12. PAP Program

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12.01 Blocking activities of *Bothrops jararaca* venom metalloproteases and serine proteases by a therapeutic antivenom

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Introduction: Snakebite is a worldwide public health problem. There are some factors that must be considered to minimize this problem, such as having qualified medical support and better reporting of recorded cases. The lack of these two makes the storage and distribution of antivenom more difficult. In view of these hindrances, the quality of the serum should be the best possible, in order to allow, when possible, improved patient treatment. Snake venoms are a complex mixture of components that act on different targets. *Bothrops* is considered the most important Brazilian snake genus, where proteolytic enzymes (around 65% of venom composition) are the main toxin components of the venom. Thus, two proteolytic classes, metalloproteases and serine proteases, were chosen for analysis in the present study. **Objectives:** The aim of this study was to determine the efficacy of the antibothropic serum widely used in Brazil, in the neutralization of *B. jararaca* venom (BjV). **Methods:** As a first step, FRETs peptides (Free Resonance Energy Transfer) were selected to be specifically hydrolyzed by metallo- and serine proteases. To achieve this, a library of FRETs sequences was tested using BjV (2 - 0.2 µg/mL) and EDTA (100 mM), PMSF (1 mM) and *o*-penanthroline (1 mM) in PBS buffer, pH 7.4. Two substrates were found: Abz-FASSAQ-EDDnp (300-700 activity units =AU; AU=FU/min/µg) and Abz-RPPGFSPFRQ-EDDnp (9,000- 16,000 AU) to measure, respectively, metallo- and serine proteases activities. After this, we measured the blocking potential of the antivenom (10 µL) to neutralize the BjV, using both substrates with a 30-min pre-incubation at room temperature. **Results and Discussion:** The metalloprotease activity was almost completely inhibited, while serine protease was weakly inhibited, showing a flaw in the action of the antibothropic serum. The poor neutralization of the serine protease activity may be due to at least two factors: a) lack of immunogenicity of these molecules or, b) degradation by another enzyme prior to venom inoculation of horses. Our findings showing that serine proteases are not blocked, contradict the literature, since these proteases have been related to a systemic action of BjV. However, these symptoms are well controlled with the administration of serum, so it could be that the role of these enzymes may be different from those given earlier. The symptoms that are not alleviated with the serum are mainly local, and so we raise the hypothesis that serine proteases may act locally activating endogenous metalloproteases involved in the tissue damage in the bite region.

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12.02 Inflammatory reaction in mice selected for high or low antibody production

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Introduction: Two mouse lines were produced by bidirectional selection according to the high (H) or low (L) antibody responsiveness against salmonella flagellar antigens (selection III). These mice have been used as important tools to understand the genetic regulation of the humoral response and its influence in processes such as susceptibility to infection, experimental arthritis and chemical tumorigenesis. These phenotypes are also influenced by inflammatory reaction and macrophage activity. **Objectives:** The aim of this work was to analyze the inflammatory capacity and macrophage activation in mice selected for different antibody production. **Methods:** Mice were inoculated s.c. with polyacrylamide beads (Biogel), and 24 h later the number of cells and the protein concentration of the exudates were analyzed. NO production was measured in cultured peritoneal macrophages in the presence or not of LPS with the Griess reagent. **Results and Discussion:** The inflammatory response induced by Biogel in H and L mice was similar regarding the levels of protein content in the exudates; however, L mice showed twofold higher numbers of migrating cells compared to H animals. Analyzing macrophage activation, we observed that no significant levels of NO were produced in control cells, whereas when LPS was present in the culture, the cells from L mice released three times more NO than cells from H mice. Despite these mice being selected for high or low antibody production they show differences in an opposite way in some phenotypes of the inflammatory response.

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12.03 Activity pattern of the bushmaster, *Lachesis muta*, in captivity at the Herpetology Laboratory

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Introduction: The genus Lachesis includes only the species *L. muta*, the largest venomous snake in the Americas, reaching up to 3.5 meters in length. This snake is commonly known as the bushmaster (in English) or surucucu, surucutinga and pico-de-jaca in Portuguese. In Brazil, it is found in regions of the Amazon rainforest and also inhabits areas of Atlantic forest and humid forest enclaves in the northeast. This species has the status of vulnerable according to the International Union for Conservation of Nature (IUCN) due to more than 93% destruction and degradation of their natural habitat. Specimens are kept at the Laboratory of Herpetology at Instituto Butantan (IB) to study the maintenance, breeding, reproduction, and the extraction of venom for the production of immunobiologics and research. **Objectives:** The study aimed to monitor the behavior of a captive male of *L. muta* by checking the patterns of its activity (start and end of activities, behaviors before and after predation events, etc). **Methods:** The behavior of the male *L. muta* kept in captivity since 2004, snout-vent length of 180 cm and weight of 3120 g, was observed in recordings made daily by a monitoring CCTV Digital DVR Stand Alone with three cameras equipped with infrared light for recording at night, located at different points in the maintenance room (5x4 m). This room has a sprinkler system that controls the humidity. The specimen of *L. muta* has a polypropylene box shelter (100 l x 60 w x 30 cm h) with a sterilized moss substrate and a ceramic shelter. Recordings were initiated on January 28, 2010. The observed behavior was recorded and data of interest were tabulated. **Results and Discussion:** Of the 152 days observed, during 55 days (36%), the snake got out of its box and moved around the room, returning to its shelter every night. In the remaining days (97 days or 64%), the snake remained coiled inside its ceramic shelter. The hours of output preference was 18:00- 20:00 and the entrance was 24:00- 02:00. The average time of activity was seven hours. The male of *L. muta* proved to be essentially nocturnal. The movement of people during the day did not affect the behavior of the snake, because on Saturdays, Sundays and holidays (days without people around), the preferred schedule of the snake was the same as those observed during the weekdays. The snake moved more when changes occurred in the room (entrance of new animals in the room or changing their positioning). The favorite activity was going up the cages of the other snakes (33%) or staying below the cages (24%). After feeding, the snake was not out of its shelter for about seven days. The interest in the female *L. muta* was higher than the interest in other snakes in the room, probably driven by reproductive interests.

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12.04 On *Segestria* Latreille, 1804 in South America with information about female genitalia (ARANEAE, SEGESTRIIDAE)

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Introduction: The genus *Segestria* Latreille, 1804 is currently represented by 21 species which can be found especially in Europe. Two species were described in South America: *Segestria pusilla* Nicolet, 1849 from Chile and *Segestria ruficeps* Guérin, 1832 from Argentina, Brazil and Uruguay. Information about female genitalia of *Segestria* is very scarce, with just a few contributions in the literature. **Objectives:** The aim of this work was to study taxonomically the South American species and obtain information about the female genitalia. **Methods:** The specimens studied are deposited in Instituto Butantan São Paulo (curator: I. Knysak), Museu de Ciências Naturais (E. H. Buckup) and American Museum of Natural History (N. I. Platnick). The description is the standard in the recent revisions in Segestriidae. To study the female genitalia, the anterior portion of the abdomen was dissected, then cleaned and digested in 85% lactic acid at 100ºC. The drawings were made with a camera lucida on a Leica MZ 12.5 stereomicroscope and the multifocal photos were taken uisng a Leica MZ 16A stereomicroscope with a Leica DFC 500 digital camera attached. **Results and Discussion:** Two males and six females of *S. ruficeps*, and one male and two females of *S. florentina* were analyzed. After comparing the male and female genitalia of the two species, we detected the synonym between them and confirmed that this species was introduced to South America from the European continent. In addition, we studied the description and drawings of *S. pusilla* Nicolet, and after analyzing some specimens of *Ariadna maxima* Nicolet, 1849, we concluded that the described specimen is a juvenile of *A. maxima* and should be synonymized in the future.

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12.05 Behavioral effects of *Tityus obscurus* scorpion venom observed in rats

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Introduction: The most important scorpion accidents in Amazônia are caused by the scorpion *Tityus obscurus* Gervais, also known as *Tityus paraensis* Kraepelin or *Tityus cambridgei* Pocock. In the west of Pará, envenomation caused by this species shows a clinical picture distinct from that described in other regions and includes myoclonus, in which patients refer to electric discharge in the body, difficult ambulation and muscular contraction. **Objectives:** The aim of this work was to observe in rats the behavioral effects of increasing doses of crude venom of the scorpion *T. obscurus*. **Methods:** Scorpions were collected in West Para, and the venom was obtained by electrical stimulation. For experiments the venom was dissolved in 0.9% NaCl (0.5, 2.0, 5.0, and 10.0 mg/kg doses) or 1.46% NaCl (10.0, and 15.0 mg/kg doses). Male Wistar rats (230-260g) were divided into 7 experimental groups and 1 control group (n=5 animal in each group). The animals were intraperitoneally injected and observed for 5-6 h. Seven days after the injection, the rats were sacrificed and had their lungs removed. **Results and Discussion:** The behavioral effects observed were immobility for a long period, strong muscular contraction in abdomen, intense respiratory difficult, wet dog shakes, palpebral ptosis, postural loss, penile erection with secretion and prostration. These symptoms generally disappeared 3-4 h after venom injection. In the morphological analysis of the lungs, hemorrhagic points were found. LD_{50} was not obtained, in opposition to what was expected, since the high doses utilized did not kill the animals. We can conclude that the venom of the scorpion *T. obscurus* has characteristics distinct from those of others of the genus *Tityus* genus, which generally exert convulsant effects and are lethal at lower doses.

