoryzomys* is well corroborated in the literature; however, the relationships within the genus are still unclear. In our data, the Amazonian clade is related to *Oligoryzomys microtis*; the clade of Atlantic forest comprises several localities of Southeast and South Brazil and is composed of representatives of *Oligoryzomys nigripes*. Distinct clades were recovered from different areas of the Cerrado. Besides, we emphasize karyotypes as important marker for this genus and that the recovered clades are related to the biomes that the animals inhabit.

Supported by: FAPESP and CNPq.

5.11 Polymorphism in codon 72 of p53 gene in women examined during routine gynecological examination in Ouro Preto, Minas Gerais, Brazil

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Introduction: The p53 gene (17p13.1) has 11 exons: the first is not transcribed. The p53 protein has 393 aminoacids, with four segments with distinct functions. The p53 protein is important in carcinogenesis by acting in the preservation of genomic integrity, the transcriptionally active p21 gene, inducing the synthesis of protein p21. p53 activates the gene GADD-45 (growth arrest DNA damage inducible) which acts in correcting DNA lesions. Protein p53 also activates genes involved in the mechanism of apoptosis and suppresses the action of anti-apoptotic genes. The E6 protein of high risk human papillomavirus is able to bind to p53 leading to its rapid degradation. The codon 72 has different alleles, causing the insertion of different amino acids in this position of the protein: arginine (Arg - GCC) and proline (Pro - CCC), generating the genotypes: Arg / Arg, Arg / Pro and Pro / Pro. **Objectives:** To evaluate the frequency of polymorphism in codon 72 of p53 gene in 348 randomly selected women in the routine gynecological examination for HPV detection. **Materials and Methods:** Analysis was performed in cervical samples obtained from 348 women of Ouro Preto, Minas Gerais. The polymorphism at codon 72 of exon 4 of gene p53 was determined by PCR, with specific primers for each allele. The PCR products were analyzed in a 2% agarose gel by electrophoresis. **Results and Discussion:** The frequencies of genotypes were: Arg / Arg 41% (141), Arg / Pro 48% (168) and Pro / Pro 11% (39). HPV was demonstrated in 15.3% (53) patients; 8.0% (28) showed substantial alterations in cytological examination. Correlating cytological alteration and the genotypic frequencies, it was possible to show: ASC-US: Arg / Arg 21.4% (6), Arg / Pro 21.4% (6), Pro / Pro 7.1% (2). LSIL: Arg / Arg 10.7% (3), Arg / Pro 14.3 (4), Pro / Pro 3.6% (1). ASC-H: Arg / Arg 14.3 (4), Arg / Pro 7.1% (2). The genotype Arg / Arg was not more frequent in samples verified as ASC-US and LSIL, but was more frequent in the samples showing cytologically more severe diagnosis.

Supported by: CNPq, FAPESP, PRONEX and FACEPE.

5.12 Bovine papillomatosis in dairy herd: preliminary evaluation in Ouro Preto, Minas Gerais, Brazil

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Introduction: Bovine papillomavirus (BPV) is a double-stranded DNA virus that induces lesions (such as warts) in the epithelium. These lesions can develop into tumors when exposed to certain co-factors. This results in massive economic losses. Bovine papillomatosis is frequent in several regions of Brazil, with dramatic impairment to cattle breeding, but without systematic evaluations of its occurrence. **Objectives**: We investigated a dairy herd in Ouro Preto – Minas Gerais, collecting lesion fragments for detection and identification of papillomavirus types. **Methods:** Lesion fragments and peripheral blood samples were collected from affected cattle. Morphological alterations in infected tissues have been analyzed by anatomopathologic studies performed in wart fragments. DNA was extracted with a Tissue kit (Qiagen), in accordance with the manufacturer´s instructions. All samples were investigated concerning the presence of papillomavirus genome by PCR techniques, using specific and generic primers. In all positive samples, enzymatic digestion was performed for confirmation of the virus type and analyzed by electrophoresis in a 2% agarose gel stained with GelRed^{tm}. **Results and Discussion:** All the samples using primers BPV-1 and FAP were found to be positive concerning the presence of papillomavirus genome. The positive products of PCR for FAP primers were confirmed by enzymatic digestion. The BPV positive products were confirmed by enzymatic digestion and sequencing. The identification of herds showing one specific BPV type is important in considering an evaluation of patterns of virus transmission and final vaccine procedures.

Supported by: CNPq, FAPESP and Fundação Butantan.

5.13 Differential display as a cost-effective alternative for the study of eukaryotic transcriptomes

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Introduction: Systemic characterization of altered gene expression has been shown to provide useful data for molecular phenotyping and classification of malignant tumors as well as for identification of potential tumor-specific therapeutic targets. For these reasons, techniques capable of detecting differentially expressed genes across transcriptomes are now considered standard investigation methods for oncology. Adrenocortical carcinoma is a rare and aggressive malignant disease for which no effective treatment, besides surgery, is available. Only a few published studies have targeted the expression profile of adrenocortical tumor tissues. Therefore, we aimed to study the transcriptomes of adrenocortical tumor cells and tissue with the use of differential display (DD) - a strategy of transcriptome characterization that requires minute amount of starting RNA and standard equipment used in molecular biology. **Objectives:** To detect relevant differences in gene expression between tumor and normal adrenocortical tissues. **Methods:** RNA from an adrenocortical carcinoma cell line and from specimens of an adrenocortical carcinoma tumor were compared to a commercial pool of normal adrenal RNA using DD, which was based on: 1) systematic radiolabeled amplification of the mRNAs 3' termini; 2) high resolution polyacrylamide gel electrophoresis followed by exposure to X-ray films; and 3) retrieval of fragments of interest for cloning, sequencing and identification. The transcription patterns of normal tissue and adrenal cortex carcinoma were compared side-by-side, using DD: those bands with difference in intensity (reflecting differential expression) were candidates for analysis. In order to reliably identify differentially expressed transcripts we adopted optical density-based criteria to select bands of interest. **Results and Discussion:** Transcripts differentially accumulated in adrenocortical tumor cells were detected and represented virtually all chromosomes. A significant portion of these transcripts represent intro-derived sequences. Differential expression of most of these transcripts has not been reported for adrenocortical tumors. Optical density-based criteria and other alternative procedures introduced to some steps of DD reduced significantly some major drawbacks of this technique. Several aspects discourage the use of DD, such as difficulty in visual comparison of hundreds of bands/gel lane and multiple clone analysis for each fragment. Besides, it is generally considered to be a labor-intensive and time-consuming technique. Other negative points are heterogeneity of gel background and low half-life of radiolabeled nucleotide. But, important advantages of DD over other techniques (e.g., microarray) should be taken into account: no requirement of previous knowledge in mRNA sequences, low RNA quantity demand, sensitivity to abundant and rare transcripts and technical simplicity and accessibility. The technical improvements introduced in our work and other considerations may render DD more atractive as a method to compare eukaryotic expression patterns.

Supported by: FAPESP.

5.14 Interaction of HPV16 L1L2 VLP with human amniotic fluid CD34$^+$/CD117$^+$ stem cells

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Introduction: The naked icosahedral capsid of HPV (human papillomavirus) is composed of 2 structural proteins called L1 and L2, which contain a double-stranded, circular DNA genome. VLPs (virus-like particles) are capsid without DNA, used as vaccines and for host-pathogen interaction studies. The infection of HPV occurs preferentially in epithelial cells; however, the presence of virus in tissue fluids is of interest from a clinical and basic research viewpoint. HPV16/18 DNA was detected in the amniotic fluid in pregnant women with cervical diseases related to HPV. The clinical meaning of HPV presence in the amniotic fluid before birth raises a great discussion around the possibility of newborn contamination. Prenatal HPV transmission was suggested when it was confirmed that surgical delivery did not protect children from mother-fetal transmission. It is important to confirm if newborns had a previous contact with HPV DNA sequences, before birth. For this, some host-pathogen interaction assays have been performed. **Objective:** This study investigated the possibility of HPV16 L1L2 VLPs interaction with cells from human amniotic fluid. **Methods:** Isolation and characterization of the cell types present in human amniotic fluid in different gestational stages were performed using cell culture. Cell samples were obtained from amniotic fluid by transabdominal amniocentesis from women clinically indicated for this procedure. Interaction assays using HPV16 L1L2 VLPs in immunofluorescence methods were analyzed by laser scanning confocal microscopy (LSCM). Anti-CD34 and anti-CD117 antibodies were used recognize stem cell markers, along with anti-transferrin receptor (anti-CD71). **Results and Discussion:** It was possible to identify fibroblasts and hematopoietic precursor stem cells in the cultures. The CD34$^+$/CD117$^+$ stem cells interacted with the HPV16 L1L2 VLPs by the internalization through CD71 receptor, confirmed by LSCM. The possibility of HPV16 infecting stem cells from hematopoietic precursor lineage can support the hematological route as a possibility of infection, which not considered in public health nowadays, mainly in the mother-fetal transmission hypothesis of HPV DNA sequences. In addition, these findings stimulate thought about the potential application of these cells for fetal therapy and tissue engineering.

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

5.15 Partial sequencing analysis of the genome of influenza A samples of subtypes H3 and H8 isolated from wild migrating birds in São Paulo State, Brazil

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Introduction: Avian influenza virus belongs to the family *Orthomyxoviridae.* In the last years, highly virulent avian influenza subtypes, H5, H7, H9 and H10 have caused outbreaks and epidemics in poultry and fatal infections in humans. The wild and migrating birds may be participating in the maintenance and interspecies transmission of the sixteen subtypes of hemagglutinin. **Objectives:** To sequence the NS1 and M1 region from two influenza A viruses isolated from wild and migrating birds and comparison to sequences of all subtypes of influenza A available from public databases in order to determine the homology between these isolates. **Methods:** The samples from species *Sporophila caerulescens* (LE 6744) and *Elaenia mesoleuca* (LE 6712) were collected in reserves and experimental field stations located in São Paulo State - Brazil, during the years 1997 and 1998. The two viral types isolated from the samples (LE 6744 and LE 6712) were identified by the hemagglutination inhibition test (HI) using the 21 antibody patterns anti-influenza A type and one for the influenza type B. Other techniques used were transmission electron microscopy (TEM) and RT-PCR. **Results and Discussion:** The HI test demonstrated that the *Elaenia mesoleuca* sample showed an antigenic relationship with 80 HAIU to A/Turkey/Ont./6118/68 (H8N4) and the *Sporophila caerulescens* sample reacted with 160 HAIU to A/Hong Kong/1/68 (H3N2), A/Equine/Miami/63 (H3N8), and A/Duck/Ukraine/63 (H3N8) antiserum. TEM revealed structures of viral particles measuring 40 to 120 nm. RT-PCR detected the specific site for influenza A virus gene: NS1 oligonucleotides amplified a 189-bp fragment and primers for a fragment of M gene amplified a 340-bp fragment. The sequencing analyses of these two isolates revealed a high homology between these two strains or NS and M genes. Sequencing of the hemaglutinin genes is under way, and it is important to confirm the subtypes and to analyze the pathogenic potential of the samples.

5.16 Lipid body formation induced by a snake venom phospholipase A_2 (PLA_2) in macrophages and the signaling pathways involved

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Introduction: Lipid bodies are cytosolic inclusions present in most eukaryotic cells, containing neutral lipids surrounded by a single phospholipid membrane and specific proteins, such as the protein related to differentiation of adipocytes (ADRP). These inclusions compartmentalize signaling proteins such as MAPK, PKC, PI3K, enzymes responsible for synthesis of eicosanoids and ADRP. Increased LB formation occurs in activated inflammatory cells and has been associated with inflammatory diseases such as atherosclerosis, obesity and diabetes. Some of them show high levels of circulating secretory PLA_2 ($sPLA_2$). MT-III, a $sPLA_2$ isolated from *Bothrops asper* snake venom, induces local inflammation and increases the activity of macrophages (MΦs), which are central cells in inflammation. **Objectives:** To evaluate the ability of MT-III to induce LB formation in MΦs and the pathways involved, analyzing: a) the number of LBs formed, b) intracellular distribution and protein expression of ADRP and c) involvement of major signaling proteins. **Methods:** Thioglycolate-elicited MΦs from Swiss mice were incubated with MT-III (6.3 µg/mL) or RMI (control) for 1 h. LB formation was assessed by both staining with osmium tetroxide (1%) followed by counting under phase contrast microscopy and electronic microscopy after conventional procedures. Cell ADRP distribution and expression were evaluated by immunofluorescence assay and Western blotting, respectively. Participation of signaling proteins was evaluated by treatment of cells with specific inhibitors before stimulation with MT-III. **Results and Discussion:** Incubation of MΦs with MT-III resulted in increased numbers of LB (4 ± 0.18 LBs/cell; control: 1.2 ± 0.11). The ultrastructural analysis showed both light and strongly osmiophilic LBs in MT-III-stimulated MΦs with some LBs in close association with endoplasmic reticulum (RE). Enlargement of both RE and Golgi cisterns were also observed. In addition, MT-III upregulated ADRP expression (369%), a marker of LB formation, at 6 h after incubation and increased the intensity of fluorescent ADRP in the cytoplasm. Pretreatment of cells with either LY294002 (1 µM) or Wortmannin (5 nM), PI3K inhibitors, reduced MT-III-induced LB formation by 58%. SB202190 (p38MAPK inhibitor, 5 µM) or $AACOCF_3$ (20 µM) or Bel (2 µM), intracellular phospholipases inhibitors ($cPLA_2$ and $iPLA_2$, respectively) or PD98059 (ERK 1/2 inhibitor, 25 µM) or H7 (PKC inhibitor, 20 µM), reduced MT-III-induced LB formation by 51, 59, 50, 45 and 67%, respectively. However, herbimycin (PTK inhibitor, 10 µM), indomethacin or etoricoxib (cyclooxygenase inhibitors, 1 µM), zyleuton (5-lipooxygenase inhibitor, 1 µM) did not alter MT-III-induced effect. MT-III is able to induce the formation of LBs in macrophages. This effect is dependent on PI3K, p38MAPK, $cPLA_2$, $iPLA_2$, ERK1/2 and PKC but not on PTK nor metabolites from cyclooxygenases-1 and -2 or 5-lipooxygenase. Moreover, MT-III is able to recruit ADRP and up-regulate its expression. These events together with activation of RE and Golgi complex may be important in LB formation induced by MT-III. These data give new insights into the role of secretory PLA_2 in inflammatory diseases involving lipid body formation.

Supported by: FAPESP and CNPq/INCTTOX.

5.17 Dental pulp stem cells as a source for neural regeneration

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Introduction: Dental pulp contains different populations of stem/progenitor cells that reside within the perivascular niche and originate from migrating neural crest cells. In the past years, studies have demonstrated the self-renewal capacity, clonogenic efficiency and multi-lineage differentiation potential of human dental pulp stem cells (DPSC). However, only recently was it demonstrated that these cells have the ability to differentiate towards functional neurons after chemical induction of differentiation (Arthur *et al.*, 2008). Previously, we showed that immature DPSC were able to undergo neuronal differention spontaneously (Kerkis *et al.*, 2006). **Objectives:** The goal of our study was to evaluate the capacity of human adult DPSC and immature DPSC to undergo spontaneous differentiation into different neural cells *in vitro*. **Methods:** Human adult and immature DPSC were characterized and maintained as previously described (Kerkis *et al.*, 2006). Differentiation towards neural cells was performed under culture conditions developed for neuronal cells in the absence of known growth factors, such as: epidermal growth factor (EGF), basic fibroblast growth factor (bFGF) and retinoic acid (RA). Anti-human antibodies: mouse anti-beta-III-tubulin, goat anti-nestin and rabbit anti-glial fibrillary acidic protein (GFAP) and others were used after cell fixation in 4% paraformaldehyde. The expression of cell specific proteins was analyzed under confocal microscopy. Morphological studies were performed using hematoxylin/eosin, as well as neutral red staining and analyzed by light microscopy. Functional tests are being carried out. **Results and Discussion:** Human adult DPSC and immature DPSC show rapid proliferation and expansion *in vitro*. They can be maintained for a long period in culture, which indicates their self-renewal potential. These cells expressed mesenchymal stem cells markers, as well as reacting positively with human embryonic stem cell markers. Undifferentiated immature DPSC cultured in basal medium already expressed neural progenitor markers, such as nestin and GFAP. These cells were able to respond to culture conditions usually used for neuronal cells, even without the use of chemical inducers, showing acquired neural cell-like morphology after eleven days of culture. The decrease in the expression of nestin and GFAP proteins was demonstrated during the process of neuronal differentiation. At the same time, the cells showed increasing expression of immature neural proteins. We observed that the cell populations that undergo neural differentiation showed terminally differentiated neuronal cell types and at the same time showed neurosphere formation. Terminally differentiated neural cells survive during a long period in culture. We showed that undifferentiated human adult and immature DPSC are already committed to originating neuron- and glial-like cells. The suggested model mimics neural stem cells growing and differentiating *in vitro* and can be possibly used to analyze the various stages of neural cell development. Dental pulp is a readily accessible source of stem cells which have a potential use in cell therapy to treat neurological disease.

Supported by: FAPESP.

5.18 Characterization of multipotent equine adipose tissue-derived progenitor cells. Clinical case reports of allogeneic cell-therapy in horses

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Introduction: In horses, stem cell therapies are a promising tool for the treatment of many injuries, which are common consequences of athletic endeavor, resulting in high morbidity and often compromising performance. We reported the isolation and characterization of equine adipose tissue-derived progenitor cells (eAT-PC) before and after crypreservation (banking of eAT-PC). The aim of this study was further characterization of eAT-PC differentiation potential and application of allogenic eAT-PC for the treatment of tendonitis in horses. **Methods:** eAT-PC was maintained under conditions previously described. Differentiation towards muscle and neuronal cells was performed following routine protocols. Mouse anti-human antibodies, anti-myosin, anti-α-actinin, anti-MyoD1, anti-beta-tubulin-III, as well as rabbit anti-human anti-nestin and anti-glial fibrillary acidic protein (GFAP) were used. Twelve animals with tendonitis received 10^7 of eAT-PC into the injured tissue under local anesthetic and ultrasonographic control. After one month, ultrasonographic control was performed again. Since our study was based on clinical cases, the animals were heterogenous for age, weight and sex, but all of them were athletic horses. All procedures were approved by horse owners under signature of a veterinary service contract. **Results and Discussion:** After the induction of myogenic differentiation, the cells showed first signs of morphological changes similar to muscle cells, at day 10. Myosin, α-actinin and MyoD1 antibodies showed positive immunostaining with the cells confirming muscle cell differentiation. Prior differentiation into neuronal lineages, eAT-PC already showed strong nestin positive immunolabelling. Neuronal differentiation was evidenced by outgrowth formation and nucleous dislocation. Neuron-like cells derived from eAT-PC reacted positively with such markers as beta-III-tubulin and GFAP. Functional test are being carried out. One month after eAT-PC application into the lesion, the formation of healthy tissue has been observed. All treated horses showed a functional recovery and were able to return to their normal activity, without lesion recurrence. Extending our previous findings, we showed that eAT-PC were able to produce smooth and skeletal muscles and neuron-like cells. Their application in horses provided functional recovery of damaged tendons, and treated animals were capable to return to their normal activity. Our findings classify eAT-PC isolated and cultured *in vitro,* as a promising tool for cell therapy, which maintain their potential even after cryopreservation. Further studies are needed in order to understand the mechanism of their action on the recovery of damaged tissues.

Supported by: FAPESP and CELLTROVET.

5.19 Increased level of chromosome aberrations in bovineS (*Bos taurus)* infected with BPV-1 and 2

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Introduction: Bovine papillomavirus (BPV) is represented by ten virus subtypes, epitheliotropic or mucosotropic, which are transmitted through contaminated blood and ephitelial contact. This virus has been related to chromosomal instabilities. **Objective:** The purpose of this study was to evaluate the levels of chromosome aberrations in samples of peripheral blood collected from cattle (*Bos taurus taurus*) to determine the presence of sequences of BPV by polymerase chain reaction (PCR). **Methods:** Sixty-one blood samples were collected from cattle showing papillomatosis (symptomatic animals) and without clinical signals (asymptomatic animals). Short-term lymphocyte cultures were performed for cytogenetic studies. Cytogenetic analyze was performed in a blind test. **Results and Discussion:** The results showed that 28 animals were not infected by BPV (Control group), 33 animals were infected by BPV types 1 and/or 2. The BPV-infected group included animals with papillomatosis and animals without detected lesions. Seventeen females (with papillomatosis) exhibited 42.71% of cells with chromosome aberrations; 16 animals (without papillomatosis) displayed 40.19%. A total of 2203 cells were analyzed: 918 showed one or more chromosomal aberrations. The chromosomal aberration rate in symptomatic and in asymptomatic animals was respectively 42.7 ± 7.8 and 40.2 ± 11, compared with an aberration rate of control group of 4 ± 2. The Kruskal-Wallis test followed by the Mann-Whitney test was used for statistical analysis ($P < 0.0001$). Significant differences were not observed between infected subgroups ($P = 0.62$). The identified chromosomal aberration types were: centric association (CA); acentric fragment (AF); telomeric association (TA); telomeric association by a single chromatid (TAcr); chromatid breaks (CtB); chromosomic breaks (CmB); gaps; aneuploidy; polyploidy; addition or loss of chromosomal segment (add or del) and early chromatid separation (EcrS). The possibility of distinguishing infected and non-infected animals by levels of chromosome aberrations establishes evidence of the virus interaction with host chromatin.

Supported by: CNPq, FAPESP, FACEPE and Fundação Butantan.

5.20 HPV detection and typing of women seen for routine evaluation in Health Department, Ouro Preto, Minas Gerais, Brazil

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Introduction: The human papillomavirus (HPV) is widely distributed in the world and virus persistent infection is recognized as an important cause, for the development of cervical cancer. Primary lesions detected by cytological examination can progress or regress spontaneously. The challenge in cervical cancer screening is to detect the risk of progression to cancer. Besides the presence of the virus, the main determinants of cervical cancer clinical progression include the involvement of high-risk viral types, viral load, integration of viral DNA in the host chromosome and interaction with different co-factors. **Objective:** We investigated the types of HPV in women of the city of Ouro Preto, MG, relating to HPV cytological alterations and the cervical cancer development. **Methods:** Patients received at the City Health Department for routine gynecological were submitted to anamnesis for assessing socio-demographic characteristics and family, sexual and reproductive history. Cervical samples were collected for cytological examination and molecular analysis (HPV). Detection and typing were done by polymerase chain reaction with primers My09 (5'-CGT CCA / AAA C / G GGA A / TAC TGA TC-3 ') and My11 (5'-GCA / CAG GGA C / AAC CAT T / T AAT GG-3 '), RFLP and sequencing. **Results and Discussion:** We evaluated 461 patients, mean age 38 years, where the majority were from the urban area (67.4%), married (46%), and with only 1 sexual partner (49.5%). Eighty-one (17.5%) presented with HPV infection, and 50 women (11%) had cellular changes in the Papanicolaou test: 28 women had cellular atypia of undetermined significance may be not neoplastic (ASC U.S.), 8 showed no cellular atypia discarding injury of high degree (Asch), 1 had non-neoplastic glandular cells (AGS-NOS) and 11 had squamous intra-epithelial lesion of low-grade (LSIL). A total of 65 had viral type identified by the method PCR / RFLP, where 60% had infections with oncogenic HPV high risk, 34% low risk HPV, 6% risk oncogenic HPV indefinite and 17% multiple viral infections. The data show the presence of different viral types in the city of Ouro Preto in women with or without various changes and cytological studies, indicating that prevalence studies should be developed previous to vaccination approaches, for the survey and understanding of the determinants of the development and progression of cervical cancer.

Supported by: CAPES, CNPq, PRONEX and FAPESP.

5.21 Expression of extracellular matrix proteins (ECM) and matrix metalloproteinases (MMP) in human dental pulp stem cells

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Introduction: Adult stem cells can be isolated from different tissues including the human dental pulp, a structure originating from the dental papillae. These cells are important in a series of studies, such as the analysis of some odontogenic tumors. **Objective:** The aim of this study was to infer the histogenesis of odontogenic myxoma (OM), a benign odontogenic neoplasia, analyzing the extracellular matrix proteins (ECM) and matrix metalloproteinases (MMP) expressed in human dental pulp stem cells. **Methods:** Three different immature dental pulp stem cell cultures (IDPSC) (DL-1, DL-2 and DL-4) were used. The proteins searched were those routinely used to characterize the OM: vimentin, type I collagen, fibronectin, tenascin and hyaluronic acid (HA). MMPs frequently highly expressed in invasive tumors (MMP-1, MMP-2 and MMP-9) were also analyzed. Immunofluorescence and enzymatic assays were performed to determine the presence of these proteins inside the cells and in the conditioned media, respectively. **Results and Discussion:** All cell lines expressed vimentin, but none of them expressed HA, a protein frequently involved in cell migration and proliferation. The DL-1 line expressed all the other ECM proteins, and the expression of type I collagen was not observed in DL-2. Fibronectin and tenascin were not observed in DL-4. All cell lines expressed all the MMPs, but the release of MMP-2 in the conditioned media was significantly higher than with others. Based on the conditions of this study, it is possible to conclude that expression of ECM proteins and MMPs in IDPSCs were similar to those found in OMs. The lack of HA expression in these cells, a protein which characterizes the tumor, needs more investigation.

Supported by: CAPES.

5.22 Effects of ovariectomy and 17β-estradiol replacement on ERK1/2 activation in rat hippocampus

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Introduction: 17β-estradiol plays a potent neurotrophic and neuroprotective role in brain (reviewed in Brann et al., Steroids 72:381, 2007). The mechanisms underlying estrogen neuroprotection are not fully understood; however, several candidate targets have been identified, for example, members of the Bcl-2 family (Nilsen and Brinton, Proc. Nat. Acad. Sci. USA, 100: 2842, 2003). Recent studies from our laboratory have shown that 17β-estradiol may help maintain long-term neuronal viability in the hippocampus by regulating the expression of Bcl-2 family members if initiated immediately after ovariectomy (Sayuri et al., Anais da XXIII Reunião Anual da FeSBE, pp. 89, 2008). 17β-estradiol has also been shown to activate extracellular signal-regulated kinase (ERK) which mediates neuroprotection in the hippocampal CA1 after global ischemia (Jover-Mengual et al., Endocrinology 148:1131, 2007). Whether ERK signaling cascade is involved in estrogen-induced expression of Bcl-2 after ovariectomy remains to be explored. **Objective:** The aim of the present study was to determine the effects of ovariectomy and 17β-estradiol replacement on ERK1/2 activation in adult rat hippocampus. **Methods:** The experimental procedures were approved by the Research Ethics Committee of Instituto Butantan (number 569/09). Hippocampi were obtained from rats in proestrus (control), rats ovariectomized for 15 days (C15) and 21 days (C21), rats ovariectomized for 15 days and then treated with 17β-estradiol benzoate for 7 days (10 μg/rat, s.c., every other day) (E7) and rats ovariectomized and immediately treated with 17β-estradiol benzoate for 21 days (10 μg/rat, s.c., every other day) (E21). Western blotting for detection of ERK1/2 and phospho-ERK1/2 was performed as previously described (Lucas et al., Biol Reprod., 78: 101, 2008). **Results and Discussion:** Ovariectomy for 15 and 21 days (C15 and C21) did not have any effect on ERK1/2 phosphorylation compared to values obtained from control animals. Similar results were obtained with 17β-estradiol replacement limited to the last week of hormonal deprivation (E7). On the other hand, 17β-estradiol replacement throughout the post-ovariectomy period (E21) induced a rapid increase in the phosphorylation state of ERK1/2 (pERK1, 199.56 ± 14.81, n=3; pERK2, 139.03 ± 19.27, n=3) ($P<0.05$) compared to values obtained from control (100%) or ovariectomized rats (pERK1: 128.59 ± 21.19%, n=3; pERK2: 105.93 ± 5.93:, n=3 and pERK1: 104.38 ± 4.38%, n=3; pERK2: 106.76 ± 4.35%, n=3, respectively, C15 and C21). These results suggest that 17β-estradiol is involved in the regulation of ERK1/2 phosphorylation in rat hippocampus if initiated immediately after ovariectomy. Neuroprotection by estrogen could be mediated in part by expression of Bcl-2 through ERK1/2 phosphorylation. Further experimental approaches will be important to clarify these events.

Supported by: PRONEX, CNPq and FAPESP.

5.23 Preliminary assessment of the use of recombinant protein l2 of bovine papillomavirus

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Introduction: Papillomatosis is an infectious disease characterized by verrucous lesions, in the skin or mucosa, which affects several species of mammals, including man. In cattle, this disease is responsible for dramatic economic losses, as in reduction in milk production and weight loss. The tumors are usually benign and often regress spontaneously. However, the lesions can process to cancer. Bovine papillomavirus types -2 and -4 (BPV-2 and BPV-4) are associated with cancers of the bladder and upper digestive tract. **Materials and Methods:** The cloning of the L2 gene and expression in a bacterial system is a viable approach for the production of immunological inputs, such as diagnostic tests or vaccines. The N-terminal portions of the protein L2 (BPV-2 / BPV-4), previously cloned, were purified by affinity chromatography system for glutathione. The protein purification resulted in 5.0 mg / mL of the protein L2-BPV-2 and 7.5 mg / mL of the protein L2-BPV-4 which were used for inoculation of calves, two doses of 1.0 mg each protein were mixed 1:1 with aluminum hydroxide. The proteins were subjected to electrophoresis in denaturing polyacrylamide gel, transferred to nitrocellulose membrane and subjected to immunodetection using the pre-and post-immune serum. The levels of antibodies were analyzed in an ELISA reader, using microplates prepared with 1.0 g of each protein. **Results and Discussion:** The immune response obtained by vaccination of calves was satisfactory, indicating the feasibility of this approach for the implementation of a vaccine-BPV in the national herd. Further analyses of specific antibodies directed against the protein L2 of bovine papillomavirus types -2 and -4 are being improved.

Supported by: FAPESP and CNPq.

5.24 Secretion cycle of primary duct after milking in the snake *Bothrops jararaca*

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Introduction: Venom gland apparatus of Viperidae snake has four distinct parts: main venom gland, primary duct, accessory gland and secondary gland connected to the fang canal. The main venom gland is well studied. This gland has a central lumen where venom is stored and the secretory cells are stimulated for a new cycle of venom synthesis after emptying the lumen either by manual extraction or biting. The venom production cycle lasts around 30 – 50 days, peaking at 4 days after milking. There are few studies about the primary duct, and we have shown that the primary duct of *Bothrops jararaca* is folded and has a pseudostratified epithelium composed of secretory cells with negative reactivity for PAS and alcian blue, horizontal cells and mitochondria-rich cells. **Objective:** The aim of this study was to determine the secretion of secretory cells of primary duct of *Bothrops jararaca* snake during the venom production cycle. **Methods:** Female *Bothrops jararaca* snakes were anesthetized with pentobarbital sodium (30 mg/kg) and the primary duct from unmilked, 1 hour milked (n=3 each), 1 (n=1), 4, 7 (n=3 each) and 15 (n=2) days milked snakes were excised after decapitation. The primary ducts were fixed and embedded in Epon resin. Histological sections with thickness of about 1 μm were stained by Toluidine Blue. Animal care and procedures used were in accordance with the guidelines of the Animal Ethics Committee of Instituto Butantan (374/2007), and by the Brazilian Institute of Environment (IBAMA, License 01/2009). **Results and Discussion:** In unmilked snakes, the secretory cells of primary duct showed a large number of vesicles with different electrodensities and a lumen full of secretion. The presence of the secretion inside the lumen began to decrease 1 day after milking and was almost empty 4 days after milking. Afterward, the lumen started to be replenished and 15 days after milking was not completely full. Just after milking, the number of vesicle decreased. Large number of vesicles can be detected only 4 days after milking and persists until 15 days after milking. Therefore, exocytosis occurs just after milking, but the replenishment of the lumen starts only 4 days after milking. Our data show for the first time the secretion of the primary duct of *Bothrops jararaca* snake after milking. These results suggest a cycle of secretion similar to that found in the main venom gland.

Supported by: FAPESP.

5.25 Isolation, culture and characterization of stem cells derived from feline adipose tissue and their multilineage differentiation potential

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Introduction: Mesenchymal stem cells (MSCs) are a promising tool for tissue regeneration, due to their particular characteristics of proliferation and differentiation into lineage-specific tissues such as bone, cartilage, fat, tendon, and muscle. In addition to the differentiation into their natural derivatives, multipotent MSCs have the potential to differentiate into other types of tissue-forming cells such as hepatic, renal, cardiac, and neural cells. Several publications have shown that stem cells reintroduced in the organism were capable to restore a tissue and its function. Although stem cells have been isolated from rodent and human tissues as dental pulp, muscle, skin and fat, very few data exist about stem cell isolation from nonrodent animals, such as dogs and cats. Adipose tissue is an attractive source of MSCs due to its abundance and ease of access with minimal donor site morbidity. **Objectives:** The aim of this current study was to provide evidence that feline adipose tissue–derived mesenchymal stem cells (fAD-MSC) can be isolated and characterized. Regarding their potential of proliferation and differentiation we directed our study to their use in cell therapy in pets. It is the essential first step toward their use in domestic cat diseases. **Methods:** First, fAD-MSCs were isolated using an explants method followed by freezing in liquid nitrogen until use. After thawing, cells were analyzed for proliferative potential, and subsequently their differentiation capacity in osteogenic, adipogenic, and chondrogenic inductive media. Differentiation was assessed by morphological criteria and immunohistochemistry. **Results and Discussion:** The cells showed similar fibroblast-like cell morphology *in vitro* before and after cryopreservation. After the induction of osteogenic differentiation, *von Kossa* staining revealed the formation of calcified extracellular matrix, and immunocytochemistry studies revealed positive immunostaining for osteocalcin (LF-32 and LF-126), as well as bone sialoprotein, confirming the osteogenic potential of these cells. The adipogenic differentiation was confirmed by oil red O staining, which revealed significant lipid droplet accumulation. Chondrogenic differentiation was observed after induced pellet culture and evidenced by histological (toluidine blue) and immunohystochemistry studies using specific antibodies, such as aggrecan and collagen type II. Under the influence of the 3 different media, fAD-MSCs were capable of advancing into all 3 differentiation pathways. In conclusion, the stem cells isolated from feline adipose tissue show characteristics similar to those of human mesenchymal stem cells. They are a suitable source of multipotent stem cells for future tissue engineering strategies and cell-based therapies.

Supported by: CELLTROVET.

5.26 New system for gene therapy delivery: crotamine kinetic in cell cycle and penetration mechanisms

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Introduction: Cell penetrating peptides (CPPs) hold great potential as delivery vectors, *in vitro* and *in vivo,* for use in research and medicine. The current use of these CPPs is limited due to a lack of cell specificity in CPPs-mediated cargo delivery, as well as insufficient knowledge of their uptake mechanism. Previously, we isolated and characterized crotamine, a rattlesnake venom compound able to penetrate into the cytoplasm and the nuclei. The interaction of crotamine with centrioles and chromosomes during cell proliferation and division suggests that its uptake depends on the cell cycle. Previously, our group demonstrated that crotamine penetrates into cells via endocytosis and interaction with heparan sulfate. **Objectives:** The aims of the present study were to determine: a) the crotamine effect in cell proliferation; b) its uptake in different cell cycle phases; c) the crotamine interaction with different syndecans (1 to 4); and d) clathrin and/or caveolin-mediated endocytosis involved in crotamine uptake. **Methods:** For this study, we performed MTT assay, immunofluorescence and flow cytometry using B16-F10, CHO-K1 and mouse peritoneal cells. Crotamine was conjugated with fluorophores FITC or Cy3 for the visualization of this CPP into the cell. For the proliferation assay, the crotamine concentration was 0.01-10 μM. To analyze crotamine kinetics into cell cycle, we synchronized the cells in G0/G1 phases and the uptake was determined every four hours, for 28 h. To verify the colocalization of crotamine with syndecans and the aforementioned proteins related to endocytosis, the following antibodies were used: anti-syndecans 1-4, anti-clathrin and caveolin. **Results and Discussion:** We observed that at a concentration of up to 1 μM, crotamine induced higher cell proliferation, up to 30% in comparison with proliferation in control culture. After cell synchronization, we analyzed crotamine fluorescence in different cell cycle phases. This CPP did not reveal any difference in treated cultures, suggesting that crotamine uptake by cells occurred independent of cell cycle phase. Crotamine did not show specificity for any syndecans tested, the colocalization of these molecules was observed on the plasma membrane and in cytoplasmic vesicles. We also observed crotamine colocalization associated with clathrin and caveolin. Our data indicates that crotamine: a) at a low concentration increased cell proliferation; b) uptake did not depend on phase of cell cycle; c) interacted with four syndecans types; and d) can be internalized concomitantly by different mecanisms: clathrin and caveolin endocytosis pathways. These data show that crotamine behaves like other CPPs such as Tat and Antp.