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12.06 Inventory and captivity maintenance of scorpion species from cities of Santarém and Belterra, PA

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Introduction: A great number of studies on scorpions from southern and northeastern parts of Brazil are available. However, little is known about the venom of scorpions from the northern part, mainly about *Tityus obscurus* which causes most of the accidents in Amazonia. This venom causes neurological symptoms different from those described for the other Brazilian scorpions. **Objectives:** We proposed to perform an inventory of the fauna and to study in captivity the maintenance of scorpions collected in Santarém and Belterra/PA to study the pharmacological effects of the venom. **Methods:** The scorpion capture methodology in Santarém was sporadic and random. In Belterra, the capture was carried out in the periurban area, and it was also random and sporadic; in the Floresta Nacional do Tapajós (on Km 83) it was carried out on five days a month, for three months, randomly and using pitfall traps. Dead animals were deposited in the scientific collections of the participant laboratories. Living animals were kept in the Laboratory of Arthropods of Instituto Butantan. Maintenance consisted of changing moistened cotton, removal of dead specimens, washing receptacles twice a week, and supplying food fortnightly. The venom was obtained through an electroportactil apparatus which excites the venom glands with adjustable electrical current intensity and tension in order to protect the animals. The venom was obtained from 10 specimens of *Brotheas sp*, 80 specimens of *T.obscurus* and 11 specimens of *T. strandii*. The venom was dried in a speed-vac apparatus and weighted. **Results and Discussion:** There were 265 specimens collected, where eight species were from the families Buthidae (85.7 %) and Chactidae (14.3 %). The family Buthidae was represented by *Ananteris balzanii* (0.7%), *Ananteris sp.* (0.7%), *Tityus obscurus* (64.1%), *T. silvestris* (7.2%) and *T. strandi* (12.9%); and family Chactidae by *Broteochactas parvulus* (1.1%), *Broteochactas sp.* (2.7%) and *Brotheas sp.* (10.6%). A predominance of *Tityus obscurus* was found in the areas. The mean quantity of venom obtained from each animal (total venom amount divided by number of extracted animals) was: *T. obscurus* 1.9 mg, *T. strandi* 0.8 mg and *Brotheas sp.* 1.2 mg. No animal submitted to the extraction died. In spite of the sampling being different for areas, it was possible to record eight scorpion species in Floresta Nacional do Tapajos. The species *Tityus obscurus* was predominant, which can explain the number of accidents in the region. Also, the greatest quantity of venom was obtained from this species, which will help in the study of this animal in the laboratory.

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12.07 Chemical and biological studies of the marine sponge *Amphimedon viridis* (Niphatidae, Haplosderida)

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Introduction: Sponges are aquatic macroorganisms which like tunicates, gastropods and bryozoans, may accumulate substances produced by microorganisms present in the same habitat. These substances usually have very complex structures that show interesting biological activities. To date, the substances isolated from the genus *Amphimedon* showed the following activities: deterrent against *Thalassoma bifasciatum*, antimicrobial against gram-positive and gram-negative bacteria, cytotoxic against P388 murine leukemia cells, and stimulatory toward guinea-pig plexus muscle contraction. **Objectives:** The aim of this study was to isolate the hemolytical and antimicrobial substances of the aqueous extract of *A. viridis*. **Methods:** *A. viridis* was collected by hand during scuba diving in Jatiúca, urban coast of Maceió, Alagoas, Brazil. The sponge methanolic extract was desalted and partitionated between water and methylene chloride. The aqueous layer was concentrated and submitted to chromatography on Sephadex G100 column (75 x 2.5 cm) with deionized water as mobile phase; after collecting a 15 mL fraction, 100 fractions of 3 mL and one of 300 mL were collected. All fractions were submitted to biologic assays of hemolytic and bactericidal activities. The last fraction showed both biological activities, and was re-chromatographed on the same system, resulting in one fraction of 15 ml, 10 fractions of 30 mL (named from A – J), followed by 100 fractions of 3 mL (numbered from 1 – 100), and one of 100 mL (named AL). All fractions were lyophilized and submitted to the same biologic assays. The fractions with small quantities and similar content were combined (C) prior to the bioassays. **Results and Discussion:** Hemolytic activity was detected in the fractions J and 1-8, and J and 1 had also strong antimicrobial activity against *E. coli* and *S. aureus*. Other fractions were bactericidal against both strains: B, 42-44 (C), 48-49 (C), 50-52 (C), 56-57 (C), 78-80 (C), 81-83 (C), 92-94 (C) and AL. Some fractions inhibited exclusively the growth of the gram-negative bacteria (*E. coli*): 10, 11, 53-55 (C) and 99-100 (C); while others inhibited the gram-positive bacteria (*S. aureus*): 7, 13, 15, 16, 19, 20, 29, 33-36 (C), 60-62 (C), 68-70 (C) and 71-75 (C). Mass spectrometry analysis of the active fractions revealed that J and 1-16 contained a polymeric mixture of different molecular weights (the highest MW detected was 5308.96 Da). The following fractions contained several non-polymeric substances. In a previous work, two polymers with membranolytic activity were isolated from *A. viridis* collected on the São Paulo State coast; however, no substance with antimicrobial activity was found.

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12.08 Study of the carbon source utilization of *Neisseria lactamica*

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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 from 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 that colonizes the newborn pharynx. Colonization by *N. lactamica,* which possibly shares antigens with *N. meningitidis*, contributes to natural development of immunity against *N. meningitidis. N. lactamica* and *N. meningitidis* release outer membrane vesicles (OMV) in cultivation. OMV is a good inducer of immune responses against surface antigens on meningococcal disease. Study of the growth of *N. lactamica* and studies about purification and productivity of OMV from *N. lactamica* may provide the key to a large scale culture and OMV production. **Objectives:** The aim of this work was to compare the *Neisseria lactamica* growth kinetics in different culture media, the carbon source consumption and OMV production. **Methods:** Shaker cultivations were carried out for a period of 8 h at 200 rpm, 36 °C. The culture media tested were: 1) Todd Hewitt broth, 2) BHI (brain heart infusion) and 3) TSB (tryptic soy broth). The carbon source consumption studies were done in MC (Catlin defined medium) with sodium lactate, or glucose, or lactose. Samples were collected every each hour and biomass was determined by optical density at 540 nm. OMV were purified from culture medium by centrifugation followed by ultracentrifugation. OMV production was determined by Lowry's method. **Results and Discussion:** *N. lactamica* grew in all culture media except in MC with lactose as a carbon source. At eighth hour of cultivation, OD_{540} reached 2.16 in BHI, 1.23 in Todd broth, 1.12 in TSB, 0.43 in MC with sodium lactate, and 0.23 in MC with glucose. The OMV yield after 8 h growth was 33.25 mg/L in BHI, 27.34 mg/L in Todd, 28.18 mg/L in TSB, 2.6 mg/L in Catlin medium with sodium lactate, 1.77 mg/L in Catlin medium with glucose. BHI, Todd and TSB are complex media that are inappropriate for vaccine production. Lactate seems to be the better carbon source tested in defined medium for *Neisseria lactamica* growth. Growth and OMV yield are still too low when compared with values obtained with complex media. A study of the nitrogen source is necessary to detect some other limiting factors to improve *Neisseria lactamica* growth.

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12.09 Potential of immature dental pulp stem cells to develop structures similar to retinal spheres and their culture *in vitro*

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Introduction: The retina is the light-sensitive eye tissue which converts captured energy in image, by highly specialized neuronal cells, responsible of sending nerve stimuli to the brain. When these cells are damaged, vision capacity is permanently lost, since these cells are unable to regenerate. Currently, the treatments aim only to decrease retinal damage instead of promoting an effective recovery of vision. Considering the difficulties of obtaining stem cells (SC) from retina, for the treatment of degenerative diseases, a constant demand of SC alternative sources is required in order to substitute injured tissue. Immature dental pulp stem cells (IDPSC) have characteristics of pluripotent SC and are able to acquire properties of almost all cell types. **Objectives:** The present work aimed to evaluate the potential of IDPSC to develop structures similar to retinal spheres, which can represent a new source of treatment to retinal degenerative diseases **Methods:** Undifferentiated IDPSC, previously established and characterized by our group, were analyzed by immunocytofluorescence to evaluate CD73 expression, which is an early photoreceptor marker. We also analyzed the expression of specialized retinal neuron antibodies such as: anti-rhodopsin, anti-calbindin, anti-PKC and anti-Phd. Further, these cells were submitted to progenitor neural differentiation using protocols developed by us. The capacity of IDPSC to differentiate towards retinal spheres similar structures was evaluated by immunofluorescence using anti-nestin and anti-β-III-tubulin antibodies. The co-culture of differentiated IDPSC with cells from retinal pigmented epithelium is being developed. **Results and Discussion:** Undifferentiated IDPSC reacted positively with CD73 and negatively with specialized retinal neurons antibodies. We also observed the positive reaction of anti-nestin and anti-β-III-tubulin antibodies in retinal spheres, indicating that these structures have a previous commitment with neural lineage, but there is still a requirement for some factors that can facilitate the induction of mature retinal characteristics. We demonstrated that IDPSC show the potential of developing structures similar to retinal spheres, with neural properties *in vitro,* which can be maintained viable for a long-term culture. Our data demonstrated that IDPSC can be an alternative source to regenerate damaged retinal tissues, maybe promoting vision recovery in blind people. Further studies are needed in order to elucidate respective roles of retinal tissue formation.

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12.10 Cloning, modeling and construction of a single-chain fragment variable (scFv) of anti-BaP1 antibody

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Introduction: Serum therapy remains the only specific treatment for envenoming by snakes, a major health hazard in tropical regions. Because of the adverse reactions it may cause, a better therapeutic antibody has been sought, and recombinant scFv antibodies could be a promising alternative due to their small size, adequacy in genetic manipulation, and low immunogenicity. BaP1 is a metalloproteinase isolated from *Bothrops asper* venom, which exerts multiple tissue-damaging activities, such as hemorrhage, myonecrosis, edema, dermonecrosis, blistering, and inflammatory response, degrades components of matrix extracellular and induces apoptosis in endothelial cells. A monoclonal antibody anti-BaP1 (MABaP1) that neutralizes BaP1 enzymatic activity and has a high affinity was previously produced by our group. **Objectives:** Our aim was to produce and to analyze the structure of a recombinant scFv using the MABaP1-secreting hybridoma as source of mRNA extraction. **Methods:** To clone its V_H and V_L Ig domains, total mRNA was isolated from hybridoma cells and transcribed into cDNAs. The amplification of V_L and V_H domains of the antibody was performed using Light and Heavy primers from Amersham Biosciences. These amplicons were cloned into pGEM-T Easy vector and sequenced. A synthetic gene devoid of rare codons for *E. coli* containing VH-linker $(G_4S)_3$ –VL was then constructed, cloned into pET20b+ vector and used to transform BL21 (DE3) bacteria. Using computer-aided homology modeling using Modeller 9v5 program, the structural/functional relevant regions of heavy and light chain CDRs were defined. In each step of modeling, about a hundred models were generated and the one with the best energy was selected. **Results and Discussion:** The cDNA sequence of V_H and V_L domains showed high homology with *Mus musculus* immunoglobulin. The modeled structure of scFv showed the common features of a classical antibody. The CDRs were identified and their antigen-binding surface exhibited electropositive and electronegative potentials that can be related to BaP1 recognition. The expression system used must be improved to increase the yield of scFv. SUMO (small ubiquitin-related modifier) containing-vector, a molecular chaperon domain, will be used to express scFv in the soluble and active form. Recombinant scFv will be tested regarding its ability to neutralize some BaP1 activities.

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12.11 Use of platelet-rich plasma (PRP) in treatment of equine septic arthritis: treatment of a serum producer at the São Joaquim Farm - Instituto Butantan

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Introduction: PRP (platelet rich plasma) has been widely used in the last times, with reports of the stimulation of the wound-healing process and repair of tendinous injuries and articulate processes in horses, but with little conclusive evidence of its effectiveness. The plasma rich in platelets is an autogenic and economic source of diverse factors of growth, with great therapeutical potential, important factors in tissue repair, due to mitogenic, chemotactic and neovascular actions. It is derived from the total blood and it can contain between 3 and 5 times the platelets of physiological levels, where in horses they can vary between 100,000 and 350,000 μl^{-1}. PRP treatment is carried out from the harvest of total blood in tubes or commercial blood stock containing anticoagulant, 3.8% sodium citrate, followed by centrifugation and activation of platelets with 10% calcium chloride and incubation at 20 and 22°C for 2 h, for the purpose of stimulating the degranulation of platelet granules. **Objectives:** The objective of the present study was to evaluate the effectiveness of the use of PRP in the treatment of septic arthritis in a serum-producing horse. **Methods:** Horse, male, 11 years old, of the diphtheric group, with a live weight of 450 kg, was referred to the Veterinarian Service of the Farm São Joaquim - Butantan Institute, located in São Roque - SP, presenting with lameness degree 4 of the right hind leg. After physical examination, the animal was medicated with conventional treatment with non steroidal antiinflammatory (flunixin meglumine), analgesic (phenylbutazone) and dexamethasone (on alternate days), with removal from the hyperimmune plasma production and lodged in a stall for movement restriction. After 30 days of treatment, improvement in lameness was not observed, with persistent swelling, pain to touch and not supported. It was opted then for treatment with PRP, applying 8.0 ml (prepared in the same farm) intra-articularly, and phenylbutazone (I.V.) and using dressing with pressure with Reparil® and NGF -5®. 10 days of the treatment with PRP, the animal came back to support the affected leg normally, showing evident recovery of the lameness picture. **Results and Discussion:** The use of PRP in the articulate disease described showed effectiveness and rapidity in the reestablishment of function in the horse, showing great importance and necessity of more studies for total briefing of its effectiveness in injuries of this type.

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12.12 Occurrence of exudative hypertrophic pododermatitis in equine serum producer of the São Joaquim Farm - Instituto Butantan

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Introduction: Exudative hypertrophic pododermatitis is a disease of chronic character that damages the hooves of horses. It is also known as chronic vegetative exudative dermovillitis and canker of frog, being characterized by its gradual growth with presence of moist secretion in frog and adjacent structures. It is believed to be of infectious origin, not totally elucidated to date. This pododermatitis does not possess predisposition of race or age of the horse and is associated with the lack of hygiene in installations, and the hooves. It can damage one or more hooves simultaneously, where its occurrence in horses is uncommon and sporadic. However, it is more common in animals lodged in humid places or that live for long periods in humid pastures. It is of great importance to get an early diagnosis and fast adoption of therapeutical measures, to prevent lesions of structures located under the dermal tissue, such as the deep digital flexor tendon, and distal phalanx. The recommended treatments vary and include the administration of antibiotics, sulphas, antimycotics, astringents, topical drugs such as 5% iodine solution, potassium permanganate, copper sulfate and formalin, and even the surgical excision of the salient tissue, where it is able to return in case the injured tissue has not been totaly removed, with significant edge. **Objectives:** The aim of this study was to quickly determine a method of treatment for this disease that is less expensive. **Methods:** One serum-producing horse of 12 years, male, weighing 450 k (live weight), of the lachetic group, was referred to the Veterinarian Sector of the Farm São Joaquim of the Instituto Butantan, located in São Roque - SP, showing lameness. At evaluation, we observed tissue proliferation and profuse bleeding from the footstep in the region of frog of the left hind leg, without occurrence of myiasis. Conservative treatment was initially instituted for about 2 months, which consisted of local application of Villate Liquor and iodine solution without success. Therefore, formation showed growth in this period. After 2 months of conservative local treatment, surgical excision of the prominent tissue was carried out. The procedure consisted of complete local antisepsis, followed by local anesthesia with 2% lidocaine without vasoconstrictor and surgical excision of tumor tissue; the treatment was more adjusted for the case in question. After excision, hemostasis was carried out with cauterization with a hot iron, and material was directed to Department of Pathology - FMVZ - USP for analysis. Pentabiotic blisters were applied in 5 doses (one on day of surgery and during 4 days). Dressing was carried out with league pressure, along with copper sulfate with cicatrizant pomade for 5 days. After these 5 days, there was only local cleaning, iodine solution application, Villate Liquor and cicatrizant pomade with copper sulfate. **Results and Discussion:** According to histopathologic findings, clinical examination of the case was compatible with exudative hypertrophic pododermatitis. After 30 days of the procedure, the animal's treatment was halted and it was returned to the breeding origin.

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12.13 Recombinant antibody (scFv) against enterotoxigenic *Escherichia coli* (ETEC) heat-labile toxin

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Introduction: ETEC pathogenic strains produce heat-labile toxin (LT) and/or heat-stable toxin (ST) that differ in their structure and function while both are used as markers for detection of infections. When compared to other methods, immunoserological assays show some advantages including high specificity and sensitivity with convenient procedures for sample preparation and assay execution. The advances in antibody biotechnology provide alternatives to obtain low-cost antibodies with desirable affinities and specificities by manipulating immunoglobulin domains. One approach consists in cloning immunoglobulin's heavy and light variable domains (HV and LV) as a single-chain fusion interspaced by a flexible linker, therefore allowing the correct interaction between the domains and preserving the antigen-binding site. **Objectives:** The aim of this work was the construction of a scFv with hybridoma cells that produce an anti-LT monoclonal antibody following its bacterial production. **Methods:** After RNA extraction from hybridoma cells and reverse transcription, coding regions of heavy and light chain variable domains (VH and VL) were PCR-amplified and fused to a linker corresponding to $(Gly_4Ser)_3$ giving rise to the scFv-LT coding region. The DNA construct was cloned into p-GEM-T Easy vector. The recombinant vector was used as template for sequencing. A synthetic gene was based on this sequence and subcloned into pET28a vector after amplification. The new recombinant plasmid was used to transform competent *E. coli* BL21(DE3) cells. Transformed cells were cultured up to 0.6 OD_{600nm}. After induction of T7 promoter-associated transcription by IPTG (1 mM, 3 h), the cells were harvested and disrupted by pressure. The inclusion bodies were isolated, solubilized with 8 M urea buffer and solubilized proteins were refolded by dilution. The refolded proteins were submitted to metal affinity chromatography using Ni-NTA resin and step-wise elution. Fractions were analyzed by SDS-PAGE. The recognition of LT by purified fractions was tested by ELISA. **Results and Discussion:** The amplification of VH, VL and scFv-LT showed fragments containing 325 bp, 340 bp and 724 bp, respectively. The target protein was identified as a major band with apparent molecular weight 30 kDa, with no biochemical activity. The design of synthetic gene was necessary to optimize the expression of recombinant protein in bacteria. The expression of the transcript showd the expected size, although with no biological activity. The lack of activity could be explained by uncorrected protein folding during the refolding process. Other refolding methods or expression strains should be used to obtain a molecule with biological activity. Once obtained this molecule could be used in the detection of ETEC infections.