Supported by: FAPESP.

5.27 Expression of laminin-5 in actinic cheilitis and human lip squamous cell carcinoma

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Introduction: The actinic cheilitis is the initial and incipient stage of lip squamous cell carcinoma, resulting from excessive and longterm exposure of the components to ultraviolet solar radiation. Laminin-5 plays important role in the development and in the invasion of squamous cell carcinoma. **Objectives:** To analyze and evaluate through immunohistochemical techniques the expression and distribution of laminin-5 in actinic cheilitis and in lip squamous cell carcinomas. **Methods:** Paraffin blocks of actinic cheilitis, superficially invasive squamous cell carcinoma and invasive squamous cell carcinoma, from Hospital das Clínicas da Faculdade de Medicina da USP, were sectioned. Immunohistochemical reactions to laminin-5 gamma-2 chain (Clone: 4G1 - Dako) were carried out, and the slides were examined with a light microscope. **Results and Discussion:** The majority of actinic cheilitis cases showed no cytoplasmic staining for laminin-5 gamma 2 chain. All cases of superficially invasive carcinoma and invasive carcinoma showed laminin 5 gamma 2 chain positivity located in the extracellular matrix and in the peripheral cells of tumor invasive front, but the expression was not homogeneous. No cancerous tissue close to invasive areas showed cytoplasmic expression in the epithelial basal layer. Thus, it is possible to conclude that expression of laminin-5 gamma 2 chain is closely related to the degree of tumor epithelium dysplasia.

Supported by: FAPESP.

5.28 Characterization of *Bothrops jararaca* genomic sequences encoding toxins with antimetastatic potential

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Introduction: Jararhagin and bothropasin are metalloproteinases present in *B. jararaca*'s venom that have the potential to be used as tools for inhibition of tumor metastasis. The cDNA sequence identity of these toxins shows 65 % identity in the pro-domain region; 74 % in the catalytic domain; 93 % in disintegrin-like domain; and 100 % in the cysteine-rich region. The published cDNAs have been obtained from mRNA of *pools* of specimens. **Objective:** This work aimed at the characterization of the genes encoding those toxins through the identification of exons and introns in the genomic DNA from a single specimen. **Methods:** Genomic DNA was extracted from the blood by current procedures and PCR *primers* were designed according to the published cDNAs for both toxins. The amplification products were cloned using the *pGEM T Easy Vector* and DH5α *E.coli* according to current procedures. The clones were sequenced using the *Big Dye Terminator Kit* and *ABI Prisma 3100 (Applied Biosystems)*. **Results and Discussion:** The total length of the jararhagin gene is about 7402 bp and that of bothropasin is 7849 bp. Twelve exons and 8 introns have already been identified and may be accessed in the GenBank database. Approximately 886 bp only are still missing to finish the whole sequence of each toxin gene. Both toxin genes had the same numbers of exons and introns, but there were several differences between the sequences of exons and introns, and also in the sizes of introns. These findings constitute the first report in the literature concerning the determination of exon and intron boundaries for jararhagin and bothropasin. The data indicate that they are encoded by two different genes that did not undergo accelerated evolution following gene duplication, since the main differences between them are found in intronic sequences. Clones containing sequences of interest, especially those encoding the disintegrin domain, may be used in the future to express protein domains particularly involved with the inhibition of tumor cell growth *in vitro* and *in vivo*.

Supported by: CAPES, PAP/SES and FAPESP.

5.29 Expression of hypoxia regulatory genes during lung inflammation produced by intestinal ischemia-reperfusion in AIRmax and AIRmin mice

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Introduction: Oxygen homeostasis is essential for survival and physiologic development of organisms. Lack of oxygen to tissue is a common underlying factor in morbidity and mortality for numerous serious medical conditions. Myeloid cells exert their functions in specialized areas of hypoxia. The group of proteins responsible for adaptation of myeloid cells in hypoxia includes the products of the *Hif-1α* gene. Hypoxia-inducible factor is a transcription factor which responds to changes in oxygen and induces the transcription of the genes such as *Vhl*, *Vegf* and pro or anti-inflammatory cytokine-encoding genes. **Objectives:** The aim of this study was to characterize the cellular and molecular mechanisms operating in hypoxia state induced by intestinal I/R on lung inflammatory reaction in two lines of mice genetically selected for maximal (AIRmax) or minimal (AIRmin) acute inflammatory response. **Methods:** Lung injury was evaluated by MPO activity, cellular infiltration in the lung parenchyma, CD11b and CD62L expression on BM, blood and lung cells and hypoxia gene expressions by real-time PCR. **Results and Discussion:** We observed an intense neutrophily in the AIRmax lung ($2.6 \pm 0.3 \times 10^6$cell/ml) and a low infiltrate in AIRmin mice with equivalent levels compared to the control groups ($0.8 \pm 0.2 \times 10^6$cell/ml). Adhesion molecules analyzed showed higher expression of CD62L in the AIRmax I/R lung than AIRmin I/R or control groups. A high expression of *Hif-1α, Vhl, Il-1β* and *Il-6* was observed in AIRmax I/R mice. Conversely, AIRmin I/R mice showed low expression of these genes. AIRmax mice display a higher acute inflammatory reaction after I/R than do AIRmin mice, characterized by massive neutrophil infiltration. High levels of *Hif-1α, Vhl, Il-1β and Il-6* were determinant for high inflammatory response in AIRmax mice. This interline difference is in accordance with selection phenotypes indicating these lines as a model for the study of *Hif* regulation in the inflammatory response after I/R.

Supported by: FAPESP and CNPq.

5.30 Yolk sac and bone marrow progenitors can be isolated from canine fetus

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Introduction: The fetus is a source of non-embryonic stem cells (SC), and these cells are easy to obtain and to expand undifferentiated, while showing high differentiation abilities. The dog model mimics important aspects of human anatomy, physiology and pathology producing pre-clinical safety results after xenotransplantation of stem cells. On the other hand, the pet is a growing market of stem cells application. **Objective:** In our present study we aimed at the isolation and characterization of yolk sac (YS) and bone marrow (BM) progenitor cells from canine fetus. **Methods:** All experimental procedures were approved by the Ethics Committee of the School of Veterinary Medicine and Animal Science of São Paulo University (No. 931/2006). Canine fetuses at 30 and 55 days gestation were obtained through an ovarian hysterectomy and under general anesthesia. The explants of YS tissues and BM cells flushed from femur bone were cultured in α-MEM + 15% fetal bovine serum. Morphology of the cells was evaluated with an inverted microscope and by transmission electron microscopy (TEM). Antibodies: goat anti-Oct3/4; mouse anti-vimentin; mouse anti-VE-cadherin; rabbit anti-nestin; mouse anti-cytokeratin. Expression of anti-CD44 antibody was analyzed by flow cytometry. Osteogenic, adipogenic, neuronal differentiation assays as well as karyotype analysis were performed according to routine protocols. **Results and Discussion:** Two days after cultivation, first fibroblast-like colonies appeared in BM cell culture as well as outgrowth of fibroblastic cells around YS explants. TEM analysis demonstrates that the cells from both sources showed two cell populations with a high nuclear-to-cytoplasmic ratio and fibroblast-like morphology. Both, YS and BM fibroblast-like cells reacted positively and uniformly with vimentin antibody, and the majority of cells were positive to nestin antibody. In addition, cells isolated from BM showed positive immunostaining with CD44 (~ 96.6 %) and cytokeratin. Several fibroblast-like cells isolated from YS were also positive for Oct 3/4 and VEGF antibodies. Both cell populations showed successful osteogenic and chondrogenic differentiation. In addition, BM-derived fibroblast-like cells were able to produce neuron-like cells. Karyotype analysis performed at passages 6 and 7 revealed a normal diploid chromosome number (2n=78). Our data suggest that undifferentiated cells can be isolated from BM and YS and maintained in culture during successive passages, showing a normal karyotype. TEM analysis indicates that BM and YS undifferentiated cells are composed of two different cell populations: i. cells show characteristics similar to very small embryonic-like (VSEL) stem cells and ii. cells show characteristics of bone marrow mesenchymal stem cells (MSC), both described previously for human BM – MSC. Unexpectedly, antigen expression profile BM of progenitor cells and differentiation capacity suggest their ectodermal commitment.

6: Animal Biology

6.01 Participation of chemical signals in the behavior of *Crotalus durissus terrificus* (Serpentes, Viperidae)

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Introduction: Pheromones are substances involved in chemical communication among animals of the same species and allow mutual and sexual recognition of individuals causing physiological and / or behavioral changes in animals that are in the range of the secretor. In snakes, the chemical communication participates in intra- and interspecific social interactions, being mediated by secretions (pheromones) from the skin and cloacal glands (present in all snakes). The functions of cloacal gland secretion seem to vary among different species, including alarm signals, sexual attraction, defense and aggression. Through the tongue flicking, the odoriferous molecules (chemical signals) are taken to the vomeronasal organ, located in the serpent's mouth on the palate. **Objectives**: This paper attempts to show if the cloacal gland secretion causes change in frequency of tongue flicking in *Crotalus durissus terrificus*. **Methods**: Eleven adult specimens of *C. d. terrificus* were used (six males and five females), all of them living more than four years in captivity. During the experiment, the animals were kept in individual wooden boxes with water "ad libitum" and were fed once a month. The stimuli were presented on a ball of cotton impregnated with cloacal gland secretion. The number of times that the snakes flicked their tongues in the first 60 s from the first tongue flick was measured. Three measurements were made, first when the box was opened (BO), second with cotton without the secretion (CWS) and third with cotton impregnated with the secretion (CIS). The cloacal gland secretion was obtained with the animal anesthetized in CO_2, by mild pressure at the base of the serpent's tail, where the glands are located. The experiments were performed in the mating season (autumn) and outside mating season (spring). To compare the results in and between the seasons we used the Mann-Whitney test with the tongue flicking differences between CWS-BO and CIS-CWS. **Results and Discussion**: In the spring, despite the increased number of tongue flicking, the difference between CWS-BO (range = -8 to 12 and average = 2.1) and CIS-CWS (r = -8 to 14 and avg = 2.9) was not significant ($p = 0.07$). When we did the same analysis in the mating season (autumn) the snakes showed a significant increase ($p < 0.0001$) between CWS-BO (r = -5 to 9 and avg = 1.1) and CIS-CWS (r = -3 to 9 and avg = 3.3). In the comparison of CIS-CWS between reproductive and non-reproductive seasons, there was no significant difference ($p = 0.29$). In both seasons, the presence of cloacal gland secretion induced an increase in the frequency of tongue flicking, indicating that this substance may have a role in communication between rattlesnakes; however, it is during the mating season that this increase becomes more effective, showing a possible influence on reproductive behavior. The fact that there was no increase between seasons may be due to a difference between the temperatures, where the average was 2°C below in autumn. More studies of how this secretion can change the behavior of males and females, and between seasons, are in progress.

Supported by: CNPq/INCTTOX.

6.02 Comparative analysis of *Culex quinquefasciatus* infected and non-infected by *Wolbachia pipientis*

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Introduction: *Wolbachia* are endosymbiotic alpha-proteobacteria harbored by terrestrial arthropods and filarial nematodes, where they are maternally transmitted through egg cytoplasm. These bacteria are capable of manipulating host reproduction yielding feminization, parthenogenesis, male killing, sperm-egg incompatibility and alteration of reproductive fitness. Effects of infection may be either advantageous or disadvantageous to the host depending on the type of interaction. *Culex quinquefasciatus* is a cosmopolite mosquito whose biting activity can disturb, cause allergies or transmit etiological agents of filariases and encephalitis. **Objectives:** In order to determine the existence of reproductive manipulation in *Culex quinquefasciatus* mosquitoes influenced by the presence of *Wolbachia* (B strain), an infected population was treated with antibiotic to obtain a colony free of *Wolbachia* infection. **Methods:** The naturally infected individuals were taken from a laboratory colony founded in 1995 with mosquitoes from Rio Pinheiros, São Paulo city. From that infected colony, 400 individuals were treated with tetracycline antibiotic to totally remove *Wolbachia* infection, yielding then a non-infected colony. Both colonies were compared regarding reproductive fitness and ovary morphology. **Results and Discussion:** The infected colony produced less eggs and with lower viability. Ovary morphology indicated that oocytes of infected mosquitoes attain maturity earlier. Crossing between infected males with non-infected females yielded non-viable eggs. Results indicate a lower reproductive fitness for infected mosquitoes, suggesting the influence of *Wolbachia* on reproductive traits. Results are also suggestive of the presence of cytoplasmic incompatibility phenomenon. The continuation of the present study will help us gain a better understanding of the genetic-physiological effects of *Wolbachia* on *Culex quinquefasciatus*.

Supported by: CAPES, CNPq and FAPESP.

6.03 Hematological values of *Crotalus durissus* (Serpentes, Viperidae) under different handling conditions at Instituto Butantan

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Introduction: The poisonous snakes in the Laboratório de Herpetologia of the Instituto Butantan are bred in a closed system (intensive handling-IH) or in an external snake farm (semi-intensive handling -SIH). Hematological information is used to detect conditions that affect the organism as a whole, aiding in the elucidation of diseases, guiding the treatment to be instituted and permitting the establishment of a more accurate prognosis. **Objectives**: To determine whether the type of handling, intensive or semi-intensive, alters the hematological response in rattle snakes (*Crotalus durissus*). The animals maintained under these two forms of handling were observed for nine months. **Methods**: Seventeen *C. durissus* adults (both male and female) with at least three years in captivity were used in the period extending from April 2007 to January 2008. Initially, all the animals were under an intensive regime and were afterward divided into two groups. One group was transferred to the external snake farm (10m x 5m x 1.7m), where the floor was partly earthen and the other part was cemented. There was a water tank and six shelters measuring 1m x 0.70m x 0.5m each; with two internal heaters in two of the shelters. The other group continued to be maintained under intensive handling at the animal house (individualized in wooden boxes measuring 50cm x 40cm x 24 cm, and lined with corrugated cardboard and *ad libitum* water). Once a month, the animals were submitted to poison extraction, and in the following week, they were fed mice or rats in a proportion of 10 to 20% of their weights. Each month, blood samples were collected from all animals of both groups for hematological tests. **Results and Discussion:** A seasonal variation in some hematological parameters was observed in both groups. From April to September, the total red blood cell count similarly decreased in both groups (April=594×10^3 cells/mm^3; September= 423×10^3 cells/mm^3). However, in November, the red blood cell count increased in the animals in SIH (586×10^3 cells/mm^3), while the increase only occurred in January for the snakes in IH (565×10^3 cells/mm^3). The white blood cells count did not vary seasonally in the SIH (6800 cells/mm^3), while in the IH group, there was a variation throughout the months, with the least numbers in June (3055 cells/mm^3) and the highest in November (8722 cells/mm^3). In both handlings, heterophilia was observed at the end of October and beginning of summer although the animals were in good health. The rattlesnakes presented few statistical differences in the different types of handling. Although the increase in the number of circulating heterophils is interpreted as an inflammatory response, in our study the results presented can have a seasonal physiological significance. The variation of the red blood cell counts during the months indicates a positive seasonal effect where exposure to light can be a positive synergistic factor. Perhaps the light that penetrated the room through the window of the IH was sufficient to maintain the seasonal hematological pattern similar to that of the SIH.

Supported by: FAPESP and CNPq/INCTTOX.

6.04 Relationship between temperature and prey size in the digestion of *Crotalus durissus collilineatus* Amaral, 1926

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Introduction: Ectothermic organisms regulate their body temperature through the heat gained and lost to the environment. Several environmental factors affect the digestion of these animals, including temperature, and then many amphibians and reptiles, including snakes, look for a warmer place after eating to optimize the process of digestion. **Objectives**: To check whether temperature and mass intake during feeding has any influence on the transit time of the ingested content. **Methods**: Ten specimens of *Crotalus durissus collilineatus* born in captivity (six females and four males) were used. The total length (TL) of the smaller specimen was 53 cm, while the TL of the larger one 74.5cm (average= 66.95 cm). The weight of the rattlesnakes used varied from 84 g to 202 g (average= 158.3g). The snakes were allocated in black acrylic boxes, inside a laboratory incubator with improved temperature control; a pump provided air supply and a fluorescent lamp connected to a timer provided a cycle of 12h light and 12h dark. In the first experiment (to determine the influence of temperature) snakes ingested mice (20% of their mass) at 30, 25 and 20°C and in the second experiment (at 25°C) the mice composed 10% and 20% of the snake's mass. A necklace with a small plastic ball was tied to the mice (a different color for each temperature) according to the temperature it would be ingested by the snake. **Results and Discussion**: In the first experiment, the average transit time (ATT) of the ingested content at 30°C was 14.7 days (standard deviation (sd = 3.8), at 25°C the ATT was 31.4 days (sd= 8.9), and at 20°C the ATT was 42.1 days (sd= 9.7) (ANOVA p = 0.05). In the second experiment, the ATT of the snakes that ingested 10% and 20% of their mass was 89 days and 31 days, respectively. One snake did not expel the plastic ball, although it has defecated. The necropsy of this animal showed that it was healthy, without gastrointestinal obstruction, despite the ball's retention. The temperature acted in a manner inversely proportional to the transit time; at higher temperatures the transit time was lower. The mass also influenced the transit time. The snakes that were fed mice half of their mass took three times longer to defecate. This study confirms through experiments that the temperature and mass of the prey ingested by *Crotalus* influence the transit time of prey in the digestive tract, without differences between males and females.

Supported by: CNPq/INCTTOX.

6.05 Incorporation of the herpetofauna specimens collected by the scientist Denise Maria Peccinini-Seale (Instituto de Biociências, Universidade de São Paulo) in the collection of the Instituto Butantan

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Introduction: Zoological collections are important material for scientific research. For this material to be maintained, updated and made available, we need the constant addition of new specimens, from various species and locations. The "Coleção Herpetológica Alphonse Richard Hoge," Instituto Butantan (IBut), has about 80,000 specimens of snakes from different regions of the world, with emphasis on neotropical fauna. Recently included is the "Coleção Referência," consisting mostly of specimens of Lacertilia, Chelonia, Crocodylia and Amphibia. The Coleção Referência was formed in order to assist in the identification of specimens coming from the reception of animals, Laboratório de Herpetologia (IBut), researchers and collections of the Institution. The material received is sorted and sent to the Coleção Referência, among other destinations of the Institute, and after scientific use, is returned to the collection. Besides to domestic destinations, the specimens are also sent to other institutions, through design and request. Dr. Denise Maria Peccinini-Seale, faculty researcher of the IB-USP, received several specimens from the receiving office, collected specimens in many scientific expeditions, including the Project Biota, and obtained specimens from scientific expeditions of colleagues for cytogenetics studies, especially with the group Lacertilia. **Objectives:** After the death of this scientist, the vast material maintained in her laboratory research was sent to the Instituto Butantan for scientific use, to be incorporated into the collection. **Methods:** With a list provided by one of her students and collaboration, specimens were screened and identified and place in four boxes with miscellaneous supplies received during May-June 2009. Due to poor conditions of fixation, the majority of specimens, originally maintained in formaldehyde solution, were rehydrated, preserved in 70% alcohol and transferred to new containers with new solutions, which hindered the identification. As the information recorded, such as local, collector and date of collection were obtained when transcribed. **Results and Discussion:** After preliminary screening and identification, specimens were identified belonging to the class Reptilia, Amphibia, Osteichthyes, Arachnida and Diplopoda, and eggs not identified. The specimens were listed to record the receipt. Of 651 specimens examined, there were 475 specimens of reptiles (470 lizards and five snakes), 168 amphibians, seven arthropods and a fish bone, and twelve eggs, two skins and four tails. Among the specimens that contained label with locality data, included were 21 cities in São Paulo, six of Pará, two of Bahia, two in Minas Gerais, one of Pernambuco and another of Amapá, collected during the last three decades. It was possible to identify the specimens and the origins for the implementation of cataloging, and the incorporation of specimens in the collection, a process that is still in progress. This material is of great value since many specimens were used for completion of course work, theses and dissertations of scientific publications, collected here to validate the specimen.

Supported by: PAP/SES.

6.06 Diagnosis and geographic distribution of species *Eunectes murinus* Linnaeus, 1758 (Serpentes, Boidae) based on specimens deposited in Coleção Herpetologica "Alphonse Richard Hoge" - Instituto Butantan

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Introduction: Snakes can vary in size, from the smallest feeding on invertebrates and being up to ten centimeters in length to the largest carnivorous constrictor being up to ten meters long with a varied diet. In the family Boidae Gray, 1825, are found the largest non-venomous snakes of the New World, boas, pythons and anacondas. The snakes of the genus *Eunectes* (sucuri) have a head covered by small scales, nostrils and eyes with vertical pupil, teeth aglyph. They are semi-aquatic and are found in watercourses, shores of rivers and flooded environments following the major river basins. This practice encourages the search for their prey, ranging from fish, birds, turtles, crocodiles and mammals of small to medium size, where they subdue their prey by constriction. The species *E. murinus* is known by the size and strength of body, the olive-green color, black spots and head with after-ocular bands, an orange one bordered by two black bands. **Objectives**: The aim of this study was to diagnose the species and examine the geographic distribution in São Paulo State. **Methodology**: For the survey of morphometric characters (standard color and design, biometrics) and meristics (pholidosis), 59 specimens were analyzed in 38 districts in the state, in Herpetology Collection "Alphonse Richard Hoge" – Butantan Institute, in order to observe and see whether or not there is a regular pattern for the species. In the analysis of master of color and design, biometrics of snout vent-length (SVL) and pholidosis, we used the statistical evaluation, Student's t-test. For the length of the side lines of the head, biometrics of tail length, distance between eyes and the eye to the nostril, the statistical test used was ANOVA of covariance between the sexes. The tests were applied to determine variation according to sex or to meet a standard for species. **Results and Discussion**: The specimens collected showed differences in some characters, while not significant in the number of scales and spots. The morphological characteristics about the spots varied as the number and color throughout the body, which does not explain a standard or not in this case for the specimens of the population sampled, suggesting a possible variation and ability to hide in the habitat where they live. With regard to the pattern of bands behind the black eyes, in some specimens the left top row shows larger sizes in length to the right, suggesting individuality in the population sampled. Males and females showed no sexual dimorphism in any of the characters sampled, except for the length of the tail, which is greater in males, due to the presence of hemipenis and retractor muscle. There was a regular pattern in the morphometric and meristic characters for the species studied. Regarding geographical distribution, the sampled localities indicated that the following for the species, the major river basins in the north, northwest and west of the São Paulo State.

6.07 Are female reproductive strategies conservative or derived in the *Bothrops atrox* group? A preliminary analysis with new data about the *Bothrops leucurus* female reproductive cycle

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Introduction: *Bothrops leucurus* is a large pitviper which inhabits the Atlantic Forest remnants in the coastal region of northeastern Brazil and the State of Espírito Santo. It also occurs in disturbed areas, such as grasslands and coconut plantations. *B. leucurus* belongs to the *atrox* species group, a monophyletic assemblage that also includes *B. atrox* and *B. moojeni*. **Objectives:** The aim of this work was to present new data about female reproductive cycle of *B. leucurus* and compare it to that of *B. atrox* and *B. moojeni*. **Methods:** A total of 98 adult females of *Bothrops leucurus* preserved at "Coleção Herpetológica Alphonse Richard Hoge" at the Instituto Butantan (IB) were macroscopically analyzed in regard to their genital tracts. The following data were recorded for each dissected specimen: (1) snout-vent length (SVL), (2) diameter of the largest ovarian follicle in the right ovary, (3) presence and number of embryos in the oviducts, and (4) occurrence of uterine muscular twisting (UMT) in the posterior region of the uterus. Data on the period of birth of the newborns were obtained from records in captivity at IB and anecdotal data available in the literature. **Results and Discussion:** Secondary vitellogenic follicles were found from April (autumn) to October (spring). Females simultaneously showing UMT and secondary vitellogenic follicles were found in May (autumn) and September (winter). Embryos were found in the oviducts from September (end of the winter) to December (summer). The number of embryos in the oviducts ranged from three to 29 (mean 11.8 ± 7.64, $N = 10$). The occurrence of UMT and secondary vitellogenic follicles in May corroborates the hypothesis that mating occurs during the autumn and that females store sperm in the UMT during the winter in the genus *Bothrops*. The occurrence of embryos in the oviducts during September shows that ovulation can occur during late winter and not only during the spring as proposed for the genus *Bothrops*. Only individuals from Bahia ovulated at the end of winter. As ovulation occurs during the spring in the population from Espírito Santo, the earlier timing of ovulation may be due to hot climatic conditions during the winter in the Northeast region of Brazil. Birth records for *B. leucurus* in captivity are only available from the beginning of the summer (December) until the beginning of the autumn (April). Ovulation occurs in July in *B. moojeni* from cerrado regions, which also have very warm conditions during the winter. *B. atrox* females ovulate in April (beginning of the dry season), much earlier than *B. moojeni* and *B. leucurus*. As a consequence, *B. atrox* parturition also starts earlier, in August, and occurs until February. This may be due to Amazon region climatic conditions where the temperature is high throughout the year. Although some reproductive strategies, such as mating during the autumn and sperm storage by means of a UMT, are highly conserved, other reproductive events, such as ovulation and the timing of parturition, can be influenced by climate conditions in the species of the *atrox* group.

Supported by: FAPESP.

6.08 What factors influence incubation time of snake eggs? Testing hypotheses on a tropical snake with labile egg retention

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Introduction: For oviparous reptiles, developmental phase inside the egg is likely to be one of the most vulnerable periods of their life cycle. Therefore, the time that eggs remain in nests may determine successful hatching. The rationale is that the longer the time in the nest, the longer is the probability of egg mortality due to predation, parasitism, mechanical injuries or inclement weather. But, what factors are able to influence the duration of incubation of reptile eggs and how do they interact with this variable? Temperature during embryogenesis is the most invoked factor influencing incubation time in reptiles. For other animals, mainly birds and invertebrates, other features such as initial egg size and egg mass also exert influence on this variable, but they are poorly studied in reptiles. Moreover, for squamate reptiles the degree of embryonic development at oviposition was never tested as a factor influencing incubation time. **Objectives:** Herein we investigated the influence of four potential factors likely to affect incubation time in reptiles: (1) incubation temperature, (2) embryonic stage at oviposition, (3) initial egg size, and (4) initial egg mass. As experimental model, we used eggs of the false-coral snake *Oxyrhopus guibei*. **Methods:** Eggs of 17 clutches of *Oxyrhopus guibei* were incubated in the laboratory. All eggs were measured and weighed soon after oviposition. One egg per clutch was dissected and embryos were collected for determination of developmental stage following standardized criteria for reptiles. The remaining eggs were housed in plastic containers with moistened vermiculite and submitted to two thermal treatments with different temperatures. High Treatment (HT) had a mean temperature of 28 ± 2 °C, whereas Low Treatment (LT) had a mean temperature of 24 ± 2 °C. At hatching, incubation time was recorded. **Results and Discussion:** In *O. guibei*, incubation period was influenced by temperature, stage at oviposition, but not by initial egg size and egg mass. Incubation time decreased significantly as stage at oviposition increased and this occurred at both temperatures. Incubation time at HT was nearly 40 days shorter than LT, although this decrease did not occur at equal proportions. This decrease obviously reflects higher embryonic metabolic rates at higher temperatures. Lastly, there were no significant correlations between initial egg size or egg mass and incubation time in either thermal treatment. Despite that these two last factors did not affect incubation time in *O. guibei*, we cannot discard this possibility occurring at the interspecific level. However, additional data for other reptiles are needed before we can generalize these results.

Supported by: FAPESP.

6.09 Egg retention and the evolution of viviparity in tropical snakes

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Introduction: The evolution of reptilian viviparity involves a set of complex events and the interaction of several factors along evolutionary time. The most accepted scenario on the evolution of viviparity in squamates suggests a gradual and progressive increase in the amount of intrauterine embryonic development before egg-laying. It is largely widespread that in oviparous squamates egg-laying typically occurs when embryos are at developmental stage 30, and the majority of species oviposits at stages 26-33. Few species oviposit soon after ovulation or retain eggs to later developmental stages. However, these observations are based mostly on egg retention in lizards of temperate zones. Therefore, we need additional data on snakes to generalize the phenomenon of egg-retention in Squamata. **Objectives:** This work addressed the following questions: (1) What is the degree of embryonic development at oviposition in tropical snakes? (2) Does embryonic stage at oviposition differ between Xenodontinae and Dipsadinae snakes? (3) Does the habit of a given species exert influence on stages at oviposition? **Methods:** For investigating stage at oviposition we used eggs of several gravid snake species donated to Instituto Butantan between 2006 and 2008. One egg by clutch was dissected at the time of oviposition and embryos were collected. We examined stages of embryonic development at oviposition from 83 eggs of 22 species from Brazil. For comparison with lizards and thus providing insights into squamates in general, we staged embryos following criteria of Dufaure and Hubert for the lizard *Lacerta vivipara*. **Results and Discussion:** Sampled snake species laid eggs with embryos between stages 26 and 34, which represents a considerable degree of intrauterine embryonic development. Egg-laying occurred with embryos at the end of organogenesis (stage 26) and the beginning of late growth (to stage 34). This pattern is similar to that seen in lizards and suggests a high degree of conservatism in the duration of intrauterine embryonic development in Squamata. A few species retain eggs until late development, which suggests that a high degree of egg-retention may result in problems for the embryos, the mothers or both. Physiological, morphological, ecological and phylogenetic constraints may be acting on the limit of egg-retention. Stage at oviposition did not differ according the habit of a given species. Xenodontine snakes lay eggs with embryos at more advanced stages than do dipsadine. This fact may explain why viviparity evolved in the xenodontine and did not in dipsadine snakes. Therefore, this work brings the phenomenon of egg-retention to tropical snakes and strengthens the idea of intermediate stages between typical oviparity and viviparity so that this evolutionary transition occurs. This was the first extensive work on egg-retention comprising different groups of tropical snakes. Further studies should focus on morphological and physiological features in both oviparous and viviparous species and on embryonic responses to extended egg-retention and incubation conditions to provide important insights into the evolution of egg-retention and viviparity in squamate reptiles.

Supported by: FAPESP.

6.10 Management and rehabilitation of lizards and turtles, wild and exotic, in the snake receiving annex - Casa Vital Brazil, Herpetology Laboratory, Instituto Butantan

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Introduction: The office for receiving snakes of the Laboratório de Herpetologia, IBu, has received for over a century wild and exotic herpetological fauna from collections, inventory and rescues of fauna, donations and owners of animals of different origins. Over the years, several species were received, in most cases, snakes, and lizards, amphisbaenas, turtles, crocodilians and amphibians, primarily due to the pet market, apprehensions of the IBAMA, including overcrowding in zoos and breeding facilities. The reception selects and sends out all received specimens, subjected to various procedures, requiring security and training of officials and technical staff. **Objectives:** To keep and to rehabilitate specimens of lizards and turtles from exceeding the serpents received and to promote new educational activities and handling of these species. **Methods:** Due to the increase in turtles and lizards in the receiving office, a project was designed in 2006 to revitalize the old serpentarium, currently disabled. This project has an area designed to house the animal for forty days and to manage these species with the aim of further exhibition of the animals for public visitation. In 2007, the changes were made in the courtyard outside the Casa Vital Brazil, to protect the species received. The infrastructure necessary for the initiation of activities was financed by funds from research projects in collaboration with other researchers of the institution. This allocated space in the Casa Vital Brazil, where the species are maintained in a healthy state, is semi-open with a forty-day site for rehabilitation of the specimens coming debilitated. **Results and Discussion:** The healthful specimens are lodged in enclosures, where they are fed with varied diet. Debilitated specimens are given special care in separate enclosures with heating and forced feeding. Specimens are observed daily with regard to development, behavior and daily mortality rate. In the case of death, if necessary, the animals are submitted to necropsy and remitted to the Reference Collection, annex to the main collection. Currently, 84 individuals are kept, 20 lizards, 64 turtles, juveniles and adults, 67 wild and 17 exotic, the following species: *Iguana iguana* (8), *Tupinambis meriane* (11), *Tropidurus* gr. *torquatus* (1) *Apalone* sp. (1), *Hydromedusa tectifera* (3), *Geockelone carbonaria* (21), *Geockelone denticulata* (1), *Trachemys scripta elegans* (14), *Trachemys dorbgyny* (14), *Trachemys* sp. (4), *Phrynops geoffroanus* (1), *Phrynops vanderhaegei* After more than two years of management of species and maintenance of enclosures, we see different reproductive behavior, mating and copulation in *Geochelone* and of posture, feeding behavior, food and territorial dispute in *Tupinambis,* among others. This is necessary to reactivate the old serpentarium to shelter the species that are well adapted and in good health, disseminating research activities in the management of reptiles, in addition to snakes, in the Laboratório de Herpetologia. Thus, we intend to increase the educational activities and public visitation at the Instituto Butantan.

6.11 Evaluation of seasonality and composition of the group of *Tomodon dorsatus* in São Paulo State, Brazil

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Introduction: Despite the great diversity and endemism of snakes in Brazil, it is difficult to find data on ecology and natural history for most species, even the most common and widely distributed. In the region of São Paulo state, it is the case of the "sword-snake" - *Tomodon dorsatus* belonging to the Colubridae family. This snake is found in central-southern Brazil, inhabiting regions of humid tropical forest, including riparian forests in open formations, and can still be found in disturbed areas. **Objectives:** To investigate using data collection and donation, the seasonal abundance of individuals of different ages of *Tomodon dorsatus* in the state of São Paulo. **Methods:** We used records of the specimens that came from the reception of the Institute Butantan, São Paulo, between January 2006 and December 2008 to obtain data on the group composition (adult or offspring). The pattern of seasonal activity and recruitment were inferred from the total number of specimens and offspring, respectively, brought each month to the Laboratory of Herpetology of Institute Butantan. **Results and Discussion:** During the study, a set of 589 individuals of this species belonging to the state of São Paulo were received. In terms of season, this species was collected mainly in the hot and dry period, in the months from October to December, the average of the highest number of catches of this species was recorded in November ($N = 32.0$), October ($N = 25.0$) and December ($N = 25.0$), and the lowest one in June ($N = 4.3$) and May ($N = 9$). In relation to the group composition, it was observed that in a set of 589 specimens, 514 were adults and 75 were offspring, the average of the highest number of adults were recorded in November ($N = 30.0$) and December ($N = 23.3$), and the offspring were in August ($N = 9.0$) and September ($N = 6.0$). This study demonstrates the presence of the species throughout the year, showing there is a significant difference ($p < 0.05$) in the number of adults collected in the dry season (June to September) and rainy season (October to May). These data differ from a previous study addressing the seasonality in areas of the Atlantic Forest in São Paulo State, which did not show significant differences in the number of individuals collected at each of the seasons. Tolerance to climatic conditions, food availability, reproductive cycle and phylogenetic constraints are considered the main factors responsible for the observed patterns in snakes. Other factors should also be considered to explain the differences in activity patterns of *Tomodon dorsatus*, since this species is malacophagous. During the study, the Institute Butantan received a larger number of animals in adulthood, the highest number of offspring arrived in August and September, confirming the data in the literature for recruitment at the end of the dry season and at the beginning of the rainy season.

Supported by: CNPq.