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12.14 The role of sympathetic outflow in regulating protein synthesis in the mouse submandibular gland

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Introduction: Venom gland of *Bothrops jararaca* snake is an oral exocrine gland related to salivary glands. We have shown that stimulation of noradrenergic innervation by venom extraction is a key activator of venom gland. However, data in the literature show that sympathetic outflow has only a role in stimulating the synthesis and secretion of the saliva proteins in mammals. The new function of the sympathetic nervous system was discovered just because the venom gland can assume distinct quiescent and activated stages, in contrast to the salivary gland that is constantly in the activated stage. **Objectives:** The aim of this study was to verify whether sympathetic outflow is also able to activate mouse submandibular gland by analyzing the protein profile of submandibular glands obtained from mice treated with reserpine or reserpine and isoprenaline plus phenylephrine (adrenoceptors agonists). **Methods:** Adult Swiss male mice (25-30 g) were divided into 3 experimental groups: 1) control – treated with vehicle (n=4); 2) treated with reserpine (n=4) for 6 days (0.5 mg/kg – i.p.); 3) treated with reserpine for 6 days (0.5 mg/kg – i.p.) and phenylephrine plus isoprenaline (20 mg/kg – i.p.) in the last day of treatment (n=4). Extracts of submandibular glands were prepared and the protein profile was analyzed by SDS-PAGE and the density of the bands was quantified using Quantity One software. **Results and Discussion:** In analyzing the protein profile of these extracts, we observed that bands of approximately 96, 44, 39, 37 and 35 kDa had their density reduced after treatment with reserpine when compared to the control group. Administration of phenylephrine (alpha-adrenoceptor agonist) and isoprenaline (beta-adrenoceptor agonist) in reserpine-treated mice partially restored the effect of reserpine, and the protein profile was similar to that of the control group. The alterations observed in the protein profile of mouse submandibular glands after reserpine treatment and after administration of adrenoceptors agonists are similar to those found in *Bothrops jararaca* venom gland. Thus, our results suggest that sympathetic outflow is also important to keep the mouse submandibular gland in activated stage and both alpha- and beta-adrenoceptors are involved in this process. These data also show that venom gland of Viperidae snake is an attractive model to study physiological regulation of exocrine glands.

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12.15 Plasma FVIII and protein C purification by IMAC

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Introduction: Coagulation factor VIII (FVIII) is the protein deficient in the severe inherited bleeding disorder called hemophilia A, while patients deficient in protein C (PC) are at risk of deep vein thrombosis. These deficiencies are treated with replacement using the corresponding protein concentrate. Most of the licensed plasma-derived concentrates are produced from the Cohn method, which requires expensive equipment. 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 the purification of FVIII and protein C from human plasma using an ion-exchange followed by immobilized metal ion affinity chromatography (IMAC). FVIII and PC coelute in the anion-exchange column. Using IMAC as the second step, it was observed that FVIII and PC are well separated by IMAC- Cu^{2+}. The efficient separation of FVIII and PC can be explained by the higher number of histidine residues present on the surface of the FVIII protein, which leads to a tighter binding to the metal ions present in the matrix, in relation to protein C. The analysis of the amino acid sequences has shown that FVIII has 75 histidine residues, while PC 15. **Objectives:** The aim of this work was to purify FVIII and PC using other metal ions such as Ni^{2+}, Zn^{2+}, Co^{2+} and Fe^{2+} to extend our knowledge in the use of IMAC. **Methods:** Plasma was directly applied to an anion-exchange column. The elution fraction is purified by IMAC using Ni^{2+}, Zn^{2+}, Co^{2+} and Fe^{3+} as metal ligands. Analytical Methods: Bradford, for protein content; chromogenic, for FVIII and PC activities. **Results and Discussion:** Purification with IMAC- Zn^{2+} was demonstrated to be as efficient as with Cu^{2+} to separate FVIII and PC. IMAC- Zn^{2+} showed the advantage of allowing the desorption of these proteins with lower concentrations of imidazol. IMAC-Ni^{2+} was not efficient for separating these two proteins due to the weak interaction of these proteins with the metal. IMAC- Co^{2+} and Fe^{3+} could not be used under the experimental conditions employed, because the metal ions were stripped from the column by the buffer solutions used. These twp metals are more weakly bound to the resin than the other divalent ions and were easily washed off by citrate buffer, even using this reagent at a concentration 3 times more diluted than the maximum concentration recommended by the manufacturer. On the other hand, because we are purifying coagulation factors, the use of citrate buffer, which is a metal chelating compound, is important to prevent clotting during the purification. Our results indicate that IMAC- Cu^{2+} and IMAC- Zn^{2+} are the more suitable ones for the purification of FVIII and PC and further experiments are being carried out with these two ions in IMAC columns.

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12.16 Biofilm formation by bacterial strains from environmental monitoring of clean rooms

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Introduction: Biofilms are considered microorganism communities enclosed in self-produced extracellular polymeric matrices, commonly attached to surfaces. The nature and the physiological attributes of biofilms confer an inherent resistance to antimicrobial agents such as antibiotics and disinfectants. Bacterial biofilms are a persistent problem in many processes in the pharmaceutical industry. **Objectives:** The aim of this work was to investigate biofilm formation on abiotic surfaces (polystyrene) by strains isolated in environmental monitoring of clean rooms. **Methods:** A total of 24 bacterial strains were isolated from classified environments of Butantan Institute, through air sampling obtained by M-Air T sample using the active sampling method Split to Agar. Bacterial strains were identified by standard biochemical tests and commercial identification system (API). Biofilm formation was investigated after 18 h of incubation in 96-well microtiter plates, using a colorimetric assay with crystal violet and as measured at 595 nm in an ELISA plate reader. Cell aggregation at the liquid-solid interface was investigated in cultures grown at 37° C for 48 h in Luria broth (LB) with and without NaCl with shaking at 210 rpm. **Results and Discussion:** The bacterial species isolated were Gram-positive (8 strains – 2 *Bacillus* spp., 3 *Staphylococcus epidermidis* and 3 *Micrococcus* spp.), fermentative Gram-negative bacilli (3 *Morganella morganii*) and non-fermentative Gram-negative bacilli (13 strains – 2 *Pseudomonas fluorescens*, 1 *Pseudomonas putida*, 1 *Pseudomonas stutzeri*, 3 *Burkholderia cepacea*, 3 *Acinetobacter baumanii*, and 3 *Stenothophomonas maltophilia*). The strains studied were capable of producing biofilm on polystyrene. Ten strains showed low biofilm formation (OD with values between 0.000 and 0.183); nine strains showed a superior potential (OD greater than 0.732 – mainly *Pseudomonas spp* and *S. epidermidis*), and three displayed moderate biofilm formation (OD with values between 0.366 and 0.732). We observed that LB medium with NaCl results in a firm, strong and marked aggregation, where rigid pellicles could not be disrupted by shaking in 8.33% of the strains. The communities that can produce rigid pellicles constitute a protected mode of growth, allowing survival in a hostile environment. The present results showed the importance of determining the microorganisms present in classified environments involved in the pharmaceutical industry, as well as their capacity for biofilm formation, reinforcing the importance of applying efficient control measures against these microorganisms.

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12.17 TNF-α and MAP kinases (p38 and ErK) are associated with IL-12 production during the activation of macrophages by *Bordetella*

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Introduction: Bioactive interleukin IL-12p70 is critical for host defense against a variety of pathogens. This cytokine is generated endogenously, especially by macrophages and dendritic cells, as two subunits designated p35 and p40, which are independently regulated. IL-12 enhances phagocytic and bactericidal activities and acts directly in the development of Th1 response. *Bordetella pertussis* and *Bordetella parapertussis* are closely related endemic human pathogens, which cause whooping cough. Both species express differences in LPS while *B. parapertussis* is a mutant in pertussis toxin gene expression. These differences may reflect on the immune response and control of the infection. There is no agreement on the significance and essential requirement of IL-12 to control both *Bordetella* strain infections. Several studies suggest that there is no cross protection between these bacteria. **Objectives:** Due to scarce literature concerning macrophage infection with both bacterias, the present work was designed to find out some signaling mechanisms responsible for control of IL-12 synthesis, especially TNF-α and MAPkinases (p38 and ErK), during the murine bone marrow-derived macrophage (BMDMO) activation by *B. pertussis* and *B. parapertussis*. **Methods:** BMDMO obtained from the femurs and tibiae of C57BL/6 mice were differentiated in complete RPMI 1640 supplemented with supernatant of L929 murine fibroblast cell culture containing macrophage colony-stimulating factor (M-CSF). On day 7, adherent cells were pretreated with anti-TNF-α (10 mg/mL), ErK inhibitor (PD 98059, 20 mM) or p38 inhibitor (SB 202190, 10 mM) for 1 hr prior stimulation with soluble protein from *B. pertussis* and *B. parapertussis* (30 ug/mL). Supernatants were removed after 20 h, for determination of IL-12 by ELISA. **Results and Discussion:** Our results showed that the neutralization of TNF-α and the inhibition of p38 MAPkinase reduced the production of IL-12p40 protein level in contrast to ErK inhibition after stimulation with both antigens. Theses results indicate that p38 MAPkinase and TNF-a are required for the induction of IL-12p40, while ErK MAPkinase negatively regulated p40 induction. Thus, IL-12p40 pathway regulation synthesis is mediated by TNF-a and p38 MAPkinases during the activation of macrophages by *B. pertussis* and *B. parapertussis*.

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12.18 The *Bothropoides jararaca aff.* complex (Serpentes, Viperidae): comparison of venoms from continental and insular populations

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Introduction: The species *Bothropoides jararaca* shows great morphologic and ecological variations in their characteristics mainly due to wide geographical distribution, both on the South American continent and coastal islands. The geographical isolation of these populations can also produce variations in the chemical composition and biological activity of their venoms. **Objectives:** This study analyzed venom variability among *B. jararaca aff.* specimens from three coastal islands in the state of São Paulo compared to venoms of mainland populations from the South and Southeast regions of Brazil. **Methods:** Samples of the venoms of specimens from Island of Búzios - SP, Island of Cardoso - SP, Island of Moela – SP, Afonso Claudio - ES and São Bento do Sul - SC were collected and analyzed for protein content, eletrophoretic profile (SDS-PAGE), zymography, lethality (LD_{50}) and proteolytic activity with casein. The National Reference Venom (NRV) of *B. jararaca* was used as standard of comparison. **Results and Discussion:** In the measurement of protein content, the venoms showed similarities among themselves: approximately 1000 µg of protein/1000 µg of dry venom. The eletrophoretic profile of proteins revealed that there are more protein bands in NRV and continental samples than in the coastal ones. The insular venoms contained fewer or no proteins in the range of 43, 30 and 14 kDa. Venoms zymography from mainland samples showed gelatinolytic enzyme activitiy in the bands of molecular mass between 43 and 94 kDa, whereas the insular samples showed less activity for the same bands. The degree of venom toxicity varied significantly among all samples. Samples of Espírito Santo (38.40 µg) and Island of Moela (52.64 µg) were the most toxic compared to the venoms of Santa Catarina (74.62µg), Island of Cardoso (77.93µg) and Island of Búzios (97.14µg). The caseinolytic activity also indicated significant differences between Espírito Santo (94.7 U/mg), Island of Búzios (90.4 U/mg) and Island of Cardoso (111.3 U/mg), but all of them were quite similar to NRV (113.8 U/mg). Santa Catarina (64 U/mg) and Island of Moela (66.2 U/mg) samples were less active concerning caseinolytic activity. The differences in venom activities suggest that a process of adaptation and therefore evolution may have occurred in the populations studied due to geographic isolation. In fact the species from Island of Moela has already been identified and described as a new one. Further experimental analyses are underway to better understand the variability and the role of such venoms in the evolutionary context of the *Bothropoides jararaca* complex.

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12.19 Protection of mice mediated by a whole-cell pneumococcal vaccine against challenge with virulent strain of *Streptococcus pneumoniae*

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Introduction: Diseases caused by *Streptococcus pneumoniae* represent an extremely important health problem worldwide in terms of morbidity and mortality. The high incidence of pneumococcal disease, despite adequate therapy, emphasizes the importance of effective vaccine strategies. Instituto Butantan is developing a killed whole cell pneumococcal vaccine (WCV) derived from the unencapsulated mutant Rx1E PdT Δ*lytA* of *Streptococcus pneumoniae*, originally a serotype 2 strain, autolysin negative, carrying a kanamycin resistance and a pneumolysin defective gene. This new proposal intends to present to the immune system more conserved and not serotype-dependent antigens in native configuration, probably enhancing the coverage and diminishing the limitations for serotype-specific replacement. **Objectives:** In this study, we determined the antibody response and survival against systemic *S.pneumoniae* challenge of mice immunized with WCV. **Methods:** The *Streptococcus pneumoniae* Rx1E PdT Δ*lytA* vaccine strain (obtained from Dr. Richard Malley - Children's Hospital, Harvard Medical School, Boston) was cultured and processed in the Pilot Special Laboratory for Recombinant Biological Products - Instituto Butantan, to obtain the WCV under GMP conditions. Groups of 10 female BALB/c mice were subcutaneously immunized with experimental lots of WCV (Lot 005 and Lot 006), diluted in Ringer lactate and using aluminum hydroxide as adjuvant (1.2 mg/ml). Two doses of the vaccine were tested (1 μg or 10 μg/animal, in a volume of 200 μl) in two injections, with 2-week interval. Controls received just the adjuvant in Ringer lactate. Two weeks after the last immunization, the animals were bled for IgG evaluation, challenged with live encapsulated *S. pneumoniae* A066 strain (1.2×10^4 cells/0.5 ml, i.p) and observed for death for 10 days. **Results and Discussion:** Both lots of WCV induced significant IgG antibodies, with an apparent dose-response against the entire vaccine. The lower dose (1 μg) of the WCV Lot 005 induced 28.6% of protection against the challenge, and the higher (10 μg) 85.7%. There was no protection against the challenge after immunization with 1 μg of WCV Lot 006, but 60% survival was observed with 10 μg of this same vaccine. Our results suggest that in addition to immunogenicity assays, this mouse-protection model can be considered a useful tool in determining the parameters of quality control of the new pneumococcal vaccine under development at Instituto Butantan.

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12.20 Assessment of maternofetal transfer of anti-rotavirus serotype G9

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Introduction: Rotaviruses are members of the virus family Reoviridae and constitute the most important cause of severe gastroenteritis among infants and young children worldwide. It is well known that the transplacental transfer of IgG antibodies from the mother to the fetus is important for the protection of the infant in the first months of life. Transplacental transfer, together with breast-feeding, mitigates in part the deficiencies of the newborn's (NB) antibody production. Little is known about the transfer of anti-rotavirus antibodies to the NB, including the IgG subclasses involvement. **Objectives:** The aim of the present work was to analyze pairs of serum samples from mothers and their newborn's cord blood for the presence of IgG antibodies reactive with rotavirus serotype G9, circulating in Brazil, and to estimate the antibody transfer percentage. **Methods:** Fifty pairs of serum samples were collected at the Hospital Israelita Albert Einstein (HIAE) at the time of delivery. Rotavirus G9 and control antigens were obtained by ultracentrifugation. The detection of IgG anti-rotavirus G9 antibodies was performed by ELISA using a human serum pool as positive control. The titer was determined as the reciprocal of the dilution giving an absorbance of 0.5. The ELISA titers were used to calculate the transfer percentage of serum antibodies from mother to NB. **Results and Discussion:** ELISA results performed with the fifty pairs of samples showed that the titers of mothers and NB sera varied in the same wide range (mothers: from 5.66 to 149.32, mean of 68.60 and NB: from 7.02 to 165.75, mean of 69.18). The mean of the percentages of antibody transfer from mother to newborn was 107.2 %. Only one pair of samples showed a discrepant result, with a value of 260.95% of antibody transfer. We analyzed a great number of samples to better evaluate the phenomena. Our results are in accordance of other reports on transplacental transfer of antibodies reactive with other pathogens. Similarly, some cases of very high percentage transplacental transfer have been observed in other works with bacterial antigens. As an active process, transplacental transfer is dependent on neonatal Fc receptor (FcRn) present in the syncytiotrophoblast. The result observed here may be due to the number of this receptor. It could also be a compensatory mechanism, as the anti-rotavirus antibody titer in this sample was very low. The detection of transplacental transfer of serum antibodies directed against the rotavirus is the first step to study the biological activity of these antibodies in rotavirus neutralization and essential for the planning of strategies to improve the protection of newborns.

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12.21 Relative position of the internal organs in *Bothropoides jararaca* (Viperidae, Ophidia) in relation to their snout-vent-length

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Introduction: In Brazil the genus *Bothropoides* comprises 11 species of snakes. The species *Bothropoides jararaca* is the most common in southeastern Brazil and is found from southern Bahia to Rio Grande do Sul, where it is responsible for most snakebites in its distribution area. The venom of *B. jararaca* is one of the most studied in various research centers. The knowledge of the snake's anatomy kept in captivity is helpful when interventions, such as surgeries, ultrasound and clinical examinations are necessary. **Objectives:** This study aimed to analyze the visceral anatomy of *B. jararaca* determining the position of the internal structures in relation to the snout-vent length (SVL) of the animals. **Methods:** Ten females and ten males of jararaca, all recently dead, coming from various cities of São Paulo state were dissected and 24 internal structures examined for their position in centimeters and percentage with respect to the snake's snout-vent length. **Results and Discussion:** The average of the male's snout-vent-length (SVL) was 85.6 cm and the average of their total length (TL) was 100 cm, while the females had a SVL of 99 cm and a TL average of 113 cm. The Females exhibited higher values than the males in all the data analyzed, except for the relative size of the tail, which was longer in males. In general, the relative anatomic position of the organs in both sexes showed similar results. The heart (positioned at 31-34% of the SVL) and most of the organs of the respiratory system are located in the first third of the body, with only the air bag and the liver (34-54% of the SVL) in the central region of the body. The gonadal structures are located in the last third of the body (right ovary: 61-72% of the SVL; left ovary: 61-72% of the SVL; right testicle: 69-72% of the SVL and left testicle: 76-79% of the SVL). The digestive tract occupies the fullest extent of the snakes. As expected, all paired organs on the right side are more elongated and have a more cranial position in relation to the left side (example: right kidney: 80-94% and left kidney: 84-94% of the SVL; right adrenal: 67 - 72% and left adrenal: 76-80% of the SVL), except for the thymus.

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12.22 CB_1 and CB_2 cannabinoid receptors are involved in the effect of crotalphine, an opioid-like analgesic peptide

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Introduction: Although morphine and other opioid-like drugs are considered the main option for the treatment of moderate to severe pain, the use of opioids is limited because of the undesirable effects. Therefore, efforts have been made in the search for new analgesic compounds. Recently, our group demonstrated that crotalphine, a 14-amino acid peptide synthesized based on the structure of the natural analgesic factor isolated from the venom of the South American rattlesnake *Crotalus durissus terrificus*, features a long-lasting analgesic activity when evaluated in experimental models of acute and chronic pain. This effect is mediated by activation of peripheral k- and d-opioid receptors. Despite showing opioid activity, the amino acid sequence of crotalphine displays no homology to any known opioid peptide. Furthermore, preliminary results indicate that crotalphine does not directly activate opioid receptors. **Objectives:** Since behavioral and molecular studies have demonstrated a great interaction between opioid and cannabinoid systems, the aim of this work was to evaluate the involvement of cannabinoid receptors in the antinociceptive effect of crotalphine. **Methods:** All procedures were approved by the Institutional Animal Care Committee of the Butantan Institute. Hyperalgesia was induced in male Swiss mice by carrageenin (Cg, 100 μg/paw) and in male Wistar rats by prostaglandin E_2 (PGE_2, 100 ng/paw). The antinociceptive effect of crotalphine (0.04, 0.2, 1 and 5 μg/kg, p.o.), ACEA (CB_1 agonist, 5, 10, 20 and 50 μg/paw, i.pl.) or AM1241 (CB_2 agonist, 5, 10, 20 and 50 μg/paw, i.pl.) was determined using the paw pressure test in rats or an electronic pressure-meter test for mice. The involvement of cannabinoid receptors in the antinociceptive effect of crotalphine was investigated using selective antagonists of CB_1 (AM251, 5, 10 and 80 μg/paw) and CB_2 (AM630, 5 and 50 μg/paw) receptors, injected by intraplantar route. The activation of CB_2 cannabinoid receptors was confirmed by imunoblotting assays using conformation-state sensitive antibodies (Proteimax Biotechnology, Brazil). **Results and Discussion:** The results demonstrated that crotalphine, ACEA and AM1241 induce antinociception in both models of pain evaluation. Both CB_1 and CB_2 receptor antagonists inhibited the antinociceptive effect of crotalphine and of their respective agonists. In addition, crotalphine increased the activation of CB_2 receptors in the skin tissue of the rat paw. These results indicate that peripheral CB_1 and CB_2 receptors are also involved in antinociception induced by crotalphine.