6.12 Morphological variability in urban populations of the mosquito *Aedes scapularis* (Diptera; Culicidae)

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Introduction: The mosquito *Aedes scapularis* is a culicid of public health interest, since it has vectorial competence for several arboviruses and filarias. It is widely distributed in the Americas and is capable of living in urban and suburban environments. In the state of São Paulo it was incriminated to transmit the Rocio virus in the 1970s. This species is suspected to be a species complex, and knowledge of it lacks populational characterization to elucidate this conjecture. Wing morphometrics is a useful tool for populational and cryptic species characterization. Such approach should be employed to investigate this biological problem of *Ae. scapularis*. **Objectives:** To investigate possible wing variation among three urban populations of *Ae. scapularis*. **Method:** Adults of *Ae. scapularis* were collected in three localities in the state of São Paulo: a)Parque Ecológico do Tietê (PET); b) Butantã (BUT); c) Pariquera-Açu (PAR). Sampling site PET is 15 km away from BUT, and both of them are situated respectively at the east and west sides of São Paulo city, whereas PAR is 200 km away from São Paulo city. Mosquitoes were fixed in 70% alcohol, and the wings were mounted and coverslipped for digital photography. In wing images, positional coordinates of 18 landmarks were taken. From coordinates matrix, shape variables were computed and then used to compare the three populational samples, using principal components analysis and discriminant analysis. Softwares used were TPS pack (J. Rohlf) and BAC (J.P. Dujardin). **Results and Discussion:** Principal component analysis showed that wing shape is slightly distinct among the three populations, but it is not possible to diagnose them with 100% accuracy. Discriminant analysis revealed that Mahalanobis distances are lower between samples from São Paulo city (PET, BUT) than they are between PAR and the other samples. Results indicate that *Ae. scapularis* showed regional wing differentiation, which may be a result of microevolutionary processes. Data are also suggestive of a direct correlation between geographic distance and wing dissimilarity. The tool "Geometric morphometrics" appears to be promising for morphological characterization of variability in mosquitoes and may help in evolutionary studies of Culicidae of medical interest.

Supported by: FAPESP.

6.13 Case report: observation of depraved appetite in *Hydrodynastes gigas* (Serpentes, Colobridade, Xenodontidae) in the exhibition of the Museu Biológico – Instituto Butantan

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Introduction: The genus *Hydrodynastes* is positioned within the subfamily Xenodontinae. The snakes are large, reaching up to 2.50 m in length, and have semi-aquatic habits. *Hydrodynastes gigas* species is distributed in Suriname, French Guiana, Venezuela, Peru, eastern Bolivia, southern Paraguay, northern Argentina and northern, southern and central Brazil. *H. gigas* feed on fish, amphibians, snakes, birds and mammals. **Objective:** To report on deviant eating behavior in *H. gigas* when submitted to living in the Biological Museum. **Methods:** A male specimen of *H. gigas,* from Ilha Solteira, state of São Paulo entered the Quarantine of the Biological Museum on July 25, 2006 and transferred to be exposed to the institution after a routine protocol on December 13, 2006. On exposure, food was offered twice and comprised *Lithobates catesbeiana* species of amphibians and the fish *Xiphophorus maculates,* alternating with rodents (*Mus musculus).* However, by direct observation it was possible to establish a differential feeding behavior in H. gigas. **Results and Discussion:** The specimen was fed for the two years and nine months, with a mixture of 51% fish and amphibians and 8% to 41% rodents. There was a depraved appetite of H. gigas in captivity, with the intake once of *Sphagnum sp*. (moss) used to compose the substrate, and the ingestion of a pasty consistency of their feces deposited on dry land area of the enclosure was noted, both in first half the month of February 2009. In early March, foraging was seen by the snake enclosure, entering a pool of water and ingesting, for the second time, their feces. Little is available in the literature on the natural history of *H. gigas* and information is based on captive animals, including maintenance, food and reproduction. Species with wide geographical distribution may have differences in feeding habits, being mainly related to the different availability of prey in each region. Cases of coprophagia in reptiles are rare, and there are few reports. Coprophagia in reptiles is observed in species of lizards *Iguana iguana*, in which the pups eat the feces of young adults to obtain the bacterial flora of microorganisms, this fact is due to the young in their diet cannot digest and absorb all the nutrients needed for their development. In the genus *Geochelone,* young adults have an omnivorous diet, but reports indicate that people can feed on their own feces or that of other animals. Cases of coprophagia and depraved appetite are not common in the literature, the described case of specimens of *H. gigas* may be due to stress of captivity, curiosity or even nutritional deficiency. For captive reptiles while offering favorable environmental conditions, the behavior of the animal is not always what is expected, resulting in a shift in behavior or nutritional syndrome, so it is appropriate to report the event, as happened in a confined environment, where reptiles, especially snakes, are more susceptible to environmental conditions imposed by captivity.

6.14 Use of silver sulfadiazine 1% for *Varanaus griseus* in wound healing – a case report

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Introduction: The lizard (desert monitor) *Varanaus griseus* is a species of monitor originally from North Africa and Western Asia. Its diet includes small mammals, birds, eggs and insects, and it will even tackle challenging prey such as hedgehogs, tortoises and venomous snakes. The use of ointment-based healing of 1% silver sulfadiazine has been reported in traumatic injury in *Varanus griseus*, with the presence of blood, edema and difficulty of healing. **Objectives**: The aim of this study was to determine the use of silver sulphadiazine in the process of healing in reptiles. **Methods:** The exotic lizard, *Varanaus griseus*, which belongs to the collection of the Biological Museum of the Instituto Butantan, showed laceration in the ventral region and cranial to the cloaca. The injury swollen with bleeding was caused by friction inside of enclosure that had stones. The wound affected the skin and subcutaneous tissue. The first recommended treatment was based on the use of hydrogen peroxide and nitrofurazone ointment (Furacin), lasting for four months without resolution of the problem. Thus, the wound was again evaluated and it was decided to use 1% silver sulfadiazine ointment twice a week. **Results and Discussion:** During a period of 6 months, the used of silver sulfadiazine resulted in complete healing and restoration of epithelial tissue. The ointment is a soft white cream, soluble in water, with a broad spectrum of antimicrobial agent, which acts as a bactericide in a variety of Gram-positive and Gram-negative bacteria and some fungal species. According to the manufacturer, this product is indicated for the treatment of wounds with great potential for sepsis, burns, varicose ulcers, infected surgical wounds and decubitus scabs. It is also an excellent topical medication used for tegumentary disorders, such as lacerations and bites by prey. Recent studies have shown that silver sulfadiazine is one of rare antibiotics used topically that does not prevent the proliferation of keratinocytes, while the majority of agents (neomycin and nitrofurazone) hinder the healing process. In reptiles, the use of 1% silver sulfadiazine showed good results as a bactericidal and healing of open wounds, showing it unnecessary to use other therapeutic measures in the healing process.

6.15 Ultrasonographic evaluation of the reproductive status of captive *Eunectes murinus* (Linnaeus, 1758)

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Introduction: *Eunectes murinus* has several regional names, but the most known are "sucuri" and anaconda. They are snakes of semi-aquatic habit, live along the banks of rivers following large river basins of tropical America. Considered the largest snakes in South America, it can reach 12 meters in length. These snakes are viviparous and complete the embryonic development in the uterus during pregnancy. As the physical examination is limited in reptiles, it has a complicated semiological process of internal organs. Ultrasonography is an easy method of assessment of organs and increases visibility causing minimal discomfort to animals because it is not an invasive procedure, principally the reproductive system. **Objectives:** The objective of this study was to check the reproductive system of captive anacondas through of use ultrasound. **Methods:** The two largest *E. murinus* studied live in the Biologic Museum of Institute Butantan with two other small snakes of the same species (a male and a female). In the period 2006 to 2009, six ultrasound examinations were performed, with portable ultrasound, to observe the reproductive system of two big female anacondas. Ultrasound examination was carried out in the following periods: December 2006 (summer), February 2007 (summer), June 2007 (winter), March 2008 (summer-autumn), December 2008 (summer) and April 2009 (autumn). **Results and Discussion:** We observed that in both females analyzed follicles decreased in size over the reproductive season. *E. murinus* number 4365 of ± 4 meters showed follicles of 2.7-3.7 cm (December 2006), 2.4-3.0 cm (February 2007), 2.1 cm (June 2007), 1.0-1.2 cm (March 2008), 2.5 cm (December 2008) and 1.1-1.2 cm (April 2009). *E. murinus* number 4389 of ± 3 meters showed follicles of 1.6 cm (December 2006). 1.2 cm (February 2007) and 1.9 cm (June 2007). According to the literature, anacondas have seasonal reproduction. Copulation occurs at the end of autumn and start of winter. In the nature reproductive aggregations occur simultaneously with vitellogenesis in spring. Pregnancy is in summer and birth occurs in the dry season (autumn - winter). The female sexual maturity occurs when they reach an average size of 3.5 meters length. Analyzing the size of follicles (vitellogenic process) in certain specific seasons, we may conclude the presence of seasonality in the reproductive process of the anacondas.

6.16 Egg sacs of *Peucetia rubrolineata* (Oxyopidae) and *Lycosa erythrognatha* (Lycosidae) predated by Mantispidae (Neuroptera, Insecta)

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Introduction: The interaction between spiders and mantispids is well documented in the literature, but little is known about the Brazilian species. Among the few reports, there are records of interaction between mantispids and *Trechalea* sp, *Trechalea manauensis* (Trechaleidae), *Parawixia bistriata* (Araneidae) and *Psecas chapoda* (Salticidae). **Objectives**: To report records of significant ecological relationship between two types of predators, spiders and mantispids, which can help the better understanding of the complexity of the invertebrate interactions. **Methods**: Six egg sacs of *Peucetia rubrolineata* collected at Salto-SP and six egg sacs of *Lycosa erythrognatha* from the reception of the Laboratory of Arthropods of the Butantan Institute were kept under laboratory conditions until eclosion of spider nymphs. The egg sacs were observed regularly and at eclosion the occurrence of spiderlings and/or parasites was recorded. **Results and Discussion**: One egg sac of *P. rubrolineata* collected in October 2008 were predated by two mantispds, one of them identified as a female of *Zeugomantispa virescens* (Rambur, 1842). From one egg sac of *L. erythrognatha* collected in June 2009, emerged two mantispids: a female of *Dicromantispa gracilis* (Erichson, 1839) and a male of *Zeugomantispa virescens*. The predators left the egg sacs through a circular opening and made the last molt outside egg sacs, which were totally destroyed. This is the first record of two species of mantispid infesting one single egg sac. Until now, the only record of interaction between two species of Mantispidae and one spider species were described for spiders carrying two different larvae, but not inside the egg sac. Species of the subfamily Mantispinae are specialized predators of spider eggs and the first-instar mantispids use two strategies to locate spider eggs: Larvae can burrow directly through the silk of egg sacs they find, or they can board and be carried by female spiders prior to sac production, entering the sac as it is being constructed. Both *P. rubrolineata* and *L. erythrognatha* exhibit parental care with the eggs and, in this case, the damage to the spider is not limited to loss of eggs, because during the development of the neuropteran, the spider continues providing care, ensuring the safe development of the predator. Of the few records of mantispds that predate spider eggs in Brazil, are two of *D. gracilis*, and two of *Z. virescens,* suggesting that these may be the main species in this type of interaction.

Supported by: CAPES.

6.17 Behavioral characters in Mygalomorphae phylogeny

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Introduction: Mygalomorph spiders are famous due their large size, being commonly found as pets. The phylogenetic relationships among their families are not clearly determined. The number of available characters for phylogenetic analysis is limited due to their morphological homogeneity. Little research has focused on Mygalomorphae's natural history, making the understanding of their evolutionary history difficult. Recently, behavioral characters have been used in phylogenetic analysis. Our research introduces behavioral characters to determine the phylogenetic relationships among these spiders. **Objectives:** The main goal was to understand the evolutionary history of the group, including behavioral characters in their phylogenetic analysis. We also wanted to test the validity of using the structure of end-products (webs) resulting from behaviors in these kinds of analysis. **Methods:** As a result of an extensive review of the literature, 8 web characters were delineated in mygalomorphs. The characters were incorporated in a morphological matrix and a total evidence analysis was performed. **Results and Discussion:** The resulting tree was better resolved than the strictly morphological one. Web related characters show a clear evolutionary sign, and some of them evolved in a correlated fashion. Burrow construction is an ancestral feature of spiders and appears correlated with silk-lining behavior. Coyle's hypothesis that sheet webs derived from silk lines is not supported by our analysis. Contrary to some authors' suggestions, in our study the end-products proved to be a reliable source of characters for phylogenetic reconstructions, besides making it possible to better understand the evolution of the behaviors that gave rise to them. From these results, we suggest that not only behavior, but also the structures resulting from it, are a good source of data for phylogenetic analysis.

Supported by: CAPES.

6.18 Residual yolk in neonates of snakes *Bothrops jararaca*

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Introduction: Higher vertebrates expedite the growth of their offspring by providing post-natal parental care, especially by feeding the young. Parental care is uncommon among reptiles, the females contribute to their newborn offspring's nutritional status by depositing yolk in the egg. The portion of yolk that remains unutilized at the time of hatching (referred to as "residual yolk") is drawn into the body of the hatching before it emerges from the egg. There are few data available from residual yolk in snake hatchlings. This study was undertaken to determine whether maternal size, clutch and egg size, and breeding timing have any influence on residual yolk mass of hatchlings in *B. jararaca*. **Methods:** Gravid females of *B. jararaca* (N=13) were used. The maternal SVL (cm), mass (g), mass of clutch (g), mass of eggs (mg), and total number of eggs were recorded. The clutches were categorized as early, mid and late depending on stage of development. At hatching, the sex, body mass (mg), SVL (cm), amount of residual yolk (mg) and fat mass (mg) were recorded. **Results and Discussion:** The residual yolk was observed in only one female with embryos (N=22) at more advanced stages of development without the presence of egg yolk (embryos: SVL= 25.3 ± 0.6; mass= 11.2± 0.6; fat mass = 0.59 ± 0.1; residual yolk = 1.48 ± 0.2). These animals were compared with other embryos without residual yolk (N= 10) but large amount of yolk in the egg (embryos: SVL=19.1 ± 0.9; mass = 5.4 ± 0.5; fat mass = 0.23 ± 0.1, yolk egg = 2.6+ 0.7). Preliminary analyses demonstrated that offspring sex had no effect on the quantity of residual yolk (t= 0.41, P=0.34, 1.52 ± 0.26 and 1.42 ± 0.32 for males and females, respectively). In relation to the fat and weight of mother, it was observed that fat in the early and late embryonic development was 0.8%, in the mid the percentage of fat increase to 3.5%. The relationships of the quantity of yolk eggs with weight of embryos were observed in the early with 66.0%, and more advanced stages was lower, 25.0%. In relation to the weight of the embryo, there was an inverse relationship, with early representing 20.0% and the end reaching 65.0% of their weight. The amount of residual yolk removed was an average of 13.3% in relation to embryo mass and 5.26% in relation to fat. In the embryos where stage of development was lower (N=10), there was no presence of residual yolk, only the yolk egg (50.2%) and fat (4.2%). No significant correlation was observed between egg mass and quantity of residual yolk, SVL embryos and residual yolk, and in relation to the size of the egg yolk and residual yolk, there was also no observed difference. The residual yolk may act as a 'plug' to prevent infection penetrating though the umbilical opening into the hatchling's peritoneal cavity. Residual yolk may enhance survival rather than growth and crucial to first days until the offspring find natural food sources. The residual yolk in *B. jararaca* was present only in embryos ready for birth, but was not detected in others with embryonic development in less advanced stages, beneficial for immediate post-hatchling activity and survival. The amount of residual yolk removed *B. jararaca* was averaged, 13.3% of hatchling mass. This study revealed that there is no relationship between residual yolk mass and egg mass embryo, embryo SVL and size of the egg yolk.

6.19 Recovery of toad (*Rhinella icterica*) parotoid macroglands after mechanical compression

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Introduction: Toads have a pair of parotoid macroglands in the post-orbital region, which are responsible for venom secretion for passive defense against predators. These macroglands are composed of juxtaposed alveoli, each one filled with a large syncytial granular gland with a thick epithelial duct forming a plug. When the toad is bitten by a predator, the venom is expelled from the parotoid in the form of jets inside its mouth, causing serious pharmacological effects. After venom expulsion, the empty secretory syncytia collapse inside the alveoli and the connective tissue around it is expanded, replacing the volume primarily occupied by the venom. The process of alveoli recovery, through gradual venom refilling is not known, both from the morphological and physiological viewpoint. **Objectives:** The aim of this work was to carry out a morphophysiological study of the parotoid alveoli recovery in *Rhinella icterica* after mechanical compression, simulating a predator bite. We followed the main morphological alterations during the process of glandular recovery. **Methods:** Specimens of *R. icterica* had both parotoids manually compressed. The animals were sacrificed and the parotoids were dissected in successive 5-day intervals, initiating with 2 h until the 40th day. Control specimens did not have their parotoids compressed. All the parotoids were fixed in Bouin fixative and were processed for histology in paraffin. The sections were stained with HE and Mallory's trichrome. **Results and Discussion:** The results indicated that even after an intense manual compression is applied, many alveoli remain full. The alveoli effectively affected by compression are observed with their syncytia totally collapsed, and with an extensive hemorrhagic area around them. From the 5th day, many cells are seen infiltrated in the connective tissue, mainly around the hemorrhagic areas. The syncytia already show signs of recovery, showing conspicuous nuclei with large nucleoli. From the 15th day, secretion is already present inside the recovered syncytia, although in small volumes when compared with the intact syncytia. The hemorrhagic areas are less extensive. On the 30th day, the recovered syncytia are filled with larger secretion volumes and there are still small numbers of infiltrated cells within the connective tissue. On the 40th day, syncytia in final recovery steps are observed together with others which remained in the initial steps described above. Even after an intense manual compression is applied, it seems that there are a number of alveoli that remain intact. This seems to be an important fact for toad survival, since after an attack the animal does not release all its venom stock from the parotoids, remaining protected in the case of new attacks. The presence of hemorrhage and the large amounts of inflammatory infiltrate around the syncytia in the first recovery stages indicate that the glandular tissue, as well as the connective tissue around it, suffer a serious injury when compressed. The recovery process is demonstrated by the gradual refilling of the compressed alveoli; the total process must last more than 40 days. Some alveoli, probably the ones which suffer the worst injuries, do not seem to recover and thus will not return to a functional state.

Supported by: CNPq and Fundação Butantan.

6.20 Reproductive ecology of a fossorial snake (*Phalotris lativittatus*) from Brazilian Cerrado

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Introduction: *Phalotris lativittatus* is a rare, fossorial medium-sized snake distributed endemically in Cerrado vegetation areas of São Paulo State. Data on feeding habits are unknown, but like congenerics, it is likely that diet may comprise other elongated fossorial vertebrates. Reproductive data are also unknown for the species and are, in general, lacking for all Elapomorphini snakes. This is due in part to the fossorial habits of the group, which makes observations difficult. **Objectives:** This work aimed to study several aspects of the reproduction of *P. lativittatus*. **Methods:** Dissections of 42 preserved specimens (28 females and 14 males) together with captive observations on egg-laying provided information on several reproductive aspects such as: size at sexual maturity, sexual dimorphism, reproductive cycles, clutch size, oviposition, hatching time, hatchling size. **Results and Discussion:** Males attain sexual maturity at about 409 mm snout-vent length (SVL) and females at 510 mm SVL. Mature females are larger than males, but with smaller tails. Although both testis volume and width of the deferent duct have showed higher values during the rainy season than in dry season, they did not differ statistically. Detailed histological surveys of testes and deferent ducts may help to elucidate reproductive cycle of males. The female reproductive cycle is seasonal, with vitellogenesis occurring in the rainy season (from spring to summer). Egg-laying was observed in early summer and hatchings occurred from late summer to mid-fall. Fecundity is low (mean = 4.5 eggs) but relative clutch mass appears to be high. This feature in fossorial snakes appears to be recurrent and may represent a strategy of maximizing the reproductive output minimizing the costs associated with locomotion of gravid females inside underground tunnels. Hatchling size averaged 234.3 mm and is similar to that of preserved immature specimens examined (mean = 280.3 mm).

6.21 Molecular phylogeny of *Bothrops neuwiedi* group based on cytochrome-*b* and ND4 mitochondrial genes

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Introduction: The genus *Bothrops*, widely distributed in Central and South America, comprises about 42 species arranged in several groups: *alternatus*, *atrox*, *jararaca*, *jararacussu*, *lanceolatus*, *pictus, taeniatus* and *neuwiedi*. Previously, four species had been recognized in the *neuwiedi* group: *B. neuwiedi*, *B. andianus*, *B. erythromelas* and *B. iglesiasi*, with *B. neuwiedi* composed of twelve subspecies - the *neuwiedi* complex. After a taxonomic review based on morphological traits, seven full species were recognized: *B. neuwiedi*, *B. diporus*, *B. lutzi, B. mattogrossensis*, *B. pauloensis*, *B. pubescens* and *B. marmoratus.* Nowadays, the *neuwiedi* group is composed of nine species, if we consider *B. andianus* and *B. erythromelas* within the group. The monophyly of *neuwiedi* has been supported by molecular data, although the studies always show insufficient sampling, and in some cases *B. andianus* is included in the analysis solely based on hemipenial morphology. Recently, a new classification was proposed, in which *Bothrops* is split into five genera, with *jararaca* and *neuwiedi* groups synonymized to *Bothropoides*. This phylogenetic study did not include all the species of *neuwiedi*, and the combined data (molecular + morphologic, except for *B. andianus* and *B. mattogrossensis*) recovered *B. neuwiedi*, *B. diporus*, *B. pauloensis* and *B. erythromelas* as a clade, but also recovered *B. andianus* and *B. mattogrossensis* out of the *neuwiedi* group. **Objectives:** In our study the aim was to investigate phylogenetic relationships of *B. neuwiedi* group based on mitochondrial DNA sequences of cytochrome-*b* and NADH dehydrogenase subunit 4 (ND4) genes. **Methods:** We used 378 bp of the cytochrome-*b* and 744 bp of ND4 from 111 individuals sampled from 78 localities of 14 states of Brazil, covering a wide distribution and eight species (*B. andianus* was not included). Sequences were edited/aligned with CodonCode Aligner and phylogenies were reconstructed using TNT1.1 for Maximum Parsimony (MP), and Mr. Bayes 3.1.2 with GTR+I+G substitution model for Bayesian inference (MB). Each mitochondrial gene was analyzed either separately or combined with 1122 bp. **Results and Discussion:** Our results recovered *neuwiedi* group as a well supported clade both in MP and MB; *jararaca* was the sister group of *neuwiedi*. Within *neuwiedi*, *erythromelas, pubescens, diporus* and *lutzi,* these were always recovered as monophyletic taxa, unlike with other groups (*e.g., pauloensis, neuwiedi, mattogrossensis, marmoratus*) that appeared polyphyletic. Reconstructed phylogeny of mitochondrial DNA evinces remarkable geographical congruence and reveals similar topologies of the trees both in MP and MB. However, topologies are only partially congruent with the current taxonomy which is based on morphological traits. Perfect agreement in terms of monophyly is observed in *B. erythromelas*, *B. lutzi, B. pubescens* and *B. diporus*. The non-monophyletic status of some species could be the result of recent differentiations of neuwiedi group, in which groups that are geographically distant are cohesive and well defined, and, on the opposite side, sympatric groups, not reproductively isolated, show introgression events.

6.22 Feeding preference and tactics of subjugation of prey by *Erythrolamprus aesculapii* in captivity

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Introduction: Ophiophagy is a habit found in several species, among them is *E. aesculapii*, an opistoglyphous colubrid snake. This species feeds on other colubrid snakes. In this work, we examined if *E. aesculapii* shows preference for specific colubrid snakes, and the differences in the way of subjugation of prey, according to their potential capacity to cause injury to the predator. **Methods:** *E. aesculapii* kept in captivity were fed five species of colubrid snakes. Analyzed items: number of ingested prey; preference for some species; site of the bite; way of ingestion; tactics used to subdue prey (live prey; use of body loops; biting/poisoning). Newborns of *B. jararaca* were also offered (N=5) to determing if *E. aesculapii* would demonstrate interest for poisonous snakes. **Results and Discussion:** A total of 75 experiments were carried out (52% ♂, 48% ♀). Of that total, 70.7% of the prey were ingested. Males ingested made up 82.1%, females 58.3%. Most snakes started the ingestion from the tail of prey. Two species (*O. guibei* and *L. miliaris*) had 100% of acceptance as food. The others: *P. Patagoniensis* 52.6%, *S. mikanii* 69.7% and *T. dorsatus* 57.1%. *E. aesculapii* did not show a pattern when the prey was subdued, biting any part of the body. After the first bite, we observed different tactics in subjugation: aglyphous and non aggressive prey (*O. guibei,L. miliaris* and *S. mikanii*) were simply bitten and ingested alive. The opistoglyphous (*P. patagoniensis*, *T. dorsatus*) started to protect themselves as soon as they were caught, biting *E. aesculapii* whose took body handles trying to immobilize the head of colubrids, moving the prey to the side of their mouths, trying to bite them with their differentiated tooth to inoculate the poison. *E. aesculapii* did not show interest in *B. jararaca* as food in any of the attempts. The species preferred by *E. aesculapii* showed a non aggressive behavior. *E. aesculapii* bites the prey in any part of body and the most of ingestions starts at the tail, and subdued as rested as active prey, using smell to find them. Aglyphous prey tried to escape while they were swallowed, but in any moment they struggled or tried to bite, not causing a great energy expense by the predator for subjugation; differently of the opistoglyphous prey. These prey are considered non poisonous, but they have the Duvernoy's gland and produce a secretion of variable intensity, being potentially capable of causing injuries. They offered resistance during subjugation, biting strongly and struggling a lot, forcing *E. aesculapii* to use the body to immobilize them and bite to inject poison. That tactics showed efficiency, in some cases the prey seemed to be softened, struggling and biting less than before, making the ingestion easy. Although they received a lot of bites (obviously some poison) *E. aesculapii* did not show any poisoning symptom. In spite of *B. jararaca* having approximately the same weight as the colubrids used as prey to *E. aesculapii*, they showed no interest but seemed to take care, which strike a lot when predators get closer. Some specimens of *E. aesculapii* were bitten by *B. jararaca*, but they did not show reaction. We do not know if the quantity of poison injected by the newborns would be enough to induce some reaction. This work is in progress, other species are being offered as food, and other parameters will be analyzed.

6.23 Diet and feeding behavior of *Tupinambis merianae* ("teiú") kept captive at the serpent receiving annex, outdoor courtyard of Casa Vital Brazil, Herpetology Laboratory, Instituto Butantan

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Introduction: Popularly known as "teiús", *Tupinambis* sp of Teiidae's family, they are the biggest lizards in the New World, and can reach approximately 140 cm of snout vent-length. They are characterized by their long and pointed heads, strong jaws with small teeth, pink bifida and long tongue. In general, their color is dark black with white or yellow spots and white ventral region. They are distributed throughout South America, living in almost all the Brazilian territory, in the coast areas of Guiana, Venezuela, Colombia, Argentina, Paraguay and Uruguay. They are all terrestrial, living in the clearings and on the edges of forests near anthropic environments. "Teiús" are usually seen during the summer – because in the winter they keep burrowed and inactive. They are omnivores and eat vegetable material, mollusks, arthropods, small vertebrates and invertebrates, meat and eggs. **Objectives**: The aim of this work was to evaluate the preference and the feeding behavior of the *Tupinambis merianae,* kept captive while analyzed, relating feeding behavior variations to the seasons of the year, limitation of area, offers and food preferences during the daylight among cubs and adults. **Methodology:** This work was produced at the Annex of the Reception, External Courtyard of Casa Vital Brazil, Laboratório de Herpetologia from January 2008 until July 2009. The animals kept at that place commonly arrive at the Reception donated by the population or through apprehensions by the governmental body. A sorting is made and then the animals are sent to the Courtyard and fort days, when they are examined by the veterinarian in charge and then are micro chipped. Eleven individuals were kept inside boxes lined with cardboard, supplied with water, one individual was kept free in the Courtyard, and all of them were observed. All observed specimens had a record of their routine and diet that contains data of start at the place, food items offered and consumed and other observations. For each day of the week a diversified diet was defined containing fruits, vegetables, arthropods, quail eggs, and newborn and adult mice. In the days that the animals were kept in the den - due to a strong cold - no food was offered to them, only to those that kept active. **Results and Discussion:** Beyond the animal management routine, it was observed that the diet preference of the analyzed adult specimens was for meat, especially mice (5 or 10 grams) and newborn ones preferred by the juveniles. More often the cubs accepted – more than adults - different food items, eating occasionally fruits, invertebrates and eggs. The juveniles ate more quantity than adults, according to their sizes and especially on cold and rainy days. The analysis revealed that the *Tupinambis merianae* stay almost inactive during the winter. However, it was noted that the juveniles, because of their phase of growth and for having an accelerated metabolism, eat more often according to the offer. During the warmest seasons they stayed very active, keeping their diet preference for endothermic prey. In captivity, their places are restricted and there is no need to hunt for food. This prioritizes the preference for live foods that stimulate at a higher level, the chemical sensitivity of the individuals.

Supported by: PAP/SES.

6.24 Redescription and new distribution records of *Acanthoscurria paulensis* Mello-Leitão 1923 (Araneae: Mygalomorphae, Theraphosidae)

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Introduction: Mello-Leitão in 1923, described nine species of the genus *Acanthoscurria* Ausserer from Brazil. However, the majority of the descriptions are based on characters such as: coloration pattern and eye arrangement currently considered of small taxonomic importance. Several holotypes cannot be located and are considered lost, as in the case for the male holotype of *Acanthoscurria paulensis* based on a male from Pirassununga, São Paulo, Brazil. **Objectives:** To re-describe *A. paulensis* based on a male from the type locality and describe the female, for the first time. **Methods:** The examined material is deposited in the collection of the Instituto Butantan, São Paulo, Brazil. Female epigynum was dissected and cleared in lactic acid for observation of internal structures. The drawings were made on a Leica MZ 12.5, with a camera lucida. **Results and Discussion:** The study of the specimens deposited in the Instituto Butantan enabled us to establish the synonymies of *Acanthoscurria atrox* Vellard and *Acanthoscurria guaxupe* Piza with *A. paulensis*. The descriptions of *A. paulensis* and *A. atrox* are both detailed and agree in general aspect, color and measurements. Mainly the morphology of the male papal bulb, of great taxonomic importance, showing a long embolus ending in a shelf due to two well-developed keels, is the same and allowed us to confirm that *A. atrox* is a junior synonym of *A. paulensis*. The specimens examined from the two type localities, Pirassununga, São Paulo and Campo Grande, Mato Grosso do Sul, Brazil corroborate the synonymy. The comparison between the holotype of *A. guaxupe* and the *A. paulensis* specimens confirmed the synonymy of the latter with *A. paulensis*. The characters cited by the author: number of spines on tibial apophysis, varying between 8-14 and the relation of measurements of carapace and tibia/patella of leg I or IV, cannot be considered distinguishable characters, as confirmed by Schiapelli & Gerschman de Pikelin. The distribution range of *A. paulensis* is increased to include the states of Mato Grosso, Goiás, Minas Gerais, Mato Grosso do Sul, Espírito Santo, Paraná and Rio Grande do Sul.

Supported by: CNPq/INCTTOX.

6.25 Working memory in web-building spider

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Introduction: Different from insects, spiders have a highly condensed nervous system in two compact ganglia. One of them is the brain. Spiders' CNS apparently contains 30,000 neurons. Cursorial spiders have more neurons, about 100,000. Spiders can show "intricate" activities such as planning more effective ways to catch prey, and can memorize information about previously captured prey and details about prey size. **Objectives:** The aim of this work was to investigate the existence of predatory memory in Orbiculariae spiders. This group is known as web builders. We also investigated the evolution of memory traces in a comparative analysis. **Methods:** We studied 5 species that represent four families from the Orbiculariae group. It is divided into three guilds (1) orb-web, (2) wandering sheet and (3) space web. In our first test, we offered each spider a first prey (p1) and after the capture of this prey item we offered a second one (p2); while spiders captured p2 we removed p1. In a second test, we attracted the spider to the periphery of the web right after it captured p1, and then removed p1 while it was distracted, outside of the hub. During this displacement we removed p1 from web. The memory improved in this predatory context is the working memory and reference memory. Working memory in animals is defined as a memory for training and specific events, whereas reference memory is related to a general context of the environment, in this case, the web. **Results and Discussion:** In the first experiment, all spiders but Zozis geniculata (Uloboridae) searched for the removed prey when p2 was offered: the spiders walked around the web and pulled web strands for several minutes. In the second experiment, all spiders searched for the removed p1, and searching behavior was more intense in this second experiment. We do not interpret the absence of searching in Zosis (experiment one) as an absence of working memory in this spider, since it shows working memory in the second experiment. Instead, we suggest that Zosis has a different organization of the memory system, that it does not recall memories (of p1, exp1) while it has an adequate stimulus (p2) in its chelicerae. Searching time (exp.1) decreases from the basal to the more derived spider families. This could be possibly explained by cost reduction associated with the reduction of searching behavior. Nevertheless, searching times in the second experiment increased from basal to derived spiders. This could be explained by changes in search image usage. The use of search images for relevant aspects of prey features imply more organized neuronal nets, suggesting that these spiders have a more complex nervous system.

Supported by: CNPq.

6.26 Prevalence of *Hepatozoon* sp. (Apicomplexa, Hepatozoidae) in boas (*Boa constrictor*) donated to the Instituto Butantan

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Introduction: The genus *Hepatozoon* is the most common group of intracellular protozoa found in snakes. It is a blood parasite transmitted to vertebrates through the ingestion of an infected invertebrate (definitive host) or through the ingestion of an infected intermediate host. In reptiles, the gametocytes infect red blood cells. The main invertebrate vectors of *Hepatozoon* sp are mosquitoes, flies, triatomides, lice, fleas, ticks and mites. The *Hepatozoon* sp infecting reptiles are apparently well-adapted, causing little or no disease in their natural hosts, but can be very important in the ecology of the species. **Objectives:** To determine the percentage of boas (*Boa constrictor*) donated to Instituto Butantan infected with *Hepatozoon* sp and their respective parasitemia. **Methods:** In 2008 and 2009, blood samples were collected with needles and disposable syringes by caudal vein puncture, and blood smears were prepared on slides for microscopy, air-dried and then stained with May-Grünwald-Giemsa modified by Rosenfeld. Interpretation of the slides was done using a light microscope with a magnification of 100X and the parasitemia was analyzed using a magnification of 1000X. **Results and Discussion:** Thirteen snakes were initially examined, of which four animals (30.77%) were found to have infection wiht *Hepatozoon* sp. The parasitemia was determined by counting approximately 700 cells (red blood cells), and detecting the presence of gametocytes. Parasitemia varied from 0.14% to 1.69%. The gametocytes found did not displace the nucleus of the red blood cell. The percentage of infected animals is in accordance with the results obtained by other authors who reported infection with *Hepatozoon* sp in recently captured snakes with a 38.9% positivity for *B. c. amarali*. A study analyzing 906 recently captured snakes donated to CEVAP – UNESP, Botucatu, reported that the species with the most significant infection rates were: *Bothrops jararaca* (21.8%), *B. c. amarali* (19.1%) and *Crotalus durissus* (15.8%). Contrary to earlier studies, displacement of the red blood cell nucleus by the gametocyte was not observed in our study, probably because it was a different species of *Hepatozoon*. The prevalence study of this parasite is important since information on this parasitosis is scarce and it is necessary to know the health status of the animals that arrive in captivity.

6.27 Snakes from Vale do Paraíba region (São Paulo State, Brazil)

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Introdução: The Paraíba Valley (PV) "Vale do Paraíba" is a natural west-east "corridor" situated between the Serra do Mar and Serra da Mantiqueira in the southeastern Brazil. This São Paulo State region has 34 municipalities with a wide range of anthropization beginning in colonial times, although important natural remnant areas still exist. The altitude of these municipalities range from 454 to 2000 meters, and at least five native forest types can be found: ombrophylous dense forest, semideciduos seasonal forest, ombrophylous mixed forest, riparian forest and cerrado. **Objectives:** The snakes from PV region were surveyed largely on the basis of Instituto Butantan (IB) and Universidade de Taubaté records, scientific literature, and fieldwork. **Methods:** The snake records from Instituto Butantan were reviewed as well as from the Herpetological Collection "Alphonse Richard Hoge" (IBSP) between 1988-2008. Fieldwork has been performed since 2007 in some remnants of Mata Atlântica by opportunistic sightings. **Results and Discussion:** A total of 7,640 snakes were recorded. The fauna as listed consists of 66 species; family Anomalepidae: *Liotyphlops beui;* family Boidae: *Boa constrictor amarali*, *Boa c. constrictor* (introduced), *Epicrates cenchria;* family Colubridae: *Apostolepis assimilis*, *Atractus pantostictus*, *A. reticulatus, A. serranus, A. zebrinus, Boiruna maculata, Chironius bicarinatus, C. exoletus, C. flavolineatus, C. quadricarinatus, C. plumbea, C. quimi, C. rustica, E. cephalostriata, E. melanostigma, E. undulata, Elapomorphus quinquelineatus, Erythrolamprus aesculapii, Helicops modestus,, Liophis almadensis, L. atraventer, L. jaegeri, L. lineatus, L. meridionalis, L. miliaris, L. poecilogyrus, L. typhlus, Mastigodryas bifossatus, Oxyrhopus clathratus, O. guibei, O. rhombifer* (doubtful), *Philodryas aestiva, P. olfersii, P. patagoniensis, Pseudoboa serrana, Sibynomorphus mikanii, S. neuwiedii, Simophis rhinosthoma, Spilotes pullatus, Taeniophalus affinis, T. occipitalis, Tantilla melanocephala, Thamnodynastes hipoconia, T. nattereri, T. strigatus, Tomodon dorsatus, Tropidodryas serra, T. striaticeps, Uromacerina ricardinii, Xenodon merremii, X. neuwiedii;* Family Elapidae: *Micrurus corallinus, M. decoratus, M. frontalis, M. lemniscatus;* Family Viperidae: *Bothrops alternatus, B. fonsecai, B. jararaca, B. jararacussu, B. neuwiedi, B. pauloensis* and *Crotalus durissus.* Not surprisingly, the municipalities closer to IB sent the largest number of snakes: Santa Isabel n=1101 (14.4%), Jacareí n=800 (10.5%). Surprisingly, São José do Barreiro, one of the most distant municipality from IB sent n= 758 (9.9%). The most representative species were *Crotalus durissus* (40.7%), *Bothrops jararaca* (21.5%), *Sibynomorphus neuwiedii* (7.3%) and *Oxyrhopus guibei* (6.1%) corresponding to 75.6% of the total. Species of open areas (*Xenodon merremii*, *Mastigodryas bifossatus* and *Simophis rhinosthoma*) were drastically reduced. The PV is a wide region with several different ecosystem remnants. The result of secular history of devastation can be testified by satellite images, showing a predominant clearing mosaic. On the other hand, important preserved remnants (federal and state parks, research stations, or even private areas) still exist. The importance of these remnants can be stressed by new recently described species, although protected areas in open formations are mandatory.

6.28 Comparative skin morphology of the dart-poison frog *Ameerega picta* and the frog *Leptodactylus lineatus*, regarding the elucidation of a possible relation of mimetism

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Introduction: Amphibian skin has many vital functions, such as respiration, communication and defense. Two types of glands are associated with these functions: mucous and granular (venom) glands. Their morphological structure and the composition of their secretions vary broadly between species. Highly toxic secretions are often associated with conspicuous skin color patterns, which are recognized and avoided by predators. This is the case of many frogs from the family Dendrobatidae, including *Ameerega picta*. The skin color pattern of this species is quite similar to that of *Leptodactylus lineatus*, which presumably acquire protection against predation through mimicry. **Objectives:** We aimed to compare morphological aspects of the skin of these two species, emphasizing the cutaneous glands. **Methods:** Skin fragments from the head, dorsum, abdomen and limbs were removed and embedded in paraffin and historesin. For histological study, fragments embedded in paraffin were stained with hematoxylin-eosin and those embedded in historesin with toluidine blue-fuchsin. For histochemical characterization of the skin, we applied bromophenol blue for proteins, PAS for polysaccharides, alcian blue pH 2.5, for acid polysaccharides and von Kossa technique for calcium detection. **Results and Discussion:** In both species, epidermis is composed of 3-4 cell layers, the outermost *stratum corneum* formed by flattened cells. The dermis is composed of the *stratum spongiosum*, where chromatophores, vessels and glands occupy the loose connective tissue, and the *stratum compactum*, where parallel-arranged collagen fibers compose a dense connective tissue. A layer of subcutaneous tissue rich in vessels internally coats the skin. A calcified layer is observed among the *strata* of the dermis. *A. picta* possesses a single type of granular gland, elliptical in shape, whose alveolus is filled with irregular secretory granules, non-protein, containing carbohydrates. *L. lineatus* shows spherical granular glands of two types, the first possessing spherical protein granules and the second containing granules of heterogeneous shape and size with non-protein content, containing carbohydrates. *A. picta* shows two types of mucous glands which differ in diameter and lumen size, possessing secretory cells whose granules are composed of carbohydrates. *L. lineatus* has a single type of mucous gland, characterized by a broad lumen and secretory cells containing carbohydrates. The glandular profiles of these species differ greatly. Although it is assumed that *L. lineatus* is non-toxic or, at least, less toxic than *A. picta*, this species has a large number of granular glands of two kinds, which may produce dissimilar secretions. On the other hand, included in a group of venomous frogs, *A. picta* possesses only one type of granular gland, suggesting a less diversified chemical defense system for protection against predation. Considering this species, this presumable incoherence may indicate that, regarding cutaneous glands and secretions, the investment in chemical defense would be more qualitative than quantitative in dendrobatid frogs.

Supported by: CAPES, FAPESP, CNPq and Fundação Butantan.

6.29 Influence of methodology in the rearing of *Grammostola mollicoma* (Theraphosidae, Mygalomorphae) in captivity

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Introduction: Mygalomorph spiders are important in population control of insects and small vertebrates, and they can also be useful as bioindicators of population diversity. *Grammostola mollicoma*'s distribution range reaches southern Brazil, Uruguay, Paraguay and Argentina. Studies concerning the biology and behavior of this genus are scarce. **Objectives:** The aim of this study was to follow the development of two *Grammostola mollicoma* groups kept in different vivariums, considering the advantages and disadvantages of the employed methods. **Methods:** After spending two years in captivity, a female, from Rio Grande do Sul, Brazil, produced an egg case in December 2008. After three months, 262 spiderlings hatched and were divided in two groups. One of them, containing 112 specimens, was kept in individual polypropylene pots of 10 x 6 cm; the other group containing 150 specimens was subdivided in 10 vivariums of 26 x 17 x 14 or 40 x 27 x 13/3 with egg carton substrate, each containing 15 spiders. Their diet consisted in *Tenebrio molitor* larvae and young *Grylus* sp. offered twice a week. Their development was evaluated by measuring their weight every 30 days and their exsuviae size. **Results and Discussion:** To date, the specimens kept in individual vivariums showed a uniform growth, while those kept in the common vivariums showed an unequal growth pattern. We also observed that the spiders in the common vivariums did not show territoriality although cannibalism did occur. The individual vivariums provide a safer environment for the spiders to grow in, while the common vivariums provide a more real environment, close to their natural habitat, since it enables competition.

6.30 Relative litter size in *Bothropoides* (*Bothrops*) *jararaca* (Serpentes, Viperidae)

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Introduction: The litter and newborn sizes are important factors that can increase chances of parental gene perpetuation. However, due to a series of costs that are always linked to reproduction, a high maternal reproductive investment may sometimes be prohibitive to snakes. The relationship between litter and newborn sizes, and the correlation between female´s size and the number of newborns per litter are known for many species and seem to be related to different habits, phylogeny and reproductive strategies. The determination of the maternal factors that can influence the litter and newborn sizes are important for the knowledge of the reproductive biology of snakes. **Objectives**: This study aimed to determine the correlations between female and litter size, and between litter size and newborn size in *Bothropoides (Bothrops) jararaca*. **Methods:** During this study, data of 70 pregnant snakes from various districts of the State of São Paulo and Santa Catarina were collected. The snakes were weighed and had their length measured before and after parturition. After birth, the litters were counted, sexed and had their weight and length measured. The relationships between female size and parental effort were then established and analyzed by linear regression. **Results and Discussion:** As previously described, the number of newborns per litter exerts a low influence on the newborn size ($p < 0.05$, $R^2 = 0.\ 15$). Female length also does not show a great correlation with litter size ($p < 0.05$, $R^2 = 0.12$), and unlike previous data for other species, the mother´s size does not seem to negatively influence the relative body mass invested on litters. The parental effort in *B. jararaca* is higher than many other viviparous snakes. This fact can be explained at least partly to their secretive habits and low energy consumption, which would allow this species, as well as the other snakes of the genus, to have a large reproductive investment, without excessively decreasing their survival ability.

Supported by: CNPq/INCTTOX.

6.31 Preferred temperature in *Bothrops moojeni* (Serpentes: Viperidae)

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Introduction: The study of preferred temperatures is essential to understand the snake's thermal biology. Although some studies have been reported, most of them focused on temperate and diurnal species. Thus, little is known about the tropical and sub-tropical nocturnal species. **Objectives:** The purpose of this study was to determine the preferred thermal range of the nocturnal snake *Bothrops moojeni*. **Methods**: In this experiment, 29 specimens of *Bothrops moojeni* were used. The animals were placed individually in a thermal arena and video-monitored for 120 h. The Tset interval was defined as the temperature range reached between the first and third quartile of the temperatures found during the period the experiment. The *t* test was used to compare thermal ranges between day and night and between sexes. For all statistical tests, the level of significance was set at $p < 0.05$. **Results and Discussion:** The results showed that the preferred thermal range of *B.moojeni* changes depending on the photoperiod (20.93°C to 22.20°C in daytime and 22.81°C to 24.42°C in nighttime), being similar to other crotalinae snakes. No significant difference was detected between male and female thermal ranges. Just like most other crotalinae, *B.moojeni* is considered mainly a nocturnal, low activity and ambush snake, which probably exerts a great influence on the temperatures searched by these animals. The search for lower temperatures during the day suggests that energy economy may play an important role in the ecology of *B.moojeni*. Moreover, higher temperatures during activity seem to increase the chances of success in prey capture and defensive ability in these animals.

Supported by: FAPESP.

6.32 Does the reproductive season of *Crotalus durissus* (Serpentes, Viperidae) impose differential reproductive costs between males and females?

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Introduction: Reproduction is energetically expensive for both sexes, but the magnitude of expenditure and its relationship to reproductive success differ fundamentally between males and females. Reproductive costs could take several forms in reptiles, but two major categories may be distinguished: costs associated with survival and costs associated with fecundity. The costs associated with survival are those that affect the likelihood of survival of reproductive individuals. The costs associated with fecundity influence the energy available for the future reproductive event. *Crotalus durissus* shows a seasonal reproductive cycle. The deposition of yolk in the ovarian follicles and mating occur during the autumn, the storage of sperm in the female reproductive tract during the winter, the end of deposition of yolk in the ovarian follicles and fertilization during the spring and finally, parturition in the summer. Males show intraspecific competition during ritual combat. They fight to access receptive females, which release particles of pheromones (vitellogenin) in the air. The inference of reproductive costs associated with survival can be achieved through surveys of mortality rates between males and females. These deaths may be caused by natural predators, trampling by humans or sighting. **Objectives:** The aim of this study was to evaluate the costs associated with reproductive survival between males and females of *Crotalus durissus* by means of a seasonal evaluation of the data of snakes arrival at Instituto Butantan (IB) (= abundance). **Methods:** The sexual dimorphism of the tail was used to determine the sex of *Crotalus durissus* adults individuals received during the years 2007 and 2008 at IB. The number of specimens received during each season was recorded to analyze possible differences in the activity patterns between males and females in relation to this species' reproductive cycle. The X^2 significance test was applied to evaluate differences between the number of males and females in all seasons. **Results and Discussion:** A total of 1,357 specimens were received. The X^2 test showed a marked difference in the autumn. However, during the winter, the difference in the abundance rates between males and females was not significant. The results show that the male reproductive strategies during the autumn increase the rate of sighting due to a prolonged search for females, fighting and mating. The greater number of females during spring and summer may be related to the vitellogenic process and pregnancy, factors that increase body mass and the rate of thermoregulation, thus making them less able to escape from predators and consequently, more sighted. During the winter, the results can be attributed to lower temperatures during this season which force males and females to stay in shelters in order to reduce travel expenditure as they are ectotherms. These data confirm the hypothesis that *Crotalus durissus* reproductive strategies impose differential survival costs for males and females, as reflected by differences on their abundance rates, characterized by strong seasonality of reproductive events.

Supported by: CNPq.

6.33 Infection of *Aedes aegypti* (Diptera: Culicidae) with avian malaria parasite *Plasmodium gallinaceum*: evaluation of its effect on wing shape

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Introduction: The family Culicidae includes mosquito species of epidemiological importance, some of them being vectors of etiological agents of zoonoses. Among such pathogens, protozoans of the genus *Plasmodium* are recognizably relevant to public health. Parasites may be capable of producing physiological, genetic and morphological and changes in their hosts. The detection of morphological changes in a mosquito species caused by infection with a parasite, may be helpful in the diagnosis of infected mosquitoes. **Objectives:** The present study aimed to evaluate possible effects of the infection by *Plasmodium gallinaceum* on wing shape of the host *Aedes aegypti*. **Methods:** Infected and non-infected individuals from parental and F1 generations were compared between them. Samples used consisted of 110 infected and 127 non-infected specimens. Mosquitoes were colonized under standard conditions from April to May, 2009. Wing shape was used as the comparative biological parameter, assessed through geometric morphometrics. *Aedes aegypti* female wings were mounted in a slide-coverslip and digitally photographed. Positional coordinates of each of the 18 anatomic points were taken. From these data, discriminant analyses and determination of Mahalanobis distances were computed using TpsDig V.1.40 software, and principal component analysis was performed using PADwin Version 89. Graphs and histograms were designed to depict the biological features analyzed: wing shape and bilateral wing asymmetry. **Results and Discussion:** Wing shape was similar in either infected or non-infected samples. Moreover, bilateral asymmetry was present in similar intensities in both samples. Principal components as well as canonical variables of the two samples overlapped in all graphical outputs, hindering attempts to distinguish infected from non-infected mosquitoes. Phenograms of Mahalanobis distances split infected and non-infected samples, whereas parental and F1 generations were clustered. Apparently, it is not possible to diagnose *Plasmodium*-infected *Aedes aegypti* individuals based on wing shape patterns. The present data suggest that these pathogens do not interfere with wing shape determination, at least when only two generations are analyzed. Thus, the influence of other pathogens on wing shape of culicids needs to be investigated.

Supported by: FAPESP and CNPq.

7: Education and Science Diffusion

7.01 Ethnozoology aspects of the species *Eunectes murinus* (sucuri) Linnaeus, 1758 in São Paulo State, Brazil

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Introduction: The largest non-venomous constrictor snakes of the New World belong to the family Boidae and are popularly known as boas, pythons, sucuri or anaconda, which has several regional names. The genus Eunectes is distributed in South America, and Brazil has a wide distribution, covering the state of São Paulo, except in the coastal zone. The species E. murinus is known by the size and strength of the body, the olive green color with its oval black spots, the large format of the head and the presence of an orange band bordered by two black spots. Due to these characteristics of the species, usually the communities of the regions of occurrence are not confused, and information reflects the common knowledge often acquired over generations. The capture of reptiles by the usual methods such as active search, drop traps and hunting, among other methods has not been very effective, and it is recommended that a larger number of samplings be performed, collections, such as night operations and obtaining information from the local populations. The help and involvement of communities to acquire data on the species is of great value. **Objective**: The aim of this study was to focus on the ethnozoological aspects of this species, knowing that the snake is embedded in the daily life of the communities sampled, indicating the status of the species is maintained in urban and rural areas occupied by people in the state of São Paulo. **Methods**: For the collection of ethnozoology data, a free questionnaire was prepared. Its application has a dynamic of its own, whose goal was to address various socio-cultural aspects of human-animal relationship - family stories and fantasy stories. Contact and informal interviews were conducted with urban and rural populations in 12 localities in the state of São Paulo. **Results and Discussion**: Ethnozoological aspects suggest that humans tend to entertain feelings of harm, danger, irritability, aversion and contempt for certain animals, including snakes. The representation or categorization that society is in this case and fauna of snakes is an essential part of the historical references to which they are accustomed. A total of 36 reports showed that the respondent usually refers to snakes giving features that confuse the senses in negative qualities and attributes of beauty. The species studied is present in everyday life, where the observation of its habits is directly related to the environment in which they are. Some respondents highlighted the size or shape of the anaconda and issued opinions about their behavior in occasional encounters with humans, demonstrating familiarity in relation to their habits. The naturalness with which they speak of encounters indicates a relationship that is not always ruled by fear, but by curiosity and fascination with regard to aesthetic-descriptive aspects and entertainment. This information is reflected in knowledge and beliefs of humans, as we have seen in the sample and is therefore important from a socio-cultural and economic aspect, in that it promotes the recovery of other knowledge and other cultures, besides amassing knowledge that is usually consistent with postulated academic knowledge.

7.02 Learning activity in science museums

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Introduction: Museums have in common the character of cultural heritage conservation and extroversion regardless of their kind and origin context. Although historically the social role of these institutions have been changing in impact and reasons, a dimension becomes clear: museums, based on the model known today, are educational spaces, organized with human knowledge historically constructed, shared and re-produced by active subjects. How do the museums lead this process, considering their audience as composed of individuals who give value and meaning to this heritage? Intending a discussion on understanding the social role of museums in terms of appropriation and re-production of culture, we used the historical-cultural approach, based on the ideas of Vygotsky, Leontiev and Davydov and we focused on the process of learning concepts and practices. We have assumed *a priori* that science museums are places where the learning process is present but not necessarily the learning activity. We distinguished, therefore, "learning" from "learning activity", considering that the latter should be investigated. **Objectives:** We aimed to understand how the learning activity is structured in science museums. **Methods:** An institution was chosen for analysis, the Museu Biológico do Instituto Butantan, and we tried to understand its long-term exhibition through a historical perspective. For this, we analyzed documents and institutional and personal collections related to science education and science communication practices held by the Instituto Butantan since its creation in 1901. On a higher scale, the analysis of cycles and microcycles of learning activity was developed from the point of view of visitors and monitors. For that, semi-structured interviews with museum guides and visiting families were recorded on audio and video. **Results and Discussion:** The macrocycles of expansive learning founded helped us to understand the current exhibition not only as a product of the anxieties and assumptions of the team of professionals involved, but as a result of activities developed over a whole century, which currently affect the interactions between audience and institution. The theoretical approaches used in this research, including the concept of "practice communities," offered important tips for organizing the educational activities in science museums, especially related to the positioning of the museum object as mediator artifact. Elements such as the use of germ-cell models and inquiry situations, the selection of nuclear concepts and practices, the promoting of the ascending from abstract to concrete, the movement between actions and operations, the use of the proximal development zone, and the social and semiotic mediation, were described as being important for the professional praxis of museum educators. With the relationship between activity theory and learning in museums, it is expected that this research may contribute to the understanding of museums as "mediator" structures which facilitate the many possibilities of interaction between the individuals and culture.

7.03 Learning in science museums: the young visitor at the Museu de Microbiologia

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Introduction: Science museums have historically prioritized actions focusing on scholarly public. However, different audiences attend these facilities. The Museu de Microbiologia receives a significant number of families, children and non-scholarly groups. Facing such demand, the studies aimed at learning about this public in order to plan appropriate educational activities are important. Research about small children (4 to 6 years old) visiting museums are little tackled in the literature in this field. However, they are crucial because the scientific literacy at this age contributes to the improvement of children's understanding of the world. For the development of activities regarding microbiology we have to consider some characteristics of small children such as the need for visualization, manipulation and play. **Objectives:** The aim of this work was to survey the main educational and communicational strategies that a science museum can use to bring youths closer to scientific culture. **Methods:** The first phase of this research, already completed, consisted of semi-structured interviews with children visiting the museum or attending the nursery school. The second phase, in progress, consists of the establishment of a children's exhibition and the analysis about youths' ways to explore it. In the first phase, qualitative research was prioritized in perception studies. The data were obtained from audio and video recorded interviews and children's drawings. **Results and Discussion:** Most young visitors understood that there are organisms not visible with the naked eye; some did not think about the possibility of using instruments to make them visible; many of them mentioned the microbe size, but only a few used the scale concept; most described their shapes and colors. While the nursery school children recognized microorganisms in different environments (water, air, living things, compost), the museum visitors usually associated them with houses and dirt. Most considered microbes as "bad beings" and generally remarked that they exist in the body (hands and feet). Rare were the children who perceived the relationship between microbes and food. The data allowed the establishment of the thematic axes to be explored in the new exhibition: scale, biodiversity, biological function, relationship with human beings and with food. These axes should be presented in the exhibition devices with different difficulty levels and should facilitate the interaction within the groups. We believe that the new expographic space will bring more elements to investigate which communicational and educational strategies can draw the empirical knowledge of the youths closer to scientific culture.

Supported by: FAPESP.

7.04 Darwin and Wallace's vision of Brazil

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Introduction: In 2008 the Museu Histórico presented the exposition "Darwin and Wallace's vision of Brazil" during the Semana Nacional de C&T, presenting the subject Evolution and Diversity. With the Laboratório de Herpetologia and História da Ciência, the team of the Museum chose to participate in the commemorations of the 150 years of the Theory of the Evolution of the Species which were happening in all Brazil during the period. **Objective:** The objective was to argue the relation between the concepts of evolution and half-environment, based on it uses and applications in the study of sciences in Brazil, as for example the model of the Theory of the Shelters. In a historical perspective, we revise the trajectory of both naturalists in Brazil, arguing the impression they had had of the nature and the culture in the coast and the Amazon region, also debating its appropriations in the diverse areas of the knowledge, as in social sciences. **Methods:** The exposition was composed of illustrative and informative panels, educational game and space simulating the Rio Negro with taxidermied animals of the Museu de Zoologia da USP. The intention of the educational action was to promote the argument of concepts that had historically derived from the theories of evolution. For the students of average education we detached the relation between biodiversity, evolution and society, and for the children of basic education we created a camouflage game. To divulge and to interact with the other educational spaces that had also participated in the commemoration, the Museum closed the exposition with informative panel detaching some other events that were happening. Each educator was responsible for a group. The characteristics of the students (purpose and time of the visit, number and age of students) determined the action of the educator, where in some cases a guided tour was selected, with a passage previously defined. In other cases, the tour was free and the educator only introduced the exposition calling the attention to some applications of the evolution concept. This same methodology was used with the other public of the museum. **Results and Discussion:** The museum received 80 schools and the visits from other segments of the public, during 30 days in September and 31 in October. The majority of the schools did not have knowledge about Semana de C&T, but when being informed that the activity was part of a national program, the schools wanted to participate and to help the educators, resulting in a positive evaluation how much the exploitation and interest, over all in the case of average education. In the case of the visitor who did not visit the exposition with a school, the educators did not interfere, interacting only when requested. For the Museu Histórico the execution of the exposition was a chance in correlating significant subjects for the Institution to a historical boarding, arguing the assimilation of concepts of natural life for social disciplines.

7.05 Preparation and production of temporary exhibition "Expo-Frog: a leap to extinction" at the Museu Biológico of the Instituto Butantan with qualitative assessment of public opinions

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Introduction: The Museu Biológico do Instituto Butantan (MIB) was established with development itself. The purpose of keeping the museum has always been to disseminate environmental education related to venomous animals. In the search for renewal and promotion of dissemination as an institution, the MIB has invested in temporary exhibitions, as the "Expo-Frog: A leap of Extinction", whose main guideline was the conservation of the group of amphibians. The choice of theme is related to the international movement to preserve these animals, being considered in 2008 the "Year of the Amphibians." **Objectives:** The objective of this study is to describe the production of "Expo-Frog", from the choice of and preparation of animals exposed information boards, and to evaluate the spontaneous perception of the visitor on this exposure. **Methods:** The study began with literature on amphibians. The assembly of the "Expo-frog" followed the pattern adopted by the MIB, on the earlier temporary exhibitions, with the use of information boards and dioramas. The preparation of the panels was the most expensive: the selected information and images that address the issues raised in the exposure: characteristics of amphibians, biodiversity, threats to survival and curiosities. Considering these aspects, set - the preparation of 6 panels. Some species of amphibians have been selected for exhibition in order to demonstrate the diversity of the group, so were mounted 7 biodioramas that "doing" the workings of their selected species. The exhibition was mounted in the building of the MIB corridor located in the final portion of the permanent exhibition in a space of 6.0 m long by 3.5 m wide. To assess the perception of the public visiting spontaneously, was used in summative evaluation, a study that examines the interaction between exposure and public. Therefore, the chosen methods were the timing and tracking & semi-structured. 22 visitors were observed in timing & tracking and among them 13 were selected for semi-structured interview. **Results and Discussion:** The temporary exhibition "Expo-frog: the one jump of Flameout!" began on 20/10/2008 and ended 20/12/2008 days, part of this period was the school recess. Evaluation with spontaneous visitors was conducted during a week in the month of December 2008. It was observed that visitors felt more attracted by biodioramas, especially those that contained three copies of *Dendrobates tinctorius*, very showy animal, which demonstrates the success of being exposed animals, the panels were not perceived in the exhibition. This experience suggests new directions regarding museographic aspects and museum for the next temporary exhibition and the MIB as a whole, especially for text information, to make the exhibition more attractive to visitors. These changes suggest the expansion of the role of the MIB as institution for disseminating scientific and environmental education related to venomous animals.

7.06 Dissemination of information and education in the Instituto Butantan, yesterday and today

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Introduction: The hygienist Republican thought that the twentieth century beginning gave to Vital Brazil and to Instituto Butantan a scenario leading to discussion of dissemination of scientific knowledge. The exchange of snakes antivenoms, as well as questionnaires and instructions for prophylaxis, was introduced to the state of São Paulo not only the scientism of specific serum, but mainly the hygiene public policies and public health. Vital Brazil with his assistants offered courses, conferences, murals, lectures and other means dissemination science for population health education. In 1918, was the first class of school group's directors in the Public Hygiene Elementary Course. Trained by the Instituto Butantan, should be knowledge multipliers to return to their cities. Moreover, the Institute formed its staff, largely composed of young doctors and medical students. Today, the Butantan works with various forms of knowledge dissemination and its products and research are recognized worldwide. **Objective:** Among the various practices of research about dissemination and education conducted in the Institute's Museum – Biológico, Histórico and Microbiologia - win highlighted the activities developed, in general, with the school audience. Aiming to find language that exceeded the limits of museums, integrating historical knowledge, architectural, biological (and microbiological), geographical, sociological, artistic and economic, in May 2009, the Museu Histórico attended the 7a Semana Nacional dos Museus, performing monitoring with pre-scheduled schools. **Methods:** The direction was developed from initial research which developed in the Instituto Butantan archive and by informal reports from the Butantan employee. Four central themes were identified: History, Economic, Scientific and Architectural. The direction crossed the quadrilateral composed between the Museu Histórico, the Prédio Central, the Complexo Bioindustrial and the Centro de Difusão Científica, also passing through some intermediate buildings where we discussed issues related to Brazilian public health, development and affirmation of science and spatial distribution. The activity was realized with five schools and led by three Museu Histórico educators. **Results and Discussion:** With the monitoring development was possible to notice how the spaces outside of the Institution are not known by school groups which attend the Butantan, reducing the students understanding about the importance of the park for science since the beginning of the twentieth century and motivating new educational activity creation to be realized once a month with previously scheduled school. Help from materials (films, photos and tables of comparative time) made during the activity allowed the possibility of integrating with external monitoring visits to three museums, something essential for a better use of time and space. The implementation of the activity by the Divisão Cultural of the Instituto Butantan contributes effectively to the purpose of science dissemination and the scientific knowledge produced in different areas of work within the Institution.

7.07 Mythical and fantasy approaches to snakes reported by visitors to the Museu Biológico - Instituto Butantan

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Introduction: Legends, myths, cases, superstitions and beliefs have as central theme, the snakes. In the history of mankind, it is common to find representations of animals and appreciation over time, differently in cultures. In the case of snakes, interpretations vary as to adjectives, but by observed facts or information, they are always protagonists of narratives and popular superstition. **Objective**: To note the analogy of visitors, about the reports of the main mythical approaches and fantasy reports related to snakes, learning their origin and establishing relations between cultural and scientific knowledge. **Methods**: A semi-structured questionnaire was given to obtain the reports of visitors to the Museu Biológico. Interviewees were chosen at random or when citing a legend, myth and beliefs about snakes. Interviews were conducted on January 21 and 22, February 11 and 12, and March 18 and 19 of 2006, respectively Saturdays and Sundays, days on which there are a large number of visitors from various places. The interviews were conducted emphasizing only the objective proposed in the work. **Results and Discussion**: Ninety-two interviews were obtained with a total of 166 reports; these included 51 men and 41 women, divided by regions of Brazil. With 60% of visitors living in the Southeast, a high number was expected since the Instituto Butantan is located in this region, followed by the Northeast 17%, South 11%, Mid-West 9% and North 3%. The interviewees described that the reports were told to them by well known individuals and often by members of their family; some were read or experienced. Several authors describe the snakes in various legends, myths and superstition as a popular symbol of good, evil, wisdom, fertility, immortality, and misfortune among other qualities. The work at the Museu Biológico do Instituto Butantan produces satisfactory results, where it was found that the interviews had 25 entries for stories with snakes. With the results mentioned in this work, we can adapt the information for each region and this without the intention of demystifying its legends, myths and beliefs, including scientific knowledge of events or experiences. No matter the age, sex, level of education or even the region of origin, there will always be fantastic and mythical stories about snakes, which is an animal that attracts us, whether it has received qualities over time. Investigations by the popular information and overlapping scientific knowledge will help prevent accidents and make better use of the knowledge for public health.

7.08 Workshop on history: legacy and memory of Belterra's community, Pará State, Brazil

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Introduction: In April 2009, activities concerning patrimonial education were developed with teachers of the public elementary schools and the general community of Belterra/Pará. This represented the official outset of activities on History, proposed in the context of the Projeto Butantan na Amazônia and further extended to the Projeto Instituto Nacional de Ciência e Tecnologia em Toxinas (INCTTOX). **Objective:** To involve and to touch the general community about the importance of rescuing cultural patrimonies, thus motivating them to raise historical documents and to recover the history of local health and popular practices about poisonous animals. **Methods:** in May 2009 workshops on History were developed in two steps. In the first one, people from the Belterra's municipality, which were participating in the Feira de Agricultura Familiar, were randomly invited to select images of natural and cultural patrimonies that were considered relevant to the History of the region. They were asked to fix those figures in a map so as to offer a spatial distribution of these places. All participations were filmed to be presented, in a second step, to the teachers of public schools located in the urban area. At this time, they were the central subjects of the activity, creating a list of places of social and economical importance that should be preserved. From this point of discussion it was possible to introduce the concept of immaterial patrimony, which is unattainable but cultural as well, and therefore representative of an identity. **Results and Discussion**: Both community participants and teachers identified historical buildings as being important, such as the water tank, the main square and houses built at the time of Ford Company administration. There were also considered as a patrimony the Floresta Nacional do Tapajós and river beaches. Some people identified the rural union trade and the local hospital as patrimonies to be preserved. In fact, health was said to be a patrimony of the population. At this point, a debate concerning traditional knowledge to treat diseases, snake bites and other animal injuries has risen. Many testimonies were given, most referring to constraints and contradictories involving this subject. There were no doubts that this knowledge represents a cultural patrimony to be preserved. However, they were aware that it is not supported by science and their role as educational agents introduce a further difficulty regarding this issue. All material was left at the Education and Culture Secretaries' disposal, to be used with other teachers, students and parents. During the field work, we found out that it is necessary to better articulate historical and ethnological approaches to go deep into this subject. In this way, these data indicate that traditional knowledge has historical origins, which are identifying elements of a community. Medical anthropology and ethnozoology are areas to be incorporated in the Butantan's actions, whether in the Amazon or other regions, at least in terms of health approach. We should develop more workshops with different communities taking into account historical, patrimonial and traditional knowledge of these populations.

7.09 Museu de Microbiologia and the dissemination of microorganism diversity

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Introduction: As an activity of the "Week of Science and Technology, 2008", whose theme was "Biodiversity," the Museu de Microbiologia do Instituto Butantan held the temporary exhibition "The invisible world of microbes: Biodiversity and Art" which addressed the issue of microorganism diversity in a creative and differentiated way. The microbes, the smallest beings that are known, are found in almost all environments - sea, land, underground, hot springs, glaciers - and in living beings, like plants and animals (including human beings), playing the most varied roles. Fungi, bacteria, viruses and protozoa have different forms, exhibit many strategies for survival using various substances and are related to humans in multiple ways. They are highly diverse, making it difficult to approach this issue in an exhibition. **Objectives:** The aim of this project was to develop a temporary exhibition exploring the microorganism universe, emphasizing their biodiversity and biological function in a creative, fun and consistent way. **Methods:** Several expographic resources, interactive and contemplative ones, were used in this exhibition. In order to observe the difference between desert and forest environments, regarding the number and diversity of bacterial species, fluorescent materials were used in two dioramas and were detected by a black light. Relevant texts in posters complete the activities information. Fabric mini-panels bringing curiosities about the microorganism biodiversity were hung from the ceiling and were available for handling after pulling them. The comparison between the estimated number of bacterial species described in the scientific literature with the estimated number of other living species, such as insects and mammals was demonstrated by a three-dimensional graph fitted with glass tubes. The DNA, extremely important in the study of biodiversity, was exposed as a model built with jelly candy. The art was one of the strategies used to illustrate the variety of shapes the microbes may have. A workshop of "light painting" was prepared to encourage the artistic creativity of the visitors, creating microorganism images with light. **Results and Discussion:** About 40,000 people have visited the exhibition, including 7,000 students. The expographic strategies used in the exhibition attracted the public in variable degrees of interest, as observed by the museum educators. The interactive apparatus was the most exploited by the public. The DNA molecule built with jelly candy intensively stimulates the public, especially children, encouraging many questions about the relationship between microorganisms and DNA. The most attractive activity in the exhibition was the workshop of "light painting" providing a collective moment, and propitiating discussion on this subject. In general, it was observed that the expographic strategies used in this exhibition were satisfactory, reaching the goal of bringing to the public a little knowledge of the microorganism diversity and also entertainment and art.

Supported by: FAPESP and Fundação Butantan.

7.10 Discourse construction and conceptual learning at the Museu de Microbiologia: serum and vaccine concepts

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Introduction: Studies aimed at examining the transformation of scientific knowledge produced in academics into the knowledge produced in the museum environments are increasing considerably. The Didactic Transposition concept used in this research is described at museums as Museographic Transposition: the movement to build an educational message, when the knowledge validated in academic and scientific spheres are modified, simplified in its complexity and transformed into "knowledge to be taught." But, this transformation reaches another step when it comes to the classroom where the teacher transforms this "knowledge to be taught" into an education content (the "knowledge taught"). Thus, for teaching a particular element of knowledge, this element should undergo some transformations. In the museum environment, it is important to understand how this transposition facilitates the meaning-making process by visitors. **Objectives:** The aim of this work was to a) investigate the discourse construction of the Museum's long term exhibition regarding serum and vaccine concepts, b) examine the process of museographic transposition and c) analyze the discourse understanding by visitors. **Methods:** We performed the following data collection processes: survey of exhibits that contain references to the studied topics, interviews with the exhibition curators, survey of the main transformations presented in the serum and vaccine concepts (in context, time, origin and authors), and semi-structured interviews with randomly selected visitors before and after the visit (stimulated recall method). Three of these interviews were transcribed. **Results and Discussion:** We identified 15 exhibits that showed the concept of serum and/or vaccine. The transformation processes mainly observed were decontextualization and changes in contemporaneousness. The process of naturalization was rarely observed. Analysis of the interviews demonstrated that the "visitor identity" and the "learning environment" affected the types of conversational elaborations by visitors. Although more observations are needed, the initial data suggest that the process of museographic transposition of the studied concepts (serum and vaccine) interferes in the visitors conversational elaborations. However, rather than approximate the visitor to this knowledge, the exhibition discourse seems to create a greater distance. Finding the "errors" and "updating problems" of the Museu de Microbiologia long-term exhibition was not the intent of this research. The purpose was to take the Museographic Transposition not as a "necessary evil" to be supplanted, but rather as an important phase in the science museum education process, which must be conducted with awareness, consistency and intentionality.

Supported by: FAPESP.