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12.23 4th 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 aimed at complementing the training of new university graduates involved in the health area. In the Instituto Butantan (IBu) this program lasts 2 years and consists of 40 hours/week of activities under 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 researchers from different laboratories of the Institute. In this course, pertinent themes are covered by scientific researchers, specialists and also 2nd year PAP-students. In addition, the course focuses on the integration of the grant-holders to the different areas of the Institute (Production, Development, Research and Museums). At the end of the course, the students must 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 fourth course offered in 2010. **Methods:** The activities lasted 2 weeks and were divided into hands-on and theoretical classes distributed in an 8 hours/day workload. In this period, all of the Divisions of the IBu were presented, and 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 the preparation of solutions were presented. At the end of the course a representive was elected by their colleagues, and a questionnaire was answered by the grant-holders in order to find out their opinion about the course. **Results and Discussion:** In 2010, 46 new graduates with different trainings (35 biologists, 4 veterinarian scientists, 3 pharmacists, 2 biomedical scientists and 2 nurses) were received by the program, and among them 75% did not belong to IBu. The questionnaire was answered by 44 students and the average of the course grade was 8.1. The program was considered totally satisfactory to all of the grant-holders, as well as the content and workload. The helpfulness and attentiveness of teachers and coordination staff were totally satisfactory to them. The main subjects of interest were laboratory techniques (64%), biosafety guidelines (61%), biotherium (61%), bioethics (61%), sterilization (59%), routine equipment operation (57%), pharmacologic assays (52%), museums (50%) and reagents and solutions preparation (50%) Moreover, organization chart (46%), SUS (34%) and São Joaquim Farm (38%) 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-holders evaluated the course positively. Although satisfaction does not assure the learning process, certainly it stimulates the performance of the grant-holders in their laboratory activities.

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12.24 Comparing BPV-1 and BPV-2 E6 genes

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Introduction: Papillomaviruses (PVs), members of the family *Papilomaviridae*, are double-stranded circular DNA viruses. PVs induce benign epithelial tumors in skin and mucous membranes. These tumors can undergo malignant progression associated with co-factors. BPV-1 and -2 are included in the genus *Delta-papillomavirus* and induce skin fibropapillomas and urinary bladder tumors in cattle. Also, BPV-1 and -2 are the only reported papillomavirus infecting different species: horses, donkeys and mules can develop sarcoid tumors. BPV-1 and -2 E6 oncoproteins interact with different cellular proteins, resulting in cell metabolic pathway alteration and, eventually, malignization. **Objectives:** The aim of this study was to clone the E6 BPV-1 and -2 genes in a bacterial system and to compare the sequences obtained. **Methods:** Specific primers were designed and PCR was used to amplify E6 BPV-1 and -2 gene sequences, using the genomic viral DNA previously cloned in pAT153 vector as template. The PCR products were cloned into pCR4-TOPO and in pET28a vectors. The recombinant plasmids were used to transform *E. coli* DH5α and BL21 cells by the heat shock method. Colonies with recombinant plasmid were selected for plasmid purification and sequencing. Sequences were compared using *BioEdit Sequence Alignment Editor*. **Results and Discussion:** E6 BPV-1 and 2 gene sequences were successfully cloned in transformed *E. coli* cells, an adequate system for papillomavirus gene cloning. The percentage of similarity verified by sequence comparison were: 87.4% between E6 BPV-1 and -2 Genbank sequences; 94.6% between cloned E6-1 and E6 BPV-1 sequence available in GenBank; 99.0% between cloned E6-2 and E6 BPV-2 available in GenBank and 85.0% between cloned E6-1 and E6-2. The percentage of similarity found by aminoacids comparison was: 83.9% between E6 BPV-1 and -2 GenBank sequences; 92.0% between E6-1 cloned and E6 BPV-1 available in GenBank; 96.3% between cloned E6-2 and E6 BPV-2 GenBank; 80.4% between sequence proteins generated from E6-1 and E6-2 cloned sequences. Despite the verified high similarity, further sequencing with full gene coverage can reveal differences possibly relevant to the development of diagnostic devices and vaccines.

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12.25 PCR identification 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 in six main pathotypes, based upon 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) that encodes the bundle-forming pilus (BFP). The genes responsible for production of A/E lesions are located on a pathogenicty island called locus of enterocyte effacement (LEE). Intimin, a 94k-Da outer membrane protein encoded by *eae*, is responsible for the intimate adherence between the bacteria and enterocyte membranes. The EAF plasmid, which harbors *bfpA*, is not essential for A/E lesions, although the presence of regulatory genes can increase expression of the chromosomal LEE genes. **Objectives:** The aim of this work was the molecular identification of 30 *E.coli strains* isolated from children with diarrhea in Ribeirão Preto, São Paulo, Brazil, previously identified as atypical EPEC. **Methods:** All samples were grown in Luria-Bertani (LB) broth and incubated overnight before DNA extraction by CTAB method. Plasmid DNA were performed using alkaline lysis. We verified the presence of *eae* and *bfp* by PCR in the following conditions: *eae* amplification cycle: 95° 5 min, 40X (94°C 1 min, 55°C 1 min, 72°C 1 min), and 72°C 8 min; *bfpA* amplification cycle : 95°C 5 min, 30 X (95°C 1 min, 56°C, 1 min, and 72°C 2 min) and 72°C 8 min. Amplified fragments of samples were evaluated by 0.8% agarose gel electrophoresis in Tris-borate-EDTA buffer and ethidium bromide staining. **Results and Discussion:** Previous identification of these strains was performed using techniques that are not the "gold standard" for these pathotypes, and showed some inconsistent results. Using molecular techniques, we performed new identifications and showed different results for almost 50% of the strains. Of the 30 samples analyzed, 5 were *bfpA* positive and 9 were *eae* negative. These results showed that the previous analysis was not reliable. At least five samples classified as atypical EPEC, actually can be classified as typical EPEC because they harbor *bfpA*. The most problematic issue is that 9 strains did not harbor the *eae* gene and certainly cannot be classified as enteropathogenic *E. coli*. This study clearly demonstrates that we need to use adequate techniques to perform pathogen identification.

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12.26 Characterization of gometoxin-3 of *Acanthoscurria gomesiana* venom

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Introduction: There is an increasing interest in the pharmacological application of antimicrobial peptides (AMPs) to treat infections due to the growing problem of pathogenic organisms resistant to conventional antibiotics. AMPs are widely distributed in nature and represent an ancient host defense mechanism present in organisms across the evolutionary spectrum. Over 1200 AMPs have been reported so far in the literature, of which a third have been isolated from arthropods, including arachnids. The AMPs of the first type are mainly detected in the hemolymph of various arthropods such as sacrotoxin in the housefly, cecropins in *Lepidoptera* and spinegirin in termites. The AMPs of the second type were found in the venom glands of bees, wasps, ants, scorpions and spiders such as *Lycosa carolinensis*, *Cupienius salei* and *Oxyopes kitabensis*. **Objectives:** The aim of this study was to characterize gometoxin-3, antimicrobial peptides of the venom of the spider *Acanthoscurria gomesiana*. This work could be useful in the scope of future pharmaceutical use. **Methods:** The venom was obtained from venom glands of *A. gomesiana* spider, which were macerated with water, centrifuged and the soluble part was dried by vacuum centrifugation and reconstituted with acidified water (TFA - trifluoracetic acid 0.05%). The soluble part was separated by 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:** We recently isolated three molecules with antimicrobial activity in the venom of *A. gomesiana*: Gometoxin-1, 2 and 3. Gometoxin-3 with anti-*M. luteus* activity and molecular weight of 1658.9 Da was reduced, alkylated and cleaved by trypsin and subjected to mass spectrometry (MALDI-TOF-MS). After obtaining the masses of the fragments, they were analyzed in the databases PROTEIN BLAST and MASCOT, where similarity was found with a fragment of a neurotoxic molecule, called huwentoxin, Chinese spider *Ornithoctonus huwena* (family Theraphosidae), as well as the spider *Cupiennius salei* (family Ctenidae), and the scorpion *Tityus discrepans* (subfamily Tityinae). Analysis of gometoxin-3, showed that this peptide is linear without disulfide bounds. The complete characterization and sequencing of gometoxin-3 are in progress.

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12.27 Noradrenergic regulation of RAS aminopeptidases in the rat pineal gland

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Introduction: The pineal gland has a circadian rhythm of melatonin (MEL) synthesis and secretion that is regulated predominantly by the sympathetic nervous system via noradrenergic fibers originating in the superior cervical ganglion. The complexity of the gland function is denoted by the presence of a local renin-angiotensin-system (RAS) that modulates melatonin synthesis. The pineal gland is the brain structure that contains the highest renin-like activity and an extensive enzymatic system for the production of the RAS active peptides. Classically, angiotensin II (Ang II) has been considered as the RAS effector peptide, but it is not the only active peptide. Several of its degradation products, including angiotensin III (Ang III) and angiotensin IV (Ang IV) also possess biological functions. These peptides are formed via the activity of several aminopeptidases (APases), particularly aminopeptidase A (APA) and aminopeptidase B and N (APB and APN). Ang IV acts through its own receptor, an insulin-regulated-aminopeptidase (IRAP), inhibiting its enzymatic activity. **Objectives:** Our aim was to determine the role of these APases (APA, APB, APN, IRAP) in the regulation of pineal RAS by investigating whether these APases are regulated by norepinephrine which is the nocturnal stimulus of melatonin synthesis **Methods:** Pineal glands of 30 healthy male Wistar rats (250 g) were maintained in culture for 3 days and then stimulated with 1 μM norepinephrine (Nor) or not. Enzyme activity of the soluble fraction (SF) and solubilized membrane-bound fraction (MF) were fluorimetrically evaluated in triplicate samples. Protein was spectrophotometrically measured in triplicate by the Bio-Rad protein assay. **Results and Discussion:** In this study, the activities of the regulatory RAS APases were identified and quantified, demonstrating the presence and effectiveness of the local system in the pineal gland. In SF, previous studies have shown, in the gland, the presence and activity of APB, APN and APA. Regarding the latter, our data disagree with the literature, because in SF our results did not identify the activity of APA, but only in the MF. In MF, all regulatory RAS APases studied had their activity identified, which, in the pineal gland, has not yet been reported. Regarding IRAP in MF, the evaluation of its activity in the gland had not yet been demonstrated either, and in accordance with the present study, its activity is present both in SF and in MF. Only in MF, did noradrenergic stimulation show significant variation in IRAP activity relative to control group, suggesting the presence of circadian rhythmicity regulated by sympathetic stimulation.

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12.28 Kefir: A natural candidate for use as a food preservative and an antimicrobial agent against infectious diseases

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Introduction: Kefir is a beverage derived from milk fermentation by a symbiotic association of bacteria and yeast, which has been used extensively as a food complement and remedy against infectious diseases. However, it has been observed that the healing property of kefir varies according to its fermentation cycle, manipulation procedure and source. **Objectives:** The aim of this study was to determine which cycle of kefir fermentation is responsible for the ability to inhibit bacterial proliferation and its capacity to retain this property in different storage conditions. Partial characterization of the molecules responsible for this inhibitory activity was performed. **Methods:** The bacterial inhibitory activity of kefir was determined by incubating kefir obtained from different cycles of fermentation with Gram negative pathogens for 18 h at 37°C. The bacterial growth was determined by reading the culture O.D. at 600 nm. Kefir was lyophilized, re-suspended in water and maintained for a period of 12 months at different temperatures -20°C, 4°C and 37°C while its ability to inhibit bacterial growth was determined as described above. Kefir was also diluted in half with TBS and kept uncovered at room temperature for three months. The size of the molecules responsible for bacterial growth inhibition was determined by centrifugal filter devices (Centriprep) and dialysis using membranes with different molecular weight cut offs and by SDS-PAGE. Fractions obtained by Centriprep size fractions (YM-10-YM-30) were submitted to temperatures ranging from 37 to 100 °C and treatment with proteinase K and trypsin. Their capacity to inhibit bacterial adhesion to Hep-2 cells was also investigated. **Results and Discussion:** The results demonstrated that the ability of kefir to inhibit bacterial proliferation starts to be significant in its 3rd cycle of fermentation. Kefir still maintains its ability to inhibit bacterial proliferation after being lyophilized, re-suspended in water, and kept for 12 months at -20°, 4°C and 37°C. The molecular weight of the molecules responsible for the inhibition of bacterial proliferation is < 10 kDa. It was also observed that these molecules were able to inhibit the adhesion of a high concentration of pathogenic bacteria (10^8) to epithelial cells. They were resistant to protease and temperatures up to 90°C. In addition, TSB with kefir was preserved against contamination for three months at R.T. In summary, due to its resilience under different storage conditions and its ability to inhibit bacterial proliferation and adhesion to epithelial cells, kefir has the potential to be used as a natural food preservative and an antimicrobial agent against infectious diseases.

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12.29 A new antistasin-family member from *Haementeria depressa* leeches: cloning and expression in *Pichia pastoris* system

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Introduction: Several inhibitors similar to antistasin have been described from different animals, mainly from leech species, and therefore, the antistasin family was created. Antistasin was described from the salivary glands of the Mexican leech (*H. officinalis*). Members of the antistasin family may act on different enzymes as well as factor Xa, thrombin, elastase, etc. Therostasin is a member of this molecule family which has been studied from the *Theromyzon tessulatum* salivary glands. This protein is a tight-binding inhibitor of mammalian factor Xa (K_i = 34 pM), it is a cysteine-rich protein (16 cysteine residues) with a molecular weight of about 9 kDa, consisting of 82 amino acid residues. Our group has studied several compounds with activity in the hemostatic system, from a cDNA library of the *Haementeria depressa* salivary complexes. We selected 2 clones showing similarity to the therostasin molecule, one of which showed 55% identify with therostasin, but was previously expressed unsuccessfully in prokaryotic system (*E.coli* – BL21DE3). **Objectives:** The aim of this work was to carry out a new cloning in pPIC9K plasmid and to express this molecule in a eukaryotic system (*Pichia pastoris*), to define the best methods of expression and purification, to obtain a recombinant molecule with activity, and also to define the target enzyme of its inhibition. **Methods:** The selected clone for this study (H05D10_pGEM11Zf) was subcloned in a pGEM-Teasy system (Promega) to final cloning in pPIC9K plasmid between Eco RI and Not I cloning site. The sequence was confirmed, and the new vector was then linearized by Sac I digestion, followed by transformation into the yeast *P. pastoris* (GS115 strain). The expression was done in BMG (Buffered Minimal Methanol Medium) and BMGY (Buffered Methanol-complex Medium) at 28° - 30ºC, and afterward, some methods of purification including dialysis, heparin sepharose and gel filtration chromatographies were carried out. The different steps of expression and purification were monitored by SDS-PAGE and the activity was initially assayed on FXa using specific chromogenic substrate (S2765). **Results and Discussion:** The complete sequence from H05D10_pPIC9K was successfully obtained. The best conditions for expression were seen using BMG medium when compared to BMGY medium at 28ºC. The heparin-sepharose chromatography was the most efficient method for the purification, where the recombinant protein of about 14kDa (SDS-PAGE) was eluted in approximately 300 mM NaCl. The molecule described here was able to inhibit factor Xa using a chromogenic assay, proving to be a new antistasin family member with potential to be explored in further investigations about its structure and role as a new anticoagulant.

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12.30 Comparative skin morphology in toads from Atlantic forest and Caatinga

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Introduction: The skin is considered an essential organ for the amphibians since it is essential for major vital activities such as breathing, defence and reproduction. The presence of glands in the skin, the mucous glands and the granular (or venom) glands is a characteristic shared among all modern amphibian species. The genus Rhinella (family Bufonidae) is constituted by species indiscriminately known as "sapos-cururu". These toads are well adapted to the life in dry environments, since they in general need body waters such as pools or ponds only during the reproductive period. The skin of these amphibians is basically formed by the epidermis and the dermis, which comprises the *stratum spongiosum* where the glands are inserted, and the *stratum compactum*, mainly formed by collagen fibers. *Rhinella icterica* and *R. jimi* occur, respectively, in the Atlantic Forest, a humid environment, and in the Caatinga, a dry and hot environment. These species can serve as models in comparative studies of cutaneous adaptations, focusing on the defense against desiccation and against predation. **Objectives:** The purpose of this work was to carry out a quantitative and qualitative histological study, in different regions along the body of *R. icterica* and *R. jimi*, aiming to make correlations with their biology and natural history. **Methods:** Skin samples of 8 specific regions of 3 females of each species were fixed in Bouin, embedded in paraffin, sectioned and stained in HE. The following measurements were made: thickness of the total skin, cornified layer, epidermis, *stratum spongiosum*, dermal calcified layer (known as Eberth-Kastschenko layer) and stratum compactum, and the diameter of the mucous and granular glands. All measurements were obtained by the use of the *Image-Pro Express* software. Comparison between measurements was made by using variance analysis for one factor (ANOVA) and later analysis by Dunn's test. The results were considered significant when $p<0.05$. **Results and Discussion:** For most sampled regions, there was a significant difference between the species, both for the thickness of cutaneous layers and for the gland diameters. The distribution of glands along the body was also significantly different. The results suggest that *R. jimi*, besides showing a thicker skin, have larger venom glands. This species also possess a larger number of glandular accumulations, distributed in the form of warts in the dorsal region. Despite the calcified dermal layer often being related to water saving, no significant difference in this aspect was obtained between the two species. The data indicate that, in the specific case of bufonids, the cutaneous adaptations show the tendency to be more associated with the defense against predators than against desiccation.