7.11 History and institute memoires workshop: the collective construction of a timeline of Instituto Butantan

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Introduction: This paper proposes a reflection on the institutional memory and history of the Instituto Butantan, which functions daily due to the congregation of individual functions. Each of these individuals have their own emotions, yearnings and memories, and their interaction among themselves and their surrounding society constitutes an institutional history; this too is inclusive of the History of Instituto Butantan, which is made every day with the contribution of each and every one. The reflection regarding memory is about collecting all these individual contributions for the construction of a collective and social memory. **Objective:** The objective of the History and Institutional Memory Workshop was to create and to develop a timeline of Butantan, through a group work that includes the actual workers, from both the Institution and the Foundation. Based on this activity, we intended to define the relationships between the individual and collective memory and between the individual and the institutional memory. **Methods:** The participation of the group motivates the collective reasoning, carried out through the methodology of Oral History. The source used was based on narrative and memory, both individual and social. Starting with the individual memory, which can also be defined as a construction of the present, in order to compose the timeline of that group; each and every element brought was able to be collected and transformed into recorded implements of an institutional narrative. The Workshop was developed with two stages: first, the Round of History – which consists of individuals' stories about the first contact with the Instituto Butantan –, secondly, the construction of the institutional timeline, developed through four previously defined topics– external context, internal context, personages and staff (this last referring one to the contact of that individual with the Institution). **Results and Discussion:** The Workshop was completed twice, and both times resulted in the construction of an institutional timeline. The first time was made among educators of the Museu Histórico and the second time, it was made among a bigger group, that included the educators, the employees of the Museu Histórico, the team of the Laboratório de História da Ciência, representatives of the Laboratório de Herpetologia, the Museu Biológico and the members of the Assessoria de Imprensa. As expected, we noticed that each one presented different facts and visions of the subject, resulting in different identity for each group. The construction of the timeline presented some variables concerning the time of contact between the individual and the Institution, the type of bond, and the internal relations of work, among others. The existence of gaps in the institutional history, observed in determined periods of the history of Butantan deserves a greater reflection, and therefore, it is necessary to question if these facts are silenced or unknown, as well as why do they happen. Aside from that, this study was intended to test the applicability of this methodology in order to construct a timeline for the exposition of the Historical Museum and based on that, utilize the potential modifications on these topics, to apply as an organized INCTTOX (Projeto Instituto Nacional de Ciência e Tecnologia em Toxinas) Project activity, in the Amazon, discussing the History of the Health in Belterra based on the means of involvement among the members of the community.

7.12 The program "Cultura é Currículo" and educational strategies

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Introduction: Since 2008, Instituto Butantan has integrated the program "Cultura é Currículo" established by the Secretaria de Educação of the Estado de São Paulo in partnership with FDE – Fundação para o Desenvolvimento da Educação. The Project makes it possible for students from state schools to visit cultural institutions and the intention is to integrate the activities developed with the programming of the schools. The activity "Lugares de Aprender" is included in Instituto Butantan, where it is dedicated to the study of living beings and involves a series of basic study. The diffusion of the scientific production is commitment of the IB since its foundation and, considering the importance of the educational action for the promotion of this knowledge, the team of the historical museum is committed to the development of activities that introduce the students with guided visits. **Objective:** The objective of this activity is to stimulate the children to compose a report of the process of the production of serum and vaccines, through current and historical icons approaching the knowledge of science in their lives. **Methods:** The didactic material distributed by the FDE consists of a booklet with activities for classroom and a film. This material offered the conceptual basis for the adopted pedagogical strategy in the Museu Histórico. Moreover, a questionnaire created for the educator was made after the visit, to evaluate the visit, identifying by which methods instrumentalism reflects the concrete-symbolic universe of the students. The category of the living beings does not encompass all the activities carried out by the IB, such as the production of serum and vaccines and the research directed to the public health. As parts of a puzzle, students will be make mobile posters creating two lines parallel in the display panels. The first line will present the procedures of the first laboratories of the IB. The second will be dedicated to environments of current research and production. The intention is that the children follow the stages of the production of the serum and vaccines, tracing comparisons between the past and the present. **Results and Discussion:** The educational actions of the Museums reflect the concern with the diffusion of the scientific knowledge and these procedures guarantee the learning of educators and students. Analyzing the questionnaires, we detected that half of the accompanying teachers were unaware of the Program "Cultura é Curriculo" or had not used the distributed material. Therefore, to assure a productive visit, the proposal of the illustrated guide and the intervention of the educator allowed to create important resources for the understanding of the objectives and activities developed for the IB. To divulge the activities of the IB is the responsibility of museums as places of education. Therefore, the Museu Histórico stimulates the reflection between our past and our gift through the changes that have occurred in the operational way of the production of serum and vaccines and of the laboratories. Our intention is to adapt our actions with the needs of the public, keeping our responsibility with the education and the spreading of the generated knowledge between education and learning.

7.13 Instituto Butantan and its archival treatment

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Presentation: The production and accumulation of documents are inherent practices of the work of institutions or people and reveal important characteristics of their own work. The document production of Instituto Butantan for a period was created by institutional functions, mainly in the direction that divulged in annual reports its activities (ongoing and ending). But, after some years, with the Institute's environment the volume of the documents increased, the installations became crowded, and a large record management began. **Objective:** Some scientists believe that scientific records are found in articles and specialized publications, so it is important to preserve the intermediate stages of a research to understand the steps of the investigation. But, before taking actions to preserve the scientific memory inside the Institute, it was necessary to produce a diagnostic to know the old practices about the document treatment. **Methods:** To know the institutional politics about the archive, some interviews were made to listen to the opinion and action of the workers with regard to document preservation as being important to the institution's memory. The choice of the candidates was based on their position and activity developed to value document preservation. On the one hand, this decision provided closed responses to some questions so that the candidates have common interests about the historical aspect, but as this research intends to identify problems in a record management system, it was interesting to understand the reason why it is still not a reality. The questionnaires were answered by directors and others of the interest area. **Results and Discussion:** According to the answers, the inauguration of Museu Histórico with the reunion of antique records was the only significant experience that existed in the institution about the preservation of historical documents. There were no sections or professional specialized staff that could respond to the record management. The lack of policies for the treatment of historical archives biased the control about documental production, since historical archives are formed with documents provided from current documents. The inexistence of workers sensitization projects make the historical documents unknown for the researchers, and also, many scientists keep documents produced in the laboratory in their personal archival, making the access more difficult. Despite that IB is a state institution, the Sistema de Arquivos do Estado de São Paulo –SAESP still does not make this clear to the candidates, so it does not happen in an extensive way. With this information, it is important to have a more detailed evaluation, with all the institute sections and one member of the Arquivo Público do Estado de São Paulo, to clarify what SAESP is and also planning ways to make it a part of the Institute routine, helping with ongoing and completed productions and the preservation of the institutional memories. SAESP actuation is a big step to direct institutions that do not have a specialized professional or a space for a documentation archives center to do the records management.

7.14 Herpetological and related contributions in "Memórias do Instituto Butantan": a compiling and updating approach

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Introduction: The technical/scientific journal "Memórias do Instituto Butantan" (MIB) had its first published issue in 1918. In 1994, this journal ceased as a formal scientific periodic. Nowadays, abstracts of the "Reunião Científica Annual," a yearly regional meeting at Instituto Butantan, are published. **Objectives:** Compilation and updating the herpetological and related bibliography at MIB, assembling a database for perpetuation, accessibility and improvement of its valuable information. **Methods:** Issues of MIB available in the library of the Instituto Butantan, São Paulo, Brazil. **Results and Discussion:** Four hundred seven papers on this subject were published. Among several diversified topics such as Morphology (Anatomy, Histology, Cytology), Taxonomy, Natural History, Behavior, Epidemiology, Physiology, Ecology, Bioterroism, and Antivenom-production, we highlight forty-three new species described. Twenty-four herpetological surveys in all Brazilian regions as well as in foreign countries (Colombia, Costa Rica, Guyana, Iran, Madagascar, Suriname, Venezuela) are accessible. All herpetological and related contributions published in MIB were chronologically indexed and cross-references assembled. Currently, there are two indexed Brazilian journals available specifically for the herpetological community (*Phyllomedusa* and *South American Journal of Herpetology*). The former MIB conceived as a formal scientific publication has been recognized for its importance to herpetology, since it is still widely used as a reference bibliography. The book "The Venomous Reptiles of the Western Hemisphere" authored by Jonathan Campbell and William Lamar in 2004, a comprehensive reference for herpetological research, quoted eighty-six articles published in the MIB. Besides this scientific relevance, we must further recall that a comprehensive historical approach also deserves consideration, since several icons of Science, including Nobel-prize winning authors are present in the MIB papers. In this way, we propose the extensive digitization of the MIB as well as its permanent accessibility in the Instituto Butantan website.

8: Others

8.01 Occurrence of lymphangitis in serum-producing horses on São Joaquim farm of Instituto Butantan

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Introduction: Lymphangitis is an inflammatory process of the lymph vessels and lymph nodes that affect the limbs. It has a cosmopolitan distribution and has no predilections for age, breed or sex. This inflammation normally happens after bacteria and fungi have entered the body through a cut or wound distal to the limbs. There have been some reports of protein imbalance as a predisposing factor for its occurrence. It is more common in the hind limb especially in the distal hock-joint region. Inflammation occurs in lymph vessels with their total or partial obstruction, making it difficult or impossible to drain lymph fluid from the affected limb. The limb exhibits an increase in volume and temperature, pain and wounds that discharge purulent material. A life-threatening or inadequately treated wound can become chronic leading to fibrosis. **Objectives:** The aim of this investigation was to determine what happened with serum-producing horses. **Results and Discussion:** Two serum-producing horses, one belonging to the anti-rabies group and other to the anti-elapid venom group of the São Joaquim Farm. There were male, castrated, weighing approximately 450 kg, cross breed, and had lameness of the right hind limb, swelling of the coxal to the shank, no apparent wounds, sensitivity on palpation, hyperthermia (39 °C) and tachycardia (HR 50 beats / min). Lymphangitis was diagnosed. They were treated with hydrotherapy to reduce the swelling associated with administration of an anti-inflammatory and antibiotic for 28 days. After this period, the horses had resolution of symptoms and return to routine activities. This is the first reported case of lymphangitis at the São Joaquim Farm.

Supported by: Fundação Butantan.

8.02 Occurrence of *Bothrops jararaca* bite in a horse on São Joaquim farm of Instituto Butantan

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Introduction: In Brazil, 88-89% of the snake bites in human beings come from snakes of *Bothrops spp* genera. This type of accident also occurs in animals, showing more common reports in the canine species. There is considerable variation in effect of the several species of venomous snakes among domestic animals. In experiments carried out by Araujo & Belluomini (1962), horses, sheep and cattle showed a greater sensitivity to snake venom, and according to Rosenfeld (1971), carnivores seem to be more resistant to the venoms in relation to the other species. In the place of the bite a great edema occurs and spreads to other parts, and 12 h after its injection, necrosis can occur at the site of the bite. In general, blood coagulation time is increased, and because of that hemorrhages occur in body cavities and in the parenchyma of some organs, and it may happen even after appropriate therapy. In domestic animals such as horses and cattle, when the bite occurs in the limbs, the clinical signs are lameness, difficulty in locomotion, immobility, decubitus and inability to stand. Many snake bite accidents result in death. The sooner the specific serotherapy is started, the better the prognostic is for the animal's life. **Objectives:** The aim of this investigation was to determine what happened with the horse belonging to São Joaquim Farm. **Results and Discussion:** A horse, male, castrated, eight years old, showed sudden behavioral changes. An employee of the farm told that a *Bothrops jararaca* was next to him. Minutes after that, the horse showed lameness in the right front limb, edema in dorsal region of the hoof coronet, and increase in heart rate (90 beats per minute) and in respiratory movements (40 movements per minute). Coagulation test showed increased coagulation time (over 25 minutes). He was treated with six vials of Instituto Butantan specific intravenous antibothropic serum. Two hours later, he received three more vials of serum in the same way. Also, he was managed with antibiotic and anti-inflammatory for five days. After 15 days the horse had completely recovered, showing normalized vital functions being able to come back to carry out its normal function.

Supported by: Fundação Butantan.

8.03 Establishment of the comet assay for the freshwater snail *Biomphalaria glabrata* (Say, 1818)

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Introduction: The comet assay is a method developed to detect breaks in the DNA. In the test, cells are embedded in agarose and lysed by detergents in high salt and the DNA released electrophoresed. Cells with an increased frequency of DNA breaks display increased migration of DNA toward the anode. This is a promising test for studies on genotoxicity, DNA repair, environmental and human monitoring. **Objective:** This research aimed to establish the comet assay in hemocytes of *Biomphalaria glabrata* to assess the capability of this system to detect mutagenic pollutants. **Methods:** Twelve snails per group were selected (control and exposed to 60-Co gamma radiation). Snails were irradiated with single doses of 2.5, 5, 10 and 20 Gy at 2.82KGy/h. After the exposure, the hemolymph was collected and suspended in 0.5% of agarose low melting point (LMP), the mixture with low melting agarose was placed on a microscope slide previously coated with a 1.5 % normal melting agarose. The slides were placed in lysis solution of pH 10 for 600 min. After lysis, the electrophoresis tank was filled with slides and covered with the electrophoresis buffer to allow the expression of damage. The slides were kept in the buffer for 30 min, and then electrophoresis was carried out at 23V and 150mA for 30 min. After running, the cells were neutralized with buffer of pH 7.5. Next, the cells were stained with 0.002% ethidium bromide and visualized with a fluorescence microscope at 400x. The visual analysis was carried out by classifying comets in categories (0 to 3) according to the extent of DNA migration. **Results and Discussion:** The results showed a genotoxic effect of radiation at all doses in a dose-dependent manner. The comet assay in *B. glabrata* was demonstrated to be simple, fast and reliable in the evaluation of genotoxic effects of environmental mutagens.

8.04 Effect of methylecgonidine (AEME) on melatonin synthesis in the rat pineal gland

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Introduction: The pineal gland synthesizes the hormone melatonin at night induced by noradrenergic stimulation. The activation of α- and β-adrenoceptors increases cAMP, which increases activity of the enzyme arylalkylamine-N-acetyltransferase (AANAT). The gland also receives parasympathetic innervation, with acetylcholine being an inhibitor of melatonin synthesis. Methylecgonidine (AEME) is a byproduct of cocaine, when used in the form of crack. Its effects on the central nervous system are even more intense than those of cocaine itself. There is evidence showing the action of AEME on the cholinergic system, which could lead to an effect on melatonin synthesis. **Objectives**: The aim of the present work was to study the AEME effects on the melatonin synthesis in the rat pineal glands *in vitro* and *in vivo*. Also investigated was the possible cholinergic action of AEME and on the activity of AANAT. The nocturnal profiles of melatonin synthesis and of the expression of their receptors (MT1 and MT2) in the cerebellum were evaluated in *in vivo* experiments. **Methods:** Pineal glands were isolated from male Wistar rats and maintained in culture for 48 h in BGJb medium. The glands were stimulated by noradrenaline (1 μM) in combination with AEME at different concentrations (1 μM and 100 μM), that was added 30 min or 48 h before noradrenaline addition, or with 10 μM atropine. For the *in vivo* experiments, the rats were injected with AEME (2.0 mg/kg) for 7 consecutive days and then were sacrificed in order to evaluate the melatonin synthesis in the pineal gland and the expression of melatonin receptors (MT1 and MT2) in the cerebellum. Melatonin was quantified by HPLC, AANAT activity was determined by radiometric assay, and melatonin receptors were analyzed by RT-PCR. **Results and Discussion:** AEME reduced melatonin synthesis at every concentration used, independent of incubation time (Nor 1 μM = 10.56±0.61 ng/gland; Nor + AEME 1 μM = 7.56±0.69 ng/gland; Nor + AEME 100 μM = 7.96±0.78 ng/gland; Nor + AEME 100 μM (48 h) = 7.93±0.72 ng/gland). Atropine did not reverse the AEME effects on melatonin synthesis, showing that its actions seem to be independent of muscarinic cholinergic receptors. AANAT activity was not altered by AEME. The MT1 receptors in the cerebellum were increased by AEME treatment. However, no differences were observed for MT2 receptor expression. Melatonin was also reduced by AEME in the in vivo experiments, with a more marked reduction at the end of the dark period.

Supported by: CAPES and FAPESP.

8.05 A bioinformatics tool to visualize peptides found by mass spectrometry in protein structures

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Introduction: Advances in mass spectrometry (MS) and in bioinformatics tools over the past decades have made it possible to characterize proteomes and peptidomes of complex biological samples such as venoms, blood sera and cell lysates, among others. Peptides found in biological fluids may result from proteolytic processing of proteins that may be triggered by endogenous mechanisms, or even by activation of proteases during manipulation in the laboratory. In some cases, the cleaved peptides come from a protein whose 3D structure is deposited in public databases and viewing the peptide positions may clarify if the peptide is internal or external or is located near some binding site in the structure, helping to build more realistic hypothesis about the mechanism of the proteolytic processing. However, it is not uncommon that the 3D structure (PDB) of the target protein is not available, as is the case of many venom toxins, making it necessary to search for a similar protein which is expected to have a similar structure. The peptides from the target protein cannot match exactly their homologs in the similar protein so a homolog peptide search must also be performed. Last, the homolog peptides must be highlighted in the PDB. Manually performing these tasks is very time consuming, while an automated computational tool may do it in few minutes. This work shows an automated method of finding the similar protein PDB and the homolog peptides and highlighting them on a 3D plot. It is available through an Intranet web server to which MS results processed by MASCOT are uploaded, processed and the resulting PDBs are downloaded. **Objectives:** The objective of this work was to develop a bioinformatics tool to extract peptide sequences obtained by MS and by the search engine MASCOT (Matrix Science), to find the most significant match of crystallographic protein structure by similarity search, and to align the peptides and redraw the structure highlighting the identified peptides. **Methods:** MS data analysis by MASCOT is processed to retrieve the match protein and identified peptide sequences. The match protein sequence is submitted to Blastp. The most similar protein with PDB is selected. The PDB is downloaded from the RCSB-PDB repository. The primary structure of the similar protein is retrieved from the PDB file. The homolog peptides are found by a Longest Common Subsequence (LCS) algorithm using Blosum62 matrix. The PDB is edited to highlight the homolog peptides in the similar protein. 3D view is obtained using PyMol or other protein viewer. **Results and Discussion:** The program can be accessed through http://172.25.60.101 IP address. We performed runs of peptide fractions of *Bothrops cotiara* venom analyzed by MS to evaluate the functionality of the tool. The resulting spectra were analyzed by MASCOT which identified several L-amino acid oxidase (LAAO) sequences, and one *Bothrops neuwiedi pauloensis* LAAO as the best match. There is no PDB file for this enzyme. Application of the program returned chain A, LAAO from *Agkistrodon halys pallas* as the most similar enzyme with PDB file and indicated the peptides found by MS.

Supported by: CAPES and FAPESP.

8.06 Participation of neutrophils in antinociception induced by glycogen, evaluated by the paw pressure model

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Introduction: Several pieces of evidence demonstrate the involvement of neutrophils in the modulation of painful response during the acute inflammatory process. However, the participation of these cells in pain generation is still conflicting since neutrophils have been proposed to play a role in either induction or inhibition of painful response. Glycogen-induced peritonitis has been shown to induce antinociception in mice submitted to the abdominal contortion test, and secretion of calcium-binding protein S100A9 by neutrophils mediated this effect. **Objectives:** To investigate involvement of neutrophil in nociception control after administration of glycogen in rats pre-treated with fucoidan, a selectin adhesion molecule inhibitor. In addition, the leukocyte migration profile was investigated in several periods of time, in which painful response was assessed after intraplantar injection of glycogen in rats pre-treated with fucoidan. **Methods:** Male Wistar rats were injected intravenously with fucoidan (5 mg/kg, 500 µL/animal) 15 min before intraplantar injection of 5% (w/v) glycogen solution (100 µL/animal) or saline, and after different periods of time the paw pressure test was evaluated. In another experimental procedure, rats were injected with fucoidan 15 min before intraplantar injection of glycogen, and after different periods of times euthanized in order to the collect the plantar tissue for histological analysis. **Results and Discussion:** Glycogen induced antinociception in rats at 2, 4, 6, 8 and 12 h. Pre-treatment with fucoidan reversed the antinociception observed at 2, 4 and 6 h after glycogen injection and induced hyperalgesia at the same periods of time. Rats pre-treated with fucoidan and tested 8 h after glycogen injection only showed reversal of the antinociceptive effect. When the nociceptive test was evaluated 12 h after glycogen administration, the pre-treatment with fucoidan failed to diminish antinociception. Histological analysis demonstrated an increase in migration of polymorphonuclear cells between 2 and 8 h after glycogen administration, but migration of polymorphonuclear cells was inhibited in rats pre-treated with fucoidan and injected with glycogen over the initial periods of time after glycogen injection. The predominant cells accumulated in the footpad after glycogen administration were neutrophils. Pre-treatment with fucoidan, a selectin inhibitor, not only reversed glycogen-induced antinociception but also induced hyperalgesia. Besides, fucoidan reduced neutrophil migration in rat footpads after glycogen injection. Thus, glycogen induces antinociception in rats evaluated by the paw pressure model, likely by accumulation of polymorphonuclear cells, particularly neutrophils. The possible participation of S100A9, found in high concentrations in neutrophil cytosol, in this phenomenon is under investigation.

Supported by: FAPESP and Fundação Butantan.

8.07 Development of a MATLAB application for processing kinetic data of animal cell experiments

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Introduction: Experimental raw data is normally difficult to be analyzed without some processing. In general, spreadsheets like Microsoft Excel are used to do this treatment. **Objectives**: The aim of this work was to uniformize and speed up data treatment using an application developed in MATLAB. **Methods**: Starting from original data of experiments with animal cells, a MATLAB application was developed to import data from Microsoft Excel spreadsheets with these data. It is important to emphasize that the data are imported to MATLAB directly from Microsoft Excel and after the treatment the resulting data of that analysis can be exported back to an Excel spreadsheet so that the user can use them in the way he/she is used to, as in making plots. In this MATLAB application, the source data are fitted and smoothed with automatic generation of a specific growth rate plot, using a spline algorithm. The application still allows the user to make small adjustments in the experimental points. **Results and Discussion**: The use of that application results in less time spent for this treatment and also in a standardization of results. This new method, using a MATLAB application, shows better results in its analysis, besides presenting the information in a clearer way to be understood by anybody. Besides, the results are standardized and obtained in a shorter time, using a single program.

Supported by: CNPq.

8.08 Ethnoherpetology in the Vale do Paraíba, São Paulo State, Brazil: relationship between rural populations and the *Bothrops jararaca* species

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Introduction: The "Vale do Paraiba" is considered a geological fault sided by the "Serra do Mar" and "Serra da Mantiqueira," which contain the "caipira" culture. The prefix *Ethno*, before the name of a discipline, such as herpetology, means the relation between the man and the environment, that is, the understanding of herpetology in a community. Ethnobiological approaches occurred in Brazil starting in the 1980s, presenting themselves as a recently discussed subject in the country. The species in question is one of the most important epidemiologic agents in the area, whose genus is responsible for 90% of the ophidian accidents in Brazil's southwest and one of the most aggressive snakes of the Viperidae family. **Objective**: The objective of this study is to analyze the regional and scientific knowledge about the natural history of the *Bothrops jararaca* species in some cities in the Vale do Paraíba, in the state of São Paulo. **Methods**: The choice of the study areas follows historical logic. Lagoinha preserves the agropastoral activity derived from the period of coffee plantation in the Valley. Roseira has been clearly impacted by the construction of President Dutra Highway, with industries that are near the traditional rural properties. Taubaté is the most ancient representative in the Valley and was the departure center for exploratory expeditions. The research was divided into two different phases, from March to November, 2009. First, a semi-structured questionnaire was applied, with a recorded interview, containing 20 questions about ecology and biology of *B. jararaca.* Afterward, some photographs were shown so that the interviewees were able to identify some snakes in order to map possible mistakes in the recognition of the species in question. A previous survey of the snakes from the sampled area has been verified in the books of the Supplier Register of Animal Reception of Instituto Butantan, over the last 20 years. **Results and Discussion**: To date, 80% of the interview activities have been made, as well as the survey from 1988 to 2003, which will be complemented up to 2008. We have determined the addition of 29 species, up to now: 1.3% to the Boidae family; 20.8% Colubridae; 0.4% Elapidae and 77.5% Viperidae. Of all those species, 20% are represented by *B. jararaca*. The killing of those snakes seems to be an attribute of courage in the area. Knowledge about viviparity and predatory activity seems to be mysterious to some rural residents. However, seasonality and the search for shelter are more clarified, justified by the unimodal reproductive pattern and the accumulation of rubble in the surroundings of the houses, creating microhabitats for the *jararacas*, known by the inhabitants of the area as *urutu*. The *jaracussu* nomenclature may occur in the more melanic individuals. *Urutu-mestiço* classifications have also occurred in Roseira, which would be a supposed cross between the rattlesnake and the *urutu*, known popularly as *cascaverana* in Lagoinha. With the development of this methodology, we intend to have a better interaction between the rural population and the scientific community, being able using this approach to map the origins and characteristics of the traditional herpetological knowledge of this sampled area.

8.09 Effect of Piperaceae amide on schistosomiasis vector *Biomphalaria glabrata* (Say, 1818)

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Introduction: Schistosomiasis occurs in 54 countries mainly in South America, the Caribbean, Africa and east of the Mediterranean. In Brazil, 5-6 million people are infected and 30 thousand are exposed to risk of infection. One of the more efficient methods to control this disease is the application of molluscicides which eliminates or reduces the intermediate host population. Concerning environmental preservation, the high cost and recurrent resistance of snail to the synthetic molluscicide have stimulated the study of molluscicides of plant origin. The species from the Piperaceae family has a diversified and bioactive compounds such as essential oils, unsaturated amides, pyrones, flavonoids, monoterpenes, sesquiterpenes, arylpropanoids and lignoids. **Objective:** In the present study the molluscicide and ovicide actions of an amide provided by the *Piper* genus (Piperaceae) were determined against the adult snail *Biomphalaria glabrata* and embryos of blastula, gastrula, trocophore and veliger stages. **Methods:** The Piperaceae amide was evaluated at concentrations lower than 10 ppm in snail to obtain LC_{90} (lethal concentration causing 90% mortality) and then submitted to evaluation of ovicidal action. **Results and Discussion:** The amide showed 100% molluscicide effect at concentrations lower than 8 ppm and 100% ovicide effect at concentrations lower than 3 ppm in all the stages of embryonic development.

Supported by: FAPESP and CAPES.

8.10 Quantification of thimerosal in vaccines without pretreatment, using flame atomic absorption spectrometry (FAAS)

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Introduction: Since the 1930s, thimerosal at high concentrations is used to prevent microbial growth in biological compounds, including many vaccines. It is an organomercurial molecule, and its composition is about 50% mercury in weight. The metabolism or degradation of this molecule results in ethylmercury whose environmental or clinical toxicity is not firmly established yet, but it bears a chemical similarity to methylmercury. **Objectives:** This work proposed a methodology for high concentration organic mercury analysis using the flame atomic absorption technique, without any sample pretreatment in DTP, Td and DT vaccines, Diphtheria and tetanus toxoids, and whole cell pertussis vaccine. **Methods:** The experiments were carried out in a HR-CS-AAS ContrAA 700 (Analytikjena) equipped with a 2D FFT-CCD detector and Echelle grating double monochromator. As continuum light source, a shot-arc xenon lamp was set for 253.65 nm (Hg line). The C_2H_2/air flame worked at 40 L h^{-1} flow rate. A stock solution of 1000 ppm thimerosal USP-RS was used as standard for the assays. All dilutions were performed only with Milli-Q water. All samples were provided by Instituto Butantan. **Results and Discussion:** The aspiration was studied in different rates, ranging the burner height as well. The signals were analyzed in a response surface graph and the best parameters were selected. The analytical curve showed linear correlation ($r^2 > 0.995$) and the standard solutions were stable for up to three days. For the analysis of selectivity and recovery assays, the standard additions method was employed for each sample, and the slopes of the curves obtained were compared to the slope of the analytical curve, using a *t*-test and hypothesis theory. This approach can give information about interferences of matrices, but results did not show such interferences. The developed method showed good precision and recovery rates, ranging below 5.0%, which is required for the AAS technique itself. The LOD and LOQ were, respectively, 1.27 mg L^{-1} and 4.63 mg L^{-1}.

Supported by: Fundação Butantan.

8.11 Validation of sterilization process by ionizing radiation of bromobutyl rubber stopper used as a component of the primary packaging of vaccines produced by Instituto Butantan

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Introduction: The sterilization by ionizing radiation of medical products and pharmaceuticals is being increasingly used. This is due to logistical reasons, or aimed to optimize and increase the efficiency of processes. Similarly, the use of such technique can be justified on the incompatibility of the material in relation to other sterilizing methods. The Instituto Butantan adopted this method for the sterilization of bromobutyl rubber stoppers, used in the primary packaging of multi-dose vaccine, which was made possible through the outsourcing process in a company that has a multipurpose radiator. This work describes the validation of the sterilization process by ionizing radiation of bromobutyl rubber stopper component of the primary packaging of vaccines produced by the Instituto Butantan. **Objective:** To perform the sterilization process validation of bromobutyl rubber stopper used as a component in the primary packaging of vaccines produced by Instituto Butantan, in accordance with requirements in the Resolution RDC no. 210/03 of the Brazilian Health Surveillance Agency (ANVISA). **Methods**: Three lots of bromobutyl rubber stoppers near the expiration date were sterilized. After determining the total microbial load "bioburden" which challenged the process, the stoppers were submitted to a radiation dose of 25 ± 5 kGy, and finally tested in the fungal and bacterial sterility test by the technique of direct inoculation in culture fluid (*fluid thioglycollate* medium and soybean-casein). Twenty different points were determined for the dosimetric mapping (use of dosimeters to evaluate the received dose) positioned by geometric analysis, as: 15 points for distinct biological evaluation using the bioindicator *Bacillus pumilus* (1.17×10^6 CFU/strip) and 5 samples at different points for the sterility test. All items were distributed in three sequential containers. Three cycles of sterilization were performed on three different days (one cycle for each batch of stopper). **Results and Discussion:** All samples tested were satisfactory in the bacterial and fungal sterility test, bioindicator showed no growth and the dose received by the product was within the stipulated acceptance criteria; the highest dose received was 28.8 kGy, the lowest 21.0 kGy and the average of 25.1 kGy sterilized in three lots. The results showed reproducibility between the cycles, obtaining the average coefficient of variation of 4.00% and an average standard deviation of 1.00 kGy. Therefore, the sterilization process of bromobutyl rubber stoppers by ionizing radiation is effective and reproducible, and it is concluded that this process is validated and can be routinely performed under the conditions in which it was evaluated.

Supported by: Fundação Butantan.

8.12 New occurrence of *Amblyomma romitii* Tonelli-Rondelli, 1939 (Acari: Ixodidae) in Pará State, Brazil

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Introduction: *Amblyomma romitii* Tonelli-Rondelli was described based on six females, one male and seven nymphs collected on a capybara (*Hydrochaeris hydrochaeris* Linnaeus), from British Guyana (now Guyana). The area of distribution of the species includes Guyana, French Guyana, Suriname, Venezuela and Brazil. In 1955, it was synonymized with *A. extraoculatum* Neumann; however, based on the type species it was redescribed and validated recently. **Objective:** The aim of this study was to record the second occurrence of this species in Brazilian territory. **Methods**: In April 2009, specimens were sent to the Laboratorio de Parasitologia do Instituto Butantan: one male, two females and two nymphs, collected on a capybara from the city of Rurópolis, southwestern Pará State (04°05'45 "S and 54°54'33" W). Based on an illustrated key, the ticks were identified as *A. romitii*. One of the females was engorged and it was kept in an incubator (BOD) at 27°C and humidity of 80-90%, where it got the posture. The other specimens were fixed in 70% alcohol and were deposited in the Acari collection from Instituto Butantan. **Results and Discussion:** The only confirmation of *A. romitii* in Brazil was a male with hypostome fractured, captured on humans in the state of Pará in 1957. No other report of its occurrence was published since that time in Brazil. This is then the first record of this species found on capybara in this country. Larvae and nymphs were obtained including an engorged female, and studies about the life cycle of this species are in progress.

Suported by: FAPESP and CNPq.

8.13 Ticks of the Neotropical region: illustrated key for immature stages of the genera Argasidae and Ixodidae

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Introduction: Determination of the genera of the tick families Argasidae and Ixodidae in the larval and nymphal stages have been a taxonomic problem, mainly because the few keys for the Neotropical region are chaetotaxic and rarely show illustrations. **Objective:** Considering the importance of the immature stages in the transmission from pathogenic agents for human and animals, the aim of this study was to help the classification of the genus of larvae and nymphs of ticks. **Methods:** Classification of the immature stages, larvae and nymphs, was based on optical and scanning electron microscopy. **Results and Discussion:** Illustrated keys for immatures of the different tick genera of both families that occur in the Neotropics were proposed. Generic diagnosis and updated listings of species for each genus were also included.

Supported by: CNPq and FAPESP.

8.14 Occurrence of heart failure with presence of ascites in a horse on São Joaquim farm of Instituto Butantan

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Introduction: Congestive heart failure (CHF) is a rare disease. It is known as a clinical syndrome characterized by limited cardiac, neuro-humoral activity increased, sodium retention, tissue edema and the presence of transudate in serous cavities. Most cardiac lesions in horses are not serious enough to cause CHF. However, it can occur in foals and adult horses as a result of severe degenerative valvular disease, valvulitis, dilated cardiomyopathy, myocarditis or myocardial necrosis, bacterial endocarditis, effusion, congenital malformation and vascular rupture or obstruction of the pulmonary artery. The most common cause of CHF in horses is valvular heart disease. It can develop quickly or gradually. The most common clinical signs are ventral, preputial, chest and limb edema, indicating widespread venous distension that is a sign of CHF on the right. On physical examination of the animal, there is elevated jugular venous pressure, increased pulse and pathological jugular filling, tachycardia at rest (over 60 beats per minute), tachypnea, pericardial effusion, ascites, lethargy and weight loss. **Objectives:** The aim of this investigation was to determine what have happened to a horse belonging to the São Joaquim Farm. **Results and Discussion:** A male horse, castrated, was found in the paddock with edema in the chest, in the abdomen and of the foreskin. On physical examination there were pale mucous membranes, tachycardia (65 beats per minute), weight loss and apathy. Specific therapy to decrease the edema and to improve the clinical condition of the animal was done. He showed reduced edema, but the clinical picture remained the same. After four days, the horse died, and was necropsied. The internal organs were jaundiced, and there were large amounts of fluid in the abdominal cavity, and presence of fibrin in many arteries, in veins and in the pericardium. It was determined that the fluid in the abdomen was ascites, which is a rare event in horses.

Supported by: Fundação Butantan.

9: PIBIC Program

9.01 Lipoic acid affects renal function and aminopeptidases in mice envenomed by *Crotalus durissus terrificus*

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Introduction: Hyperuricemia and alterations of renal protein and aminopeptidase (AP) activities of soluble (SF) and membrane-bound (MF) fractions have been found in the kidneys of mice envenomed by the snake *Crotalus durissus terrificus*. **Objectives:** This study analyzes the effect of lipoic acid (LA) po (0.1 mg/g bw) on renal AP and in the severity of acute renal failure induced by 80% LD50 ip of *C. d. terrificus* venom (*vCdt*) (0.0512 μg/g bw) in mice. **Methods:** Measurements of classical renal function parameters by spectrophotometry and AP assay by fluorometry. **Results and Discussion:** The lethality (33-40%, n=10-12), hypercreatinemia (>1.9 mg/dL, n=6-8) and hyperuricemia (>1.6 mg/dL, n=6-8) did not differ between *vCdt* and *vCdt*+LA. However, LA mitigated the effect of *vCdt* on protein content in MF of renal cortex (1.8±0.1, n=12, control; 7.9±0.2, n=6, *vCdt*; 2.1±0.1, n=6, *vCdt*+LA) and medulla (1.6±0.1, n=12, control; 3.6±0.7, n=6, *vCdt*; 1.5±0.1, n=6, *vCdt*+LA), on basic AP (16537±6059, n=12, control; 9582±842, n=6, *vCdt*; 22255±2161, n=5, *vCdt*+LA) and neutral AP (12959±1286, n=12, control; 22645±1476, n=6, *vCdt*; 12619±1094, n=6, *vCdt*+LA) in SF of renal cortex and neutral AP in SF of renal medulla (13302±1871, n=12, control; 45637±2416, n=6, *vCdt*; 14512±1233, n=6, *vCdt*+LA). The present data suggest that LA could be beneficial for the treatment of *vCdt* nephrotoxicity.

Supported by: FAPESP and CNPq/PIBIC.

9.02 Morphology and structural organization of Gené's organ in ixodid ticks

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Introduction: The process of oviposition in ixodid ticks was found to be a sequence of exactly coordinated events in which Gené's organ plays the important role of waxing the eggs with a substance capable of protecting them from desiccation and microbial attacks. There are few studies about the morphology of this organ. This study was performed in order to confirm if Gené's organ is either the only producer of the wax substance or if it is responsible for the storage. **Objective:** To study the morphology of Gené's organ of *Amblyomma cajennense* (Fabricius) using light microscopy. **Methods:** Four egg-laying females of *A. cajennense* on the third day of the oviposition period were used. Scutum was removed with a microscalpel by cutting across the dorsal shield at the most anterior part, just distal to the basis capitulum to expose Gené's organ. The organs were collected and immediately immersed in Bouin, dehydrated in 95% ethanol and embedded in historesin. Sections (3 µm) were stained with 1% methylene blue and examined in a DM LS (Leica) light microscope. **Results and Discussion:** Light microscopy revealed that Gené's organ in ixodid ticks is formed by a simple columnar epithelium and the lumen is filled with an amorphous mass. Muscle fibers can be also observed, consistent with the nearness of retractor muscles by which the cuticular sac of Gené's organ everts. It suggests that it is glandular; however, more studies are in progress to confirm it.

Supported by: CNPq/PIBIC and FAPESP.

9.03 Paracrine interactions between astrocytes and pinealocytes in the glutamatergic modulatory effects on melatonin synthesis

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Introduction: The pineal gland is a small endocrine gland present in all vertebrates. It synthesizes the hormone melatonin which is produced and secreted by the pinealocytes. Melatonin synthesis is controlled by cyclic environmental factors and it is almost restricted to the dark period. In this way, melatonin signals to the internal millieu if it is day time or night time and even the seasons. There are glutamatergic receptors in the astrocytes and pinealocytes membranes of the rat pineal gland. Glutamate causes an inhibitory action on melatonin synthesis, and this effect is dependent on the interaction between astrocytes and pinealocytes. **Objectives:** The objective of this work was to investigate the types of glutamate receptors, both ionotropic and metabotropic, which are involved glutamate's inhibitory action on melatonin synthesis. **Methods:** Young male Wistar rats were sacrificed by decapitation; their pineal glands were isolated and dissociated by the Papain Dissociation System kit. The pinealocytes in association with astrocytes (co-culture) were kept in culture and then were submitted to the pharmacological treatments for 5 h. Afterward, the glands were kept frozen at - 80°C until melatonin was assayed by HPLC with electrochemical detection. The cells in co-culture were stimulated with norepinephrine (1 μM), indispensable to the modulation of melatonin synthesis, in association with glutamate or specific ionotropic (NMDA, AMPA) or metabotropic receptors agonists (type I: DHPG, type II: l-CCG). **Results and Discussion:** The cell group stimulated with norepinephrine and AMPA did not show alteration of melatonin production when compared with the control group, stimulated only with norepinephrine. The cell group stimulated with NMDA showed reduced melatonin synthesis when compared with the control group. DHPG and L-CCG, agonists of type I and type II metabotropic receptors, respectively, did not display any effect on melatonin synthesis. Glutamate induced the known inhibitory effect. The data showed that only the NMDA ionotropic receptors seem to be involved in the glutamatergic inhibitory effect on melatonin synthesis.

Supported by: CNPq/PIBIC.

9.04 Effects of *Bothrops jararaca* venom and lipoic acid on renal function, oxidative stress and aminopeptidases in mice

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Introduction: The nephrotoxic effect of *Bothrops jararaca* venom (*vBj*) can alter important enzymes in renal physiopathology, besides stimulating oxidative stress. Moreover, antioxidants have been proposed for the therapy of renal damage. **Objectives**: This study aimed to evaluate the relation of classical parameters in renal function, aminopeptidase activities (APs) and oxidative stress with acute renal failure (ARF) induced by *vBj* venom and treated with lipoic acid (LA). **Methods:** The LD50 of *vBj* (2.08 µg/g bw) was used to induce ARF in mice and LA (0.1 mg/g bw) was used as a treatment. APs in renal soluble (SF) and membrane-bound (MF) fractions were measured by fluorometry, and classical renal function parameters and GSSG/GSH by spectrophotometry. **Results and Discussion:** LD50 of *vBj* caused hypercreatinemia (>1.4 mg/dL), hypercreatinuria (72.2±1.6mg/dL), hyperuricemia (2.0±0.13mg/dL), decrease in hematocrit (17±1.3%), protein (51.7±0.15 mg/dL) and urea (21.7±7.3 mg/dL) in plasma, hypo- (275.6±2.8) and hyper- (2650.0±28.8) osmolality (mOsm/Kg) respectively in plasma and urine, and GSH decrease, GSSG increase and consequently increase of GSSG/GSH ratio in renal cortex and medulla. In general, *vBj*, LA or *vBj*+LA affected the APs examined in renal tissue in a pattern that suggests deleterious effect, except that normal levels of neutral AP in *vBj*+LA were restored in SF of renal medulla and MF of renal cortex. LA also restored uricemia and normal ratio of GSSG/GSH in renal cortex and medulla without effect on hypercreatinemia, but with substantial proteinuria (3.0±0.7, control; 2.5±0.07, *vBj*; 8.2±0.22, *vBj*+LA). The present data include alteration in renal AP activities among mechanisms and consequences of *vBj* nephrotoxicity and demonstrate that benefits of LA in the therapy of this envenomation could be limited by the induction of proteinuria.

Supported by: FAPESP and CNPq/PIBIC.

9.05 Characterization of proteolytic enzymes in saliva of chronic obstructive pulmonary disease (COPD) patients: search for inhibitors in low-molecular-weight fraction of *Bothrops jararaca* venom

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Introduction: Chronic obstructive pulmonary disease (COPD) is an inflammatory and progressive disease characterized by a decrease in the diameter of airways which increases their resistance to airflow. Incidence, morbidity and mortality rates of COPD have increased continuously in recent decades. The major risk factors for COPD are cigarette smoking, age, passive smoking and hyper-reactive airway. A common feature of COPD disease is the chronic inflammation in the airways (influx of inflammatory cells into the lung, such as macrophages and neutrophils) and the development of extensive tissue remodeling during the course of the disease. In some cases this remodeling causes destruction of healthy lung tissue, leading to emphysema. For this reason, there is an increased interest in the role of MMPs (MMP-2 and MMP-9) and neutrophil elastase in COPD. All cited proteases probably contribute to the migration of inflammatory cells into the lung, as well as to the remodeling, and sometimes even to destruction of lung tissue. This hypothesis is supported by several studies with both bronchoalveolar lavage (BAL) fluid and sputum (produced spontaneously or induced) of COPD donors. **Objectives**: The aim of this work was to investigate the activity of metallo- and serineproteases in saliva of COPD patients in comparison with saliva obtained from healthy donors. In addition, this study also aimed at the search for inhibitors in the low molecular weight (LMW) fraction from *B. Jararaca* venom specific for MMP-2, MMP-9 and elastase. **Methods**: Saliva samples from 20 COPD and seven healthy donors were analyzed using 30-300 UA (UA= units of activity; UF/min/μg) of each sample and 5 μM of the FRET substrate, Abz-FRSSRQ-EDDnp. The measure of the total metalloproteinases and serineprotreinases activities was obtained by the use of EDTA (100 mM) and PMSF (0.5 μM), respectively. The LMW fraction (100 μg) was used to block 50-100 UA of each sample and 0.7 μM of the FRET substrate described above. **Results and Discussion**: All saliva samples used in the present study showed high specific activity values toward the Abz-FRSSRQ-EDDnp substrate, but we could not find any differences between the COPD and healthy samples. The results concerning the total activity levels of metallo- and serineproteases in the saliva samples also revealed no differences between the two groups studied here. For both groups, the use of EDTA blocked around 35% and PMSF was able to inhibit 63% of the total activity measured. The use of the LMW fraction from *B. Jararaca* venom resulted in good inhibition levels when the COPD saliva samples were analyzed (49%), but a similar inhibitory power could be found in the samples from the healthy group (57%). Taken together, the results led us to hypothesize that the saliva samples, despite their ease of collection, may not be a good source for proteolytic enzymes involved in COPD. Another explanation for our results is a possible balance between the enzymes and the antiproteases that act as their inhibitors, such as TIMP-1, TIMP-2 (MMP-9 and MMP-2 inhibitors, respectively) and α1-AT (elastase inhibitor) in the saliva samples.

Supported by: CNPq/PIBIC.

9.06 Gene polymorphism of gyroxin from *Crotalus durissus* rattlesnake venom

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Introduction: There are a great number of biochemical compounds in snake venoms, where the proteases are in large amount and show many activities. These activities are classified into two groups: serine proteases and metalloproteinases. Regarding the serine protease genes, many cDNAs were described, but just one genomic sequence from batroxobin: a toxin from the *Bothrops atrox* venom. The gyroxin is a serine protease from *Crotalus durissus* venom, which shows many enzymatic activities such as amidasic, esterasic and fibrinogenolytic. Only gyroxin cDNAs sequences from venom glands are known. The attainment of gyroxin gene partial sequence confirmed that the amplified sequences in our work are from the gyroxin gene. It also allows a preliminary study about the evolution at the exons and introns of gyroxin genomic sequences. **Objective:** Analysis of the gyroxin gene partial sequences attained by PCR amplification. **Methods:** PCR was accomplished using the primers F4gyr/R2gyr, which hybridize with exon 3 and exon 4, respectively. The amplified sequence was cloned in the pTZ57R/T vector. The plasmids were digested with the Eco RI and Hind III to confirm the presence of inserts and to subclone the gyroxin sequence. The plasmids were quantified by spectrophotometry and sequenced using the Big Dye Terminator V 3.1 Cycle Sequencing kit and the ABI Prism 3100 Genetic Analyzer from Applied Biosystems. The sequences were analyzed at BLAST (NCBI), and aligned with the ClustalW2 program. **Results and Discussion:** Five clones were analyzed after cloning the 1.9 kbp sequence. The Eco RI and Hind III double digestion identified two distinct clones, pTZ-gyr1.9-5 and pTZ-gyr1.9-7. Both sequences showed 89.1% similarity. B1_4 cDNA (EU360952.1) was compared to gyr1.9-7 and gyr1.9-5 sequenced and showed identity of 98% and 85%, respectively. Regarding the B1_3 cDNA (EU360951.1), 90% similarity to gyr1.9-7 sequence was obtained and 85% to gyr1.9-5. Besides *Crotalus* and *Bothrops* genera, isoform 6 mRNA from *Sistrurus catenatus* snake also displayed similarity of 91% and 89 % in relation to gyr1.9-7 and gyr1.9-5 sequences, respectively. Comparison to the gyroxin B1_3 cDNA showed synonymous and nonsynonymous mutations in exons 3 and 4, with a predominance of nonsynonymous at exon 4. It was observed that the intron-exon boundaries are conserved in gyr1.9-5, gyr1.9-7 and batroxobin genes with similarity of 86% and 83%, respectively. The gyr1.9-7 sequence showed more conservation of the exon 3 sequence than of the exon 4. In this case, the mutations increase the number of nonpolar amino acids and decrease the number of charged amino acids. The analysis of *Trimeresurus flavoridis* serine protease cDNAs and *Crotalus durissus* gyroxin cDNAs showed accelerated evolution which was confirmed with our results. This fact may change the biological activity of these serine proteases. Accelerated evolution also occurs in other toxins and it was first shown for phospholipase A_2 from *Trimeresurus flavoviridis* and *T. gramineus*. The new biological activities of toxins may occur to permit adaptation to environmental changes.

Supported by: FAPESP and CNPq/PIBIC.

9.07 Digestive astacin-like enzyme and astacin-like inhibitor from *Nephilengys cruentata*

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Introduction: Astacins (EC 3.4.24.21) are metallopeptidases (family of zinc-endopeptidases) which show a consensus motif HEXXHXXGXXH and was first identified in the crayfish *Astacus astacus*. To date, more than 200 members of this family have been identified in species ranging from bacteria to humans including disintegrins and matrix metalloproteinases involved in diseases such as cancer and rheumatoid arthritis. There is no description in the literature about a natural inhibitor for this family of metallopepetidases. **Objectives:** To isolate the major astacin-like enzyme present in the hepatopancreas from the giant spider *Nephilengys cruentata* and to characterize the purified enzyme and to test hemolymph homogenate in order to identify possible inhibitory activity against astacin-like activity. **Methods:** Adult females were not fed for at least two weeks. Afterward, cannibalism among spiders was favored. Spiders were dissected, the hemolymph was collected in the presence of sodium cacodylate and phenylthiourea, and the hepatopancreas was isolated and then homogeneized in cold Milli Q water in a Potter-Elvehjem homogenizer. Homogenate samples were applied ito a Hitrap Q column equilibrated in 0.02 M Tris HCl buffer, pH 9.0, and eluted with a linear NaCl gradient. Fractions active on casein-FITC were pooled and submitted to gel filtration in a Superdex G-75 column. Active fractions were individually applied on a 15% polyacrylamide gel and submitted to electrophoresis. Hepatopancreas homogenate samples and Hitrap Q pooled active fractions were also used in zymography with gelatin as substrate. Thermal inactivation was studied by incubating the homogenate samples containing the enzymes at the specified temperatures followed by determination of residual activity after different times. Hemolymph samples were homogenized in Milli Q water and added to Hitrap Q chromatographic fractions obtained from *Nephilengys cruentata* hepatopancreas using casein-FITC as substrates. **Results and Discussion:** The astacin-like enzyme present in *Nephilengys cruentata* hepatopancreas was isolated with a yield of 100 % and a purification of at least 10 times. SDS-PAGE showed the isolation of a peptidase of 14 kDa. This peptidase was inactivated at 55°C with a half-life of 46 min, and showed a Km of 0.48% using casein-FITC as substrate and a pH optimum of 8.3. Tests of inhibition of astacin with hemolymph samples of *Nephilengys cruentata* indicated an inhibition of 70% astacin activity. Anion–exchange chromatography followed by gel filtration allowed the isolation of a peptidase present in the hepatopancreas from *Nephilengys cruentata.* The low molecular mass of this enzyme and data on inhibition with 1,10-phenanthroline and EDTA indicated that this enzyme is a metallopeptidase from the astacin family (astacin-like enzyme). The inhibition of astacin activity demonstrated a specific natural inhibitor present in hemolymph. Synthesis of specific substrates for astacin and the isolation of the inhibitor are being tested.

Supported by: FAPESP and CNPq/PIBIC.

9.08 Gometoxins: antimicrobial peptides from *Acanthoscurria gomesiana* venom

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Introduction: Infectious diseases are one of the main causes of death in human populations. For the most part, this is due to microorganisms resistant to different antibiotics. Thus, it is important to investigate natural or synthetic substances that have an antimicrobial activity, especially concerning substances that have a different mechanism of action than those of current antibiotics. In this scope, the research of antimicrobial molecules in Brazilian fauna and flora could be valuable. The study of antimicrobial molecules of arachnids is very important because this animal family is 350 million years old. Furthermore, this kind of animals lives in places which increase the development of pathogenic microorganisms. This observation tends to indicate that this research could be prolific. In arachnid toxins, mainly from spiders and scorpions, some antimicrobial peptides were identified. Concerning spiders, toxins have been extracted from venom glands of the following spiders: *Lycosa carolinensis* and *Cupiennius salei*. Some antimicrobial peptides have been discovered in scorpions including *Hadrurus aztecus*, *Pandinus imperator* and *Opisthacanthus madagascariensis*. **Objectives:** The aims of this study were to identify and characterize antimicrobial peptides in venom of *Acanthoscurria gomesiana*. This work could be useful in the scope of a future pharmaceutical use. **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 - trifluoracetic acid 0.05%). The soluble part was applied to HPLC reversed-phase chromatography on a semi-preparative or analytical Jupiter C18 column. Elution was performed with different linear gradients of ACN/TFA 0.05%. The presence of antibacterial activity was determined by a liquid growth inhibition assay against Gram-negative bacteria *Escherichia coli SBS363*, Gram-positive bacteria *Micrococcus luteus A270* and yeast *Candida albicans* MDM8. Molecular weight and purity of the molecules were analyzed by mass spectrometry (MALDI-TOF). **Results and Discussion:** After two stages of purification, four fractions with antimicrobial activity were observed: frac21 (*C. albicans*), frac22 (*C. albicans*, *E. coli* and *M. luteus*), frac30 (*C. albicans*), and frac48 (*M. luteus*). After purification and re-purification, only fractions 22, 30 and 48 were pure on analysis by mass spectrometry. The analysis revealed monocharged ions of m/z corresponding to 5,463.4, 2,731.7 and 1,659.8. These peptides were called gometoxin-1, 2 and 3 respectively. Analysis of gometoxin-3, using reduction and alkylation, showed that this peptide is linear without disulfide bonds. The complete characterization and sequencing of gometoxins are in progress.

Supported by: FAPESP and CNPq/ PIBIC.

9.09 Histopathological evaluation of mice injected with extracts of two toxic cyanobacteria strains

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Introduction: Cyanobacteria are responsible for numerous cases of human intoxications. The ingestion of endotoxins produced by cyanobacteria may cause: gastroenteritis, nausea, vomiting, fever, and eye irritation. In the laboratory, the effects of cyanobacteria toxins are studied in mice in order to classify the cyanobacteria as neurotoxin, hepatotoxin or dermatotoxin producers. **Objectives:** To perform toxicological studies of the cyanobacterial strains of the *Instituto de Botânica* algae bank, classifying the strains that produce toxins and to do further histophatological evaluation of the mice treated with the cyanobacterial extracts. **Methods:** Extract preparation: the cultured cyanobacterial cells were filtered through an AP-20 filter and freeze-dried. The resulting material was then extracted with 1) 0.1 M acetic acid (4x) by ultrasonication (4 x 10 sec., 50W) and centrifugation. The supernatant was concentrated under reduced pressure (water extract, strain SPC 422 *Pseudoanabaena galeata*); or 2) $MeOH/H_2O$ 75:25(v/v) (5x) by ultrasonication (40 x 30 sec.; 560W) and centrifugation. The supernatant was concentrated under reduced pressure (methanol extract, strain SPC 920 *Geitlerinema unigranulatum*). The extracts were kept at -20°C until they were used. The toxicity tests (i.p.) were performed in male Swiss-Webster mice (19-21 g). The mouse symptoms were observed up to 8 days after toxin administration. After death by acute intoxication or euthanasia, necropsy was performed and tissue samples were taken from liver, kidneys and lungs, fixed and used for histopathological analysis. **Results and Discussion:** The extract of the strain SPC 920 showed acute toxicity in mice, causing deaths at 10 mg/animal, within 50 min to 2 h after i.p. injection. Histological analysis showed lung abnormalities, with hemorrhagic foci and erythrocytes within some alveoli sacs. The kidneys showed fluid accumulation in the tubules, and in the liver there was an augmented space between the hepatocytes, maybe due to fluid retention. Small hemorrhagic foci were also detected in the liver. These results do not resemble the hepatotoxic toxicity induced by microcystins, which would cause strong liver hemorrhage, or by neurotoxin, lipopolysaccharide or cylindrospermopsin envenomation. The mice treated with the extract of the strain SPC 422 (20 mg/animal) did not die of acute toxicity, but showed weight loss after 7-8 days. They were euthanized and in 50% of the mice (N=6) several tumors were visualized in the liver. Liver tissue and tumor of treated animals were fixed to perform histological evaluation. Hepatocytes were found in the veins of the liver tissue, and condensed and/or fragmented nuclei were found in both (liver and tumor) samples. Microcystins may induce neoplasias, but usually with repeated doses within 20 weeks. The extract of the SPC 422 strain caused neoplasm formation in less than 10 days, in 50% of the mice. The preliminary tests for microcystin detection in this extract were negative, indicating a different class of hepatotoxin. Isolation and characterization of the toxins of these two cyanobacterial strains are in progress at the *Instituto de Botanica.*

Supported by: CNPq/PIBIC and FAPESP.

9.10 Characterization of biologicaly active peptides derived from the proteolysis of fibrinogen by bothropasin

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Introduction: Angiogenesis is a biological process by which new capillaries are formed from pre-existing vessels and is influenced by integrins expressed on endothelial cells, vascular smooth muscle cells, fibroblasts and platelets. The angiogenesis process is present in many pathologies of medical interest, such as arthritis, diabetic retinopathy and cancers, and result in the search for modulators. Many of these products are formed by degradation of extracellular matrix and blood plasma components, including a variety of bothropasin substrates. The bothropasin is a SMVP belonging to class III present in the *Bothrops jararca* venom. **Objectives:** The aim of the present work was the search for and characterization of proteolytic fibrinogen products resulting from the bothropasin activity able to act on cell migration and proliferation. Also, we studied the bothropasin itself and its disintgrin-cysteine rich domain (DC) with respect to direct action in proliferation and migration assays. **Methods:** The products of the fibrinogen digestion by bothropasin were purified by HPLC, screened by cell assays and the active ones were sequenced by MALDI-TOF mass spectrometry. The cell lines used in these assays were HUVEC and VSMC. For the migration assay using bothropasin and DC domain, the plates were previously coated with fibronectin. In the wound healing assay, a scratch was made after cell confluence using a p200, simulating a wound. The bothropasin and DC domain were added and the cells were photographed at time zero and after six hours. These same cells were subjected to an immunofluorescence assay using anti-FAK and phosphorylated anti-FAK. The same procedures were made for the scratch assay, but instead of the fluorescence step the cells were counted using the ImageJ program. The proliferation assay was performed with the BrdU Cell Proliferation Assay kit, 1000 Tests (Chemicon), and the factors utilized were bothropasin, DC domain and FGF. **Results and Discussion:** Our results show that a peptide fraction, resulting from the cleavage of fibrinogen by bothropasin, displays inhibitory activity in a variety of biological assays with different cell lines. In fibroblasts these peptides inhibit up to 50% of bFGF-triggered mitogenesis. In HUVEC and smooth muscle vascular cells, these peptides have a more reduced inhibitory effect on FGF and serum triggered mitogenesis, reducing DNA synthesis by at most 30%. The next step will be the testing of their synthetic counterparts. The results concerning the study of the bothropasin show that the migration was stimulated by this SMVP. Since bothropasin degraded the subtrate fibronectin, the observed stimulation may have resulted from fibronectin proteolytic fragments binding to specific receptors on the cell membrane, likely integrin-type, as pointed out by FAK localization changes in immunofluorescence result. VSMC proliferation was inhibited in the presence of bothropasin and DC domain, which also blocked the action of FGF. On the other hand, HUVECs were not affected by the DC domain, even at higher concentrations; this result points to a DC anti-proliferative action that is specific for muscle cells and therefore needs further investigation.

Supported by: FAPESP and CNPq/PIBIC.

9.11 Analysis of enteropathogenic *Escherichia coli* outer membrane proteins by two-dimensional electrophoresis

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Introduction: *E. coli* is a versatile pathogen in animals and humans. EPEC has been identified as a main causative agent of acute diarrhea in developing countries. **Objectives**: The goal of this work was to verify the reproducibility of the two-dimensional (2D) electrophoresis protocol of OMPs extracts derived from one strain of EPEC (9100-83, O125:H6). **Methods**: 2D electrophoresis was performed by a two-step protocol, the first dimension by focusing of 13 cm, pH 4-7 strips (IPGphor III, GE Healthcare) and the second dimension by 15% acrylamide SDS-PAGE electrophoresis (SE 600 Ruby, GE Healthcare). **Results and Discussion**: Data from three 2D electrophoresis gels were compared by ImageMaster Platinum software (GE Healthcare). A total of 98 spots were observed in the first gel in the range of 13 kDa to 93 kDa and pI 4.03 to 6.66, 52 spots with molecular weight (mw) between 18 kDa and 90 kDa, pI 4.01 to 6.91 in the second and 44 spots (mw 17 kDa - 92 kDa, pI 4.12 – 6.63) in the third. Three high similarity regions were visualized in all gels. One between 30 kDa and 45 kDa, pI 5.11-5.24 with two main spots, region two with six major spots (24 kDa-30 kDa, pI 4.82-4.94), and region three with two spots (20 kDa, pI 5.47-6.06). One area (14 kDa-20 kDa, pI 5.00-6.91) was significantly distinct comparing the first gel (12 spots) to the other two (only 2 spots). All gels had quite similar spot ranges of mw and pI. Data from literature showed 100 spots from outer membrane extracts. The lower numbers of spots of gels 2 and 3 in relation to the first gel suggested a probable proteolysis process in the storage period of the corresponding strips at -20°C. The general profile of OMPs from EPEC (9100-83) by analysis of three gels was reproducible. However, future assays are necessary and they will determine the proteins that will be identified by mass spectrometry.

Supported by: FAPESP and CNPq.

9.12 Study of the plasticity of immature stem cells from human tooth pulp

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Introduction: Stem cells are cells that are able to self-renew and to differentiate into a specific cell line. The stem cells may be embryonic or of adult origin. Adult stem cells are less problematic to use than embryonic stem cells, because their use does not require the destruction of embryos. The use of adult stem cells in developing research in tissue engineering and regenerative medicine is important and has advantages, since the differentiation is more controlled. Furthermore, when introduced into the organism, they hardly produce tumors. Our group was able to establish a protocol that allows the original isolation, extraction, cultivation and differentiation of immature stem cells from tooth pulp, thus holding the know-how to manipulate these cells. This project will use two specific lines of stem cells from human tooth pulp, DA1 and DL7 which respectively refers to the second molar of a patient of 15 years and the primary tooth of a child of 5 years. **Objectives:** To study the capacity of proliferation, the plasticity and the mechanism of chondrogenic and osteogenic differentiation in vitro of stem cells from tooth pulp. **Methods:** Growth curve was the method performed to assess cell proliferation. The cells were cultured under appropriate conditions, with daily change of shift. The analysis was done by immunofluorescence in immature stem cells from tooth pulp using specific antibodies to embryonic and mesenchymal stem cells. For the analysis of the differentiation process, we used various antibodies and specific staining and morphological analysis enabling a more comprehensive characterization of the differentiation of the immature stem cells from tooth pulp for osteogenic and chondrogenic lineages. For chondrogenic differentiation the cells were grown in suitable conditions and concentrations with the use of a means of inducing chondrogenic differentiation. This inducer was renewed daily for 21 days. **Results and Discussion:** On the graph, it can be observed that both strains studied (DA1 and DL7) have considerable potential for change in proliferation after the first five passages, reaching amounts unviable to continue the growth curve after transition 15.

Supported by: CNPq/PIBIC.

9.13 Effect of crotoxin on secretory activity of peritoneal macrophages during tumor progression

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Introduction: Several lines of evidence indicate that the crotalid venom modulates macrophage function, stimulating hydrogen peroxide and nitric oxide production, anti-candidacidal activity and glucose and glutamine metabolism of these cells. Crotoxin (CTX), the major toxin of *Crotalus durissus terrificus* venom induces an inhibitory effect on tumor growth and modulates, particularly, the functions of macrophages, cells essential for innate defense mechanisms. One of the functions of macrophages is to provide a defense mechanism against tumor cells. These cells can be involved in both tumor killing and stimulation of tumor development, depending on stage of the macrophage activation and the development stage of the tumor. Functional plasticity is a well-known characteristic of the mononuclear phagocyte system, and the paradigm of *M1* and *M2* polarization identify the two extremes of the whole spectrum of macrophage functional activities. In the beginning of tumor progression, *M1* macrophages are avidly phagocytic and secrete or release reactive nitrogen intermediates-RNI/ROI and cytokines TNF-α, IL-1β and IL-6. On the other hand, when the tumor is established, tumor-associated macrophages (*M2* macrophages) show these abilities to be decreased. **Objectives:** The aim of this work was to investigate the effects of CTX on peritoneal macrophages secretory activity obtained from Walker 256 tumor-bearing rats in two different protocols: 1) CTX injected during the establishment of the tumor (to investigate the action of CTX on *M1* macrophages) and 2) CTX injected on the 5th day after the injection of Walker 256 tumor cells (to investigate the action of CTX on *M2* macrophages). **Methods**: Male Wistar rats were inoculated subcutaneously in the right flank with 1 mL of sterile suspension of $2x10^7$ Walker 256 tumor cells and immediately after were treated with CTX (18 μg in 300 μl per rat) or saline, in the same volume (control), s.c. administered. In other groups, rats were inoculated subcutaneously in the right flank with 1 mL of sterile suspension of $2x10^7$ Walker 256 tumor cells, and after five days of this injection, animals were treated with CTX (18 μg in 300 μl per rat) or saline, in the same volume (control), s.c. administered. Animals from control groups were inoculated subcutaneously in the right flank with 1 mL of PBS, treated with CTX or saline. Peritoneal macrophages were obtained on the 14th day after tumor cell injection, and H_2O_2 release and NO production were measured and levels of IL-1β, IL-6 and TNF-α in the culture supernatants were determined by ELISA using kits from R&D Systems. **Results and Discussion**: In both protocols evaluated, CTX stimulated the H_2O_2 release and NO production and the secretion of the cytokines IL-1β and TNF-α by peritoneal macrophages obtained from both Walker 256 tumor-bearing rats and non-tumor-bearing rats. For the first time, it was demonstrated that CTX modulates, *in vivo*, secretory activity of peritoneal macrophages and this effect is induced by a single dose of CTX. These results contribute to the elucidation of mechanisms involved in the CTX antitumor effect and bring new prospects for the development of a new substance with therapeutic properties.

Supported by: FAPESP and CNPq/PIBIC.

9.14 Purification of factor X from the blood of the snake *Bothrops jararaca*

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Introduction: The clotting factors II, VII, IX and X as well as the inhibitors protein C and protein S belong to the group of vitamin K-dependent proteins. This group of proteins plays a key role in blood coagulation. Coagulation factor X (FX) plays an important role in the regulation of blood coagulation by converting prothrombin into thrombin. Human FX has molecular mass about 62 kDa and consists of two polypeptide chains, light (17.5 KDa) and heavy (45 KDa). Prothrombin (FII) is the precursor of the enzyme thrombin that converts fibrinogen into fibrin and amplifies its self-generation. Human FII shows molecular mass of 72 kDa and consists of only one polypeptide chain. Protein C (PC) inhibits coagulation by selectively inactivating the active forms of factor V and factor VIII. Human PC has a molecular mass of about 62 kDa and consists of two polypeptide chains, light (21 kDa) and heavy (41 KDa). **Objectives:** The aim of this study was to purify *Bothrops jararaca* (*B. jararaca*) FX, FII and PC. **Methods:** The purification process consisted of barium chloride precipitation, two sequential steps of ammonium sulfate fractionation (40 and 67% saturation) and HiTrap DEAE Fast Flow chromatography. Along all the purification steps, protein concentration was determined by absorbance at A_{280}. Protein activity was measured using specific chromogenic substrate. The fractions were analyzed by SDS-PAGE (10%). **Results and Discussion:** The three proteins were concentrated by barium chloride precipitation and ammonium sulfate fractionation and partially purified by HiTrap DEAE Fast Flow chromatography showing that this process can be suitable to separate these proteins simultaneously. The perspectives for this work are to improve the purification process in order to get higher purity proteins and to compare them biologically and biochemically with other animals' FX, FII and PC.

Supported by: FAPESP and CNPq/PIBIC.

9.15 Characterization of a putative outer membrane lipoprotein of *Leptospira interrogans*

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Introduction: Leptospirosis is a widespread zoonosis caused by pathogenic spirochetes belonging to the genus *Leptospira.* The wide distribution of *Leptospira spp* results from their ability to colonize the renal tubules of mammalian hosts, including humans, wildlife, and many domesticated animals. Transmission to humans involves either direct or indirect contact with the urine from chronically infected animals. The genome of *L. interrogans* serovar Copenhageni has been sequenced and *in silico* analysis allowed identification of predicted outer membrane lipoproteins that could be involved in pathogenesis and immune responses. The development of a vaccine and of an effective diagnostic assay is important to the treatment and control of this disease. **Objectives:** The aim of this project was the cloning, expression, purification and characterization of the probable lipoprotein encoded by the gene LIC10704 identified in *L. interrogans* serovar Copenhageni genome. **Methods:** The gene was amplified from genomic DNA by PCR and cloned into the expression vectors pAE and pLIPO, which provide expression of non-lipidated or lipidated recombinant proteins. The recombinant 6xHis-tagged proteins were purified by metal affinity chromatography, and characterized by circular dichoism spectroscopy (CD). The recombinant purified proteins were used in immunization and challenge assays, and their capacity to mediate attachment to ECM components was evaluated by binding assays. **Results and Discussion:** LIC10704 encoded proteins were expressed in *E. coli* BL21-SI (pAE) or *E. coli* BL21-C43 (pLIPO) and purified from the soluble fraction (pAE) or detergent fraction (pLIPO). The purified proteins exhibited a single major band of 23.5 kDa (pAE) or 23.7 kDa (pLIPO) in SDS-PAGE. Their structural integrity was assessed by CD spectroscopy, which revealed a predominant α-helical secondary structure. PCR analysis demonstrated that the gene is conserved among different pathogenic serovars of *Leptospira.* Mouse polyclonal antiserum against the recombinant protein (pAE) was produced with a high antibody level detected by ELISA. However, the recombinant proteins do not display extracellular matrix binding properties, and probably they are not a leptospire antigen involved in adherence to host tissues. Hamsters immunized with recombinant proteins were not protected against mortality upon challenge with a lethal inoculum of *L.interrogans* serovar Copenhageni. This result indicated that LIC10704-encoded protein is not a vaccine candidate against leptospirosis.

Supported by: FAPESP and CNPq/PIBIC.

9.16 Multiplex RT-PCR for typing and subtyping of influenzavirus A

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Introduction: Influenza virus is a common childhood disease with the highest morbidity occurring in preschool children. The period of influenza virus circulation is associated with increased medical practitioners' consultation, hospitalizations, and excess deaths. The virus shows high genetic and antigenic variability, mainly in its surface glycoproteins, such as hemagglutinin and neuraminidase, which are responsible for the subtyping of *Influenzavirus A*. Due to the segmented nature of its genome, the virus can display rearrangements, generating new viruses, different from those in circulation, for which the population does not have immunity. The only way to prevent the flu and its possible consequences is through vaccination. Great efforts are made worldwide for the monitoring and characterization of circulating virus. It makes possible the detection of emergent viruses, such as avian influenza (H5N1) and influenza A (H1N1), as well as the choice of samples that will be part of the composition of the vaccine in the following year. The fast and specific detection of influenza viruses has significant importance in influenza monitoring and control programs. In Brazil, few data on infections of influenza in children are available, who are considered the most important vectors in transmission of the virus. **Objectives:** The aim of this work was to standardize RT-Multiplex-PCR technique, using specific primers to HA and NA genes in order to subtype *Influenzavirus A*. This method was also used to characterize the isolated virus from children hospitalized in University Hospital of the University of Sao Paulo (HU-USP), during the year of 2006. **Methods**: Nasopharyngeal aspirates from 500 patients with acute respiratory illness, ranging from infants to 5-year-olds, were collected at the HU-USP and tested using duplex RT-PCR for detection of influenza A and B. The multiplex RT-PCR was used to subtype *Influenzavirus A*. **Results and Discussion:** Twenty-three samples were positive by duplex-RT-PCR. Among these, 4 (17.4%) were positive for *Influenzavirus B*, and 19 (82.6%) for *Influenzavirus A*. During the year of the study, influenza A (H3N2) was the prevalent subtype (65%). A rapid detection and subtyping method are necessary to obtain detailed information about the prevalence of different subtypes of influenza virus and mainly to establish effective control measures.

Supported by: CNPq/PIBIC.

9.17 Skin and cutaneous glands during the development of the toad *Rhinella granulosa* (Amphibia, Anura). Ultrastructural aspects

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Introduction: Amphibian skin is a highly specialized organ with a wide spectrum of functions such as for the preservation of the whole body, defense against injury and microorganisms, sensitivity, diffusion of molecules, and it varies according to environment and life stage. **Objectives:** We aimed to describe the ultrastructural differentiation of the skin cells and cutaneous glands, during larval and juvenile stages of the toad *R. granulosa.* **Methods:** Tadpoles and newly metamorphosed toads of *R. granulosa* were collected in Angicos, RN, Brazil, during the rain season (February, 2004). Larvae were staged according to Gosner (1960). Skin samples of juvenile toads and of larval individuals were fixed in Karnovsky and osmium tetroxide, and embedded in Epon resin. Ultrathin sections (70 nm) were stained with uranyl acetate and lead citrate, and examined in a LEO 906E transmission electron microscope. Larval stages 25, 43, and newly metamorphosed toads were analyzed. **Results and Discussion:** At the early larval stage 25, the skin is composed by 2 layers of epidermal cells resting on a thick acellular basal membrane, the adepidermis. Basal cells show a well-developed system of intermediate filaments – the Eberth figures – which provide stability and flexibility to the epidermis. The superficial layer has 2 types of cells: a) cells with numeorus apical granules corresponding to the PAS positive granules observed in histological sections; and b) cells with remnants of cilia. Other types of cells are the Merckel cells with neurotransmitter granules, and vacuolated cells. At stage 43, the adepidermis is almost totally invaded by fibroblasts, capillaries, melanocytes , and projections of cutaneous glands, becoming a true dermis. Gradual cornification of the external layers is observed. Cutaneous glands are not fully developed. Granular glands are composed of a syncitial secretory epithelium with abundant, rough and smooth endoplasmic reticulum, in active stage of synthesis and storage of granules. The newly metamorphosed toads show a cornified epidermis with a sloughing external layer. Granular and mucous glands are well developed. In fully differentiated granular glands the atrophied cytoplasmic organelles are segregated to the syncitium periphery by the numerous maturing lamellar vesicles that are merged in an electron-dense cytoplasm. The amphibian skin is a dynamic system adapted to each phase of the animal life and is greatly influenced by thyroid hormones in the final larval stages. The cell ultrastructure reflects the varied functions required for the animal adaptation and survival during larval development. Cutaneous glands, important for the toad defense, are present and differentiated by the time of metamorphosis.