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12.31 Adjuvant activity of dioctadecyldimethyl ammonium bromide (DODAB) on immune response in mice to crotoxin isolated from *Crotalus durissus terrificus* venom

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Introduction: In Brazil, the South American rattlesnake *Crotalus durissus terrificus* (C.d.t.) is responsible for a part of the ophidian accidents registered annually and by the lethality observed in the absence of serum therapy. The crotoxin (CTX) is the main component of the C.d.t. venom and has toxic activity. It has been shown that both venom and CTX exert an inhibitory effect on the immune system. In contrast, some molecules have the ability to stimulate the innate immunity and consequently to promote robust adaptive immunity to distinct antigens such as dioctadecydimethyl ammonium bromide (DODAB). **Objectives:** According to this, we evaluated the ability of DODAB to enhance the humoral and cellular immune responses to the CTX isolated from C.d.t. venom. **Methods:** For this, groups of BALB/c mice were immunized with CTX (5 μg/animal) in DODAB (2 mM); CTX adsorbed in aluminum hydroxide (Alum-1 mg/animal) or CTX emulsified in Marcol-Montanide via sc. After 14, 21 and 28 days of the immunization the mice were bled and on day 21 received the antigenic booster of CTX (5 μg). Anti-CTX IgG1 and IgG2a production was measured by ELISA. Other groups of mice were immunized as mentioned above, and after 8 days they were sacrificed for the cell suspension preparation from the antigen draining lymph nodes. Cell viability was evaluated and the suspensions were incubated *in vitro* for 48 h with the toxin, concanavalin A (ConA) or anti-CD3 mAb. After the incubation period, the supernatants were collected for cytokines measurement by ELISA. The cells were also stimulated with the toxin, ConA or anti-CD3 mAb for 72 h to evaluate the T cell proliferative response. **Results and Discussion:** We observed higher anti-CTX IgG1 and IgG2a production in mice immunized with CTX/DODAB or CTX/Marcol-Montanide when compared to those obtained in CTX/alum-immunized mice. The analyses of cell viability showed the same percent of cell death in all suspensions obtained from distinct groups of immunization. High levels of IL-10 and IFN-γ were observed in cell cultures from CTX/DODAB or CTX/Marcol immunized mice when stimulated with ConA. The presence of IL-5, IL-4, IL-12 and IL-13 was not detected in the supernatants of all cell cultures evaluated. The ConA or anti-CD3 mAb induced high proliferative response of T lymphocytes obtained from all groups of mice. DODAB and Marcol induced strong anti-CTX immune response when compared to alum adjuvant. CTX/alum immunization seems to induce preferential Th2 response to the toxin, whereas DODAB and Marcol-Montanide promote the generation of both Th1 and Th2 responses.

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12.32 Preliminary data on the snakes' type material rescued after fire at the Instituto Butantan herpetological collection

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Introduction: Instituto Butantan Herpetological Collection began with Dr. Vital Brazil at the end of the 19th century in Botucatu, SP. In the 20th century, already in Instituto Butantan, to obtain raw material for the production of anti-venom, he made one of the best publicity campaigns ever by calling the population to exchange snakes for anti-venom. This permutation is inactive today, but the population still responds to it by bringing ca. 8000 specimens/year. This effort together with researchers' contributions produced the world's largest snake collection, with over 77,000 registered specimens. Unfortunately, a great part of it was lost in the tragic fire on May 15, 2010, which destroyed the Herpetological and Arthropods collections facilities. The type species (holotypes, paratypes, lectotypes, paralectotypes, neotypes and syntypes) are the most important specimens of a collection, for they aggregate scientific name to the biological entity and have been used on the original description or were carefully designated by revisers after it. It was also the world's biggest collection of Brazilian types. Rescuing already began on May 15 with the help of firemen, students, employees and volunteers and was extended until July 4, 2010. Anything still with an ID number was selected, regardless of its conservation state and the intact ones were taken even without identification. There is no quantitative or qualitative evaluation of what is left yet, due to the current impossibility to screen the material. **Objectives:** Here, a first inventory of the rescued types is presented. There is still material to be screened, so the numbers may increase. **Methods:** After the rescue, ca. 100 containers of 50 L were filled up with specimens and part of the type material was separated from the other ones during rescue, which enabled us to do this first estimation by comparing the ID numbers with our records and with the original descriptions. **Results and Discussion:** About 150 different species or subspecies were present at the type collection, 21 were rescued (13.3% of the total). Regarding the number of types, there were more than 1120 registered specimens. So far, 299 specimens were rescued (26.7% of the total). Besides rescuing, we also intend to gather information about the types and other specimens by requesting researchers and students, which possess data and images of them, to send copies in order to include in records and make them available for consultants. Facing the expectation of total loss of the type material, the certainty that these numbers are yet initial as well as the effort to gather information, we hope to minimize the damage, so our consultants will keep on counting on Institute Butantan to carry out their projects.

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12.33 MAIP-1, the monoclonal antibody against PAS-1, neutralizes the anti-inflammatory effect of *Ascaris suum*

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Introduction: Helminth parasites stimulate regulatory mechanisms that are associated with suppression of the host immune responses. We have recently demonstrated that *Ascaris suum* experimental infection, the adult worm whole extract (ASC), the body fluid (BF) or the purified suppressive protein (PAS-1) highly suppresses LPS-induced acute inflammation due the stimulation of regulatory T lymphocytes. On the other hand, in our laboratory we produced a monoclonal antibody named MAIP-1 that specifically recognizes PAS-1, the suppressive protein from *Ascaris suum*. **Objectives:** The aim of this study was to investigate the ability of MAIP-1 to neutralize the anti-inflammatory effect of *Ascaris suum* infection or their products **Methods:** Air pouches made on the shaved back of BALB/c mice with 2.5 mL of sterile air, twice, were stimulated with 1 ug LPS. Three hours after stimulation the exudates were collected with 2.5 mL of PBS and the magnitude of inflammation was evaluated by cell migration and measurement of inflammatory cytokines (TNF-a, IL-1b and IL-6). The protocol consisted of four groups of mice that received 2500 eggs of *Ascaris suum* by intragastric route, or 300 mg of PAS-1, BF or ASC into the air pouches. Control groups were injected with PBS. The infected group received different doses of MAIP-1 or mouse IgM intravenously and the other groups into the air pouches. **Results and Discussion:** Our results showed that the *Ascaris suum* infection or their products highly suppressed inflammatory leukocyte migration and pro-inflammatory cytokine release. On the other hand, MAIP-1 was able to neutralize this anti-inflammatory effect. These results demonstrated that the suppressive effect of the *Ascaris suum* infection, the body fluid or the whole extract is due to the presence of the protein PAS-1. Moreover, our results showed that the monoclonal antibody MAIP-1 recognizes the functional epitope of PAS-1.

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12.34 Analysis of the reproductive behavior of *Bothrops spp.* and *Bothropoides spp.* in captivity

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Introduction: The species of the genera *Bothrops* and *Bothropoides* are viviparous and seasonal with biennial reproduction, reproducing only once every two years. The number of newborns for offspring varies according to the size and weight of the mother. The birth of the newborns occurs in summer, which is the period of the year that will provide more nourishment and a favorable environment for its metabolism and development. **Objectives:** The objective of this study was to analyze the reproductive behavior of six species of the genera *Bothrops* and *Bothropoides* , making a comparison between a captive group and one that lives in a natural habitat. **Methods:** We studied two species of the genus *Bothrops (B. moojeni* and *B. jararacussu),* and four species of the genus *Bothropoides* (*B. erythromelas, B. jararaca, B. pauloensis,* and *B. neuwiedi)*. The study was carried out in Americana - Sao Paulo, in a Conservationist Breeding. For each species, we kept one female with one or two males. The specimens were kept in captivity and observed from May/2008 to July/2008. The reproductive events were documented by photographs, and the data obtained were analyzed and compared with literature data. **Results and Discussion:** The specimens mated between May and June, with the majority of births being reported in February. We also observed specimens with mating and births at different dates, which characterizes in the species of the genera *Bothrops* and *Bothropoides* a change in its reproductive behavior. Thus, the conditions of captivity may have influenced the change in mating season.

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12.35 Rabies virus glycoprotein and messenger RNA expression in S2 cells cultivated at different temperatures

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Introduction: The rabies virus glycoprotein (RVGP) is the major antigen of rabies virus. In order to show its antigenic conformation, RVGP has to undergo complex post-translational modifications. Therefore, its expression in eukaryotic cells is mandatory for the synthesis of the antigen with the required characteristics for biological activity. *Drosophila melanogaster* S2 cells have been successfully utilized for RVGP expression in different culture conditions. **Objectives:** The aim of this study was to evaluate the influence of different culture temperatures in the expression of RVGP and its messenger RNA (*RVGPmRNA*) in recombinant S2 cells. **Methods:** S2AcRVGP-2k cells were kept in T flasks with serum-free culture medium for 15 days at 28ºC or 25ºC for temperature adaptation. Cells were then inoculated in Schott flasks with 20 mL of culture medium, 100 rpm shaking, for three days for adaptation to growth in suspension at the respective temperatures. Suspension cultures were further inoculated in triplicates and followed for 120 h. The cultures were kept at 28ºC or 25ºC during whole cultivation period. In an alternative protocol, the temperature was sharply decreased from 28ºC to 25ºC after 48 h of cultivation. The cell concentrations were determined by hemocytometer counting. RVGP concentrations were determined by ELISA and *RVGPmRNA* by qRT-PCR. Glucose, lactate and glutamine concentrations were analyzed by biochemical methods (YSI-2700). **Results and Discussion:** The decrease in culture temperature of S2 cells results in the decrease of exponential growth rate. Consequently, the cell cultures at 28ºC reached the end of exponential phase at 72 h while the cultures at 28ºC/25ºC and 25ºC reached it at 96 h. The metabolic analysis showed no differences in the glucose and glutamine consumption or in the lactate production among the cultures. We observed a reduction in the cellular and volumetric RVGP concentrations when the cultures were maintained at 25ºC. The *RVGPmRNA* levels showed increasing values between 72 h to 120 h for all cultures, but did not indicate a direct correlation with RVGP concentration levels or productivity. The *RVGPmRNA* levels were most likely related to the culture phase. When cells showed maximum growth rate, lower relative concentrations of *RVGPmRNA* were found. At the cell stationary growth phase, the relative *RVGPmRNA* concentration increased. This profile is considered as a consequence of a decrease in the levels of other messenger RNAs, much more than ones linked to the cell cycle. The increased amounts of *RVGPmRNA* in relation to total messenger RNA, did not generate an increase in RVGP levels because cells usually have less translation activity when entering the stationary culture phase.

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