Supported by: CNPq/PIBIC.

9.18 Antibacterial activity of extract of marine sponge *Amphimedon viridis*

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Introduction: The emergence of new infectious diseases and the multiple-drug resistant strains of bacteria, besides the indiscriminate use of antibiotics, created an urgent need for the development of new strategies to treat bacterial infections and for the development of new antimicrobials. Many natural products from marine sources are endowed with promising antibacterial activities. **Objectives:** The objective of this study was to evaluate the antibacterial activity of extracts obtained from the marine sponge *Amphimedon viridis*. **Methods:** The water extract (AvA) and methanol-water extract (AvM), prepared from the marine sponge *Amphimedon viridis*, collected in the Maceió cith (Alagoas), were initially tested against *Escherichia coli* and *Staphylococcus aureus*, using the agar diffusion method for antimicrobial susceptibility, following Kirby and Bauer methodology. The extracts that showed inhibitory activity against these two bacterial strains were also tested against other bacterial species and *Candida albicans* using the same methodology. **Results and Discussion:** The AvA extract did not show an inhibition halo, whereas the AvM extract, at a concentration of 1 mg/ml and 5 mg/ml inhibited the bacterial growth of these two species. At 2 mg/ml, the AvM extract showed an inhibition halo to Gram-positive bacteria: *Enterococcus spp*, *Streptococcus agalactiae*, *Streptococcus pyogenes*, *Micrococcus luteus*, and *Staphylococcus epidermidis*, but did not inhibit the growth of *Bacillus spp.* With respect to Gram-negative bacteria, such as *Proteus mirabilis*, *Klebsiella oxytoca*, *Acinetobacter baumanii* and *Pseudomonas aeruginosa*, the AvM extract showed low capacity of inhibition and did not inhibit the growth of *Stenotrophomonas maltophilia* and *Candida albicans.* This extract may contain halitoxin (which shows diverse toxic and cytotoxic activities) or other active substances in its composition and will be purified to study its antibacterial activity.

Supported by: FAPESP and CNPq/PIBIC.

9.19 How can biological variables influence the incidence of *Bothrops jararaca* snakebites in São Paulo State, Brazil?

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Introduction: Snakes of the genus *Bothrops* are responsible for almost 90% of all accidents occurring in Brazil. *Bothrops jararaca* is responsible for almost 93% of all *Bothrops* accidents. **Objectives:** The aim of this study was to evaluate the influence of biological variables in *B. jararaca* snakes which cause snakebites in São Paulo State. **Methods:** *Bothrops jararaca* specimens that have caused accidents from 1995 to 2008 (N=3053) are preserved at "Collection Vital Brazil" at Institute Butantan. All these snakes were dissected, examined, and measured. Snake stomachs were dissected to check whether or not they had stomach contents. **Results and Discussion:** These data revealed that newborn males of *Bothrops jararaca* fed mainly in spring, whereas female newborns fed mainly in winter. *B. jararaca* male newborns had 128 identifiable items in their stomachs (86% endothermic preys and 14% exothermic preys), whereas female newborns had 110 identifiable items in their stomachs (89% endothermic preys and 11% exothermic preys). A large number of accidents occurred during the day, between 6:00 am and 6:00 pm (67%). However, many accidents occurred during the night period, between 7:00 pm and midnight (19%). Adult snakes caused more accidents during the day period. Our data show that 71% of the accidents are caused by newborns, whereas 29% are caused by adults. Adult females (53%) caused more accidents than adult males (47%). Males caused more accidents during the autumn, whereas females caused more accidents during the summer. Newborns (male and female) caused more accidents during the spring and summer. Analyzing the female reproductive status of the snakes that caused accidents, 45.6% were non reproductive females, and 16.5% were reproductive. However, pregnant females were found in late spring. Newborns are more abundant in autumn. Seasonal patterns of accidents are different between *B. jararaca* adults and newborn. However, newborn caused more accidents than adults. Preliminary analysis shows that females cause more accidents than males. Adult males cause more accidents during the autumn, whereas adult females cause more accidents during the summer. These periods coincide with this species' reproductive pattern, mainly for females which must feed heavily during this period, so they can have enough energy for vitellogenesis. The spermatogenic activity begins in the spring, with spermiogenesis occurring in spring-summer and spermiation in the autumn. During the autumn, males are searching for females (mating period), and therefore, the increase of risks of accidents is higher.

Supported by: CNPq/PIBIC.

9.20 Microvesicles in *Crotalus durissus terrificus* venom (Serpentes-Viperidae) originate from microvilli

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Introduction: Microvesicles 40-80 nm in diameter are consistently observed in the tubular and venom gland lumina of viperid snakes associated with secretory cell apical microvilli. They have been isolated from *Crotalus durissus terrificus* venom by ultracentrifugation and characterized morphologically. Intramembranous particles detected by freeze-fracture indicate intramembranous proteins in microvesicles. Similar vesicles, observed in *Glodyus blomhoffii blomhoffii*, were supposed to originate from multivesicular bodies, and they show dipeptidyl peptidase and ecto-5'-nucleotidase activities, which may disturb hemostasis. **Objectives:** To standardize a fractionation protocol for the recovery of microvesicles from *C. d. terrificus* venom by ultracentrifugation, and to analyze the microvilli-microvesicles relationship. **Methods:** Four milliliters of venom from 18-20 specimens of *C. d. terrificus* maintained in the Laboratory of Herpetology were used in five experiments. Venom was submitted to a first centrifugation varying from 150 *g* to 32,500 *g* at 10ºC to eliminate sloughed cells and cell debris, and afterwards the supernatants were ultracentrifuged from 100,000 to 200,000 *g* for 60 min in a refrigerated Sorvall OTD 75 B with an AH650 rotor. The resulting pellets were fixed in Karnovsky aldehydes and in osmium tetroxide, and embedded in Epon resin. Ulltrathin sections and negative stained preparations were examined in the LEO 906E transmission electron microscope. **Results and Discussion:** The best recovery of venom microvesicles was achieved with an initial centrifugation at 3,600 *g* for 15 min, followed by an ultracentrifugation at 190,000 *g* for 1 h. Clustered microvesicles and fragments of secretory cell apical cytoplasm with microvilli and associated microvesicles pelleted even at the lowest centrifugation speed. Microvilli fragmentation and microvesicle budding from microvilli are often observed in ultrathin sections of pellets. Amorphous material was observed outside the microvesicles. Negative stained images from pellets also showed clusters of microvesicles and microvesicles associated with microvilli. Negative staining of the final supernatant showed numerous electron-dense granules, sized from 0.3 to 0.5 µm, but no microvesicles. Our electron microscope images provide show that the bulk of microvesicles found in *C. d. terrificus* venom originates from microvilli, by fragmentation or membrane budding. Their release into the venom, in association with fragments of apical cytoplasm of secretory cells, likely represent a way to eliminate excess membranes, while, at the same time, they expose proteins that may have biological or biochemical effects.

Supported by: CNPq/PIBIC.

9.21 Piperacea extracts as bait to control *Achatina fulica* and *Rumina decolatta* (Gastropoda: Stylommatophora)

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Introduction: Terrestrial mollusks are commonly known as snails and slugs. Some of them are used as food and they have great nutritional value, including vitamins and minerals. They can accomplish an important function on flower pollination and dissemination of seeds and spores through their mucus or feces. On the other hand, they cause damage to agriculture, mainly exotic species, injuring crop plantations and ornamental plants. Furthermore, they can be intermediate hosts of some parasites. The control of these animals in nature is difficult. There are two kinds of chemical substances used as bait: carbamate and metaldehyde. But, they bring risks to the environment and have a high production and application cost. In this manner, studies with plant extracts are being done to find more options in the population control of gastropods. **Objectives:** The aim of this project was to analyze the molluscicidal effect of different plant extracts from the family Piperaceae on *Achatina fulica* Bowdich, 1822 and *Rumina decollata* Férussac, 1821 to achieve a less damaging option to the environment. **Methods**: We used five young snails of each species in duplicate, exposed to 1000 ppm of extract through ingestion for 24 h and were observed for nine days. The tested extracts were: leaves of *Piper crassinervium*, *Piper diospyrifolium*, *Piper fuligineum*, *Piper gaudichaudianum*, *Piper tuberculatum* and *Photomorphe umbellatum*, besides the fruit of *P. crassinervium* and inflorescence of *P. tuberculatum*. A commercial molluscicide, Pikapau®, based on metaldehyde (3%) was used as positive control. **Results and Discussion**: Despite that *R. decollata* was more sensitive to metaldehyde (70% mortality), *A. fulica* showed higher mortality after exposure to plant extracts, so that *P. crassinervium* (leaf) was the most effective (30%). Also recorded were changes in the feeding behavior of the animals after eating the extracts. *P. crassinervium* (leaf and fruit), *P. tuberculatum* (inflorescence), *P. gaudichaudianum* (leaf) an *P. diospyrifolium* (leaf) inhibited feeding of *A. fulica*, but only *P. fuligineum* (leaf) recorded this response on *R. decollata*, while the extracts of *P. gaudichaudianum* (leaf), *P. diospyrifolium* (leaf), *P. tuberculatum* (inflorescence) and *P. crassinervium* (fruit) stimulated feeding of *R. decollata*. Therefore, the response to ingested plant extracts was different between the species, which means that their physiology and behavior were distinct and must be considered in field attempts of population control of these snails.

Supported by: CNPq/BIPIC and Fundação Butantan.

9.22 Expression of iNOS and arginase mRNA in murine macrophages stimulated *in vitro* with *Bordetella pertussis*

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Introduction: L-arginine metabolism results in the production of nitric oxide (NO) and arginase, molecules involved in the control of several infections and inflammatory process. iNOS and arginase compete for the same substrate, L-arginine and co-regulate the function of each other. The activities of both enzymes are regulated in macrophages by cytokines and microbial components while the concentration of iNOS and arginase at the site of inflammation can be responsible for modulating the level of immune response. The macrophage is one of the main defense cells in the organism and also regulates the activity of the inflammatory cell including the activity of T lymphocytes. *Bordetella pertussis* is the causative agent of whooping cough and its prevalence in the vaccinated population is increasing in many countries. The mechanisms that regulate the expression of iNOS and arginase in activated macrophages with *B. pertussis* are unknown. **Objective:** We examined the expression of iNOS and arginase I mRNA in macrophages activated *in vitro* by *B. pertussis*. **Methods:** Mouse bone marrow macrophages (BMDMϕ) from C57/BL6 mice were cultured and differentiated for 7 days in RPMI supplemented with 10% of FBS, 20% supernatant of L929 fibroblasts, 50 U/ml penicillin, 50 μg/ml streptomycin and non essential aminoacids. BMDMϕ was activated with soluble protein from *B. pertussis* (30 μg/ml) in the presence or absence of IFN-γ for 6 and 24 h. At the indicated time points, total cellular RNA was prepared with Trizol reagent (Invitrogen), according to the manufacture's instruction. Total RNA (2 μg) was converted to cDNA. PCR was performed using sense and antisense primers, respectively, for iNOS: AGGAAGAAATGCAGGAGATG and CACCTGCTCCTGGCTCAAG (253-bp); arginase: TTCTGGGAGGCCTATCTTAC and CCAAGGTTAAAGCCACTGCC (162-bp); β-actin: ACTACATTCAATTCCATCAT and CGATCCACACAGAGTACTTG (196-bp). PCR products were separated by electrophoresis on 2% agarose gels and visualized by staining with ethidium bromide. **Results and Discussion:** Relative levels of mRNA for iNOS and arginase I were determined by semi-quantitative RT-PCR at 6 and 24 h after macrophage stimulation. iNOS and arginase I mRNA was present at a very low level prior to treatment. After stimulation with *B. pertussis* antigen for 6 and 24 h, both mRNA increased markedly. These results show that components of *B. pertussis* induce the expression of mRNA for iNOS and arginase, but not the synthesis of NO. The mechanisms involved in NO regulation are under investigation.

Supported by: FAPESP, CNPq/PIBIC and Instituto Butantan.

9.23 Inventory of ground dwelling spiders (Arachnida, Araneae) from the Parque Estoril, São Bernardo do Campo, São Paulo State, Brazil

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Introduction: Brazil is responsible for 20% of the world's spider fauna. Nevertheless, knowledge of the ground-dwelling species is poor, and studies concerning this particular habitat in the state of São Paulo are rare. **Objective:** The aim of this study was to analyze the ground dwelling species of spiders from Parque Estoril, São Bernardo do Campo, during two years, and comment on their seasonality patterns. **Methods:** Twelve samplings, using pitfall traps, were carried out every two months for two years. In each sampling period, one hundred traps of 500-ml plastic cups were installed for 5 days. Each trap with spiders was considered an independent sample. The resulting material was sorted and identified to family and morphospecies level. **Results and Discussion:** This work provided an important database of soil spider fauna to compare with another sample from the Atlantic Forest. A total of 4340 spiders were collected, 3395 adults and 947 juveniles belonging to 25 families and 77 species. The seven most abundant species were *Sphecozone castanea* (Millidge, 1991), *Meioneta* sp.1, *Sphecozone* sp.2, *Vesicapalpus simplex (*Millidge, 1991*)*, Zoridae sp.1 and sp.2, Linyphiidae sp. 1. The families Theridiidae (18 species), Linyphiidae (17 species) and Araneidae (8 species) showed the most richness. The Nemesiidae were represented by only three juveniles. A total of 25 (32.5%) singletons, 13 (16.8%) doubletons, 31 uniques (40.2%) and 12 duplicates (15.6%) were found. The Bootstrap method yielded the lowest richness estimates and Jack 2 the highest, with 89 and 122 species respectively. While Chao 1 showed a stable result with 98 species.

Supported by: CNPq/PIBIC.

9.24 Convulsant effect of intraperitoneal administration of whole venom of *Tityus serrulatus* scorpion: electroencephalographic, behavioral and histopathologic aspects

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Introduction: Clinical data have shown that scorpion venom can induce convulsion, mainly in children. These data confirmed results obtained in our early studies, where rats received intrahippocampal injection of TsTx toxin isolated from *Tityus serrulatus* scorpion, which resulted in behavioral and electroencephalographic seizures and neuronal damage. We have shown that i.v. injection of whole venom of scorpion causes convulsions in adult and newborn rats. In view of these facts, there are some questions we want to research further: Is it possible that convulsion caused by systemic injection of whole venom of scorpion induces late epilepsy? Could treatment with anticonvulsivants given just a few hours after the beginning of convulsions prevent late epilepsy? **Objective:** The aim of this study was to study the acute convulsant effects of i.p. administration of *Tityus serrulatus* scorpion venom in male and female adult rats. An electroencephalografic, behavioral and histopathological study was performed. **Methods:** Surgery to implant electrodes in the hippocampus area to record eletroencephalografic analysis was performed. After three-four days, the whole venom of *Tityus serrulatus* scorpion in 0.75, 1.0, 1.5 and 2.0 mg/kg doses was administered (i.p.). Behavior and electroencephalographic activity of rats were observed for six hours uninterruptedly. One week later, rats were anesthetized with carbon dioxide to do a perfusion. They were then decapitated and their brain was removed and preserved in formaldehyde for one week. Brains were processed for histological analyses. The cells of CA1, CA3, hillus and dentate gyrus of hippocampal formation were counted on an area of 100 um^2. **Results and Discussion:** Electroencephalographic recordings were characterized by isolated spikes and epileptic discharges in all groups of rats that had received different doses of venom (0.75, 1.0, 1.5, 2.0mg/kg). The behavioral modifications were characterized by paralysis, "wet-dog shake," intense salivation, convulsion, and respiratory and locomotion difficulties. These behavioral modifications were shown in all groups of rats that received different doses of the venom (0.75, 1.0, 1.5, 2.0 mg/kg). A dose of 1.5 mg/kg of whole venom caused death in 100% of rats. Histopathologic analysis of brains of these rats did not show injuries in the hippocampus and in any other area of the brain. These results showed that the systemic venom injection in adult rats was able to induce central effects such as convulsion and epileptiforme activity. These data are in concordance with neurotoxic symptoms observed in severe envenomation in human.

Supported by: CNPq/PIBIC.

9.25 Exploring the proteome and the peptidome of South American viperid venoms

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Introduction: Snake venoms are complex mixtures of components that have a diverse array of actions on prey. Most of these components are active proteins and peptides that work to kill or to immobilize the prey as well as to assist in their digestion. Proteomic approaches have been extensively used to study the complexity of viperid venoms and various mass spectrometric techniques allow an accurate analysis of venom proteins and peptides. These techniques have shown to be important tools in the study of the proteome and the peptidome of snake venoms. **Objectives:** The main goal of this study was to explore the proteome and peptidome of South American viperid venoms. **Methods:** Venoms were provided by the Herpetology Laboratory (Instituto Butantan, SP, Brazil). *Bothrops cotiara* venom was chromatographed (FPLC system) on a gel filtration column (Superdex 75 10/300 GL; GE Healthcare). Fractions containing proteins up to 30 kDa were further submitted to cation-exchange chromatography (MonoS HR 5/5; GE Healthcare). Absorption of column eluates were detected at 225 nm and 280 nm, and the proteins were analyzed by SDS-PAGE. Protein identification was performed by *in gel* trypsin digestion followed by liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) analysis in an ion trap mass spectrometer (LTQ-XL, Thermo Scientific). Mass spectra of peptides were submitted to database search (MASCOT 2.2.04, Matrix Science) restricted to Serpentes taxonomy. *Bothrops jararaca* venoms (from newborn and adult specimens) were fractionated by gel filtration (Superdex 75 10/300 GL; GE Healthcare). Fractions containing peptides were submitted to RP-HPLC (Shimadzu) on a C_{18} column (Discovery 10mm x 250mm, Supelco, particle size 5 µm), to isolate peptides. Peptide fractions were manually collected according to absorbance values at 215 nm and analyzed by MALDI-TOF mass spectrometry (MALDI-TOF, GE Healthcare). **Results and Discussion:** The complexity of Bothrops venoms has been assessed by our group using various chromatographic and mass spectrometric approaches. Previously, we had detected the presence of a proteinase of ~30 kDa with high affinity for heparin in the venom of *B. cotiara*. Here, we isolated the enzyme that was identified as a serine proteinase (SVSP) by *in gel* trypsin digestion and mass spectrometry. The functional activity of this SVSP indicates that it is active upon platelets. To compare the peptidomes of venoms from newborn and adult specimens of *B. jararaca*, the peptide fractions were analyzed by LC/MS/MS and the peptide spectra revealed similarities and differences, and some of these peptides were initially identified as bradykinin potentiating peptides; however, the presence of unknown peptides was detected and these should be further characterized.

Supported by: CNPq/PIBIC and FAPESP.

9.26 Effect of *Crotalus durissus terrificus* venom (CdtV) on the formation of multinucleated giant cells in chronic inflammatory process: immunohistochemical analysis of F-actin and phosphotyrosine

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Introduction: In human envenoming by South American rattlesnakes there are no inflammatory reactions at the site of the bite. Experimentally, the CdtV inhibits the acute phlogistic activity induced by thioglycolate and carrageenan, showing significant anti-inflammatory action. Still, this venom shows a dual action on macrophage functions, inhibiting the ability of spreading and the phagocytic activity of this cell, and stimulating its bactericidal activity by increasing the release of hydrogen peroxide and nitric oxide. Macrophages are key cells of acute inflammation and the main cellular component of chronic inflammation. In chronic cases the macrophages fuse into multinucleated giant cells and, subsequently, differentiate into epithelioid cells, forming the granuloma. This process of cell differentiation depends on the participation and the dynamics of actin filaments (F-actin) and signaling proteins, such as phosphotyrosine which mediates this process. Previous studies showed that CdtV changes the patterns of F-actin expression and phosphotyrosine in macrophages subjected to an acute inflammatory stimulus and, when applied prior to a chronic inflammatory stimulus this venom reduces the formation of multinucleated giant cells. **Objectives:** To assess the effect of Cdt venom on the rearrangement of F-actin and phosphotyrosine in animals subjected to a chronic inflammatory stimulus. **Methods:** For the induction of chronic inflammation, round glass cover slips were implanted in the subcutaneous tissue of Swiss mice pre-treated with CdtV or with saline (control animals). Immunohistochemical assays with antibodies against F-actin and phosphotyrosine were performed on cover slips removed 4, 7 and 21 days after implantation and analyzed with a confocal microscope. **Results and Discussion:** The immunostainings for F-actin were significantly inhibited in cover slips removed 4 and 7 days after implantation in the groups pre-treated with CdtV, when compared to control groups. In cover slips removed 21 days after implantation, this difference was not observed. Regarding phosphotyrosine, differences were not observed in the staining of cells of groups treated with CdtV or with saline, in any of the times studied. These results differ from those of acute inflammation, in which an increased expression of F-actin and reduced phosphotyrosine was observed in macrophages collected from animals pre-treated with CdtV. Inhibition of F-actin expression in the implants of 4 and 7 days observed in this study were positively correlated with the inhibition of the giant cell formation observed previously in cover slips implanted in the same period and stained with hematoxylin/eosin. Taken together, these data suggest that CdtV has a significant inhibitory action on the progression of the chronic inflammatory response.

Supported by: CNPq/PIBIC and FAPESP.

9.27 Description of adults and redescription of the larvae of *Carios mimon* (Kohls, Clifford & Jones, 1969), a tick species currently included in the Brazilian fauna

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Introduction: *Carios mimon* (Kohls, Clifford & Jones, 1969) is an argasid tick parasite of Chiroptera, originally described from larvae collected on *Mimon crenulatum* (E. Geoffroy, 1810) of Bolívia and on *Eptesicus brasiliensis* (Desmarest, 1819) of Uruguay. Later, it was recorded on other species of bats from Argentina, and recently, it was included in the Brazilian fauna. This tick is known only through the description of the larval stage, this makes its morphological separation from other closer species belonging to the genus *Carios* difficult. **Objective:** To describe males and females and to redescribe larvae of *C. mimon*. **Methods:** Females of *C. mimon*, collected together with nymphs of different instars, in the municipally of Araraquara, SP, were maintained in colony using laboratory animals as hosts. Redescription of larvae and description of males and females were based on optical and scanning electron microscopy. **Results and Discussion:** Among other characteristics, the species *C. mimon* could be separate from the others by the presence of cheeks and by the absence of humps on tarsus I.

Supported by: CNPq/PIBIC and FAPESP.

9.28 Melatonin's protective role against the oxidative effects of methylecgonidine and phospholipases from the venom of *Micrurus lemniscatus*

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Introduction: Melatonin is part of a system that generates circadian biological oscillations. This hormone is an indolamine (N-acetyl-5-metoxytryptamine) derived from the amino acid tryptophan. Its actions may occur by direct molecule-molecule interaction or through cell receptors. Acting on membrane receptors (MT1 and MT2), melatonin can induce the expression of the antioxidant enzymes superoxide dismutase and glutathione peroxidase preventing oxidative stress. The heating of cocaine base during smoking of crack produces methylecgonidine, also called methyl ester anhydroecgonine (AEME). AEME is a potent inducer of cell death. *Micrurus lemniscatus* is a snake from the family Elapidae. The predominant clinical manifestations of Elapid snake bite are related to the neurotoxic and myotoxic actions of the venom, causing blockade of the peripheral nervous transmission. Two PLA2 neurotoxins, Mlx-8 and Mlx-9, isolated from the venom of *Micrurus lemniscatus*, induced neuronal death in primary culture of embryonic hippocampal neurons. **Objectives:** We examined the protective effect of melatonin, which is reported to be a powerful antioxidant, on the actions of the phospholipase toxins (Mlx-8, Mlx-9) and the cocaine metabolite AEME, studying cell viability. **Methods:** Hippocampal neurons were isolated and maintained in culture for 24 h in Neurobasal medium. The cells were incubated with different concentrations of AEME (0.1 mM, 1 mM and 10 mM), Mlx-8 (10 ng/mL), Mlx-9 (100 ng/mL and 1000 ng/mL) and melatonin (10^{-7} M and 10^{-9} M). Viability was measured by the MTT test. **Results and Discussion:** AEME (0.1, 1 and 10 mM) induced a decrease in cell viability. The toxin Mlx-8 (10 ng / ml) induced only a small reduction in the cell viability. The cells incubated with Mlx-9 (100 and 1000 ng / mL) showed significant reduction in cell viability. Melatonin at 10^{-9} M reversed the induction of neuronal death by AEME (0.1 and 1 mM) which was not observed in relation to Mlx-8 (10 ng / mL) and Mlx-9 (100 and 1000 ng / mL). Melatonin at 10^{-7} M did not show any protective effect. Thus, melatonin showed protection at the physiological concentration (10^{-9} M) against the neurotoxic effects of AEME, but did not against the Micrurus venom toxins. It is possible that the mechanisms of cell death induction of both toxins and AEME are different.

Supported by: CNPq/PIBIC.

9.29 Collagen type I induces apoptosis by caspase-3 pathway in mice bearing melanoma and breast adenocarcinoma

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Introduction: Tissue microenvironments play an important role in maintaining normal cell behavior. Moreover, type I collagen is a prevalent component of the stromal extracellular matrix; its expression is spatially and temporally regulated during mammary ductal formation and dermal differentiation, suggesting that it plays important roles in development. The primary function of collagen fibers is to add strength to the connective tissue and can have a stimulatory or inhibitory effect on cell proliferation, and organized fibrillar structure inhibits normal and malignant cell proliferation. **Objective:** To evaluate the inhibitory effects of type I collagen on cell proliferation and apoptosis in murine B16F10 melanoma and Ehrlich breast adenocarcinoma in C57BL/6J and Balb-c mice. **Methods:** We determined the inhibitory concentrations (IC50%) of collagen type I in B16F10 melanoma, Ehrlich breast adenocarcinoma and normal fibroblasts by the colorimetric method - MTT. Activity of collagen in induced cell death pathway caspase-3 was tested by an enzymatic-fluorogenic method. C57BL/6J and Balb-c mice were implanted with $5x10^4$ B16F10 melanoma cells and $1x10^4$ breast adenocarcinoma of Ehrlich. The treatment was performed with collagen at different concentrations, administered by intraperitoneal route for 30 days. **Results and Discussion:** The interaction of collagen in the stroma as an intrinsic defense mechanism to establish a breast tumor is likely to produce morphological changes in epithelial and stromal cells of breast. The inhibitory concentrations (IC50%) were 18.6 mg/ml for B16F10 melanoma and 49.9 mg/ml for breast adenocarcinoma. Microscopic analysis of tumor cells showed multicellular aggregation and loss of adhesion in extracellular matrix. The treatment by collagen showed cytotoxic effects and morphologic changes but cell proliferation and sites of collagen by dermal fibroblasts. Collagen had significant activity as a substrate for active site specific of proteolytic cleavage of caspase 3 in mitochondrial intrinsic pathway. It is an inducing agent of apoptosis, where activity proved to be selective for cell tumor effects and no toxicity in normal cells such as fibroblasts. Animals bearing the B16F10 melanoma and Ehrlich adenocarcinoma treated with collagen showed significant reduction of tumor mass, respectively 79% and 68%. The survival rate calculated by the Kaplan-Meier test showed that treatment with the samples of collagen induced a significant reduction in mortality rate and reduction in number internal metastases. Collagen significantly reduced tumor growth, active caspase-3 and formation of metastases in experimental models of melanoma and breast adenocarcinoma. Collagen type I and hydrolysate peptide products are a promising new weapon for cancer treatment.

Supported by: CNPq/PIBIC.

9.30 Hippocampal effects of toxins isolated from pool 5 of *Tityus bahiensis* scorpion venom in rats

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Introduction: In Brazil, one of the most dangerous scorpions is *Tityus bahiensis*. Nevertheless, there are few studies about the effects of this venom, mainly in the central nervous system. Scorpion venoms are composed of neurotoxins, which are polypeptides of low molecular weight. In previous studies performed in our laboratory, it was demonstrated that the venom of *T. bahiensis* is very convulsive, with different characteristics from *T. serrulatus*. Six fractions of this venom were studied and four of them showed interesting results in the observed parameters (behavior and electroencephalographics alteration and neuronal loss). Fraction P5 was chosen to develop this work due to its ability to cause behavioral and electroencephalographic convulsion and large neuronal loss. **Objectives**: Evaluation of the histological effects after intrahippocampal injection of toxin obtained from pool 5 of *T. bahiensis* venoms. **Methods**: The crude venom, with adequate treatment, was applied in a Sephadex G50 column. Chromatographic profile showed six distinct fractions. Toxic fraction P5 was separated on reverse-phase column by HPLC. The profile revealed five peaks. Three of them were used in this study. Four groups of Wistar male rats (n=6) weighing 250 g were submitted to stereotaxic surgery to implant cannulas in the hippocampus. One day after the surgery, the animals were injected with 1 μl of Ringer solution (control group) or with solution of toxin I-V, IV-V or V-V at a concentration of 1 μg/μl. Seven days after the injections, the animals were sacrificed and perfused. The brains were removed and prepared for histological analysis. Statistical analysis was done by ANOVA test followed by Tukey Kramer, with $p < 0.05$. **Results and Discussion**: When animals were injected with toxins I-V and IV-V, there was a significant decrease in cell number only in the CA1 area contralateral to injection site. No significant differences were found in other hippocampal areas; however, toxin IV-V caused neuronal loss in CA1 ipsilateral area in half of observed animals without effect in the others. In animals injected with toxin V-V no neuronal loss was observed in any area. The toxins of pool 5 of *T. bahiensis* scorpion act on the central nervous system causing neuronal loss in the hippocampus on the opposite side of injection. Thus, these toxins may be useful tools for the study of hippocampal ways.

Supported by: CNPq/PIBIC and FAPESP.

9.31 Secreted autotransporter toxin (Sat) in atypical enteropathogenic *Escherichia coli*

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Introduction: *Escherichia coli* is an incredibly diverse bacterial species with the ability to colonize and persist in numerous niches both in the environment and within the host. *E. coli* strains associated with the human host are classified as commensals, enteric pathogens or extraintestinal pathogens, more frequently including the strains associated with urinary tract infection. These differences depend on the set of virulence genes and clinical properties of each pathotype. Enteropathogenic *Escherichia coli* (EPEC) constitutes one of the six diarrheagenic *E. coli* categories, which was divided into two groups, typical EPEC and atypical EPEC, whose basic difference (as compared to typical EPEC) is the absence of the EPEC adherence factor plasmid (pEAF), and wider heterogeneity of virulence factors. The diffusely adhering *E. coli* (DAEC) is one of the human pathogenic *E. coli* strains responsible for recurrent urinary tract and gastrointestinal infections. Studies have shown that some DAEC strains harbor virulence factors found in uropathogenic *E.coli* (UPEC) strains, such as the secreted autotransporter toxin, Sat. Sat belongs to the subfamily of serine protease autotransporters of *Enterobacteriaceae*. This family, which has a specific and distinct function, has been indentified only in pathogenic bacteria, and includes a variety of other virulence toxins. **Objective:** To investigate Sat toxin expression in isolates of aEPEC, whose gene *sat* was amplified by multiplex PCP. **Methods:** Cytotoxicity assays in HEp-2 cells were performed with the bacterial culture or supernadant of the bacterial culture from isolates 589 (O5:H2) *sat/pic/east*; 1887 (O111:H38) *sat/hly*; 2294 (O9:H33) *east/sat* and 3170 (O145:H2) PCR negative. DAEC strain C1845 was used as a prototype strain to study the expression of Sat. **Results and Discussion**: Our results show that isolates positive for the *sat* gene were unable to produce cellular alterations in HEp-2 cell cultures. On the other hand, cells incubated with the concentrated supernatant from bacterial cultures (50 and 100 μg/mL), containing only molecules over 50 kDa, showed high levels of vacuolization, after 5 h of incubation. A partial cell detachment was only detected in cultures incubated with concentration of 250 μg/mL, for 24 h. These results suggest for the first time that aEPEC may express the Sat toxin, important for infection by both UPEC and DAEC. The study of the expression and activity of bacterial toxins is of essential importance for the understanding of the pathogenesis of the bacterial infections. In particular, the finding of the expression of the Sat toxin in aEPEC will certainly contribute to the understanding of the pathogenesis of infections by this class of *E. coli*, still very poorly known.

Supported by: CNPq/PIBIC and FAPESP.

9.32 Hematological parameters of the snakes *Oxyrhopus guibei* and *Xenodon neuwiedii* (Ophidia:Colubridae)

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Introduction: Hematological values usually express the physiological conditions of the animal. Hematological changes are often early indicators of some pathology and aid in disease diagnosis. In snakes, the hematological studies are more frequent in venomous species than in non-venomous ones, probably due to their medical importance, and usually, venomous snakes are kept in captivity to supply venoms for anti-venom production. **Objectives:** The aim of this study was to establish the reference range of hematological data of two species of Brazilian non-poisonous snakes, recently wild-caught and sent to Instituto Butantan. **Methods**: One milliliter of blood was collected in 0.1% EDTA from ventral caudal vein and 1 ml from the abdominal aorta of *Xenodon neuwiedii* (n=19) and *Oxyrhopus guibei* (n =26) snakes. The total red blood cells (RBC), white blood cells (WBC) and thrombocytes (TBC) were determined manually with the improved Neubauer counting chamber, using Natt and Herrick solution (1:200). The packed cell volume (PCV) was determined by the microhematocrit method (Ht) and hemoglobin(Hb) concentration was determined by the cyanomethemoglobin method. The differential white blood cell count was done in blood smears stained with May-Grunwald-Giemsa and pro-erythrocytes (PE) were counted in smears stained by New methylene Blue. **Results and Discussion:** The mean values (X±SD) for *X. neuwiedii* and *O. guibei* were respectively: RBC x10^9/L (455.4±125.9 and 476.6±138.8); WBC x10^9/L (4.4±1.9 and 4.8±2.1); TBC x10^9/L (7.6±2.8 and 7.3±2.7), mean corpuscular volume fl. (523.9±60.5 and 524.1±98.4), mean corpuscular hemoglobin pg. (138.5±27.6 and 147.9±50.4), mean corpuscular hemoglobin concentration % (25.6±5.3 and 27.8±6.7), Ht % (23.8±6,7 and 24.3±6.0); Hb g/dl (6.3±2.0 and 6.8±2.3); PE x10^9/L (23.5±18.6 and 21.5±25.3). The following types of white blood cells were described respectively for Xn and Og respectively (x10^9/L): lymphocytes (1.7±1.0 and 1.8±0.7); azurophils (1.9±0.8 and 1.9±1.0); heterophils (0.6±0.4 and 0.8±0.9) and basophils (0.4±0.3 and 0.4±0.2). There were no significant differences in caudal vein hematotological parameters between the two species of snakes and also, between males and females in *X.neuwiedii* snakes. However, hematocrit and hemoglobin values were significantly higher in *O. guibei* males. In the comparison between the two forms of collection, tail vein and abdominal aorta, there were significant differences in hematocrit and CGE in the snake *O. guibei* ($p < 0.05$). Intracellular parasites were not found in any of the specimens studied. Our results are similar to reference ranges of hematological data obtained for other snakes, such as the families Viperidae and Boidae. In some *X. neuwiedii* snakes, there were morphological changes of heterophils such as cytoplasmic extension, not yet described in literature, but its function unknown. The hematological changes observed in both species are probably associated with factors that influence their physiological state such as stress of capture, seasonality, and food, among others.

Supported by: CNPq/PIBIC and FAPESP.

9.33 *Tityus serrulatus* venom modulates the immune response of mouse spleen cells

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Introduction: Human accidents caused by scorpion venom represent a significant public health problem in Brazil, according to the Health Department. *Tityus serrulatus* venom (TsV) is responsible for most of scorpion envenomations in Brazil. This venom causes various neurotoxic, circulatory and muscular effects. Inflammatory properties of this venom have been described in humans and mice. As lymphocytes play an important role in the inflammatory response, it is important to understand if TsV can affect these cells. **Objectives:** The aim of this study was to evaluate the activity, *in vitro,* of TsV and its components on the proliferation and cytokine production of mouse spleen cells. **Methods:** Whole TsV and 11 fractions obtained by gel filtration chromatography were used in this study. For analysis of TsV activity on proliferation, spleen cells from naive mice were labeled with CFSE and incubated with TsV and the different fractions, in the presence or absence of ConA. After 72 h at 37°C, cells were labeled with antibodies and analyzed by flow cytometry. For the analysis of cytokines, spleen cells were stimulated with phorbol myristate acetate plus ionomycin or ConA and incubated with TsV and each fraction. $IFN\gamma^+$ cells were detected by FACS analysis of intracellular staining. Cytokines in cell supernatants were detected by ELISA or CBA analysis. **Results and Discussion:** TsV induced (10.9%) spleen cell proliferation ($CFSE^{low}$ cells) when compared to untreated control cells (2.2%). Proliferation was mainly detected in B220+ (8.5%), compared to CD3+ (0.7%) cells. Moreover, some TsV fractions also induced CD3+ and CD19+ cells, and others were able to inhibit proliferation induced by conA. TsV inhibited the production of intracellular IFNγ (0.6%) compared to untreated PMA/ionomycin 24h stimulated cells (7.4%). The analysis of the activity of TsV on kinetic production of cytokines showed that TsV induced the production of TNFα (48 h), but inhibited IL-10 production induced by conA. The analysis of the activity of each fraction on the kinetic production of cytokines showed that all induced TNFα, but most of them inhibited IL-2. Moreover, four fractions of low molecular weight inhibited IFNγ production induced by conA after 24h incubation. TsV and its components did not affect the production of IL-4 and IL-5. In this study, we demonstrated that TsV and its components are able to modulate immunocompetent cells, *in vitro*, especially T and B cells either by induction or inhibition of proliferation and cytokine production. The identification of the TsV components responsible for these activities is currently being investigated.

Supported by: CNPq/INCTTOX and CNPq/PIBIC.

9.34 On the genus *Pycnothele* Chamberlin (Araneae: Mygalomorphae, Nemesiidae)

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Introduction: The genus *Pycnothele* was described by Chamberlin based on the type species, *P. perdita* Chamberlin, male and female from Mendes, Rio de Janeiro, Brazil. Currently, the genus comprises five species: *P. perdita*, *P. singularis* (Mello-Leitão) and *P. piracicabensis* (Piza) from Brazil; *P. auronitens* (Keyserling) from Brazil and Uruguay and *P. modesta* (Schiapelli & Gerschman) from Argentina and Uruguay. Pérez-Milles & Capocasale in 1988 and Goloboff in 1995, made revisions of the genus but did not include all known species. **Objectives:** To revise the genus *Pycnothele*, with emphasis on the Brazilian species. To enhance the distribution range of all species of the genus. To describe two new species from Brazil and transfer *P. piracicabensis* to the genus *Rachias* Simon. **Methods:** The examined material is deposited in several Brazilian and two foreign collections. Female spermathecae were dissected and cleared in clove oil for observation of internal structures. The drawings were made on a Leica MZ 12.5, with a camera lucida. **Results and Discussion:** The examined material enabled us to redescribe the Brazilian species, enhance the distribution range of *P. perdita*, *P. singularis* and *P. auronitens* and record the occurrence of *P. modesta* in Brazil for the first time. In addition, two new species are described, *P.* sp. 1 from Araraquara, São Paulo and *P.* sp. 2 from Arapongas, Paraná and Ribeirão Grande, São Paulo, both in Brazil. Based on the study of the characters of the genus, *P. piracicabensis* is transferred from *Pycnothele* to the genus *Rachias*.

Supported by: CNPq/PIBIC.

9.35 Comparative study of rDNA 28S of diploid and tetraploid *Odontophrynus americanus* (Amphibia, Anura)

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Introduction: Ribosomal cistrons are represented by hundreds of copies of rDNA units per haploid genome in eukaryotes. These multiple copies have essential role in the cells to provide appropriate supplies of rRNAs 18S, 5.8 S and 28S for ribosome assembly. Previous study on the 18S rRNA of *Odontophrynus americanus* provided primary sequence and secondary structure comparisons between diploid (2n) and tetraploid (4n) specimens, confirming their close evolutionary relationship. The intergenic spacers (IGS) of *O. americanus* 2n and 4n were also cloned and sequenced. Although the same structural elements were present in both IGSs, the highest level of repeated elements in IGSs 4n sequences suggested that the ancestor IGSs must have been subjected to several rounds of internal duplication. **Objective:** This work aimed to complement previous studies on the 28S rDNA 2n and 4n sequencing in order to establish comparisons concerning the molecular evolution of these polyploid amphibians. **Methods:** Clones previously constructed (POA EK 211, EK 417 POA, POA 211 KB, 417 KB POA, POA 211 BB, 417 BB POA, POA BH 211, HK 211 and POA POA 417 BK) were plated in LB agar medium containing ampicillin, and the recombinant plasmids were obtained through mini preparations. The inserts were subsequently sequenced (*Big Dye Terminator Kit* and *ABI Prisma 3100, Applied Biosystems*). **Results and Discussion**: The sequences were compared with, and complemented most data previously obtained. As compared to *O. americanus* 2n, the conserved region of the 28S rDNA region showed 62 deletions, 46 insertions and 33 substitutions in *O. americanus* 4n and 29 deletions, 11 insertions and 11 substitutions in *Xenopus laevis*. So far, the divergent domains of *O. americanus* 4n showed 6 deletions, 2 insertions and 5 substitutions, and *X. laevis* showed 136 deletions, 65 insertions and 220 substitutions, as compared to *O. americanus* 2n. The final confirmation of the results depends on further sequencing experiments. The complete sequence of the 28S rDNA would help to understand the mechanisms involved in the molecular evolution of ribosomal cistrons and would also contribute to the understanding of the control of gene expression in polyploid amphibians.

Supported by: CNPq/PIBIC and FAPESP.

9.36 Comparison of the litter spider fauna (ARANEAE) from sixteen areas, sampled with soil traps in the Atlantic Forest

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Introduction: Of a total of 109 known spider families, 67 occur in Brazil, and approximately 70% include species that live exclusively in the litter layer. The study of the composition of the spider community aims to compile taxonomical and statistical data that will characterize this community in the environment. The information obtained from these studies constitutes the basis of more complex ecological studies. In this study we analyzed the material collected with pitfall traps, during the development of the BIOTA/FAPESP project, and deposited in the collection of the Butantan Institute. The resulting data will be analyzed taxonomically and ecologically in order to obtain information on the still poorly studied soil spider fauna. **Objectives:** To examine the material from the areas sampled during the BIOTA/FAPESP project and to compare them in order to enhance our knowledge of soil-dwelling spiders. **Methods:** The material was collected with pitfall traps made out of 500-ml plastic cups with 7-cm opening and filled up to 1/3 with 80% alcohol. Approximately 170 traps were placed in each area and left for 5 days. The identification data were copied into Excell worksheets for future analysis. **Results and Discussion:** From a total of 16 areas, 10,255 spiders were collected, belonging to 45 families. Twenty-two families have been sorted to morphospecies but we will emphasize Linyphiidae, Oonopidae and Amaurobiidae, since the number of specimens is high (n>36). Linyhiidae was the most abundant family with 62 species. Of these, 49 are new to science and 11 could not be placed in any known genera. The Floresta da Cicuta yielded the highest number of adult Linyphiidae (385 specimens), followed by Caraça (364 specimens). The genus *Sphecozone*, with 737 adult specimens, was represented by *S. castanea*, *S. novaeteutoniae*, *S. labiata* and 8 new species. The genus *Moyosi,* with 122 adult specimens, was represented by *Moyosi prativaga* and 7 new species. From the family Oonopidae, 561 specimens were collected, belonging to 29 species: *Ichnothyreus peltifer, Oonops reticulatus, Triaeris stenapis* and 26 new species. The areas with the highest abundance of Oonopidae were Cicuta, with 152 adult specimens, and Caparaó, with 97 adult specimens. From the family Amaurobiidae, 218 specimens were collected, distributed in 7 morphospecies of which one belongs to the subfamily Macrobuninae, and 6 could not be placed in any known genera. The area with the highest number of species was Caraça, with 98 specimens, all belonging to Amaurobiidae sp. 6, and the area with the highest diversity was Foz do Iguaçu, with 7 specimens belonging to 3 morphospecies.

Supported by: CNPq/PIBIC.

9.37 Proteomic analysis of the effects of the platelet-aggregating enzyme PA-BJ, a serine proteinase from *B. jararaca* venom, on platelets

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Introduction: PA-BJ is a serine proteinase isolated from *Bothrops jararaca* venom that induces platelet aggregation on platelet rich plasma and on washed platelet suspensions. It is a 30-kDa protein that contains one N- and one O-glycosidically linked carbohydrate moiety. The effect of PA-BJ on platelets is mediated by the thrombin receptors PAR1 and PAR4. It induces calcium mobilization in platelets and desensitizes platelets to the action of thrombin and the SFLLRN peptide. In vitro, PA-BJ cleaves the recombinant exodomain of the receptor PAR1 at peptide bonds Arg41-Ser42 and Arg46-Asn47 resulting in the inactivation of the tethered ligand. **Objectives:** This study had two main objectives, namely to achieve an improved method to isolate PA-BJ using less chromatographic steps than the previously published method and to analyze the global effects of PA-BJ on platelets using proteomic approaches. **Methods**: For the isolation of PA-BJ, the venom was submitted to cation-exchange chromatography on a HiPrep 16/10 SP XL column using an Äkta purifier FPLC system, and bound proteins were eluted with an increasing gradient of NaCl. Fractions that contained PA-BJ as analyzed by SDS-PAGE and activity on platelets were further chromatographed on a Mono S HR 5/5 column resulting in the elution of two groups of chromatographically distinct forms of PA-BJ which were re-chromatographed on the Mono S column. To test the effects of PA-BJ, platelets were washed with Tyrode solution, and the suspensions of washed platelets were incubated with 10 nM thrombin, 127 nM PA-BJ or without any agonist (control sample) at 37°C for 8 min in cuvettes, and the aggregation was recorded using an aggregometer (490 Four Channels, Chronolog). The effects of agonists on platelet proteomes were evaluated by two-dimensional gel electrophoresis (2DE). Platelet proteins (250 μg) were separated in the first dimension by isoelectric focusing using a pH gradient of 4-7 and in the second dimension on 12% SDS-polyacrylamide gels. The gels were stained with colloidal Coomassie blue, analyzed by the software Image Master 2D Platinum (GE Healthcare), and some spots were identified by mass spectrometry and database search. **Results and Discussion:** The previously published method for the isolation of PA-BJ involved five isolation steps and resulted in the isolation of one PA-BJ form. Here, using three chromatographic steps we isolated six chromatographically distinct forms of PA-BJ differing slightly in isoelectric points as observed by their elution profiles. The presence of forms of this serine proteinase in the venom is likely due to different degrees of glycosylation that can be detected by their slightly distinct mobilities in SDS-PAGE indicating different molecular masses. PA-BJ had significant effects on washed platelets, inducing up to 70% aggregation at a concentration of 127 nM. Differential spots were observed on the 2D-gel of PA-BJ-activated platelets which were not present on the control and thrombin 2D-gels. These spots were identified as cytoplasmic beta-actin. Other protein spots common to the three 2D-gels were also identified.

Supported by: **FAPESP and CNPq/PIBIC.**

9.38 Evaluation of the adjuvant activity of dioctadecydimethyl ammonium bromide (DODAB) on mouse humoral immune response to crotoxin isolated from *Crotalus durissus terrificus* venom

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Introduction: Crotoxin (CTX) is the main toxic component of the *Crotalus durissus terrificus* venom (C.d.t.), a rattlesnake of South America. Furthermore, the CTX exerts an inhibitory effect on the immune system. In contrast, substances called adjuvant have the ability to stimulate the innate immunity and consequently to promote robust adaptive immunity to distinct antigens. Dioctadecydimethl ammonium bromide (DODAB) has been reported as a novel and potent adjuvant. **Objective:** We evaluated in mice the adjuvant activity of DODAB on humoral immune response against CTX isolated from C.d.t. venom. **Methods:** CTX was purified from C.d.t. venom by anion-exchange chromatography in FPLC system. Afterward, CTX adsorption onto DODAB was quantitatively determined for establishing ranges of maximal adsorption by ZetaPlus-ZetaPotential Analyzer. Next, BAB/c mice were immunized s.c. with CTX (5 μg/animal)/DODAB (2 mM) or CTX (5 μg/animal) adsorbed onto alum (1 mg/animal). After 14, 21 and 28 days, the mice were bled and on day 21 received the antigenic booster of CTX (5 μg). Anti-CTX antibody production was measured by ELISA. **Results and Discussion:** On days 14 and 21 after immunization, higher anti-CTX IgG1 and IgG2a antibody production in serum from mice immunized with CTX/DODAB was demonstrated compared with the CTX-alum immunized mice. In the secondary antibody response (28 days after immunization), the specific anti-CTX IgG1 and IgG2a antibody production was 7 and 2 times higher in serum from CXT/DODAB immunized mice compared to CTX/alum immunized mice, respectively. The adsorption of CTX onto DODAB resulted in a stable complex, which induced the activation of the immune system resulting in high levels of specific antibodies. Furthermore, DODAB was more efficient in promoting anti-CTX antibody response compared with alum adjuvant.

Supported by: CNPq/PIBIC, FAPESP, CAPES.

9.39 Genotoxicity of textile dye Remazol Orange 3R in snail *Biomphalaria glabrata* (Say 1818) using the alkaline comet assay

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Introduction: Synthetic dyes represent an important group of xenobiotic chemicals. Among textile dyes, approximately 10-15 % of world production is lost to the environment during synthesis, processing and application. The presence of these compounds in aqueous ecosystems may cause negative impacts, deteriorating water quality. Furthermore, these dyes and/or their degradation products may be toxic and mutagenic. The single cell gel eletrophoresis, or comet assay, has been the major tool in the evaluation of genotoxic effects in genetic toxicology. **Objective:** The aim of this work was to evaluate the genotoxic effects of synthetic textile dye Remazol Orange 3R in hemocytes of *B. glabrata* in the alkaline single gel eletrophoresis assay. **Methods:** Snails were exposed for 7 days to four different dye concentrations (100 mg/L, 500 mg/L, 1000 mg/L and 2000 mg/L) and the solutions were renewed every 48 h. To perform the comet assay, about 100 µL of hemolymph containing hemocytes of each animal were collected by pedal stimulus, and then, added to 500 µL of LMP agarose 0.5% (w/v), mixed, and placed on two microscope slides pre-coated with NMP agarose 1.5% (w/v). The slides were immersed in a lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% Triton X-100 and 20% DMSO, pH 10.0), kept at 4 °C and protected from the light for at least 12 h. They were subsequently incubated in freshly prepared alkaline buffer (300 mM NaOH and 200 mM EDTA, pH>13) for 10 min for DNA unwinding. Electrophoresis (30 min at 300 mA and 23 V (0.74 V/cm) was performed in the same buffer. After electrophoresis, the slides were neutralized in 400 mM Tris-HCl (pH 7.5) and fixed for 10 min in alcohol. Prior to examination, the slides were stained with 20 µg/ml ethidium bromide, and 100 cells per slide (200 per animal) were analysed using a Zeiss Axioplan epifluorescence microscope. The extent of the DNA damage was determined by visual anaysis. **Results and Discussion:** DNA damage was measured as percent number of comets (classes 1, 2 and 3) and normal cells (class 0). There was no increase in DNA migration with any of the concentrations. The trypan blue exclusion test showed no cytotoxicity for all the concentrations tested. Further studies with the hydrolysed form of the dye, the form found in industrial effluents, will give important information on the risks of environmental exposure.

Supported by: CNPq/PIBIC.

9.40 Efficacy of Vaxcine as an oral adjuvant for a conjugated vaccine against O111 polysaccharide

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Introduction: In order for an oral vaccine to be effective, it is very important that the antigens employed be delivered in a highly efficient carrier system. Vaxcine is an oil-based formulation which has been used successfully as an oral antigen carrier, and whose components are all approved for human use. However, its ability to generate an immune response against polysaccharide antigens via the oral route has never been tested. **Objective**: The objective of this work was to determine whether an O111 polysaccharide conjugated vaccine incorporated in Vaxcine is able to induce an IgA response in the mucosa and in the blood against the polysaccharide. **Methods**: Balb/c female mice (6-8 years old) were immunized orally twice with an O111-LTB conjugated vaccine incorporated in Vaxcine as an adjuvant and delivery system. One day before, and 7 and 21 days after immunization, stool and blood samples were collected to determine by ELISA technique the presence of IgA and IgG antibodies against O111 polysaccharide. **Results and Discussion**: The results showed that incorporation of the conjugated vaccine in Vaxcine protected the conjugate against degradation during its passage through the digestive tract and also increased the antibody responses against the polysaccharide. The results obtained in this work indicate that the Vaxcine formulation has the potential to be used as an oral delivery system and as an adjuvant for conjugated vaccines containing polysaccharide antigens.

Supported by: CNPq/PIBIC.

9.41 Distribution of mygalomorph spiders at different altitudes in the Serra do Japi, São Paulo State, Brazil

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Introduction: Mygalomorph spiders, known in Brazil as "aranhas-caranguejeiras," is a diverse taxon having 2,628 species distributed in all continents, except Antarctica. Fifteen families are recognized; 11 have representatives in Brazil and 8 in the state of São Paulo: Actinopodidae, Barychelidae, Ctenizidae, Cyrtaucheniidae, Dipluridae, Idiopidae, Nemesiidae and Theraphosidae. Little is known of their biology and ecology. Habits are rarely recorded and almost nothing is known about the influence of altitude in the distribution of mygalomorphs. **Objectives**: We are making an inventory of mygalomorph species that occur at three different altitudes in the Serra do Japi, Jundiaí, state of São Paulo. The faunal composition will be compared between the sampled areas. **Methods:** The collections were carried out for a week in each season for a period of one year. To date, two expeditions were conducted: in the summer and the autumn. The specimens were sampled in two areas at each altitude of roughtly 800 m, 1,000m and 1,200m, totaling 6 areas. Four collecting methods were used in all areas: 50 pitfall traps with preservative medium; four diurnal one hour samplings; nocturnal collection in four transects of 60 m^2 and one 1 m^2 of litter sample. **Results and Discussion:** After two expeditions, a total of 147 mygalomorph individuals consisting of 49 adult males and 98 females or immatures were collected. Four families were recorded and nine species belonging to eight genera were identified: Dipluridae, 1 species, 7 individuals; Idiopidae, 2 species, 32 individuals; Nemesiidae, 4 species, 104 individuals; Theraphosidae, 2 species, 4 individuals. The majority of the individuals sampled belong to the Nemesiidae (70.14%) and the Idiopidae (22.22%). The collecting method that resulted in the capture of the highest number of individuals was the pitfall traps (135 specimens collected or 92.2%). Comparions among the samples of each altitude will be made after the conclusion of the expeditions.

Supported by: CNPq/PIBIC, CAPES and FAPESP.

9.42 Immunohistochemical expression of collagen IV, fibronectin and laminin in actinic cheilitis and human lip squamous cell carcinoma

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Introduction: Actinic cheilitis is a pathologic condition affecting mainly the lower lip and it is caused by chronic and excessive exposure to the ultraviolet radiation of sunlight. Actinic cheilitis can also develop into squamous cell carcinoma of the lip. The basement membrane (BM) is a ubiquitous extracelular matrix that separates organ parenchymal cells from the interstitial stroma. Profound changes occur in the distribution and quantity of the epithelial BM during the transition from benign to invasive carcinoma. **Objectives**: To evaluate by immunohistochemical technique the expression and distribution of collagen IV, fibronectin and laminin in actinic cheilits and human lip squamous cell carcinoma at different histological grades. **Methods:** Paraffin-embedded tissues of actinic cheilitis and lip squamous cell carcinoma provided by Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo were submitted to immunohistochemistry for collagen IV (Clone CIV 22, Dako:), fibronectin (Clone: A0245, Dako) and laminin (polyclonal, Dako). Slides were examined by light microscopy. **Results and Discussion**: The majority of cases of actinic cheilitis showed no expression of collagen IV, fibronectin and laminin in epithelial BM, and immunoexpression of these proteins was seen only in endothelial BM. Slides of superficially and invasive squamous cell carcinoma showed immunostaining for these proteins in epithelial and endothelial BM. However, in some cases there was a lack of immunoexpression in small areas of tumor islands and strands, but it was not possible to define a pattern of staining for these proteins. The lack of immunostaining observed in actinic cheilitis is probably due to abnormal elastotic material seen in the dermis, a process called solar elastosis, which is caused by sun exposure.

Supported by: FAPESP and CNPq/PIBIC.

9.43 Monosodium glutamate effects on melatonin synthesis

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Introduction: The pineal gland synthesizes melatonin during the night, which participates in the internal temporal organization. Melatonin has a signaling role, indicating night and day by its presence and absence, respectively, in body fluids. There are other sites of melatonin synthesis, such as the retina and gastrointestinal tract. Monosodium glutamate (MSG), when administered neonatally, induces a metabolic syndrome due to a hypothalamic lesion, in particular the arcuate nucleus, characterized by obesity, growth reduction, insulin resistance and type 2 diabetes. Moreover, hippocampal neurons from CA1 region are lesioned and the amacrine, ganglion and bipolar cells of the retina are degenerated. **Objectives:** The aim of the present work was to analyze the nocturnal melatonin profile in the pineal glands and retinas of male adult Wistar rats injected with monosodium glutamate (MSG) during the neonatal period. **Methods:** MSG was administered subcutaneously at a dose of 4 mg/g of body weight in Wistar rats just after birth for 8 days. Animals were sacrificed at the age of 3 months, during the dark period. **Results and Discussion:** The results showed that with 3 months of age, the MSG rats were obese and, according to the literature data, probably with insulin resistance. In fact, glycemia was greater in MSG rats than in controls, suggesting the presence of type 2 diabetes. Melatonin synthesis in the pineal gland was elevated in MSG rats only in the ZT=14.5 and did not differ in the retina. The alterations observed may be a consequence of central lesions or peripheral disturbances that result from them. The elevation of pineal melatonin synthesis may be due to a hyperinsulinemia or elevated corticosterone levels. In the retina, it is possible that there was no lesion in the photoreceptor cells responsible for melatonin synthesis.

Supported by: CNPq/PIBIC.

9.44 The suppression of acute inflammation is mediated by *Ascaris suum*-primed regulatory T cells

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Introduction: Helminth parasites stimulate regulatory mechanisms that are associated with suppression of host immune responses. We have recently demonstrated that *Ascaris suum* experimental infection downregulates pulmonary allergic inflammation and LPS-induced acute inflammation. **Objective:** We investigated the effect of *Ascaris suum*-primed leukocytes, $TCD4^+CD25^-$ and $TCD4^+CD25^+$ cells, from mouse lymph nodes and the immunomodulatory cytokine IL-10 on the LPS-induced inflammation. **Methods:** C57BL/6 wild type and IL-10 knockout mice were infected by intragastric route with 2,500 *Ascaris suum* embryonated eggs. On day 8 after the infection, the mesenteric lymph nodes were removed in order to isolate $TCD4^+CD25^-$ and $TCD4^+CD25^+$ cells by magnetic activated-cell sorter (MACS). The cells were adoptively transferred to recipient mice by intravenous route, and on day 12 the air pouches made with sterile air on the back of the recipient mice were LPS-injected. Control group received only PBS in their pouches. Three hours after the stimulation, the air pouch exsudates were recovered for the measurement of inflammatory cytokine levels by ELISA and quantification of cellular migration. **Results and Discussion:** Our results demonstrated that adoptive transfer of $CD4^+CD25^+$ Treg cells from WT infected mice, but not from IL-10-deficient donor mice, resulted in a decrease of leukocyte recruitment and inflammatory cytokine levels (IL-1β, IL-6 and TNF-β), but an increase in IL-10 and TGF-β cytokines. On the contrary, transfer of $TCD4^+CD25^-$ cells did not show suppression of leukocyte influx or cytokine levels into the air pouches. We showed that the suppression of the LPS-induced inflammation was only observed after adoptive transfer of $CD4^+CD25^+$ Treg cells from *Ascaris suum*–infected WT mice, suggesting that primed-Tcells producing IL-10 are crucial for the suppressive effect. Cells from knockout mice or non-infected mice had no effect on LPS-induced inflammation. In conclusion, our results demonstrated that *Ascaris suum* infection primes $CD4^+CD25^+$Treg cells which play an important role in the suppression of LPS-induced inflammation. Furthermore, IL-10 was implicated as an effector molecule of Treg cell-mediated suppression.

Supported by: CNPq/PIBIC.

9.45 E6 gene cloning of bovine papillomavirus type 2 in *Escherichia coli*

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Introduction: Bovine papillomatosis is an infectious disease showing lesions in skin and/or mucosa in mamals, including humans. These lesions cause dramatic hazards for milk and meat production. Papillomaviruses are double-strand DNA viruses and the related lesions can regress or progress to cancer. In cattle, BPV-2 causes cutaneous fibropapillomas, bladder cancer and its clinical manifestation, enzootic hematuria. **Objectives:** Cloning of E6 BPV-2 gene in a bacterial system where expression and purification of E6 protein could allow the production of biotechnological inputs. **Methods:** In this work, specific primers were designed and the polymerase chain reaction (PCR) was used to amplify the E6 BPV-2 gene, using the genomic DNA of BPV-2 previously cloned in pAT153 vector as template. The PCR product was sequenced, analyzed to confirm E6 BPV-2 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-2 gene was successfully cloned in transformed *E. coli* cells, allowing viral protein expression and its purification by chromatographic affinity columns. Therefore, papillomavirus antigen production in prokaryotic system such as *E. coli* could be useful for further developments in diagnostic and vaccine research.

Supported by: CNPq/PIBIC and FAPESP.

9.46 Mutation spectra induced by 5-aminolevulinic acid in bacterial system

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Introduction: 5-Aminolevulinic acid (ALA) is a heme precursor accumulated both in inborn and acquired hepatic porphyria, such as acute intermittent porphyria (AIP), tyrosinosis and lead overload. Increased hepatocellular carcinoma (HCC) incidence in patients with AIP has been reported by several authors and has been hypothesized to be related to ALA and its derivatives. *In vitro*, ALA undergoes enolization and subsequent metal-catalyzed aerobic oxidation yielding reactive oxygen species, which can cause oxidative damage to DNA and proteins which could be involved in the initiation and promotion of cancer. We demonstrated that ALA is able to cause single strand breaks in plasmid and calf thymus DNA *in vitro,* and to increase the steady state level of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodGuo) in liver DNA of ALA-treated rats. Besides, ALA was also shown to promote the formation of 2,6-diamino-4-hydroxy-5-formamidopyrimidine, 8-oxo-7,8-dihydro-2'-deoxyadenosine, 5-hydroxy-2'-deoxycytidine (5-OHdCyd), 5-hydroxy-2'-deoxyuridine, cytosine glycols, 5-(hydroxymethyl)uracil and 5-formyluracil in isolated calf thymus DNA. The level of 5-HdCyd, which was also measured in liver DNA, was found to increase upon treatment of rats with ALA. Another mechanism that could be involved in the triggering of cancer is the alkylating property of the final oxidation product of ALA, 4,5-dioxovaleric acid (DOVA). We established that DOVA is an efficient alkylating agent of the guanine moieties within nucleoside and isolated DNA. Diastereoisomeric adducts were produced through the formation of a Schiff's base involving the N^2 -amino group of 2'-deoxyguanosine and the ketone function of DOVA. ALA and DOVA were shown to be mutagenic in Salmonella/microsome mutagenicity assay and Chromotest. **Objectives:** The main objective of this work was to determine the mutation spectra promoted by ALA in a bacterial system, contributing to the elucidation of the mechanism involved in DNA damage promoted by ALA. **Methods:** Competent *Escherichia coli* DH10b strain was transformed with plasmid pAC189, which contains the *supF* gene. The plasmid DNA was extracted and treated with different concentrations of ALA. MBL50 strain was then transformed with ALA-treated plasmid DNA and plated in selective medium containing X-gal, IPTG or *L*-ara. Survival rate of transformed bacterial colonies was calculated. Mutants were selected, and *supF* gene was sequenced to obtain the mutation spectra. **Results and Discussion:** An ALA dose-dependent decreased colony survival rate and an increased mutation rate were observed. Preliminary results showed that the predominant mutagenic event was base substitution involving G:C pair (71.4 %), followed by G:C-A:T transition (33.3%), G:C-C:G (28.6 %) transversion and A:T-G:C (14.3 %) transition. Further analysis is under investigation to obtain more mutants and determine wide mutation spectra and possible hot-spots. These results showed the possible mutagenic events involved in the mechanism of DNA damage induced by ALA and its derivatives, which could act as endogenous weapons, and are consistent with the hypothesis that these compounds could be associated with deleterious processes involved in the development of HCC in symptomatic AIP patients.

Supported by: FAPESP and CNPq/PIBIC.

10: PAP Program

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

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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

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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

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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

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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*

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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

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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

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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

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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

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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

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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

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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

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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

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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)

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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

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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

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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

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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

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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

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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

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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

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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*

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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

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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

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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

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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

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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

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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

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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

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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

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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)

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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

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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

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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*

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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

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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

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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

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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

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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?

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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.

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Memórias do Instituto Butantan (Secretaria de Estado da Saúde).
São Paulo, SP, Brasil, 1918 - 1990, 52 (Supl. 1-3)
Em apenso, a partir de 1990, 52 (3): BOLETIM DE BIOTECNOLOGIA
1991, 53 (1) (Supl. 1, 2)
1992, 54 (1, 2)
1993, 55 (1, 2) (Supl. 1)
1994-1995, interrompido
1996, 56 bienal, nova forma
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2006, 63 anual
2007, 64 anual, CD-ROM
2008, 65 anual, CD-ROM
2009, 66 anual, CD-ROM

ISSN 1982-3045

EDITORIAL

The 66th volume of "Memórias do Instituto Butantan" presents the proceedings of the XI Annual Scientific Meeting of Butantan Institute, organized by the Division for Scientific Development. The theme of this 11th edition of the Instituto Butantan's meeting was "Scientific Research and Public Health Challenges".

The scientific program addressed important issues regarding emerging and reemerging problems of public health, and the directions of the scientific research in order to triumph over these challenges. Three round-table, discussions and conferences were planned in order to instigate productive discussions on our current public health problems and innovative research approaches suitable for this matter.

Also, the meeting included three poster sessions, Young Scientist Awards in four categories (scientific initiation, PAP program, master and doctoral degrees) and two special sessions about the memories of Instituto Butantan, also linked to this year's theme.

This 66th volume brings together all the abstracts of the posters presented in the Poster Sessions, organized in ten different areas: Venoms and Envenomations, Biochemistry, Immunology and Vaccines, Microorganisms, Cellular Biology and Genetics, Animal Biology, Education and Scientific Diffusion, Others, Scientific Initiation (PIBIC) and PAP Program. The impressive number of submitted posters indicates the involvement of our scientific community with the Annual Scientific Meeting. These abstracts are representative of the scientific research that was carried out at Instituto Butantan in the last year. Therefore, this "Memórias do Instituto Butantan" electronic issue is an important source of scientific information and dissemination.

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XI Annual Scientific Meeting of Instituto Butantan

December 02-04, 2009

Scientific Research and Public Health Challenges

Scientific Program

Wednesday	**12/02/2009**
08:00	**Poster Session I** Venoms and Envenomations Biochemistry Scientific Initiation (PIBIC)
09:00 - 09:30	**Opening Session**
09:30 - 12:20	**Round Table I – Public Health: what we have to deal with** Coordinator: Otávio Azevedo Mercadante – Instituto Butantan **Participants:**
09:30 - 10:10	Clélia Maria Sarmento de Souza Aranda – Coordenadoria de Controle de Doenças, Secretaria de Estado da Saúde de São Paulo
10:10 - 10:30	**Coffee break**
10:30 - 11:10	Wanderson Kleber de Oliveira - Coordenação Geral de Doenças Transmissíveis, Secretaria de Vigilância em Saúde, Ministério da Saúde
11:10 - 11:50	Eliseu Alves Waldman – Faculdade de Saúde Pública, Universidade de São Paulo
11:50 – 12:20	**Discussion**
12:20 – 13:00	**Interval**
13:00 – 15:00	**Poster Session I**

15:00 – 17:30	**Young Scientist Award - Scientific Initiation and PAP Program** **Award Committee:** Yara Maria Corrêa da Silva Michelacci – Disciplina de Biologia Molecular, Universidade Federal de São Paulo Viviane Abreu Nunes Cerqueira Dantas - Escola de Artes, Ciências e Humanidades, Universidade de São Paulo Antonio Carlos Marques - Instituto de Biociências, Universidade de São Paulo
15:00 – 16:00	**Scientific Initiation Award** Coordinator: Yara Cury – Laboratório de Fisiopatologia, Instituto Butantan
16:00 – 16:30	**Coffee break**
16:30 - 17:30	**PAP Program Award** Coordinator: Ida Sigueko Sano Martins – Laboratório de Fisiopatologia, Instituto Butantan

Thursday	12/03/2009
08:00	**Poster Session II** Immunology and Vaccines Microorganisms PAP Program
09:00 – 12:00	**Round Table II – Innovative techniques in the service of public health** Coordinator: Paulo Lee Ho – Instituto Butantan **Participants:**
09:00 - 09:30	Arthur Gruber – Instituto de Ciências Biomédicas, Universidade de São Paulo
09:30 - 10:00	Roberto Gomes de Souza Berlinck – Instituto de Química de São Carlos, Universidade de São Paulo
10:00 – 10:30	**Coffee break**
10:30 - 11:00	Lygia da Veiga Pereira – Instituto de Biociências, Universidade de São Paulo
11:00 – 11:30	Mauricio Martins Rodrigues – Centro Interdisciplinar de Terapia Gênica, Universidade Federal de São Paulo
11:30 - 12:00	Discussion
12:00 – 13:00	**Interval**
13:00 – 15:00	**Poster Session II**
15:00 – 17:30	**Young Scientist Award – Master and Doctoral Degrees** **Award Committee:** Catarina Segreti Porto - Departamento de Farmacologia, Universidade Federal de São Paulo Marimélia Porcionatto - Disciplina de Biologia Molecular, Universidade Federal de São Paulo

Carlos Roberto Ferreira Brandão - Museu de Zoologia, Universidade de São Paulo

15:00 – 16:00 Master Degree Award
Coordinator: Ana Lúcia Tabet Oller do Nascimento – Centro de Biotecnologia, Instituto Butantan

16:00 – 16:30 **Coffee break**

16:30 - 17:30 Doctoral Degree Award
Coordinator: Roxane Maria Fontes Piazza – Laboratório de Bacteriologia, Instituto Butantan

Friday	**12/04/2009**
08:00	**Poster Session III** Immunology and Vaccines Cellular Biology and Genetics Animal Biology Education and Scientific Dissemination Others
09:00 – 12:00	**Round Table III – National Institutes of Science and Technology (INCTs): generation of knowledge and responses for public health** Coordinator: Osvaldo Augusto Brazil Esteves Sant´Anna – Instituto Butantan **Participants:**
09:00 - 09:30	George Alexandre dos Reis - Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro
09:30 - 10:00	Glaucius Oliva - Instituto de Física de São Carlos, Universidade de São Paulo
10:00 - 10:30	**Coffee break**
10:30 - 11:00	Jorge Kalil – Faculdade de Medicina, Universidade de São Paulo
11:00 - 11:30	Osvaldo Augusto Brazil Esteves Sant´Anna – Laboratório de Imunoquímica, Instituto Butantan
11:30 - 12:00	Discussion
12:00 – 13:00	**Interval**
13:00 – 15:00	**Poster Session III**
15:00 – 15:40	**Instituto Butantan flashbacks** **Public health in the 20th century: the role of Instituto Butantan** Nelson Ibañez – Laboratório de História da Ciência, Instituto Butantan
15:40 – 16:10	**V Photograph Award**

16:10 – 16:40	**Special Session** **The relationship between Brazil and France in the establishment of Instituto Butantan** Carlos Eduardo Sampaio Burgos Dias - Laboratório de História da Ciência, Instituto Butantan
16:40 – 17:30	**Young Scientist Award**
17:30	**Closing Session**